



**Training Manual on  
DUS Test in Cotton  
with reference to PPV & FR  
legislation, 2001**

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## Dus Testing of crop varieties-Principles and Procedures

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### Introduction

Crop variety improvement research and development based on conventional breeding as well as biotechnological methods (GM varieties) require considerable investments, in terms of both scientific manpower and financial expenditure. Therefore, in order to attract investment and encourage progress and development, plant-breeding programs have to be protected from misuse, and ensure appropriate incentives to the breeders. Thus, an effective system of Plant Variety Protection (PVP) will not only be a safeguard against unlawful commercial exploitation of the new varieties but also stimulate the development of new varieties.

The International Union for the Protection of New Varieties (UPOV), with 54 member countries (as in 2004), provides and promotes an effective system of Plant Variety Protection (PVP) with the aim of encouraging development of new plant varieties for the benefit of mankind in member states. India is not yet a member. UPOV has developed guidelines for testing of more than 200 plant species before granting them protection. These Test Guidelines are used as standard reference document world wide in relation to the description of plant varieties and testing of plant varieties to be qualified for Protection in UPOV member states.

### Plant variety protection

General Agreement on Tariffs and Trade (GATT) recognized Agriculture as 'an enterprise of investment and profit making' and included it in the negotiations for the first time in Uruguay Round (1986-1994). This round led to the establishment of world Trade Organisation (WTO) in 1995 of which India is a signatory. The provisions of the Trade Related Intellectual Property Aspects (TRIPS) of WTO require that plant varieties need protection, either by parenting or by a 'sui-generics' system or by a combination of both (Mauria,2000). The Indian Patents Act of 1970 does not permit patenting of plants or varieties. Patents on plants, considered a strong form of protection, are available in advanced countries like USA, Japan under certain conditions. But in developing countries, patents are replaced by PVP (Plant Variety Protection) in recognition of the fact that 'variety development' involves improvement of already existing ones and not de novo creation. Involves improvement of already existing ones and not de novo creation. Accordingly, India had enacted its own PVP law, "Protection of Plant Varieties and Farmers' Rights Act" in 2001, with provisions for protecting both breeders and farmers rights. This act provides protection of new varieties including extant and farmer's varieties. The grant of plant breeders rights (PBR) entitle the breeder (or his successor, agent, licensee) to exclude others from producing, selling, marketing, distribution, export or import of propagating material of protected varieties for a period of 15 years. The act also permits a breeder to use a protected variety for research purpose. The act allows the farmer to save, sow, resow, exchange, share or sell farm produce including seed of a protected variety.

### Dus Testing

Thus, under the "Protection of Plant Varieties and Farmers' Rights Act", a new plant variety can be registered and protected for a specific duration; 15 years for annuals and 18 years for vines and trees. Registration and protection can be granted to a variety only if it conforms to the criteria of Distinctness, Uniformity and stability. It means that the new variety has to Distinct-

Uniform-Stable (DUS) in its characteristics. This requires the examination of the variety if it conforms to the standards of DUS test. The examination of a variety for DUS generates a description of the Variety, using its relevant characteristics. This examination of a variety is either conducted by the Plant Variety Protection Authority ('Official testing') or by the breeder seeking protection ('Breeder testing'). In some countries (Japan, New Zealand), both government or official testing and breeder testing are done. Official testing is common in European countries. Under breeder testing (as in USA, Australia), the applicant has to conduct the tests and demonstrate to the PVP examiner that his new variety meets the criteria of distinctness, uniformity and stability.

### National Test Guidelines

The principles and methods on various aspects of DUS testing of new crop varieties have been documented as National Test Guidelines (NTG). NTG has been developed for 35 crops by the National Core Committee constituted by ICAR. NTG contains details on plant material required, conduct of tests, methods and observations, grouping of varieties, characteristics and symbols, table of characteristics, literature and technical questionnaire. Usually the DUS examination requires at least two independent growing cycles. Not less than two centers have been identified for each crop for conduction the tests.

### Distinct-Uniform-Stable (DUS):

**Distinct** means a variety should be clearly distinguishable by one or more essential characteristics from any other existing variety. The variety is deemed **Uniform** if it is sufficiently uniform in its relevant characteristics, subject to variation that may be expected from the particular features of its population. The basis of assessment is normally the number of off-types in the variety, judged on the basis of a population standard and an acceptable probability fixed in the corresponding species. To identify off-types in a population, generally visual observation on characteristics may suffice. However, in a few cases/ crops, it may be necessary to make measurements of each plant to apply statistics to decide or not whether a plant is an off-type. In most of the crops, acceptance probability of 95% has been suggested. For vegetatively propagated and self-pollinated varieties, the following standard has been suggested:

Sample size	Off-types (permissible)
<5	0
6-35	1
36-82	2
83-137	3

Generally, cross-pollinated varieties exhibit wider variation within the variety. Relative tolerance limits can be found by comparing with comparable varieties. The standard deviation or variance may be used as the criteria for comparison. Recently, UPOV has proposed a statistical method called 'Combined Over Year Uniformity (COYU)' that takes into account variations between years for dealing uniformity in measured (quantitative) characters. The variety is said to be **Stable** if its relevant characteristics remain unchanged after repeated propagation. Though it is not usually possible to assess stability with in a period of 2 or 3 years, the variety can be considered stable if is shown to be uniform.

### Characterization

The requirement of distinctness, uniformity and stability are assessed on the basis of characteristics. The characteristics are a feature of whole plant or part of plant. Such

characteristics may be morphological, biochemical, molecular or any other nature. The table of characteristics chosen by experts forms the main part of test guidelines and of DUS testing. In Genetic resources, the term 'characteristic' is known as descriptors (with descriptor states) and describing a plant based on such descriptor is known as 'characterization'. (Table of characteristics for DUS testing in sugarcane is given in end)

**a) Morphological characterization:** This is based on botanical or morphological descriptors or characters of the plant or plant part.

### Types of characteristics

**1. Qualitative characteristics:** Truly qualitative characteristics show discrete discontinuous states and are stable, heritable and uniformly expressed in all environments (Ex. Shape, Flower colour, etc)

**2. Pseudo-qualitative characteristics:** Here, the range of expression is at least partly continuous varying in more than one dimension. In some cases, intermediate states of expression such as 'weakly expressed' are included between 'absent' and 'strongly expressed'. (Ex. Pubescence, Pigmentation, etc.).

Qualitative characteristics are assessed visually while quantitative characteristics are usually measured. The following types of assessments are recommended:

VG: Visual assessment by a single observation of a group of plants or parts of plant.

VS: Visual assessment by observation of individual plants or parts of plants.

MG: Measurement by a single observation of a group of plants or parts of plant.

MS: Measurement of a number of individual plants or parts of plant.

**a) Grouping characteristics** can be universally used, either individually or collectively, for grouping the similar varieties. These characteristics are considered to be most reliable in distinguishing or discriminating varieties.

**b) Biochemical characterization:** Isozyme based descriptors have been widely used for identification of crop varieties because of their reliability (Smith & Smith 1992, Cooke 1995). UPOV has also included electrophoresis of isoenzymes in maize, soybean, sunflower and of seed proteins in barley, wheat as additional characters for establishing distinctness of varieties. Selection of an appropriate electrophoresis technique provides a potential tool for variety identification, DUS test or grouping of varieties.

**c) Molecular characterization:** Biotechnology has widened the possibilities for applying such technologies to the problem of characterization, varietal identification and protection (Smith, 1995). The two commonly adopted approaches in the use of molecular markers are essentially either probe based such as RFLP (Restriction Fragment Length Polymorphism), or amplification based like RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), STMS (Sequence Tagged Microsatellites), etc. At present, molecular markers are not being used in DUS testing anywhere, but they are reliable, fast and cost effective to discriminate an EDV (essentially derived variety). Data from these methods may help resolve disputes on identity of germplasm or hybrids (Santhy et al, 2003)

### DUS test design

The use of experimental design with respect to the number of growing cycles, lay out of the trial, number of plants to be examined and method of observation is largely determined by the number and nature of varieties to be examined in a particular trial. In DUS trials, because of the presence of only one treatment factor (variety), the following designs are used

- 1. Completely Randomised Design-** if total number of test varieties is small. Several varieties are examined in a number of replications.
- 2. Randomised complete Block Design-** the number of plots per block equals the number of varieties and all varieties are placed in each block. The advantage is that Standard Deviation between plots does not contain variation due to difference in blocks.
- 3. Randomised incomplete Block Design-** in case of large number of varieties. Here, the number of plots per block is less than the number of varieties. In Poland, performed analysis of variance of the results of experiment concerning seven characters in pea varieties showed that randomized complete block and completely randomized designs were more effective than incomplete block (Pilarczyk, 1999).

### Reference collection

To test whether a candidate variety meets the DUS criteria, it is compared with varieties whose existence is a matter of common knowledge. To satisfy the requirement of distinctness, a candidate variety must be clearly distinguishable from all other existing varieties. These varieties are called the Varieties of Common knowledge, which includes:

- 1) Protected varieties
- 2) Varieties listed in official register
- 3) Varieties, subject of an application for protection
- 4) Varieties listed in any commercial document in which varieties are offered for marketing in its territory as propagating or harvested material, specially where there is no official registration system.
- 5) Ecotypes and land races
- 6) Publicly available varieties within plant germplasm collection (genetic resources, old varieties, etc.)

Hence, the competent authority before conducting DUS testing, is expected to collect, establish and maintain the collections of these Common knowledge varieties, in the form of viable seeds or of vegetative plant material of varieties. These will form the 'Reference Collection'. Theoretically, varieties in common knowledge have to be considered on a world wide basis and it is necessary to examine DUS criteria in relation to all varieties of common knowledge known worldwide. But, in practice, this can never be realized, as there are limitations in assembling all varieties on a national basis and every nation has to define strategy to produce a National Reference Collection for each crop. Therefore, it is obvious that the list of common knowledge varieties for a given species/crop will include a very large number of entries. It is also very important to ensure the authenticity of the collections as well as the source of collections. The issues on setting up and use of reference collections for DUS testing has been given by UPOV (1997)

Maintenance of a reference collection of known varieties is essential for efficient DUS testing. For vegetatively propagated species such as rose, potato, sugarcane, though regeneration of varieties is easy, the clones have to be field-maintained in a disease free condition and without loss of vigour. *In vitro* conservation could be an alternative and serve as a backup collection. In case be an alternative and serve as a backup collection. In case of other crops, viable seeds of reference varieties are placed in cold storage. The quantity of seed of some crops to be stored are given below

Crop	Seed quantity (g)	Crop	Seed quantity(g)
Cotton variety/hybrid	4000	Groundnut	7000
Cotton parental lines	4000	Pigeon pea	2000
Rice-variety/hybrid	3000	Soybean	3000
Rice-parental lines	1500	Linseed	500



After the establishment of the reference collection, documentation on the passport and characteristics (morphological, isozyme or molecular) is essential. A proper database on the collection has to be created so that searching and identifying varieties most similar to be candidate variety for examination of distinctness is made easy. Wherever necessary, those similar varieties may be grown and directly compared with the candidate variety in case of doubt or dispute.

### Use of statistical procedures in DUS testing

DUS tests are conducted to compare the varieties and the absolute determination of the characteristics. The measurement or observation of the characteristics are analyzed and based on analysis results, decision is taken on DUS criteria. In DUS testing, an experimental unit is a plot with certain plant population. The plot is a subdivision of the field about which the varieties are randomized. Within a plot, the observations are recorded on some characteristics on certain plants in each replication for estimating the variability of the variety. The mean of the individual plant observations in a plot can be considered as the plot measurement for that character. To address the variation of the data within a variety, the following statistical procedures may be adopted

1. Frequency Distribution or Histogram can be used to describe the values of measurement of quantitative characters.
2. Population mean, Variance and Standard deviation: The mean is the sum of all the expressions of the characters in the population divided by total number of observations. The square root of variance is called standard deviation. For quantitative normally distributed characters, the means of two varieties can be calculated. The comparison of two varieties can be made by computing least significant difference (LSD) between two means. If the difference between two means is greater than LSD, the two means are said to be different.
3. Combined-Over-Years-Distinctness (COYD): This is a procedure for computing maximum distance for establishing DUS. For testing of varieties for distinctness based on measured characters, there is a need to establish a minimum distance between varieties. The pair of varieties showing difference greater than the minimum are said to be regarded as Distinct in respect of that character. The method helps in analyzing quantitative data for open pollinated crops where intra-varietal variation occurs. In most countries, tests are conducted in one testing center for 2 or 3 years. For distinctness, the difference between pairs of varieties is tested on character-by-character basis. To determine the minimum difference analysis of variance is used to calculate LSD for comparing variety means. If the over years/locations mean difference between two varieties is greater than the LSD, then the varieties are considered to be distinct in respect of that character. If more consistency between years/location occurs for the position of different varieties, the minimum distance, which is required for assessing distinctness, will be smaller. On the other hand, if there is a strong interaction, the minimum difference will be enlarged. In COYD analysis, the stability of the relative varieties value is taken into account. This combines the information from different environmental conditions rather than considering centers separately. The basic values to be used in the analysis are the annual location variety means. For bulk sampling which gives at least one value for each variety per year / location, it will usually still be possible to use COYD method for distinctness for any degree of bulking as long as at least one value is recorded for each variety in each year/ location and that the bulk samples are representative for the variety. This method replaces a previous method (ANOVA based on individual experiment) in which analysis was year-by-year or location-by-location basis.
4. Combined-Over-Years-Uniformity (COYU): When the uniformity is judged based on measurement; the standard deviation (SD) can be used to summarize the spread of

observations. A new variety can then be tested for uniformity by comparing its SD with that of reference variety. However, in some species or crops, varieties with large plants tend to be less uniform than those smaller plants. If the same standard is applied to all varieties then it is possible that some will have to meet very strict criteria while the other face standard, which are easy to satisfy. Above-mentioned problem with SD is addressed by the use of COYU. This procedure adjust the relationship that exists between uniformity, as measured by plant-to-plant SD, and the expression of the characteristics, as measured by the variety mean, before setting a standard. The main advantages of COYU are that all varieties can be compared on the same basis and that information from several years of testing may be combines into a single criterion.

### Statistical software available

Many computer softwares for statistical procedures in DUS testing are in use abroad. Few examples of countries and name of programs used in DUS testing are listed in the table below

Country	Program Name	Functions
Japan	KIRI	General Data base software
Poland	POWT3 POWT5	-Analysis of categorical data -Analysis of variance (for cumulative records)
England	DUST	-Analysis of data from DUS trials
Germany	SAS-COYD SAS-COYH VERA	-Distinctness tests with 1-3 year trial results -Homogeneity test with 2-3 tear trial results -Generates randomized designs for variety trials with up to 225 entries.
Denmark	SAS	-for calculation of data from DUS & VCU trials
Netherlands	CIS  SCIL-image	-Oracle database with applications for DUS & VCU trials data storage and analysis -Image analysis package

### Literature

- Cooke R.J.1995. Review: Gel electrophoresis for the identification of plant varieties. Journal of Chromatography 698:281-299.
- Mauria S.2000. DUS testing of crop varieties-a synthesis on the subject for new PVP opting countries.Plant varieties and Seeds. 13:69-90.
- Pilarczyk W.1999. On efficiency of resolvable incomplete block design in DUS trials on French bean varieties. UPOV TWC/17/2.
- Santhy V., Mohapatra T., Dadlani M., Sharma S.P. & R.P.Sharma. 2000. DNA markers for testing distinctness of rice varieties. Plant vars and seeds 13:141-148.
- Smith J.S.C 1995. Identification of cultivate varieties by nucleotide analysis. In Identification of food grain varieties, Wrigley C.W. (ed.). AACC, USA.
- Smith O.S & J.S.C.Smith. 1992. Measurement of genetic diversity among maize hybrids. A comparison of isozyme, RFLP, pedigree and heterosis data. Maydica 37:53-60.
- UPOV 1997. Thoughts on the setting up and use of reference collection for DUS testing. Document TWA/26/5.
- UPOV. 2002. Use of statistical procedures in distinctness, uniformity and stability testing. Document TGP/8.5.
- UPOV.2000. The combined over years distinctness and uniformity criteria. Document TWC/18/10.

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## DUS testing of crop varieties- a synthesis on the subject for new PVP-opting countries

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The minimum requirement of an effective sui generic system for the protection of plant varieties under the GATT Agreement establishing the WTO points towards the available system under the UPOV. The UPOV Convention provides a system based on DUS testing of crop varieties, accepted and in operation in a large number of countries. A number of new PVP-opting countries are also becoming UPOV members to provide the required effective system. Realizing the present-day need for detailed DUS characterization of crop varieties, a synthesis on the subject is attempted. It discusses the criteria for DUS and important technical initiatives in UPOV, besides covering the discussions on new technologies like isozyme electrophoresis and DNA-profiling in DUS testing, the issue of essentially derived varieties, and developments in analysis of DUS data. The Conclusion section presents some essential elements to be considered for developing a DUS/PVP system in new PVP-opting countries.

### Introduction

The question of Plant Variety Protection (PVP) (or Plant Breeders' Rights-PBR) was brought into worldwide focus by the Agreement on Trade Related (Aspects of) Intellectual Property Rights (TRIPs), which is the part of the General Agreement on Tariffs and Trade (GATT) establishing the World Trade Organization (WTO) in 1995. It is largely a universal agreement with 131 countries being the contracting parties. Article 27.3(b) of the Section on TRIPs in GATT (GATT 1994) provides that (contracting) parties shall provide for the protection of plant varieties either by parents or by an effective sui generic system or any combination thereof. Thus, provision for an effective sui generic system by the contracting parties is the minimum requirement. A legislative framework for PVP is to be provided by the contacting parties in the specified time frame, by 1 January 2000 in developing countries (except least developed countries). The effectiveness of any sui generic system developed by any contracting parties is to be reviewed under the Agreement from November 1999. Being signatory to the Agreement, many developing countries, which were not hitherto having any such system, have either already initiated some form of system or are discussing the issue for putting a system in place.

Options for a sui generic system have been discussed by Leskien & Flitner (1997) but in the absence of any specified criteria, for judging the effectiveness, mentioned in TRIPs Section of the GATT Agreement, all thinking and developments point toward the system provided by the inter-governmental International Union for Protection of New Varieties of Plants (commonly known as UPOV –based on its initials in French - Union Internationale pour la protection des Obtentions Vegetables). UPOV provides a system already accepted and in operation in a large number of countries, and is making efforts to receive recognition under WTO. The Director General of the United Nations World Intellectual Property Organization (WIPO) is the Secretary General of UPOV.

The UPOV Convention has two main functions viz. i) it prescribes minimum rights that must be granted to plant breeders by its member States, that is to say, it specifies a minimum scope of protection; and ii) it establishes novelty, distinctness, uniformity and stability, and the requirement of a suitable denomination, as the standard criteria for the grant of protection. The UPOV Convention was signed in Paris in 1961, it entered into force in 1968. It was revised in Geneva in 1972, 1978 and 1991. The 1978 Act entered into force on 8 November 1981. The 1991 Act entered into force on 24 April 1998. A concise description of technical criteria for protection is provided in UPOV (1996).

Currently, UPOV has 46 member States with only two States i.e. Belgium and Spain bound by the UPOV's 1961 Act (as amended by the Additional Act of 1972), 29 States bound by the 1978 Act and 15 States bound by its latest 1991 Act. Among these, there are only two States from Africa (Kenya, South Africa) and 2 only from Asia (China, Japan). A number of Latin American developing countries have also now become UPOV member now and most of the UPOV member States are in the process of amending their laws to conform to the 1991 Act.

Both the 1978 and 1991 Acts have been discussed and debated for implementation in countries which so far are not members of UPOV. One important difference relevant in the present context between the 1978 and 1991 Acts is that, in the UPOV (1978) Act, a protected variety can be modified in a very limited respect e.g. by reselection, mutation, the addition of a gene etc. and provided variety it can be separately protected without any obligation to the breeder of the protected variety. The UPOV (1991) Act provides that varieties that are "essentially derived" from a protected variety in this way can still be protected but cannot be marked without the permission of the breeder of the protected variety from which they are derived. Varieties are "essentially derived" for this purpose only when they are virtually entirely constructed upon the basis of the protected varieties from which they are derived. Discussions are also alive on the issue of unprotected varieties already in public domain.

Realizing that the UPOV (1991) Act will ultimately come into force to have its impact on global agriculture, many developing countries like India, and countries of the Andes region (Seiler, 1998), also included this clauses in their draft legislations considering their own strengths in variety development, testing and commercial release. Obviously, the requirements of Distinctness, Uniformity and Stability (commonly referred to as DUS or sometimes as DHS, wherein "Uniformity" is substituted as "Homogeneity") under UPOV to grant PVP have to be first understood on extant crop varieties of a country. This is necessary because for granting PVP certificates to new varieties, consideration has to be given to obsolete varieties no more in trade as they are of common knowledge, even if they are known by incomplete and imprecise description in literature. Besides, the criteria of common knowledge in granting a PVP certificate cannot be limited to national borders, and similar varieties known anywhere in the world may have to be taken into account. In a new PVP-opting country, its extant varieties can thus initially serve as basic materials to study DUS followed by establishing DUS in new crop varieties alongside the references developed from extant varieties. These extant varieties can thus serve as example varieties, for comparison for character states of particular characteristics, and for studying DUS on new crop varieties. In DUS testing, the principal test is of Distinctness, and detailed examination of diagnostic characteristics of crop varieties and their parental lines has become imperative, to establish this requirement.

With the above background, the following is an effort to synthesize at one place the available information on DUS testing of crop varieties, which may be helpful particularly for the new PVP-opting countries where DUS testing systems would have to be established ab initio. It includes the important discussions in UPOV, which is playing an important role in assisting its member States and new PVP-opting countries in their endeavor of developing appropriate systems for meeting the requirements. It needs to be noted that while there may not be much difficulty in establishing a system de nova in any new PVP-opting country by largely adopting from already established systems in many developed countries, there are still many unresolved issues, which UPOV continues to deliberate upon to continually guide its member States and other interested countries / organizations. The author's personal understanding, developed while attempting DUS testing in India for the first time following UPOV Test Guidelines using Indian public maize inbred, was the incentive for attempting this synthesis.

## Need for detailed examination of Diagnostic characteristics of crop varieties

In early days, all over the world, a small list of descriptors was sufficient to distinguish between crop varieties in use. However, in the recent decades, the world witnessed the emergence of large and highly competitive variety development programmes, particularly in the developed countries and in some of the developing countries. At the global level, a large number of new candidate varieties are being generated for testing every year, thus underlining the need for establishing their clear-cut diagnostic features. The technology-rich developed countries had obviously realized this requirement much earlier and had, accordingly, tuned their systems to meet the requirement. In most of the developing countries now considering implementing a PVP-system, while certain diagnostic features for released crop varieties are generally known and followed in seed certification procedures, accurate identification keys, giving detailed description on a comparative basis with clear-cut features of distinctness are, in general, lacking; and thus cases of confusion in seed certification and quality control, if such systems are existing, are also not uncommon. The example of India, who benefited greatly from the Green Revolution is cited and compared here. The country has an established system on variety development, testing and release; and over 2600 crop varieties are already notified for commercial cultivation.

In India, the variety testing and release system, undertaken through a crop commodity-specific coordinated varietal evaluation system with a large network of cooperating centers in public and private sectors, basically concentrates on generating data on parameters like yield, quality, reaction to important diseases and pests under field conditions and artificial epiphytotics, performance under different agronomic management schedules etc.(Tandon, 1992). It is thus more akin to a VCU (Value for Cultivation and Use) test in the European Economic Community (EEC) countries aimed at stimulating plant breeders to produce varieties, which are an improvement over existing varieties (Bould, 1992) done by the Government-designated authorities. The latter test requiring a detailed botanical examination using a standard list of descriptors and is intended to remove any confusion in naming new varieties, which was a major problem in EEC countries in earlier times. Besides meeting the requirement of registration of the new variety, the botanical description is also used for awarding PVP, for which uniqueness is an essential requirement.

The need for a detailed examination of diagnostic characteristics thus becomes imperative in new PVP-opting countries to maintain identity of released and notified varieties and their parental lines. With respect to India, Sharma (1991) has indicated that no system of variety registration exists in India apart from the rather vague variety release proposals provided by the breeders and there is a need for establishing a DUS testing system. Such work additionally assists in protecting morphologically, and often agronomically, similar but distinct varieties when a PVP system is established de novo in a country. The requirement also finds support from the work of Singhal & Prakash (1992) who have identified a high degree of resemblance in morphology in recently developed wheat varieties in India. Virk & Witcombe (1998) have also stated that the selection strategy employed in the All India Coordinated Crop Improvement Programmes, the system of multi-locational testing of new varieties, concentrates on selection for yield with emphasis in selection on one important adaptive trait i.e. flowering time, towards which there is strong stabilizing selection.

A similar, or even less intensive, system is the general phenomenon in most of the developing countries. In the USA, the entire responsibility for identity of seed material and DUS testing rests with the breeder, and the records provided by the breeders are maintained in PVP offices providing PVP-certificates. In Australia, New Zealand and a few other countries, systems marginally different than of EEC countries are in existence.

Also, the technical criteria for the grant of rights differs from one country to another and even the variety concept is not seen in the same light in all the countries. The technical standards

and testing procedures likewise depend largely on the expertise of the officials concerned. This lack of harmonization has caused problems, especially when a breeder sought protection for his variety in several countries.

Obviously, the developed countries and their private seed sector considered availability of effective PVP systems as necessary in developing countries in order to safeguard the interest of plant breeders as well as to play an important role in the global agriculture. This was made possible by getting included the requirement of an effecting systems for plant variety protection through the multilateral negotiations under GATT. As contracting parties in WTO, and recognizing current efforts by UPOV for harmonization of procedures, a general description of UPOV's criteria is first provided for such new PVP-opting countries.

### **UPOV's criteria for Distinctness Uniformity and Stability**

A technical examination performed according to standardized principles established by UPOV comprises a comparative growing trial which involves sampling, observation and measurement, processing and evaluation. These trials are conducted either by the national government authorities themselves or on their behalf by specialized bodies or, to varying degrees by the applicants or breeders themselves. In UPOV, the crop-specific test guidelines for conduct of DUS tests are supported by a general introduction to these test guidelines (UPOV, 1979). This general introduction to crop-specific test guidelines is presently under revision (UPOV, 1999a), and is likely to be oriented for guidelines on DUS testing procedures as a whole rather than just supporting the crop-specific Test Guidelines, thereby also aiming to provide guidance on DUS testing where there are no UPOV Test Guidelines available (e.g. for new species). Nevertheless, the majority of the points on conduct of DUS tests are well-accepted principles. Accordingly, a brief introduction on UPOV's criteria for Distinctness, Uniformity and Stability is synthesized here to serve the immediate requirement of new PVP-opting countries.

#### **Distinctness**

According to Article 7 of UPOV (1991) Act, a variety shall be deemed distinct if it is early distinguishable from any other variety whose existence is a matter of common knowledge at the time of the filling of the application. Two varieties have to be considered distinct if the difference i) has been determined at least in one testing place, ii) is consistent.

In the case of true qualitative characteristics, two distinct varieties show expression, which fall into two different states. In the case of other qualitative handled characteristics (i.e. some visually assessed quantitative characteristics), a degree of continuity has to be taken into account in establishing distinctness, and thus a different character state not be sufficient to establish distinctness.

When distinctness depends on measured characteristics, the difference has to be considered clear if it occurs with one percent probability of an error, for example, on the basis of the method of the Least Significant Difference (LSD). The differences are consistent if they occur with the same sign in two out of three growing seasons. In order to take into account the variation between years, UPOV has developed a more sophisticated method, the Combined Over Years Distinctness (COYD) method. It is supplemented by a further LSD method for cases where certain standards required for the COYD analysis cannot be met. Its main use is for measurements in cross-fertilized varieties, but, if so desired, it can also be used for measurements in vegetatively propagated or self-fertilized varieties.

If a normally visually assessed quantitative characteristic is the only distinguishing characteristic in relation to another variety, it should be measured, in case of doubt, if this is possible with reasonable effort. A direct comparison between two similar varieties is advised since pair-wise comparisons show the least bias. Distinctness can be established if consistent differences (significant differences with the same sign) are found in pair-wise comparisons, but if

they can be expected to recur in the following trials. The number of comparisons has to be sufficient to allow a comparable reliability as in the case of measured characteristics. In some cases, differences between two varieties may be observed in several separately assessed characteristics but the establishment of distinctness depends on using a combination of such data. Currently, combined characteristics may only be used where they have clear biological meaning and the degree of reliability is comparable with that provided for measured or normally visually observed characteristics.

In identification of characteristics for establishing distinctness, UPOV has obviously put those characteristics, in individual Test Guidelines, on which sufficient knowledge and experience has been gained. Nevertheless, it is also indicated that the tables of characteristics provided in the Test Guidelines are not exhaustive and may be enlarged by further characteristics if this proves to be useful. Such characteristics should obviously have discriminating properties. Different degrees of uniformity are not accepted as a characteristic for distinctness.

It must also be mentioned here that the crop-specific Test Guidelines are harmonized for making descriptions and include a list of characteristics, and their character states, which are indicated by different numerical Notes on a 1-9 scale. For the decision on distinctness, uniformity and stability, these guidelines only represent the first step. The Test Guidelines are silent on the minimum distance (discussed later) required in each characteristic and thus a decision on distinctness can never be based on the description resulting from the Test Guidelines. However, to make the first step meaningful and allow a first idea on the possibility of distinction, the following is advised to be observed.

- i. In true qualitative characteristics, each character state is clearly separated from the other without any transition; the minimum distance is therefore always one Note. There are, however, only very few true qualitative characteristics.
- ii. In quantitative characteristics which are observed visually, it should be aimed at setting up a scale of states- if possible as a rule with a difference of two Notes- which could lead to a clear difference ( this is meant by the requirement that the states be meaningful). However, these two Notes are no absolute standard for the minimum distance. Depending on the testing place, the year or other environmental conditions, variety collection or special pair of varieties, the minimum distance may be more or less than two Notes, e.g. three, four or five Notes in a characteristic affected to a larger degree by the environment; or may be one only or even inside one Note, distinction may be possible. It is up to the expert doing the observations to take the necessary precautions. The variety description based on the Test guidelines should therefore never be used alone for the decision on distinctness and a general yardstick of two Notes is only an aim for the experts who draft the Test Guidelines but never for the user.
- iii. Characteristics which are handled like qualitative characteristics, but which are not really qualitative characteristics, should be handled in such a way that possible fluctuations are taken into account when distinctness is assessed. Therefore, one cannot automatically presume that the minimum distance is one Note. The sequence of the states should in such characteristics rather be chosen in such a way that as a rule a minimum distance is two Notes could be expected. Accordingly, the states may be for instance for growth habit; erect (1), semi-erect (2), prostrate (3) in one species; and erect (1), semi-erect (3), intermediate (5), semi-prostrate (7), prostrate (9) in another species; and for a third species the states may be set up again in a different way. The same reservations as for quantitative characteristics apply, however, and the description based on the Test Guidelines should not be used alone to take a decision on distinctness.

In practice, some countries regard consecutive states of true qualitative characteristics to be distinct (1 & 2), while only every second state of a quantitative characteristics is regarded as distinct (1 & 3, 2 & 4). The majority of the UPOV member States do not follow this idea. There is a frequent misinterpretation of the use of the Test Guidelines, which may stem from the title of the



Test Guidelines. The function of the Test Guidelines is mainly for description purposes. Experts have to be avoiding mixing description and distinction of a variety. It is possible that two samples of plant material could have different descriptions but is not sufficiently distinct to be from two varieties eligible for protection. Therefore, the yardstick of two states of expression in quantitative characteristics is for the drafter of the Test Guidelines and not for the user. Test Guidelines are, as stated, merely guidelines and not instructions for the testing at a certain place.

### **Uniformity (Homogeneity)**

According to Article 8 of UPOV (1991) Act, the variety shall be deemed to be uniform if, subject to the variation that may be expected from the particular features of its propagation, it is sufficiently uniform in its relevant characteristics. In effect, it means that variation shown by a variety, depending upon its breeding system, must be as limited as necessary to permit accurate description and assessment of distinctness and to ensure stability. This requires a certain tolerance, which will differ according to the reproductive system of the variety. The approaches to vegetatively propagated varieties, truly self-pollinated varieties, mainly self-pollinated varieties; cross-pollinated varieties, synthetic varieties and hybrid varieties are thus necessarily very different.

In case of vegetatively propagated and truly self-pollinated varieties, the maximum acceptable number of off-types in samples of various sizes is specifically provided in the Test Guidelines. For mainly self-pollinated varieties, a higher tolerance is admitted and the populated standard for the calculation of the maximum number of off-types allowed for vegetatively propagated and truly self-pollinated varieties is generally doubled.

In the case of cross-pollinated varieties, including synthetic varieties, which normally exhibit wider variations within the variety, relative tolerance limits are used through comparisons with comparable varieties already known. For measured characteristics, a variety is considered not to be homogeneous in the measured characteristics concerned, if its variance exceeds 1.6 times the average of the varieties used for comparison (UPOV, 1979). In order to take into account variations between years, the Combined Over Years Uniformity (COYU) method has been developed, which is a further development of the earlier mentioned COYD method used for distinctness. In the case of visually assessed characteristics, the number of plants visually different from those of the variety should not significantly (5% probability of an error) exceed the number found in comparable varieties already known.

Single cross hybrid varieties have to be treated as mainly self-pollinated varieties, but an additional tolerance has to be allowed for inbred plants. It is not possible to fix a percentage as the decisions differ according to the species and the breeding method. The maximum number of off-types tolerated is proposed to be fixed and provide in the Test Guidelines of that crop. For other categories of hybrids, a segregation of certain characteristics is acceptable if it is in agreement with the formula of the variety. If the heredity of a clear-cut segregating characteristics is known, this characteristic has to be treated as a qualitative characteristic. If the described characteristic is not clear-cut, it has to be handled as in the case of other kinds of characteristics of cross-pollinated species with high-inbred depression or non-uniform parent lines, only relative uniformity standards are advised to be applied. It is for the national authorities to take the decision where the parent did not show uniformity and it follows from this that the formula of the hybrid must be known to the testing authority.

In individual crops' Test Guidelines, parameters like sample size, population standard, acceptance probability and maximum number of off-types permitted are also indicated to meet the requirements of Uniformity. Any plant is to be considered an off-type if it differs in the expression of any characteristic, of the whole plant or part of the plant, from that of the variety, taking into consideration the particular species. An admixture is considered an off-type. However, plants that are very different from the variety may be disregarded as long as their number does



not interfere with the test (dependent on the judgment of the expert). An interim period for adjustment to adoption of Test Guidelines is also provided for new PVP-opting countries.

### **Stability**

According to Article 9 of UPOV (1991) Act, a variety shall be deemed stable if its relevant characteristics remain unchanged after repeated propagation or, in the case of particular cycles of propagation, at the end of each such cycle.

It is not generally possible during a period of two to three years (testing time required) to perform tests on stability which lead to the same degree of certainty as the testing of distinctness and homogeneity. Generally, when a submitted sample has been shown to be homogenous, the material can also be considered stable. Nevertheless, during the testing for distinctness and homogeneity, careful attention has to be paid to stability. As far as necessary, stability has to be tested by growing a further generation or new seed stock to verify that it exhibits the same characteristics as those shown by the previous material supplied.

### **Important Technical Initiatives in UPOV**

UPOV is continually striving to harmonize the systems developed in individual countries. Thiele-Wittig (1992) reviewed the development of technical work is handled by five Technical Working in UPOV. In UPOV, the technical work is handled by five Technical Working Parties, four on different crop-commodity groups (viz. agricultural crops, fruit crops, ornamental plants and forest trees, and vegetables), and one for automation and computer programs (TWC). Besides, there is a Working Group on Biochemical and Molecular Techniques and DNA-profiling in particular (BMT). The Council of UPOV has appointed a Technical Committee to which the Working Parties report.

UPOV's main achievements in the technical field cover important topics viz. common understanding on basic principles for the testing of new varieties, harmonized and adopted individual test procedures on about 170 genera or species, cooperation between member States and distribution of tasks, cooperation with applicants and breeders in the growing tests, discussion on use of new technologies and data analysis methods including on new types of characteristics like electrophoresis, colour measurements, image analysis etc.

The general introduction to the test guidelines and crop-specific guidelines on conduct of DUS tests provide the basic information. Crop-specific test guidelines mainly cover the traditional morphological and physiological characteristics studied in field testing of materials. Other characteristics obtained with the help of new, generally laboratory-based technologies, which have recently been considered in a few species, have to fulfill the same requirements as other traditional characteristics before being accepted for DUS testing. There should be an accepted standardized method for observation of the characteristic and it should lead to reliable and repeatable results.

Important subjects discussed by TWC include inventory of databases and their structure, inventory of existing hardware, inventory of data processing functions applied and/ or required in the plant variety protection offices, methodology for the testing of homogeneity in cross-fertilized and self-fertilized crops, evaluation of combined over years analysis for distinctness and uniformity testing, and content and format of descriptions of varieties to facilitate automation. Information from UPOV could hitherto be obtained only on paper, but initiatives have begun in the recent past for computerization of the information bank available with UPOV and its member States.

## DUS data from new technologies

### Isozyme electrophoresis

A survey on use of rapid variety identification techniques in laboratories of the International Seed Testing Association (ISTA) revealed that (bio) chemical tests were most frequently used in the small number of responding countries (van der Burg & van Zwol, 1991). The study inter alia concluded that although rapid techniques were receiving more attention from seed testers and other users, surprisingly little development work have been done in this area during the last twenty years. The conclusions also stated that if electrophoresis and DNA-fingerprinting techniques like RFLP analysis are to be used as rapid identification techniques, more specific attention will have to be given to the development of simple and standardized laboratory protocols.

The observed general absence of use of electrophoresis as a routine procedure in a number of ISTA-recognized laboratories despite availability of enormous published literature on development of standardized protocols for electrophoresis technique and their utility (in establishing variety identity, genetic diversity analysis and other aspects) led UPOV to undertake detailed studies, through its technique in relation to the award of PVP's (Thiele-Wittig, 1992). As a result, barley and maize as supplementary but non-mandatory characteristics for establishing variety distinctness.

A general discussion on the use of electrophoresis with respect to variety identification and plant breeder's rights has been presented by Cooke (1989) for autogamous species and Gilliland & Almgard (1978) and Bailey (1983). The literature is, in fact, eplete with publication on use of electrophoresis for genetic identification, classification or distinctness testing, or characterization or description of varieties / genetic resources (Konarev et al., 1979, 1987; Goodman & Stuber, 1980; cooke, 1984, 1988, 1995; Draper, 1987). Isozyme data have been used in preparation of keys for classification of lines / populations (presence / absence of bands, identifying major groups), providing representative phenograms, determining allozyme frequency of different loci, identifying rare alleles, understanding heterozygosity / homozygosity at different loci, accounting for residual variability in developed lines, explaining unexpected variation in genetic purity analysis etc. Evaluation of genetic purity of seed lots using electrophoresis has also been reported, e.g. in maize by Motto et al. (1991), etc. Publications like Smith (1984), Smith & Smith (1987) and Higginbotham et al. (1991) have specifically dealt with multivariate and cluster analysis of isozyme data in maize, whether alone or in combination with other data. Electrophoresis studies from various angles are supported by publications on standardization of methods and on interpretation and scoring of gels (Tanksley & Orton, 1983; Bourgoin-Greneche & Lallemand, 1993). Standardized protocols are provided in respective crop-specific UPOV Test Guidelines wherever electrophoresis is included for studying DUS.

In UPOV, it is now held that the requirements for inclusion of electrophoresis-based characteristics should include a good knowledge of the genetic background, a standardized method and a positive result of a ring test between member States on the method. The characteristics are intended to be used as a last report if other characteristic fail to establish distinctness (UPOV, 1994a). Unlike many morphological or physiological characteristics, which are marked with an asterisk (\*) in crop-specific Test Guideliness, meaning a 'compulsory' characteristic for recording observation in every growing period of examination (except when the state of expression of a preceding characteristic or regional environmental conditions render this impossible), the characteristics derived by using electrophoresis are placed in an annexure to the Test Guidelines, thereby creating a special category of characteristics, because the majority of UPOV member States consider that it is not appropriate to establish distinctness solely on the basis of a difference found in a characteristic derived y using electrophoresis. Such characteristics should, therefore, only be used as a complement to other differences in morphological or physiological characteristics. In interpretation of electrophoretic bands, each locus should form one characteristic and each allele one state expression.

Isozyme-based descriptors were included because of the advantages of biochemical tests over morphological methods for variety identification. Notwithstanding the chances of sampling or technical error, the biochemical tests are rapid, relatively cheap, eliminate the need to grow plants to maturity and are largely unaffected by the growth environment. In addition, the codominant nature of the genes controlling their expression means that heterozygotes can be recognized. Further, morphological traits encompass a range of complexity regarding the genetic basis of their control, as these traits, in general, have not been genetically mapped. Therefore, morphological characteristics in several instances cannot form the basis for a very objective determination of difference or distinctness. However, even analysis of the usually highly polymorphic isozymes / proteins may not always provide a means of identifying varieties unequivocally. It was thus agreed that new methods (like bands in the case of electrophoresis) not used for the testing of distinctness should not be used for the screening of varieties for the layout of trials unless there was a strong correlation between such results and a morphological or physiological characteristic used in the Test Guidelines.

In discussion of electrophoresis characteristics in Test Guidelines, the Maize Section of ASSINSEL (International Association of Plant Breeders for the Protection of Plant Varieties) in France played an important role. It firmly and unanimously opposed the introduction of isozymatic markers into the Guidelines (UPOV, 1994b). While this Section recognized the value and good repeatability of this method in maize, it considered them to be inappropriate because the additional cost would not be trivial in either time or effort as many breeders do not routinely use isozymes as a breeding tool, and the apprehension that isozyme techniques will only be the first of many of biomolecular type to be officially used in maize DUS studies (as other techniques like DNA-profiling may subsequently find use as a routine measure in DUS testing of maize varieties). This group formally inter-alia demanded that enzymatic markers should be additional and non-mandatory, and used with the agreement of the breeder in cases where morphological and / or physiological markers are not sufficient. The breeder would later on bear the consequence of his decision in his breeding and maintenance programme.

### **DNA-profiling**

DNA-profiling offers significant advantages over biochemical tests which assay gene products and which may be profoundly influenced by tissue-specificity and the development state. Also, compared to morphological and biochemical traits, a thorough sampling of genomic and genetic diversity is possible in DNA-profiling. The drawbacks of DNA-profiling are time, expense and technical difficulty (Ainsworth & Sharp 1989).

A similar view of the consequences for the breeder is held for the use of DNA-profiling for DUS testing (UPOV, 1994c). Realizing the large resolution power of DNA-profiling, it was generally accepted in UPOV that although the member States at present are not able to use DNA-profiling for DUS testing, it could be considered as one possibility in future as complementary information. DNA profiles could identify genotypes, which had been proved to be distinct by other means and could give much information, which could be used to choose the best reference variety. The decision would continue to be made on the basis of the expression of the genotype e.g. morphological or physiological characteristics. There existed thus, two sets of characteristics, one used for establishing distinctness and another set of additional characteristics used only for identification. The DNA-profile would thus just be help and not basis for establishing distinctness.

In UPOV, even though the majority of the earlier reports centred on the RAPD and RFLP methods of DNA-fingerprinting, in its fourth session, the BMT Working Group also considered the AFLP method to be of better repeatability and more reliability owing to its seemingly unlimited capacity to produce data (UPOV, 1997a). In this meeting, the RAPD method was left aside as, in general, its origin is not known, whether from the expressed or non-expressed part. The same band could also result from different loci UPOV (1997 a) has also particularly indicated that while

RFLPs and SSRs could cover the whole genome, the use of AFLPs and SSRs made it possible to avoid the use of radioactive material and thus was better for the environment. Besides, it also mentions that while RFLPs and SSRs could cover the whole genomes; the SSR are more discriminative, reliable and repeatable, could potentially be standardized more easily and there already existed good hardware and software for the method.

It is thus clear that the development of each of the methods as well as the search for new methods is progressing very fast, and even DNA-profile may come in use in DUS testing in future. So far, as already stated, use of electrophoresis for DUS testing in a few crops has been included to provide supplementary information. For cross-fertilized crops, many experts, including all experts from the breeders, are entirely opposed to such a use. UPOV has thus set up a special sub-group to continue discussions on the possible use of electrophoresis for DUS testing in cross-fertilized crops but encountered difficulties and the topic is not being pursued for the time being.

### **Other possible methods**

Other methods like cytology, isoelectric focusing, high performance liquid chromatography, colour intensity measurement, image analysis, pedigrees and heterosis data can also be used to understand distinctness or identity of individual materials as well as the genetic relatedness among different lines.

Each method obviously has its own limitations or advantages. Since morphological data are affected by environmental interaction, descriptions must be made with sufficient replications, and in these circumstances, valid comparisons are only possible for descriptions taken at the same location during the same season (Smith & Smith, 1988). However, this view may not hold good especially for truly qualitative characters. Laboratory-based methods would obviously also require appropriate sample sizes for valid comparisons besides the standardized procedures for testing, some of these techniques provide the opportunity to compare precise genotypes with those that would be expected on the basis of stated pedigree. Pedigree data provide an estimate of relatedness that is based on all genes but estimated relationships can be inaccurate since they are based on the assumption of an equal contribution of genes from both the original parents, that formed the  $F_1$  hybrid, from which the progeny inbred lines were derived following several generations of selection and self-pollination (Kempthorne, 1969; Delanny et al., 1983). Furthermore, not only do pedigree data take no account of selection, they may be unavailable or in error. In heterosis data, the precise locations and magnitudinal effects of numerous widely spread loci in the genome are not known. Also, heterosis data have shown relationships between lines that closely mirror those to be expected on the basis of known pedigree. For these reasons, heterosis is generally considered to be an indicator of genetic relationships, at least across a relatively limited range of germplasm as would usually to be the case with elite breeding materials (Moll et al., 1965).

Notwithstanding the merits and demerits of different methods used in variety identification (or diversity analysis), the criterion of variety individually mandates that the usefulness of various traits as genotype descriptors and the level of distance at which uniqueness will be recognized be investigated. Therefore, the objectives of any study on DUS characterization and diversity analysis should be to compare the abilities of different methods to describe and to reveal associations among varieties and their parental lines.

### **Essentially derived varieties and minimum distance between varieties**

Plant Breeders' Rights or Plant Variety Protection Regimes allow the use of protected for further breeding (Breeders' exemption). In some cases, this has, however, led to problems e.g. mutants in ornamentals and fruits. Accordingly, the UPOV (1991) Act introduced the principle of essential derivation would considerably reduce the present pressure on the minimum distance

between varieties, as it will discourage plagiaristic approaches (Thiele-Wittig, 1992). The high-resolution power of isozymes and DNA-profiling characteristics would lead to reduction of the minimum distance required between varieties. Accordingly, 'it was understood that the distance should not be reduced because of the increased power to distinguish. The tool and the power of the tool should be seen separately.' (UPOV, 1994c).

After an in-depth discussion on the issue of minimum distance between varieties, it was preferred 'to search for objective assessment of the genetic distance, crop by crop, discusses the threshold for each crop and try to reach a common agreement among breeders. The advantages and disadvantages of each of the methods, their limits and the way of calculating and interpreting the results should be discussed and fixed crop by crop.' (UPOV, 1994a). The same principle will apply to the use of a colour meter for measurement of intensity of pigmentation in a plant part or image analysis (Draper & Keefe 1989) in future leading to more accurate measuring of morphological characteristics, which can also result in smaller variety distances.

The debate on minimum distance between varieties so far remains inconclusive for obvious reasons. Thus, Baltjes & Ghijsen (1992) have suggested, 'For the time being, electrophoresis and molecular techniques could be used to screen the reference collections in order to minimize the testing work. It might also be possible to label new varieties with a kind of bar code, like an electrophoregram or DNA fingerprint, in order to facilitate identification.' However, it is just an opinion of these authors and is not the position held by UPOV.

### **Analysis of DUS data**

A number of publications on computer management systems based on well-known statistical procedures for analysis of data from DUS trials or for crop variety selection have appeared in the literature (Kelly, 1968; Patterson & Talbot, 1974; Tonkin, 1974; Weatherup, 1974, 1994a; Eade & Law, 1983; Richards et al., 1989; Jarman & Hampson, 1991). Other publications related to this subject cover aspects like role and use of biometrics in DUS testing (Baltjes, 1986a), use of artificial classification in crop variety distinctness testing (Higgins & Evans, 1986), concept of distinctness in PBRs (Schneider, 1986), block design for variety trials (Patterson, 1978, 1984; Weatherup 1980, 1994a, b; Patterson & Weatherup, 1984), analyzing data over many sites and seasons (Silvey & Fiddian, 1972; Silvey, 1978), variability in results of a large number of trials for guidelines in planning future series of trials (Patterson et al., 1977) and technical aspects of plant breeders' rights (Baltjes & Ghijsen, 1992). UPOV has also deliberated on aspects of analysis of DUS data in its different documents.

