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GENETIC IMPROVEMENT OF COTTONSEED OIL

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1. Introduction

Refined cottonseed oil is one of the best edible oil, which is used in many parts of the world including USA, China, Uzbekistan, India and Middle East. Genetic improvement in the seed oil content without reduction in the lint yield will be an added advantage. Apart from fibre cottonseed is also an important source of edible oil. In U.S.A. it ranks third in volume, next only to soybean and corn oil with annual production averaging 1 billion pounds (4,50,000 tonnes approx.). Cottonseed oil is among the most unsaturated oils. Others include safflower, corn, soybean, rapeseed and sunflower seed oils (Chart 1). Cottonseed oil roughly has a ratio of 2:1 ratio of polysaturated to saturated fatty acids. It generally contains 70% unsaturated and 30% saturated fatty acids. In unsaturated group, it contains 18% oleic and 52% polyunsaturated linoleic acids. The oleic acid is monosaturated and linoleic acid is polysaturated fatty acid. The saturated fatty acids include palmitic and stearic acids. Cottonseed oil is one of the few oils considered acceptable for reducing saturated fat intake. Cottonseed oil is described by scientists as being “naturally hydrogenated” because of the levels of oleic, palmitic, and stearic acids which it contains. These make it stable frying oil without the need for additional processing or the formation of trans fatty acids. Another of Cottonseed Oil’s benefits is the high level of antioxidants-tocopherols (Table 1) that contribute to its long life in the cooker or on the shelf. Studies show that these natural antioxidants are retained at high levels in fried products, preserving their freshness and creating longer shelf life. Although the development of cottonseed industry had its beginnings with the invention of the cotton gin in 1793 which made the availability of large supplies of cottonseed possible, it was in the early twentieth century only that cottonseed was utilized in commercial quantities for production of oil and decorticated cottonseed cake. In India tradionally, the cottonseed utilization has been as an animal feed and it was in 1914 that the first cottonseed oil mill was established at Navsari by Indian Oil Company. Since than the industry has made rapid strides to establish for itself a place in the national economy. This bulletin deals with genetic improvement of cottonseed oil in terms of quantity and quality.

Table 1: Tocopherol contents in various edible Oils

Oil Crop	Table	Contents (mg/100 g)			Alpha-Tocopherol equivalent
		α	β	γ	
Canola	66	19	43	4	23
Corn	104	26	75	3	33
Cottonseed	65	35	30	-	38
Olive	13	12	1	-	12
Palm	26	6	11	9	8
Peanut	13	9	4	1	9
Rapeseed	67	22	19	26	24
Soybean	104	10	70	24	17
Sunflower	65	62	3	-	62

(Source: The National Cottonseed Products Association Guide to Edible Oils.)

1. Genetical Studies

In cotton, genetical studies have been limited mainly to yield contributing characters and fibre properties and a very little work has been reported on seed oil improvement. The genetical studies made on seed oil improvement related to variability, heterosis, combining ability and gene action, correlations and G x E interactions are briefly discussed below:

2.1 Genetic Variability

Genetic variability for cottonseed oil has been studied and reviewed by several workers in the distant past and more recently (Kohel 1978; Cherry et al., 1981; Dani 1990) in the genetic stock of all the four cultivated species. A wide range of genetic variability has been reported in four cultivated species. Kohel (1978) reported a wide range of variation (15-33%) for seedoil content in global collection of upland cotton. In Indian conditions also a broad range of variability has been reported (Table 2).

Table 2. Extent of variability for seed oil % observed in genetic stock of four cultivated species

Sr. No.	Species	Genotypes Studied	Extent of variability	Reference
1.	G.arboreum	337	125-22.9	Singh and Singh (1983)
2.	G.herbaceum	96	13.5-20.4	Singh and Singh (1983)
3.	G.arboreum	765	14.4-24.5	Singh (1988)
4.	G.barbadense	162	14.0-25.8	Singh (1988)
5.	G.hirsutum	914	12.9-29.8	CICR, 1989
6.	G.barbadense	242	14.0-25.8	CICR, 1989
7.	G.arboreum	149	15.1-23.4	CICR, 1989
8.	G.herbaceum	346	13.5-20.4	CICR, 1989
9.	G.hirsutum	176	10.2-26.1	Dani et al.,(1997)
10.	G.arboreum	79	14.8-25.8	Dani et al., (1997)
11.	G.hirsutum	-	16.1-24.4	Pande (1998)
12.	G.barbadense	-	17.9-25.6	Pande (1998)
13.	G.arboreum	-	14.6-22.1	Pande (1998)
14.	G.herbaceum	-	16.9-22.4	Pande (1998)

