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# GLANDED AND GLANDLESS COTTONS



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## GLANDED AND GLANDLESS COTTONS

### INTRODUCTION

Cotton refers to those species of the genus *Gossypium* which bear spinnable seed coat fibre. In the genus *Gossypium*, 50 species have been identified so far. Of these four species, viz. *Gossypium*, *hirsutum*, *G. barbadense*, *G. arboreum* and *G. herbaceum* are cultivated and rest are wild. Based on the presence or absence of gossypol glands, cotton genotypes are of two types, viz. glanded and glandless. Both types have their own merits and demerits. This bulletin deals with various aspects of glanded and glandless cottons.

### A. GLANDED COTTONS

Black dot like structures which are present in cotton leaves. Stem, calyx, bracts, boll surface etc. are called gossypol glands. Gossypol glands are found in the genus *Gossypium*, to which cotton belongs, and other related genera such as *Cienfugosia*, *Thespesia* and *Kikia*. Gossypol glands are also known as *lysigenous* glands, resin glands, dot glands, epidermal glands, oil glands and secretion cavities. These glands contain gossypol, a toxic phenolic compound which imparts dark colour to cotton seed oil and is toxic to non-ruminant animals including poultry. Gossypol can be removed by liquid cyclone process, from the oil but not from the cake or meal.

### DISTRIBUTION

Gossypol glands are found in all parts of cotton plant such as leaves, stem, calyx, bracts, corolla, style, stigma, ovary surface and pollen grains. In older plants, glands are also found in the phloem rays of the bark. The intensity of gossypol glands differs between different species or races of *Gossypium*. The highest density of gossypol glands, among cultivated species, is found in *G. barbadense*.

### STRUCTURE AND DEVELOPMENT

Anatomically, pigment glands are composed of *lysigenous* intercellular space in the form of large central cavity, containing yellow to orange oily substances surrounded by a single layer of flattened epithelial cells. In green tissues of the most *Gossypium* species, the epithelial cells are red or purple because they contain high level of *anthocyanins*. These glands are designated as *holocrine* glands, because excretion product is usually discharged by disintegration of group of internal cells. Excretion cells accumulate the excretion products but they do not transport them to the exterior. Most primary tissues lack symmetrical arrangement and secondary tissues may lose this during development.

The first sign of the pigment glands in the embryo appears soon after fertilization and it coincides with the differentiation of the embryo tissues. Well defined circles of cell mark the boundary of the developing glands. After a number of changes, the pigment glands are left as a large central cavity surrounded by layers of flattened cells. Within a few days after the development of glands, gossypol formation begins. Synthesis of oil starts after several days. Both oil and gossypol appear at increased rate from first to fiftieth day when the seeds are matured and bolls open.

Root and seed glands contain gossypol and *anthocyanins*, while those in other tissues contain *anthocyanins*, *queratin* and *quercetin* alongwith small amount of gossypol. Variation in the content of

these *terpenoids* among glands of different *Gossypium* species and tissues has been reported by various workers.

### SHAPE, SIZE AND DENSITY

The shape and size of gossypol glands on surface of various plants parts in upland and Asiatic cottons have been reported by various workers. The shape of gossypol glands is spherical on leaf, bract and ovary surface, elliptical on stem and stigma surfaces, and spherical and oval on calyx surfaces. The glands of elliptical shape are the biggest in size and those of spherical the smallest. However, “leaky” glands with diffused pigmented substances into surrounding tissues are found occasionally.

The density and size of gossypol glands vary in different parts of the plant. Variations are also observed between different species and races of cotton. The gossypol glands of *Gossypium barbadense* are more dark and conspicuous as compared to those in other cultivated species. However, there are races and varieties which are glandless in nature, partly or fully. Gossypol glands density per unit area and gossypol contents were studied in leaf, bracts, calyx, stigma and style and seed in 40 genotypes each of four cultivated species. The highest density of gossypol glands and gossypol content in seed were observed in *G. barbadense* (Table 1 a and b).

**TABLE-1: Range of gossypol gland density per unit area and gossypol content in different plant parts of four cultivated species of cotton.**