Therefore, a good amount of research has gone into planning of and deriving meaningful results from trials aimed at developing statistically-sound detailed DUS characteristics of developed crop varieties. However, universally accepted and adopted single-run computer programs for easy applicability is only a recent development in UPOV to find a place in all UPOV member States.

Use of univariate method of statistical analysis is obviously the first choice in understanding aspects like within variety uniformity, comparisons between varieties, evaluation of individual characteristics, determining the essential characters and identifying the minimum character set. This is because separations made by univariate methods can be more readily explained in terms of simple plant features such as height, time of anthesis or silking (in maize), presence / absence of pigmentation in plant parts etc. Further, univariate analysis can provide information on discriminating power of individual characteristics. However, in many cases, assessment of the combined effect of several characteristics is desired to discriminate between pairs of varieties, which cannot be separated by univariate tests. In such problem cases, multivariate tests are required to enable such separations to be made objectively (Weatherup, 1980).

Weatherup (1980) has shown the application of multivariate tests to a set of specimen data obtained from Italian Ryegrass (*Lolium multiflorum*) using the statistics Coefficient of Racial Likeness, Mahalanobis distance, Canonical variate analysis and Cluster analysis, and



devised a DUST computer system, which was further improved in Weatherup (1994a). In Weatherup (1994b), the multivariate Mahalanobis Generalised  $D^2$  statistics was proposed as an alternative to repeated univariate tests on the measured characteristics in varietal distinctness testing. Weatherup (1994b) defines a method which assists in the interpretation of distinctness based on  $D^2$ . It is also cautioned that only a small number of characteristics, typically just two, are needed to establish distinctness using  $D^2$  and the use of more characters can lead to fewer significant differences between varieties.

UPOV (1994a) has also identified the potential of multivariate tools-mainly for distinctness, but also for uniformity, and foresees an important role of multivariate techniques in the field of essentially derived varieties, and also in the interpretation of data generated with biochemical and molecular techniques. From its discussion on use of multivariate analysis, the following conclusions are listed.

- i. Multivariate analysis would come into play, in respect of two varieties (a 'problem pair'), when these varieties cannot be distinguished using the CYD analysis and the crop expert feels that they are distinct.
- ii. Multivariate analysis can lead to a significant ( $P < 0.01$ ) difference only if the most significant difference (in the 'best characteristics') is close to the distinctness threshold of COYD.
- iii. Multivariate analysis will (if at all) lead to a significant difference using two or at most three characteristics.

Use of multivariate approaches will obviously come when varieties cannot be separated by univariate tests. While multivariate approaches of analysis of DUS data are still under discussion in UPOV's relevant technical working parties Baltjes & Ghijsen (1992) reported that many breeders on smaller differences on individual characters provided that three are several characters just falling short of the present UPOV criterion of significance at LSD 1% probability level. Such inclination is obvious because the total impression of a variety is considered more important than distinctness on the basis of just one simple character, and breeders and variety experts have realized difficulties in establishment of distinctness of a variety on the basis of one character based on LSD value at 1% probability level in two out of three years. For example, there may be two varieties, though classified identically, are distinct. That is, these may be two varieties falling within the same class but the observed difference between them may exceed the LSD 1% value during the examination. That is, there are limitations to application of the very concept of LSD. Nevertheless, in view of such reported criticisms of the LSD criteria, use of multiple range test (e.g. Newman-Keuls, Duncan) has been advised (Baltjes & Ghijsen, 1992).

Inclination for smaller differences in establishing distinctness is also there because the two out of three criterion does not take into account a difference which just fails to achieve the 1% significance level. To overcome this weakness, Patterson & Weatherup (1984) proposed the use of 't-score criterion' which allows a range of actual t-values between the 5% and 0.1% significance level to be used. Several Technical Working Parties in UPOV have also asked for a more simple test, like t-test, in comparison to Combined Over Years (COY) analysis as often only data from one year are available. Patterson & Weatherup (1984) also advised on use of COY analysis as over years LSD values tend to be more stable. The COY method simultaneously takes into account the difference between variety means and variance ratio based on continuous probability levels rather than fixed probability levels (Baltjes 1985, 1986b). With consistent characters, the COY analysis gives the highest proportions of separations, and the inconsistency of characters over years is indicated by high values of the variance ratio, which can differ considerably from location to location. However, in an international system, it is to be expected that with respect to continuity of the proportion of positive decisions, a change of distinctness criterion would cause problems that are more serious. Accordingly, a way to overcome this



problem is to apply Modified Joint Regression Analysis (Digsby, 1979) according to the Laidig and Muller (1985) model.

Separate documents on COYD and COYU analysis have been completed, and their application in different crop species, cross-fertilized or self-fertilized, is under deliberation in UPOV's Technical Working Parties. COYD and COYU analysis programs are now integrated in recently revised DUST9 software on analysis of DUS trials' data to understand its application in member States. DUSTW, a prototype version of DUST9, is available from Dr. (Ms) Sally Watson, Biometrics Division, DANI, New Forge Lane, Belfast BT9 5PX, UK.

The main advantages of COYD analysis (UPOV, 1997b) are: (a) it combines information from several seasons into a single criterion in a simple and straightforward way, (b) it ensures that judgments about distinctness would be reproducible in other seasons ( in other words, the same genetic material should give similar results within reasonable limits from season to season,) and (c) the risks of making a wrong judgment about distinctness are constant for all characters. Like COYD, the main advantage of COYU analysis (UPOV, 1997b) is that all varieties can be compared on the same basis and information from several years of testing may be combined into a single criterion.

This is so far a discussion on statistical analysis of field-based DUS trials' data. Obviously, the difficulties may become more acute when laboratory-based data, like that of isozyme electrophoresis or DNA-profiling, are also included in inferring distinctness. In this context, the paper of Staub et al. (1996) on 'Plant Variety Protection: a consideration of genetic relationships' is relevant. In this paper, application and depiction of genetic distance estimations, as the subject of essential derivation, is comprehensively covered. It is relevant to mention its conclusion here:

- i. Multivariate techniques have proven useful for identifying patterns in large data sets. However, the statistical analysis of difference among entities can be difficult. Confidence intervals can be calculated for individual entities and used for comparison. One-way analysis of variance can also be applied on a comparison-by-comparison basis. The question, however, that will always be raised in causes of varietal infringement is the probability of making Type I (varieties are different when in fact they are the same) errors. Statistical estimates of error, although scientifically valid, may not provide the type of precision that the judicial system may demand (i.e. beyond a reasonable doubt).
- ii. Quantification of genetic difference based on any molecular descriptor is subject to sampling or technical error. Sampling error can be minimized by scoring a large number of individuals and by replication. A PVP application can be strengthened when molecular markers are used in conjunction with stable, well-documented phenotypic descriptors that describe the distinctiveness of a variety.
- iii. In cases of alleged infringement, DNA-profiling can provide estimates of genetic distinctness. In such cases, it is essential that the variances of genetic distance estimates be provided to allow for critical judicial examination of varietal relatedness. The worth and validity of such information will likely require a case by case appraisal of historical evidence, pedigrees analysis, and an assessment of statistical probabilities of allelic frequency. In the last analysis, vigorous legal interpretation will only be possible when cumulative biological evidence is weighed against existing law. Plant protection law will be refined as precedents are made.

In view of the above, it can be said that although considerable progress has been made in statistical analysis of DUS data, the chances that wrong decisions can still be made do remain. The statistical tests only indicate the probability that there is a difference, not how much difference. If the requirement is to test for a pre-set minimum distance, the chances of rejecting true distinct varieties increase disproportionately (van der Heijden, 1992). To overcome only such problems for the time being, the UPOV's BMT Working Group has decided on two kinds of characteristics-one used for establishing distinctness and another set of additional characteristics

used only for identification (UPOV, 1994c). That is, the decisions for distinctness of a variety would continue to be made based on expression of the genotype e.g. morphological or physiological characteristics. That is the generally followed approach in most of the UPOV member States in present times.

#### **Developing a DUS/PVP system in new PVP-opting countries**

After the arrival of WTO, many developing countries, who hitherto did not have any PVP-system, have become UPOV member States. These have finalized their PVP-laws in consultation with the UPOV Secretariat. That is the reason for indicating in the Introduction that for judging the effectiveness of any sui generic system, all thinking and developments point towards the system provided under UPOV. An effective PVP system as desired under Article 27.3(b) of GATT (1994) would obviously require fulfillment of minimum requirements of a DUS system and provision for allowing action against infringement of protection rights. In the endeavor, distinctness or identity of each material is obviously a crucial requirement to be affected through an appropriate technical, legal and administrative system in place. Guidance from the UPOV Secretariat can thus greatly help in putting an internationally accepted system in place. Some basic issues are discussed below for developing a DUS/PVP system in the new PVP-opting countries.

#### **Finalization of National Test Guidelines for DUS**

In providing internationally harmonized test guidelines, UPOV has done a useful service considering the acute difficulty in preparing test guidelines for worldwide application. Thus, the general and crop-specific DUS test guidelines from UPOV can serve as the basic material for new PVP-opting countries to finalize their National possible selection of a relevant set of characteristics by crop experts. Nevertheless, new characteristics can be added to increase the possibility of establishing distinctness or identification of any crop variety. It must, however, be kept in view that finalization of NTGs should ideally be a one-time exercise to serve a country for a long period to avoid the need subsequently to include new characteristics. One basic rule to be followed is that no characteristic can be used for distinctness for which uniformity and stability cannot be tested and guaranteed. The minimum difference between two varieties has not to be reduced to a point making it impossible for a breeder to keep his variety uniform and stable in that small margin. Addition of more characteristics can also undermine the protection system in reducing the scope of protection to almost zero. Moreover, an additional burden should not be placed retroactively on existing varieties as uniformity of existing varieties has to be kept under consideration. This concern may, however, be addressed in the review of the General Introduction i.e. during finalization of the revised general introduction for conduct of DUS tests. Besides, acceptable statistical requirements are also to be kept in view for inferring 'clear distinguishability'. Additional identification of each material can, however, be established using new laboratory-based technologies as discussed earlier and suggested by Baltjes & Ghijsen (1992).

In so far as 'uniformity' of crop varieties in specific features of distinctness is concerned, this has to be explained in terms of variability, if any, by providing information on tolerance limits and extent of 'off types'. The criteria of 'stability', as earlier stated, derives its basis from 'uniformity' in the specific features of distinctness and its establishment using a different seed stock. Thus, the requirement of 'distinctness' and 'uniformity' should emanate from the NTGs. For the purpose, the finalization of characteristics and character state and other criteria for testing, like sample size, population standard, acceptance probability, maximum number of off-types permitted etc. are a critical requirement in crop-specific NTGs. It is preferable to select characteristics, which are least susceptible to environment. Selection of character states for each characteristic should keep in view the requirement of clear differentiation in different states of expression. It should also be possible to observe and evaluate the finalized characteristics and character states with reasonable effort and expenditure, and the breeder must also be able to

maintain his variety uniform and stable in those characteristics with reasonable effort. The requirement of 'reasonable effort' is, however, under discussion in UPOV, but it may not be a requirement in the new General Introduction.

In finalization of characteristics and character states in NTGs, it is advisable to include compulsory characteristics marked with asterisk (\*) in the UPOV's test guidelines because this set of characteristics provides a common basis to facilitate comparison of variety descriptions across borders. If laboratory-based tests or tests for disease / pest resistance are to be included in such NTGs, whether as a mandatory requirement or to be provide supplementary information, the availability of a standardized and agreed method would also need mention in those specific NTGs. Therefore, crop experts are needed to provide crucial input for finalization of NTGs.

In practice, the UPOV test guidelines are taken over in many member States entirely without any change. In other member States, all characteristics with an asterisk and a selection of those without an asterisk are taken over. Further characteristics, whether the traditional morphological or physiological features, or based on new methods, are also added in some cases. In principle, the UPOV Test Guidelines are broadly accepted and guaranteed on account of the broad participation in their preparation and continuous updating, which also proves their quality. In finalizing the NTGs, it must also be ensured that they play a certain role in court cases on infringements, as they represent considered opinion for testing of the species concerned.

#### **Documentation on existing crop varieties**

Once the NTGs are finalized with involvement of crop experts, comparative descriptions of existing crop varieties in a country are needed to establish their distinctness or identity. This can serve a dual purpose, to keep an idea on the extent of their usage in cultivation as also to provide earlier varieties as reference materials for testing distinctness or identity of new crop varieties. Although an application for protection or for entry into any official register anywhere in the world causes a variety to be regarded as a matter of common knowledge and UPOV provides the needed assistance in regularly updating the worldwide Plant Variety Database, there are limitations in pooling crop varieties available worldwide to test new materials and this restricts the selection of reference varieties to a national basis, or may be to a regional basis. This is also borne out from the questionable adaptability and exteriorization of expression of characteristics of crop varieties from altogether different agro-climates as well as the necessity for applying for protection separately in each country.

The number of possible example varieties as reference material will continuously increase over time, thereby creating difficulties in selecting the right references for testing new crop varieties. UPOV has recognized this problem of coping with the large number of example varieties so as to strike a balance between the risks of not including a variety and the workload involved in unnecessarily including some varieties. A possible method for the setting up and use of reference collections for DUS testing is attempted in UPOV (1997c). It is also felt that to find the closest varieties the use of electrophoresis or other new methods could be more useful rather than the restriction of comparisons to traditional characteristics of national / regional reference collections. Thus, the whole screening exercise has to be a balance of risks between what should ideally be done and what is reasonable and financially possible. In any case, characteristics of description and distinctness have to be initially provided by the breeders themselves to assist the DUS testing system, which can lead to build up of comparative descriptions and selection of references in cooperation with the breeders. In the endeavour, if new technologies are also to be used for inferring distinctness or identity, or identifying references, a system in cooperation with breeders is again a critical requirement. While it is also felt in UPOV that methods not included in Test Guidelines should only be admitted for screening if a strong correlation existed between the characteristics in question and morphological or physiological characteristics in the Test Guidelines, the use of new methods is considered helpful to provide supplementary proof for

protection of indigenous materials provided that it leads to establishment of additional proof of identity beyond doubt.

### **Establishment of a DUS testing system**

The choice has to be made between utilization of available human resources and infrastructure with minimum additional inputs or establishment of a new and independent institution with requisite technical and administrative capability. In the former case, care has to be taken to avoid dilution of the main objectives of the existing infrastructure, which should not ideally be relegated to a low priority at the cost of meeting the new requirements of DUS testing. In the latter case, an autonomous institution or industry could be created to conduct carefully controlled DUS evaluation trials and the official authority can make use of the created facility. Again, in cases of minor crops involving very few varieties, the task can be assigned to crop breeders only with a system to check the trials by official authorities. In both the alternatives, a close cooperation with breeders or applicants for protection remains a necessary requirement, to precisely understand precisely the properties of their varieties and as a mechanism to assist the government-authorities who may face difficulties in carrying out the task at least in the initial stages.

The developed DUS testing system would also have appropriate sites identified for testing of each crop. The process of identification of sites could consider selection of agro-ecologically different locations to ensure appropriate expression of character states of different characteristics, and should ensure that highly adaptive traits do not get affected. The system would also have accredited laboratories for lab-based DUS characteristics if these are considered for inclusion in NTGs. The VCU test for consumer protection may also need integration with the DUS test system in order to reduce the workload and expenditure. However, these two types of tests have different objectives and databases for VCU and DUS purposes are independent in all countries. Nevertheless, they do have some elements in common in countries like France and Poland (UPOV, 1999b). In the system developed, there could also be separate mechanisms for recognition of heterogeneous groupings and provision for simple 'identifiability' of open-pollinated heterogeneous varieties can be one element in a national system. Switzerland, for example, has set up a 'second register' for highly heterogeneous groupings of cereals ('landraces') (Blumlein, 1996). Any finalized DUS/PVP system could also consider including appropriate elements of its economic viability to ensure its sustainability.

Considering the UPOV (1991) Act, whereby varieties of all botanical taxa become eligible for protection within a period of five years after its coming into effect in a particular country, the overall task indeed becomes enormous. Accordingly, the member States are increasingly considering the adoption of systems of cooperation with breeders and applicants or with the competent authorities in other member States. This is becoming a common practice among developed countries, like in EEC, not only to share the workload but also to gain from each others' strengths. Each new PVP-opting country has thus to identify its possible place in such cooperative arrangements, without which it may be difficult to meet the increasing international requirements. In any cooperative arrangement, the parties would also need to display similarities in approach and system. UPOV is now considering convincing developing countries of the advantages of the 1991 Act (UPOV, 1999c). Matching requirements to safeguard indigenous interests as well as to meet mutually and internationally accepted procedures have to be in-built in the system.

In conclusion, if any country has decided to opt for a PVP-system and the required DUS/PVP system has to be put in place, it would be useful to harness advantages from strengths of specific countries or agencies, and international conventions like UPOV, which alone has so far provided the only widely accepted and ready-made model of a sui generic system. UPOV has taken the needed initiative by organizing regional seminars in different parts of the world and by supporting participation of developing countries in such seminars. Proceeding of these seminars

provide useful learning on current situation in different countries and practical aspects for development of a DUS/PVP system. In its draft programme for the 2000-2001 biennium (UPOV, 1999c), UPOV is proposing to further expand its initiative of technical assistance and cooperation. Such initiatives could, however, become much more meaningful if there also is the reciprocal initiatives, with involved participation from the new PVP-opting countries. These interactions can particularly dwell on the ground realities in the area of plant variety development and protection in developing countries to arrive at feasible solutions for each new PVP-opting country. Introduction of an appropriate PVP/DUS system has become one essential requirement with the arrival of WTO, whereby intellectual property regimes in agriculture increasingly become a universally accepted phenomenon.

#### References:

- Ainsworth, C.C. & Sharp, P.J. (1989) The potential role of DNA probes in plant variety identification. *Plant Varieties and Seeds* **2**, 27-34.
- Bailey, D.C. (1983) Isozymic variation and plant breeders' rights. In: (Eds S.D. Tanksley & T.J.Orton), *Isozymes in Plant Genetics and Breeding Part A*, pp.425-440. Elsevier, Amsterdam.
- Baltjes, H.J. (1985) Simulation of the combined over years analysis. UPOV, Geneva. Working document for the UPOV Technical Working Party on Automation and Computer Programs, UPOV, Geneva.
- Baltjes, H.J. (1986a) Role and use of biometrics in DUS testing. Proceedings VIth meeting of the Eucarpia Section Biometrics on Plant Breeding, Birmingham, U.K.1985, pp.170-188.
- Baltjes, H.J (1986b) On the interpretation of the combined over years analysis results. Working document for the UPOV Technical Working Party on Automation and Computer Programs, UPOV, Geneva.
- Baltjes, H.J. & Ghijsen, H.C.H. (1992) Technical aspects of the plant breeders' rights. *Plant Varieties and Seeds* **5**, 175-182.
- Baltjes, H.J., Klein Geltink, D.J., Nienhuis, K.H. & Luesink, B. (1985) Linking distinctness and description of varieties. *Journal of the National Institute of Agricultural Botany* **17**, 9-19
- Blumlein, G. (1996) Biologische Vielfalt fuer den oekologischen Landbau oder oekologischer Landbau fuer die biologische Vielfalt, epd – Endwicklungspolitik – Materialien 1/96.
- Bould, A. (1992) Variety registration and protection in the UK and countries of the European Community. In: Proceedings of the Indo-British Workshop on Plant Breeders' Rights, seed Certification and Storage. Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi.
- Bourgoin-Greneche, M. & Lallemand, J.L. (1993) Electrophoresis and its application to the description of varieties – a presentation of the techniques used by GEVES. Ed GEVES, Guayancourt.
- Clapham, D. & Almgard, G. (1978) Biochemical identification of cultivars leads to award of plant breeders' rights. *Agricultural and Horticultural Genetics* **36**, 88-94.
- Cooke, R.J. (1984) The characterization and identification of crop cultivars by electrophoresis. *Electrophoresis* **5**, 59-72.
- Cooke, R.J. (1988) Electrophoresis in plant testing and breeding. *Advances in Electrophoresis* **2**, 171-261.
- Cooke, R.J. (1989) The use of electrophoresis for the distinctness testing of varieties of autogamous species. *Plant Varieties and Seeds* **2**, 13-13.
- Cooke, R.J. (1995) Gel electrophoresis for the identification of plant varieties. *Journal of Chromatography A* **698**, 281-299.
- Delanny, X., Rodgers, D.M. & Palmer, R.G. (1983) Related genetic contributions among ancestral lines to North American soybean cultivars. *Crop Science* **23**, 944-949.
- Digby, P. (1979) Modified joint regression analysis for incomplete variety x environment data. *Journal of Agricultural Science, Cambridge* **93**, 81-86.



- Draper, S.R. (1987) ISTA Variety Committee: report of the working group for biochemical tests for cultivar identification, 1983-86. *Seed Science and Technology* **15**, 431-434.
- Draper, S.R. & Keefe, P.D. (1989) Machine vision for the characterization and identification of cultivars. *Plant Varieties and Seeds* **2**, 53-62.
- Eade, A.J. & Law, J.R. (1983) The determination of distinctness in the self-pollinated cereals utilizing electronic data storage and retrieval. *Journal of the National Institute of Agricultural Botany* **16**, 231-248.
- GATT (1994) Final Act Embodying the Results of the Uruguay Round of Multilateral Trade Negotiations, in Marrakesh on 15<sup>th</sup> April, 1994. General Agreement on Tariffs and Trade, 1994.
- Gilliland, T.J. (1989) Electrophoresis of sexually and vegetatively propagated cultivars of allogamous species. *Plant Varieties and Seeds* **2**, 15-25.
- Goodman, M.M. & Stuber, C.W. (1980) Genetic identification of lines and crosses using isozyme electrophoresis. *Proceedings of the Annual Corn and Sorghum Research Conference* **35**, 10-31.
- Higginbotham, J.W., Smith, J.S.C. & Smith, O.S. (1991) Quantitative analysis of two-dimensional protein profiles of inbred lines of maize (*Zea mays* L.). *Electrophoresis* **12**, 425-431.
- Higgins, J. & Evans, J.L. (1986) The use of artificial classification in crop cultivar distinctness testing. In: (Ed. B.T. Styles) *Infraspecific Classification of Wild and Cultivated Plants*, pp. 277-282. The Systematics Association Special Volume 29. Oxford University Press, UK.
- Jarman, R.J. & Hampson, A.G. (1991) The use of a computer management system for testing candidate cereal varieties for distinctness, uniformity and stability and the award of plant breeders rights. *Plant Varieties and Seeds* **4**, 161-168.
- Kelly, A.F. (1968) The work of the Systematic Botany Branch. *Journal of the National Institute of Agricultural Botany* **11**, 246-252.
- Kempthorne, O. (1969) *An Introduction to Genetic Statistics*. Iowa State University Press, Ames.
- Konarev, V.G., Gavriljuk, I.P., Gubareva, N.K. & Peneva, T.I. (1979) seed proteins in genome analysis, cultivar identification and documentation of cereal genetic resources: a review. *Cereal Chemistry* **56**, 272-278.
- Koranyi, P. (1989) Simple purity checking of maize (*Zea mays* L.) lines and hybrids by protein monomer analysis. *Seed Science and Technology* **17**, 161-168.
- Laidig, F. & Müller, G. (1985) Analyse on unvollständigen Zwei-Wege-Tafeln mit der 'Modifies-Joint-Regression' Method. Bundessortenamt, Hanover.
- Leskien, Dan & Flitner, Michael (1997) Intellectual property rights and plant genetic resources: options for a sui generis system. *Issues in Genetic Resources* No.6. International Plant Genetic Resources Institute, Rome.
- Moll, R.H., Lonnquist, J., Velez Fortuno & Johnson, E.C. (1965) the relationship of heterosis and genetic divergence in maize. *Genetics* **52**, 139-144.
- Motto, M., Salamini, F., Reggiani, R. & Soave, C. (1979) Evaluation of genetic purity in hybrid corn (*Zea mays* L.) seed production through zein iso-electrophoretic patterns. *Maydica* **24**, 223-233.
- Orman, B.A., Lawrence, G.D., Downes, P.M., Phillips, D.S. & Ripberger, C.J. (1991) Assessment of maize inbred genetic purity by isozyme electrophoresis. *Seed Science and Technology* **19**, 527-535.
- Patterson, H.D. (1978) Routine least square estimation of variety means in incomplete tables. *Journal of the National Institute of Agricultural Botany* **14**, 401-412.
- Patterson, H.D., Silvey, V., Talbot, M. & Weatherup, S.T.C. (1977) Variability of yields of cereal varieties in U.K. trials. *Journal of Agricultural Science, Cambridge* **89**, 239-245.
- Patterson, H.D. & Talbot, M. (1974) A computing system for cereal variety trials. *Journal of the National Institute of Agricultural Botany* **13**, 142-151.
- Patterson, H.D. & Weatherup S.T.C. (1984) Statistical criteria for distinctness between varieties of herbage crops. *Journal of Agricultural Science, Cambridge* **102**, 59-68.
- Patterson, H.D., Williams, E.R. & Hunter, E.A. (1968) Block designs for variety trials. *Journal of Agricultural Science, Cambridge* **90**, 395-400.
- Patterson, L.J. (1984) Statistical criteria for uniformity in wheat, barley and oats. *Journal of the National Institute of Agricultural Botany* **16**, 443-451.



- Richards, M.C., Morgan, O.W., Oskoui, K.E. & McGregor, M.J. (1989) CUE (Cultivar Expert), a computer program for crop variety selection. *Plant Varieties and Seeds* **2**, 179-191.
- Schneider, F. (1986) The concept of distinctness in PBRs. In: (Ed. B.T. Styles) *Intraspecific Classification of Wild and Cultivated Plants*, pp. 393-397. The Systematics Association special Volume 29. Oxford University Press. UK.
- Seiler, Achim (1998) Sui generic system: obligations and options for developing countries. *Biotechnology and Development Monitor* **34**, 2-5.
- Sharma, S.P. (1991) Plant variety testing system in UK-lessons for India. *Seed Research* **19**, 101-105.
- Silvey, V. (1978) Methods of analysing NIAB variety trial data over many sites and seasons. *Journal of the National Institute of Agricultural Botany* **14**, 385-400.
- Silvey, V. & Fiddian, W.E.H. (1972) The interpretation of genotype x environment interaction in cereal variety assessment. *Journal of the National Institute of Agricultural Botany* **12**, 477-485.
- Singhal, N.C. & Prakash, S. (1992) The characterization and identification of wheat cultivars – integrated approach. In: *Proceedings of the Indo- British Workshop on Plant Breeders' Rights, seed Certification and Storage*. Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi.
- Smith, J.S.C. (1984) Genetic variability within US hybrid maize: multivariate analysis of isozyme data. *Crop Science* **24**, 1041-1046.
- Smith, J.S.C. & Smith, O.S. (1987) Associations among inbred lines of maize using electrophoretic, chromatic and pedigree data I. Multivariate and cluster analysis of data from 'Lancaster Sure Crop' derived lines. *Theoretical Applied Genetics* **73**, 654-664.
- Smith, J.S.C. & Weissinger, H.H. (1984) Rapid monitoring of purity in seed lots of hybrid maize: modification of current technologies. *Maize Genetics Cooperative Newsletter* **58**, 103-105.
- Staub, Jack E., Gabert, August & Wehner, Todd C. (1996) Plant variety protection: a consideration of genetic relationships. *Horticultural Science* **31**(7), 1086-1091.
- Tandon, J.P. (1992) An appraisal of variety testing and release system in India. In: *Proceedings of the Indo-British Workshop on Plant Breeders' Rights, Seed Certification and Storage*. Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi.
- Tanksley, S.D. & Orton, T.J. (1983) *Isozymes in Plant Genetics and Breeding, Part A and B*. Elsevier, Amsterdam.
- Thiele-Wittig, M. –H (1992) Development of technical work in UPOV. *Plant Varieties and Seeds* **5**, 129-142.
- Onkin, M.H. (1974) A computing system for analysis of data from distinctness tests. *Journal of the National Institute of Agricultural Botany* **13**, 225-243.
- Turner, M.R. (1992) A review of the seed and variety control system in UK. In: *Proceedings of the Indo-British Workshop on Plant Breeders' Rights, Seed Certification and storage*. Division of seed Science and Technology, Indian Agricultural Research Institute, New Delhi.
- UPOV (1978) Publication No. 295 (E) of 1992 on International Convention for the protection of New Varieties of Plants of December 2, 1961; as revised at Geneva n November 10, 1972; and on October 23, 1978. UPOV, Geneva.
- UPOV (1979) Revised general introduction to the guidelines for the conduct of tests for distinctness, homogeneity and stability of new varieties of plants. Document TG/1/2. UPOV, Geneva.
- UPOV (1991) Publication No.221(E) of 1992 on International Convention for the protection of New Varieties of Plants of December 2, 1961; as revised at Geneva on November 10, 1972; on October 23, 1978; and on March 19, 1991. UPOV, Geneva.
- UPOV (1994a) Progress report on the work of the Technical committee and Technical Working Parties. Document C/28/10. Council, 28<sup>th</sup> Ordinary Session-Geneva (Nov 9, 1994). UPOV, Geneva.
- UPOV (1994b) Remarks on the draft test guidelines for maize. Document TC/31/5(June 6, 1994). UPOV, Geneva.

- UPOV (1994c) Working group on biochemical and molecular techniques and DNA-profiling in particular. Document BMT/2/9. Second Session – Versailles, France (Mar. 21-23, 1994). UPOV, Geneva.
- UPOV (1996) Technical criteria for protection. In: National Seminar on the Nature and Rationale for the Protection of Plant Varieties under the UPOV Convention- organized by the UPOV in cooperation with the Ministry of Agriculture, Govt. of India at New Delhi (Sep.12,1996). Document UPOV/ DEL/96/2. UPOV, Geneva.
- UPOV (1997a) Working group on biochemical and molecular techniques and DNA-profiling in particular. Document BMT/4/21. Fourth Session – Cambridge, UK (Mar.11-13, 1997). UPOV, Geneva.
- UPOV (1997b) Users notes for combined-over-years distinctness and uniformity procedures. Document TWC/15/7. 15<sup>th</sup> Session – Budapest (June 3-5, 1997). UPOV, Geneva.
- UPOV (1997c) Thoughts on the setting up and use of reference collections for DUS testing. Document TWA/26/5. 26<sup>th</sup> Session – Montevideo (Nov. 10-14, 1997). UPOV, Geneva.
- UPOV (1999a) Revised working document for the preparation of a new general introduction for the conduct for distinctness, uniformity and stability of new varieties of plants. Document TC/35/13. Technical Committee, 35<sup>th</sup> Session – Geneva (Mar.22-24, 1999). UPOV, Geneva.
- UPOV (1999b) Summary of UPOV questionnaires concerning DUS and VCU databases and computer systems. Document TWC/17/3. 17<sup>th</sup> Session- Helsinki (June 29-July 2, 1999). UPOV, Geneva.
- UPOV (1999c) Draft program and budget for the 2000-2001 biennium. Document C/33/4. Council, 33<sup>rd</sup> Ordinary Session – Geneva (Oct. 20, 1999). UPOV, Geneva.
- Van der Burg, W.J. & van Zwol, R.A. (1991) Rapid identification techniques used in laboratories of the International Seed Testing Association: a survey. *Seed Science and Technology* 19, 687-700.
- Van der Heijden, G.W.A.M (1992) Differences between varieties. UPOV technical working party on automation and computer programs. 10<sup>th</sup> Session- Wageningen 1992. UPOV, Geneva.
- Virk, D.S. & Witcombe J.R.(1998) An analysis of varietal testing 1. Introduction. In: (Eds John R. Witcombe, Daljit S. Virk & John Farrington), *Seeds of Choice- Making the Most of New Varieties For Small Farmers*, pp. 3-6. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi.
- Weatherup, S.T.C. (1974) A computer program, DUST, for analysis of data from distinctness, uniformity and stability trials. *Journal of the National Institute of Agricultural Botany* 13, 244-251.
- Weatherup, S.T.C. (1980) Statistical procedures for distinctness, uniformity and stability variety trials. *Journal of Agricultural Science, Cambridge* 94, 31-46.
- Weatherup, S.T.C. (1994a) Distinctness, uniformity and stability trial (DUST) analysis system-users manual. Department of Agriculture for Northern Ireland, Biometrics Division, Newforge Lane, Belfast, BT9 5PX.
- Weatherup, S.T.C. (1994b) Use of Mahalanobis distance to measure varietal distinctness. *Plant Varieties and Seeds* 7, 107-119.

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## Protection of Plant Varieties and Farmers' Rights Act-Implementation and Likely Impact on Indian Seed Sector\*

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### Introduction

The Lok Sabha passed the Protection of Plant Varieties and Farmers' Rights (PPV&FR) Bill on 9 August 2001 and the Rajya Sabha on 28 August 2001. It was assented to by the President of India on 30 October 2001. It has thus become a law of the land [PPV&FR Act (No. 53 of 2001)], to be implemented to guard the interests of the (i) breeders of plant varieties, and ii) farmers, who have been considered not just users of improved varieties but also as conservers and developers of their own varieties. This law has also attempted to regulate the activities of other players in the seed multiplication chain so that while rights on plant varieties are duly honoured, the availability of quality seed to the farmer masses is also ensured. In conjunction with the revised Seed Bill, 2004 that is expected to be passed in the near future, it is considered that the Indian seed sector, and consequently agriculture production in India, will witness a qualitative change in the years to come. Therefore, all concerned with the Indian seed sector look forward to the implementation of these laws. The manner of implementation will determine the nature of impact on the seed sector.

Several activities related to the implementation of PPV&FR Act were started, these activities gained momentum after 2001 when the law was finally passed. Broadly, the activities cover the following aspects: i) preparation of rules, regulations and procedures of administration, ii) establishment of the Authority to undertake the task, and iii) a system to properly characterize the existing varieties which will facilitate the testing of new varieties for the purpose of award of rights.

This law is exhaustive, comprises of 97 Sections. The intent is not to present an analysis of each section of the law. This paper builds the scenario of implementation and impact only from some of the salient considered provisions. Salient provisions are analyzed in the first section of this paper. The analysis brings out broad issues of implementation. The second section highlights the issue of implementation of farmers' rights. In the third section, a few studies on impact of plant variety protection elsewhere are highlighted to build up a background for discussion on the likely or possible response of this law to contribute to a qualitative improvement in the Indian seed sector. The latter is the fourth section before the conclusion section.

### Salient features of the Law

These can be divided into three parts. The first part considering the interests of the breeders includes: i) Researchers' rights for use of an initial variety for conducting experiment / research (Section 30), ii) Essentially derived variety [Section 29(i)], in conjunction with the requirement of authorization for repeated use under Section 30 proviso and Section 23(6) proviso; and iii) Extant variety [Section 2(j)]. The second part covers the provisions favouring the farmers in the law. These provisions are: iv) Farmers' Rights (Chapter VI), v) Benefit Sharing (Chapter IV), and vi) compulsory License (Chapter VII). An immediate thought that comes seeing these salient provisions is that interests of the breeders are safeguarded only in small sections, whereas issues concerning interests of the farmers are described in full chapters. It will, however,

be seen from the provisions that the law is fairly balanced to equally safeguard the interests of both breeders and farmers and is not tilted in favor of the farmers. The third part of the salient features relates to the Central Government and the Authority to be established under this law. This includes: vii) General functions of Authority (Section 8), viii) Gene Fund (Section 45), ix) Framing of schemes etc. (Section 46), and x) Central / State Government as the Owner of Rights on 'extant' varieties (Section 28(1) proviso). A few other sections are also cited, wherever considered relevant.

### **1. Researcher's Rights (Section 30)**

It says- "Nothing contained in this Act shall prevent- (a) the use of any variety registered under this Act by any person using such variety for conducting experiment or research; or (b) the use of a variety by any person as an initial source of variety for the purpose of creating other varieties: Provided that the authorization of the breeder of a registered variety is required where the repeated use of such variety as a parental line is necessary for commercial production of such other newly developed variety".

The preamble of this Section along with sub-section (a) and (b) highlights that even cosmetic changes are encouraged to create new varieties and rights can be awarded to breeders on their applications of new varieties provided they fulfil other requirements, like that of novelty, DUS (D-Distinctness, U-Uniformity and S-Stability) and suitable denomination in Section 15 on "Registrable varieties"; and of 'expected performance under given conditions' under sub-section (2) of Section 39 on 'Farmers' rights'. The proviso to Section 30, however, indicates that authorization of the breeder of an earlier registered under the Law is required in cases where repeated use of that registered variety is required as a parental line for Commercial production of the new applicant variety. This largely restricts the requirement of taking authorization from another breeder to those few cases of new applicant hybrids wherein an earlier registered variety of the other breeder is used as a parental line for commercial production of the applicant hybrid. Thus, whereas Section 30 proviso protects the commercial interest of only a particular kind of earlier, Section 39 (2) attempts only to protect the farmer as a consumer of seed of a variety protected under the law.

### **2. Essentially Derived Variety [Sec.2 (i)]**

The definition is: "essentially derived variety", in respect of a variety (the initial variety), shall be said to be essentially derived from such initial variety when it-(i) is predominately derived from such initial variety, or from a variety that itself is predominately derived from such initial variety, while retaining the expression of the essential characteristics that result from the genotype or combination of genotypes of such initial variety; (ii) is clearly distinguishable from such initial variety; and (iii) conforms (except for the differences which result from the act of derivation) to such initial variety in the expression of the essential characteristics that result from the genotype or combination of genotype of such initial variety;

A variety has thus to be inferred "essentially derived" with respect to the 'initial variety'. The 'initial variety' may be a protected variety or may not be a protected under this law. While some elaboration of what constitutes as 'essentially derived variety' is required in rules and regulations of the law, an essentially derived variety is, in general, as good as a new variety in terms of the requirements of benefit sharing under this law. This is so because Section 26 (1) on "Determination of benefit sharing by Authority" considers novel, 'extant' as well as 'essentially derived' varieties for the purpose. It is so also because of the wider scope under Section 30 on "Researcher's rights". The only exception is proviso to Section 30 discussed above. In addition, proviso to sub-section (6) of Section 23 on "Registration of essentially derived variety" requires for mutual agreement between such two breeders. It means a record of such mutual contractual understanding may be additionally required. Sub-section (e) of Section 18 on "Form of application" also necessitates the requirement of complete passport data (pedigree details etc.) in

applications of varieties, which additionally helps in determining the essentially derived nature of an applicant variety. The requirement of complete passport data should not be considered a serious objection, since the practice in other countries requires information on the pedigree, breeding methods and selection criteria used in developing the candidate variety.

### **3. Extant Variety [Sec.2 (j)]**

The definition covers already notified varieties under Section 5 of the Seeds Act, 1966; a 'farmers' variety', a 'common knowledge' variety, or any other variety in the 'public domain'. The term 'initial variety' in the definition of 'essentially derived variety' could thus mean any indigenous and naturalized variety for benefit sharing.

Sub-section (2) of Section 15 on "Registrable varieties" requires that an 'extant' variety shall be registered under this Act within a specified period if it conforms to such criteria of DUS as shall be specified under the regulations. Clause (a) of sub-section (2) of Section 8 on "General functions of Authority" requires that the registration of 'extant' varieties is subject to such terms and conditions as may be prescribed. Therefore, it highlights the requirement of (i) development of 'special' DUS criteria for 'extant' varieties, and (ii) the delineation of terms and conditions for registration of 'extant' varieties. The terms and conditions need to be defined. The two possibilities for defining terms and conditions could be: (i) concerning the use of 'extant' varieties for ensuring benefit sharing, and (ii) ensuring that availability of seed of those 'extant' varieties to the farmers remains undisturbed as before.

Regarding the development of 'special' DUS criteria for registration of 'extant' varieties, the definition of a 'variety' and practices in other countries need to be reviewed. The UPOV Convention (International Union for the Protection of New Varieties of Plants-55 member countries as on 15 June 2004) as well as laws of a few countries like Switzerland, Australia, UK and Ireland provides scope even for protection of heterogeneous have been protected, Leskien and Flitner (1997) have suggested the DI criteria (D- Distinctness, I-Identifiability) for heterogeneous varieties. The requirement is thus to finalize this criterion for 'extant' varieties. While the criteria and practices for protection of varieties in such countries can be a helpful guidance, it may be necessary to limit this criterion only to the extent of describing the specific attributes of an 'extend' variety, and the extent of presence of such specific attributes in the seed sample of that 'extant' variety. Thus, for 'common knowledge', 'public domain', and 'notified' 'extant' varieties as per Section 2(j), this criterion should require comparatively better description for identifiability of these varieties. It is suggested so because additional burden of precision in description or uniformity should not be imposed retroactively on 'extant' varieties. In case of farmers' varieties in particular, the criteria can be relatively simple, requiring only one or two specific economic attributes, along with information on extent of presence of such attributes.

### **4. Farmers' Rights (Chapter VI)**

The preamble of Section 39 (1) on Farmers' rights states: "Notwithstanding anything contained in this Act –", thereby giving wide amplitude to honour Farmers' rights. However, clause (iii) of sub-section (1) of Section 39 on Farmers' rights restricts the recognition and reward from the "Gene Fund" (Section 45) only to those farmers who are engaged in conservation of genetic resources and their improvement and preservation. This is again subject to the proviso that the materials selected and preserved have been used as donors of genes in varieties registrable under this Act. Likewise, subsection (1) and (2) of Section 41 on "Rights of communities" requires a procedure and a clear proof of evidence of the contribution of the people of a particular village or local community in the evolution of a variety registered under this law.

Clause (iv) of sub-section (1) of Section 39 states that 'a farmer shall be deemed to be entitled to save, use, sow, resow, exchange, share or sell his farm produce including seed of a variety protected under this Act in the same manner as he was entitled before the coming into force of this Act, provided that the farmer shall not be entitled to sell branded seed of a variety



protected under this Act'. The explanation to this sub-section provides the meaning of 'branded seed'. The intent to allow the farmers to reuse in next season, exchange, share or sell his farm produce is thus clearly subject to entitlements of a farmer before the coming into force of this Act. In effect, it means that the law only allows leaving undisturbed the earlier practices of the farmers. Any significant violation will require strict proof of evidence of the particular farmer's earlier business.

The administrative requirement for a farmer to successfully get compensation in sub-section (2) of Section 39 if the breeder's variety does not provide the expected performance needs elaboration. Seed has to be sold along with the crop production technology capsule indicating on label of the seed packet the likely production in farmers' crop growing conditions. Work on simple and feasible mechanisms for the purpose is thus a necessity.

Section 42 is on "Protection of innocent infringement". The language in its preamble – 'Notwithstanding anything contained in this Act'- again provides the intent of the law to protect innocent infringement at any cost. Sub-section (ii) of this Section will, however, require the farmer to prove this innocent infringement in the courts of law, which is certainly not an easy proposition for the poor farmer masses.

## **5. Benefit Sharing (Chapter IV)**

Sub-section (1) of section 26 on "Determination of benefit sharing by Authority" states, 'On receipt of the certificate of registration under sub-section (8) of section 23 or sub-section (2) of section 24, the Authority shall publish such contents of the certificate and invite claims of benefit sharing to the variety registered under such certificate in the manner as may be prescribed.' Since Section 23 is on 'essentially derived varieties', and Section 24 covers varieties 'other than essentially derived varieties', it is clear that benefit sharing applies to registered novel, extant as well as essentially derived varieties (as earlier referred in the discussion on 'essentially derived variety'). The intent is, therefore, to safeguard the interest of indigenous wealth of 'extant' varieties so much valued under this law.

Under sub-section (3) of section 26 on 'Determination of benefit sharing by Authority', the Authority shall send a copy of the claim of benefit sharing to the concerned breeder of the registered variety. Under sub-section (7) of section 26, the amount of benefit sharing determined shall be recoverable as an arrear of land revenue by the District Magistrate within whose local limits of jurisdiction the breeder liable for such benefit sharing resides. Sub-section (5) of section 41 on "Rights of communities" also reiterates the requirement of recoverability of benefit sharing as an arrear of land revenue with respect to the case of communities. Under sub-section (5) of section 26, the guideline to dispose of claim of benefit sharing is indicated. In this sub-section, the determination of amount of benefit sharing is based on: a) the extent and nature of the use of genetic material of the claimant in the development of the variety relating to which the benefit sharing has been claimed, and b) the commercial utility and demand in the market of the variety relating to which the benefit sharing has been claimed. The functionality of this provision on benefit sharing, therefore, needs further elaboration. With respect to the nature and use of genetic material in development of a variety registered under this law, the determination shall have to keep in view the provision of Research's rights under Section 30. Regarding the issue of commercial utility and demand in the market of the variety, a requirement that cannot be assessed in advance, unless the variety actually sells in the market, a procedure has to be developed.

Heisey et al. (2001) writes, 'Even in industrialized countries, though some seed production or certification data may be available, data on individual crop varieties grown in farmers' fields are usually not consistently or routinely collected across entire countries. Where possible, estimates may be based on seed sales or possibly marketing data; but in some cases, it is practical to rely on expert opinion.' Clearly, the requirement is to develop some simple and feasible procedure.

## 6. Compulsory License (chapter VII)

This chapter addresses the issue of authorization by the Authority to get the production of seed of a registered variety undertaken in situations when the reasonable requirements of the public for seed or other propagating material of the variety has not been satisfied. In general, the sections in this Chapter are structured in such a manner that the Authority pays equal attention to the responsible concerns of the right holders as well as the interested party seeking a compulsory license. Broadly speaking, doubts in functionality of this chapter are not apparent. However, guidelines for compulsory licensing need to be formulated.

## 7. General Functions of the Authority (Section 8)

Sub-section (1) of Section 8 makes it a mandatory duty of the Authority to promote such measures, as it thinks fit, which i) encourage the development of new varieties of plants, and ii) protect the rights of the farmers and breeders. In sub-section (2) of Section 8, these measures are listed. These *inter alia* include: i) developing characterization and documentation of varieties registered under this Act; ii) documentation, indexing and cataloguing of farmers' varieties, iii) compulsory cataloguing facilities for all varieties of plants, and iv) collecting statistics with regards to plant varieties including the contribution of any person at any time in the evolution or development of any plant variety in India or in any other country, for compilation and publication. The giganticity of the task thus needs to be appreciated and acted upon, more so for giving due recognition to 'extant' varieties, which also include farmers' varieties.

## 8. Gene Fund (Section 45)

Sub-section (1) requires the Central Government to constitute a Fund to be called the National Gene Fund to which will be credited the: a) benefit sharing received from the breeders of varieties registered under this Act, b) the annual fees collected by way of royalty, c) the compensation received to honour rights of communities, and d) the contribution from any national and international organizations and other sources. Sub-section (2) requires the Gene Fund to be applied for meeting: a) any amount to be paid by way of benefit sharing as determined by the Authority, b) the compensation payable to communities, c) the expenditure for supporting the conservation and sustainable use to genetic resources including *in situ* and *ex situ* collections, and for strengthening the capability of the Panchayat in carrying out such conservation and sustainable use; and d) the expenditure of schemes relating to benefit sharing under Section 46 (Framing of schemes etc). The manner of working should thus take all necessary steps that allow growth in the kitty of token National Gene Fund that has been initially proposed in the budget proposal of the Authority.

## 9. Farming of Schemes etc. (Section 46)

This Section is the last section in the chapter on farmers' rights. According to clause (f) of its sub-section (2), such schemes formulated may provide for the utilization of benefit sharing for the purpose related to breeding, discovery or development of varieties. Clearly, the requirement of actions that allow growth in the National Gene Fund shall have a bearing on promotion of breeding, discovery, or development of varieties from the Fund.

## 10. Central / State Govt. as the owner of right (Section 28 (1) proviso)

Section 28 is on 'Registration to confer right'. Sub-Section (1), which is subject to other provisions of this Act, talks of a certificate of registration for a variety, issued under this Act, which certificate shall confer an exclusive right on the breeder or his successor, his agent or licensee, to produce, sell, market, distribute, import or export the variety. The proviso attached to this sub-section, however, provides that in the case of an 'extant' variety, unless a breeder or his successor establishes his right, the Central Government, and in cases where such 'extant' variety is notified for a State or any area thereof under Section 5 of the Seeds Act, 1966, the State

Government, shall be deemed to be the owner of such right. This proviso limits the right of the Central or State Govt. only to 'extant' varieties notified under Section 5 of the Seed Act, 1966. Again, the condition attached is that the Central/ State Government shall be deemed the owner of such right only in cases where the breeder or his successor has not been able to establish his right.

