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# Conventional and molecular breeding approaches for seed oil and seed protein content improvement in cotton

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Cotton (*Gossypium* sp.) is the leading fibre crop in the world, and secondarily important source of vegetable edible oil and protein meals. Cotton seed meal is used principally as a protein concentrate feed for livestock. Moreover to flavor stability, cotton seed oil also has superior nutritive qualities; it has a 3:1 ratio of unsaturated to saturated fatty acids, which meets the recommendation of many health professionals. Cotton seed oil is rich in essential fatty acids such as palmitic and stearic (saturated), oleic and linoleic (unsaturated) acids. Several researchers reported cotton seed oil and protein concentration in cotton is controlled by multiple genes and is strongly influenced by the environment. Numerous studies indicated significant negative correlation between oil and protein content and also seed oil and protein content are predominantly controlled by non-additive gene action. In biotechnological features of cottonseed oil and protein; genetic mapping provides an essential tool to understand the genetic architecture of quantitative traits at the molecular level. Recently DNA markers linked to QTL controlling kernel oil percentage, kernel protein percentage and amino acids was identified in cotton. These detected QTL for seed quality traits in cotton are expected to be useful for future breeding programmes targeting development of cotton with improved oil and protein content. Marker assisted introgression and genetic transformation techniques will be used for transfer of specific genes/alleles would also undoubtedly increase the efficiency of cotton seed oil and protein content focused breeding programs in the future.

**Key words:** Cotton, molecular breeding, seed oil and seed protein

## INTRODUCTION

Cotton (*Gossypium* spp.) is an important fibre crop and plays a vital role as a cash crop in commerce of many countries. Cotton, also known as “King of fibres” plays a remarkable role in Indian economy. The cotton seed, which is byproduct, is an important source of edible oil. Cottonseed oil is cooking oil extracted from the seeds of cotton plant of various species, mainly *Gossypium hirsutum* L. and *Gossypium herbaceum* L. Cotton seed is the second largest source of vegetable oil in the world. The five largest producers (China, 27%; United states, 12% ; India, 11% former Soviet Union, 10%; Pakistan

9%) of cotton seed oil from 1995 to 2003 accounted for 70% of global output (Song and Zhang, 2007). The refined cotton seed oil is one of the best edible oil, which is used most of the world including USA, Uzbekistan, and China etc., Nowadays, the refined cotton seed oil was started to be used as an edible purposes in India and Pakistan.

Cottonseed oil is typically composed of about 26% palmitic acid (C16:0), 15% oleic acid (C18:1), and 58% linoleic acid (C18:2) Liu et al. (2002). The relatively high level of palmitic acid provides a degree of stability to the oil that makes it suitable for high-temperature applications, but is nutritionally undesirable because of the low-density lipoprotein cholesterol-raising properties

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of this saturated fatty acid (Cox et al., 1995). Linoleic acid, which is the most important one, is present to the extent of 51 per cent (Shaikh et al., 1996). Although cottonseed oil has recently been shown to lower total serum cholesterol compared with corn (*Zea mays*) oil (Radcliffe et al., 2001), it ensured thus by lowering the level of the desirable high-density lipoprotein cholesterol without reducing the level of the undesirable low density lipoprotein cholesterol, presumably because of its significant content of palmitic acid.

The processed cotton seed oil is the fifth leading vegetable oil in the world. Refined cotton seed oil is free from phenolic compound; gossypol and it can be directly used as cooking medium. Chemical analysis showed that by and large cotton seed oil and groundnut oil have similar physiochemical properties except free fatty acids which indicate the better keeping quality of cotton seed oil. Cotton seed oil is generally considered as healthy vegetable oil. It is cholesterol free and hence termed as "Heart oil". In India nearly entire cotton seed oil being utilized for edible purposes and mostly for Vanaspati, only small quantity (5-10 %) is used for manufacturing soaps (Ashokkumar, 2006). It has high level of antioxidants (Vitamin E) that contribute to its long life in the cooking or on the shelf. Breeding for the improvement of cotton seed oil has not made much progress. Marked differences were observed in oil composition between varieties within the species. There is urgent need to utilize such improved varieties of cotton seed oil in cotton cultivated countries for edible purposes. If we use the extra quantity for edible oil, it may fulfill global need. Further it is essential to have varieties/ hybrids with more oil content (25%) for oil extraction.