Plant parts	<i>G.hirsutum</i>	<i>G.barbadense</i>	<i>G.arboreum</i>	<i>G.herbaceum</i>
<b>(a) Gossypol gland density</b>				
Leaf	6.43-20.33 (11.73)	9.97-29.11 (17.55)	6.15-16.65 (10.52)	8.36-18.06 (13.33)
Bracts	0.33-1.46 (0.93)	2.18-7.31 (3.99)	0.45-2.32 (1.31)	1.98-4.10 (3.31)
Calyx	2.63-5.06 (3.80)	6.11-24.33 (14.88)	2.76-5.75 (4.30)	3.80-7.73 (5.32)
Stigma and Style	4.97-14.10 (9.53)	7.11-18.51 (11.33)	6.11-13.17 (9.83)	4.11-19.13 (11.23)
Ovary	24.70-59.93 (39.66)	56.35-85.35 (65.24)	30.11-82.26 (63.77)	41.44-80.60 (61.87)
Seed(L.S.)	9.37-18.09 (12.33)	9.57-24.44 (14.00)	6.10-19.17 (13.55)	8.62-17.21 (10.68)
<b>(b) Gossypol content (%)</b>				
Leaf	0.21-0.53 (0.39)	0.29-0.68 (0.47)	0.19-0.48 (0.33)	0.59-1.02 (0.83)
Bracts	0.10-0.33 (0.19)	0.20-0.49 (0.36)	0.18-0.52 (0.25)	0.14-0.42 (0.22)
Calyx	0.10-0.28 (0.13)	0.29-0.53 (0.48)	0.35-0.58 (0.46)	0.37-0.51 (0.44)
Stigma and Style	0.18-0.48 (0.30)	0.25-0.77 (0.39)	0.17-0.63 (0.45)	0.14-0.59 (0.47)
Ovary	0.10-0.95 (0.63)	0.33-0.86 (0.63)	0.28-0.65 (0.43)	0.32-0.60 (0.41)
Seed(L.S.)	0.39-1.05 (0.68)	0.73-1.49 (1.11)	0.30-1.25 (0.69)	0.58-0.79 (0.69)

Figures in parenthesis indicate mean.

## GENETICS OF GLANDED TRAIT

The glanded condition is a dominant trait which is governed by three dominant genes ( $G1_1$ ,  $G1_2$ , and  $G1_3$ ) with minor modifiers ( $G1_4$  and  $G1_5$ ). The amount of gossypol in cotton seed depends mainly on the combination of  $G1_2$  and  $G1_3$  alleles. However,  $G1_2$  is more active than  $G1_3$ . the seed gossypol level in  $G1_2 G1_2 / g1_3 g1_3$  is about twice as high as in  $g1_2 g1_2 / G1_3 G1_3$ . the gene  $G1_6$  is similar to gene  $G1_1$  (Table-2). The role of different genes in governing glanded condition is presented in Table-2.

**Table- 1: Genes controlling glanded condition in cotton.**

Genes / Loci	Main features/ effects
$G1_1$	Determines glands on stem, petioles and carpel walls.
$G1_2$ and $G1_3$	Determine glands on cotyledons, leaves, stems, petioles and carpel walls.
$G1_4$ and $G1_5$	Behave as modifiers and have minor effects on number and distribution of gossypol glands.
$G1_6$	Has similar effect as $G1_1$ .

## CHEMICAL NATURE

The predominant constituent of pigment glands is gossypol. Gossypol is a highly reactive, dimeric sesquiterpenoid, antioxidant, antipolymerizing, polyphenolic, biologically toxic group of related secondary plant metabolites known as terpenoid. Several workers have reviewed the role of gossypol, particularly with reference to insect resistance and proceeding quality of cotton seed.

## BIOSYNTHESIS

Recent studies have shown that gossypol is derived from mevalonic acid viz E.E. farnesyl diphosphate (EE FDP) Fig.1. The gossypol monomer, hemigossypol was identified in 1975 and its conversion to gossypol by peroxidase has been demonstrated. Desoxyhemigossypol was identified in the same year and its non-enzymatic conversion to hemigossypol has been carefully studied (Fig.1.). other intermediates have not been identified.

A cadinene has been proposed as the biosynthetic precursor of 2, 7 dihydroxycadalene and germocrone D has been proposed as an intermediate in the biosynthesis of S.candine. A 1,3 hydride shift has been established during the biosynthetic conversion of EE-FDP to candalene including gossypol. The conversion of EE-FDP to nerolidylidiphosphate is reasonable postulate. The role of NDP in the biosynthesis of cadalene has not been established, nor has the intermediary of germacrone D in the biosynthesis of delta cadinene been proven.

## ROLE IN PEST AND DISEASE RESISTANCE

In cotton, gossypol plays a dual role. On one hand, it confers resistance to insect pests and diseases while on the other hand its high concentration in seed is undesirable when used for human or animal consumption in different forms. Gossypol, a phenolic compound which is found in the gossypol glands of cotton, plays an important role in conferring resistance against certain insect pests and diseases. It has antiviral activity and confers resistance in cotton against tobacco budworm, cotton bollworm, spiny bollworm (*Earias insulana*) and cotton leaf worm (*Spodoptera littura*). It also confers resistance against root knot nematode (*Meloidogyne incognita*) and fungi *Verticillium dahliae* and *Fusarium oxysporum*. Gossypol confers resistance against insect pests by way of antibiosis. In other words, it imparts antibiosis type of resistance mechanism to the host plant.