The extent of variability has been reported to be higher in tetraploid cotton than in diploid. The extent of variability observed in germplasm of four cultivated species indicated ample scope for genetical improvement of cottonseed oil through hybridization followed by directional selection.

The variability has also been observed for various saturated and unsaturated fatty acids (Valick and Zukalova 1978; Ermakov et. Al. 1979). In India, range of variability for different fatty acid profiles in cultivated cotton species has been studied by various workers (Table 3). Extent of variability witnessed in fatty acid profiles in above mentioned studies for all the cultivated cotton species could well be utilized in developing lines with high polyunsaturated and monosaturated fatty acid contents through appropriate breeding techniques.

Table 3. The range of oil content and fatty acid content in four cotton species

Sr.No.	Name of the species	Extent of variability				Reference
		Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	
1.	G.arboreum	23.1-25.9	2.3-3.4	20.8-26.3	41.1-50.6	Pande (1998)
2.	G.herbaceum	20.5-23.4	3.2-4.4	17.5-20.8	51.3-55.1	Pande (1998)
3.	G.hirsutum	23.1-28.0	2.4-3.4	14.7-20.9	47.6-55.4	Pande (1998)
4.	G.barbadense	24.4-25.5	2.6-3.0	18.7-19.7	50.0-51.7	Pande (1998)
5.	G.arboreum	8.90-21.2	1.1-2.9	16.5-30.7	30.3-59.3	Dani et al., (1997)
6.	G.hirsutum	8.83-24.4	1.2-4.5	10.3-30.2	20.6-58.0	Dani et al., (1997)

Some of the promising germplasm lines of cultivated cotton species for seed oil content evaluated at Central Institute for Cotton Research, Nagpur (CICR 1989) are given in Table 4.

Table 4. Seed oil Content and Oil Index in Different Cultivated Cotton Species

Sr. No	Name of the Germplasm lines	Oil %	Oil index
G.hirsutum			
1	ACALA-5-1	29.8	1.90
2	ALEPPO-1 X UGANDA 1-121-17-174	29.4	1.99
3	ACALA Q-6-1	28.0	1.90
4	ALEPPO-1 EMPIRE GLANDESS	27.3	2.98
5	B-58-1290	27.3	2.46
6	ACALA 1517-BR-2	26.6	1.90
7	X-82	26.6	2.31
8	5-44	26.5	2.09
9	561	26.5	2.33
Sr.No.	**Name of the Germplasm lines**	**Oil %**	**Oil index**
G.hirsutum			
10	Z 421	26.5	2.05
11	A 12	26.4	1.85
12	AC 6277-64-65	26.3	2.05
13	ACALA MORRELL	26.3	2.30
14	AMBASSADOR	26.3	1.80
15	M-4	26.3	2.42
16	AC 117	26.2	2.30
17	AC 130	26.1	1.77
18	ALEPPO 40	26.1	2.79
19	AC 69	26.0	1.98
20	A (59) 26	25.5	2.14
G.barbadense			
1	EC 97635	25.8	2.52
2	EGYPT-1	24.3	2.80
3	EC 98252	24.1	2.40
4	EC 97634	24.1	3.14
5	St.-Kitts	24.0	2.25
6	Marrad	23.9	-
7	5230	23.5	2.40
8	K-4831-56015	23.3	2.15
G.arboreum			
1	H 474	24.5	1.18
2	18 B	23.7	1.29
3	DESI 72	23.4	1.28
4	INDICUM-1290	23.3	1.15
5	VERUM 262	23.3	1.04
6	W-31-O-B	23.2	1.24