Cottonseed meal is left after oil extraction and used as a source of fodder protein in the livestock industry, but the sphere of its use in agriculture is limited. Constituting nearly half of a seed's weight, the meal contains 23% high biological-value protein. Limiting its more widespread use is the presence of gossypol which binds with the proteins. The digestibility of the protein is diminished and consequently, is its assimilability in the animal. For years, scientists have tried to breed cotton with gossypol levels safe for consumption. In the 1950s they succeeded, but because the toxin was missing from leaves as well as seeds, the plants proved defenseless against pests. With the help of a new technique called RNA interference, or RNAi, a gene-silencing mechanism succeeded in lowering the gossypol level in seeds only with minimum or no change in the rest of the plant (Ganesan et al., 2006).

In this situation the conventional and molecular breeding techniques have to use the evaluation of seed oil and seed protein content in all the species of cotton and developing varieties/hybrids with high seed oil and seed protein along with higher yield is have to essential requirements. The available literatures on cotton (*Gossypium spp.*) in respect of seed oil and protein

content have been presented below.

### Genetic variability of seed oil and seed protein

In the absence of conscious selection for seed quality components in the past, the levels of oil and protein in seeds of present day cultivars of cotton have remained largely unchanged over decades. Differences existing among cotton cultivars for seed oil and protein content are considered natural consequences of unselected characters, which can be improved through selection. Variation between plants has been shown to exert sizable influence on oil quality and quantity in some oil seed crops. In this condition an attempt was made to review the available information of variability studies in cotton seed oil and protein content were summarized (Table.1).

A study made by Pandey (1977) indicated the variability for oil content in improved strains of *G. hirsutum*. In his results oil content was ranged from 14.5 to 22.0 with mean of 19.2 per cent. Dani (1988) reported that seed oil percentage in  $F_1$ s was higher (21.20 – 26.30) as compared to the parents (21.48 – 24.16). Thiagarajan and Ramaswamy (1982) identified varietal variation and seasonal influence on the pattern of accumulation of oil and protein in the developing cotton seed. The authors suggested possible genetic improvement of seed oil content in diploid cotton through hybridization and selection.

Kohel et al. (1985) compared cotton germplasm collections for seed protein content and concluded that sufficient variability existed for seed quality. Dani and Pundarikakshudu (1986) investigated the changes in cotton seed protein and oil with repeated harvests. The studies showed that the varietal differences for seed protein percentage and protein content per seed were highly significant at second harvest. In spite of highly significant differences due to stages of harvest, the cultivar x stage interactions was not significant for percentage of protein in both *G. hirsutum* and *G. arboreum*. Selection among single cross derivatives would be more effective as compared to the progenies of double cross and back cross derivatives for improving the seed oil content (Dani, 1986). Dani and Kohel (1987) found significant difference in oil content per seed due to time of boll set in both high and low oil lines. Oil content showed a less increase in early set bolls, followed by more increase and finally a sharp decline in the boll set during the last two or three weeks in *G. hirsutum* L. Oil percentage was positively associated with lint yield, only in the bolls set during fourth week.