The following could be the interpretation of this provision. All the breeders who have their varieties notified under the Seeds Act, 1966 must establish their right on these 'extant' varieties. Regarding the varieties notified under the Seed Act by the ICAR-SAU (Indian Council of Agricultural Research- State Agricultural University) system, this autonomous system must establish its rights at the earliest by fulfilling other basic requirements under this law. Considering that ICAR-SAU system is technically a non- government system in the present context of interpretation of what constitutes 'government', this law requires to honour the rights of ICAR-SAU system to the extent provided in this provision.

Regarding the non-notified but finished variety products, which are already common to farmers' cultivation, owners of such varieties can establish that these varieties are 'common knowledge' or 'in public domain' to fall under the definition of 'extant' varieties under Section 2(j). This shall be a useful step for expecting benefit sharing proceeds in future because the 'novel' and 'essentially derived varieties' under this new law shall also require pedigree details, as inferred earlier. In addition, under Section 24(6) (iii), the protection period of 15 years is allowed for such 'extant' varieties. Section 24(6) (iii) shall also allow a protection period of 15 years for registered farmers' varieties, and the Government can be the custodian of registered farmers' varieties to harness benefit-sharing proceeds and honour farmers' rights. With this approach, the regulation and use of farmers' varieties could also be made in accordance with the provisions of the chapter II on "Regulation of Access to Biological Diversity" of India's Biological Diversity Act, 2002.

In nutshell, it can be said that the discussion on salient features has brought to the fore a requirement of clarity on several technical issues, which is essential for successful implementation of the Law. The finalization of rules, regulations and procedures of administration of the Law has to thus consider these and many other smaller (but even more vital) issues, which shall emerge when every provision of the Law is considered for implementation. Wherever required, the provisions are to be elaborated, guidelines provided and the functionality ensured. It should also be ensured that the procedures of administration are simple and feasible. Salient issues pertaining to implementation of DUS testing have not been made a part of this article. However, another paper of the author (Mauria, 2000) does provide some idea on this part.

### **Implementation of Farmers' Rights**

In exploring the feasibility of farmers' rights, Srinivasan (2003a) examines the Indian law and analyses three approaches to implement farmers' rights. He concludes:

"----- IPR-based approaches to farmers' rights are not only likely to involve severe operational difficulties, far beyond the present administrative capacity of developing countries to handle; they are also unlikely to provide significant returns to farming communities. ----- Conservation projects supported by community gene funds may be a better way to address concerns regarding the preservation of agro-biodiversity. But again, the expectation that levies on breeders' IPR-related returns can be major source of revenue for these funds is unrealistic.

These three approaches to farmers' rights, earlier discussed in Blakeney (2002) are: i) situating traditional practices of farmers as exceptions to exclusive rights of plant breeding under existing IPR laws, ii) modifying existing IPR laws to permit farmers themselves claim exclusive rights in plant varieties they cultivate informally, and iii) recognizing farmers' rights not through IPRs but through benefit sharing mechanisms (e.g. payments, technology transfers to compensate their contributions to plant genetic diversity).

The third approach of charging a levy on the sales of protected varieties, which is considered practically feasible, was earlier discussed in Swaminathan (1996). However, breeders do not fail to point out that a levy on the sale of protected varieties to raise funds for a National Gene Fund would ultimately be passed on to farmers in the form of higher prices for seed (Srinivasan, 2001). Again, Swaminathan (1996) observes, “taxing seed sale alone may imply farmers funding Farmers’ Rights”, and proposes an across the board one per cent levy on the sale of all agricultural commodities. A legal person may raise another question: “Farmers’ rights in this proposal are not a juridical concept but merely a compensation mechanism. Should ‘Rights’ be reduced to mere compensation mechanism?”

The above studies perhaps require a further examination of the concept of farmers’ rights. One question could be: can there be more collective international action to uplift this important issue from the present ‘concept’ stage. Under the International Treaty on Plant Genetic Resources for Food and Agriculture, which has now become a legally binding Treaty, the responsibility to recognize farmers’ rights has been left to national governments. In that case, the levy approach could be one direction suitable for countries like India to implement farmers’ rights. At the same time, use of farmers’ varieties registered under Indian law can be negotiated to realize farmers’ rights, as already discussed in the section on “Central / State Govt. as the owner of right (Section 28 (1) proviso)”. The nation thus needs to have a designated depository of indigenous farmers’ varieties that are economically important, may be from the breeders’ viewpoint as donors of useful characters / genes. The economic worth of farmers’ varieties will be a determining factor in making them a source of revenue to realize farmers’ rights.

#### **Impact of Plant Variety Protection in other countries**

The conclusion from any study in developing countries could be more relevant to the Indian situation. Only one report by Jaffe and Wijk (1995) could be found in the literature. It was entitled: “The Impact of Plant Breeders’ Rights in developing Countries: Debate and Experience in Argentina, Chile, Colombia, Mexico, and Uruguay”. Plant Breeders’ Rights (PBR) legislation was introduced in Argentina, Chile and Uruguay in the 1970s and 1980s. In the 1990s, these three Southern Cone countries have strengthened and enforced their PBR protection. All five countries became UPOV members during 1994-97. It must, however, be cautioned that these countries have not addressed to the question of farmers’ rights, and thus even this example may not be relevant to forecast the likely impact on Indian seed sector. Nevertheless, it is worthwhile to cite just the last two conclusions of this informative study. These are:

“1. The limitation of the PBR system is that it has been designed to support the production of those farmers which have the opportunity to operate under relatively favourable circumstances. In developing countries, in general, this is a relatively small group of farmers; the majority of farmers work in marginal areas under often adverse conditions. PBR legislation is not a suitable instrument to make breeding technology available for these farmers. Other additional measures are necessary to support the breeding for resource-poor farmers.

2. It seems worthwhile for all countries who (have to) consider the introduction of PBR protection to study the effects of this protection on the seed industry, seed diffusion and technology transfer, prior to the adoption of legislation. Early identification of potential winners and losers enables the design of a PBR law, or a law which resembles such, that is adjusted to national needs. Moreover, additional measures could be considered to mitigate or prevent some undesirable effects which result from PBR protection.”

In the studies on impact in case of developed countries, no study could be found which separates the impact of other mechanisms, like general measures of support to farmers, and stronger intellectual property regimes (e.g. gene patents), to arrive at a clear impact of implementation of plant breeders’ rights alone. A study on effects of US Plant Variety Protection Act (PVPA) on wheat genetic improvement (Alston and Venner, 2000) finds no evidence of increase in private

investment in wheat breeding. This study finds that PVPA has served primarily as a marketing tool. Srinivasan (2003b) explores concentration levels on the ownership of intellectual property rights over plant varieties worldwide. The paper analyses data from 30 UPOV-member countries. It concludes : a) high level of concentration in individual countries, even greater in smaller UPOV countries, b) lower concentration level in France because of participation of public sector (INRA) and large cooperatives in variety development, c) concentration mainly through merges and acquisitions (mainly in 1990s) and not through share of new certificates, and d) concentration not because of the same set of varieties to develop countries, thus underlying the necessity of adaptive research to develop location-specific varieties for each country. It can thus be inferred that continued presence of a strong and vibrant public sector is an absolute necessity to serve as a check on excessive trend towards privatization.

### **Likely response and impact of Indian Law**

The real impact of Indian Law on farmers' practices and on seed industry is difficult to anticipate until it actually influences agriculture management in the country. Even becoming a UPOV member may not be a real alternative unless there are signs of positive impact of UPOV law on agriculture management in developing countries. The singular study of Jaffe and Wijk (1995) about impact of PBR in developing countries is a study much earlier in time; it even raises many questions when considered in the Indian context. This study also concludes a requirement of 'additional measures' to mitigate or prevent some undesirable effects which result from PBR protection. Therefore, a law based on special social, economic and political circumstances of a country can alone attempt to deliver country-specific requirements. There cannot be a single attempt for all kinds of countries, particularly on subjects that affect masses.

The impact of the law must, however, be anticipated to appropriately conclude this topic. It could be viewed in terms of 'extant' varieties (which include farmers' varieties) on the one hand and 'novel' varieties on the other hand. Regarding the 'extant' varieties, depending upon the development of a simple criterion of their description and implementation of proper schemes to document, conserve, and utilize 'extant' varieties, the applications can come in large numbers. Regarding the 'novel' varieties, applications may come only from the public sector, or may be for some hybrid varieties from the private sector because the private sector would be able to control the repeated use of its parental lines. The private sector may also like to apply for its earlier non-notified 'extant' varieties / hybrids by establishing that they are 'common knowledge' varieties, or are already in the 'public domain', to gain recognition for its contribution to Indian agriculture in the past. The requirement of deposit of sample of parental lines of registered varieties under section 27 is, however, again a difficulty, though it is a practice in many countries. This is stated because the country could not even ensure the required deposit of seed samples from the private sector under the New Policy on Seed Development (implemented in 1988), which did not involve any regime on plant variety protection.

At this point, the Seed Bill' 2004, which was mentioned in the introduction, should, however, be brought again. The provisions in this Bill do reinforce the intention of the law on production of plant varieties and farmers' rights. Section 2(14) elaborates the meaning of "misbranded" seed. Section 2 (29) defines a "variety" and also explains "essentially derived variety" and "extant variety" as in the PPV&FR Act. Section 13 (3) provides opportunity for grant of provisional registration to all varieties, which are available in the market on the date of commencement of this Act, thereby reiterating the requirement of regularization of 'common knowledge' and 'public domain' varieties as per the PPV&FR Act. Section 20 is on compensation to farmers when farmers fail to get the expected performance under given conditions. Section 25 (a) mentions the requirement of "identifiability", its clause (e) provides wide amplitude, requiring compliance for "such other requirements as may be prescribed". The overall impression thus one gets from the two laws is that they are indeed mutually reinforcing. A successful implementation of the intention of the Government may take some more time but the endeavor of the



Government, as evident from these two instruments, is basically to streamline the Indian seed sector for ultimate food security in the nation.

The documentation, conservation, and use of 'extant' varieties (which includes farmers' varieties) have particularly become an issue of prestige for the country. The particular challenge is to streamline the collection, conservation, and management of farmers' varieties for greater revenue generation to honor farmers' rights, an issue that alone will lead the nation to exercise a sovereign right on its agro-biodiversity, allowed under the Convention of Biological Diversity. Indeed, there are difficulties, but they have to be surmounted to completely reform the Indian seed sector. A sincere implementation will also allow us to learn new lessons. Amendments can be proposed at a shorter interval of time to modify the law, to ensure a still more positive impact on agriculture management in the country.

## Conclusion

As the law has been framed fully considering the overall circumstances of the nation, the functioning of the law has to be ensured at any cost. It is felt that this pioneering law has contributed to an important purpose of effectively pushing the requirement of review for an effective *sui generis* system for protection of plant varieties under Article 27.3 (b) of TRIPs to a much later time. Helfer (2002) does not expect a complaint over plant varieties in the WTO Dispute Settlement Mechanism, particularly within the next 5-10 years. A period of 5-10 years should be sufficient for needed reforms in Indian seed sector. However, the compliance to patent regime under TRIPs is required by 2005. The nation needs to ensure that the patent regime in 2005 does honour the already elaborated national interests in plant varieties. So far, the amendments in India's Patent Act, 1970 have kept the plant varieties as non-patentable subject matter.

There is one more possibility that the history of TRIPs is repeated in case of protection of plant varieties also. India may have to become a UPOV member, if UPOV law becomes a near universal agreement based on prior bilateral agreements with a larger number of developing countries. This is another reason for ensuring a successful implementation of this pioneering law.

In the end, it must also be submitted that the salient considered provisions have been interpreted in this paper based on literal rule of interpretation, the only possibility in the absence of judicial precedence. Therefore, any miss in interpretation of vital points of law shall be gratefully accepted. It must again be reiterated that each country and its circumstances are unique to itself. While the quantum of literature available is useful for guidance, this literature has elements of variance, in thoughts and consequently the conclusion drawn, from the Govt. of India's perspective, which has obviously considered both, the national circumstances and the international developments, while legislating on this law. At the present stage of socio-economic development, the nation has rightly avoided making plant variety protection law a marketing tool as inferred in the study of Alston and Venner (2000).

## References

- Alston, Julian, M. and Raymond J. Venner (2000). The Effects of the U.S. Plant Variety Protection Act on Wheat Genetic Improvement. EPTD Discussion Paper, International Food Policy Research Institute, Washington DC.
- Blakeney, M. (2002). Protection of Plant Varieties and Farmers' Rights. 24 Eur. Intell. Prop. Rev. 9-19.
- Heisey, Paul W., C.S. Srinivasan and Colin Thirtle (2001). Public Sector Plant Breeding in a Privatising World. Resource Economics Division, Economic Research Service, US Department of Agriculture. Agriculture Information Bulletin No. 772.
- Helfer, Laurence R. (2002). Intellectual Property Rights in Plant Varieties: An Overview with Options for National Governments. FAO Legal Papers Online #31.

- Jaffe, Walter and Jeroen van Wijk (1995). The Impact of Plant Breeders' Rights in Developing Countries: Debate and experience in Argentina, Chile, Colombia, Mexico and Uruguay. Inter- American Institute for Cooperation on Agriculture, University of Amsterdam, Amsterdam, The Netherlands.
- Leskien, Dan and Michael Flitner (1997). Intellectual Property Rights and Plant Genetic Resources: Options for a *Sui Generis* System. Issues in Genetic Resources Institute, Rome, Italy.
- Mauria, S. (2000). DUS testing of crop varieties – a synthesis on the subject for new PVP- opting countries. *Plant Varieties and Seeds* 13:69-90.
- PPV & FR Act (2001). The Protection of Plant Varieties and Farmer's Rights Act (No.53 of 2001). Department of Agriculture and Cooperation, Ministry of Agriculture, Govt. of India, Krishi Bhavan, New Delhi.
- Seed Bill (2004). Department of Agriculture and Cooperation, Ministry of Agriculture, Govt.of India, Krishi Bhavan, New delhi.
- Srinivasan, C.S. (2001). International Experience of Plant Variety Protection: Lessons for India. Ph.D. Thesis, Department of Agriculture and Food Economics, University of Reading (UK), August 2001, unpublished.
- Srinivasan, C.S. (20023a). Exploring the Feasibility of Farmers' Rights. *Development Policy Review* 21(4): 419- 447.
- Srinivasan, C.S. (2003b). Concentration in Ownership of Plant Variety Rights: Some Implications for Developing Countries. *Food Policy* 28:519-546.
- Swaminathan, M.S. (1996). *Agrobiodiversity and Farmers' Rights*. New Delhi: Konark Publishers Pvt. Ltd.
- The Biological Diversity Act (2002). (No. 18 of 2003). The Ministry of Environment and Forests, Govt. of India, Paryavaran Bhavan, New Delhi.

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## Criteria for Plant Variety Protection: Establishment of Dus on the basis of Qualitative and Quantitative Characters

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Distinctness, Uniformity and Stability (DUS) trial are experiments with two goals 1) comparison of varieties and 2) absolute determination. In DUS trials, certain number of characteristics of the plants is observed to assess DUS. The measurements or observations are analyzed and the results are used to make statements about DUS.

### Resources required for DUS testing

#### Staff

- Scientist in charge of DUS test
- Separate technical staff/ examiners for each species or group of Species
- Persons responsible for secretariat work
- Limited number of casual labour to assist the examiner in seasonal work
- Limited number of casual labour to assist the examiner in seasonal work

#### Facilities

- Land for conduct of DUS test
- Public / private gardens (roses etc.)
- Orchard (trees)
- Glasshouse facilities
- Growth chambers
- DUS test guidelines
- Reference collection

### DUS testing options

- Government testing
- Breeder testing
- Combination of both
- Foreign test reports

### I. Government testing

#### 1. Testing on government farms

It is a system of trial under the Central Plant Variety Protection Office. PVP office follows normal guidelines for DUS testing procedures. Appropriate testing fees are charged from the applicant, since properly conducted trials are costly to run. However, the cost of paying the PVP office to conduct the trial is less than what it had been costing the breeders to conduct their own trial.

#### 2. Official testing on the breeders premises

Under the arrangements, an applicant seeking plant variety protection is required to establish and maintain on his farm a DUS growing trial of a new variety. It must be conducted according to the procedures laid down by the PVP examiner. At the appropriate stage of the plant growth the examiner / suitable, qualified staff will visit the test site.

Distinctness of a new variety will be assessed against similar varieties because of description of the variety and coloured photographs taken. In most cases, the examiner will seek the advice of an expert in the distinctness of a new variety.

### 3. Testing contracted to another organization

When the PVP office does not have DUS test facilities, it may contract the public / private organization, capable of conducting the tests, for doing this job for example, UK Plant Variety Protection Office is getting the testing of varieties of wheat, barley, faba beans, rape seed / mustard etc. done through National Institute of Agricultural Botany, Cambridge. DUS is conducted by the staff of contracting organization on its own property.

### 4. Central testing by Research Institutes

Particularly for fruit varieties, testing for distinctness and assessing uniformity present difficulties if the test is held at applicant property because of fruit crops is assessed by a Government fruit Research Institute. Government Institute conducts the test at research orchard containing large collection of existing varieties of the crop concerned. The test is carried out by the expert staff of the Institute according to the guidelines specified by Plant Variety Protection Office.

#### II. Breeder Testing

Breeder him/ his self has the responsibility to conduct a correctly designed DUS trial. For this purpose, test breeder is required a detailed description and establish to the satisfaction of PVP office that new variety is distinct, uniform and stable. Distinctness from any very similar varieties must generally be established in a side-by-side growing comparison. The applicant must demonstrate that the new variety meets the PVP standards for uniformity and stability.

#### III. Combination of Both

Some countries use both government as well as breeder testing of the varieties depending on the species concerned. International Union for the Protection of New Varieties of Plant (UPOV) considers both systems acceptable as long as breeder testing fulfills certain conditions which would normally be fulfilled in Govt. testing.

#### IV. Foreign test reports

This is used for agricultural varieties, many ornaments and fruit trees. Some times the use of foreign test reports is not feasible. Variety description based upon plants grown in one country may differ to a great extent from a description prepared in another country. Similarly, a variety uniform in one country may not be uniform in another. Secondly, there may be varieties grown in one country are not available in other countries and when DUS is conducted in another country it can not take such varieties into comparison when deciding on distinctness. However, these problems do not occur with all kinds of plants. For examples, have indoors or glass houseplants, the problem arising from climate difference are largely avoided. In such cases, it is technically valid, and to the advantage of DUS conducting authorities to use the reports from other countries. Test reports can also be used if other country concerned is a breeding centre for the species and the testing authority has expertise that we do not have. Some countries, with a similar climate, may have bilateral cooperation in the testing of varieties. The effect of this agreement is that one country does the testing of all candidate variety for one species, while the other country does the same for another species, thus sharing and reducing the workload for both and avoiding unnecessary parallel testing.

#### DUS testing system in operation

Government testing - European Union Countries  
Breeder testing - USA, Canada, Australia  
Combination of both - New Zealand, Japan  
Potato, Grasses, White cloves (Govt. testing)  
Fruit varieties (Central testing by Research Institute)  
Minor agricultural crops-Breeder testing

#### Types of scales of data

DUS depends on the level of scales of data, which are recorded for the characteristics. Scale may be quantitative or qualitative.

## Quantitative scaled data

The data that are recorded by measuring or counting is said to be quantitative scaled data. This data can have continuous or discrete distribution. Continuous data results from measurement. Discrete quantitative data result from counting.

### Example

Continuous : Plant length in cm. – measurement

Discrete : Number of stamens (1,2,3,4 and so on).

In discrete quantitative data there are no real values between two neighbouring units but is allowed to compute an average, which is in between these units, Quantitative scales can be subdivided into ratio scales and interval scales,

i) Ratio scale: Ratio scaled data may be continuous or discrete

Example – Ratio- length to width

Giving an index number (ratio), it is combination of two characteristics. In UPOV terms, it is called as combined characteristics.

ii) Interval Scale: Interval scale data may be distributed continuously or discretely. Continuous interval scale data is the relative “measurement in °C”. There is no example for this kind of scale in technical guidelines of individual crops. Discrete interval scales “time of beginning of flowering as date”.

## Qualitative scaled data

Qualitatively scaled data are data, which can be arranged, in qualitatively different categories. Usually they are based on visual assessment. Qualitatively scaled data is further divided into ordinal (qualitative underlying quantitative variables) and normal scales.

i) Ordinal scale

Qualitative data (qualitative underlying quantitative variables) in which discrete can be arranged in an ascending or descending order

Example: intensity of anthocyanin

Ordinal scale consists of numbers which correspondence to the scales of expression of the characteristics (notes). Expression varies from one extreme to the other. Thus they have a clear logical order and not possible to change this order. An ordinal scale does fulfill the condition to calculate the arithmetic mean value, which is the quality of intervals throughout the scales.

### ii) Nominal scale

Nominal scale qualitative data are data without any logical order of the discrete categories.

### Example

Sex of plant: dioecious female (1), dioecious male (2)

Monoecious unisexual (3), monoecious

Hermaphrodite (4)

Leaf blade: non-variegated (1), variegated (9)

A nominal scale consists of numbers which correspond to the state of expression of the characteristics in the test guidelines as notes. Characteristics with only two categories (alternative characteristics) are a special form of nominal scales.



### Type of Scales

Type of Scale		Description	Distribution	Data recorded
Quantitative	ratio	Constant distance with exact zero point	Continuous	Absolute measuring
			Discrete	Counting
	interval	Constant distance without zero point	Continuous	Relative measurement
			Discrete	Date
Qualitative with-underlying quantitative variables	Ordinal	Ordered expressions with varying distances	Discrete	Visually assessed notes
Qualitative	nominal	No order, no distance	Discrete	Visually assessed notes

Process for establishing distinctness with homogenous varieties and a large reference collection

#### Main steps

- In Office –Pre-distinctness
- Study of technical questionnaire (TQ)
- Use of grouping characteristics
- Selection of a set of comparable varieties

#### Conditions

- Full information on the origin and structure of variety
- Correct description of all requested characteristics
- Reference to well known varieties
- Any additional information on a specific trait of the variety
- Possible use of a morphological distance combining the TQ

#### Characteristics

Depending on the species, possibility to consider firstly reference varieties, which are largely used or known as having good performance in the area where the application is made

#### First growing cycle

First official full description of the variety based on DUS test guidelines

Description: Check of the breeder description.

#### Conditions

- Good trial with 2 locations where possible
- Observation on any particularity of the variety along with the cycle.

#### In the Office

Study of first official description

#### Distinctness

- Comparison with the reference variety grown in the same cycle.
- Not grown in the same cycle
- Elimination of the clearly distinct varieties
- Selection of the closest varieties
- Organization of the next cycle lay-out

### Conditions

- Good trials with two locations when possible
- Observations of any particularly of the variety along with the cycle
- Possible use of morphological distance
- Rejection (of new first cycle) for any variety with a wrong TQ description
- Contact with applicant to get any information on the distinctness from the closest variety.

### Second growing cycle Description

- Second official description as for the first cycle plus any additional characteristics mentioned by the applicant
- Direct comparison of the candidate and the closest varieties

### Conditions

- Possible use of specific lay-out to compare the varieties (side by side, row plots,)
- Possible use of panel of experts
- Visit of the trials by the applicant

### In the Office Distinctness

The variety is clearly distinct (plus Uniformity and Stability)

### Decision

- Positive report
- Final description
- The variety is not clearly distinct from one or several reference varieties
- With no difference observed and no claim from the applicant

### Rejection

With no difference observed and claim from the applicant with additional reliable information

### Third growing cycle

With a set of small differences but not consistent over the two first cycles and experts convinced that the candidate variety is original

If supporting evidence —————> acceptance

If no supporting evidence —————> third growing cycle

### Third growing season Distinctness

Direct comparison of the candidate and the similar reference varieties  
Description (complement)

### Conditions

- As for the second growing season, plus
- Direct comparison in different locations
- Possible use of mixtures and coded samples in the applicant's premises
- Possible use of morphological distance
- Possible use of "supporting evidence" characteristics
- Contact with other DUS services

### In the Office Decision

- If clearly distinct based on
- Consistent differences among 3 cycles
- Or a small differences + positive judgments of experts + "supporting evidence" characteristics

### Acceptance

If none of these conditions

### Rejection

Example: Wheat variety 'Torlesse' in New Zealand  
Analysis of Distinctness during 1999/2000 and 2000/2001

**Source:** Bill Whitemore (2002) Conduct of the DUS testing in New Zealand Presented at National Seminar on the Protection of Plant Varieties under the UPOV Convention at New Delhi, Feb. 18-19, 2002. PP. 1-7.

#### **Analysis of the Distinctness of Torlesse wheat-199912000 and 2000/01**

Torlesse is an alternative, medium maturity, medium height wheat with scurs.

Other alternative / spring wheats with scur are Amethyst, Belfield Commando, Domino, Kotare, Millbrook and Tancred., Torlesse is distinct from Amethyst as it has different grain colour (red vs purple), from Domino and Millbrook as it has a different stem pith thickness (same vs none), from Kotare as it is shorter (medium vs tall), and from Tancred as it is later maturity (medium vs early). This leaves Belfield and Commando as the most similar varieties

#### **Distinctness**

Torlesse was found to be distinct from Belfield as

It has greater ear glaucosity (7/vs/1;5/vs/1)

It has a different ear shape (tapering vs parallel)

It has a wider glume shoulder (6/vs/4;7/vs/5)

It has longer ears (7/vs/5; 7/vs/5)

Torlesse was found to be distinct from commando as:

It has a lesser intensity of auricle anthocyanin (1/vs/7; 1/vs/5)

It has earlier ear emergence (3, 4 days)

It has shorter plant (41vs/6; 31vs15)

It has shorter ears (7/vs/9; 7/vs/8)

This is followed by a summary report on the examination and final recommendation

**Distinctness:**Torlesse wheat is distinct from any other wheat in New Zealand whose existence is known to us. It most closely resembles commando, but has earlier emergence

**Uniformity and Stability:** All plants appeared uniform in their growth characteristics. No off-types were observed.

**Recommendations:** Recommended for grant.

#### **Testing distinctness in cross-fertilized species**

For testing of varieties for distinctness based on measured characteristics we need to establish a minimum distance between varieties. The pair of varieties showing difference greater than the minimum might be regarded as “distinct” in respect of that character. There are several possible ways for computing minimum distance for establishing distinctness, uniformity and stability. Some of them are: to be less uniform than those smaller plants. If the same standard is applied to all varieties, it is possible that some will have to meet very strict criteria while face standard, which are easy to satisfy.

#### **Testing stability**

According to the PVP Act the variety must be stable in its essential characteristics. It is not generally possible during a period of 2 to 3 years to perform tests on stability, which lead to the same certainty as the testing of distinctness and uniformity. Generally, when a submitted sample has been shown to be homogenous, the material can be considered stable. As far as necessary, stability has to be tested by growing a further generation of new seed stock to verify that it exhibits the same characteristics as those shown by the previous material supplied.

Information compiled in the article have been gathered from following UPOV documents

- I. TG/36/7(2000). Draft or outlines for documents complementing the general introduction to the assessment of Distinctness, Uniformity and stability in new varieties of plants.
- II. TG /1/2(1979). Revised general introduction to the guidelines for the conduct of tests for DUS of new varieties of plants.
- III. TW A/29/8(2000). Process for establishing distinctness.
- IV. TGP/8.SDraft I. Special methods of DUS examination.
- V. Bill Whitemore (2002). Conduct of the DUS testing in New Zealand. Presented at National Seminar on the Protection of Plant Varieties under the UPOV Convention at New Delhi, Feb., 18-19, 2002.

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## Protection of Plant Varieties and Farmers Rights act, 2001 and DUS test-an over view

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Any property created by human intellect which can be incorporated in tangible objects and reproducible in different locations and rights granted on such finding is called intellectual property right. Depending on the nature and tangibility of the intellectual property, different type of rights such as patent, copy rights, trademarks, industrial designs, plant breeders or farmers rights, protection of undisclosed information, protection of database etc., are granted by the respective competent authority. India being the signatory and founding member of world trade organization; thrust on plant variety protection of undisclosed information, protection of database etc., are granted by the respective competent authority. India being the signatory and founding member of world trade organization; thrust on plant variety protection has been envisaged under the provisions of Trade Related aspects of intellectual property rights (TRIPS), which is an integral part of WTO. The member countries of WTO have freedom in formulating their own system of plant variety protection either patent or an effective sui generic system under the provisions of article 27.3 (b) of TRIPS agreement. Accordingly, India opted for the sui generis system of plant variety protection that paved the way for enactment of the protection of plant varieties and farmers rights bill 2001. The rationale behind the adoption of sui generis system in India is that it is rich in biological resources with greater amount of diversity. This concern more on the equity share of rights of farmers, rights of village community and rights of village community and rights of genera, species and varieties, level and period of protection, sustainable development of agro biodiversity with benefit sharing arrangement.

The section 14 of protection of plant varieties and farmers rights act provide immense opportunity for the registration of genera, species an extant variety, a farmers variety and a new plant variety provided it should confirm to the criteria of **Novelty, Distinctness, Uniformity and Stability**. As per the Act

### Novelty

Means at the date of filing of the application for registration for protection, the propagating (or) harvested material of such variety has not been sold or otherwise disposed of by the breeder or his successor for the purpose of exploitation earlier than one year in India, out side India, in the case of trees (or) vines earlier than six years or in any other case earlier than four years.

### Distinctness

It means that if the new variety applied fir protection is clearly distinguishable by at least one essential characteristic from any other variety whose existence is a matter of common knowledge in any country at the time of filing of application. Filing of an application for the granting of a breeders right to a new variety or for entering such variety in the official register of varieties in any convention country shall be deemed to render that variety a matter of common knowledge from the date of the application in case the application leads to granting of the breeders right.

### Uniform

A variety is considered as uniform if subject to variation that may be expected from, the particular features if its propagation it is uniform in its essential characteristics.

### Stable

A variety is stable if its essential characteristics remain unchanged after repeated propagation or in the case of a particular cycle of propagation at the end of each such cycle.

### Application for registration of plant variety protection

Application for registration of a new plant variety can be made independently or jointly by any person claiming to be a breeder, any successor of breeder of the variety, any person being the assignee of the breeder of the variety, any farmers or group of farmers or community of farmers, any person authorized in prescribed manner by a person and an university or publicly funded agricultural institution claiming to be the breeders of the variety.

A new variety submitted for protection may not considered for registration if it is not capable of identifying itself, consists solely of figures, liable to mislead to cause confusion concerning the characteristics, no different from every denomination, likely to deceive the public or cause confusion in public regarding identity, likely to hurt religious sentiments, prohibited for use as a name of emblem for any purpose and having the name of geographical location.

### IPR: Intellectual Property Right

- IP is the property created by the human intellect-which can be incorporated in tangible objects and reproducible in different locations
- Depending on the nature and tangibility of the IP, different type of rights, called intellectual property rights (IPR), are granted by the state **Types of IPR**
- Patent
- Copy rights
- Trade marks
- Industrial designs
- Layout designs
- Plant breeder's or farmer's rights
- Protection of undisclosed information
- Protection of database
- Geographical indications

### IP Protection in Indian Agriculture

The Indian Patents Act 1970 does not provide patent for:

- Method of agriculture or horticulture
- Any process for medicinal, surgical, curative, prophylactic or other treatment of animals or plant
- Any living form including microorganisms
- Products obtained from chemical processes
- All innovations from biological research

### Implications of WTO

The issue of plant variety protection has been brought into focus under the provisions of trade related aspects of intellectual property (TRIPs) rights which is a part of agreement on agriculture under world trade organizations (WTO). India is a signatory and founder member of WTO. This casts an obligation on the member countries to provide for a system of plant variety protection

### TRIPs

Article 27.1-Patents shall be made available for all inventions, whether products or processes in all fields of technology. Article 27.3- Shall provide for the protection of plant varieties either by patents or by an effective sui generis system. Accordingly, India adopted sui eneris system of plant variety protection, which paved the way for enactment of the Protection of Plant Varieties and Farmer's Right's Bill 2001.



### **The rationale behind sui generis system**

- India is rich in biological resources
- Greater amount of diversity
- Equity share of
  1. Right's of farmer's
  2. Right's of village community
  3. Right's of researches

This system also provide wider flexibility in protection of genera, species and varieties, level and period of protection, sustainable development of agro biodiversity with benefit sharing arrangement

### **The protection of plant varieties and farmer's right's Act, 2001**

#### **PVP authority**

- The authority shall be a body corporate by name
- The head of office of the authority shall be as notified by Govt. of India
- The authority shall consist of a chairperson and 15 members
- Chairperson- appointed by GOI
- Members
  1. DDG (Crop Sciences)
  2. Director (NBPGR)

#### **Mandate of PVP authority**

1. Registration of plant varieties
2. Characterization and documentation of registered varieties
3. Documentation, indexing and cataloguing of farmers varieties
4. Providing compulsory cataloguing facility for all plant varieties
5. Ensuring that seeds of all registered varieties are made available to farmers
6. Collection of comprehensive statistics on plant varieties.
7. Maintenance of national register of plant variety

#### **Plant Varieties Registry**

1. Registrar general of plant varieties to be the chief executive functionary
2. There may be number of registrars required
3. Register called the national register of plant varieties-kept at the head office of the registry

#### **Registration of plant varieties and EDV**

Application for registration

- a. Genera and species as specified by GOI
- b. Which is an extant variety
- c. Which is a farmers variety

#### **Applicant**

- a. Any person claiming to be the breeder
- b. Any Successor of the breeder
- c. Any person being the assignee of the breeder
- d. Any farmer or group of farmers or community of farmers
- e. Any university or publicly funded agricultural institution

A new variety shall be registered under this act if it conforms to the criteria of Novelty, Distinctness, Uniformity, and Stability

#### **Novel**

Novel, if, at the date of filing of the application for registration for protection, the propagation or harvested material of such variety has not been sold or otherwise disposed of by or with the consent of its breeder or his successor for the purpose of exploitation of such varieties-

1. In India, earlier than one year or
2. Outside India, in case of trees or vines earlier than six years, or in any other case, earlier than four years before the date of filing application

#### **Distinct**

Distinct if it is clearly distinguishable by at least one essential characteristic from any other variety whose existence is a matter of common knowledge in any country at the time of filing of the application.

### **Stable**

Stable, if its essential characteristics remain unchanged after repeated propagation or, in the case of a particular cycle of propagation, at the end of each such cycle

### **A new variety shall not be registered**

1. If not capable of identifying such varieties
2. Consist solely of figures
3. Liable to mislead to cause confusion concerning the characteristics
4. Is not different from every denomination
5. Cause confusion public regarding identity
6. Likely to hurt religious sentiments
7. Is prohibited for use as a name of emblem for any purpose
8. Comprising of geographical name

### **Application**

Every application for registration under section 14 shall:

- a. Be with respect to a variety
- b. Denomination assigned to such variety by the applicant
- c. Be accompanied by an affidavit sworn by the applicant that such variety does not contain any gene or gene sequence involving terminator technology
- d. Be in such form as may be specified by regulation
- e. Contain a complete passport data of parental lines from which the variety has been derived along with geographical location (genetic material) and information relating to the contribution (farmer / village community / institution/ organization in breeding, evolving or developing a variety)
- f. Accompanied by statement containing a brief description of the variety bringing out its characteristics of NDUS as required for registration
- g. Accompanied by fees as prescribed
- h. Contain declaration that genetic material required for breeding, evolving or developing the variety has been lawfully acquired
- i. Be accompanied by other particulars as may be prescribed
- j. Every application shall be filed in the office of the registrar

### **Conduct of test**

- Every applicant shall make available prescribed quantity of seed of a variety for registration, for conduct of test along with parental material conforming to the standards
- During conduct of test, seed viability and quality shall remain unaltered
- The applicant along with application should deposit prescribed fees
- Tests should be conducted in prescribed manner

### **Acceptance of application**

- a. Registrar will accept the application after making enquiry, it finds the information as prescribed in regulation
- b. Amendment / reject

### **Advertisement of application**

1. Advertise in local newspaper
2. any person with three months from date of advertisement of an application-may give notice in writing in prescribed manner to registrar
3. Opposition
  - a. Person opposing the application is entitled to breeders right as against the applicant
  - b. Variety is not registrable under this act
  - c. The grant of certificate of registration may not be in public interest
  - d. The variety may have adverse effect on the environment

- The registrar will serve a copy of notice within two months from receipt of application
- Applicant shall send his counter statement
- Registrar will serve a copy of counter statement
- Registrar shall issue a certificate of registration and seal with seal of registry

#### Technical questionnaire

- Information on the origin, maintenance and reproduction of the variety
- State of expression for each of the grouping characteristics
- Similar varieties and the differences from them
- Additional information of distinguishing the variety
- Resistance to pest and diseases
- Special conditions required for examination of the variety
- Other useful information

#### Facilities

- Land for conduct of DUS test
- Public /private gardens (roses etc.)
- Orchard (trees)
- Glass house facilities
- Growth chambers
- DUS test guidelines
- Reference collection

#### Staff

- Scientist in charge of DUS test
- Separate technical staff / examiners for each species of group of species
- Persons responsible for secretariat work
- Limited number of casual labour to assist the examiner in seasonal work

#### DUS testing options

1. Government testing
2. Breeder testing
3. Combination of both
4. Foreign test reports

#### DUS testing system in operation

Government testing	-European Union Countries
Breeder testing	-USA, Canada, Australia
Combination of both	-New Zealand, Japan
Potato, Grasses, White cloves	-Govt. testing
Fruit varieties	-Central testing by Research Institute
Minor agricultural crops	-Breeder testing

#### Distinctness of a candidate variety is established by:

1. Morphological characters eg. UPOV guidelines
2. Additional phenotypic characters
  - Yield
  - Sugar content
  - Disease resistance
  - Oil quality (Erucic acid etc.)
  - Fertility behaviour / combining ability (for autogamous parental lines)
3. Additional non-phenotypic convincing evidence eg. Electrophoretic characteristics

These should be used in combination with phenotypic characteristics,

- Only if characteristics listed above fail to establish sufficient distinctness
- With the agreement of the applicant
- If a test procedure has been agreed upon between the competent authority and the applicant

There should be,

- A standardized methodology
- Agreement on which band could be used
- An agreed format for incorporation of the characteristics in to the guidelines and data base

#### **Procedures of filing PVP application in ICAR system**

Primary application forms

Form 1- application-undertaking in respect of bonafides of invention

Form 2-complete specification –declaration to the effect that ICAR is the authorized signatory

Submit the application through proper channel to ADG (IPR)

#### **DUS test guidelines for cotton**

a. Seed submission

1kg of seed in each year of testing

2kg of seed for storage in the reference collection

Or

4kg of seed in one single seed submission

b. During conduct of test, seed viability and quality shall remain unaltered

c. The applicant along with application should deposit prescribed fees

d. Tests should be conducted in prescribed manner

#### **Designs of layout**

Design : RBD

Replication : 4

Row length : 6 m

Row to row spacing : 90cm

Plant to plant spacing : 60cm

No. of rows / replication : 5

#### **DUS test centres**

- CICR, Nagpur
- CICR, RS, Coimbatore
- CCS HAU, Hisar (NSP centre)

UAS Dharwad (NSP centre)

- PAU, Ludhiana

#### **Special test centres on contractual basis**

NBPGR for testing transgenic cotton

Surat for testing herbaceum species

#### **Test duration**

Minimum 2 similar period

#### **Preparations for implementation of PVP&FR, 2001**

- Subordinate legislation
- National test guidelines
- Extant varieties
- Developing a system

#### **Progress**

- NTGs of identified crops
- Documentation of extant-notified varieties
- Human resource development
- Rules & regulations
- SFC Memo of authority

#### **Perceived difficulties in implementation**

- Mechanism for 'workable benefit sharing policies'
- Workable means to deal with sovereign rights in an international sitting
- Participation of small farmers in commercial situations
- Varieties with 'technology capsule'
- Expected performance under given conditions

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## Conduct of DUS Test in Cotton: Planting material, duration, plant population, experimental design, method and observations

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The Protection of Plant Varieties and Farmers' Rights Act, 2001 envisages that protection for new variety shall only be granted after careful examination of the candidate variety. The prescribed examination should be adapted to the special requirements of each species and crop variety. The PVP&FR also encourages that a new variety shall be registered under this act if it conforms to the criteria of novelty, distinctness, uniformity and stability.

### Novel

Novel, if, at the date of filing of the application for registration for protection, the propagating or harvested material of such variety has not been sold or otherwise disposed of by or with the consent of its breeder or his successor for the purpose of exploitation of such varieties-

1. In India, than one year or
2. Outside India, in case of trees or vines earlier than six years, or in any other case, earlier than four years before the date of filing application

### Distinctness

A variety is distinct if it is clearly distinguishable by at least one essential characteristic from any other variety whose existence is a matter of common knowledge in any country at the time of filing of the application

The variety must be clearly distinguishable by one or more important characteristics from any other variety whose existence is a matter of common knowledge at the time when the protection is applied for. Common knowledge at the time when the protection is applied for. Common knowledge may be established by reference to various factors such as: cultivation or marketing already in progress, entry in an official register of varieties already made or in the course of being made, inclusion in reference collection, or precise description in publication.

### Uniformity

A variety is uniform, if subject to the variation that may be expected from the particular features of its propagation, it is uniform in its essential characteristics

The variety is deemed uniform if, subject to the variation that may be expected from the particular features of its propagation, it is uniform in its relevant characteristics. Relevant characteristics include at least all characteristics used as a basis for distinctness or included in the variety description established at the date of grant of protection of that variety.

For vegetative propagated and self-pollinated varieties the basis of assessment is normally the number of off types in the variety, judged on the basis of a population standard and an acceptable probability fixed in the corresponding species. In particular, for cross-pollinated species the basis of assessment is the variation in comparable variety (relative uniformity).

### Stability

A variety is stable, if its essential characteristics remain unchanged after repeated propagation or, in the case of a particular cycle of propagation, at the end of each such cycle. It is not usually possible during a period of two or three years to perform test on stability. Generally, when a submitted sample has been shown to be in uniform, the material can also be considered stable. Careful attention has to be paid to stability when testing for distinctness and uniformity. Where appropriate, stability is tested by growing a further generation from new seed stock to be supplied by the applicant to ensure that it exhibit the same characteristics as those shown by material supplied previously.



### **Planting materials for cotton**

The required quantity and quality material is specified for each crop. The quantity of planting material should cater the need of two years of DUS tests and deposit in reference collection. It is recommended that plant material should not have undergone any treatment. If the seed has been treated full, details should be supplied to the authorities. Quantity of planting material recommended in National Test Guidelines of cotton is 4000 grams in only one submission for Variety, hybrid and parental line

1. The Plant Variety Protection (PVP) Authority decides when, where and in what quantity and quality the plant material required for testing the variety is to be delivered. Applicants submitting material from a country other than India must make sure that all customs formalities are complied with. The minimum quantity of seed to be supplied by the applicant:
2. The seed should meet the minimum requirements for germination capacity, moisture content and physical purity prescribed for certified seed in India. Especially for storage, which requires a higher standard, the applicant should state the actual germination capacity that should be as high as possible but not less than 75%.
3. The plant material must not have undergone any treatment unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

### **Duration of DUS tests**

New varieties of plants must be tested for DUS at least for two similar growing seasons.

### **Test Locations**

Usually the DUS examination required more than one independent growing cycle for studying the consistence of results. There are several options for multiple growing cycles.

- The candidate varieties are studied in a given location, over at least two successive seasons.
- For many crops, it is possible to complete two growing cycle in the same year. The two growing cycles should be independent of each other.
- For some crops such as fruit trees, the same plants are examined over successive years. The condition of independence of growing cycle is also satisfied in this case.
- For plants grown in green houses, provided the time between the sowing is not 'too short' and the trial is randomized, at least partly, cycles can overlap and still be compared as independent.
- In some circumstances, authorities can allow one growing season. Such a possibility is mentioned in crop specific guidelines.

### **Test Locations for cotton**

The DUS test locations for cotton are

- Central Institute for Cotton Research, Nagpur
- Central Institute for Cotton Research, Regional Station, Coimbatore
- National Seeds project Unit, University of Agricultural Sciences, Dharwad
- Department of Seed Science & Technology, CCS HAU, Hisar
- PAU, Ludhiana

### **Conduct of tests in cotton**

- The minimum duration of tests should normally be two independent similar growing seasons with reference to the ecosystem of the variety submitted for DUS test.
- The test should normally be conducted at two test locations. If any important characteristics of the variety can not be seen at these places, the variety may be tested at an additional place.

- The test should be carried out under conditions ensuring normal growth. The size of the plot should be such that plants or parts of plant may be removed for measuring and counting without prejudice to the observation which must be made upto the end of the growing period. Each test should include a minimum of 150 plants, which should be divided among 4 replications. Separate plots for observation and for measuring can only be used if they have been subjected to similar environmental conditions.

#### Test plot design

No. of rows	: 5
Row length	: 6 m
Row to row distance	: 90 cm
Plant to plant distance	: 60 cm
No. of replications	: 4

Observations should not be recorded on plants in border rows.

Additional tests for special purpose may be established.

#### Methods and observations

- The characteristics described in the table of characteristics should be used for the testing of varieties, inbred lines and hybrids for DUS.
- For the assessment of distinctness and stability, observations should be made on 40 plants or parts of plants, which should be divided among 4 replications (10 plants per replication). The number of apparent plant should not exceed 4 in 40.
- For the assessment of uniformity of characteristics on the plot as a whole (visual assessment by a single observation of a group of plants or parts of plants), the number of aberrant plants or parts of plants should not exceed 8 in 150.
- All leaf characteristics should be observed on 4<sup>th</sup> leaf from the top.
- For the assessment of colour characteristics, it is recommended that Royal Horticultural Society (RHS) colour chart be used.

#### Reasons to use more than one location

Breeders throughout the world now are pressing the Plant Variety Protection Authorities for the availability of the DUS test results within the shortest possible time. One of the possibilities being explored as the conduct of DUS test at more than one location in the same season. There are other different reasons why authorities may have more than one location.

- Varieties of different geographical regions may require different agro-climatic growing conditions. Different locations can be used in order that the ad-hoc growing conditions are met.
- Some offices might have a primary location, backed by a safety location. Normally, only the data from primary location will be used, but in case of this location has major problem then the second one will be available to prevent the loss of one-year results.
- Some office may have more than one location for a given crop for testing candidate varieties at all these locations. Each location in this case is considered as completely different and separate examination. Each location has a result. When all examination result in the positive conclusion, the variety is accepted for PVP.
- Even UPOV is currently exploring the circumstances in which more than location might be used in order to obtain independent growing cycles in a given year. In such cases, the locations must have different environments,
- In order to provide double check for consistency, some offices systematically grow the varieties in more than one location (usually two). In this case, the consistency over cycle for each location and the consistency between locations are checked.

#### Sample size for DUS Testing

The UPOV recommendations to put 60 plants (3 times 20) into a DUS trial are not a general rule. The question is not: What is the optimal sample size in DUS testing for specific crop over all characteristics? For qualitative characteristics distinctness procedure are not the basis to

determine the optimal sample size up to now. However, for uniformity point of view the optimal sample size can be calculated. Maximum of determined sample size is from the statistical point of view the optimal. The sample size depends on a number of factors:

- Precision at the stage of individual single plants (within plots)
- Precision at the stage of replication (over the plots)
- Precision for years or cycles (over the years or cycles)
- Uniformity of a variety within the species
- Type of characteristics in respect of variability within the variety, over the plot and over the year/cycle.

It is difficult to determine the optimal sample size. Per characteristic, per stage and per type of testing, it is possible to give formulas for calculating the number of plants or the number of plots, but it is not clear how to combine all these individual calculations. Another difficulty is that the crop expert has not enough information about the variation when he starts the work with a new crop and when he has to establish new guidelines.

#### **Type of experimental design**

Because of the presence of only one treatment factor (variety) in DUS trials, simple designs are most used.

##### **i. Completely randomized design**

When several varieties are examined in a number of replications, the varieties can be completely randomized over all plots in the field. This design is only recommended if the total number of plots is small.

##### **ii. Randomized complete block design**

It is the most used experimental design in DUS trials. In CBD, number of plots per block equals the number of varieties and all varieties are placed in each block. The advantage of this design is that SD between plots does not contain variation due to differences in blocks.

##### **iii. Randomized incomplete block design**

In this design, the number of plots per block is less than the number of varieties. Such design may be appropriate when the number of varieties is very large. In this case, the block size for a randomized complete block design would become so large that the plots within a block would be too heterogeneous.