7	57-94	23.2	1.52
8	091	23.2	1.52
9	SARGUJA NL-WF	23.1	1.32
10	X-89	23.1	1.09
11	WESTRN BANI	23.0	1.24

Sr.No.	**Name of the Germplasm lines**	**Oil %**	**Oil index**
G.herbaceum			
1	LS EARLY 2	20.4	1.3
2	BALUCHISTAN	19.9	1.4
3	SUJ 4-3-3	19.6	1.2
4	179-1-10 p-1	19.4	1.2
5	369255	19.3	0.9
6	SEG 22-3-13	19.1	0.9
7	SEG 22-3-13-1	19.1	0.9
8	SM 4-3-135	19.0	0.9
9	72-13	19.0	0.9

Heterosis

The level of heterosis for seed cotton oil has been studied by various workers over mid parent, better parent and standard check (Dani 1984, Singh and Narayanan 1989,). The level of heterosis for this trait has been reported to be low to medium by various workers. Since, in India, about 50% of the cotton area is covered by hybrids even a 2-3% increase in seed oil content will lead to substantial increase in cottonseed oil production. The research work conducted so far on heterosis for oil content suggests only little hybrid vigour for the trait, but still hybrids owing to their higher productivity potential for cottonseed will eventually always give higher seed oil yield per hectare.

Combining Ability and Gene Action

Combining ability is an important measure of studying gene action. Combining ability for seed oil content has been studied by various workers (Dani 1991). Both GCA and SCA variances were found important in expression of seed oil content. This suggests that seed oil content is governed by both additive and non-additive gene action (Kohel 1980; Singh et.al. 1985; Ramos 1985). In a study by Ramos 1985, heritability estimates for oil content ranged between 40% to 53%.

Maternal Effects

Since seed and embryo size in cotton are determined predominantly by maternal parent (Van Heerden 1969), various workers have studied maternal effects as source of variation for seed oil content in cotton (Kohel 1980; Dani and Kohel 1989). Ji Xinag et al. 1995 reported maternal genetic effects to be more important than direct effect for seed oil percentage. They further suggested that varieties could be developed for high seed oil percentage by direct selection based on maternal plants.

2.5 Correlations

To bring a simultaneous improvement in oil trait alongwith fibre traits of economic importance, the direction and magnitude of association among these variables are to be worked out thoroughly so as to decide proper breeding tactics and selection procedures. Table 5 gives a brief account of association between seed oil content and various morphological and economic traits.

The correlation of seed oil content was found to be positive with earliness, seed size, okra leaf and long fruiting branches and negative with protein content and saturated fatty acid contents. Which suggested that improvement in seed oil content would result in some improvement in fatty acid profile of the seed oil.

Table 5. Association between seed oil content and various morphological and economic traits

Sr.No.	Oil Trait	Associated Trait	Relationship	References
1.	Oil %	Seed size, Lint yield, Fibre Length and Strength	Insignificant Negative	Turner et al., (1976)
2.	Oil %	Seed Size	Positive	Bourley (1982), Liu et al., (1994)
3.	Oil %	Seed Index	Insignificant Negative	Kohel (1980)
4.	Oil %	Seed Index	Independent	Malik and Baluch (1986)
5.	Oil %	Fibre Length	Positive	Rakhmanov et al., (1978)
6.	Oil %	Protein Content	Negative	Pande and Thejappa (1975), Chen et al., (1986), Malik and Baluch (1986)
7.	Oil %	Okra Leaves, Long Fruiting Branches	Positive	Liu et al., (1994)
8.	Oil %	Earliness, Lint Yield	Positive	Dani (1984)
9.	Oil %	Linoleic Acid	Positive	Gubanova (1989)
10.	Oil %	Palmitic, Stearic and Oleic Acid	Negative	Gubanova (1989)
11.	Oleic Acid	Linoleic Acid	Positive	Dani et al., (1997)

2.6 Environmental Interactions

A wide array of variability for seed oil content in cottonseed has been reported by various workers world-wide. This is a very interesting and useful phenomenon but with one bottleneck that a sizeable proportion of this variability for cottonseed oil content observed has its reasons beyond genetics. Climatic factors like rainfall and temperature, management factors like irrigation and mineral nutrition, biotic and abiotic stresses, and interaction of all these factors with genetic constitution of a line are known to affect the cottonseed oil content and quality. Several workers so far have studied the effects of these factors, which are presented in Table 6. some of the factors mentioned in these studies are uncontrollable and unpredictable and hence could not be manipulated, while rest of the factors particularly management aspects could favourably be altered so as to increase cottonseed oil % with high polyunsaturated fatty acid profile.