Disruptive selections for earliness resulted in simultaneous improvement in seed oil content and seed oil index besides seed cotton yield, boll number, halo length and ginning percentage as reported by Narayanan et al. (1988) in the upland cotton. Seed oil content estimated by Singh (1988) using Nuclear Magnetic Resonance was found to vary from 14 to 25.8 percent

**Table1.** Genetic variability of seed oil and seed protein content in cotton

Name of the Species	Oil/protein	Range (%)	Mean (%)	Remarks	References
<i>G. hirsutum</i>	Seed oil	14.5-22.0	19.2	-	Pandey (1977)
<i>G. hirsutum</i>	Seed oil	18.1-20.3	-	Winter season	
<i>G. hirsutum</i>	Seed oil	18.4-20.9	-	Summer season	Thiyagarajan and Ramasamy (1982)
<i>G. hirsutum</i>	Seed protein	15.1-18.9	-	Winter season	
<i>G. hirsutum</i>	Seed protein	14.5-19.8	-	Summer season	
<i>G. arboreum</i>	Seed oil	12.5-22.8	-	-	Singh and Singh (1983)
<i>G. herbaceum</i>	Seed oil	13.5- 20.4	-	-	Singh and Singh (1983)
<i>G.arboreum var nadan</i>	Seed oil	14.0- 25.8	-	162 germplasm lines	Narayanan <i>et al.</i> (1988)
<i>G. hirsutum</i>	Seed oil	33.0-42.0	-	-	Gubanova (1989)
<i>G. barbadance</i>	Seed oil	39.0-45.0	-	-	
<i>G. hirsutum</i>	Seed oil	21.5 -24.2	-	-	Dani (1988)
<i>G. barbadense</i>	Seed protein	-	32.7	300 accessions	Liu <i>et al.</i> (1994)
<i>G. barbadense</i>	Seed oil	-	40.9	300 accessions	Ming <i>et al.</i> (1994)
<i>G. hirsutum</i>	Seed oil	18.2-24.5	-	8 cultivars	Azhar <i>et al.</i> (1999)
<i>G. hirsutum</i>	Seed oil	11.4-16.0	-	12 genotypes	Preetha (2003)
<i>G. arboreum</i>	Seed oil	17.6 -19.5	-	6 races	
<i>G. lobatum</i>	Seed oil	22.8	-	wild species	Gotmare <i>et al.</i> (2004)
<i>G. stocksii.</i>	Seed oil	10.2	-	wild species	
<i>G. hirsutum</i>	Seed oil	19.3-21.0	18.8	4 cultivars	
<i>G. hirsutum</i>	Seed oil	17.7-22.1	20.0	7 accessions	Ashokkumar and Ravikesavan (2009)
<i>G. hirsutum</i>	Seed protein	19.4-27.7	24.0	4 cultivars	
<i>G. hirsutum</i>	Seed protein	20.8-26.9	23.5	7 accessions	
<i>G. arboreum</i>	Seed oil	14.4-18.7	-	9 cultivars	Sharma <i>et al.</i> (2009)
<i>G. hirsutum</i>	Seed protein	20.2-38.6	-	9 accessions	
<i>G. hirsutum</i>	Seed oil	27.5-30.2	-	6 cultivars	Khan <i>et al.</i> (2010)

in 162 germplasm lines of *G. arboreum* var *nadan*. Hybridization and directional selection of high oil lines was suggested for improving seed oil content. Gubanova (1989) reported that variation was greater in *G. hirsutum* (33-42 per cent oil) as compared to 39-45 per cent in *G. barbadense*, but the varieties with highest oil content belonged to *G. barbadense*. Bhale *et al.* (1989) observed variability in lint per seed, seed index and seed oil index were relatively higher in the oil cultivars L 147 and B 1007. Dani (1989b) suggested plant to row selection to more effective than bulk selection for the improvement of oil content and lint content. A mean protein content of 32.7 per cent was noticed among the 300 accessions analysed by Liu *et al.* (1994) in *G. barbadense*. Twenty two accessions have been identified to have higher protein content of 37 per cent. Protein content was relatively high in accessions with small seeds, normal leaves and short fruit branches. Series with highest protein content (33-52 per cent) were established among accessions of nine series introduced from different ecosystems. Ming *et al.* (1994) analysed 300 accessions of *G. barbadense* for oil content and found the mean oil content to be 40.99 per cent. He also suggested oil content

was relatively high in cultivars with large seeds, okra type leaves and long fruit branches.