#### **Plot elements**

The plot is the smallest sub-division of the trial and the unit on which the varieties and the soil and plant conditions should be focused. Therefore, the trial elements: plot size, shape of plots, alignment of the plots, barrier rows and border strips, protective stripes should be arranged accordingly. Plot size and shape of the plots depend also on the soil conditions and on sowing and harvesting machinery. Narrow and long plots are preferred from the technological point of view. The best length to width ratio lies between 5.1 to 15.1 and depends on the plot size and the number of the varieties. The larger the number of varieties in the trial the narrower the plots.

#### **Number of replications**

The number of replications can be calculated with the help of a statistical formula which include several components such as deviation between plots, difference between the variety means which has to be significant, percentage point of Student's t-distribution, degree of freedom in correspondence to the "between plot" standard deviation, number of varieties, number of blocks, type I error and type II error. Blocking is practiced to overcome the differences in fertility. To minimize the standard deviation for computing variety SD, the plots are arranged in blocks. The blocks are arranged in such a way that plots within block have comparable fertility and the fertility differences are between the blocks.

#### **Grouping of varieties**

- The collection to be grown should be divided into groups to facilitate the assessment of distinctness. Characteristics that are suitable for grouping purposes are those which are

known from experience not to vary, or to vary only slightly, within a variety and which in their various states are fairly evenly distributed within the collection.

- It is recommended that the competent authorities use the following characteristics for grouping varieties.
  - (i) Leaf: shape (characteristic 5)
  - (ii) Flower: petal colour (characteristic 17)
  - (iii) Bo1l: shape (longitudinal section) (characteristic 23)
  - (iv) Fibre: Length (characteristic 27)

It will be a very difficult task to assess the candidate variety for distinctness against all the varieties in reference collection or in common divided into groups based on grouping characteristics. These are characteristics, which are known from experience not to vary, or to vary only slightly, within a variety. A candidate variety falling into one group may be sufficiently distinct from varieties in other groups that there is no need to compare them in trials.

#### **Reference collection**

Each country maintains the reference collection for conducting DUS testing of variety submitted for protection. It may contain both living material and descriptive information's. A variety is included in reference collection only if seed / plant material is available to make a technical examination. Theoretically, the full reference collection to be used for comparison for any candidate variety is the world -wide known collection of varieties of same species and crop. However, in practices the number of varieties in reference collection can be reduced by selecting varieties from similar environmental regions. The selection can usually be further narrowed down to only the most closely similar varieties supplied by the breeder in Technical Questionnaires.

#### **Types of characteristics for DUS testing**

##### **i) Truly qualitative characteristics**

The characteristics, which are expressed in discontinuous states with no arbitrary limit on their number, are called truly qualitative characteristics. These states are self explanatory and independently meaningful. Each state is clearly different from the others and as a rule; the characteristics are less susceptible to environment.

##### **Example**

Sex of plant: dioecious female (1), dioecious male (2), monoecious unisexual (3), monoecious hermaphrodite (4)

Type of flower: single (1), semi-double (2), double (3)

Ploidy: diploid (1), tetraploid (4), hexaploid (6) octoploid (8)

Resistance: no resistance (1), resistance to one or several races (2), resistant to all races Colour: colourless (1), single coloured (2), bicoloured (3), multicoloured

##### **ii) Quantitative characteristics**

These are the characteristics, which are recorded on a one- dimensional scale and show continuous variation from one extreme to the other. They are divided into a number of states of expressions for the purpose of description. The division is made only for description and not for distinctness purpose. The test guidelines do not specify the difference needed for distinctness. The state of expression should be meaningful for DUS assessment. The whole range is divided into nine states, which are normally equally spaced and measurable on one-dimensional scale. Some truly quantitative characteristics may be handled as qualitative when only a condensed range is used instead of full range of nine states. In case of all the quantitative characteristics, the full scale 1, 2, 3, 4, 5, 6, 7, 8, 9 is applicable. However, for practical purposes of presentation only notes 2, 5, 7 or 1, 3,5,7,9 are given in test guidelines to indicate' that the quantitative scale is applicable.

##### **Example**

Intensity of pubescence: weak (3), medium (5), strong (7) (quantitative characteristics)

Pubescent: absent (1), present (9) (qualitative characteristics)

### iii) Pseudo-qualitative characteristics

The range of expression is at least partly continuous varying in more than one dimension. These cannot be defined by just two ends of a linear range. These characteristics do not fit the definition of truly qualitative characteristics, but are treated as qualitative when it is more reasonable to disregard continuous variation for practical purposes and the states created are meaningful and sufficiently different from each other.

#### Example

Shape: ovate (1), elliptic (2), round (3), obovate (4)

Expression: absent or very weakly expressed (1), weakly expressed (2), strongly expressed

Growth habit: upright (1), pendulous (2)

#### Selection of characteristics

The characteristics must be important for the description of varieties and therefore, for the assessment of DUS, such characteristics may be biochemical or of another nature. These characteristics are selected from the point of view of suitability for description morphological, physiological, and for DUS testing of varieties and not for their commercial value. Characteristics of commercial value such as yield are usually affected by environment. The superiority or usefulness of a variety is not criterion for protection. It is for the user of the variety and not for the testing authorities to decide on its superiority or usefulness.

#### Basic requirement of characteristics for DUS testing

- a) Capable of precise definition
- b) Produce consistency and repeatable results for existing varieties
- c) Allow uniformity requirements to be fulfilled
- d) Clearly defined in the observation and evaluation of results
- e) Allow a clear differentiation among the varieties
- f) Least susceptible to environment influence

Characteristics like, disease resistance, chemical resistance (herbicide) as well as characteristics based on chemical constituents may be included provided they are precisely tested.

#### Categories of characters

##### Grouping characteristics

These characteristics can be universally used for grouping varieties.

These are sufficiently independent of environmental influences in all regions.

Level of expression of these characteristics should be sufficient for establishing distinctness. In DUS, tests groups are formed in such a manner that a candidate variety will only be compared to varieties in its groups. All varieties in reference collection similar to the candidate variety must be in that group. In most agricultural species, the groups are actually formed in the trials while in fruit tree species the groups appear on paper only since trees cannot be rearranged annually according to new candidate varieties.

##### i) Asterisked characteristics

Authorities consider these characteristics important for testing of DUS. These characteristics should be based as a matter of routine for all varieties in every growing period. These should always be included in variety description except when the regional environmental conditions render this impossible. They are marked with asterisk (\*) in test guidelines. A characteristic should only receive an asterisk status if it a) important for description b) it is needed as minimum information c) all expert agree to asterisk at least d) the range of example varieties remain the same in different countries in case the expression changes from country to country e) for a pest or disease resistance characteristics, it has only "absent, present" states,

##### ii) Standard characteristics

The characteristics that are considered appropriate by the authorities for testing DUS but not considered necessary by all the countries included in test guidelines without an asterisk. Standard characteristics not included in test guidelines. These are those characteristics suitable for testing of DUS but are important only in one or few countries. These are needed only very rarely for distinctness is not included in test guidelines.



#### **iv) Supporting evidence characteristics**

These characteristics are not considered sufficient on their own to establish distinctness but may provide supporting evidence for other differences, which are used for distinctness. They are not included in test guidelines, but if they meet certain requirements, are included in an Annex to test guidelines. These are not used as routine characteristics but only at the request of applicant of the candidate variety and if a test procedure has been agreed upon between the competent authorities. In case of electrophoresis, supporting evidence characteristics are that there has to be good knowledge of genetic background, a standard method and a positive result in a ring test method between member countries.

#### **Explanation on the table of characteristics**

A separate chapter "Explanation and Methods" follows a table of characteristics in the test guidelines. It describes explanations, drawing, photographs or the methods, which are necessary for the understanding of the different characteristics mentioned in the table of characteristics.

#### **Technical questionnaire**

A proforma containing Technical Questionnaire about the candidate variety is to be submitted by the applicant seeking plant variety protection.

The applicant is asked.

- Name of species
- Applicant (name and address)
- Proposed domination or breeder's reference
- Information on origin, maintenance and reproduction of the variety
- Type of material
- State of expression for each of the grouping characteristics
- Most similar varieties and the difference of candidate variety from these varieties
- Any additional information, which can help to distinguish the variety
- Resistance to pests and diseases
- Any special condition required for examination of the variety
- Any other useful information

#### **Methods and observations**

The recommendations are given on

- The number of plants, or parts of plants, that should be observed when assessing distinctness and stability.
- The maximum number of aberrant plants permitted when assessing the uniformity of characteristics of plot as a whole.
- The permitted tolerance when assessing uniformity on single ear panicle rows, plants or parts of plants.

#### **Recommendation and Grant**

By the end of the second year, all the necessary recordings from the growing trials should have been taken. The examiner analyzes the data and prepares his recommendation on whether or not each candidate variety meets the DUS criteria.

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## Testing of varieties for Distinctness, Uniformity and Stability

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In order to provide incentive for development of plant varieties to public and private sector research organizations and to fulfill obligations under Trade Related Intellectual Property Rights (TRIPs), India has enacted legislation for the Protection of Plant Varieties and Farmer's Rights in 2001. This act provides protection of new varieties including extant and farmers' varieties. Novelty, Distinctiveness, Uniformity and Stability are the essential requirements for grant of protection to all the varieties.

The concept of distinctness, uniformity and stability are fundamental to the characterization of a variety as a unique creation. . "Distinct" means that the variety must be clearly distinguishable by one or more important morphological, physiological or other characteristics from any other variety whose existence is a matter of common knowledge at the time of application. "Uniform" means that the variety must be sufficiently uniform or homogeneous having regard to the particular features of its sexual reproduction or vegetative propagation. "Stable" means that the variety must remain true to its description after repeated reproduction or propagation or, where the application prescribes a particular cycle of reproduction or multiplication, at the end of each cycle.

### Procedures

#### 1. Application (Technical Questionnaire)

The breeder seeking protection for his new variety is required to submit an application form and a detailed technical questionnaire. On the basis of this information, supplemented by further inquiries as necessary, the PVP authority determine whether it is dealing with a prima facie valid application. Although the breeder is required to describe the characteristics of his new variety to the best of his ability, his description is not regarded as defining the limits of his claim to registration. Its main function in practice is to guide the authorities in carrying out trials on the living plant material, including the selection of suitable control varieties for purpose of comparison.

#### 2. Seed or a Candidate Variety

The breeder is required to supply prescribed quantities of seed or other reproductive material when required for sowing of planting. The growing tests are normally conducted for a minimum of two successive growing seasons under the control of authority. The layout of the tests, their location and duration, the numbers of centres, and the nature of the observations to be made on the plants are all prescribed in regulations and working rules laid down by the authority. These regulations and rules differ from one species to another. The working arrangements for tests are flexible and can be changed in the light of experience or to deal with special cases. Fees are payable by applicants to cover a proportion of the costs of the systems.

#### 3. Reference Collection

In order to establish distinctness, it is usually necessary to compare the candidate variety with existing varieties. For this purpose, the test centres maintain reference collections of authentic samples of seed of existing varieties. The candidate varieties and appropriate varieties from the reference collection are compared in field and laboratory tests. The number of comparisons depends on the species, but where sufficient information is available, comparisons are limited to groups or varieties, which are similar to the candidate variety.

#### 4. No. of Years or Testing

Tests for DUS normally take two years for self-pollinated crops but cross-pollinated crops may require three years.

## 5. Characters used for DUS

In many cases, the distinctness of self-pollinated crops can be established using characters which can be assessed by visual examination and whose expression falls into clearly defined discrete states. Because of the genetic diversity of cross-pollinated crops many of the varietal characteristics are on a continuous scale of expression and require measurement. Usually, distinctness can be determined only on the basis of statistical analysis. It is normal to require the difference between two varieties to be statistically significant at equal to or less than a probability of 0.01 or 0.05, depending on species, for the same characteristic in two out of three years. For herbage species combined over years analysis is used using a significance level equal to or less than a probability of 0.01.

The number of characters examined tends to be governed by the number of readily identifiable characters associated with the crop, the amount of effort required to record those characters, and the value of those characters in distinguishing varieties. Fewer characters are used in crops, which rely on precise measurement and statistical analysis to establish distinctness than in crops whose characters can be recorded simply by observation. The cost of precisely measuring characters is high so that there are cost advantages in keeping the number of characters as low as possible. Simply observed characters are much cheaper to record and the number of characters has little effect on the overall cost. The number of characters examined in the test of distinctness depends on the crop. An indication of the range of characters as per UPOV (International Union for the Protection of New Varieties of Crops) is shown in the following table.

Crop	No. of Characters	Characters with * mark
Wheat	33	20
Rice	30	5
Sunflower	40	13
Peas	58	27
Maize	34	8
Onion	27	12

\*Mandatory characters to be recorded.

## 6. Tests for Uniformity

The definition of uniformity of a variety takes into account its reproductive system. In the authority guidelines a variety is required to be sufficiently uniform, depending on its breeding system, to allow accurate description and assessment of distinctness, and to ensure stability. For self-pollinated species up to 1% off-types are normally tolerated in an except cereals. For cereals all the seeds from 100 individual ears are sown in separate rows. Variant ear rows are tolerated up to a maximum of 3 in 100.

For cross-pollinated crops a variety is sufficiently uniform if the standard deviation for each character examined for distinctness is not repeatedly greater than the pooled standard deviation of the same characters in comparable control varieties, in the some season and test, at a probability of 0.0 1. For visually assessed characteristics the uniformity of the candidate variety is checked against known comparable varieties.

## 7. Testing of Stability

It is not generally possible during a period of 2-3 years to perform test on stability, which lead to the same certainty as the testing of distinctness and homogeneity. Generally, when a submitted sample has been shown to be homogeneous during the test, the material can also be

considered stable. Stability has to be tested by growing and further generation from the new seed stock to verify that it exhibits the some characteristics as those shown by the previous material supplied.

#### **8. DUS Test Centers:**

There are several factors to be considered in deciding where the DUS: tests should be carried out. These include:

- i. where the species can best display its characteristics; examination at one site may reveal characters, which are less obvious at another site;
- ii. where there is least risk of damage; the plots can be at risk from pests, diseases and the weather;
- iii. where most of the seed crop and main crop are grown; this gives DUS testing a link with the region where the characters will be expressed most often, through a high volume of seed certification and commercial crop production;
- iv. where there is earliness of the site; early results enable breeders and the testing authorities to plan the next season's activities more effectively;
- v. where there is ease of breeders' access; breeders like to see how their varieties are performing in the test and to discuss any problems with the testing authority staff.

#### **9. Biochemical and Molecular techniques for DUS testing**

Currently there is a lot of interest in the use of protein electrophoresis and DNA profiling techniques for DUS testing. Protein electrophoresis has been included as a supplementary tests in the DUS test guidelines of crops such as wheat, maize and barley. Research is being undertaken for inclusion of these tests in the guidelines for other crops also. Molecular techniques have not yet been included in the UPOV guidelines.

However, a lot of research is being carried out under the aegis of the Biochemical and Molecular Techniques (BMT) group of the UPOV for exploring the potential of these techniques for distinctness and uniformity testing. These methods have the advantages of being rapid, reliable, stable and high discriminatory power and multiplex ratio. The disadvantages of these methods include the erosion of minimum distances between cultivars, lack of information (in terms of the appropriate techniques to be used and harmonization with morphological characters) for developing appropriate guidelines and requirement of high skill and sophisticated infrastructure. These issues need to be addressed before these techniques can be applied for DUS testing. The future applications of biochemical and molecular techniques are (i) in the establishment of essential derivation of EDV's (ii) as supplementary tests for DUS testing and (iii) for prescreening for selecting reference varieties for PBR tests.

#### **10. The way ahead**

- i. The clauses in the Act in respect of rights of the farmers to sell seeds of protected varieties and benefit sharing may not induce the private sector to increase research for investment in plant breeding particularly in self-pollinated crops. They may seek protection only hybrid varieties of high value low volume seed crops. Therefore in major cereals, pulse and oilseed crop, the public sector research organization will continue to play major role in plant breeding and seed production.
- ii. The Institutes/Research organizations engaged in plant breeding shall have to streamline their maintenance breeding programme as to make available pure seed along with the application, which shall be used for DUS tests and kept as reference sample for at least 7 years.
- iii. ICAR is expected to organize DUS tests and shall have to take steps for preparation of the Indian DUS test guidelines, data base of characters of extant and farmers varieties and create facilities for conduct of DUS tests. A well-conceived programme of training of staff for conduct of DUS tests, data compilation, analysis and its interpretation shall have to be organized at the earliest.

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## Guidelines for DUS Testing In Cotton

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Plant breeders have been struggling since long for legal protection of their rights to get benefit of new varieties developed by them. In this connection, a union called the Union for Protection of New Plant Varieties (UPOV) was constituted by member countries in 1961. After several changes the UPOV Act of 1978 came into force in 1981. The UPOV Act, 1991 has not been accepted so far. This act provides legal rights to the original breeder or owner of a variety for commercial production, marketing and export of his variety. In India, Plant Variety Protection (PVP) and Farmers' Rights Act, 2001 has been approved by the Government of India to provide legal protection to new varieties. It is likely to be enacted in 2005. This article deals first with PVP related aspects and then with DUS test guidelines in of cotton.

### Requirements for protection

There are some basic requirements for protection of new varieties. These requirements include (1) distinctness, (ii) uniformity, (iii) stability, and (iv) novelty. The first three requirements were as per UPOV Act 1978 and are known as DUS. The fourth was included in UPOV Act, 1991. These are briefly discussed below.

(i) Distinctness: The new variety must be clearly distinguishable in one or more characters from previously available varieties. It may differ in morphological quality, agronomic or any other character

(ii) Uniformity: The variety should be pure and look phenotypically similar. It should be homogeneous

(iii) Stability: The variety should give stable performance in different generations and over regions and seasons. In other words, it should give stable performance under different agro-climatic conditions.

(iv) Novelty: It refers to newness of a variety. The variety should be new one and should not have been commercially cultivated for more than one year before granting protection under PVP Act.

### Period of Protection

The period of protection varies with plant species. For field crops, the minimum period of protection is 15 years; whereas, for forest trees, fruit trees, ornamental trees, shrubs and vines it is 18 years.

### Breeders' Privilege

The legal rights provided to breeders to use protected material for further research is referred to as breeders' privilege or research exemption or breeders' exemption. The provision of breeders' privilege is in UPOV Act, 1978 but not in UPOV Act, 1991.

### Farmers' Rights

It refers to legal rights that are provided to farmers to save, use, exchange, share or sell his farm produce of a variety. Here the sale is restricted to non-commercial sale. This provision is only UPOV Act, 1978.

### Type of Varieties

In connection with plant variety protection (PVP) Act, various terms such as extant variety, candidate variety, reference variety, example variety and farmers' variety are frequently used. Hence, knowledge of these terms is essential. These are defined below:

(i) Extant variety: All released and notified varieties which have Variety not been protected are called extant varieties.

(ii) Candidate variety: A variety that has to be registered under Plant variety Protection Act is referred to as candidate variety.

- (iii) Reference variety: All released and notified extant varieties which are in seed production chain are known as reference varieties.
- (iv) Example variety: A variety that is used for comparison for a particular character is called example variety.
- (v) Farmers' variety: A variety that has been developed by a farmer and used for commercial cultivation for several years is called farmers variety

### Advantages of PVP

PVP has several advantages. It will provide incentive to breeders. They will get advantage of production and marketing of their varieties. It will lead to fast development of seed industry and improvement in quality due to competition. It will help in procurement of good material by way of purchase resulting in enrichment of the plant genetic resources.

### Disadvantages of PVP

PVP has some disadvantages. It will encourage monopolies for genetic material with special traits. The holder of PBR will produce less seed than market demand to increase price to get more price. It will inhibit free exchange seed and will encourage unlawful practice. This will lead to reduction in genetic diversity leading to narrow genetic base and narrow adaptation.

### Need for DUS Testing

The testing of DUS characters is useful in four main ways, identification of varieties, (ii) for registration of varieties and plant variety protection (PVP) Act, (iii) for varietal information system and classification of varieties into different groups, and (iv) for creating data base for plant viz. (i) for genetic resources.

### DUS Testing in Cotton

Cotton is a major fibre yielding crop of global significance. It is cultivated in tropical and sub-tropical regions of more than 70 countries. Major cotton producing countries are China, U.S.A., India, Pakistan Uzbekistan, Australia, Greece, Turkey, Argentina, Mexico etc. In India, Punjab, Haryana, Rajasthan, Madhya Pradesh, Maharashtra, Gujarat, Andhra Pradesh, Karnataka and Tamil Nadu are major cotton growing states.

Cotton is an often cross-pollinated crop. The average outcrossing is 5-6 % which occurs through insects mainly by honey bees and bumble bees. Wind pollination is not possible because cotton pollen is heavy and sticky. Cotton seed is an important source of vegetable oil and protein. Cotton earns about one-third of foreign exchange through export of lint, yarn, fabrics and garments. It also provides employment to millions of people in farming, seed production, marketing, ginning and pressing textile industries, export and import.

### Characters for DUS Testing

As per national DUS-Test Guidelines, in cotton, 41 characters have been decided for DUS testing. These traits are related to plant (5), stem (2), leaf (9), bract (2), flower (6), boll (8), fibre (6) and seed (3). These characters are of two types, viz. (i) oligogenic characters, and (ii) polygenic characters. The oligogenic characters have high heritability, whereas polygenic characters have low to medium heritability.

#### i) Essential characters

The characteristics are again classified into two groups viz. (i) essential characters, and (ii) optional characters. Observations have always to be recorded on essential traits. In cotton, there are 20 essential characteristics as follows:

1.	Leaf shape	Palmate (normal), semi-digitate (semi-okra), digitate (okra) and laceolate (super okra)
2.	Leaf size	Small, medium and large
3.	Leaf colour	Light green, green, light red and dark red
4.	Leaf: Pubescence	Absent, medium and strong
5.	Leaf nectaries	Absent and present
6.	Bract type	Normal and frego



7.	Petal colour	White, cream, yellow, pink, red and bicolour
8.	Petal spot	Absent and present
9.	Position of stigma	Embedded and exerted
10.	Anther colour	White, cream, yellow and purple
11.	Boll size	Small, medium and large
12.	Boll shape	Round, oval and elliptic
13.	Boll surface	Smooth and pitted
14.	Boll opening	Open, semi-open and close
15.	Fibre length	Very short (<20 mm), short (20.5-24.5 mm), medium (25-29 mm), long (29.5-33.5 mm) and extra long (>35.5 mm)
16.	Fibre strength	Weak (<20 g/tex), medium (20.1-25.0 g/tex) and strong (>25 g/tex)
17.	Fuzz colour	White, cream, brown and green
18.	Ginning percent (%)	Low (<31), medium (31-35), high(38-40) and very high (>40)
19.	Ginning percent (%)	Low (<31), medium (31-35), high (38-40) and very high (>40)
20.	Density	Naked, semi-fuzzy and fuzzy

(ii) **Optional characters**

There are 21 characters that are optional. These characters may or may not be recorded. List of optional characters alongwith their categories is presented below:

1.	Hypocotyl:pigmentation	Absent and present
2.	Plant:time of flowering (50% of plants with at least one open flower)	Early (<45 days),medium (45-60 days) and late (>60 days)
3.	Plant: stem pigmentation	Absent and present
4.	Plant: stem hairiness	Absent, sparse, medium and strong
5.	Leaf: lobe number	One, three, five and seven
6.	Leaf: appearance	Cup and flat
7.	Leaf: gossypol glands	Absent and present
8.	Leaf: petiole pigmentation	Absent and present
9.	Bract: number of serration	Few, medium and many
10.	Flower: Sepal pigmentation	Absent and present
11.	Flower: filament pigmentation	Absent and present
12.	Boll: Bearing habit	Solitary and cluster
13.	Boll: Colour	Green and red
14.	Boll: Prominence of tip	Blunt and pointed
15.	Boll: weight of seed cotton / boll	Small (<3.0 g), medium (3.1-5.0 g.) and large (>5.0 g)
16.	Plant: growth habit	Determinate and indeterminate
17.	Plant: height	Very short (<61 cm), short (61-90 cm), medium (91-120 cm), tall (121-150 cm) and very tall (>150 cm)
18.	Seed: size (100 seed wt.)	Very small (<5.1 g), small (5.1-7.0 g), medium (7.1-9.0 g), bold (9.1-11.0 g) and very bold (>11 g)
19.	Fibre: fineness (micronaire value)	Very fine (<3.0), fine (3.0-3.9), medium (4.0-4.9), coarse (5.0-5.9) and very coarse (>5.9)
20.	Fibre: uniformity	Poor (<40), average (40-45) and good (>45)
21.	Fibre: maturity (%)	Poor (<70), average (70-80) and good (>80)

### Characters for Grouping

Highly heritable characters are used for classification of varieties into different groups. In cotton, following characteristics have been recommended for grouping of varieties.

1.	Leaf shape	Palmate (normal), semi-digitate (semi-okra), digitate (okra) and laceolate (super okra)
2.	Petal colour	White, cream, yellow, pink, red and dark-red
3.	Boll shape	Round, oval and elliptic
4.	Fibre length	Very short, short, medium, long and extra-long

### DUS Testing Centres

In cotton, four DUS testing centres, viz. Nagpur, Coimbatore, Dharwad and Hisar have been decided. The Nagpur and their centres will deal with testing of *G.hirsutum* and *G. arboreum* species. Coimbatore centre will test *G.hirsutum* and *G.barbodense* species and Dharwad centre will test varieties of *G.hirsutum* and *G.herbaceum*.

### Conduct of Test

The test should be conducted at least at two locations for two normal seasons. Each test should be based on minimum sample of 150 plants which should be divided into four replications. The test plot design should be as given below:

Number of rows	:	5
Row length	:	6 m
Row to row distance	:	90 cm
Plant to plant distance	:	60 cm
Number of replications	:	4

In all, observations should be recorded on 40 plants or plant parts (10 in each replication). Leaf characters should be recorded on 4<sup>th</sup> leaf from the top. For assessment of colour characteristics, it is recommended that Royal Horticultural Society Colour Chart be used.

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## Morphological characterization of Cotton Varieties

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Cotton is the most important commercial crop of India and is otherwise called as the "**White Gold**" because of its commercial value. Due to the sincere efforts of the breeders of the country, several high yielding varieties as well as hybrids have been developed in our country satisfying varying agro-climatic condition of the country matching the Textile Industry requirements. In earlier days, only the public institutions were involved in developing and commercializing varieties and hybrids. But of late, several private industries including multi-national companies are involved in developing the varieties and hybrids. Especially, after the liberalization of commercial market in the post-GATT scenario, several other companies are also diversifying their business.

Characterization of cultivars becomes essential especially following the enactment of the "**Protection of Plant Varieties and Farmers' Rights (PPV&FR) Bill**" to maintain identity of released and notified varieties and the parental lines of hybrids. Crop plants may be characterized in three different ways viz., i) Morphological characterization through descriptors, ii) Biochemical and Physiological characterization using various biochemical and physiological parameters (for example proteins and enzymes) and iii) through molecular markers (like RFLP, RAPD etc.).

A list of descriptors has been developed in all the important crop plants by various organizations including IPGRI to characterize the crop cultivars morphologically. Though very few in number, these descriptors were originally used to characterize the germplasm accessions and to distinguish the cultivars in use. However, in recent times, the world is witnessing competitive variety development programmes, especially in developed world. World wide a large number of varieties and hybrids are being developed at an exponential rate and it is necessary to develop suitable diagnostic characteristics to distinguish these candidate varieties. Especially, it is even more essential on the part of India after the introduction of PPV&FR Act.

This paper briefly discusses about the morphological characterization of cotton cultivars using various descriptors. These descriptors are easy to characterize and evaluate, evaluation is done mostly under field condition, it does not require costly equipments, requires less skill, their expressions are mostly stage specific, are highly influenced by environment and only limited variability is available in cotton.

Unlike other major field crops, cotton has a very limited variability in respect of morphological characters, which can distinguish the cultivar easily. Based on UPOV test guidelines, a draft test guideline was developed in cotton and circulated to several cotton breeders, who are actively involved in breeding cotton cultivars. Incorporating suggestions received from the cotton breeders of the country, Government of India has developed a National Guideline for the Conduct of Tests for Distinctness, Uniformity and Stability in cotton.

In the national test guideline, all the details of be required material to for the conduct of DUS test, how to conduct the test, test plot design, methods of recording the observations etc. Since these aspects have already been covered by other lecture, the discussion will be concentrating on the characterization part alone. For the assessment of distinctness and stability, observations should be made on 40 plants or parts of plants, which should be divided into 10 plants per each replication of four. The number of apparent plant should not exceed 4 in 40.

For assessing the uniformity of characteristics on the plot as a, whole (visual assessment by a sing observation of a group plants or parts of plants), the number of aberrant plants or parts

of plants should not exceed 8 in 150. For recording the all the leaf characteristics, fourth leaf from the top of plant should be used as per the stage of plant mentioned in the table of characteristics. For assessing the colour characteristics, Royal Horticultural Society colour chart should be used.

The morphological characteristics (41 in number) may be classified into two groups viz., essential and optional characters. The essential characters are to be recorded always and there are 20 such characters in cotton viz., Leaf Shape, Leaf size, Leaf Colour, Leaf Pubescence, Leaf nectaries, Bract Type, Petal Colour, Petal spotting, Position of Stigma, Anther Colour, Boll Size, Boll shape, Boll Surface, Boll Opening, Fibre Length, Fibre Strength, Fuzz Colour, Lint Colour, Ginning Percent, Density of Fuzz. The 21 other characters are optional which may or may not be recorded. They are Hypocotyl Pigmentation, Days to 50% Flowering, Stem Pigmentation, Stem Hairiness, Leaf Lobe Number, Leaf Appearance, Leaf Gossypol Glands, Leaf Petiole Pigmentation, Bract Number, Sepal Pigmentation, Filament Pigmentation, Boll Bearing Habit, Boll Colour, Boll Prominence of Tip, Boll Weight, Plant Growth Habit, Plant Height, Seed Size, Fibre Fineness, Fibre Maturity, Fibre Uniformity.

The characters identified to assess the DUS of cotton are listed in Table of characteristics.

S.No.	Characteristics	States	Notes	Stage of observation	Type of assessment
1.	Hypocotyl pigmentation	Absent	1	Seedling	VS
		Present	9		
2.	Plant: time of flowering (50% of plants with at least one open flower)	Early (<45 days)	3	50% of plants with at least one open flower	VG
		Medium (45-60 days)	5		
		Late (>60 days)	7		
3.	Plant: stem pigmentation	Absent	1	Peak flowering	VS
		Present	9		
4.	Plant : stem hairiness	Absent	1	Peak flowering	VS
		Sparse	3		
		Medium	5		
		Strong	7		
5.	Leaf: shape	Palmete (Normal)	1	Peak flowering	VS
		Semi-digitate (Semi-okra)	2		
		Digitate (Okra)	3		
		Lanceolate (Super-okra)	4		
6.	Leaf: Lobe Number	One	1	Peak flowering	VS
		Three	2		
		Five	3		
		Seven	4		
7.	Leaf: size (Width at maximum point)	Small	3	Peak flowering	VG
		Medium	5		
		Large	7		
8.	Leaf: Colour	Light Green	1	Peak flowering	VS
		Green	2		
		Light Red	3		
		Dark Red	4		
9.	Leaf: Pubescence	Absent	1	Peak flowering	VS
		Medium	5		
		Strong	9		

10.	Leaf: Appearance	Cup	1	Peak flowering	VS
		Flat	2		
11.	Leaf: Gossypol glands	Absent	1	Peak flowering	VS
		Present	2		
12.	Leaf: nectaries	Absent	1	Peak flowering	VG
		Present	9		
13.	Leaf: Petiole Pigmentation	Absent	1	Peak flowering	VS
		Present	9		
14.	Bract: Type	Normal	1	Peak flowering	VG
		Frego	2		
15.	Bract: Number of Serration	Few	3	Peak flowering	VS
		Medium	5		
		Many	7		
16.	Flower: Sepal Pigmentation	Absent	1	Peak flowering	VS
		Present	9		
17.	Flower: Petal colour	White	1	Peak flowering	VS
		Cream	2		
		Yellow	3		
		Pink	4		
		Red	5		
		Bicolour	6		
18.	Flower: Petal Spotting	Absent	1	Peak flowering	VS
		Present	9		
19.	Flower: Position of Stigma	Embedded	1	Peak flowering	VG
		Exserted	2		
20.	Flower: Filament Colouration	Absent	1	Peak flowering	VG
		Present	9		
21.	Flower: Anther Colour	White	1	Peak flowering	VS
		Cream	2		
		Yellow	3		
		Purple	4		
22.	Boll: Bearing Habit	Solitary	1	First boll bursting	VS
		Cluster	2		
23.	Boll: Size (width of boll at maximum point)	Small	3	Before boll bursting	VG
		Medium	5		
		Large	7		
24.	Boll: Colour	Green	1	Before boll bursting	VS
		Red	2		
25.	Boll: shape (Longitudinal section)	Rounded	1	Before boll bursting	VG
		Ovate	2		
		Elliptic	3		
26.	Boll: Surface	Smooth	1	First boll bursting	VG
		Pitted	2		
27.	Boll: Prominence of Tip	Blunt	1	First boll bursting	VG
		Pointed	2		
28.	Boll: Opening	Open	3	First picking	VG
		Semi-open	5		
		Close	7		
29.	Boll: weight of seed cotton / boll	Small (<3.0 g)		First picking	VS
		Medium (3.1 – 5.0 g)			
		Large (>5.0 g)			

30.	Fibre: Length	Very short (<20 mm)	1	First picking	VS
		Short (20.5-24.5 mm)	2		
		Medium (25.0-29.0 mm)	5		
		Long (29.5-33.5 mm)	7		
		Extra long (>33 mm)	9		
31.	Plant: Growth habit	Determinate	1	Final picking	VG
		Indeterminate	2		
32.	Plant: Height	Very short(<61 cm)	1	Final picking	MS
		Short (61-90 cm)	3		
		Medium (91-120 cm)	5		
		Tall (121-150 cm)	7		
		Very tall (>150 cm)	9		
33.	Seed: Fuzz colour	White	1	Harvest maturity	VS
		Grey	2		
		Brown	3		
		Green	4		
34.	Seed: Size (100 seed weight)	Very small (<5.1 g)	1	Harvest maturity	MG
		Small (5.1-7.0 g)	3		
		Medium (7.1-9.0 g)	5		
		Bold (9.1-11.0 g)	7		
		Very bold (>11 g)	9		
35.	Fibre: Colour	White	1	Harvest maturity	VS
		Cream	2		
		Brown	3		
		Green	4		
36.	Fibre: Strength	Weak (<20 g/tex)	3	Harvest maturity	
		Medium (20.0-25.0 g/tex)	5		
		Strong (>25.0 g/tex)	7		
37.	Fibre: Fineness (Micronaire value)	Very fine (<3.0)	1	Harvest maturity	
		Fine (3.0-3.9)	3		
		Medium (4.0-4.9)	5		
		Coarse (5.0-5.9)	7		
		Very coarse (>5.9)	9		
38.	Fibre: Uniformity	Poor (<40)	3	Harvest maturity	
		Average (40-45)	5		
		Good (>45)	7		
39.	Fibre: Maturity (%)	Poor (<70)	3	Harvest maturity	
		Average (70-80)	5		



		Good (>80)	7		
40.	Ginning %	Low (<31)	3	Harvest maturity	MG
		Medium (31-35)	5		
		High (36-40)	7		
		Very high (>40)	9		
41.	Seed: Density of fuzz	Naked	1	After ginning	VG
		Semi-Fuzzy	2		
		Fuzzy	3		

The type of assessment of characteristics indicated in the table is as follows:

**MG:** Measurement by a single observation of a group of plants of parts of plants

**MS:** Measurement of a number of individual plants of parts of plants

**VG:** Visual assessment by a single observation of a group of plants of parts of plants

**VS:** Visual assessment by observations of individual plants of parts of plants

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Ability to discriminate between and identified varieties of crops is crucial to the seed and seed related industries. In the seed programme, maintenance of genetic purity of the parental lines and the hybrid seed is the first requirement. For achieving these objectives, information on morphological characteristics of the parental lines and the hybrid is essential which can be used by the seed growers / seed corporation, seed certification agencies and also seed testing labs in order to determine the genetic purity and also to remove off types from the seed crop.

Varietal characterization becomes increasingly important in the operation of modern crop production. All sectors of seed Industry from plant breeders through the variety and seed testing authorities, the certification agencies, seed merchants, farmers and ultimately the grain buyers, processor and consumer benefit for the ability to assess varietal identity and the purity.

One of the important future obligations under WTO will be to enact Plant Variety Protection Legislation either through patenting or "Sui-generis" system or combination therefore of PBR for new varieties. As per obligation to GATT, India has enacted its own "Sui-generis" system of PVP called as Protection of Plant Varieties and Farmers Right Act, 2001. It will help

- Provide for the establishment of an effective system for protection of Plant Varieties) the rights of farmers and plant breeders.
- To stimulate investment for research and development and to facilitate growth of the seed industry.
- To ensure availability of high quality seeds and planting materials of improved varieties to farmers.

A new variety shall be registered under this act, if it conforms to the criteria:

- Novelty
- Distinctness
- Uniformity
- Stability

#### **Need for detailed examination of diagnostics characteristics of crop varieties**

In early days, a small list of morphological descriptors was sufficient to distinguish between crop varieties in use. However, in recent decades, the world witnessed the emergence of large and highly competitive variety development programmes particularly in the developed countries and also in some of the developing countries. At global level, a large number of new candidate varieties is generated for testing every year, thus underlining the need for establishment their clear-cut diagnostic features. The technology- rich developed countries had obviously realized this requirement much earlier and had, accordingly, tuned their systems to meet the requirement. In most of the developing countries now considering implementing a PVP-system, while certain diagnostic features for released crop varieties are generally know and followed in seed certification procedure, accurate identification keys, giving detailed description on a comparative basis with clear-cut feature of distinctness are, in general, lacking; and thus cases of confusion in seed certification and quality control, if such systems are existing, are also not uncommon. The example of India, which benefited greatly from the Green Revolution is cited and compared here. The country has an established system on variety development, testing and release, and over 2600 crop varieties are already notified for commercial cultivation.

In India, the variety testing and release system, undertaken through a crop commodity-specific coordinated varietal evolution system with a large network of cooperation centres in public and private sectors, basically concentrates on generating data on parameters like yield, quality, reaction to important diseases and pests under field conditions and artificial epiphytotics,

performance under different agronomic management schedules etc. it is thus more akin to a VCU (Value for Cultivation and Use) test in the European Economic Community (EEC) countries aimed at stimulating plant breeders to produce varieties which are an improvement over existing varieties. The latter test includes a detailed botanical examination using a standard list of morphological descriptors and is intended to remove any confusion in naming new varieties, which was a major problem in EEC countries in earlier times. Besides meeting the requirement of registration of the new variety, the botanical description is also used mainly but not exclusively for awarding PVP, for which uniqueness is an essential requirement.

The need for a detailed examination diagnostic morphological characteristics thus becomes imperative in new PVP-opting countries to maintain identity or released and notified varieties and their parental lines. With respect to India, Sharma (1991) has indicated that no system of variety registration exists in India apart from the rather vague variety release proposals provided by the breeders and there is need for establishing a DUS testing system. Such work additionally assists in protecting morphologically, and often agronomically similar but distinct varieties when a PVP system is established *de novo* in a country. The requirement also finds support from the work of Singhal and Prakash (1992) who have identified a high degree of resemblance in morphology in recently developed wheat varieties in India.

Obviously, the development countries and their private seed sector considered availability of effective PVP systems as necessary in developing countries in order to safeguard the interest of plant breeders as well as to play an important role in the global agriculture. This was made possible by including the requirement for an effective system for plant variety protection through the multilateral negotiations under GATT. As contracting parties in WTO, and recognizing current efforts by UPOV for harmonization of procedures, a general description of UPOV'S criteria is first provided for such new PVP-opting countries.

### **Morphological Characterization**

Pant taxonomist have traditionally involved in the detailed observation and recording of morphological descriptors and this classical approach has served the cause of variety identification. International Plant Genetic Resource Institute (IPGR) promotes a minimum set of morphological characters that entails genetic diversity at particular points in the genome corresponding to the observed characters/descriptors recognized internationally thought to be satisfactory for the custodial management of crop germplasm collections which cover most of the important crop genera (Erksine and Williams, 1980). They are the main source of description information for Plant Variety Protection (PVP) applications. In India, NBPGR is actively involved in describing the minimal descriptors for various field and horticultural crops.

The characteristics to be determined are either qualitative or quantitative (UPOV, 1979). Qualitative characters can be determined visually e.g. colour of leaf, anthocyanin pigmentation of seedlings, presence or absence of gossypols, etc., but a few available discontinuous characters result in sufficient discrimination (Higgins and Evans, 1983). Quantitative characters are visually determined, measured or counted. All measurement and counts are carried on specified number of plants as provided in the IPGRI descriptors. Varieties are distinguished on the basis of statistical difference in measured characters with further resolution based on the field observed differences. Both types of genetic variation (qualitative or quantitative) have been used to establish the distinctness criterion for cultivar identification. The minimum descriptors may vary from one crop to another (Ashri, 1973) and need to be standardized.

Morphological characters in various parts have traditionally been used to distinguish one cultivar from other (Simmonds and Shepherd, 1955; Oka, 1958; Harlan and de Wet, 1972; Pierce and Wehner, 1990) and seed characters are also valuable in the seed certification process to control seed production and seed quality standards (Harvey-Murray, 1980). Although the observation of phenotype undoubtedly represents a very successful means identification, it cannot be reliable system under all situations. For example, many varieties in a particular crop

would have emanated from a single cross which make them least different each other. The problems with morphological description are; they are subjected to environmental fluctuating, show differential expression, which depends on ontogeny, not disturbed throughout the genome, and it necessitates the growth of plant to maturity.

## DUS test guidelines for studying the morphological descriptors of all varieties, hybrids and parental lines of cotton

### I. Material required

1. The Plant Variety Protection (PVP) Authority decides when, where and in what quantity and quality the plant material required for testing the variety is to be delivered. Applicants submitting material from a country other than India must make sure that all customs formalities are complied with. The minimum quantity of seed to be supplied by the applicant:  
Variety, hybrid and parental line - 4000 grams (in only one submission)
2. The seed should meet the minimum requirements for germination capacity, moisture content and physical purity prescribed for certified seed in India. Especially for storage, which requires a higher standard, the applicant should state the actual germination capacity, which should be as high as possible but not less than 75%.
3. The plant material must not have undergone any treatment unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

### II. Conduct of tests

1. The minimum duration of tests should normally be two independent similar growing seasons with reference to the ecosystem of the variety submitted for DUS test.
2. The test should normally be conducted at two test locations. If any important characteristics of the variety cannot be seen at these places, the variety may be tested at an additional place.
3. The test should be carried out under conditions ensuring normal growth. The size of the plot should be such that plants or parts of plant may be removed for measuring and counting without prejudice to the observation which must be made upto the end of the growing period. Each test should include a minimum of 150 plants, which should be divided among 4 replications. Separate plots for observation and for measuring can only be used if they have been subjected to similar environmental conditions.
4. Test plot design:
 

No. of rows	:	5
Row length	:	6 m
Row to row distance	:	90 cm
Plant to plant distance	:	60 cm
No. of replications	:	4
5. Observations should not be recorded on plants in border rows.
6. Additional tests for special purpose may be established.

### III. Methods and observations

1. The morphological characteristics described in the table should be used for the testing of varieties, inbred lines and hybrids for DUS.

#### Morphological descriptors of cotton

Sl. No.	Characteristics	States	Stage of observation
1.	Hypocotyl pigmentation of seedlings	Absent Present	Seedling stage

2.	Time of flowering (50% of the plants with at least one opened flower)	Early (<45 days) Medium (45-60 days) Late (>60 days)	-
3.	Plant stem pigmentation	Absent Present	Peak flowering
4.	Plant stem hairiness	Absent Sparse Medium Strong	Peak flowering
5.	Leaf shape	Palmate (Normal) Semi-digitate (semi-okra) Digitate (okra) Lanceolate (super okra)	Peak flowering
6.	Leaf lobe number	One Three Five Seven	Peak flowering
7.	Leaf size (Width at maximum point)	Small Medium Large	Peak flowering
8.	Leaf colour	Light green Green Light red Dark red	Peak flowering
9.	Leaf pubescence	Absent Medium Strong	Peak flowering
10.	Leaf appearance	Cup Flat	Peak flowering
11.	Leaf gossypol glands	Absent Present	Peak flowering
12.	Leaf nectarines	Absent Present	Peak flowering
13.	Leaf petiole pigmentation	Present Absent	Peak flowering
14.	Bract type	Normal Frego	Peak flowering
15.	Bract number of serrations	Few Medium Many	Peak flowering
16.	Flower sepal pigmentation	Absent Present	Peak flowering
17.	Floer petal colour	White Cream Yellow Pink Red Bicolor	Peak flowering
18.	Flower petal spotting	Absent Present	Peak flowering
19.	Flower position of stigma	Embedded Exserted	Peak flowering

20.	Flower filament colouration	Absent Present	Peak flowering
21.	Flower anther colour	White Cream Yellow Purple	Peak flowering
22.	Boll bearing habit	Solitary Cluster	First boll Bursting
23.	Boll size (Width of the boll at maximum point)	Small Medium Large	First boll bursting
24.	Boll colour	Green Red	First boll bursting
25.	Boll shape (longitudinal section)	Rounded Ovate Elliptic	First boll bursting
26.	Boll surface	Smooth Pitted	First boll bursting
27.	Boll prominence of tip	Blunt Pionted	First boll bursting
28.	Boll opening	Open Semi-open Close	First picking
29.	Boll weight of seed cotton / boll	Small (<3.0g) Medium (3.1-5.0g) Large (>5.0g)	First picking
30.	Fibre length	Very short (< 20mm) Short (20.5-24.5mm) Medium (25-29mm) Long (29.5-33.5mm) Extra long (>33.5mm)	First picking
31.	Plant growth habit	Determinate Indeterminate	First picking
32.	Plant height	Very short (<61cm) Short (61-90cm) Medium (91-120cm) Tall (121-150 cm) Very tall (>150cm)	First picking
33.	Seed fuzz colour	White Grey Brown Green	Harvest maturity
34.	Seed size (100 seed weight)	Very small (<5.1g) Small (5.1-7.0g) Medium (7.1-9.0g) Bold (9.0-11.0g) Very bold (>11g)	Harvest maturity
35.	Fibre colour	White Cream Brown Green	Harvest maturity
36.	Fibre strength	Weak (<20g/tex) Medium (20.1-25.0g/tex)	Harvest maturity



		Strong(>25g/tex)	
37.	Fibre fineness (micronaire value)	Very fine (<3.0) Fine (3.0-3.9) Medium (4.0-4.9) Coarse(5.0-5.9) Very coarse (>5.9)	Harvest maturity
38.	Fibre uniformity	Poor(<40) Average(40-45) Good (>45)	Harvest maturity
39.	Fibre maturity (%)	Poor (<70) Average (70-80) Good (>80)	Harvest maturity
40.	Ginning (%)	Low(<31) Medium (31-35) High (36-40) Very high(>40)	Harvest maturity
41.	Seed density of fuzz	Naked Semi-fuzzy Fuzzy	After ginning

1. For the assessment of distinctness and stability observations should be made on 40 plants or parts of plants, which should be divided among 4 replications (10 plants per replication). The number of apparent plant should not exceed 4 in 40.
2. For the assessment of uniformity of characteristics on the plot as a whole (visual assessment by a single observation of a group of plants or parts of plants), the number of aberrant plants or parts of plants should not exceed 8 in 150.
3. All leaf characteristics should be observed on 4<sup>th</sup> leaf from the top.
4. For the assessment of colour characteristics, it is recommended that Royal Horticultural Society (RHS) colour chart be used.

#### IV. Grouping of varieties

1. The collection to be grown should be divided into groups to facilitate the assessment of distinctness. Characteristics that are suitable for grouping purposes are those, which are known from experience not to vary or to vary only slightly, within a variety and which in their various states are fairly evenly distributed within the collection.
2. It is recommended that the competent authorities use the following characteristics for grouping varieties.
  - i) Leaf: shape
  - ii) Flower: petal colour
  - iii) Boll: shape (longitudinal section)
  - iv) Fibber: Length

#### Reference

- Ashri, A. 1973. Divergence and evolution in sunflower lines. In: Fin.Res.Rep., ARS,USDA, Beltsville.
- Erksine, W. and J.T. Williams, 1980. The principles, problems and responsibilities of the preliminary evaluation of genetic resource samples of seed-propagated crops. FAO/IBPGR Plant Genet.Res.Newslett., 41;9 1-92
- Harlen, J.R and M.J de Wet.1972, A simplified classification of cultivated sorghum, CropSci., 12: 172 -176.
- Harvey-Murray, C.G. 1980. The identification of cereals varieties, University Press, Cambridge.
- Higgins, J.and J.L.Evans.1983. Description of faba bean cultivars, J.Nat1.Inst.Agrl.Bot., 15:480-487.