Table 6. Non-genetic causes affecting cottonseed oil content and quality

Oil Trait	Affecting Factor	Relationship	References
Oil Content	Rainfall	Positive	Hancock (1942), Dani et al., (1997)
Oil Content	Rainfall	Negative	Khan and Mian (1986)
Oil Content	Minimum temperature	Positive	Hancock (1942)
Oil Content	Seasonal variations		Kohel and Cherry (1983), Dani (1984, 1985), Dani and Pundarikakshudu (1986), Kashalkar et al., (1988),
Fatty acid Composition	Seasonal variations	Little effect	Dani et al., (1997)
Fatty acid Composition	Genotype X Location Genotype X Year		Turner et al., (1976), Cherry et al., (1978), Dani and Kohel (1987), Pande and Thejappa (1981)
Oil content and Polyunsaturated Fatty Acids	Irrigation	Positive	Dzhuraev et al., (1984)
Oil Content	Increased NPK Nutrition	Positive	Dzhuraev and Gubanova (1986), Madraimov (1984)
Oil Content	Trace Elements (Zn & Co)	Positive	Eweida et al., (1979), Kadyrov (1983)
Oil Content	Mite Infestation	Negative	Sadras et al., (1995), Sadras and Wilson (1996)
Oil Content and Unsaturated Fatty Acids	Cu and Mn Application	Positive	Sawan et al., (1993)

3. Breeding Approaches

Various breeding procedures viz. mass selection, pedigree method, backcross and mutation breeding have been used in the past for genetic improvement of seed oil content in cotton. Pedigree method was found to be very effective in improving seed oil content by 2 to 3% (Singh and Narayanan 1991). In Russia, India and China mutation breeding was found to be effective in improving seed oil content. The breeding methods for seed oil content in the past mainly focused on the following aspects:

i. Reduction in the portion of hull and linters in cotton seed and increase the kernel.
ii. Increase in cottonseed oil and protein content.
iii. Genetic enhancement of polyunsaturated fatty acid profile.
iv. Decrease or elimination of Gossypol content
v. Reduction in cyclopropene free fatty acids.

Various breeding schemes such as restricted selection (Cherry et al., 1981), complex crosses (Straumal 1981), gene introgression (Kyzalakova 1976) and mutation (Zhailov 1980; Bhat and Dani 1993) have been used for increasing seed oil content in cotton. Shroff (1976) induced naked seed genotypes in upland cotton, which were found to possess high seed oil content. The glandless mutant of upland cotton can be used for developing genotypes for obtaining gossypol free cottonseed oil that can be used for human consumption without refining (McMichael 1959). This glandless genotype was used by various workers for developing cotton genotypes with high seed oil content (Cherry et al., 1981; Kohel 1980; Ramos 1985). Dilday 1986 indicated possibilities of transferring glandless trait in the seed alone retaining the gossypol in other plant parts by introducing the concept of delayed moephogenesis for improving the seed oil quality.

4.0 Biotechnological Approach

Developments in gene manipulation techniques have provided new tools for generating novel plant phenotypic expressions otherwise not possible through conventional plant breeding techniques (MacKenzie, 1995). Herbicide tolerant and insect resistant transgenics have already made long strides in terms of achievements in several crops including cotton. Cotton being an oil yielding crop also offers tremendous opportunities where novel genetic engineering techniques could chip in with valuable contributions. Although conventional crop improvement techniques have resulted in increased oil contents in some of the advanced cultures, trends still suggested that oil content and quality cannot be enhanced to an appreciable extent using simple and direct selection among exotic genetic resources or their transfer to suitable agronomic background using conventional breeding methods.