It has been observed that high levels of bound gossypol in the meal definitely reduce the rate of growth in chicks, rats and swine. Gossypol reacts with essential amino acid lysine and reduces its absorption in the intestine.

In poultry, 0.1% gossypol content in the diet produces discoloured yolks if the eggs were to be stored over extended period of time. Symptoms of gossypol toxicity include depressed appetite and loss of body weight, and discolouration of yolk in egg laying hens. In broilers, tolerance levels of gossypol vary widely from 160 ppm to 1000 ppm. Increased levels of gossypol also lead to decreased hatchability of eggs.

In swine, the adverse effects of cotton seed meals are largely due to the presence of gossypol. The addition of iron to the ration minimizes gossypol toxicity in swine. Dogs showed no greater susceptibility to gossypol than cattle when fed quantities in proportion to their body weight. Similar doses when fed to pigs caused death on nineteenth day. Feeding experiments with rabbits and pigs revealed that within first few days rabbits gained weight but later on died.

The pigment glands are more toxic than pure gossypol. The iron gossypol combination is non toxic to mice. The cotton seed cake has long been highly valued as a protein rich concentrate for livestock (cattle and sheep). In fact, ruminants are capable of detoxifying gossypol present in the cotton seed cake effectively. In India, whole cotton seed has been a traditional cattle feed for centuries, without any adverse effects on the animal health.

In mammals, recemic gossypol (isolated from cotton seed) acts as an effective male antifertility agent (Chinese Report). In the humans, it leads to spermatocidal activity, menorrhagia and atrophy of the uterus. In rats and rabbits, dextro gossypol isolated from *Thespesia populinae* acts as an effective male antifertility agent (Indian Report). The effects of gossypol on animals are summarized below.

1. The oral toxicity of gossypol varies in rats which could be correlated with gossypol content analysed in the body of rats.
2. The pigmented glands are more toxic to rats than pure gossypol.
3. Repeated doses of gossypol at levels 10-200 mg/kg per day are fatal to dogs.
4. The acetone and water soluble fractions of cotton seed pigment gland are the most toxic material ever isolated from cotton seed.
5. The original pigment glands containing only 40% gossypol are double toxic than 90% pure gossypol.

6. Gossypol combination products are considerably less toxic than cotton seed pigment glands.
7. The sensitivity of various animals species to cotton seed pigment glands decreased in the order: guinea pig, rabbit, mouse, rat.
8. Gossypol was slightly more toxic when applied with oil than in water.
9. Various doses of pure gossypol fed in the diets of experimental animals caused proportionate reduction in the body weight of such animals.

### DELAYED MORPHOGENESIS

High gossypol provides natural defense against some insect pests and diseases. However, presence of gossypol in the seed makes the seed cake and protein unfit for human consumption. To solve both the problems, there is need to develop varieties which have high gossypol in vegetative and floral parts and gossypol free seeds. The presence of gossypol glands in leaf, stem and flower buds but their absence in the seed is referred to as delayed plant morphogenesis. This trait occurs in both *Cienfuegosia* sp. and in Australian wild cotton. The delayed morphogenesis of gossypol glands in embryo, is found in three wild species viz. *Gossypium bickii*, *G. australe* and *G. sturtianum*. These sources are being used at CICR, Nagpur to develop glanded plants with glandless seeds for imparting insect resistance and ensuring clean seed oil. Evaluation of cotton germplasm for gossypol in seed and plant parts at CICR, Nagpur has indicated existence of lines with low gossypol content in seed. Manipulation of these lines through appropriate breeding programmes would help in developing lines with nil or negligible gossypol content in the seed.

### B. GLANDLESS COTTON

The glandless cotton mutant was first identified in USA in 1959. Glandless cotton refers to those genotypes which are devoid of gossypol glands. Glandless cottons open new areas for enhancing the utility of cotton seed as they are potential source of good quality vegetable oil and protein for human consumption. A lot of work on glandless cotton has been done in USA and elsewhere. Both public and private sector breeders have developed a large number of strains and few varieties of these cottons. The first glandless strains tended to be low in yield and unsatisfactory for commercial production. Hence, work on glandless cotton was discontinued. Since cotton seed is important source of edible seed oil, glandless cottons are likely to receive more importance in the years ahead.