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## Physiological and Biochemical techniques for crop variety identification

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Varietal characterization and identification are still attracting the attention of Breeders, Farmers, Seed Industry, Certification Agencies, Seed Testing Laboratories and Breeders Right Protection Institutions with the main objectives of determining the extent to which a seed sample confirms to a given cultivar and to assure the quality of seed marketed to the consumers. Hundreds of cultivars are now included in the national varietal lists, that require increasingly detailed data to distinguish a cultivar from another, thereby making identification an essential requisite for inclusion in varietal lists.

The success of an improved variety in the farmer's field depends upon the availability of seed with genetic purity. Only high quality seeds of assured genetic purity can be expected to respond fully to all other inputs (Agrawal, 1980). Maintaining genetic and physical purity of seed are of utmost importance and will enable growers to exploit the full benefits of introducing improved varieties. When a seed lot passes from one generation to another some form of genetic and physical contamination is likely to occur that can be detected morphologically. If we are not able to detect the contamination, the contamination may go on accumulating in the population, finally leading to **deterioration** of that variety. The only legally recognized method in India for cultivar identification and genetic purity assessment continues to be **seed certification** based on field plot grow out tests conducted against a standard sample of the variety with the aid of descriptors supplied by the concerned plant breeder. Thus, the ability to distinguish and identify the varieties is of prime importance to the operation of seed certification and it is the only way that it can be guaranteed that the genetic advantages built into a variety by the plant breeder are passed on to the farmer. The out come of all the above mentioned breeding, seed testing and seed certification activities is that a farmer or seed grower is ultimately able to purchase seed that represents a high quality product.

Early, distinctness, uniformity and stability (DUS) of any cultivar have relied on morphological methods, which are subjective and which may be influenced by environmental conditions. However the morphological makers were not quite enough to expose the genetic diversity between the morphological overlap cultivars and the morphological identical accessions. The need, therefore, for new tool was disparate.

Electrophoretic makers appear to be due to neutral genes, which are not linked to any loci that appear affect the cultivar and value. They are also independent of cultivar morphology and physiology, and are largely unaffected by the growth environment. The biochemical methods have some disadvantages e.g. that they are profoundly influenced by tissue specificity and developmental stage. This disadvantage can be overcome by using the electrophoretic markers of a conservative protein e.g. seed storage proteins.

Variety identification is a pre-requisite for the effective provision of Plant Breeders Rights (PBR), which can be achieved by trade secrets, plant variety protection (PVP), or where available through utility patents. All the three forms of protection require some measure of **distinctness**. Variety identification for the attainment of plant breeders' right is a taxonomic and genetic approach to determine varietal distinctness. The chief goals are to promote the release of fresh genetic diversity into agriculture and to create an environment of continued funding for Plant Breeding Research and Genetic Resource Conservation. At the international level, variety identification and grain commodity usage become linked, because seeds are the encapsulated intellectual property, the protection of which forms an integral component of the General Agreement on Tariff and Trade (GATT).

The traditional way to assess the genetic purity of seed of established varieties of crops is **grow out test**, where the crop is grown in **isolation** and vigorous roguing during different phases of crop growth is done with the aid of morphological descriptors available for that variety under consideration. The main problem for variety identification during field inspection of the seed crop is the lack of satisfactory standard characteristics for varietal assessment. The authorities responsible for this task require stable characters for detecting the performance of registered variations and of new releases. Further characterizations such as laboratory tests like phenol test, KOH test, response of the variety to the added chemical, electrophoretic pattern and cytology allow the opportunity to improve the characterization of varieties and could provide tools to improve efficiency of field inspection.

To identify cultivars of various crops, relevant taxonomic descriptors were developed by International Union for the Protection of New Varieties of Plants (UPOV) and National body like Indian Council of Agricultural Research (ICAR) through National Seed Project (Crops). Such type of traditional (taxonomic) approach is still being employed by certification programme as well as for grow-out test to determine the genetic purity of crop species. These morphological descriptors have a traditional significance and are immediately accessible on the spot without need of any equipment.

Thus, a clear basis for distinctness testing procedure prior to variety registration can be drawn out of this. But the approach demands a field assessment, which is laborious; time consuming and very much depends on the degree of experience of the experimenter. Most of the descriptors require subjective decisions on minor distinctness upon interaction of cultivars with environment. Thus, the visible phenotype is influenced in diverse way by variation in growth conditions.

In general, it is an increasingly desirable objective to reduce or eliminate the environmental influence so that the genotype or a variety can be observed more directly. So, it is needed to develop the modern approaches, which address the question of the environmental factor. The modern approaches viz., the use of computerized systems to capture and process morphological information (**image analysis** technique) and the use of **chemical** and **biochemical** methods to analyze various components of seeds (**chemo taxonomy**) give solutions to above questions and which are currently applied to varietal identification.

Several **chemotaxonomic** tests are available to span the distinction between true morphology and chemical composition of seeds. These characteristics of seed have the value of readily providing information about any individual seed at one examination, often on the basis of colour differences. Some workers like Selvaraju and Sivasubramanian (2001) have attempted chemical test to classify sorghum cultivars based on the seed and seedling response to added chemicals. These chemotaxonomic approaches would also help in identifying the degree of heterogeneity of a sample with respect to varieties that differ in their characteristics.

Similarly an effective laboratory method of cultivar distinction relate to the analysis of seed protein composition using SDS-PAGE and isozyme profiling using electrophoretic technique have been well attempted for distinction of cultivars of many crop species. The success of electrophoretic procedures depends on the wide-ranging polymorphism of seed, seedling proteins and isozymes and the fact that these proteins represent primary gene products. Analysis of protein composition has proved to be a good indicator unless altered by growth condition only to a relative minor extent. Rapidity and repeatability of result are the foremost among its advantages. Hence, there is a need to develop protein profiles for all the cultivars for varietal characterization.

Recently, use of electrophoresis in seed purity testing has been recommended by International Seed Testing Association (ISTA) in this situation, with the advent of an array of molecular markers at DNA level for finger printing presently available and the new generation markers as a result of technology spillovers of genome projects. It may be possible to have a varietal specific finger print that reflects the stable genetic descriptor for inclusion in variety

release proposal for unequivocal identification of varieties and improved method of genetic purity testing other than those involving morphological descriptors.

Present systems of DUS testing involve the comparison of candidate variety with the existing varieties by recording the phenotypic characters, which are (mostly) morphological, physiological or pathological in nature.

The procedure for various tests for variety identification are furnished below.

## I. Morphological characterization

### 1. Seed characters in Cotton

Sl. No	Characteristics	States	Notes	Stage of observation	Type of assessment
33 (*)	Seed: fuzz color	White Grey Brown Green	1 2 3 4	Harvest maturity	VS
34	Seed: size (100 seed wt.)	Very small (<5.1g) Small(5.1-7.0g) Medium (7.1-9.0g) Bold (9.1-11.0g) Very bold (>11g)	1 3 5 7 9	Harvest maturity	MG
35 (*)	Fibre: color	White Cream Brown Green	1 2 3 4	Harvest maturity	VS
36 (*) (+)	Fibre: strength	Weak (<20 g/tex) Medium(20.1-25.0 g/tex) Strong (>25 g/tex)	3 5 7	Harvest maturity	
37 (+)	Fibre: fineness (micronaire value)	Very fine(<3.0) Fine (3.0-3.9) Medium (4.0-4.9) Coarse (5.0-5.9) Very coarse (>5.9)	1 3 5 7 9	Harvest maturity	
38 (+)	Fibre: uniformity	Poor (<40) Average (40-45) Good (45)	3 5 7	Harvest maturity	
39 (+)	Fibre: maturity (%)	Poor (<70) Average (70-80) Good (>80)	3 5 7	Harvest maturity	
40 (*)	Ginning %	Low (<31) Medium (31-35) High (36-40) Very high (>40)	3 5 7 9	Harvest Maturity	MG
41 (*)	Seed: density of fuzz	Naked Semi-fuzzy Fuzzy	1 2 3	After ginning	VG

## II. Response of Seed and Seedling to Added Chemicals

### A. Seed response to added chemicals (Physiological)

#### 1. Phenol colour reaction

##### a) Standard phenol test

Soak fifty seeds of each cultivar with eight replicates in distilled water for 16h. Then transfer these seeds to petriplates with two layer of Whatman No. 1 filter paper saturated with 1% phenol solution. Cover the petriplates and keep it in an incubator at

30°C±1°C. Observe the colour reaction after 24h. Based on the colour reaction cultivars can be grouped into no visible colour change; light brown; dark brown and black.

#### b) Modified phenol test

Modified phenol test can be carried out with the presence of Fe<sup>+</sup> and Cu<sup>+</sup>. This can be conducted similar to the standard phenol test, except that the seeds are to be soaked in 0.6% Na<sub>2</sub>CO<sub>3</sub> and 0.4% CuSO<sub>4</sub>, separately. The grouping of cultivars can be done based on the colour reaction, which remains the same as described for standard phenol test.

#### 2. Ferrous sulphate test

Soak 100 seeds of four replicates in 1% ferrous sulphate solution and keep it in an incubator for 2h after the stipulated time record the distinct colour groups.

#### 3. Potassium hydroxide response test

Soak each variety in 5% potassium hydroxide solution with four replications of 100 seeds and keep it in room temperature for 6h. Then, observe the colour development of the solution.

#### 4. Peroxidase test

Four replications of 100 seeds in each cultivar are to be soaked in 0.5% guaiacol solution in the test tubes. After 10 minutes, add 10 drops of 0.1% hydrogen peroxide. The grouping can be made as high, moderate, low and no response by identifying the solution colour as dark reddish brown, reddish brown, light reddish brown and colourless, respectively.

#### 5. Seedling response to added chemicals (Table 1 & 2)

##### a. GA<sub>3</sub> soak test

Germinate four replication of 100 seeds in roll towel and keep it in a plastic bucket containing GA<sub>3</sub> (Gibberellic acid) 100-ppm concentration and allow the seeds to germinate in a germinator at 25°C After 10 days, evaluate 10 seedlings at random for seedling characteristics.

##### b. 2, 4-D soak test

Moisten the germination paper with 50-ppm concentration solution of 2, 4-D sodium salt. Then, place four replication of 100 seeds of each cultivar on 2, 4-D moistened germination paper and allow it to germinate in between paper method. The study can be conducted in step-in-germinator (Mode1: Hoffman scientific company, USA) or Room type germinator or germination cabinets at 25°C. After 10 days, seedlings are to be observed for their root length inhibition.

#### 6. Seedling response to germination tests

##### a. Germination Test

Grow seedlings of each variety for 14 days in seed terminator at 25 ± 2°C in two replications as per ISTA (1999). The observations are to be recorded for germination per cent, shoot length, root length and seedling dry weight. The following standard formulae can be used to compute the vigour indices.

$$\text{Mean daily germination} = \frac{\text{Final germination percentage}}{\text{Total no. of days in a test}}$$

$$\text{Peak value} = \frac{\text{Final germination percentage}}{\text{No. of days required to reach maximum germination percent}}$$

Germination value = Peak value x mean daily germination.

Vigour index = Germination percent x total seedling length (cm)

##### (i) Shoot length

Measure the distance between the collar and tip of the shoot for ten normal seedlings and express the mean value of shoot length in cm.

##### (ii) Root length

Select ten normal seedlings per replicate at random from the germination test. Measure the distance between the collar and the tip of the primary root and express the mean value in cm.

### (iii) Number of lateral roots

**Numbers of lateral roots are to be counted at the end of the test.**

Image analysis for varietal characterization

## III. MODERN APPROACHES

### B. BIOCHEMICAL CHARACTERISATION OF SEED AND SEEDLINGS

#### Biochemical methods

Chemotaxonomists have recognized two groups of compounds that are generally useful for the classification of organisms.

1. Episemantic or secondary compounds (pigments, fatty acids, etc.)
2. Semantides or "sense-carrying" molecules (proteins, nucleic acids).

Although the semantides have proved to be far more useful for variety identification, particularly from the seed, there are several instances of the successful use of secondary compounds.

#### Analysis of secondary compounds

A range of different tests are available for the analysis of secondary compounds in seeds and vegetative parts of plants. The tests range from simple colour tests to complex chromatographic separation of anthocyanins, flavonoids and other compounds. Probably, the best-known example of a widely used colour test is the phenol test, used to distinguish between varieties of wheat by the differential oxidation of phenol of the seed. This test has also been used for varietal identification in rice and Kentucky Bluegrass (*Poa pratensis*). Another simple colour test is the use of acidified vanillin reagent to detect the presence of tannins in the testa of field bean (*Vicia faba*) seeds.

Two principal types of chromatography have been used, depending on the analyst's interest. Thus, gas-liquid chromatography (GLC) has been used for the separation of fatty acids from seeds of oilseed rape (*Brassica napus*). GLC has also been used for glucosinolate analysis in Brassica and related species.

The other major type of chromatography, high performance (or pressure) liquid chromatography (HPLC), has also been used for glucosinolate analysis, but has an important chemotaxonomic role in the identification of varieties of horticultural species by the separation of anthocyanin and flavonoid pigment from the flower.

#### Protein and variety identification

The successful exploitation of proteins for variety identification purposes is based on the fact that proteins are the direct products of gene transcription and translation. Proteins can thus be regarded as markers for the structural genes that encode them. The proximity of the process of protein synthesis to the primary genetic information (DNA) also greatly reduces or even eliminates any environmental interaction in protein composition.

For variety identification, it is necessary to utilize proteins that exist in multiple molecular forms (i.e., are polymorphic) and also preferably that are present in relatively large amounts and are easy to extract. For these reasons, seed proteins of all types are extremely useful for identification purposes and have been widely used. This includes albumins (Water soluble proteins, mainly enzymes), globulins (the typical salt soluble storage proteins of legume seeds), prolamins (the typical alcohol soluble storage proteins of cereal seeds), and glutelins (detergent soluble structural or enzymatic proteins).

#### The use of HPLC

Following the first report of Bietz (1983), it has been demonstrated unequivocally that HPLC will separate seed proteins of wheat, barley, oats, rice, maize and other cereals, and that the resultant protein profiles can be used to distinguish between varieties. Generally, the



analyses have been of the alcohol-soluble prolamins (storage proteins) of cereals - gliadins (wheat), hordeins (barley), zeins (maize), avenins (oats) etc., - although there are methods involving albumins and glutelins (eg., Gluteins in wheat). Several different reversed-phase (RP) HPLC systems and methods have been developed. Varieties can be distinguished from one another by the qualitative absence or presence of particular protein peaks detectable at specific points (elution or retention times) on the profiles.

The attractions of this approach, in addition to the potential removal of environmental effects and greater discrimination possibilities, include speed and automation. An HPLC separation is generally completed within an hour, and can be much quicker. HPLC does inevitably have some disadvantages,

the two primary ones being (1) the relatively high capital and operating costs and (2) the long-term reproducibility of the analyses.

### Variety identification by electrophoresis

The uses of electrophoresis for variety identification have been comprehensively reviewed summarized in recent years. Two main approaches have been recognized.

1. The direct (multi-locus) approach, in which proteins that are polymorphic and genetically encoded at multiple loci are analyzed. Cereal seed storage proteins provide a good example. They are encoded by multigenic loci and the products of single locus can comprise several electrophoretically separable bands. The criterion for distinctness between varieties is taken as the presence or absence of a particular protein band (or set of bands) occurring at a defined position or positions on the gel.
2. The indirect (single-locus) approach involving the examination of proteins in which, although polymorphic, are derived from a single locus (isoenzymes, allozymes). Varietal differences are demonstrated either as the occurrence of different isozyme phenotypes (banding patterns) in self-pollinated and vegetatively propagated species or as differences in the frequency of occurrence of isozyme phenotypes in the cross-pollinated species.

The electrophoretic markers of the seed storage proteins helps to: 1) identify between cultivars, 2) to check species identification, 3) to assist biosystematic analysis, 4) to study phylogenetic relationships of the species, and 5) to generate pertinent information to complement evaluation and passport data and thereby increase the knowledge of the genetic diversity of the materials in the germplasm collections.

### Varietal Identification of cotton genotypes through electrophoresis

The experiment on Varietal Identification of cotton genotypes through electrophoresis was initiated and several attempts has been made for the development of predictable banding pattern due to single seed protein globulin in cottonseed. In the first experiment total soluble protein from hybrids Savita (T7 x M12) and Kirti (Suman x BN) were extracted by adopting the procedure of Dadlani et al., (2002). The extract was loaded in an SDS-PAGE and run in a mini gel unit for three hours. After that the gel was removed and put in a staining solution for twelve hours. Then allowed for destaining till the appearance of bands. The observation on the gel indicates improper formation of bands due to differential movement of protein on molecules.

Tris - HCL soluble proteins in the seed can be separated using SDS-PAGE, following the method of Varier et al. (1992).

### Analysis of total soluble (Tris-HCL soluble) seedling proteins through Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Total soluble proteins of seedlings can be analysed by SDS-PAGE using the method of Varier et al. (1992).

### **Seed and seedling peroxidase isozyme profiling through Poly Acrylamide Gel Electrophoresis (PAGE)**

Peroxidase isoenzyme can be analysed by alkaline PAGE procedure described by Dadlani and Varier (1993) and staining procedure of Reddy and Gasber (1971).

### **Seed and seedling polyphenol oxidase isoenzyme profiling through Poly Acrylamide Gel Electrophoresis (PAGE)**

Polyphenol oxidase isoenzyme can be analysed by alkaline PAGE procedure described by Ravi (2000).

### **Seed and seedling phosphoglucosomerase profiling through Poly Acrylamide Gel Electrophoresis (PAGE)**

**Sn:** D - Glucose - 6 - phosphate keto isomerase (E.C.5.3.1.9)

**Reaction:** D - Glucose - 6 - p → Fructose - 6 - p

## **IV. Molecular characterisation of Cotton Hybrids and Varieties**

Isolation of genomic DNA of cotton hybrid Savita

The method described by Krishna and Jawali (1997) was followed to isolate the DNA from single seed of hybrid Savita and its parents T7 (female) and M 12 (male).

- Seeds were soaked in water for 16 hours
  - About 100 mg of seed tissue were placed in a 1.5 ml eppendorf tube after removal of the seed coat
  - The seed tissue was crushed with a spatula in 200 µl of extraction buffer
  - Then 20 µl of sodium dodecyl sulphate was added and the tubes were placed in water bath maintained at 65°C for 10 minute.
1. After cooling at room temperature 70 µl of 5 M potassium acetate was added and vortexed thoroughly and the cooled at 4°C for 20 minute, after cooling centrifuged at 4°C for 20 minute at 14000 rpm.
  2. From the supernatant 100 µl of samples were pipetted out in to a fresh 1.5ml eppendorf tube to that 25 µl of 10 M ammonium acetate and 75 µl of isopropanol were added and centrifuged at room temperature for 10 minute at 14000 rpm
  3. The supernatant were discarded and the pellets remaining at the bottom were rinsed with 70% ethanol and allowed for air-drying.
  4. The pellets were dissolved in 100 µl of Tris-EDTA buffer.
  5. The isolated DNA was verified for size intactness, homogeneity and purity by electrophoresis method and the bands obtained are presented below.

### **Amplification of genomic DNA through PCR**

PCR reaction mixture (standardized by repeated running)

Genomic DNA	-2.0
dNTP's	-1.0
Primer	-2.0
Assay buffer	-2.0
Taq polymerase	-0.3
Sterile water	-12.7

The reaction mixture was taken in PCR tubes and placed in a thermocycler machine.

#### **PCR cycles**

Steps	Process	Temperature	Time
1	Initial denaturation	94°C	2 min
2	DNA denaturation	94°C	1 min
3	Primer annealing	36°C	1 min
4	Primer extension	72°C	2 min
Step 2,3 and 4 repeated for 35 cycles			
5	Final extension	72°C	10 min
6	Storage	4°C	

Note: After the reaction, the amplified products resolved through agarose gel (1.5%) electrophoresis.

**A. Isolation of genomic DNA (Gawel and Jarret, 1991)**

**B. Random Amplified Polymorphic DNA (RAPD) analysis (Siaki et al., 1988)**

**Agarose Gel Electrophoresis (Sambrook et al., 1989)**

**Conclusion:**

Experience gained with cotton cultivars so far indicate that Polyacrylamide gel techniques are a valuable tool to identify species and cultivars. This identification is very important for plant Breeders, Certification authorities, and in genetic resource management.

**Polyacrylamide gel techniques allow us to**

- I. Identify variation among taxonomy of each species
- II. Screen the purity of ever expanding number of cultivars
- III. Variety whether or not two or more morphologically identical accession in the collection were also electrophoretically similar
- IV. Exploit the important traits of land races and wild relatives to provide increasing crop production and stabilizing yield.
- V. With the advent of an array of molecular markers at DNA level for finger printing, it may be possible to have a varietal specific finger print that reflects the stable genetic descriptor for inclusion in the variety release proposal as an improved method of genetic purity testing other than those involving morphological descriptors.

**Table-1: Seedling characters / responses of cotton genotypes to GA<sub>3</sub> test**

Sl. No	Genotype	Response to GA <sub>3</sub> (25 ppm)						Increase (%)	Response
		Control	R1	R2	R3	R4	Mean		
1.	L 803	10.7	25.0	24.7	24.6	24.8	24.8	131.8	High
2.	L 802	16.6	24.7	24.2	24.3	24.1	24.3	46.4	Medium
3.	L 801	15.8	23.2	23.3	23.4	23.4	23.3	47.5	Medium
4.	Narasimha	16.5	21.3	21.1	21.1	20.8	21.1	27.9	Low
5.	LAHH 7	16.4	23.6	23.4	23.9	23.4	23.6	49.9	Medium
6.	LAHH 8	16.9	23.4	23.6	23.3	23.8	23.5	39.1	Low
7.	L 765	15.7	27.6	27.6	27.8	27.7	27.7	76.4	High
8.	L 761	14.0	23.4	23.3	23.4	23.7	23.5	67.9	High
9.	Veena	12.9	21.3	22.0	21.8	21.6	21.7	68.2	High
10.	LAHH 4 Male	12.8	21.8	21.0	21.2	20.5	21.1	64.8	High
11.	L 603	14.2	21.4	21.6	21.5	21.9	21.6	52.1	High
12.	LK 861	14.2	25.9	26.0	25.4	25.9	25.8	81.7	High
13.	Aravinda	13.2	20.0	20.1	19.9	19.9	20.0	51.5	High
14.	L 805	11.0	20.5	20.9	21.0	20.8	20.8	89.1	High
15.	LAHH 5	15.7	23.6	23.7	23.7	23.7	23.7	51.0	High
16.	LAHH 4Female	12.0	25.9	26.1	26.5	26.5	26.3	119.2	High
17.	L 104	15.2	22.9	22.6	22.0	22.4	22.5	48.0	Medium
18.	L 804	13.0	24.5	24.6	24.1	24.0	24.3	86.9	High
19.	LAHH 4	13.5	22.5	22.6	22.4	22.4	22.5	66.7	High
20.	NDLA 2463	15.7	21.1	22.0	22.4	21.6	21.8	39.9	Medium
21.	L 232	13.0	22.2	22.4	22.9	22.1	22.4	72.3	High
22.	L389	16.7	23.0	22.7	22.9	23.0	22.8	36.5	Medium
23.	MDL 2463	14.4	22.0	22.4	22.5	22.3	24.3	54.9	High
24.	L 604	15.1	24.4	24.2	24.1	24.3	24.3	60.9	High

Source: Annual Report NSP (Crops)-2003-04

**Table-2: Distinguishing cotton varieties on the basis of seedling characteristics and chemical test**

Sl. No	Varieties	Seedling pigmentation	Control		Growth response to GA <sub>3</sub>		2,4-D test	Peroxidase test	FeSO <sub>4</sub>
			Hypocotyl length	Radicle length	Hypocotyl length	Radicle length			
1.	DS-5	Sparse	Long	Medium	Low	Medium	Pi, RLi	L.brown	Brown
2.	HD-107	Sparse	Medium	Medium	Medium	Medium	Pi, RLi	D.brown	D.green
3.	HD-123	Sparse	Medium	Medium	Low	Nil	Pi, RLi	L.brown	Brown
4.	H-777	Prominent	Long	Long	Nil	Nil	CI	NC	D.green
5.	H-974	Absent	Medium	Long	Medium	Low	Pi, RLi	NC	D.green
6.	H-1098	Sparse	Long	Long	Nil	Nil	CI	L.brown	D.green
7.	H-1117	Absent	Long	Medium	Low	Medium	CI	D.brown	Yellow
8.	H-1180	Sparse	Medium	Long	Low	Low	CI	D.brown	D.green
9.	HS-6	Sparse	Medium	Long	High	Medium	CI	NC	Brown
10.	HS-182	Sparse	Medium	Medium	High	Medium	CI	D.brown	D.green
11.	LRK-516	Prominent	Long	Long	Nil	Nil	Pi, RLi	L.brown	Brown
12.	LRA-5166	Sparse	Long	Long	Low	Nil	CI	NC	D.green
13.	LH-1556	Sparse	Short	Short	High	Low	CI	L.brown	D.green
14.	LH-900	Absent	Short	Medium	High	Low	CI	NC	Yellow
15.	F-1378	Sparse	Medium	Medium	Low	Nil	CI	L.brown	Yellow
16.	RS-875	Sparse	Medium	Short	Low	High	CI	NC	D.green

PiRLi-Plumule insensitive, root length inhibited: CI-Complete inhibition: L.brown:-Light brown  
D.brown-Dark brown D.green-Dark green

Source: Annual Report NSP (Crops)-2003-04

## Reference

- Cooke, R.J. 1988. Electrophoresis in plant testing and breeding. Pages 171- 261 in advances in electrophoresis, Vol. 2. A. Charmbach et al. (eds.)VCH Verlagsgesellschaft, Weinheim, Germany.
- Keef, P.D. and Draper, S.R. (1986). The measurement of new characters for cultivar identification in wheat using machine vision. *Seed Science and Technology*, 14, 715-724.
- Myers, D.G. and Edsall, K.J. (1989). The application of image processing techniques to the identification of Australian wheat varieties. *Plant Varieties and Seeds*, 2, 109- 116.
- Anonymous (2004). Annual report of National Seed Project (Crops)
- Sivasubramanian, S. and V. Ramakrishnan 1978. Identification of rice varieties by laboratory techniques. **Seed Res.**, 6(1): 71-76.
- Wrigley, C.W., Andran, J.C. and Bushuk, 1982. Identification of cereal varieties by gel electrophoresis of grain proteins. **Adv. Sci. Technol.**, 5: 211-259.
- Government of India, Ministry of Agriculture, New Delhi (1999): Joint Committee on the protection of plant varieties and Farmers Rights Bill

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## Molecular Markers: History, Features and Applications

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### Introduction

The world's food supply depends absolutely on achievements of plant breeders of the major food and feed crops. Plant breeders exploited the genetic knowledge generated by the geneticists to achieve their goal and remain as indispensable basis of the world's food supply. But the threat is 'dubbed genetic vulnerability' due to excessive genetic uniformity in major food crops. This warrants maintaining the so-called genetic variability in the natural population to combat the new biotypes of disease and insect pests, uniquely adapted to prevailing conditions. The existing knowledge on polymorphism between individuals is enormous **-from morphological to molecular level**. Although tremendous achievements have been made in crop improvement by exploring the genomes of individual crop species, exponential growth in the field of molecular biology widens the possibilities to exploit the polymorphisms in breeding programmes. From late 1980s, the number of publications generated was unexpectedly at a higher volume projecting that the genetic analysis in living organisms would be a "cake walk". Everybody in the field of biological science started using molecular marker technology for the purpose of the so-called "gene discovery". The hype on this technology was so prominent and started dividing scientists working in "plant biology" into various groups. Anyhow, this kind of situation is not new to science/not new in doing science. **At any point of time, any discipline in science tends to be seized by a particular methodology or "enthusiasm" and other approaches get "dumped"**. This resulted in the development of new types of molecular markers and various technological simplifications to resolve the problems associated with molecular marker technology. The recent PCR based approach, gel free visualization of PCR products and automation at various steps are boons to the molecular marker approaches adopted for genome mapping and genetic diversity analysis in plant kingdom.

### Markers at DNA level: History

#### First Generation DNA markers

The concept of using variations at DNA level as genetic markers started with Restriction Fragment Length Polymorphism (RFLP). When the DNA of different individuals are digested with restriction enzymes, differences in size of the resulting fragments of DNA can be visualized via

Southern hybridization with labelled probe (Southern 1975) . The differences are due to evolutionary changes in sequence of nucleotides in the DNA of different individuals. These genetic differences may be the result of point mutations, deletions or insertions, inversions or translocations in occurring in chromosomes. The first documentation of RFLP came from viruses (Grodzicker et al 1974) followed by a subsequent elegant demonstration made in the human  $\alpha$ -globin gene cluster (Jeffreys 1979). Since then, most organisms have been explored for the presence of RFLP and application of technology has evolved in various fields. Subsequent to RFLP, several other methods such as Variable Number Tandem Repeats (VNTR), Allele Specific Oligonucleotide (ASO), Allele specific polymerase chain reaction (AS-PCR), Oligonucleotide polymorphism (OP), Single Stranded Conformational Polymorphism (SSCP) and Sequence Tagged Sites (STS). The conventional hybridization based assays of detecting DNA level variations were replaced by the Polymerase Chain Reaction based assay have been evolved to detect variations at DNA level.

**Table 1: Chronological Evolution of DNA markers****First generation DNA Markers**

Year	Acronym	Nomenclature	Reference
1974	RFLP	Restriction Fragment Length Polymorphism	Grodzicker et al. (1974)
1985	VNTR	Variable Number Tandem Repeats	Jeffreys et al. (1985)
1986	ASO	Allele specific oligonucleotides	Saiki et al. (1986)
1988	AS-PCR	Allele specific polymerase chain reaction	Landegren et. al. (1988)
1988	OP	Oligonucleotide polymorphism	Beckmann (1988)
1989	SSCP	Single Stranded Conformational Polymorphism	Orita et al. (1989)
1989	STS	Sequence Tagged Site	Olsen et. al. (1989)

**Second Generation DNA markers**

The next generation of molecular markers responsible for various revolutions in the field of molecular genetics are microsatellites- arrays of tandemly repeated di-, tri-, tetra- and pentanucleotide DNA sequences which occur dispersed throughout the genomes of all eukaryotic organisms investigated to date. The microsatellites are otherwise called as Sequence Tagged Microsatellite Sites (STMS) or Simple Sequence Repeats (SSR). SSRs are currently considered the molecular markers of choice within the genome mapping community and are rapidly being adopted by plant researchers as well. SSRs consist of around 10-50 copies of motifs from 1 to 5 basepairs that can occur in perfect tandem repetition, as imperfect (interrupted) repeats or together with another repeat type. These repeated motifs are flanked by unique or single copy sequences, which provide a foothold for specific amplification via PCR. Primers complimentary to the unique sequences in those flanking regions can be designed to amplify single copy products. The other marker systems developed during this period include Restriction Landmark Genome Scanning (RLGS), Cleaved Amplified Polymorphic Sequence (CAPS), Degenerate Oligonucleotide Primer –PCR (DOP-PCR), Single Strand Conformation Polymorphism (SSCP), Multiple Arbitrary Amplicon Profiling (MAAP) and Sequence Characterized Amplified Region (SCAR). The usage of these marker systems was not realized as SSRs.

**Table 2: Chronological Evolution of DNA markers****Second generation DNA Markers**

1990	RAPD	Randomly Amplified Polymorphic DNA	Williams et al. (1990)
1990	AP-PCR	Arbitrarily Primed Polymerase Chain Reaction	Welsh and McClelland (1990)
1990	STMS	Sequence Tagged Microsatellite Sites	Beckmann and Soller (1990)
1991	RLGS	Restriction Landmark Genome Scanning	Hatada et al. (1991)
1992	CAPS	Cleaved Amplified Polymorphic Sequence	Akopyanz et al. (1992)
1992	DOP-PCR	Degenerate Oligonucleotide Primer - PCR	Telenius (1992)
1992	SSR	Simple Sequence Repeats	Akkaya et al. (1992)
1993	MAAP	Multiple Arbitrary Amplicon Profiling	Caetano-Anolles et al. (1993)
1993	SCAR	Sequence Characterized Amplified Region	Paran and Michelmore (1993)



### New generation DNA markers

Recent developments in molecular biology have opened the of employing various types of molecular tools to identify and use genomic variation for the improvement of various organisms. Information concerning the basis of these techniques and their applications are from the technology spill-over of several genome projects. The last ten years have witnessed the birth of an array of molecular markers with high-throughput performance coupled with shift from manual mode of detection to complete automation.

The following are the markers of recent origin with tremendous potential in understanding the variation at DNA level.

**Table 3. Chronological Evolution of DNA markers**

#### New generation DNA Markers

1994	ISSR	Inter Simple Sequence Repeats	Zietkiewicz <i>et al</i> (1994)
1994	SAMPL	Selective Amplification of Microsatellite Polymorphic Loci	Morgante and Vogel, (1994)
1994	SNP	Single Nucleotide Polymorphisms	Jordan and Humphries (1994)
1995	AFLP (SRFA)	Amplified Fragment Length Polymorphism (Selective Restriction Fragment Amplification)	Vos <i>et al.</i> (1995)
1995	ASAP	Allele Specific Associated Primers	Gu <i>et al.</i> (1995)
1996	CFLP	Cleavase Fragment Length Polymorphism	Brow (1996)
1996	ISTR	Inverse Sequence – Tagged Repeats	Rhode (1996)
1997	DAMD-PCR	Directed Amplification of Minisatellite DNA- PCR	Bebeli <i>et al.</i> (1997)
1997	S-SAP	Sequence- Specific Amplified Polymorphism	Waugh <i>et al.</i> (1997)
1998	RBIP	Retrotransposon Based Insertional Polymorphism	Flavell_ <i>et al.</i> (1998)
1999	IRAP	Inter-Retrotransposon Amplified Polymorphism	Kalendar <i>et al.</i> (1999)
1999	REMAP	Retrotransposon-Microsatellite Amplified Polymorphism	Kalendar <i>et al.</i> (1999)
1999	MSAP	Methylation Sensitive Amplification Polymorphism	
2000	MITE	Miniature Inverted-repeat Transposable Element	Casa <i>et al.</i> (2000)
2000	TE-AFLP	Three Endonuclease AFLP	Van der Wurff <i>et al.</i> (2000)
2001	IMP	Inter-MITE polymorphisms	Chang <i>et al.</i> (2001)
2001	SRAP	Sequence-related amplified polymorphism	Li and Quiros (2001)

### ISSR

ISSR marker system is another newly developed method which relies on one primer for PCR that anneals to an SSR region and amplifies region between inversely oriented adjacent SSR<sub>s</sub>. ISSR assay can be undertaken for any species that contains a sufficient number and distribution of SSR motifs and has the advantage that genomic sequence data is not required. This technique amplifies large numbers of DNA fragments per reaction, representing multiple loci from across the genome; it is an ideal method for fingerprinting varieties.

### SAMPL

SAMPL is PCR-based multiplex DNA marker system where in compound microsatellite primers are used to detect genetic polymorphisms between individuals. This assay is based on

AFLP where the template is identical to AFLP template, but the rare cutter primer is replaced by microsatellites such as  $(CA)_{72}(TA)_{22}$  or  $(GT)_{72}(AT)_2$ .

#### **SNP**

SNPs are biallelic markers, which unravels polymorphism between individuals due to change of a single variations arise because of point mutations. The detection of SNPs needs only a plus/minus assay with automation. Both gel-based and Non gel- based methods are being used to detect these abundant polymorphisms. However, adequate sequence information is necessary. In case of gel-based methods, specific primers are designed this would cause amplification of a positive allele due to exact match of primer. Mismatches in primer design and/or DNA sequence fail to give amplification of a negative allele. In case of non gel-based methods, appropriate regions are amplified and thin mismatches detected by techniques such as denaturing high performance liquid chromatography (HPLC) or Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI TOF MS). Both these techniques are extremely sensitive and can detect mismatches from a few down to only one nucleotide.

#### **AFLP**

AFLP is a multiplex PCR based method in which a subset of restriction fragments are selectively amplified using oligonucleotide primers complementary to sequences that have been ligated to each end. AFLP analysis allows the reliable identification of over 50 loci in a single reaction. This technique combines the reliability of the RFLP and ease of the PCR and thus AFLP is a new typing method for DNA of any origin or complexity.

#### **ASAP**

ASAP is another PCR method in which high-stringent annealing temperature is maintained to generate a single DNA fragment that is specific to the allele of interest in an individual that can be identified by the sequence of the decamer oligo derived from normal RAPD.

#### **CFLP**

CFLP assay relies on denaturation (melting) of DNA. The single stranded DNA will assume folded hairpin-like structures, which are unique to the nucleotide sequence. The endonuclease Cleavase I specifically cleaves the junction between single strand and double strand regions. Fragments are 5' labelled, electrophoretically separated and visualized. Polymorphic fragments indicate a mutation; their length is indicative for the position of the mutated site.

#### **ISTR**

ISTR is a PCR-based multi-locus marker system using oligonucleotide primers homologous to dispersed high copy sequences, e.g. copia like transposons. Multi-locus polymorphic amplification products are obtained with two primers, designed in a direction outward the element, and allows the amplification of the stretch connecting the elements.

#### **DAMD-PCR**

DAMD-PCR is another approach where minisatellite core sequences are used as primers for PCR amplification. It is found to reveal various degrees of polymorphism and generate individual specific DNA fingerprints which could be used for species differentiation and cultivar identification.

#### **S-SAP**

S-SAP is a dominant, multiplex marker system for the detection of variation in the DNA flanking the retrotransposon insertion site. Fragments are amplified by PCR, using one primer designed from the conserved terminus of the LTR and one based on the presence of a nearby restriction endonuclease site. Experimental procedures resemble those used for AFLP analysis. Compared to AFLPs, S-SAP generally yields fewer fragments but higher levels of polymorphism.

#### **RBIP**

RBIP is a codominant marker system that uses PCR primers designed from the retrotransposon and its flanking DNA to examine insertional polymorphisms for individual retrotransposons. Presence or absence of insertion is investigated by two PCRs, the first using one primer from the retrotransposon and one from the flanking DNA, the second using primers

designed from both flanking regions. Polymorphisms are detected by simple agarose gel-electrophoresis or by dot hybridization assays. Drawback of the method is that sequence data of the flanking regions are required for primer designing. Major advantage is that RBIP does not necessarily require a gel-based detection system but can easily be automated to gel-free procedures, such as TaqMan or DNA chip technology in order to increase sample throughput.

#### **IRAP**

IRAP is also a dominant, multiplex marker system that examines variation in retrotransposon insertion sites. Imps fragments between two retrotransposons are generated by PCR, using outward-facing primers annealing to LTR target sequences. Fragments are separated by high-resolution opposite gel-electrophoresis.

#### **MSAP**

MSAP is a modification of the amplified fragment length polymorphism (AFLP) method that makes use of the differential sensitivity of a pair of isoschizomers to cytosine methylation.

#### **REMAP**

REMAP is yet another dominant, multiplex marker system that examines variation in retrotransposon insertion sites. REMAP fragments between retrotransposons and microsatellites are generated by PCR, using one primer based on a LTR target sequence and one based on a simple sequence repeat motif. Fragments are separated by high-resolution agarose gel-electrophoresis.

#### **MITE**

MITE assay involves transposed display (TD) which is a modification of the AFLP procedure where PCR products are derived from primers anchored in a restriction sites and a transposable element rather than in two restriction sites. For this candidate primers in transposable elements are designed based on a consensus sequence generated of transposable elements.

#### **TE-AFLP**

TE-AFLP differs from traditional AFLP by reducing the number of amplified fragments not only by primer extension, but also by selective ligation. Three endonucleases together with only two sets of adapters are added to a single reaction. As a consequence, the reduced number of potential amplifiable fragments diminishes competition during PCR, permitting stringent reaction conditions and thus eliminating the need for a two-step amplification in fingerprinting complex genomes.

#### **IMP**

IMP assay is a novel PCR based approach where polymorphisms were revealed with primers designed from the terminal inverted repeats (TIR) of two adjacent MITE<sub>s</sub>. The distribution of MITE<sub>s</sub> can be established by computer-assisted database searches and structural analysis of genomic sequences.

#### **SRAP**

SRAP assay involves the amplification of the open reading frames (ORFs). It is based on two-primer amplification. The primers are 17 or 18 nucleotides long and consist of the following elements. Core sequences, which are 13 to 14 bases long, where the first 10 or 11 bases starting at the 5' -end, are sequences of no specific constitution ("filler" sequences), followed by the sequence CCGG in the forward primer and AATT in the reverse primer. The core is followed by three selective nucleotides at the 3' -end. The filler sequences of the forward and reverse primers must be different from each other and can be 10 or 11 bases long.

#### **Features of molecular markers**

The following are the deciding factors influencing the purposes of molecular markers in biological explorations.

#### **Abundance**

Genetic markers should be in abundance covering the entire genome for the development of high-density linkage maps or genome wide DNA fingerprinting.

#### **Level of polymorphism**

The appropriate genetic marker technique having high level of polymorphism should be employed in genome mapping/DNA fingerprinting. The level of polymorphism among the genetic markers depends on the type of marker, and methods used for detection.

#### **Number of alleles**

There are two possible types of markers: markers with a single alternative allele (biallelic) and several alternative alleles (polyallelic).

#### **Locus specificity**

Markers are classified two groups as single locus markers (unique location on the genome) and multilocus (several locations on the genome) markers. Single locus markers are preferred for genome mapping while the markers of multilocus nature are employed for DNA profiling. Among the single locus markers, markers of polyallelic nature are very useful for DNA profiling.

#### **Nature of alleles**

Markers of biallelic nature are considered as codominant when both the alleles are observed in the hybrid. If one of the two alleles is observed then the marker is considered as dominant. Codominant markers are more informative than the dominant markers since codominant markers can distinguish heterozygotes from homozygotes. This allows the determination of genotypes and allele frequencies at loci more precisely. Therefore, codominant markers are preferred over the dominant for genome mapping.

#### **Technical demands**

RFLP, minisatellites and PCR-sequencing require technical skills and facilities to carry out radioactive labelling. In addition, Southern blot hybridizations are part of the RFLP and minisatellite analysis. These techniques are therefore among the more technically demanding markers. Technical demands for RAPD and ISSR markers comparatively less. These days many of the markers resolved with ease using either silver staining or fluorescently labeled primers.

#### **Quantity of DNA required**

Because only small quantities of template DNA (5- 100 ng per reaction) are required, techniques which are based on the Polymerase Chain Reaction (PCR) currently are preferred. RFLPS and minisatellites require the largest amount of DNA (5- 10 µg per reaction) but Southern blots may be reprobbed several times. Intermediate quantities of DNA are needed for AFLP-analysis (0.3- 1 µg per reaction) because endonuclease restriction of the DNA template precedes the PCR-reaction. Application of PCR-based markers may be relevant when only small amounts of DNA can be extracted; e.g. when working with tiny organisms.

#### **Amenability to automation**

Currently, techniques, which can be automated, are preferred because they enable increased sample throughput. Although considerable financial investment still is required, automation may be cost-effective when techniques are applied on a routine basis. Nearly all techniques, which are based on the Polymerase Chain Reaction (PCR), are amenable to automation.

#### **Operational costs**

Wages, laboratory facilities, technical equipment and chemicals all contribute to the operational costs of the technologies. Relatively expensive chemicals include Taq-polymerase needed for PCR, restriction enzymes (particularly, frequently cutting endonucleases used in AFLP-analysis) and radioactive label. Polyacrylnide gels are more expensive to run than agarose gels and require visualization of polymorphisms by autoradiography or silver staining which are more costly compared to ethidium-bromide staining. Laborious and technically demanding markers, such as RFLPS, minisatellites and PCR-sequencing are therefore quite

expensive. Compared to these techniques the operational costs of the other markers presented vary from low to medium, depending on the methodological procedures followed.

### Development costs

Marker development may be very time-consuming and costly when necessary probes or sequence data for primer construction are unavailable. In addition, sufficient technical skills and facilities need to be present. Development of suitable probes for Southern blot hybridizations (e.g. for RFLP-analysis) requires the construction of a genomic library, the isolation of DNA fragments and the examination of various probe/restriction enzyme combinations for the ability to detect polymorphisms. Development of site specific PCR-primers (e.g. for microsatellite analysis) also requires the construction of a genomic library, which then needs to be screened to identify the fragments of interest. Subsequently, the identified fragments need to be sequenced in order to obtain the necessary data for primer construction. Therefore, the investment required for marker development should be evaluated in relation to the intended range of application of the technique. Alternatively, probes, primers and sequence data may be obtained from genome databases of other species, although the usefulness of this approach decreases with increasing evolutionary distance.

### Applications of DNA markers

The molecular markers produced a greater impact on genome mapping, gene tagging and evolutionary studies of crop plants. As far as mapping genomes and genes is concerned, the success depends on the availability of suitable base populations of  $F_2$ s backcross progenies, doubled haploids (DH), recombinant inbred lines (RIL), and near isogenic lines (NIL). Exploiting the available populations in conjunction with molecular marker techniques, molecular linkage maps have been constructed for several crop species and very many major and minor genes have been mapped with molecular markers. These "molecular and gene tags" are to be used to exercise marker aided selection, map based cloning and physical mapping of genes of agronomic importance. There are success stories on cloning genes based on their map positions. Apart from genome/gene mapping, molecular markers are employed in assessing the extent of genetic diversity in plant populations. The following section deals with various applications of molecular marker technology individually.

### Genome Mapping

The genome map of an organism summarizes much of the genetic information available for that species and can serve as a reference for the development and testing of additional genetic hypotheses. However, generation of a complete linkage map remains a daunting task, for many of suitable population. For the construction of linkage maps with molecular markers, parents are chosen that show the maximum of polymorphic loci in order to ensure the mapping of as many markers as possible. Several strategies are followed to have a mapping population. In most of the map construction,  $F_2$  segregating populations are used. These populations are the result of selfing  $F_1$ s of two homozygous inbred lines. Most of the molecular maps to date are based on segregation data from  $F_2$  progenies. In some cases, the segregating progenies of  $F_1$ s backcrossed to recurrent parent were also used to construct linkage maps. Developing a population of RIL is an alternative strategy in mapping projects. Recombinant inbred lines are developed by continuous selfing of  $F_2$  individuals until the homozygosity is achieved. Doubled haploids from anther or microspore culture are also used for linkage map construction in various crop species. Though the strategy sounds good, construction of DH population is a genotype-dependent process to the *in vitro* culture conditions.

### Mapping genes

Molecular markers offer a tool for locating genes governing agronomically important characters *via* linkage to mapped DNA sequences. Phenotypic evaluation at the whole plant level or at the cellular level provides information, which can be used to determine the chromosomal

location of the genes that confer the phenotype of interest. This is accomplished by analyzing linkage between mapped molecular markers and expression of the target phenotype in a range of related individuals. Markers linked to the genes of interest function as "gene tags" facilitating selection of favourable alleles in a breeding programme.

Like for linkage map construction, gene tagging component also needs a suitable population in which the trait to be tagged with molecular markers shows clear-cut segregation with a higher level of polymorphism for the molecular markers. The process of gene tagging involves two steps: 1) surveying parents with molecular markers for their level of polymorphism, and 2) surveying the polymorphic markers on progenies with an aim to tag the trait of interest with a molecular marker(s).

**Mapping major genes:** Establishing associations between molecular markers and simply inherited traits is comparatively easier. To date, several major genes have been tagged with molecular markers using NIL and Bulk Segregant Analysis (BSA).

**Mapping genetic loci controlling quantitative most of the traits:** In crop breeding, most of the traits that breeders concerned with are polygenically controlled. Location of polygenes in individuals by conventional analysis was difficult. The advent of molecular marker technology provides the geneticists with powerful new tools for identifying the component Mendelian loci of those complexly inherited traits. The main practical limitation to localizing QTLs seems to be the availability of suitable markers. This limitation was remedied by the construction of saturated molecular linkage maps permitting systematic searches of an entire genome for QTLs associated with various traits.