4.1 Marker Aided Selection (MAS)

The advent of new molecular markers such as RFLP, CAPS, SSR, AFLP and RAPD has demonstrated that these could be used as a powerful selection tool for enhancing selection efficiency and curtailing time and resources involved ibn traditional selection procedures. In cotton using such markers, some possibilities have been expressed, aiming towards improvement of quantitative traits (Meredith, 1994). Altaf et al., (1997), based on a study of segregating patterns of molecular markers for identifying alien introgressions and economically important traits from wild species and rare collections of cotton.

4.2 Improvement in oil content and quality

As already mentioned developmental efforts through conventional genetic interventions have not been very remunerative in increasing seed oil content. The regulation of the partitioning of carbon in plant seeds is not understood. Thus, despite various research efforts, the diversion of carbon flow from carbohydrate to lipid has not yet been achieved (MacKenzie, 1995). Even a small increase in seed oil % would add a considerable value to an oil yielding crop. Any demonstration of such genetic manipulation through genetic engineering would be applicable to most of the oilseed crops. Improving quality of oil involves reduction of saturated fatty acid contents and there is a scope for biotechnology again. It could be possible with the further efforts to enhance the production of long chain polyunsaturated fatty acids with reduction in palmitic and stearic acid content, and an increase in oleic acid content for improving functional and shelf life properties.

4.3 Improvement in Functional Properties

Cottonseed oil is extensively used in food industry for several purposes but it is generally subjected to hydrogenation, which renders it stable during cooking at high temperature and, also softens and provide mouth feel in baked products. But hydrogenation leads to production of trans fatty acids also in some amount, which are health hazards and raise the cholesterol level in the blood. To produce naturally hydrognerated cottonseed oil free from trans fatty acids, scientists have reintroduced a small amount of cotton's own DNA into its genome to switch off the gene which converts monosaturates to polyunsaturates (ICAC Recorder, 2001). This results in cottonseed oil with high level of polyunsaturated fatty acid and, hence, avoiding any need for hydrogenation.

4.4 Development of Gossypol free Cotton

The annual world-wide cottonseed yield could supply the dietary protein needs of some 240-350 million people, but presence of Gossypol is a major deterrent. Ruminant animals could tolerate the Gossypol, but it is toxic to non-ruminants. If Gossypol were not present, cottonseed oil could be made more economically and cottonseed meal could be processed for food and feed. The use of glandless cotton could produce Gossypol free cottonseed, but then insect infestation would be a big menace. To keep Gossypol in plant but away from seed Ow (2000) proposed engineering the seed specific breakdown of Gossypol. He reasoned that Gossypol in cottonseed, like all organic matter must get recycled into the basic building blocks and that would mean there should be microbial enzymes that breakdown this compound. He identified an enzyme that could degrade the Gossypol, which ultimately led to the gene and construct for expression in plants. His group has been able to produce transgenics in which size and density of Gossypol containing glands were reduced in leaves and they have to further concentrate there work on getting the gene expressed in seeds, which would be the ultimate goal.

5. Future Thrusts

In cotton, several studies have been made on seed oil content related to variability, heterosis, combining ability and gene action, correlations etc. The future research work for improvement of cottonseed oil needs to be directed towards the following thrust areas:

i. The average seed oil content in the presently cultivated varieties and hybrids is about 20-22%. The seed oil percent in germplasm has been recorded upto 27%. Thus, there is a gap of 5-6%, which could be realized through appropriate breeding techniques.

ii. All presently cultivated cotton varieties and hybrids are glanded. The oil extracted from such varieties requires refinement to make it suitable for human consumption. There is ample scope to develop high seed oil lines with glandless trait in the seed and glanded vegetative parts to bring down the cost of cottonseed oil.

iii. In the presently available cotton hybrids and cultivars, the amount of oleic acid needs further improvement.

iv. The proportion of hull in the presently available cultivars and hybrids is quite high. There is ample scope to reduce the hull content and increase the kernel portion in the cotton genotypes.

v. The modern breeding techniques such as transgenic development, molecular breeding and marker aided selection may be rewarding in developing lines with high seed oil content.

vi. There is ample scope to incorporate through biotechnology Bt gene in glandless lines to enhance seed oil quality and productivity.

6. Suggested Further Readings

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