Agarwal and Mukta Chakrabarty (2002), evaluated twenty nine advanced culture for seed oil content. They recorded Culture 3 HS (24.9) have highest oil percentage followed by 2 HS (24.4). Gopalakrishnan and Gururajan (2002) evaluated twenty genotypes and advanced germplasm lines for seed oil content. The highest seed oil content (25%) was observed in the SOCC 17 germplasm line. Supriya, Khandwa, MCU 5 and NME 70 possessed high oil content ranging between 22-25 per cent. They also observed GA 28 and GA 39 had higher oil content among the *G. arboreum* lines. In general, the *G. barbadense* germplasm lines had lower oil percentage.

Preetha (2003) studied four lines, eight testers and their 32 hybrids of *G. hirsutum* for seed oil content. Gotmare *et al.* (2004) observed a wide range of variability for seed oil content in the wild species and perennial races of *G. arboreum*. The highest seed oil content (22.89%) was observed in the wild species *G. lobatum* and the lowest (10.26%) was recorded in *Gossypium stocksii*. The content of seed oil and protein is

associated with the seed size that it has a considerable contribution to yield of the crop in cotton was reported (Pahlavni et al., 2008). Ashokkumar and Ravikesavan, (2009) studied four lines, seven testers and their 28 hybrids of *G. hirsutum* for seed oil and seed protein content. In this study results point out the hybrids showing high seed oil content had low seed protein content is decreased.

### **Correlation and path analysis studies for seed oil and seed protein**

Knowledge on genetic correlation between different characters is very essential for a plant breeder to design appropriate selection for crop improvement. The available literature pertaining to the association studies and cause and effect relationship have been reviewed here under.

Turner et al. (1976) investigated four *G. hirsutum* varieties grown at 17 sites in USA. Three varieties showed positive correlation between oil content and fibre maturity and negative correlation between oil content and percentage of immature seed. The strongest correlation was between protein content which was negative and significant with fibre quality. Genetic studies of seed oil in cotton by Kohel (1980) using parent progeny regression revealed that the heritability estimate for seed oil percentage was 35 per cent and showed the possibility of genetic improvement. Seed oil percentage was not highly correlated with seed physical properties such as seed index. Kohel and Cherry (1983) found that in *G. hirsutum* L. protein percentage increased with stratified harvests. The correlation between seed oil and protein indices have changed from a significant to a non-significant positive correlation ( $r = 0.35$ ) and to a non-significant negative correlation ( $r = 0.44$ ). A high negative correlation between seed protein and oil content was reported by Xiang et al. (1984). Six cultivars of *G. hirsutum* and two of *G. arboreum* were scored for seed quality traits by Malik and Baluch, 1986. According to them, oil content was positively correlated with kernel per cent but was independent of seed index. Hull per cent was negatively correlated with protein per cent.

Singh (1986) reported that seed oil improvement could be possible without affecting in the important characters, like seed cotton yield, ginning outturn, halo length and seed index. Seed oil content showed positive association with seed cotton yield and negative correlation with lint index in Asiatic cotton. Gubanova (1989) reported *G. hirsutum* varieties had high concentrations of palmitic acid, while oil content of *G. barbadense* varieties were correlated positively with linoleic acid but negatively with palmitic, stearic and oleic acids. Dani (1989a) from data on eight oil and fibre quality traits in six American genotypes and two Indian cultivars grown in 1984-86, indicated that the American lines were suitable for crossing to transfer high oil content to the Indian cultivars.