#### **Map based cloning of genes**

Once a gene has been chromosomally localized, the next step is to move towards it from a linked marker, creating a physical map. Construction of physical map can be accomplished by adopting Pulsed Field-Gel Electrophoresis (PFGE) and a library of overlapping pieces of DNA, obtained with Yeast Artificial Chromosomes (YAC). Map based cloning follows physical mapping of the gene of interest and in an alternative strategy for cloning the genes for which only the phenotypes are known, but not the gene products. In this approach, the actual gene of interest is identified by adding overlapping DNA pieces from the position of molecular marker. In molecular terms, this is called chromosome walking.

#### **Marker Aided Selection**

The development of molecular markers promises to overcome most of previous limitations associated with morphological markers. Tight linkage of a marker to a gene can be exploited for indirect selection of traits in a breeding programme, otherwise called as Marker Aided Selection (MAS). Two prerequisites for adopting marker aided selection in breeding programmes are: 1) a tightly linked marker to the gene concerned and 2) population which is polymorphic for the marker and the gene which are in extreme linkage disequilibrium. There are three approaches to applying MAS to plant breeding. 1) selection markers alone with no measurement of phenotype, 2) simultaneous selection on markers and phenotype, and 3) two stage selection, the first stage involving use of markers to select among seedlings and second involving phenotypic selection among surviving individuals. The potential efficiency of MAS depends upon the heritability of the trait, the proportion of genetic variance explained by the markers, and the selection method. A major practical problem in using MAS is that recombination will reduce linkage disequilibrium between the markers and genes, thus diminishing selection effectiveness. The successful application of MAS will require very tight linkages between marker and the trait.

#### **Genetic Diversity Analysis**

Study of genetic diversity is the process by which variation among individuals or groups of individuals or populations is analyzed by a specific method or a combination of methods. The data often involve numerical measurements and in many cases, combinations of different types of variables. Diverse data sets have been used by researchers to analyze genetic diversity in crop



plants; most important among such data sets are pedigree data, passport data, morphological data, biochemical data obtained by analysis of isozymes and storage proteins, and, recently, DNA-based marker data that allow more reliable differentiation of genotypes. Since each of these data sets provide different types of information, the choice of analytical method(s) depends on the objective(s) of the experiment, the level of resolution required, the resources and technological infrastructure available, and the operational and time constraints, if any. The advances in DNA marker technology really revolutionized the process of portraying diversity within plant population, crop germplasm and establishing DNA fingerprints for each genotype.

### Conclusion

A wide range of numerical of molecular marker technologies is now available for genetic studies. Of these, RAPD, AFLP, ISSR and SSR marker systems are emerging as the lead technologies. Using RAPD marker system is not felt convenient because of its inconsistency. However, RAPD assay is still used for DNA fingerprinting along with other dominant markers viz. AFLP and ISSR markers. SSR markers remain the markers of choice for genome mapping and genetic diversity analysis. Several variations of the entire above mentioned marker systems are also made available. Among the new generation of markers viz. SRAP, MITE, TE-AFLP and IMP are in the early phase of usage and are not routines in the molecular marker technology laboratories.. Though an array of molecular markers is available to the researchers, one has to decide the right choice marker for the right problem. Even when the potential utility of markers is well established, the key question is "whether these markers offer significantly enough outputs to speed up the process of crop improvement justifying the investment in this area of research?"

### References

- Akkaya MS, Bhagwat AA and Cregan PB (1992) Length polymorphism of simple sequence repeat DNA in soybean. *Genetics* 132: 1131- 1139.
- Akopyanz N, Bukanov NO, Westblom TU and Berg DE (1992) PCR-based RFLP analysis of DNA sequence diversity in the gastric pathogen *Helicobacter pylori*. *Nucleic Acid Res.* 20: 6221-6225
- Bebeli PJ, Zhou Z, Somers DJ and Gustafson JP (1997) PCR primed with minisatellite core sequences yields DNA fingerprinting probes in wheat. *Theor. Appl. Genet.*, 95: 276-283.
- Beckmann JS (1988) Oligonucleotide polymorphisms: A new tool for genomic genetics. *Bio/Technology* 6: 16 1- 164.
- Beckmann JS and Soller M (1990) Toward a unified approach to genetic mapping of eukaryotes based on sequence tagged microsatellite sites. *Bio/Technology* 8:930-932.
- Brow MA, Oldenburg MC, Lyamichev V, Heisler LM, Lyamicheva N, Hall JG, Eagan NJ, Olive DM, Smith LM, Fors L and Dahlberg JE (1996) Differentiation of bacterial 16S rRNA genes and intergenic regions and Mycobacterium tuberculosis katG genes by structure-specific endonuclease cleavage. *Journal of Clinical Microbial.* 34: 3129-3137.
- Caetano-Anolles G, Bassam BJ and Gresshoff PM (1993) Enhanced detection of polymorphic DNA by multiple arbitrary amplicon profiling of endonuclease-digested DNA: identification of markers tightly linked to the supernodulation locus in soybean. *Molec. Gen. Genet.* 24 1: 57-64.
- Casa AM, Brouwer C, Nagel A, Wang L, Zhang Q, Kresovich S and Wessler SR. 2000. The MITE family Heartbreaker (Hbr): Molecular markers in maize. *Proc. Natl. Acad. Sci (USA)* 97: 10083-10089.
- Chang RY, O'Donoghue LS and Bureau TE (2001) Inter-MITE polymorphisms (IMP): a high throughput transposon-based genome mapping and fingerprinting approach. *Theor. Appl. Genet.* 102: 773- 781.
- Flavell AJ, Knox MR, Pearce SR and Ellis THN (1998). Retrotransposonbased insertion polymorphisms (RBIP) for high throughput marker analysis. *Plant Journal*, 16: 643-650.
- Grodzicker T, Williams J, Sharp P and Sambrook J (1974) Physical mapping of temperature sensitive mutations. *Cold Spring Harbor Symp. Quart. Biol.* 39: 439-446.
- Gu WK, Weeden NF, Yu J and Wallace DH (1995) Large-scale, cost-effective screening of PCR products in marker-assisted selection applications. *Theor. Appl. Genet.* 91:465-470.

- Hatada I, Hayashizaki Y, Hirotsune S, Komatsubara H and Mukai T (1991) A genome scanning method for higher organism using restriction sites as landmarks. *Proc. Natl. Acad. Sci. USA*, 88 : 397-400.
- Jeffreys AJ, Wilson V and Thein SL (1985) Hypervariable minisatellite regions in human DNA. *Nature*. 314: 67-73.
- Jordan SA, and Humphries P (1994) Single nucleotide polymorphism in exon 2 of the BCP gene on 7q31-q35. *Human Molecular Genetics* 3: 19-15
- Kalendar R, Grob T, Regina M, Suoniemi A and Schulman A (1999) IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques *Theor. Appl. Genet.* 98: 704-711.
- Landegren U, Kaiser R, Sanders J and Hood L (1988) DNA diagnostics. *Molecular techniques and automation. Science* 241: 1077-1080
- Li G and Quiros (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. *Theor. Appl. Genet.* 103: 455-461.
- Morgante M and Vogel J (1994). Compound microsatellite primers for the detection of genetic polymorphisms. *U. S. Patent Appl.*, 08/326456.
- Olsen M, Hood L, Cantor C and Botstein D (1989) A common language for physical mapping of the human genome. *Science* 245: 1434-1435.
- Orita M, Suzuki, Y, Sekiya T and Hayashi K (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using polymerase chain reaction. *Genomics* 5:874-879
- Paran I and Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. *Theor. Appl. Genet* 85:985-993.
- Reyna-Lopez GE, Simpson J, Ruiz-Herrera J (1997) Differences in DNA methylation patterns are detectable during the dimorphic transition of fungi by amplification of restriction polymorphisms. *Mol Gen Genet* 253:703-710.
- Rohde W (1996) Inverse sequence-tagged repeat (ISTR) analysis, a novel universal PCR-based technique for genome analysis in the plant and animal kingdom. *Journal of Genetics Breed* 50: 249-261. .
- Saiki RK, Bugawan TL, Horn GT, Mullis KB and Erlich HA (1986) Analysis of enzymatically amplified beta-globin and HLA-DQ alpha DNA with allele-specific oligonucleotide probes. *Nature*. 324: 163-6.
- Soller M and Beckmann JS (1983) Genetic polymorphism in varietal identification and genetic improvements. *Theor. Appl. Genet.* 67: 25-, 33
- Telenius H, Carter NP, Bebb CF, Nordenskjold M, Ponder BJ., Tunnacliffe A (1992) Degenerate oligonucleotide-primed PCR: general amplification of target DNA by a single degenerate primer. *Genomics* 13: 718-725.
- van der Wurff AWG, Chan YL, van Straalen NM and Schouten J. 2000. TEAFLP: combining rapidity and robustness in DNA fingerprinting. *Nucleic Acids Res.* 28: 105-109.
- Waugh, R., McLean, K., Flavell, A.J., Pearce, S.R., Kumar, A., Thomas, B.T., Powell, W. 1997 Genetic distribution of BARE-I retrotransposable elements in the barley genome revealed by sequence-specific amplification polymorphisms (S-SAP). *Mol. Gen. Genet.*, **253**, 687-694.
- Welsh J and McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* 18: 7213-7218.
- Wessler S, Bureau TE and White SE (1995) LTR-retrotransposons and MITEs: important players in the evolution of plant genomes. *Curr. Op. Genet. Dev.* 5: 814-821.
- Williams JGK, Kubelik AR, Livak KJ, Rafalski JA and Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Research* 18: 6531-6535.
- Xiong LZ, Xu CG, Maroof MAS and Zhang QF (1999). Patterns of cytosine methylation in an elite rice hybrid and its parental lines, detected by a methylation-sensitive amplification polymorphism technique. *Mol. Gen. Genet.* 261, 439-446.
- Zietkiewicz E, Rafalski A and Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, 20: 176-183.

## Cotton Varieties in India - Influence of climate, soil and location of cultivation on expression of morphological characters - an overview

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Cotton is one of the important commercial crops of the world. More than one hundred countries grow cotton. Of these, 10 countries account for as much as 80 per cent of the total cotton production. World cotton production during 2004 was of the order of 20 million tonnes, of which India's share was only 3.6 million tonnes. On the other hand, India ranked first in world cotton area with 8.9 m ha followed by china (5.7), USA (5.3) and Pakistan (3.2).

Cotton belongs to the Genus *Gossypium* and comprises of 50 different species, distributed in 8 genomes. Of the 50 species, only four species are cultivated. *Gossypium, arboreum* and *G. herbaceum* belong to the old world Diploid group, whereas the New world tetraploid cultivated species are *G. hirsutum* and *G. barbadense*.

Cotton is grown in India very widely from 69 to 97°N longitude and 8 to 37°E latitude . Based on the agro climate and geographic distribution, cotton cultivation in India is divided into three zones. The details of cotton area, production and productivity in all the three zones are furnished in Table 1.

**Table 1. Cotton Scenario - India (2003-04)**

State	Area (lakh. Ha)	Production (lakh bales)	Productivity (kg/ha)
<b>North Zone</b>			
Punjab	5.5	15.0	464
Haryana	6.3	16.5	448
Rajasthan	3.0	8.0	453
<b>Total</b>	<b>14.8</b>	<b>39.5</b>	<b>455</b>
<b>Central Zone</b>			
Gujarat	20.0	55.0	469
Maharashtra	30.4	40.0	224
Madhya Pradesh	5.9	19.0	551
<b>Total</b>	<b>56.2</b>	<b>114.0</b>	<b>345</b>
<b>South Zone</b>			
Andhra Pradesh	11.4	33.0	492
Karnataka	5.5	8.0	246
Tamil Nadu	1.3	5.0	680
<b>Total</b>	<b>18.2</b>	<b>46.0</b>	<b>430</b>
Others	0.5	1.0	315
<b>Total</b>	<b>89.7</b>	<b>200.5</b>	-
Loose	-	12.5	
<b>Total</b>	<b>89.7</b>	<b>213.0</b>	<b>404</b>

The climatic requirements for the normal expression of the cotton plant are:

- (i) Mean annual temperature of over 60°F
- (ii) Annual rainfall of at least 20" with favorable distribution
- (iii) Good sunshine hours especially during boll maturation period.
- (iv) Frostless season especially in North India.

Of the different climatic factors that influence the normal growth of the cotton plant, rainfall and temperature are the most important. As much as 65 percent of the cotton cultivation in

India, depends on rainfall recorded during Northeast and southwest monsoons. Cotton is cultivated in India under four seasons.

1. Kharif (May-Sep) - North zone
2. Rabi (Oct-Feb) - Central Zone & South zone
3. Summer (March-July) - Parts of Tamil Nadu & Maharashtra
4. Rice fallows (Jan-May) - Parts of Tamil Nadu & Andhra Pradesh

Temperature normally decreases from North to South. In North, Cotton is grown during the hottest month with temperature over 105<sup>0</sup>F. By July and August there is a fall in temperature. In Central Zone, the maximum temperatures falls rapidly after the break of the south west monsoon with a mean maximum temperature of about 90 to 95<sup>0</sup>F and minimum of 65 to 70<sup>0</sup>F. In the south, the maximum and minimum temperature during the crop season range from 85 to 70<sup>0</sup>F. However, the mean maximum temperature in summer and Rice fallows areas of south range from 95 to 100<sup>0</sup>F.

Cotton needs ample sunshine during boll development and boll bursting period. In North Zone, July and August, being monsoon month, the season of grand growth and boll formation stage remains cloudy and humid with lesser hours of sunshine. Because of early winter, the cropping period is limited in North Zone. In Central zone, ideal temperature and ample sunshine during grand growth and maturity period, cool, and rain free weather during October to February are favourable for obtaining higher yields. South zone also has a clear cool dry weather, bright sunny day devoid of clouds and rains during maturity. Andhra Pradesh with well-distributed bimodal distribution of rainfall, the crop performs very well. In Tamil Nadu cotton is grown in more than one season.

#### **Soils**

The major soil types in which cotton is grown in India are black, alluvial, red and laterite soils.

#### **Black soil:**

Black soils are widely distributed in central and south zones and are best suited for Rainfed cultivation. Very high yield up to 50 q/ha have been recorded in Gujarat under irrigated cultivation and under assured rainfall conditions in Andhra Pradesh. However, the shallow black cotton (murrum) soils of Madhya Pradesh and certain parts of Gujarat have very poor fertility and water retention capacity and record very low yields.

#### **Alluvial soil:**

Alluvial soils may be of clay-loam type as is found in Punjab or sandy loam as in Haryana and Rajasthan. These types of soils are also found in delta regions of Andhra Pradesh and Tamil Nadu. They are suitable for intensive crop production.

#### **Red soil**

Red soils are found mainly in parts of Andhra Pradesh, Karnataka and Tamil Nadu. They need good irrigation and are responsive to chemical fertilizers.

#### **Laterite and coastal saline soils**

Cotton is also grown in a limited scale under laterite (Comilla cottons in Assam & Megalaya) and coastal saline soils. (Waged & Dhumad cotton in Gujarat).

To suit the needs of the varied agro climatic conditions under which cotton is grown in the country and to meet the demands of the textile industry which utilize cotton capable of spinning from 20s to 120s count of cotton, through the network of AICCIP centres, as many as 250 varieties and hybrids belonging to all the for cultivated species have been released for commercial cultivation. The species and varieties to be grown in any particular region depends on the soil and climate of the region. The most important currently cultivated and recently released varieties are given in Table 2

**Table2: Cotton growing Zones, Soils and important varieties in India**

**North zone: Soils-Alluvial, sandy-Irrigated**

State	G.hirsutum	G.arboreum	Hybrids
Punjab	F 846, F 1054, F 1378, LH 900, LH 1556	LD 327, LD 491	Intra hirsutum LHH 144
Haryana	H 777, HS 6, H1117 H1098	HD 107, HD 123	Intra arboreum AAH-1
Rajasthan	BN,RST 9, RS 810, RS 875, RS 2013	RG 8, RG 18	
Uttar Pradesh	Vikas	Lohit	

**Central zone: Soils-Black, Alluvial-Mostly rainfed**

State	G.hirsutum	G.arboreum	Hybrids
M.P	Khandwa-2, Vikram	Jawahar Tapti	Intra hirsutum JK Hy-1
Maharashtra	LRA 5166, PKV 081, RKV Rajat	AKH 4, AKA 5, AKA 7, AKA 84635, PA 183	Intra hirsutum NHH 44, Pkv Hy 2Ankur 651
Gujarat	SRT-1		Intra hirsutum H.4, H.6, H8, H10

**South Zone: Soils-Black, Alluvial, Red, Laterite-Both irrigated and Rainfed**

State	G.hirsutum	G.arboreum	Hybrids
A.P	LK 861, LS 389 Narasimha	Aravinda	Intra hirsutum RCH2
Karnataka	Sharada, Arunabha	DLSA 17	Intra hirsutum Bunny RCH2, DHH.11 Interspecific Varalaxmi, Jayalaxmi
Tamil Nadu	MCU 5, MCU 5VT, Surabhi, LRA 5166, Sumangala, MCU.7	K 10, K 11	Intra-hirsutum Savita, RCH 2, Bunny interspecific TCHB 213

G. herbaceum: Gujarat: Digvijay, V797, Gcot 13, G cot 17 and G cot 21

Karnataka: Jayadhar, DB 8-12

G .barbadense: TamilNadu Sujata, Suvin

**Diagnostic Characteristics of Crop varieties**

In early days, a small list of descriptors was sufficient to distinguish between crop varieties in use. As the world became more competitive, and the number of varieties increased manifold there arose a need for more clear- cut diagnostic features. In cotton, a meaningful system of variety testing and release is available under the ICAR system. So at the time of testing itself some basic data on yield, quality, disease and pest reaction are collected. This is more akin to a VCU (value for cultivation and use) test in the European countries. However, for the registration of new varieties and plant varietal protection apart from VCU a detailed botanical examination using a standard list of descriptors is essential. Such a system will eventually assist in protecting varieties when PVP system is established.

**Expression of Morphological Character and DUS testing.**

The soil and climate of the region are known to have an effect on the morphological expression of a character. However, it is seen that the variation in their expression is more pronounced in the case of quantitative characters rather than the qualitative character So it is essential to grow the genotypes under conditions where the full expression of morphological and agronomic characteristics are possible. Further, it is also necessary to employ proper statistical procedures to establish their distinctness. Several statistical procedures and software are now available to analyse the voluminous data gathered and arrive at meaningful conclusions

## Emerging Strategies in Cotton Breeding Programme

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In the post independence period, India has witnessed a quantitative and qualitative improvement in its cotton production. The cotton area has increased from 4.3 m. ha in 1947-48 to 7.8 million ha by 1996-97. Similarly, cotton production has also increased from 2.6 m. bales to 5.6 m. bales. There has been a further quantum jump in area and production during the past two decades. The year 2003-04 witnessed an all time increase in area (89 m. ha), production (2 l.3m.bales) and productivity (404 kg/ha). The qualitative composition of the Indian cotton crop also changed favourably during the period. Today, the Textile Industry produces yarn in the range of 6<sup>s</sup> count to 120<sup>s</sup> count, exclusively from the indigenous cotton.

The conventional breeding tools like hybridization and selection, pedigree breeding, mass pedigree selections, progeny test, intense mating, back cross breeding and recurrent selections have helped to attain this excellence. Development of the Madras Cambodia Varieties (MCU series) in Tamil Nadu, the Dharwad American Cotton (Laxmi, Gadag 1) in Karnataka, Indo American Varieties (SRT- 1) in Gujarat, Madhya Pradesh Cotton (Khandwa-2) in Madhya Pradesh and Punjab American Cotton (320 F, P2 16F, J34) in Punjab have effectively contributed to quantitative and qualitative improvement. The desi cotton varieties like V 797, Jayadhar, AK 235 and Gaorani Cotton also have helped to stabilize production under rainfed condition.

The popular improved *G.hirsutum* varieties like HS 6, F 846, LH 1556, RS 8 10 in North Zone, SRT- 1, Khandwa-2, LRA 5166, Rajat in the Central Zone, MCU 5, LRA 5166, MCU 7, LK 861 and Surabhi in the South Zone have contributed favourably towards attaining the present level of production. Similarly, desi varieties like LD 327, HD 123, RG 18, Jawahar Tapti, PA 183 and Aravinda have stabilized yields under irrigated conditions in North zone and under rainfed situations in central and south zones.

A significant landmark in the history of cotton development in India is the successful commercial exploitation of Heterosis through conventional hybrid seed production programme. Developments of intra *hirsutum* and interspecific (*G. hirsutum* x *G. barbadense*) hybrids have significantly contributed towards both quantitative and qualitative improvement in Indian cotton. Currently fifty percent of the national cotton area is reported to be grown with hybrid cotton, contributing to approximately sixty per cent of the total production.

### Emerging strategies in conventional breeding

Conventional breeding techniques are still expected to play a vital role to meet the challenges emerging out of the PVP and other acts.

#### Breeding for wider adaptability

Even though it is imperative to release different varieties to suit the varied agro-climatic conditions and to match the varied demands of the Textile Industry, the multiplicity of the varieties, mismatch between production of different quality groups and deliberate mixing of the varieties leave us with no option but to reduce the number of varieties. Hence, it is necessary to develop varieties with wider adaptability. Bulked progeny row test system evolved by the Texas Agricultural Experimental Station and introduction of agronomic differentials in the early generation of testing may help to develop varieties for different input conditions. Complex crossing in cycles of generation as suggested by Joshi may help to break unfavorable genetic linkage and accumulate additive genes and add x add interactions

#### Breeding for yield improvement

Breeding for the improvement of yield components instead of per se improvement in the seed cotton yield may help achieve a break through in productivity per unit area. High ginning out turn with optimum seed weight will lead to greater stability in yield per unit area and increased productivity of lint.



### **Breeding for newer fibre quality norms**

Introduction of high speed spinning machines is making new demands and standards for fibre quality. Hence, it is necessary to develop varieties with better strength and micronaire. The revised standards set by the CIRCOT should be adopted from the primary stages of selection. However, it is to be decided whether the stringent quality standards required for the textile mills are to be stipulated for handloom and khadi sections also. Improvement of number of fibre quality characters like short fibre content, seed coat fragments, elongation percentage, naps and motes will go a long way in making Indian cotton more competitive internationally. Incorporation of naked seeded character through conventional breeding is worth considering towards reduced seed coat fragment. Variability available for short fibre content, elongation percentage among the hirsutum genotype may also be favourably utilized through conventional breeding programme.

### **G. barbadense Improvement**

Development and release of the G. barbadense variety Suvin put India on the International Cotton map. The highest production of Suvin was in 1989-90 with 36,141 bales from about 30,000 hectares in Andhra Pradesh & Tamil Nadu. Due to combination of factors like instability in yield, competition from hybrids, variation in fibre quality, instability in pricing, long duration etc. has brought down the current area to less than 1000 hectare. Further improvement in G. barbadense varieties utilizing wide genetic base and conventional breeding methods is very important to regain India's pride of place in the ELS scenario.

### **Desi Cotton improvement**

The area under Asiatic Cotton has come down to less than 25 percent from 98 percent in 1947-48. In view of the valuable gene pool available in the Asiatic diploid cultivated species, further reduction in desi cotton area is to be arrested. Hence, it is necessary to improve the quality of desi cotton to that of at least medium staple low count G. hirsutum varieties like LRA 5166. Improved G. arboreum varieties like PA 255, AKA 8401 in Maharashtra and MPL 243 in Andhra Pradesh developed through conventional breeding methods have helped to improve the fibre quality parameters considerably. Further, introgression of G. hirsutum genes into G. arboreum have resulted in the development of improved varieties like PA 402 (Maharashtra) and DLSA 17 (Karnataka).

### **Emerging trends in Plant Breeding**

Crop Improvement is the exploitation of genetic variability followed by several generations of selection. The modern biotechnological tools available to the breeder have helped in

- i acceleration of the selection process
- ii new genetic combinations not possible through conventional breeding
- iii greater precision in the desired modification of the genome.

### **Use of doubled haploids**

Using in vitro technique, it is possible to regenerate plants from pollen or ovule. These plants, which contain only one copy of each chromosome, are called haploids. Through colchicine the chromosome number is doubled to obtain a viable doubled haploid. These are homozygous for all the genes and enable one to develop pure line varieties or inbreed much quicker than through conventional breeding.

### **Embryo Rescue**

Interspecific hybrids developed through wide hybridization often result in non-viable embryos, mainly due to incompatibility between the embryo and the mother plant. Through in vitro cultivation of ovules or embryos, it is now possible to circumvent the sterility barriers.

### **Protoplast fusion**

Fusion of protoplasts to allow for interspecific hybridization, even in cases where embryo rescue techniques have failed is yet another tool to create variability.

### Marker aided selection

Unlike morphological marker which are limited in number and which do not represent the true genetic variability, the protein or DNA markers are much more reliable. However, in the case of protein marker, the isozyme numbers are limited and expression is restricted to certain developmental stages and requires electrophoresis and special staining technique. The DNA markers are unlimited; expression not necessary for detection and all markers can be detected with a single technique.

Several techniques for molecular markers like RFLP, RAPD, AFLP, and micro satellites are now available. Using these techniques polymorphism has been detected in restricted genomic DNA of plants, which have paved the way for the development of molecular markers for cotton breeding.

There are many applications for the use of DNA markers in breeding programme. Broadly they are:

- (i) Enhancing knowledge of breeding material and systems such as Quantitative trait loci (QTL)
- (ii) Rapid introgression or back cross breeding of simple characters.
- (iii) Early or easy indirect character selection, which is important for genes that cannot be detected at an early developmental stage.
- (iv) New goals not possible through conventional breeding like pyramiding of disease resistant genes.

### Breeding for Quantitative trait loci

Many ergonomically interesting traits such as yield and fibre quality are controlled by polygenes, with every polygons contributing only a small percentage to the expression of the trait. Molecular markers will allow direct selection for genotypes, thereby providing a more efficient means for selection for fibre properties.

### Breeding for Resistance

DNA markers linked with disease resistance would enable the selection of resistant plants without the need to actually infect plants with the pathogen using DNA markers, smaller number of plants could be selected.

### Pyramiding of genes.

Single gene resistance is often rapidly broken by pathogens. Hence, it is necessary to accumulate several minor and major resistance genes into one cultivar to achieve more resistance that is desirable. The utilization of DNA markers will facilitate the selection of cotton varieties carrying different genes for disease resistance and could enable plant breeders to pyramid combinations of such genes into one variety.

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## Homogeneity in field Trials

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Five and a half decades is over, Bapuji says agriculture is the backbone of the Indian Economy. Even today, as we entered into the new millennium, the situation is still the same, i.e., agriculture is the mainstay of the villages. Not only the economy, but also every one of us looks up to agriculture for our sustenance too.

### **New problem: Scarcity of land and water**

The global availability of arable land is decreasing and will further decline from currently 0.24 ha per capita to 0.17 ha in 2020. Most striking is the situation in Asia where, 20 years from now, only 800 m<sup>2</sup> per capita will be available for crop production. A similar trend is expected in India. The per capita land availability will decline from currently 0.14 ha to 0.10 ha in 2025 (Ramanathan, 2004). Moreover, "... the quality of land (in India) likely to remain available for agriculture due to severe competition from urbanization, industrialization and civic needs, will be poor..." Of the remaining land, 9% has limited nutrient retention capacity, 23% aluminum toxicity, 15% high P fixation and 26% low potassium reserves. What has been said for land availability also applies for water. Withdrawal of water in developing countries will increase by 43% between now and the year 2020, in developed countries by 22%. But, in developing countries, the demand for domestic and industrial uses will double, reducing the supply for agriculture. In consequence, horizontal expansion in food production is hardly possible unless further deforestation and use of marginal land is accepted. The necessary increase in production has to come through higher yields and denser cropping sequences, i.e. through higher productivity of the remaining land and water (i.e., improvement in crop production environment, Fig. 1).

The traditional arable **land management practices** were developed towards managing fields uniformly and have tended to ignore the inherent spatial variability found on most farms/fields. Since the mid 1980s, a host of terms have been used to describe the concept for new improved agriculture, including farming by foot, farming by soil, variable rate technology, spatially variable, precision, prescription or site - specific crop production and site - specific management. The latest definition of Pierce and Nowak (1999) is "Improved or Precised farming is the application of technologies and principles to manage spatial and temporal variability associated with all aspects of agricultural production for the purpose of improving crop performance and environmental quality". In fact, it must be associated with lower cost of production and/or higher returns (Fig.2).

### **The Issues of Homogeneity**

#### **a) Why ?**

The current debate on biodiversity and the underlying assumption that it is a desirable feature suggesting that the world, and perhaps especially agriculture, is heading rapidly for uniformity. Some of the discussion neglects the driving forces behind this desired uniformity, such as marketing and consumer pressures, but also tends to ignore the need to manage the diversity that does exist. Crop husbandry often seems to assume uniformity. Fertilizers are applied to whole fields; herbicides, fungicides and insecticides are applied to fields often on the basis of relatively causal observation, sampling, 'gut reaction' or tradition. For most purposes it is yield and quality that are the measures of the actions that have, or have not been taken.

These two measurements (yield & quality) integrate the interactions between potential productivity that determined by the genetic composition of the host as modified by Nature and man's actions to overcome those limitations. For protected crops some of the variables are controllable e.g. the growing medium, nutrient and water supply, and to some extent temperature,

light and gaseous environment. The protected environment also requires a wider range of approaches to crop protection than is usually possible in crops grown out of doors.

Environmental pressures to decrease wasteful pesticide use continue, and ethical considerations and concerns surround the introduction of transgenic crops and materials. Adhering to such requirements is likely to be increasingly required as a 'ticket to the market place'. Like it or not the market for production for its own sake has gone. A consequence of all these pressures needs to be more precise.

#### b) What?

Homogeneity in agriculture is concerned with the application of principles/ proven practices to manage spatial and temporal variability associated with all aspects of agricultural production for the purpose of improving crop performance and environmental quality (Pierce & Nowak, 1999). Thus, this is technology enabled, information based, and decision focused.

Statistically,

**HOMOGENEITY,  $H = 1 - SD$ ,**

where, SD is standard deviations;

If  $H = 1$ , then it is highly homogeneous field as  $SD = 0$  and

If  $H = 0$ , then it is a complex system describing maximum variability in field as  $SD = 1$  (Mandal and the Ghosh, 2000)

Thus, by maintaining homogeneity, we suppose to maintain the following features of crop production viz.,

- Accuracy, which is desirable, is heart of homogeneity, and is often dictated by the available tools.
- Small-scale spatial accuracy in agriculture is unnecessary as increased accuracy increases cost against benefits.
- Temporal accuracy improves continually.
- Most importantly, taking the correct decision (Fig.2)

#### Basic Components in homogeneity

Any farming system must first address the measurement and understanding of the variability. Next, this system must use information to manage the variability by matching inputs to conditions within fields using site-specific management recommendations and mechanisms to control the accuracy of site-specific inputs. Finally, this system must provide for the measurement and recording of the efficiency and efficacy of this site-specific practices in order to assess value on and off farm. Thus, this is technology enabled, information based and decision focused.

Homogeneity in experiments is primarily concerned with the improved management of the crop production system based on obtaining information, making decisions and acting on these decisions in an appropriate way. These concepts can explain the reasons for spatial and temporal variability in crop yield.

The introduction of yield monitors, yield mapping software, global positioning systems (GPS), satellite and aerial images and geographic information systems (GIS) has made it possible to measure crop growing conditions as well as grain yield within a field at a very high spatial resolution, allowing very fine and precise description of the spatial variability. If the causes of this spatial variability can be identified then corrective action may possibly increase yield and/or reduce environmental impacts.

Beside production, other notably cost/economics play vital for achieving the crucial homogeneity so far land management strategy is concerned. The theoretical flow of income over time under alternative management scenarios are illustrated in Fig 2 (Ramasamy, 2004). In the figure, OE is the income at present from the degraded land. Thus, opportunity cost of degradation in year 0 is ES. Four scenarios are considered in the conservation/management of (land) work. These are as under:

1. 'Do nothing' - if no maintenance or conservation of the land is undertaken, the flow of decline in net income is EA; at time T, the opportunity cost of degradation would be FS.

2. While with the soil conservation, but requiring additional maintenance, exhibit decline in level of net income, which will be EB.

**Fig.2 Flow of net income scenarios over time under alternate land management scenarios**

3. Area with soil conservation not requiring any significant additional work, reflect more sustainable level of net income. But over the time, there will be gradual decrease in net income as further land restoration becomes necessary.
4. At curve D, to reach a sustainable level of net income, all degradation is treated and management changes are undertaken to ensure stabilized net income flow ED. Here the annual price, yield and cost are constants.

Thus, the basic steps in homogeneity in agriculture are assessing variation, managing variation and evaluation. While the enabling technologies facilitate precision agriculture, it is the knowledge and understanding of variability and the extent that site - specific agronomic recommendations are viable to manage this variability.

**i) Assessing variability**

This is the critical first step in homogeneity agriculture since it is clear that one cannot manage what one does not know. The processes and properties that regulate crop performance and yield vary in space and time. Adequately quantifying the variability of these processes and properties and determining when and where different combinations are responsible for the spatial and temporal variations in crop yield is the challenge facing uniformity in agriculture. Variability can be assessed by using surveys, interpolation of a network of point samples, high resolution sensing and modeling to estimate spatial patterns. Interpolation of point samples is a technique for assessing spatial variability. A network of points in some spatial arrangement is sampled and then interpolated to produce a surface. Modeling is proposed as an important tool in precision agriculture to stimulate spatial and temporal variation in soil properties, crop yield and environmental performance of cropping systems.

Techniques for assessing temporal variations also exist (Shumway, 1988), but the simultaneous reporting on spatial and temporal variation is rare and the theory of these types of processes is still in its infancy. However, Variation must be in a manageable range. Maps form one basis for precision management; real time management forms the other basis. Use of management maps is more common and these can be categorized as condition maps, prescription maps and performance maps. The Condition maps are for real - time precise management. Prescription maps are derived from one or more condition maps and form basis for Variable rate technology/site specific management. Performance maps are the records of either inputs (fertilizers, pesticides, seeds, energy etc.) or outputs (crop yield and quality) and serve as condition maps. Yield goal maps are constructed from multi year yield maps. Condition maps are generated by a) Surveys (Spatial variability), b) Interpolation of point samples, c) High - resolution sensing (yield maps) and d) Modeling (simulate and predict variations)

**ii) Managing variability**

Once variation is adequately assessed, one must match agronomic inputs to known conditions employing management recommendations that are site - specific and use accurate application control equipments.

**Homogeneity in soil resources management**

These are categorized under the following broad heads.

- i) Land use according to land capability
- ii) Management of soil physical constraints
- iii) Management of soil fertility.
- iv) Avoiding soil pollution.

**i) Land use according to capability**

The primary concern of land use planning should be to increase continuously the productive capacity of land and prevent its deterioration. It is essential that land use planning be based on a resource survey production potential of the land. The cropping pattern should be

restructured according to land capability agro-climatic condition and hydrological characteristics. What is required is assessment of the potential and constraints. The factors determining land capability are the major soil characteristics of the land eg. texture of the soil, its effective depth, permeability of surface and subsurface soil, the nutrient capital of the soil and associated land features e.g. slope of the land, the extent of erosion, the degree of wetness and susceptibility of over flowing and flooding etc. Eight classes are recognized classes I to IV are arable. They are capable of producing common cultivated crops of the region under good management conditions. Soils in classes V to VII are suitable for pastures and forestry. Soils of class VIII are neither suitable for agriculture nor silviculture.

#### ii) Management of soil physical constraints

Improper tillage, excessive use of chemical fertilizer, cultivation of similar type of crops, excessive use of irrigation water and poor drainage conditions are responsible for deteriorations in the physical condition of the soil. Inclusion of deep rooted perennials, preferably leguminous crops in cereal based cropping system, recycling organic wastes, adopting proper tillage practices for different farming situations, addition of soil amendments and creation of good drainage facilities are some of the essential improved technologies for the management of physical condition of the soil.

#### iii) Maintaining homogeneity in soil fertility (avoiding soil heterogeneity)

The Nutrient input to crop production is important because soils naturally do not supply nutrient in sufficient quantities to meet nutrient demands of commercial crops eg. cotton. Soils vary in their ability to supply nutrients to plants and crops vary in their demand for nutrients. The fact that soil supply and plant demand vary in space and time and nutrient losses through leaching, erosion and runoff also vary temporally and spatially indicating that significant opportunities may exist for precision management of soil fertility. For successful implementation, the concept of avoiding soil heterogeneity requires data on in field variability with accurate identification and interpretation, then only the variability that influences crop yield, crop quality due to environments can be managed with inputs of accurate amount applied to that specific point/patch of the field.

The inadequate, imbalances, and no-integrated use of fertilizers not only reduced the yield of crops, but it results in poor response to applied fertilizer and causes multinutritional deficiencies in many field crops. In India, fertilizer consumption (65 Kg/ha) was lesser than even the average consumption per hectare for Asian countries (144.8 kg/ha). Within India, Punjab state with consumption of 158.6 kg/ha is at the top followed by AP (131.0 kg/ha), Tamil Nadu (119.7 kg/ha), Haryana (94.4 kg/ha) and UP (68.1 kg/ha). Eastern States, Rajasthan, MP, Maharashtra etc have fertilizer consumption lower than 50 kg/ha, Developed countries in Europe have more balanced use of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O (2:1:1). The ratio in India is 1:0.38:0.15. It has been observed that cereal based cropping system (rice- wheat) removing as high as 300-400 kg / nutrients / ha has resulted in decline in organic matter content of the soil. Further quality of fertilizer should be applied based on soil-test recommendation. Hence precise knowledge on when to apply, how much to apply and where to apply is essential for achieving homogeneity in fertilizer management. Other statistical tool for lowering soil heterogeneity is **Confounding** although modern statistical principles viz., Randomization, Replication and local control takes care of many factors.

#### Nitrogen

Both deficiency and excess N leads to problems in uniformity. Fertility concerns are focused on deficiency to plants excess and loss of NO<sub>3</sub>-N, leaching, run-off and denitrification. One of the typical management strategies is with the help of Leaf Colour Chart (LCC) Fig.3

#### A Case study for N management in rice through Leaf Colour Chart (LCC)

1. Measure the colour of the top most fully expanded and healthy leaf from 10 randomly selected plants. Do not detach the leaf.



2. Take LCC readings every week, starting from for transplanted rice after seeding for direct wet-seeded rice.
3. Apply the N fertilizer when leaf colour of more than five leaves is below the critical value
  - (i) Use critical value (CV) of 4 for transplanted rice.  
If CV <4, apply N @ 35 kg ha<sup>-1</sup> each time for dry season (Kuruvai) and @ 30 kg ha<sup>-1</sup> each time for wet-season (Thaladi/Samba)
  - (ii) Use critical value of 3 for direct wet-seeded rice.  
If CV <3 apply N @ 35 kg ha<sup>-1</sup> each time for dry season (Kuruvai) and @ 30 kg ha<sup>-1</sup> each time for wet-season (Samba)

#### **Fig.3 N nutrition in rice by leaf colour chart for better crop homogeneity**

Nutrient losses by erosion, leaching, run-off varies temporally and spatially. Phosphorus and potassium have generally low temporal variability in soil tests, but still have the role in soil variability (Fig.4). Uniformity management based on proven soil fertility management philosophy is the key. Management based on replacing nutrients removed by crops by variable rate nutrient management is also very important. **Site-specific nutrient management in cotton**

The key steps for working out site-specific fertilizer recommendation (Smith et al. 1998) are as under:

1. Estimation of crop nutrient demand for a specific yield target
2. Estimation of potential indigenous nutrient supply (INS)
3. Estimation of fate / relative efficiency of nutrient from applied fertilizer
4. Calculation of fertilizer rate as a function of steps 1-3

#### **Figure 4. Soil phosphorus as determined by sampling and interpolation.**

Higher use of soil applied pesticides affect the population of beneficial microflora and fauna. This can be overcome by integrated pesticide management systems. The sewage water charged with Industrial effluents not only caused heavy metal pollution but decreased hydraulic conductivity of the soil. Therefore this water should only be used after giving proper treatments.

#### **Uniformity in Water Management**

Water is critical to crop productivity since yields generally increase linearly with water transpired by a crop. Excess water can induce nutrient and aeration stresses and encourage pests that reduce yield and quality. Three following approaches to precision water management are therefore apparent.

- i) Uniformity in irrigation rate (temporal variability reduced)
- ii) Soil-landscape irrigation (reducing spatial variability)
- iii) Drainage requirements (Provision of desired drainage)

#### **Management of climatic resource**

Climate resource accounts for 85% variation in crop yield. Climate determines the choice of the crop. Length of the growing period is primarily determined by weather elements and has little dependence on crop type. Length of growing season shows large fluctuations particularly in semi-arid tropics. In such environment, the crop genotypes with growth period matching with the length of the growing season are to be adopted for better use of resources. In the climatic resource, the precision farming technology viz., time of sowing, had profound influence on both rainfed and irrigated crops. In rain fed crop, time of sowing can be achieved by pre-monsoon sowing. The aberrant weather condition can be overcome by precised contingent crop planning and midterm corrections.

#### **Stability of crops and cropping system**

Crop diversification has been adopted for long to avoid total crop failure due to uncertain rain and sudden epidemic of pests and diseases. Improved cropping system developed to replace the existing one takes this biological stability as an important component before recommending the wide scale adoption of improved system. Apart from this, the economic stability is also an important criterion.

Besides these, uniform application of capital, labour and other intercultural /weed/pest management strategy is important in keeping desired homogeneity. Thus, all these factors contribute in making decision in agriculture (Fig.5)

Therefore, the concept of homogeneity in soil fertility management requires that within field, variability exists and is accurately identified and reliability interpreted. The higher the spatial dependence of a manageable soil property, the higher the potential for precision management and the greater its potential value. Hence, P and K fertility are very conducive to uniformity management because temporal variability is low. For N, temporal component of variability can be larger than its spatial component (Pan et al., 1997),

#### **Fig.5 Factors influencing decision, making in agriculture (Plumb, 1996)**

Making homogeneity in N management much more difficult in some cases. Weeds, insects and diseases are ever present and costly management problems to crop production. Here also, pest management is more important as public is concerned regarding the impact of pesticide use, that include health risks related to food safety, water quality, worker safety, wild life and ecosystem health. Therefore, agricultural management practices that reduces pesticide use, improve pest management or reduce risks of pesticides to human and ecosystem health are very desirable.

#### **Agronomic factors influencing homogeneity**

The major factors of crop production influencing homogeneity are described here as under the following heads and the effects of these are self- interpretative & explained before.

- ❖ Selection of site (spectral responses- Fig.6 below)
- ❖ Soil - fertility & productivity
- ❖ Optimum density
- ❖ Crop / species / variety
- ❖ Time of planting - TEMPORAL
- ❖ Plant rectangularity- SPATIAL
- ❖ Proper weed management
- ❖ Proper water management
- ❖ Proper nutrient management-N,P,K, Secondary, Micro and biofertilizer /organics
- ❖ Cropping system approaches
- ❖ Harvest parameters / criteria

#### **Importance of Crop Geometry**

Crop geometry can be most easily visualized in a row crop where it can be defined as the ratio of the distance between plants within the row to the distance in the row. Uneven crop geometry leads to the unevenness of competition. Competition may be too intensive between some plants and insufficiently intense between others. Better crop geometry can be achieved by using precise farming tools.

#### **Factors influencing optimum density and uniformity in plant population**

##### **Size of the plant**

The spread or the volume occupied by the plant at the end of log phase or at the time of flowering has influence on the spacing to be adopted for these crops. Plants of red gram, cotton, sugarcane etc. occupy larger volume of space in the field compared to plants of wheat, rice, finger millet etc. Even the varieties of the same crop differ in the size of the plant. In red gram, the cultivar LRG-30 grows to a height of 1.5 to 2.0 m with a horizontal spread or 1.0 to 1.5m while the average height and spread of ICPL 87 is 70 and 30cm respectively (Gnanamoorthy et al., 2004). Similarly in cotton, some bushy cultivar of arboreum cotton (Desi) has relatively less spread over that in hirsutum /barbadense cotton.

##### **Elasticity of the plant**

Variation in size of plant between the minimum size of the plant that can produce some economic yield to be the maximum size of the plant can reach under unlimited space and resources is the elasticity of the plant.LRG-30 red gram can produce a few pods when the plant

attains a minimum size of 20g dry weight but it can attain a size to produce a dry weight of 2000g per plant. Similarly, American or hybrid cotton has higher number of bolls over that in Desi counterparts. Instead of the weight of the plant, it is more meaningful to consider elasticity in the number of estimated by coefficient of variation (CV). The higher the CV, more is the elasticity of the plants. The elasticity of red gram for branching and number of pods are 30 and 80 per cent respectively. Elasticity of growth and yield characters of plant population range is quite high for indeterminate crops. For the indeterminate red gram varieties, the optimum population ranges 55 to 133 thousand plants ha<sup>-1</sup>. The elasticity of plant is due to branching or tillering. For determinate plants, the elasticity is less and the optimum plant population range is small as in maize, sorghum etc.

#### **Methods of planting**

Planting pattern influence the crop growth through influence on light interception, rooting pattern, moisture extraction pattern, logically and reasonably square planting will be more efficient in getting nutrient, moisture and light than rectangular planting. In wheat, reducing the rectangularity increased the yield. But in tobacco square planting is advantageous for inter cultivation (possible on both direction). At the same time, square planting is not ideal for all crops. Groundnut at 30x 10 cm spacing gives more yield than at square planting at same population. Rectangular planting is inevitable where implements are inevitable eg.Cotton. Drill sowing has to have more space i.e. rectangularity.

#### **Sowing direction**

Planting orientation is that the plants emerging in a sight trench left from the narrow points in the east - west orientation received less sunlight during the early stages of emergences. Plants sown in the east- west orientation appeared to till to the north as if to catch more sun, but this may also have been due to the prevailing wind direction. In North India, for e.g. North-south/ Bi-directional planting gives higher yield in wheat over that in east west planting because of better illumination, but not in South India (where light is not limiting).

#### **Fertilizer and fertilizer application**

Dense plant stand is necessary to fully utilize higher level of nutrients in the soil to realize potential yields. Nutrient uptake increases with increase in plant population. Higher plant population under low fertility conditions leads to development of nutrient deficiency symptoms. In this situation, identify which nutrient causes the deficiency symptoms. We can assess through sensor and corrective measure should be taken. Based on that, fertilizer can be applied only where symptom is occurred.

#### **Yield of individual plants and community**

The full yield potential of individual plant is achieved when sown at wider spacing. When sown densely, competition occurs among the plants. Yield per plant decreases gradually as plant population per unit area is increased due to efficient utilization of growth factors resulting in size and yield of the plant.

#### **Plant population and growth characters**

Higher plant density brings out certain modifications in the growth of plants. Plant height increases with increase in plant population due to competition for light. Sometimes it may happen that moderate increase on plant population may not increase but decrease plant height due to competition for water and nutrient but not light. Increase in plant height due to higher plant population is advantageous for better light interception due to exposure of individual leaves at vertical interval. Another adaptation of dense plant stands is reduction in leaf thickness. Leaf orientation is also altered due to population pressure. The leaves are effect, narrow and are arranged at longer vertical intervals under high plant densities. This is desirable architecture to intercept more light. Dry matter production per unit land area increases with increase in plant population up to a limit when the reduction in the growth of plant is more than compensated by increase in the number of plants per unit area.

### **Plant population and yield components and yield**

Decrease in the yield of individual plants at higher plant density is due to reduction in the number of ears in indeterminate plants. In determinate plants, wherein the terminal bud ends in a flower or inflorescence, the reduction in yield is mainly due to the reduced size of ears or panicles. Highly branching or tillering plants behaves as indeterminate plants are yield reduction is due to reduction in the number of seeds .Red

gram produces about 20 pods per plant at 3.33 lakh ha<sup>-1</sup> while it produces more than 100 pods per plant at 50,000 plants ha<sup>-1</sup>. Similar example can be cited for cotton also. Conversely, non-tillering or non-branching plants produce lesser yield due to reduction in size of ears as in the case of maize and sorghum (Yellamanda reddy and Sankara reddy, 1999).

### **Evaluation of Heterogeneity in Agriculture**

Technologies feasible and possible based on sound scientific principles do not necessarily establish utility or value in the process, evaluated on economics, environment and technology transfer. With regard to evaluation, it is essential that evaluation procedures are consistent with the emerging features of scientific agriculture, while not relying solely on the traditional approach used for agricultural machinery, genetics and chemicals. The person concerned/farmer is the integral part of evaluation because the assessed variability must ultimately be managed on the farm. The impacts of uniform agricultural systems will extend beyond crop production to the environment and to the very structure of our agriculture system. Consequently, evaluation needs to involve all sectors of agriculture.