Geneticists have worked out of the inheritance of glandless character and demonstrated that by selecting for two major genes, plant breeders can produce gossypol free cotton genotypes. The glandless condition is governed by two pairs of recessive genes ( $g_{12}$  and  $g_{13}$  with some modifiers ( $g_{14}$  and  $g_{15}$ ). Glandless character enhances the use of cotton seed products viz. oil, meal or cake and protein. It also reduces the cost of proceeding. A brief account of the advantages of glandless cottons is given below.

### SEED OIL

Cotton seed oil contributes about 10% to the global production of edible vegetable oils. Its quality is superior to soybean oil in terms of stability, flavour and range of uses. The major disadvantage of oil obtained from glanded cotton seed is the dark colour derived from gossypol pigments. The oil of glandless cotton is acceptable in colour without expensive process of refinement. Thus, glandless cottons, as compared to glanded varieties, have several advantages.

In such cottons the costly refining process is eliminated which leads to reduction in the processing and refining costs. Glandless cottons leads to higher recovery of refined oil yield i.e. 95 % against 92 % obtained from glanded genotypes. The oil obtained from glandless cotton can be stored for longer period without discolouration (1q to 3 months).

### SEED MEAL OR CAKE

The cotton meal is primarily used as cattle feed. The feeding of cotton seed meal with excessively high content of free gossypol to non- ruminants produces effects ranging from undesirable to toxic. Its feeding to egg laying hens results in discolouration of egg yolks and reduced hatchability. Gossypol reacts with essential amino acid lysine and reduces its availability. Thus, glandless cotton seed meals can have a higher content of availability lysine. Moreover, it has been observed that glandless cotton seed meals are somewhat higher in metabolizable energy than meals produced from glanded varieties. The cotton seed meal from glandless varieties can safely be used in the rations of swine, poultry and other monogastric animals.

### SEED PROTEIN

Cotton seed is an excellent source of protein and is currently used as cattle feed. The presence of gossypol in the cotton seed meal limits its use to mature ruminant animals only. Glandless cottons provide a valuable source of quality protein to greater variety of animals and can be utilized as a cheaper source of protein for human consumption in developing countries. There is growing need of edible protein throughout the world. Although soybean protein is currently the predominant raw material for bakery products, protein from glandless cotton seed is equally nutritious and has the advantages of blend flavour and unique textural properties. The roasted kernels of glandless seed are used for human consumption in country like USA. The seed protein of glandless cotton genotypes can be safely used in baked goods and also as animals feed.

Much energy is currently used in the processing of glanded cotton seed to remove gossypol from the oil and meal fractions. It is estimated that as compared to glanded cottons, glandless seed can save 50% of the energy required in the extraction processes. Considering the rapidly increasing cost of energy, this could be a very significant potential advantage of glandless cottons.

### PEST MANAGEMENT

The available data suggest that certain insects, especially lygus, bollworms and budworms may find glandless varieties more attractive than glanded. Such cottons are attacked even by those insects which are not usually considered serious pests of cotton. For example, Japanese beetle, which is not a cotton crop insect, has been reported feeding on glandless genotypes. Other insects including boll weevil, do not appear to discriminate between the two types. Glandless genotypes are also damaged by rodents such as rats, rabbits and squirrels. In order to minimize the insect damage in glandless cottons following three measures should be taken up.

1. Growing of glandless varieties in those areas that are relatively free of the critical insect pests.
2. Breeding glandless seeded cottons with genetic resistance to insect pests.

3. Utilising integrated pest management systems involving genetic resistance, biological control, selected cultural practices and judicious use of chemicals in such a way to make it possible to produce glandless cotton on a competitive basis with glanded types.

### PURITY MANAGEMENT

As long as glanded and glandless varieties are grown in the same area it will not be possible to produce glandless seed completely free of contamination from glanded types. Contamination may occur in the field from natural crossing as well as from mechanical mixing during harvesting, ginning and processing including handling and storage operations. For maintaining the genetic purity in glandless cottons, the following measures are required.

1. Pure seed should be used for planting every year.
2. There should be sufficient isolation distance from glanded varieties to prevent natural outcrossing by pollinating insects.
3. Glanded plants should be thoroughly rogued out.
4. Harvesting and transportation machinery must be carefully cleaned before handling the seed cotton.
5. The gin should be thoroughly cleaned before use.
6. Storage of glanded and glandless types should be done at separate places. The contamination of glanded seed in no case should be more than 2-3%.

The use of glandless cotton seed will help in (1) getting higher recovery of refined oil, (2) saving considerable amount of energy required for processing, and (3) expanding the current oil and meal markets. In the long run, new high protein products from glandless cotton seed will offer additional market opportunities. Thus, the future economic health of the cotton seed industry should be greatly enhanced by moving to glandless cotton seed. However, purity and pest management will add to the cost of cultivation of glandless cultivars.