In the correlation study conducted by Dani (1991) for six seed oil and quality traits, he found that correlation between oil content and fibre quality traits were not significant. Gururajan et al. (1992) reported a negative correlation between embryo oil and protein per cent. Taneja et al. (1993) reported significant correlation between oil content versus seed index and protein whereas significant correlation was obtained between oil percentage and total phenol. Studies of Taneja et al. (1998) disclosed the negative correlation of oil content with harvest dates and its positive correlation with seed index. Azhamd and Azhar (2000) reported oil and protein content was positively related with other yield traits and this results also disclosed protein content was affected by number of bolls and boll weight is responsible for seed oil content. Rashmi et al. (2004) observed negative correlation between seed cotton yield and both fibre length (-0.093) and oil content (-0.087) shows weak unfavorable linkage needs to be broken.

Phlavani et al. (2008) reported strong linear relationship having between seed weight and oil content. In his results showed Seed weight provided a better indication of oil content than protein content. There are no considerable relationship between seed size and protein content of seed. There was a positive and significant correlation between seed weight and oil content ( $r=0.88^{**}$ ). Ashokkumar and Ravikesavan (2010) made correlation studies for seed cotton yield and seed oil content, and found that correlation between seed cotton yield and seed oil content were negative and not significant. Path analysis revealed that seed oil content was influenced by seed cotton yield negatively. Therefore, the result of this study indicate that sympodia per plant, boll weight, number of seeds per boll, lint index, seed index and 2.5 per cent span length were affecting seed oil improvement in cotton. It was concluded that these characters are considered as significant selection criteria for seed oil improvement in cotton.

### **Heterosis studies for seed oil and seed protein**

The superiority in expression of a character in hybrids over their respective parent is known as heterosis. It is the manifestation of increase in vigour as compare to the parental values. Existence of a significant dominance variance was reported to be essential for undertaking heterosis breeding programme. Earlier researchers' investigations have been carried out to find out number of hybrids with increased oil and protein content over their parents and also exact information on heterosis has been reviewed here under (Table 2).

Dani (1988) reported heterosis for oil percentage to be positive (0.92 – 16.49 per cent over mid parent averages) in most of the cases. Oil percentage and oil index of the  $F_2$ s indicated inbreeding depression. Ji and Zhu (1988) noticed positive heterosis of 4.57 per cent for oil content

**Table 2.** Effects of gene action in seed oil and protein content in cotton

Name of the Species	Oil/protein	Gene action	Mating design	No. of genotypes used	References
<i>G. hirsutum</i>	Seed oil	Non additive	L x T	16 genotypes	Boghra et al. (1985)
<i>G. arboreum</i>	Seed oil	Non additive	-	-	Singh and Narayanan (1988)
<i>G. hirsutum</i>	Seed oil	dominance , dominance X dominance, and additive X additive	Generation mean analysis	-	Dani and Kohel (1989)
<i>G. hirsutum</i>	Seed oil	additive x additive, and additive x dominance	Reciprocal	-	Dani, (1992)
<i>G. hirsutum</i>	Seed oil	additive	Diallel	8 parents and 56 F1hybrids	Azhar et al. (1999)
<i>G. hirsutum</i>	Seed oil	Non additive	Diallel	8 parents and 56 F1hybrids	Azhar and Ahmad (2000)
<i>G. arboreum</i>	Seed oil	additive	-	3 F1hybrids	Rashmi et al. (2004)
<i>G. hirsutum</i>	Seed oil	additive	Diallel	6 parents and 30 F <sub>1</sub> and F <sub>2</sub> generation	Khan et al. (2007)
<i>G. hirsutum</i>	Seed oil	Non additive	L x T	11 parents and 28 F1hybrids	Ashokkumar and Ravikesavan (2008)
<i>G. hirsutum</i>	Seed protein	Non additive	L x T	11 parents and 28 F1hybrids	Ravikesavan (2008)
<i>G. hirsutum</i>	Seed oil	Non-additive	Diallel	6 parents and 30 F1 hybrids	Khan et al. (2009)

and negative heterosis of -3.60 per cent for total amino acid content. Marginal heterosis in F<sub>1</sub> and inbreeding depression in the F<sub>2</sub> were evident for seed oil percentage and this was found to be influenced by maternal effects. A greater degree of positive heterosis (16 to 47.3 per cent) and inbreeding depression were also observed for seed indices. Additive effects for seed oil percentage were detected in progeny of high x low, low x high and low x low seed oil parents. Dominance effects were more important than additive effects in the inheritance of both seed index and seed oil percentage (Dani and Kohel, 1989).