### **Maintaining uniformity field trials (reducing yield variations)**

To sum up, the key components in maintaining uniformity in field trials involving crop production revolves the following broad agricultural management/practices.

#### **1. Land Management**

- Tillage depth and type
- Residue management and organic matter
- Soil compaction and reduction
- pH corrections

#### **2. Soil compaction**

- Subsoil loosening (11% yield increase in many crops)
- Amelioration (Organic matter and nutrients)

#### **3. Available water**

- Ameliorating compaction (deep rooting) and more moisture retention
- Patches of less organic matter (mad subsequent organics application)
- Variation in soil texture - sandy patches (monitors moisture and irrigate at critical stage)
- Monitor the input application to sustain potential crop yield.

#### **4. Water logging**

- Slowly permeable pan (sub soiling or shallow loosen is desirable)
- If no pan, (draining selected areas using mole drainage or mole line system)

#### **5. Crop establishment/cultivar selection**

- Planting depth and population/crop geometry
- Planting date and rotation

#### **6. Soil nutrients and pH**

- Imbalances of nutrients and pH impede crop development
- Guided yield maps and soil & plant analysis
- Fertilizer application (site specific response curve, crop sap analysis, LCC)
- For pH identify areas and ameliorate

#### **7. Crop protection/weed management using sensors (patch spraying using handheld global positioning system)**

- Selection of best fungicide and pesticides
- Optimal rate and method of application (Optimized treatment maps)

## 8. Crop Harvest

Dates and moisture, quality of crops

### Applications of Results of Homogeneity experimentation

There are various applications of homogeneity in experiment trials.

The important ones are as under:

Better interpretation and reliable results

- i) Ease in application of inputs including fertilizers / irrigation / pesticides
- ii) Better growth and development of crop
- iii) Yield monitoring
- iv) Yield mapping
- v) Weed mapping
- vi) Uniform spray scheduling
- vii) Topography and boundaries consideration
- viii) Salinity mapping
- ix) Guidance systems
- x) Records and analyses.

### **Good Agricultural Practice (GAPs)**

In the context of agreed international goals to reduce hunger and promote food security, four principles of Good Agricultural Practice (Vadivel, 2004) apply to all scales of farming:

- 1) Economically and efficiently produce sufficient, safe and nutritious food;
- 2) Sustain and enhance the natural resource base;
- 3) Maintain viable farming enterprises and contribute to sustainable livelihoods;
- 4) Meet the cultural and social demands of society.

GAP provides a means to assess and decide on farming practices at step in the production process. For any given agricultural production system, a sound and comprehensive management strategy must be in place providing for the capability for tactical adjustments in response to changes in circumstances. Implementing such a management strategy requires knowing, understanding, planning, measuring, monitoring, and record keeping, with the aim of achieving production, safety and sustainability goals. Successful implementation depends upon developing the skill and knowledge bases, on continuous monitoring and analysis of performance, and the use of expert advice as required.

### **A Case study on homogeneity (Optimum plot size)**

The choice for optimum plot size has long been recognized important for efficient experimentation in the field. The relation between soil variability with plot size helps in determining the plot size. The relationship can be obtained either by the conduct of uniformity trials or by utilizing past available secondary data on soil variability and plot size from earlier conducted experiments (at least for 3 years).

Under AICWIMP (wheat), every year about 350 field trials are being conducted for the study of varietal performance at about 90 centers covering six zones of India. The results of these data collected for the last 14 years (1988-2001) on soil variability and plot size, generated from Coordinated trials (DWR, Karnal 1988-32001) revealed the relationship between soil variability (i.e.. Coefficient of variation, CV) and plot size and is explained by Smith model

$$y = a * x^{-b}$$

Where y=average soil variability, x = plot size, a= constant and b = soil heterogeneity index (Smith, 1938).

The number of replication that is appropriate for any field experiment is affected by soil heterogeneity and degree of precision desired. It is estimated by

$$r = V_1 / (p * X^{-b})$$

Where,  $V_1$  is the variance between plots of basic unit size, p is the required precision for plot size x and b is the soil heterogeneity index.

The regression model was fitted using data of 10 different plot sizes against their respective  $CV_s$ . The model obtained was  $y = 55.17 * x^{-0.8761}$  and the plotted regression is given below in FIG. 1

This curve reveals CV falls from 30 to 10 % with increase in plot size from 2 to 8 m<sup>2</sup>. Increase in plot size up to 12 m<sup>2</sup>, the reduction in CV was marginal i.e., from 10 to 8 % only and thereafter CV is stabilized. This may be concluded that a plot size of 8-12 m<sup>2</sup> may be considered optimum for conduction field trials with wheat crop, which provides high degree of uniformity and precision. Moreover, for a plot size of more than 8 m<sup>2</sup>, the number of replication required varied from 3 to 5 at 10 % precision level.(Singh and Lata, 2004).

**Applicability of the case study:** Since the data on CV for different plot sizes were averaged out over locations, the interactions of soil variability over location reduced to greater extent. So, these findings will hold good irrespective of locations.

### Conclusion

Applications of agricultural inputs at uniform rates across the field without due regard to in-field variations in soil fertility and crop conditions does not yield desirable results in terms of crop yield.

The management of in-field variability in soil fertility and crop conditions for improving the crop production and minimizing the environmental impact is the crux of homogeneity in agricultural experimentation.

### Literatures cited

- Gnanmoorthy, P., Vaiyapuri K. and Marimuthu, S. Importance of crop geometry for efficient resource utilization. In: *Precision farming*. (Ed) Muthukrishnan P, Chinnusamy, C. and Chinnamuthu, C.R., pp.122- 124. Center of Advanced Studies, TNAU, Coimbatore (T.N.), India.
- Mandal. D and S.K.Ghash 2000. Precision farming - The emerging concept of agriculture for today and tomorrow. *Current Science*. **79**. 1644- 1647.
- Pan, W.L., Huggins, D.R. Malzer, G.L., Douglas, C.L., Jr. and Smith, J.L.1997. Field heterogeneity in soil - plant nitrogen relationships: Implications for site - specific management. In: *The State of Site - Specific Management for Agriculture* (F.J. Pierce and E.J. Sadler, Eds.)ASA Miscellaneous Publication, pp. 8 1 - 99. ASA, CSSA, and SSSA, Madison, WI.
- Pierce, F.J. and Nowak, P. 1999. Aspects of Precision Agriculture. *Advances in Agronomy*., 67, 1 - 85.
- Plumb, R.T. 1996. Issues and opportunities for precision approaches to crop management. Brighton Crop Prot. Conf., - Pests and Diseases. 1996. pp.1097 - 1104.
- Ramanathan, S. 2004. Precision farming in Indian agricultural scenario. In :*Precision farming*.(Ed) Muthukrishnan, P, Chinnusamy, C. and Chinnamuthu, C.R., pp. 1-9. Centre of Advanced Studies, TNAU, Coimbatore (T.N.), India.
- Ramasamy, C. 2004. Precision farming - applications for farm planning and natural resource management. In: *Precision farming*(Ed) Muthukrishnan, P, Chinnusamy C. and Chinnamuthu, C.R., pp.122-124. Center of Advanced Studies, TNAU, Coimbatore (T.N.), India.
- Shumway, R.H. 1988. Applied Statistical Time Series Analysis. Prentice - Hall, Englewood Cliffs, N.J.
- Smith, S.A., Essington, M.E., Howard, D.D., Tyler, D.D., and Wilkerson, J. 1998. Site- specific nutrient management: Variability in cotton yields response and soil chemical characteristics. *Better Crops* **82**, 12 - 14.
- Vadivel, E. 2004. Good Agricultural Practices In: *Precision farming*. (Ed) Muthukrishnan, P, Chinnusamy, C. and Chinnamuthu, C.R., pp.174- 182. Center of Advanced Studies, TNAU, Coimbatore (T.N.), India
- Verhagen.A,H.W.G.Bootlink and J.Bouma. 1995. Site-Specific management: Balancing Production and environmental requirements at farm level. *Agric. System*. **49**:369-384.
- Yellamanda Reddy, T and G.H.Sankara Reddi. (1999).Principles of Agronomy.Kalyani Publishers, New Delhi.

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## Water and Weed Management in Cotton

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India Ranks first in acreage with about 20 per cent of the world cotton growing area but contributes only 12 per cent to the global cotton production. The most limiting agronomic factor for lower production level is "Water" and the 70 percent of our cotton area is under rain fed. Hence, there is ample scope for enhancing the cotton production substantially by adopting the scientific water management techniques.

Next to water, the most important agronomic factor governing cotton production is weed management. Cotton is very sensitive to weed competition due to its slow initial growth as well as wider row spacing gives greater chance for severe weed infestation. Yield loss due to uncontrolled weed infestation was reported to range between 50 - 85 per cent (Joshi, 1997).

### Water Management in Cotton

#### Irrigation scheduling

This depends on the soil moisture storage, climate and stage of growth of the crop and as such varies from place to place. Depending on climate and total growing period, cotton needs 700 to 1200 mm of water to meet its maximum water requirement. In early vegetative period of crop (up to 60 DAS) water requirement is only 30% but during flowering and boll development, it is about 60%. The experiments conducted at different parts of our country indicated that the cotton crop needs to be irrigated at 50% to 75% depletion of available soil moisture.

#### Critical stage approach of irrigation scheduling:

For Every crop, there are some growth stages known as "Critical" or moisture sensitive periods. In these stages, any moisture stress leads to irrecoverable yield loss. When irrigation water is available in sufficient quantity, irrigation is scheduled when soil moisture is depleted to critical moisture level. If the water supply is limited, irrigations are to be supplied compulsorily at the most moisture - sensitive stages. In cotton, the flowering and boll formation stages are most sensitive for moisture stress and any moisture stress during such critical stages results in premature boll shedding, poor boll development, and low yield. It is proved that for cotton, commencement of sympodial branches (9- 10 weeks) flowering (14- 15 weeks, boll formation (18 weeks) and bursting are distinctly critical stages for moisture in cotton.

#### Common methods of irrigation

##### Check basin method

In this method, the size of the basin is about 2m X 2m to 4m X 4m or it may be rectangular. Accurate leveling is not necessary. For small streams, this method can be suitable.

##### Furrow irrigation

This method is most common for cotton in south zone. It is most suitable to deep soils (Clay loam and loam) with nearly level or moderate slope. Furrow irrigation is not suitable for very coarse sandy soil.

##### Alternate furrow irrigation

In this method, crop is planted just like in the conventional method and there is no variation in spacing but variation in water application. Here water is applied at every given irrigation in alternate rather than in all furrows (irrigating odd and even furrows alternatively)

##### Skip Furrow/pair Row Irrigation

Skip furrow irrigation is a modified method of furrow irrigation. In this method, the distance between the two rows of cotton is 60 cm and the gap adjacent to the rows is 90 cm (if the normal row space is 75 cm). The space available in between parts of rows in skip furrow can be intercropped with pulses. If the conventional row spacing is 90cm (as in the case of hybrid), the distance between two rows planted in skip furrow / Pair row is 60 cm and the adjacent to the furrow is 120 Cm.

## **Advanced Irrigation Methods**

### **Drip Irrigation**

In this method, water is applied at low rate over a long period of time and directly into the plant root zone so as to keep the soil at or nearer to field capacity. Drip irrigation has proved to be an efficient and economical method in many developed countries. However, in India drip irrigation is practiced mainly for plantation and orchards crops and has potential to be used for cotton crop for the saving of irrigation water.

The experiments conducted at Central Institute for Regional Station, Coimbatore revealed that the increase in seed cotton yield due to drip fertigation was 34.5% over conventional irrigation and the water use efficiency for drip irrigation during summer season at Coimbatore ranged from 16.3 to 35 kg/ha cm as against 4.9 to 8.3 kg/ha cm for flood irrigation. (Nalayini and Shanmugham, 2002).

In another experiment conducted recently under Technology Mission on cotton, Scheduling of irrigation through drip at 0.8 Etc was on par with 1.0 Etc but found significantly superior to conventional irrigation. The yield increase due to drip irrigation ranged from 28.9 to 61.5% than conventional furrow irrigation (Anon, 2002-03).

### **Advantages of Drip Irrigation**

1. Due to drip system, saving of water by 50 to 75%. The saved water can be utilized for bringing additional area under irrigation.
2. Fertilizer can be precisely applied at the root zone. There by wastage is minimized and saving of 25% fertilizer than conventional method.
3. The intensity of weed competition is lesser than conventional method.
4. Uniform germination, early vigour and yield enhancement than conventional irrigation.

### **Sprinkler Irrigation**

This method is specially suited to shallow soils of uneven topography, where leveling is not practicable and in areas where labour and water are scarce. This method is advantages compared to the surface methods as water can be applied at any controlled rate and a uniform distribution with high efficiency can be ensured. It is very popular in advanced countries and it is not extensively used in our country due to initial high investment. This method can be used for all soils except very fine texture soil.

### **Weed Management in Cotton**

The cotton crop is widely spaced with slow initial growth and these factors are favourably utilized by weeds to dominate the crop during early days.

### **Critical Period of Crop-Weed Competition**

Crop yield levels obtained by managing the weeds during this period should provide crop yields close to those obtained in weed free yield.

The critical period of weed control in cotton is between 20 and 60 DAS. The weeds emerging after 60 DAS will not cause significant yield loss but for having cleaner harvest, the field may be kept weed free depending upon the availability of labour.

### **Weed control methods**

#### **Manual weed control**

Manual weed control is an efficient method, though it is laborious and expensive. If labour is available in plenty and the weather condition permit to go for manual weed control, it is considered to be very efficient than chemical method as it does not only remove weeds, but associated benefit like better aeration to crop growth is ensured.

#### **Chemical weed control**

#### **Pre-emergence weed control**

Pre-emergence herbicides are applied before the crop or weeds have emerged, In annual crops, this is normally done after planting the crop, but before the emergence of weeds. Since the advent of herbicide molecule, many selective herbicides are successfully field tested to

kill the germinating weeds selectively without affecting the cotton crop. Herbicides like fluchloralin 1 kg (pre plant incorporation), Pendimethalin 1 to 1.5 kg pre-emergence spray on third day offer better weed control upto one month.

Recently conducted AICCIP experiment revealed that the ready mix of pendimethalin + clomazone, marketed as galaxy at 2 lit/ha controlled broad leaved weeds as well as grassy weeds in cotton, (AICCIP, 2003)

#### Post-emergence weed control

Pre-emergence herbicide offers weed control only for a limited period and hence the late emerging weeds escape from killing. Most of the annual grasses and small seeded broadleaved weeds can be effectively controlled by many of the presently available herbicides. However, perennial weeds are not effectively controlled and needs alternate method of weed control.

Cotton being a widely spaced crop, there is lot of scope to use non-selective herbicides for the effective control of established weeds especially the perennial weeds like *Cynodon dactylon* and *Cyperus rotundas* which are difficult to control by other means. Directed application of glyphosate at 1 kg ai/ha can be safely made between crop rows with plastic hood (shield) to prevent contact of glyphosate with the crop.

#### Biological method

Weed smothering nature of some of the intercrop can reduce the weed population appreciably when compared to sole crop. In cotton, by adjusting the geometry through paired row technique, intercrops like cowpea, green gram, black gram can be grown between two paired rows to smother the weeds during critical period of weed crop competition.

#### Integrated weed Management

Integrated approach of weed management with chemical + mechanical + cultural means will be more effective and economical in sustainable cotton production system.

#### Polyethylene Mulching - a new tool for efficient water and weed management

Mulching has been practiced in India since long time using mainly the crop residues like straw, thrash, leaves etc., But of late plastic mulches have come into use for efficient moisture conservation and weed suppression. At CICR, Regional Station, Coimbatore, the technique of growing cotton under polyethylene mulching has been standardized (Nalayini et al., 2004).

The water requirement of poly mulched cotton was 52.46 ha cm as compared to 88.46 ha cm for non-mulched bed planting, but still the polymulched cotton maintained higher available soil moisture and the water use efficiency under polyethylene mulching was 38.39 kg seed cotton /ha cm of water as against 10.07 kg seed cotton recorded with non-mulch.

Polymulching maintained weed free cotton upto harvest and the favourable microclimate under polyethylene mulching was reflected in yield attributes like more number of heavier bolls and finally the enhanced yield to the tune of 2.32 fold (Table 1) than normal planting (Nalayini et al, 2004.)The seed index and lint index also enhanced numerically due to polymulching.

**Table 1. Yield attributes, seed cotton, yield, seed index, lint index of cotton cv LRA 5166 due to polyethylene mulching.**

Treatments	Bolls/plant	Boll wt.g/boll	Sympodia / plant	Seed cotton yield kg /ha	Seed index	Lint Index
T1-100 micron	26.9	3.92	18.1	2039	9.17	4.67
T2-75 micron	6.2	4.10	18.2	2113	8.97	4.63
T3-50 micron	25.8	4.05	18.1	2104	8.67	4.57
T4-30 micron	23.7	3.94	18.0	2010	9.17	4.63
<b>Mean for Mulch</b>	<b>25.65</b>	<b>4.00</b>	<b>18.1</b>	<b>2067</b>	<b>9.00</b>	<b>4.63</b>
T-5 Control	15.1	3.50	15.9	890.7	8.93	4.53
SEd	2.12	0.17	0.79	123.4	0.23	0.20
CD (p=0.05)	4.89	0.40	1.82	284.4	NS	NS

The fibre quality parameters like 2.57 span length and fibre quality index (fibre length x fibre strength /  $\sqrt{\text{micronaire}}$ ) were influenced significantly due to polymulching.

**Table2. Fibre quality parameters of cotton cv LAR 5166 due to polyethylene mulching.**

Treatments	2.5% span length	FS (g/tex)	Micronaire	FQI	Ginning %
T1-100 micron	29.46	23.8	3.78	361.2	34.0
T2-75 micron	29.93	24.4	3.89	370.4	35.0
T3-50 micron	29.43	24.2	3.88	361.3	24.7
T4-30 micron	30.00	23.9	3.74	371.3	34.7
<b>Mean for mulch</b>	<b>29.71</b>	<b>24.1</b>	<b>3.82</b>	<b>366.1</b>	<b>34.6</b>
T5-Control	27.76	23.6	3.77	338.5	33.3
SEd	0.34	0.55	0.12	9.65	0.60
CD (p=0.05)	0.78	NS	NS	22.24	NS

### Advantages of Polyethylene Mulching

1. Better retention of soil moisture.
2. Complete control of evaporation.
3. Cuts down the water requirement.
4. Opaque mulches prevent weed growth.
5. Prevents Stalination.
6. Higher soil temperature for faster mineralization.
7. Encourages plant growth promoting rhizobacteria.
8. Pest and disease control.
9. Improve crop production efficiency.

### References

- AICCIP - 2003-04, AICCIP Annual report 2003-04, All India Coordinated cotton improvement project, Coimbatore.
- Anon. 2003 -03, CICR Annual report 2002 - 2003, Central Institute for Cotton Research pp, 144.
- Nalayini. P and Shanmugam.K.2002. Efficiency of drip irrigation for summer cotton (Gossypium spp.) In: Second International Agronomy Congress, Nov 26- 30, 2002, New Delhi (Extended Summaries Vol. 2) pp, 1343 - 13144.
- Nalayini.p, Rajendran .T.P, Anandhan. R, Sankaranarayanan. K and Anderson A, Kumar. 2004. Polyethylene mulching - A new technique for better water management and efficient weed control in cotton. In International Symposium on 'Strategies for Sustainable Cotton Production - A Global Vision'. 2 crop Production, 23 - 25 November 2004, University of Agriculture Sciences, Dharwad, Karnataka.

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## Management of Cotton Diseases in DUS Test Experimental Plots

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Cotton (*Gossypium* spp.) known as 'white gold' is an important commercial crop of the world. There are four cultivated species viz, *G. herbaceum*, *G. arboretum*, *G. hirsutum* and *G. barbadense*. India is the only country where all the above four species are being cultivated. Cotton is being grown over 32.6 million hectares in the world producing about 20.6 million tonnes of lint (ICAC recorder 2004).

Cotton is a crop of warm climate and requires regular supply of water, either natural in the form of rainfall or assured, through canals from the above surface and/or from under ground sources. Although cotton is not a water loving plant, it requires a regular supply of water for maintaining growth and balance between vegetative and reproductive phase. Water stressed seed or plant, will have poor growth leading to low yield as well as exposure to diseases. About 55% of the world cotton area is under irrigation and the balance is rainfed.

Although India occupies first place in area with 8.0 million hectares, its productivity is only 376 kg/ha compared to the world average of 642 kg/ha (ICAC recorder 2004). This is mainly because 70% of the cotton is cultivated under rainfed conditions. In India, the cotton is being grown in three distinct zones viz., North (Punjab, Haryana and Rajasthan), Central (Gujarat, Maharashtra and Madhya Pradesh) and South (Orissa, Andra Pradesh, Karnataka and Tamil Nadu).

In the North, Cotton is sown during April-May, in Central June-July and in South July-September. In Tamil Nadu, the summer cotton as well as rice fallow cotton are sown during January-February. In view of the differing agro-climatic conditions, the prevalence of diseases also varies. Only few diseases are common for all the regions and the rest are specific to individual zones.

### Diseases of cotton

The cotton crop is affected by various diseases caused by organisms such as fungi, bacteria and viruses that grow on and with in the plant tissues. These organisms often cause stunting of the plants, defoliation, reduced vigour and yield and sometimes death. Seeds and seedlings attacked by these pathogens often die, while older plants usually survive but perform poorly.

Diseases can also be caused by environmental changes such as too much or too little of water or fertilizer, air pollutants and chemical injury such as those caused by herbicides and their residues. The diseases caused due to environmental changes become localized and do not spread where as diseases caused by organisms are contagious and can be spread by wind, water or vectors. We discuss here the diseases of cotton caused by various organisms (Table 1) and their management through adoption of various modern techniques.

### Seedling diseases

Seedling diseases cause an estimated average annual yield loss of about five per cent. Several fungi are responsible for this disease. However, cultural and environmental factors that delay seed germination and seedling growth may predispose the seed and seedlings to diseases (Koening, 2004). Seedling diseases occur more frequently under cool, wet conditions and seem to be more prevalent on sandy soils with low-organic-matter soils and other factors such as planting too deep, poor seed bed conditions, compacted soil and nematode or insect infestations (Heydari and Misaghi, 2004) may increase the problem.

Several species of fungi can cause seedling diseases, but the primary agents are *Rhizoctonia solani*, *Rhizoctonia bataticola* (*Macrophomina phaseolina*) *Pythium* spp., *Phoma exigua* (*Ascochyta*), and *Fusarium* spp. These disease-causing organisms can attack the seed before or at germination. They also can attack the young seedling before or after emergence. Symptoms include seed decay, decay of the seedling before emergence, partial or complete girdling of the emerged seedling stems, and seedling root rot. A soft and watery rot characterizes seed and seedling diseases. Damaged seedlings that emerge are pale, stunted, slower growing and sometimes die within a few days. Examination of infected seedlings may reveal dark lesions on the stem and root. Often the taproot is destroyed, and only shallow-growing lateral roots remain to support the plant. The "sore shin" phase of seedling disease is characterized by reddish brown, sunken lesions at or below ground level. These lesions enlarge, girdle the stem and cause it to shrivel. Seedling diseases do not usually kill the entire seedling population, but rather result in uneven, slow-growing stands with gappiness in the rows necessitating replanting. The most common fungi associated with seedling diseases are *Pythium* spp. and *R. solani*. Often both fungi can be found on the same seedling. The same fungus may cause seed decay, seedling root rot or both. However, *Pythium* spp. and *Fusarium* spp. usually attack the seed and below-ground parts of young seedlings, while *R. solani* usually causes sore shin. *Rhizoctonia solani* and *P. exigua* may attack seedlings from the time they emerge until they are about six inches tall. After this stage, the stem becomes woody and subsequent infection rarely occurs unless the stem is injured (Koenig, 2000). The herbicides pendimethalin (Stomp) and prometryn (Gesagard, Prometrix) that are currently being used on cotton may cause significant increase in the incidence of *R. solani*-induced cotton seedling damping-off in the field (Heydari and Misaghi, 2004).

#### **Foliar diseases**

##### **Bacterial leaf blight (*Xanthomonas axonopodis* pv *malvacearum*)**

Dark green, watersoaked, angular lesions of 1 to 5 mm across the leaves and bracts, especially on the undersurface of leaves. Hence called angular leaf spot. Sometimes extensive dark green, watersoaked lesions along the veins known as vein blight. Symptoms are usually more prevalent on lower leaves than on upper leaves. Lesions dry and darken with age and leaves may be shed prematurely resulting in extensive defoliation. Black lesions on the stem which girdle and spread along the stem or branch known as black arm. Dark green, watersoaked, greasy, circular lesions of 2 to 10mm across the bolls, especially at the base of the boll under the calyx crown. As the boll matures the lesions dry out and prevent normal boll opening.

Pathogen inoculum either may be present in the field on infected crop residues from a previous season or it may be introduced at planting within infected seed. Lesions on cotyledons may be initiated by inoculum within the seed during germination. Inoculum from infected crop residues may be splashed onto the foliage and into the growing point of young seedlings where it can survive saprophytically on leaf surfaces. When environmental conditions are favourable the bacteria enter the plant via the stomata or wounds. Symptoms of bacterial blight develop when the temperature is over 25°C and relative humidity exceeds 85%. As lesions develop, bacteria exude out onto the leaf surface for further dispersal through wind driven rain. The pathogen is able to enter the seed when mature, open, blight-infected bolls are exposed to wet weather prior to harvest.

##### **Alternate leaf spot (*Alternaria macrospora*, *A. alternata*)**

*Alternaria macrospora* causes brown, grey brown or tan lesions, 3- 10mm in diameter, especially on lower leaves. Sometimes with dark or purple margins and with concentric zones. The environment is most favourable within the crop canopy and therefore *Alternaria* leaf spot should be most severe on lower leaves and least severe on the upper leaves unless the upper leaves have been affected by premature senescence). Plants with a high boll load are more susceptible than plants with a low boll load. When a susceptible crop is exposed to a favourable environment, defoliation occurs rapidly. Affected leaves develop an abscission layer, senesce



and drop to the ground. Circular dry brown lesions up to 10mm across may also be seen on the bolls. *A. alterate* causes usually purple specks or small lesions with purple margins on leaves and bolls. Epidemic development is therefore either favoured by repeated heavy dews or extended periods of wet weather. Under ideal conditions, the pathogen kills the surrounding leaf tissue and produces more spores on the surface of the lesions within a few days. Numerous spores are produced on defoliated leaves on the ground under the crop.

#### **Grey mildew (*Ramularia areola*)**

The disease generally appears on older leaves as the plants reach maturity, in the form of irregularly angular, pale translucent spots, 1- 10mm (usually 3-4 mm) in diameter and with a definite and irregular margin formed by the veins of the leaf (called areolate). The lesions are light to yellowish green on the upper surface. As the spots grow older, the leaf tissues turn yellowish brown while a whitish frosty growth appears chiefly on the under surface but occasionally also on the upper surface. This is the conidial stage of the causal fungus. Lesions occur on the bracts subtending the bolls. As the leaf becomes chlorotic, the lesion turns reddish brown and defoliation takes place. Early and severe defoliation leads to premature boll opening and immature lint (Srinivasan, 1994, Hillocks, 1992 and Watkins, 1981). The consistent association of *A. macrospora* leaf spot with conditions less than optimal for growth of the host, specifically wind damage and inadequate nutrition or drainage (poor soils) in South Africa (Watkins, 1981).

#### **Leaf Curl virus disease (CLCuV - Gemini virus)**

The initiation of disease is characterized by Small Vein Thickening (SVT) type symptoms on young upper leaves of plants. The disease is further characterized by upward curling of leaves, which occur because of the uneven growth of veinal tissues on the abaxial side of the leaves. Later, formation of cup shaped or leaf laminar out growth called enations appear on the underside of the leaf. In severe cases and in plants affected at early age, reduction of inter-nodal length leading to stunting and reduced flowering/fruitletting is observed (Sheoraj et al., 2002).

#### **Wilts**

Cotton wilts are caused by pathogens such as *Fusarium oxysporum* f.sp. *vasinfectum*, *Verticillium dahliae*, non-pathogenic factors such as stem or ash weevil etc. (Table 2).

#### **Management of cotton diseases**

A plant disease occurs when there is an interaction between a plant host, a pathogen and the environment (Fig. 1). Most plants are immune or completely resistant to almost all pathogens. However, some pathogens have developed the ability to overcome the natural resistance mechanisms of particular hosts. The host is then regarded as being susceptible to that pathogen and the pathogen is described as being virulent. When the environmental conditions are conducive, the virulent pathogen attacks the susceptible host and the disease develops. Therefore, any disease management strategy should focus on the host, the pathogen and/or the environment. Hence, an 'Integrated Disease Management' involves the selection and application of a harmonious range of control strategies that minimize losses and maximize returns.

The following are some of the strategies that can be adopted for the management of the diseases.

1. Exclusion of the pathogen from the area - 'quarantine'. The pathogen from entering particular area where the disease is not prevalent.
2. Elimination of alternate hosts/weed hosts. The pathogens of *Verticillium* and *Fusarium* wilts, *Alternaria* leaf spot, bacterial blight and leaf curl virus have many weed/alternate hosts (e.g. Alternate hosts like Bhendi for CLCuV and its vector should not be grown between March to June to avoid build up of virus and vector).
3. Crop rotation with non-host crops.
4. Crop residue management to eliminate the pathogens being carried over (e.g. The CLCuV infected plants should be uprooted immediately and burnt).

5. Provision of balanced nutrition (e.g.) potassium deficiency results in increased susceptibility to *Alternaria* leaf spot and application of potassium increases the natural resistance of the host.
6. Application of biocides through seed treatment and/or foliar sprays for control of *Alternaria* leaf spot, grey mildew etc.
7. Control of insect vectors - diseases caused by virus (CLCuV) can often be controlled by controlling the vector (White fly - *Bemisia tabaci*) that carries the pathogen
8. Applications of biocontrol agents, that antagonize, inhibit or compete with the pathogen.
9. The application of biocontrol agents or systemic activators to turn on the host plants natural defense mechanisms.

#### Use of good quality seeds

Seed is the basic input for any commercial venture of agriculture. It is needless to mention the importance of good quality seed. In cotton, for obtaining good quality of seed, acid deviating with commercial sulphuric acid (100 ml/kg of seed) followed by seed treatment with either bio-fungicides or any systemic chemicals is being advised. This helps in the near total elimination of disease of seed and seedlings.

#### Use of resistant varieties/cultivars

Use of disease resistant lines/hybrids is the basic tenet of any IPM programme. Accordingly, many disease resistant materials have been developed and released for commercial cultivation. The following are the resistant variety/hybrid to the respective diseases.

Disease	Variety / hybrid
Verticillium wilt	MCU 5VT, Surabhi, Savita (Hybrid)
Bacterial leaf blight	MCU 10, L 604, L 389
Grey mildew	GMR 5, GMR 9 (resistant lines)
<i>Alternaria</i> leaf spot	CCH4 (resistant line)
CLCuV disease	RS 810, LHH 144, LH 1556, H 1117, F 1861, LRA 5166, Anjali, CP 15/2

#### Cultural practices

There are number ways to incorporate cultural practices in the integrated disease control system. As a general approach, the farmers should take steps to sow only high quality seed materials. Seeds having above 80% germination will have vigorous growth and there by they do not suffer from infection due to soil borne organisms. The farmers can have good stand.

1. Crop rotation is another important aspect which should be taken into consideration especially for diseases like *Verticillium* wilt. Converting *Verticillium* infested fields to paddy crop will greatly reduce the microsclerotial population in the soil. It is also known that growing *Chrysanthemum* will be inhibitory to *Verticillium*.
2. Time of sowing is also important. If the farmers are able to take up sowing during warmer temperature (i.e. at 65<sup>o</sup>F temperature and above) there will be better germination and seedling growth.
3. Irrigation management is an important factor involved in disease control. Timing and duration of irrigations should satisfy crop water requirement without allowing for excess water. Over watering will favour soil borne pathogen, where as use of over-head sprinkler systems will favour diseases affecting leaves. Accordingly, the farmers should manage crops.
4. Excessive application certain organic manures like poultry manure will induce high vegetative growth. Dense crop growth is conducive for foliar diseases like *Alternaria* leaf spot and grey mildew.
5. Field sanitation is another essential part of disease management. The main source for the development and spread of the foliar diseases is only through previous year's crop residues

and weed hosts near the fields. Hence, destruction of the crop residue as well as weed hosts around the field is essential.

6. Incorporation of composts in to the soil is a fundamental cultural practice in organic cotton production. Composts increase the soil fertility and help in disease management. The disease control is possibly effected through
  - (i). Successful competition for nutrients by beneficial microorganisms
  - (ii). Antibiotic production by beneficial microorganisms
  - (iii). Successful predation against pathogens by beneficial microorganisms and
  - (iv). Activation of disease resistant genes in plants by composts.

One can enrich composts through incorporation beneficial microorganisms like *Trichoderma* spp., which compete against pathogens and antagonize them. It is well known that application of town compost having high percentage of cellulolytic materials will increase the population of *Trichoderma* spp. there by helping in the management of *Verticillium* wilt as well as root rot due to *Rhizoctonia solani*.

#### **Chemical control**

Carbendazim 50 WP is an effective fungicide for the management of grey mildew, *Cercospora* leaf spot and boll rot. The recently introduced triazole compounds viz, propiconazole, hexaconazole, cyproconazole and tebuconazole and prochloraz (imidazoles) Benzothiazole group chemical Bion (Benso (1, 2, 3) thiadiazole -7-carbothioic acid S-methyl ester) are effective broad spectrum fungicides which can be used in the management of grey mildew, *Alternaria* leaf spot and other diseases of cotton (CICR annual report, 1998-1999 and Chidambaram and Johnson, 2002).

#### **Biological control**

Biological control is an important area of focus in the discipline of Plant Pathology. Every major university with department of Plant Pathology has one or more faculty members conducting basic and/or applied research on biological control organisms (Gardner and Fravel 2002). Biological control agents (BCA<sub>S</sub>) have been found among the most abundant plant associated microbial genera such as PGPR - Plant Growth Promoting Rhizobacteria (*Bacillus*, *Burkholderia*, *Pseudomonas*, *Streptomyces*) and the fungal genera *Trichoderma*. While synthetic toxins have their place in disease control, there is growing awareness that Biologically Based Pest Management (BBPM) fitting in the existing IPM strategies provide more environmental friendly and economically viable alternatives for agriculture. Whether acting by competitive exclusion, biochemical antagonism or induction of host defenses, BCA<sub>S</sub> must be well adopted for survival and functional activity in the photosphere (Gardner and Fravel, 2002).

Biopesticides are cheaper than synthetic toxins by 50 per cent. They are ecofriendly, have a high cost benefit ratio and do not induce resistance in plant pathogens. The advantages of biological agents as seed treatment are i) the saprophytic nutritional status of biocontrol agents makes large-scale production feasibility, ii) small Amounts of inoculum requirement, iii) simple methods of application, iv) independent of every sources for survival, v) systemic spread along the surface of the developing root system vi) antagonistic activity on the root surface during the economically important phase of early root infection by the pathogens, vii) ecofriendly and viii) no resistance development in the pathogen.

Their versatile metabolism, fast growth, active movement and ability to readily colonize the root surface make the rhizobacteria suitable for seed bacterization In addition, some of the PGPR have the added advantage of plant growth promoting activities also (Shetty and Raj, 2003). However, some of the disadvantages of the biopesticides are i) narrow spectrum of activity, ii) inconsistent performance in practical agriculture, iii) environmental sensitivity and iv) short shelf life.

#### **Transgenic cotton for disease resistance**

The mycoparasitic fungi like *Trichoderma virens* are proving to be rich sources of antifungal genes that are being utilized for developing transgenic plants resistant to fungal

pathogens. Scientists are able to transform cotton and tobacco plants with a CDNA clone encoding a 42 kDa endochitinase from *T. virens*. When the homozygous T2 cotton plants with high endochitinase were tested against *R.solani* and *A. alternata* they showed significant resistance to both pathogens (Emani et al., 2003).

### References

- Chidambaram, P. and Johnson. 1. 2002. Grey mildew In: *Twenty five years achievements in cotton pathology at CICR (1976 - 2001)*. Published by The Director, CICR, Nagpur. pp. 29 - 39.
- CICR annual report, 1998- 1999. *Central Institute for Cotton Research annual report*. pp. 53 - 54.
- Emani, C., Garcia, J.M., Finch, E.L., Pozo, M.J., Uribe, P., Kim, D.J., Sunilkumar, G., Cook, D.R., Kenerley, C.M. and Rathore, K.S. Enhanced fungal resistance in transgenic cotton expressing an endochitinase gene from *Trichoderma virens*. *Plant Biotechnology Journal*, **1(5)**: 321.
- Gardner, B. M. and Fravel. D. 2002. Biological control of plant pathogens, research, commercialization and application in USA. Online. *Plant health progress* doi. 10.1094/PHP-2002-0510-01-RV.
- Heydari, A. and Misaghi, I.J. 1998. Interactions between herbicides and cotton seedlings dumping-off in the field. In: *Cotton.. A college of agriculture report*, 1998. College of agriculture, The University of Arizona, Tucson, Arizona-85721. ([http://ag.arizona.edu/pubs/crops/az\\_1006/az10061\\_0b.html](http://ag.arizona.edu/pubs/crops/az_1006/az10061_0b.html)).
- Hillocks, R. J. 1992. Cotton Diseases. CAB International
- ICAC Recorder. 2004. Cotton: World Statistics. The *International Cotton Advisory Committee technical bulletin*, November 2004.
- Koenning, S. 2004. Cotton seedling diseases In: Cotton disease information note no.1. Extension pathology report. Arizona state university
- Sheoraj, Monga, D. and Gupta, K.N. 2002. Leaf curl 1n: Twenty five years achievements in cotton pathology at CICR (1976- 2001). Published by The Director, CICR, Nagpur. pp. 16 - 25.
- Shetty, H.S. and Raj. S N. 2003. Plant growth promoting rhizobacteria for beneficial agriculture - current concepts and future outlook. In: *Sixth International workshop on Plant .Growth Promoting Rhizobacteria symposium conducted at Indian Institute of Spices Research, Calicut, Kerala on october5, 2003*. pp. 40-47.
- Srinivasan, K.V. 1994. Cotton diseases. Published by Indian Society for Cotton Improvement, Bombay. p.312.
- Watkins, G.M. 1981. Compendium of cotton diseases. American Phytopathological Society.

**Table 1. Major diseases of cotton and their distribution.**

Disease	Organism	Distribution
<b>Seed and Seedling diseases</b>		
a. Seed infection	<i>Alternaria, Aspergillus, Colletotrichum, Fusarium, Rhizopus and Xanthomonas axonopodis pv malvacearum</i>	India
b. Shore-shin disease	<i>Rhizoctonia solani/ R.bataticola</i>	Egypt, USA, Morocco and India (Andhra Pradesh, Maharashtra and Tamil Nadu)

c. Damping off	<i>Pythium</i> , <i>Phytophthora</i> , <i>Rhizoctonia solani</i> , <i>R.bataticola</i> , <i>Fusarium solani</i> , <i>F. moniliforme</i> , <i>F.o. f. sp. vasinfectum</i> , <i>f.</i> <i>roseum</i> , <i>Ascochyta gossypii</i> , <i>Glomerella gossypii</i>	India (Bihar, Madhya Pradesh, Tamil Nadu, Andhra Pradesh and Central India), USA
	<i>Xanthomonas axonopodis pv</i> <i>malvacearum</i>	North and Central India and not common in South India
d. Root rot	<i>Macrophomina phaseolina</i> ( <i>R.bataticola</i> )	USA, Venezuela, Trinidad, Uganda, Zaire, Egypt, Sudan, Greece, Israel, Pakistan and India(Punjab, Rajasthan, Utter Pradesh, Bihar, Gujarat, Andhra Pradesh and Tamil Nadu)
	<i>Sclerotium rolfsii</i> (Collar rot)	South USA, Island of St. Vincent in Caribbean, Peru in South America, El Salvador in Central America, New South wales in Australia and India (Maharashtra, Madhya Pradesh, Andhra Pradesh and Tamil Nadu)
	<i>Thielaviopsis basicola</i> (Black root rot)	South western US, Peru, Egypt, Uzbekistan and Australia
<b>a. Fungal diseases</b>		
<i>Alternaria</i> leaf spot	<i>Alternaria macrospora</i> , <i>Alternaria alternata</i>	Tanzania, India (Karnataka, Gujarat Andhra Pradesh and Tamil Nadu) and Australia
Grey mildew	<i>Ramularia areola</i>	USA, Caribbean countries, Central America, Brazil, Egypt, Central Africa, Madagascar, Uganda and India (Madhya Pradesh, Bihar, Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu)
<b>Minor diseases</b>		
<i>Helminthosporium</i> leaf spot	<i>Helminthosporium speciferum</i>	Puerto Rico, Zaire, Peru, The Philippines and India (Haryana and Punjab)
<i>Ascochyta</i> blight	<i>Ascochyta gossypii</i>	Southern US, Zaire and Central and east Africa
<i>Curvularia</i> leaf spot	<i>Curvularia lunata</i>	India (Maharashtra)
<i>Cercospora</i> leaf spot	<i>Cercospora gossypina</i>	West Indies, Egypt, China and India
<i>Myrothecium</i> leaf spot	<i>Myrothecium roridum</i>	India (Punjab, Haryana and Gujarat)
<i>Rhizoctonia</i> leaf spot	<i>Rhizoctonia solani</i>	Louisiana, El Salvador and India (Tamil Nadu and Maharashtra)
Tropical rust	<i>Phakopsora gossypii</i>	Mexico, Southern USA, India, Indonesia, West Africa, The

		Philippines, New Guinea and West Indies
<b>b. Bacterial Disease</b>		
Bacterial blight	<i>Xanthomonas axonopodis</i> pv <i>malvacearum</i>	Mexico, USA, Sudan, Tanzania and India (Tamil Nadu, Karnataka, Maharashtra, Madhya Pradesh, Andra Pradesh, Punjab, Haryana and Rajasthan)
<b>c. Vascular wilts</b>		
<i>Fusarium</i> wilts	<i>Fusarium oxysporum</i> f.sp. <i>vasinfectum</i>	Egypt, China, Southern US, West Indies, Southern France, Yugoslavia, Bulgaria, Greece, Israel and India (Maharashtra, Gujarat, Madhya Pradesh, Punjab, Haryana, Andra Pradesh and Karnataka)
<i>Verticillium</i> wilt	<i>Verticillium dahliae</i>	Most of the countries except Egypt ( <i>G. barbadense</i> ). India (Karnataka and Salem and Madurai districts of Tamil Nadu )
<b>d. Viral diseases</b>		
a. Leaf crumple	Virus	USA, Philippines and India (Maharashtra)
b. Leaf Curl	Virus	Nigeria, Sudan, Pakistan and India (Rajasthan, Haryana and Punjab)
c. Stenosis	Virus	Western India (Including Gujarat, Andra Pradesh, and Karnataka), China and Haiti

**Table 2. Wilts of cotton (Hillocks, 1992 and Watkins, 1981)**

<b>Fusarium wilt</b>	<b>Verticillium wilt</b>	<b>Sudden wilt</b>	<b>Insect damage</b>
Caused by <i>Fusarium oxysporum</i> f.sp. <i>vasinfectum</i>	Caused by <i>Verticillium dahliae</i>	Unknown etiology	Stem weevil ( <i>Phempherulus affinis</i> ) and ash weevil ( <i>Myloccerus</i> sp) damage
Plants may be affected atg any time throughout the season	Most common late in the season or after wet and/ or cool weather	Occurs after wet weather or water logging	Any time
Favoured by mean temperatures above 23°C	Favoured by means temperatures below 23°C	Favoured by cultivation prior to irrigation and warm weather	--
Plant death, wilting, yellowing, stunting, defoliation, some attempted re-growth	Leaf mottling, death of leaf tissue between the veins and around margins, defoliation sometimes	Sudden wilting followed by defoliation and some re-growth	Sudden drooping of leaves followed by wilting
Brown / chocolate discolouration of vascular tissue throughout the entire main stem	Dark brown, tan to black discolouration of vascular tissue throughout the entire main stem	Some browning of vascular tissue in the lower stem- especially under the back	Galls at the base of plants due to stem weevil. Rotted damage due to grub of ash weevil
Areas of reduced or	Stand usually not	Stand usually not	--



patchy stand usually spreading in the direction of irrigation	affected. Diseased plants scattered throughout stand	affected- isolated plants together in a row-especially in low patches or near tail drain	
Soil-inhabiting, spread with soil and plant debris-especially in irrigation water	Soil- inhabiting, spread with soil and plant debris-especially in irrigation water	Soil- inhabiting, spread with soil especially in irrigation water or flood water	--
Survives as singly celled, thick-walled chlamyospores (7-13 microns)	Survives as multi-cellular, thick-walled microsclerotia (30-60 microns)	Survives as a saprophyte living on plant debris in soil	--
Can be seed borne	Not seed borne	Not seed borne	--
Host range- <i>Sesbania</i> , Pea and dwarf <i>amaranths</i>	Egg plant, sunflower, soybean, potato, tomato etc., and weed hosts	--	--

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## Pest management in Cotton in DUS experimentation

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Cotton crop in India is damaged by more than 170 insects right from germination to the final picking of the produce- seed cotton (kapas). However, only a few reach pest status causing considerable damage to the crop and many species even though seen over a long period, do not cause any economic loss. If the pest is not properly identified and the necessary pest management strategies are not adopted, the yield loss may be as high as 70-80 per cent.

The important, insect pests are the sap feeding insects (thrips, jassids, aphids and whiteflies) the bookworms (Spotted, American and Pink) and the leaf feeding insects (cotton leafworm, leafroller and semiloopers). The sap feeding insects may have piercing and sucking type of mouthparts (thrips aphids and jassids). According to their incidence in the phenology of the crop, their biology and damage are provided

### Sap Feeding Insects

#### 1. Cotton thrips: *Thrips tabaci*, *T. palmi*, *Scirtothrips dorsalis*

Thrips are generally one of the main early season cotton pests with lacerating mouth parts. They initially damage the cotyledons and then several other plant parts and the types of damage vary according to the parts of the plant attacked. Most damage occurs during early vegetative stage of the crop, when the nutritional quality of tissues is ideal for these insects.

#### Damage

Both adults and nymphs usually remain on the under surface of leaves, lacerate the tissues and suck the cell sap. Cotyledons are damaged immediately after emergence. The appearance of glistening white silvery patches on the under surface of the leaves is the characteristic initial symptom. The leaves become thickened, blistered and bronzed due to continuous feeding. The browning and bronzed symptom appears on the upper surface also. Cotyledonary leaves fall prematurely affecting the plant growth. When the apical meristem is damaged, the proper furling of leaves is hampered, growth is affected and the plants become stunted. Affected young leaves get thickened, curl at the edge and get distorted. Heavy infestation causes leaves to turn coppery brown or reddish and the affected leaves curl and fall prematurely. On the under surface of the affected leaves, tiny black specks - which are the faeces of the thrips can be seen particularly near the veins. Feeding on developing bolls makes them turn brown due to development of necrotic patches. Thickening of boll rind can also be noticed when bolls are attacked, boll opening is affected.

#### Description

Adult thrips are small, 1-2.0 mm long and elongate and they possess a pair of fringed wings (could be seen clearly under microscope). Nymphs are similar to adults in appearance except that they are wingless. Both nymphs and adults can be seen in large numbers on the under surface of leaves. They are yellow, straw yellow to dark brown in colour.

#### 2. Cotton jassid- *Amrasca devastans*

Jassids (leafhoppers) are the important sap feeding insects in all the cotton growing regions of India. Among the 10 different species recorded on cotton, the most important and widely distributed species is *Amrasca devastans* (*A. biguttula biguttula*).

#### Host range

It is a polyphagous pest. In addition to cotton, the important alternate host is okra. The other host plants are castor, brinjal, sunflower, cucurbits and many malvaceous weeds.

### Damage

Nymphs and adults remain usually on the under surface of the leaves, particularly at the base of the vein and suck the sap of the mesophyll layers. While feeding, they introduce a toxin through saliva that impairs the photosynthesis in proportion to the number of nymphs present. The initial symptoms are the paling of the green colour to yellow at the edges of the leaf, followed by downward curling of the leaf edges and bronzing. Due to continuous feeding, the leaves become brick red with the development of necrotic patches between the veins causing drying up of leaves. In severely infested fields with susceptible cultivars, the crop gets blighted and appears as though it is burnt with fire, with dried leaves which drop off. This type of symptom is known as 'hopper burn'. When the damage occurs in early stage of the crop, the plants succumb to the injury. Plants at preflowering stage are most susceptible. In grown up plants, the growth is retarded the plant become stunted and fruiting bodies such as square, flower and boll may shed. Attack during bolling stage reduces the boll size and boll opening and the quality of the fibre. Even presence of 3-4 nymphs / leaf can cause severe 'hopper burn' leading to heavy yield loss.

### Methods of suppression

Insecticides are commonly necessary when the jassid population reaches 2-3 nymphs / leaves although for glabrous varieties, the threshold may be lowered to one per leaf if the cotton crop is highly susceptible or jassid population build up. The choice of insecticide to be selected depends upon the age of the crop and the density of jassid population.

Application of systemic insecticide methyl-o-demeton gives good reduction of jassids upto 45 days. When the crop is above 50 days old and the jassid population is high, acephate can be used. The systemic insecticide, dimethoate is ineffective against jassid and aphids. Similarly, the contact insecticides chlorpyrifos, quinalphos and endosulfan are not effective in cotton crop. Very often, the cotton fields treated with these insecticides have more jassid population. DUS plots shall never have insecticides such as monocrotophos, imidachloprid, Acetnmidiprid etc. that have growth promoting action in plants.

### 3. Cotton aphid- *Aphis gossypii*

Aphids (plant lice) are widely distributed in all the cotton growing regions of India. Earlier, it was considered as a minor pest, but now causes serious damage particularly in cotton fields after use of synthetic pyrethroids, which induce resurgence of this pest. Aphids are found particularly underside of the leaves in large number.

### Damage

#### Direct

The degree of damage depends on the period of attack and the size of the population, insecticide previously used and the weather conditions. A dry weather with prolonged drought favours the fast build up of this pest. Aphids remain in colonies on the under surface of leaves and terminal shoots and suck the plant sap and the general vigour and growth of the plant is arrested. Initially, the edges of the leaves curl downward and crinkle, the tender portions fade gradually and the whole plant becomes stunted. The attack is severe in younger plants. Heavy infestation in old plants particularly after the use of synthetic pyrethroids reduces the overall vigour and growth of the plant. Flowering and boll formation are also reduced. Shedding of fruiting bodies is noticed. Boll opening is hampered and the fibre quality on is affected.

#### Indirect

The excess sugar in the plant sap (phloem sap) on which the aphids feed is excreted in the form of 'honeydew', which is deposited on the foliage forming a sticky glistening substance. This can be seen as fine drops on the underlying vegetation. Depositions of honeydew over the foliage interfere with respiration of the leaves and provide substrate for the growth of the black sooty mould. The sooty mould interferes with the photosynthetic activity of the plant by hindering light absorption by chlorophyll. Further, if attack occurs or persists later in the season during boll opening period, the honeydew may drip on to the open bolls and is one of the causes of

'stickiness' of lint; sooty mould which develops over the honeydew also discolors the seed cotton and thus reduce the market value.

#### **Description**

Aphids are small, ovate (1-2 mm) soft bodied, sluggish moving gregarious insects with slender legs and antennae. They are characterized by a pair of tubular process called 'cornicle' projecting backward and upward from near the end of the abdomen.

In a colony, both winged (alate) with two pairs of long transparent wings and wingless (apterous) forms can be seen. Aphids are variable in colour and colonies are always made up of individuals of different colours, green to dark green, yellowish, brown orange yellow, lemon yellow and sometimes very blackish brown. Size also varies considerably (apterous 0.9 to 1.8 mm, alate 1.0 to 2.0 mm). Infested plants can be easily noticed by the presence of ants, moving around the plant to feed on sweet 'honeydew' excreted by the aphids.

Usually, winged females are the first to arrive on a young plant. They reproduce by giving birth to young ones (nymph) without mating. This type of reproduction is called 'parthenogenetic viviparity'. Nymphs resemble adults in all respects except for size and immature abdominal segments. Wingless females are slightly large and more globular than the winged forms. Multiplication is carried out by the wingless females. Winged forms appear in the colony only when the dispersal of the colony is needed and are developed due to crowding and poor food quality. Sexual reproduction and egg stage are not usually observed in South India.

As overlapping continuous generations are observed, 50 generations have been recorded in a year. The average life span of an individual aphid is about 20-25 days and a female can give birth to 80- 100 nymphs at the rate of 3-5 nymphs per day depending upon the nutritional status of the plant and insecticides applied on the crop. In synthetic pyrethroid treated fields, the reproductive capacity (fecundity) and the longevity of the individual is greatly high.

#### **Host range**

*A. gossypii* is present in all cotton growing area of the world. It is polyphagous and recorded on 300 host plants. The most important crop hosts are cucurbits, okra, hibiscus, legumes and numerous ornamental lots.

#### **Favourable condition**

The favourable condition for the pest multiplication is dry weather with prolonged drought and a moderate temperature of 25-30<sup>o</sup> C. Heavy plant canopy, higher nitrogenous fertilizer and improper usage of insecticide also favour the pest build up.

Heavy rain reduces population directly by washing them away. Further, high humidity enhances the appearance of entomopathogenic fungi, which in turn reduce the aphid population.

#### **Methods of suppression**

Sowing the cotton seeds treated with the insecticide, imidacloprid or thiamethoxam (5- 10 g /kg of seed) gives protection to the crop from the sucking pests particularly aphids, thrips and jassids for about 45 days.

When the pest infestation is observed, application of methyl-o-demeton in the early phase of crop growth and acerbate in late stages of crop growth during flowering and boll formation stage.

When the pest is seen in patches in the field usually around the shade places, spot application of insecticide is advisable rather than the whole field application.

#### **4. Cotton whitefly – *Bemisia tabaci***

The cotton whitefly, an occasional pest of cotton in India has emerged as a major pest in several states in recent years. Severe outbreak was first noticed in Guntur region of Andhra Pradesh during 1984-85 and now continues to be in all the cotton growing regions. It is believed that continuous drought; excessive application of nitrogenous fertilizers and the indiscriminate use of synthetic pyrethroid insecticides have induced the resurgence of this pest.

### Host range

It is polyphagous with an extremely wide host range. It can breed and feed on over 400 host plants of cultivated and non-cultivated species. Among the cultivated crops, cotton, brinjal, tobacco, sunflower, okra, tapioca, potato, sweet potato, tomato, pulses, cucumber etc. are the important hosts.

Its weed hosts, to mention a few include *Abutilon indium*, *Solanum nigrum*, *Sida cordifolia*, *Urena lobate*, *Lantana sp* and *Tribulus terrestris*.

### Damage

#### Direct

The nymphs and adults remain in colonies on the surface of leaves and suck the sap. Due to continuous feeding chlorotic spots develop on the leaves which later coalesce and the leaves become reddish, brittle and finally drop off prematurely. This results in reduced nutrition to the plant leading to stunting, shedding of fruiting bodies and reduction in the size of bolls. The bolls are also forced to burst prematurely leading to poor quality lint. The oil and protein contents of seeds are lowered. In North Zone, this insect acts as a vector for the spread of Cotton Leaf Curl Virus (CLCuV) disease.

#### Indirect

In addition to the direct damage, upper the 'honey dew' excreted by this insect drops on the upper surface of lower leaves and bolls which favour the development of black sooty mould fungus on the leaves which in turn interferes, with the photosynthesis of leaves. Heavy fungal growth on honeydew-covered leaves leads to premature leaf drop.

Honeydew deposition on open bolls causes stickiness. Sticky cotton interferes with picking, ginning and spinning and hence sticky cotton fetches low price. Sticky cotton shall interfere in the quality of seed cotton, meant for DUS purpose.

#### Management of whitefly

As the Pest is assuming great importance, the following integrated approach is suggested for its management.

- Cotton should be grown only once in a year in the proper season.
- Late sowing should be avoided. In Tamil Nadu, late sowing the first week of September suffers more and sowing taken during the middle of August will be free from whitefly. Similarly in Andhra Pradesh sowing made prior to the first week of July had less infestation.
- Cotton should be rotated with non-host crops like cereals so as to avoid continuous food supply to the pest. Immediately after last picking, cotton stalks should be removed to avoid carry over of the pest during off- taken after the following integrated of whitefly Season.
- Alternate weed hosts should be removed from the field and neighboring areas.
- Adopt recommended spacing spraying for the cultivar and closer spacing always conducive for this pest.
- Adopt paired row planting to facilitate easy inter cultivation and spraying operations in addition to saving of water.
- Judicious use of nitrogen and irrigation should be practiced to check excessive vegetative growth and consequent pest buildup in the system. Balanced application of fertilizers with P and K is needed.
- The appearance, activity and the population buildup of whitefly should be monitored by setting yellow sticky traps.
- Ineffective insecticides, vegetative growth inducing insecticides (monocrotophos, acephate) and insecticides having high toxicity to natural enemy should be avoided.

- The use of synthetic pyrethroids at frequent intervals and at very early vegetative phase of the crop should be avoided. It should be used only during peak flowering and boll formation stages depending upon the bookworm density. It should not be repeated unless warranted.
- Repeated use of acerbate and monocrotophos also cause resurgence of this pest.
- Extending of crop growth beyond its duration it's additional fertilizers and irrigation is to be avoided to prevent the cycle of the pest.
- Application of fish oil resin soap (2%) and neem oil (0.5 %) is found effective in suppressing the population. While using neem oil alone, teapot or soap solution at the rate of 1 ml per litre of water has to be added for emulsification of the oil and for better contact of the spray fluid it's the foliage.
- Application of methyl-o-demeton (1 litre/ ha) in the early phases of crop growth and spraying triazophos (2.5 litre / ha) in the late stages of crop growth will be useful to manage this pest.
- Triazophos combined with either neem oil or fish oil resin soap is more effective. Five hundred litres of water per ha in the early stages and 750- 1000 litres per ha in the late stages may be used for spraying with knapsack sprayer. For power sprayers, 200 - 250 litres of water may be needed.
- Use either high volume or low volume sprayers and ensure thorough coverage of the under surface of the foliage where the insects remain in colonies. Avoid use of Heli or Garden sprayer and spraying highly concentrated insecticides.
- The insecticides are to be applied *en bloc* in a particular locality as and when needed
- Avoid scheduled application of insecticides and sub lethal dosage of insecticides as it hastens the development of resistant strain of insect population.

#### LEAF FEEDING CATERPILLARS

Several species of leaf feeding caterpillars occur on cotton and damage the foliage right from early vegetative stage till harvest of the Kapas. But most of them are of minor importance and seldom cause serious damage.

##### 1. Cotton leaf worm: *Spodoptera litura*

*S. Litura* (*Prodenia litura*) also known as tobacco cutworm and castor leafworm, is a highly polyphagous insect and a very common pest of various agricultural crops. However, occurring sporadically, it can cause economic losses to cotton and many other crops such as tobacco, castor, cabbage, cauliflower, pulses, cereals and many other crops. In addition, it can survive on several other weed plants also. One hundred twelve cultivated plants belonging to 44 families and several weeds are listed as host plants.

##### Damage

The larvae which hatch out from egg mass remain gregarious for about 3-5 days and feed on the undersurface of the leaves by scrapping the epidermal layer. Later, the whole surface of the leaf is scrapped leaving the veins alone. At this stage, the typical feeding symptom and the skeletonised leaves can be identified from the distance. Beyond third instar, the larvae move to other leaves and feed on the leaves causing extensive defoliation. The larvae also feed on squares, flowers and bolls inflicting severe loss to the reproductive parts. In case of severe attack, only the stem and shoot will be standing in the field. The larvae have the habit of feeding during early morning hours and night. During daytime, they hide in the cracks of the soil or under the debris.

##### Seasonal occurrence

The pest can be seen throughout the year. In cotton, it causes considerable damage during peak flowering and fruiting stage. Cloudy weather, continuous rain and high humidity with high night temperature favour the pest outbreak. During 1997, severe outbreak of this pest was recorded in parts of Andhra Pradesh on cotton and other crops during November- December causing considerable yield loss.



## Management

- Monitor the moth activity by erecting pheromone traps
- Grow castor as trap crop along the borders as the moth prefers to lay eggs on these plants rather than on cotton. Monitor the trap crop regularly for the egg masses and gregarious early instar larvae by identifying the feeding damage (lace like leaves). Collect and destroy them along with the affected leaves.
- Remove and destroy the early instar larvae found in the cotton crop also. The affected plants can be identified even from distance by the lacerated leaves.
- Hand collection of grown up larvae
- Use *Spodoptera* NPV virus @ 200- 400 LE / ha.
- Use poison bait rice bran (12 kg) jaggery (1 kg) and chlorpyrifos and water. The bait balls can be spread in the field in the evening hours, so that the caterpillars coming out of hiding place feed on the baiting materials and get killed.
- Spray neem seed kernel extract (NSKE) when the infestations are low. The insecticide chlorpyrifos or quinalphos can be used in later stage.
- Avoid closer spacing and excessive application of nitrogen.

## 2. Cotton leafroller-*Sylepta derogata*

It is a sporadic pest on cotton. Besides cotton it also infests other malvaceae plants such as Hibiscus sp, okra, Abutilon and Sida. Though, it is a minor pest, in extreme cases, the grown up caterpillars are capable of causing such serious damage that the cotton plants are completely defoliated affecting the growth of the plant and yield.

### Damage

The early instar larvae feed on the under surface of leaves. Later, grown up larvae roll the leaves into 'trumpet shaped' structure fastened by means of silken threads. Initial infestation frequently occurs in shady places. The caterpillars by remaining inside the rolls feed outside on the marginal portions of leaves. In severe cases of attack, the whole plant becomes completely defoliated and in each plant, 5-7 typically rolled leaves can be seen.

### Management

The larvae and pupae are parasitised by several parasitoids. Generally, the natural enemies keep the leafroller under check. When infestation is noticed, the rolled leaves can be collected and destroyed. As the pest always appears in patches, the chemical control if needed can be directed against those areas where the pest population is concentrated and not extended over the whole field, unless other pests have to be controlled at the same time. The insecticides used for bookworm management or cotton leafworm can be used for this pest also.

## 3. Semi-loopers: *Anomis flava*, *Acontia graelsi* and *Tarachae nitidula*

Several species of semi loopers feed on the leaves. However, they are minor in importance and seldom cause any serious damage to leaves as the cotton plant produces leaves in excess especially in the late vegetative phase of the crop growth. The larvae feed on the leaves, causing at first the so called "windowing" and latter perforated leaves which are very typical. In cases of severe attacks the leaves are eaten away right down to the main veins and rarely young shoots and squares are attacked.

Moths are varying in color, depending upon the species. Body is about 14- 16 mm long with a span of 30-40 mm. Adult longevity is about 15 days.

### Host plants

In addition to cotton, it breeds on many malvaceous plants such as okra, Hibiscus spp and Abutilon.

### Management

Larvae are heavily parasitised by several parasitoids particularly *Apanteles* sp. and *Bracon* sp. In general, the damage caused by loopers does not attain excessive proportions warranting any insecticidal spray.

### Other caterpillars

In addition to these semi loopers, the caterpillars such as the red hairy caterpillars, *Amsacta spp.*, leaf perforator *Bucculatrix* sp, hairy caterpillar *Euproctis spp.*, cutworm *Laphygma exigua*, the leafwebber *Phycita infusilla* and castor hairy caterpillar *Pericallia ricini* also damage the leaves.

Further, the cutworms *Agrotis* spp also damage the seedling in early stage of crop growth.

### Cotton bollworms and their management

#### Spotted bollworm: *Earias vitella* Spiny bollworm: *Earias insulana*

The spotted bollworms are widely distributed in India. They damage the shoots as shoot borer in the early stage of the crop growth and fruiting bodies such as square, flower and bolls in later stage. They were the major bollworm complex causing as high 60-70 % of boll damage prior to the introduction of the synthetic pyrethroids and at present they are not as serious as the American bollworm, *Helicoverpa armigera*.

#### Host range

In addition to cotton, the most important alternate host is okra (*Abelmoschus esculentus*) which is the most preferred host than cotton. The other reported host plants are malvaceous plants of the genera *Abelmoschus*, *Malva*, *Malvastrum*, *Abutilon*, *Sida* and tiliaceous plant *Corchorus*.

#### Damage as shoot borer

*Earias* is distinguished from other bollworms by its marked stem boring habit in the vegetative phase of crop growth prior to square formation. The larvae bore into the tender terminal shoot and burrow downward inside the stem feeding the soft tissues. The shoot above the damaged part withers, droops and dries up. As the terminal shoot is affected, the axillary buds develop giving a bushy appearance to the plant and as such, the whole architecture of the plant is changed. This type of shoot boring habit is usually noticed only in cotton and not in other host plants.

#### Damage as fruit borer

When square, flower and bolls are developed, the larvae damage them by feeding usually on the bolls, (the borehole is attacked by the excreta). The larvae damage several squares and flowers only partially feeding on them. When the square is damaged, the bracts (calyx) enclosing the flower bud get 'flared up' which could be easily identified. There are reports that the saliva of the caterpillar contains some toxins, which on entering the ovary damage the square even without feeding. Several 'flared up' squares can be seen with slight puncturing of the ovary. Damaged squares, flower buds and young bolls drop down and older attacked bolls remain attached to the plant and often get infected by fungi, which in turn leads to the development of prematured inferior worthless fibre and the affected hard lobules (locs).

### Management

#### Chemical

Application of neem products (neem seed kernel extract / neem oil) alone or in combination with insecticide is found to be effective. When the pest infestation is above the threshold level (5- 10 % on the reproductive parts) any one of the following insecticides are suggested; endosulfan, quinalphos or chlorpyrifos depending upon the crop age and pest infestation level.

#### American bollworm: *Helicoverpa armigera*

*Helicoverpa (=Heliiothis) armigera* commonly called American bollworm is one of the most destructive pests of cotton and many other crops in India. Its severe and widespread outbreak in cotton causing as higher as 30-40 % yield loss in Andhra Pradesh in the prime cotton growing regions of Guntur and Prakasam districts occurred during 1987. This was mainly attributed to the development of resistance to several insecticides particularly the synthetic pyrethroids that were introduced during 1982. Similarly, during 1997 in the non-traditional cotton growing areas of

Warangal, Khammam and Karim nagar districts of Andhra Pradesh, a sever outbreak of this pest occurred causing an yield loss of 25-30 per cent.

Many farmers who obtained an yield of 20-25 q of seed cotton per hectare hardly realized 2-3 q /ha More than 150 farmers mostly tenant farmers, who were unable to beat the loss, committed suicide because of indebtedness and related economic problems, drawing national and international attention. *H. armigera* for the past several years continued to remain as a serious threat to cotton production in most of the cotton called the American growing countries.

#### **Host range**

*H. armigera* is a polyphagous pest attacking several cultivated and wild plants. World wide, it has been recorded from 60 cultivated and 70 wild host plants including weeds. The important crop plants in addition to cotton are pigeon pea, chickpea, soybean, almost all pulses, sunflower, groundnut, tobacco, sorghum, ragi, maize, tomato and okra.

#### **Distribution**

*H. armigera* is one of the widely distributed pests occurring almost throughout the tropical and subtropical regions from the Cape Verdi Island in the Atlantic through Africa, Asia and Australia to South Pacific islands and from Germany in the North to New Zealand in the South. However, it is called as American bollworm, it does not occur in America, but it is most serious in Australia, Pakistan, China and India.

#### **Seasonal occurrence**

The pest is observed to occur throughout the year. During summer, it breeds on vegetable, pulses, other crops and wild hosts. Five to eight generations are seen in a year. In cotton, the maximum activity is observed to occur during the peak flowering stage of the crop, when the crop is 80- 100 day old. Maximum damage on cotton in Southern India occurs during October to December. Thereafter, the pest moves to pulses and other crops.

During 1997, a very heavy incidence of this pest in certain parts of Andhra Pradesh was reported during November due to unseasoned continuous heavy rainfall. In Tamil Nadu, the pest incidence is high during November- December.

#### **Management of *H. armigera***

*H. armigera* is a pest of major importance in all the cotton growing regions of our country. Its high mortality, polyphagy, rapid and high reproductive potential, capability to develop resistance to synthetic insecticide, make them very difficult to control. More than 70 % of the pesticide used in most of the cotton growing regions are targeted against this single pest and such heavy dependence often leads to environmental pollution and socio-economic problems as noticed in Andhra Pradesh during 1987 and 1997. Hence, an integrated pest management strategy has to be adopted to contain this pest.

#### **Cultural methods**

- Cotton should be grown once in a year and monocropping of cotton should be avoided
- Crop rotation should be adopted and intercropping such as cowpea, soybean and pulses should be encouraged to increase the natural enemy buildup. This will avoid early application of insecticides.
- Growing short duration, jassid tolerant varieties / hybrids is desirable. As jassid susceptible cultivars receive early application of pesticides, the early predatory and parasitic activity is hampered. Hybrid like Savita and short duration jassid resistant cultivars (LRA 5166, LRK 516) having tolerance to jassid are desirable.
- Alternate weed host should be removed and destroyed.
- Recommended spacing for each cultivar should be adopted. Closer spacing favours pest and disease buildup. Paired row planting can be adopted for easy inter cultivation and pest control operations.
- Sowing cotton seeds treated with the insecticide imidacloprid helps to avoid early use of systemic insecticide upto 40-50 days.

- Judicious use of nitrogenous fertilizers and irrigation should be practiced to check excessive vegetative growth and consequent pest buildup in the cotton growing areas.

### Monitoring and Scouting

Monitor the moth activity by erecting pheromone traps. The trap catches needs to be confirmed by scouting the egg for field infestation / egg laying.

For scouting, 20-30 plants per hectare have to be selected at random and the presence of eggs has to be monitored. The insecticides are to be applied based on the economic threshold level (ETL). ETL for *H. armigera* is 0.5 to 1.0 egg or larva / plant or 5 % of the damaged squares.

### Insecticides

The following insecticides are recommended. Endosulfan, quinalphos, chlorpyrifos, indoxocarb and spinosid according to stage of the crop. When the crop is 40-55 day old, endosulfan can be used. In later stages, other insecticides are recommended.

In the early phase of crop growth, neem products such as neem seed kernel (NSK) or neem oil can be used alone or in combination with recommended insecticides. Neem products can be used in later stage along with insecticide. The use of ineffective insecticides, insecticides that induce vegetative growth (acephate, monocrotophos) insecticides having high mammalian toxicity (methomyl, monocrotophos) are to be avoided.

All the synthetic pyrethroids available at present induce resurgence of aphids, whiteflies and mealy bugs. Hence, use of synthetic pyrethroids at early stages of crop growth has to be avoided. Pyrethroids may be used in restricted way during peak flowering boll formation stage. They should not be repeated unless warranted. The sprayers having poor delivery system (Akela and ULV) should be avoided. Power operated mist blowers are to be used.

The insecticides are to be applied *en bloc* in a particular locality as and when needed. Two to three days after each spray, the surviving larvae have to be hand picked and destroyed so as to avoid insecticide resistance problem.

### Mechanical control

*H. armigera* beyond third instar (above 7 day old) is not amenable to any insecticides. Hence, labour force has to be employed and the larvae and the affected plant parts are to be collected and destroyed. Extending crop growth beyond its duration with additional fertilizers, irrigation and growth inducing chemicals as well as ratooning should be avoided. After the harvest, the plants are to be removed or ploughed into the field and field sanitation should be strictly maintained.

### Pink bollworm: *Pectinophora gossypiella*

The pink bollworm (PBW), *Pectinophora gossypiella* is one of the most important pests of cotton affecting the yield, quality and seeds in a number of ways.

It was first recorded in India in 1918 and is now known to occur in all the cotton growing regions of the world except in parts of Uzbekistan (Russia). Though, widespread occurrence of PBW is noticed in all the cotton growing areas in India, it is more serious in North-Western Indian states of Punjab, Haryana, Maharastra and parts of Tamil Nadu, Karnataka and Andra Pradesh.

### Host range

The major host other than cotton is okra (*Abelmoschus esculentus*). It also attacks jute, Abutilon, Sida and other malvaceous plants.

### Damage

The PBW larvae attack squares, flowers and bolls. Infested squares are usually shed and the flowers do not open. Boll infestation results in bad and premature opening with cotton in one or more loculi completely or partially damaged. Ginning percentage, oil content and seed viability are reduced appreciably.

However, PBW is a late season pest centering its attack on the bolls, damage to squares and flowers occur early in the season before the green bolls are formed. In the infested squares and flowers, the larvae mainly feed on anthers and style and after boring into the top of the ovary. As

a result, the squares shed and attacked flower buds do not open, showing a characteristic symptom of 'rosetted' flowers.

### **Rosette flowers**

The young larva after entering the flower bud web up the tips of the petals together around the time of blooming. These infested flowers do not open normally, but have the peculiar rosette appearance – hence known as rosetted flower. In the infested flower, the larva feed on anthers, style and rarely ovary. These flowers may drop or form bolls, which may be damaged by the larva to complete the development. Usually only one larva is seen in a flower.

### **Boll damage**

Bolls in all stages of growth / age are attacked, but most preferred are those one-half to three fourths grown (15-20 days old). More than one larvae per boll can be seen. Three to five per boll is common and as much as 30 larvae per boll has been recorded.

The neonate larvae on hatching enter the boll and entry hole may not be visible from the outer surface as it is extremely small. The larvae upon entering feed and burrow through the lint and reach the seed and mainly feed on immature seeds. In the young bolls, the larvae may destroy the entire contents, while in older bolls; they partially damage to bolls by making typical holes in the septa from one loc, to another loc. The excreta and the frass remain inside the boll itself. The partial damage to bolls leads to bad opening with hard locs. Through the entry hole, pathogenic fungi also enter which also stain the lint. Damage to bolls lower both quantity and quality of lint and seed. Having fed on the tissues of bolls the fibre length, strength, fineness, colour, seed germination and oil content are reduced, thus lowering the market value of the produce.

### **Management**

A satisfactory control of this pest could be achieved by adopting cultural, mechanical and chemical methods.

#### **Cultural**

Cultural control plays a key role in keeping down the number of PBW larvae carrying over between cotton crop

- After the harvest of the kapas, the stalks should be removed and disposed properly. If possible, cotton debris left in the fields should be collected and burnt.
- As long as the plants are carrying any fruiting bodies, the PBW will be able to continue multiplying so the crop must be terminated after harvesting of the kapas.
- Once the crop is harvested, the field should be deep ploughed to destroy any long cycle larvae in the soil and bury all the crop residues particularly fallen seed cotton and remaining bolls.
- Remove wild host plants growing around cotton fields during non- cotton season. Volunteer cotton growing in old fields from seed cotton that has germinated should be uprooted and destroyed. Alternate hosts must be destroyed.
- Acid delinted cottonseeds alone should be grown in areas where larval diapause is common.

#### **Monitoring**

From squaring to harvest, PBW moth activity, eggs and larvae should be monitored at least once in a week. Ideally, male moth activities are monitored with gossypure traps. 'Rosette' blooms are useful in determining the larval infestation in the field.

#### **Rosette flower**

The larvae remaining in the rosette flowers in the early period have to hand collected and destroyed. This will greatly the population buildup which will attack the developing bolls later.

#### **Chemical**

During the early stages of the cotton, insecticides should be used as little as possible to allow natural enemies to establish. When insecticidal sprays are needed for the control, great

care should be taken with regards to the choice of the insecticides. Many of the insecticides used for the control of PBW particularly the synthetic pyrethroids can lead to outbreaks of other secondary pests, which in turn may require chemical control. Use of synthetic pyrethroids is to be avoided in areas where, cotton leafcurl virus disease is problem as it causes resurgence of the whitefly, the vector of the disease. The insecticides viz., chlorpyrifos, quinalphos and synthetic pyrethroids (cypermethrin / alpha cypermethrin) are found effective.

Controlling effect of these insecticides has been most likely due to mortality of moths and the first instar larvae rather than the larvae, which have entered into the bolls. Hence, it is better to undertake the spraying in the evening hours.

### **Red cotton bug: *Dysdercus cingulatus*, *D. koenigi* and *D. similis***

Red cotton bugs also called as cotton stainers occur in all the cotton growing areas of our country. They are generally gregarious, bright red coloured and occur during peak bolling and bursting stage period. They are not serious pests but at time cause considerable damage to developing seeds impairing the seed viability as well as staining the lint leading to stained cotton.

#### **Host plants**

Red cotton bugs are primarily found on most of the malvaceae plants. Although it can live on other plants too. Among the secondary hosts, the kapas tree (*Cieba pentandra*) is the most important alternate host plant.

#### **Damage**

Both nymphs and adults, particularly the adults feed on the fully developed bolls making use of their long stylets. They bore the wall of bolls to feed on the seed. While feeding, the bugs leave the saliva as result of which the cotton fibres inside the bolls turn yellowish brown. As the lint gets stained due to their feeding, these bugs are called as cotton stainers. The feeding injury does not leave any external sign of damage, but the extend of damage becomes apparent only at bursting stage. Severe attack on young bolls (less than 20 day old) leads to boll shedding. When bolls above 20 days are attacked, their normal size is reduced than the healthy ones. The fibre gets weakened, remains attached to the boll wall as it opens to form a web rather than fluffing out. Feeding on seed reduces seed viability and weight. Poor seed quality affects the oil content also. The gregarious nymphs feed mostly on seeds and the deposition of excreta by them spoils the lint color.

In addition to these direct damages, during the feeding process, they also transmit the fungus *Ashbya (Nematospora) gossypii* into the green bolls with the salivary fluid. The development of mycelium of these fungi inside the boll leads to internal 'bollrot' which damages the whole boll leading to heavy loss.

#### **Stage of occurrence**

The extent of damage to the crop depends on the number of stainers present. They occur during the boll bursting stage and continue to remain till the final harvest of the seed cotton. After the harvest of the crop, they move to other host plants.

#### **Management**

In general, red cotton bugs are not serious of cotton. The insecticides used for the control of other insects keep them under check. If any specific control is needed, any one of the contact insecticides (endosulfan, quinalphos) needs to be applied.

### **Dusky cotton bug: *Oxycarenus laetus* and *O. hyalinipennis***

The lygaeid bugs also referred to as cotton seed bugs in the cotton during the bursting stage of the crop and they feed on seeds. They occur in all the cotton growing areas.

They are small, slender, sluggish insects with black or dark brown coloured membranous wings. They are gregarious in nature found on open bolls and produce powerful bad smell when crushed or disturbed. They may be carried from the field to the storage place along with the kapas.



### Damage

Both nymphs and adults can be seen in the burst bolls as well as in half opened bolls. They feed on the seed and do not damage the lint directly except by discoloration if they are crushed during picking or ginning. When the population is large, the seed weight is reduced to the tune of 15-20%. The seed germination is also impaired.

### Management

Usually it does not require any specific control measures. If needed contact insecticides used for the bollworm management reduces the pest population.

### Cotton stem weevil: *Pempherulus affinis*

This is a serious pest in certain cotton growing areas of Tamil Nadu and parts of Coimbatore and Salem districts in both winter and summer are affected. However, it has been reported in order states like Andhra Pradesh, Karnataka and Gujarat, no economical damage was noticed.

### Damage

In endemic areas, the damage to the crop may be high as 90-100% gall damage and plant mortality due to wilting upto 80% is not uncommon. The small female weevil usually lay eggs near the collar regions of the stem. The grub upon hatching feeds on the tissues of the stems by making circular tunneling around the stem leaving the dark intact. In the affected plants near the collar region, a small swelling called 'stem gall' may be seen. Sometime, the gall may not be visible, but when the bark is split open the typical circular tunnel like feeding injury will be visible clearly.

### Alternate hosts

In addition to cotton, it affects certain malvaceous plants such as abutilon sp., *Corchorus* sp., *Sida* sp., *Triumfetta rhomboids*, *Malvastrum* sp. and *Urena lobata*. Among these *T. rhomboids* reported to be most preferred alternate host plant. For the successful management of this pest both prophylactic and curative methods are to be adopted.

### Chemical control

Chemical control has to be adopted in endemic areas. As the pest in the early stages attack the crop when it is 15-20 day old, the initial first application of insecticides should be done during this period to kill the adult, which come for egg laying.

### Other beetle pests

The other important coleopteran pests which attack cotton are the ash weevil / grey weevil, *Myloccerus* spp. Which are commonly found on brinjal. The adults feed on the leaves and make typical notching type of feeding symptom. Nevertheless, the grubs when they feed on roots cause severe wilting of the whole plant. This damage usually appears during November- January in winter cotton in Tamil Nadu.

The shoot weevil *Alcidodes affaber* causes partial shoot wilting. The incidence is rather high on glabrous varieties and hybrids like DCH 32. The jewel beetle *Sphenoptera* also causes similar type of wilting to the plant.

The surface weevil, *Attactogaster finitimus* rarely causes damage during early vegetative phase of the crop growth.

### Precautions in the choice and application of insecticides

Many molecules such as imidachloprid, Thiomethoxam, acetamiprid, monocrotophos and many of the newer molecules that have hormonal action for growth promotion. The distinctiveness of genotypes could be modified due to their application. The crop is raised for recognizing distinct morphological characters and not for seed cotton yield and hence need to be only protected from such insect that affect early growth and establishment of the corp.

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## Perspectives of Seed Industry under Protection of Plant Varieties and Farmer's Right Act, 2001 and National Seed Policy 2002

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### National Seed Policy – 2002

Indian Agriculture has realized spectacular achievements in the past fifty years. Seed is the most important and critical input in agriculture on which the efficacy of other agriculture inputs is dependent. The seed sector has made impressive progress over the last three decades. The government of India had announced an National Agricultural Policy to be implemented during the Tenth and subsequent plans to achieve the objective of “Food and Nutritional Security” with emphasis on development / production / distribution of improved varieties / hybrids, global competitiveness of Indian seed sector and export promotions. To sub serve the agricultural policy an National Seed Policy was formulated and issued to meet opportunities and challenges in the seed industry.

The main objectives of the National Seed Policy are

- i. Provision of an appropriate climate for the seed industry to utilize available and prospective opportunities
- ii. Safeguarding of the interests of Indian farmers
- iii. Conservation of agro-biodiversity.
- iv. A regulatory system of a new genre with quality assurance mechanisms coupled with facilitation of a vibrant and responsible seed industry.

### National Seed Policy – 2002; Thrust areas in seed industries Varietal Development and Plant Variety Protection

Appropriate policy framework and programmatic intervention will be adopted to stimulate varietal development in tune with market trends, scientific- technological advances, suitability for biotic and abiotic stresses, locational adaptability and farmers' needs. An effective sui generis system for intellectual property protection will be implemented to stimulate investment in research and development of new plant varieties and to facilitate the growth of the Seed Industry in the country. The rights of farmers to save, use, exchange, share or sell farm produce of all varieties will be protected, with the proviso that farmers shall not be entitled to sell branded seed of a protected variety under the brand name, The rights of researchers to use the seed/ planting material of protected varieties for bonafide research and breeding of new plant varieties will be ensured. Equitable sharing of benefit arising out of the use of plant genetic resources that may accrue to a breeder from commercialization of seeds/ planting materials of a new variety will be provided. Plant Genetic Resources for Food and Agriculture Crop will be permitted to be accessed by Research Organizations of the Seed Companies from public collection as per the provisions of the 'Material Transfer Agreement' of the International Treaty on Plant Genetic Resources and the Biological Diversity Bill.

### Seed Production

Public Sector Seed Production Agencies will continue to have free access to breeder seed under the National Agriculture Research System. The state Farms Corporation of India and National Seeds Corporation will be restructured to make productive use of these organizations in the planned growth of the Seed Sector. Private Seed Production Agencies will also have access to breeder seed subject to terms and conditions to be decided by Government of India. State Agriculture Universities / ICAR Institutes will have the primary responsibility for production of

breeder seed as per the requirements of the respective states. Seeds of newly developed varieties must be made available to farmers with minimum time gap. Seed producing agencies will be encouraged to tie up with Research Institutions for popularization and commercialization of these varieties. Seed banks will be established for stocking specified quantities of seeds of required crops/ varieties for ensuring timely and adequate supply of seeds to farmers during adverse situations such as natural calamities, shortfalls in production, etc. Seed Banks will be suitably strengthened with cold storage and pest control facilities. Seed growers will be encouraged to avail of Seed Crop Insurance to cover risk factors involved in production of seeds. The Seed Crop Insurance Scheme will be reviewed so as to provide effective risk cover to seed producers and will be extended to all traditional and non-traditional areas covered under the seed production program. \

#### **Quality Assurance**

The National Seeds Board (NSB) will be established in place of existing Central Seed Committee and Central Seed Certification Board. The NSB will have permanent existence with the responsibility of executing and implementing the provisions of the Seeds Act and advising the Government on all matters relating to seed planning and development. All varieties, both domestic and imported varieties, which are placed on the market for sale and distribution of seeds and planting materials, will be registered under the Seeds Act. However, for vegetable and ornamental crops a simple system of varietal registration based on “breeders’ declaration” will be adopted. The Board will undertake registration of kinds/ varieties of seeds that are to be offered for sale in the market, on the basis of identified parameters for established value for cultivation and usage (VCU) through testing / trialling. Registration of varieties will be granted for a fixed period on the basis of multilocational trials to determine VCU over a minimum period of three seasons, or as otherwise prescribed as in the case of long duration crops and horticultural crops. Samples of the material for registration will be sent to the NBPGR for retention in the National Gene Bank.

#### **Transgenic Plant Varieties**

All genetically engineered crops/ varieties will be tested for environment and bio-safety before their commercial release, as per the regulations and guidelines of the Environment Protection Act (EPA), 1986. Transgenic crops/ varieties will be tested to determine their agronomic value for at least two seasons under the All India Coordinated Project Trials of ICAR, in coordination with the tests for environment and bio-safety clearance as per the EPA before any variety is commercially released in the market. After the transgenic plant variety is commercially released, its seed will be registered and marketed in the country as per the provisions of the Seeds Act. Transgenic varieties can be protected under the PVP legislation in the same manner as non-transgenic varieties after their release for commercial cultivation. If the seed or planting material is a product of transgenic manipulation, it will be allowed to be imported only with the approval of the Genetic Engineering Approval Committee (GEAC), set up under the EPA, 1986.

#### **Seed Distribution and Marketing**

For promoting efficient and timely distribution and marketing of seed throughout the country, a supportive environment will be provided to encourage expansion of the role of the private seed sector. Efforts will be made to achieve better coordination between state government to facilitate free Inter-State movement of seed and planting material through exemption of duties and taxes. A mechanism will be established for collection and dissemination of market intelligence regarding preference of consumers and farmers. A National Seed Grid will be established as a data-base for monitoring of information on requirement of seed, its production, distribution and preference of farmers on district – wise basis. Access to term finance from commercial Banks will be facilitated for developing efficient seed distribution and marketing facilities for growth of the seed sector. National Seed Board can direct a dealer to sell or distribute seeds in a specified manner in a specified area if it is considered necessary to the public interest.

### **Import of Seeds and Planting Material**

All imports of seeds will require a permit granted by the plant protection advisor to the Government of India, which will be issued within the minimum possible time frame. All import of seeds and planting materials, etc. will be allowed freely subject to EXIM policy guidelines and the requirements of the Plants, Fruits and Seeds (Regulation of import into India) Order, 1989 as amended from time to time. Import of parental lines of newly developed varieties will also be encouraged. All importers will make available a small sample of the imported seed to the Gene Bank maintained by NBPGR.

### **Export of Seeds**

Government will evolve a long term policy for export of seeds with a view to raise India's share of global seed export from the present level of less than 1% to 10% by the year 2020. Establishment and strengthening of seeds Export Promotion Zones with special incentives from the Government will be facilitated. A data bank will be created to provide information on the international Market and on export potential of Indian varieties in different parts of the world.

The Government of India trusts that the National Seeds Policy will receive the fullest support of State Governments/ Union Territory Administrations, State Agricultural Universities, plant breeders, seed producers, the seed industry and all other stakeholders, so that it may serve as a catalyst to meet objectives of sustainable development of agriculture, food and nutritional security for the population, and improved standards of living for farming communities. The National Seeds Policy will be a vital instrument in attaining the objectives of doubling food production and making India hunger free. It is expected to provide the impetus for a new revolution in Indian agriculture, based on an efficient system for supply of seeds of the best quality to the cultivator. The National Seeds Policy will lay the foundation for comprehensive reforms in the seed sector. Significant changes in the existing legislative framework will be effected accompanied by programmatic interventions. The policy will also provide the parameters for the development of the seed sector in the Tenth and subsequent plans. The progress of implementation of the policy will be monitored by a High Level Review Committee.

### **Perspective of Seed Industry under Protection of Plant Varieties and Farmer's Right Act, 2001**

In order to provide for the establishment of an effective system for protection of plant varieties, the rights of farmers and plant breeders and to encourage the development of new varieties of plants it has been considered necessary to recognize and protect the right of the farmers in respect of their contribution made at any time in conserving, improving and making available plant genetic resources for the development, it is necessary to protect plant breeders' rights to stimulate investment for research and development for the development of new plant varieties.

Such protection is likely to facilitate the growth of the seed industry which will ensure the availability of high quality seeds and planting material to the farmers. India having ratified the Agreement on Trade Related Aspects of the Intellectual Property Rights has to make provision for giving effect to Agreement. To give effect to the aforesaid objectives the Protection of Plant Varieties and Farmers' Right Bill was introduced in the parliament, and received the assent of the President of India on 30<sup>th</sup> October, 2001.

The main features of the *Sui-generis* system for protection of plant varieties and farmers rights are as follows.

This Act, called the Plant Varieties and Farmer's Rights Protection Act, extends to the whole of India and will come into force on such date as the Central Government may appoint by notification in the official gazette. Only varieties of such genera ecies, notified for this purpose by the Central Government from time to time will be covered for protection under this Act. The

Central Government shall establish a Plant Varieties and Farmer's Rights Protection Authority, consisting of a Chairperson and 15 members for the purpose of this Act. This Authority may appoint such committees of experts as necessary for the efficient discharge of the Authority shall be the Chief Executive of the Authority. The main functions of the Authority are ; 1) registration of plant varieties, 2) characterization and documentation of registered varieties, 3) documentation indexing and cataloguing of farmers' varieties, 4) providing compulsory cataloguing facility for all plant varieties from India and abroad, 5) ensuring seeds of all registered varieties are made available to the farmers, 6) collection of comprehensive statistics on plant varieties, and 7) maintenance of national register of plant varieties.

A new variety shall be registered if it conforms to the criteria of novelty, distinctness, uniformity and stability. Novelty means if, at the date of filling of the application for registration for protection, the propagating or harvested material of such variety has not been sold or otherwise disposed of by or with the consent of its breeder or successor for the purpose of exploitation of such variety for use as seed in India (earlier than one year) or outside India (earlier than six years in case of trees or vines. Or, earlier than four years in any other case). A new variety shall not be registered if it consists solely of figures or comprises solely or partly of geographical name. Every application for register shall contain a complete passport data of the parent lines from which the new variety or its propagating material has been derived. Every applicant shall along with application for registration make available to the Register such quantities of seeds of the new variety or its propagating material for the purpose of conducting tests to evaluate whether seeds of such variety or propagating material along with parental material conform to the standards as may be specified.

The certificate of registration shall be valid for nine years in the case of trees and vines and six years in the case of other crops and may be reviewed and renewed for the remaining period on payment of fees subject to the condition that the total period of validity shall not exceed (a) in the case of trees and vines, eighteen years from the date of registration; (b) in the case of extant varieties, fifteen years from the date of notification of that variety; (c) in order cases, fifteen years from the date of registration. Any person or group of persons or any government or non – governmental organization may on behalf of any village community stake a claim on the ground that such community or people have contributed significantly to the evolution of the plant variety or its propagating material which has been granted protection under this Act. The Authority, if satisfied, may grant compensation to be paid and make, appropriate direction regarding the distribution of such compensation.

Any person, group of persons (whether actively engaged in farming or not) or any government or non- government organization can or behalf of any village or local community in India, file in any centre any claim attributable to the contribution of the people of that village or local community in the evolution of any variety for the purpose of staking a claim on behalf of such village or local community. No registration of a plant variety shall be made under this Act in case where prevention of commercial exploitation of such variety is necessary to protect public order or public morality or human, animal and plant life and health or to avoid serious prejudice to the environment. Having regard to public interest, the central government may also denotify genera/ species, upon denotification. New varieties belonging to these genera/ species not be eligible for protection. Nothing contained in this Act shall prevent the use of any plant variety or propagating material registered under this Act by any person using them for conducting experiment or research, and also the use of a variety as an initial source of variation for the purpose of creating other varieties.

Nothing contained in this Act shall also affect a farmer's traditional right to save, use, exchange, share or sell his farm produce of a variety protected under this Act except where a sale is for purpose of reproduction under a commercial marketing arrangement. At any time, after the expiry of three years from the date of issue of certificate or registration of variety, any person interested may make an application to the Authority alleging that the reasonable requirements of

the public for seeds or propagating material of the variety have not been satisfied or that the variety is not available to the public at a reasonable price and praying for the grant of a compulsory license to undertake production, distribution and sale of the seed or propagating material of that variety. The Authority, if satisfied after giving an opportunity to the registered breeder of such variety to file opposition and after hearing the parties, may order such registered breeder to grant a license to the applicant upon such terms and conditions as it may deem fit. Any person/ institution who applies any false denomination to variety or its propagating material or provides false information in the application be punishable with imprisonment for not less than 3 months but it may extend to two years and with fine which shall not less than rupees fifty thousand but which may extend to rupees ten lakhs. More severe penalties have been provided for subsequent offences by the same offender.

#### **Farmers' right**

Farmers means any person who –

- I. cultivates crops by cultivating the land himself; or
- II. cultivate the crops by directly supervising the cultivation of land through and person; or
- III. conserves and preserves, severally or jointly, with any person any wild species or traditional varieties, or adds value to such wild species or traditional varieties through selection and identification of their useful properties.

This new law recognizes the farmers not just as a cultivator but also as a cultivator but also as a conserver of the agricultural gene pool and a breeder who has bred several successful varieties. The Act makes provision for such farmer's varieties to be registered, with the help of NGOs so that they are protected against being scavenged by formal sector breeders.

#### **Breeders' rights**

Breeder means a person or group of persons or a farmer or group of farmers of any institution which has bred, evolved or developed any variety.

Breeders' rights over the varieties they have developed are more than adequately protected by the draft legislation. On registration, the breeder has rights of commercialization for the registered variety either in his/ her own person or through anyone he designates. These rights include the right to produce, sell, market, distribute, import or export a variety, in short, full control over formal marketing.

#### **Rights of researchers**

The Bill has provisions for researchers' rights, which allows scientists and breeders to have free access to registered varieties for research. The registered variety can also be used for the purpose of creating other, new varieties. The breeder cannot stop other breeders from using his / her variety to breed new crop varieties except when the registered variety needs to be used repeatedly as a parent line. In that case, authorization is required.

The legislation provides for the granting or compulsory license to a party other than holder of the breeders certificate if it is shown that the reasonable requirements of the public for seeds have not been satisfied or that the seed of the variety is not available to the public at reasonable price, the authority shall determine the duration of the compulsory license granted but in any case the license can not exceed the total remaining period of the protection of that variety. Compulsory license however will not be awarded if the breeder can demonstrate reasonable grounds for his inability to produce the seed.

#### **Conclusion**

In India the seed production program is in the hands of organized and unorganized sector (farmers). In fact most of the farmers are resource poor and do not have experts hand in their command. They need technical and financial support to produce quality seeds.



The liberal farmer's right provided on the use of seed of protected varieties and Researcher's right provide in the Act may restrict the increased research investment from the private sector.

Transgenic crop has been released after thorough Bio-safety measures. The release and the distribution of this crop must be regularized through proper channel and with strict supervision.

The registration of new plant varieties by PVP authority will be based on the criteria of novelty, distinctiveness, uniformity and stability. This test will be conducted very precisely with much technical knowledge to avoid confusion. Varieties and hybrids which are unscrupulously released without being subject to DUS test will lead to considerable reduction in the targeted production causing insufficiency.

The private seed industry is playing a greater role in production and supply of quality seeds to the extent of 65.0%. It is worthy to mention both public and private sector have done good job in improving the production of the hybrids and vegetables accounting 50% of the total production of seeds in India. Private companies take lead in recent years in establishing the R&D with strong research base and greater availability and access to the germplasm. These companies are keen to use the emerging new technologies with high risk and investments. They are gearing up to the challenges with increased efforts on skill and knowledge and they need to be encouraged.

The public and private seed agencies are to be treated alike in availing levies and tax concessions, subsidies pertaining to production, distribution and financial support in maintenance of seed banks.

The Government and the private representatives should work in close coordination to meet the challenges in the seed industry for mutual benefits and this interaction between this two can put the seed industry on par with best in the western world.

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--- *End of the Manual* ---