Patterns of inter and intra seasonal variations were noticed in 10 elite cultivars. Dani (1989b), study showed a decrease in oil content at the third harvest in each year. Effects of years, cultivars, harvests and their two factor interactions were highly significant for oil content. A small increase in oil content in the F<sub>1</sub> over parents was recorded. Rakhmankulov (1991) reported that oil content of hybrids was usually high when both the parents had high oil content. Kapoor et al. (1994) reported that the range of seed oil content was higher in F<sub>1</sub> (16.42 to 24.7 per cent) than parents (5.49 to 23.80 per cent) of the 22 crosses evaluated 15 exhibited positive midparental

heterosis, 13 had heterobeltiosis and standard heterosis. In F<sub>2</sub> generation of the 10 superior heterotic F<sub>1</sub> combinations, inbreeding depression ranging from 3.7 to 17.2 per cent was noticed indicating limited scope for improving seed oil content in the commercial hybrids by selection. Resorting to special techniques like recurrent selection was also recommended from this study.

In a study of 11 intra *hirsutum* hybrids, their 17 parents and 2 controls by Varghese *et al.* (1995) suggested to select the hybrids based on mean values rather than extent of heterosis for seed oil content. Kowsalya et al. (1999) conducted an experiment using 40 cross combinations of *Gossypium hirsutum* to find out the magnitude of inbreeding depression in seed oil. Seed oil content in F<sub>2</sub> ranged from 18.15 per cent in MCU 7 x Q/6-1 to 24.60 per cent in MCU 9 x ISC 78. All the combinations showed inbreeding depression in F<sub>2</sub> generation with a mean value of 15.03 per cent for seed oil, indicating less scope for improving this trait. Genetic architecture of cotton seed oil was studied by Shanthi et al. (2000). Parents, F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub> and BC<sub>2</sub> of three crosses involving cytoplasmic male sterile lines TC CMS 4A, TC CMS 8A and JC MSBN and three restorers, December III (3), MEX 635-5 and Athens, were evaluated. The results

suggested that improvement in seed oil content might be accomplished by one or two cycles of recurrent selection followed by pedigree breeding.

Manimaran (2003) Studied 12 F<sub>1</sub> hybrids for seed oil content. Among the 12 F<sub>1</sub> hybrids the F<sub>1</sub> hybrids of H 26 X TCH 1641 was positive over midparent with a value of 9.26 per cent. The same hybrid H 26 X TCH 1641 also expressed positive heterotic effect over the better parent with a value of 7.98 per cent. Rashmi et al. (2004) Studied 3 F<sub>1</sub> hybrids for seed oil content, none of the F<sub>1</sub> hybrids showed significant superiority over mid-parent, better parent or standard check. Ashokkumar (2006) Studied 28 F<sub>1</sub> hybrids for seed oil and seed protein content in upland cotton. Among them the seed content has significant positive relative heterosis over mid parental value was displayed by eight hybrids. For seed protein content the twelve hybrids recorded significant relative heterosis ranging from 6.32 per cent to 35.40 per cent. Heterobeltiosis was significant positive in six hybrids and the range exhibited was from 6.22 per cent to 29.66 per cent. Standard heterosis was positive sixteen hybrids range was from 8.23 per cent in MCU 12 x TCH 1646 to 52.26 per cent in MCU 5 x SOCC 17 for seed protein content. Ganapathy and Nadarajan (2008) reported that hybrid combinations viz., Sahana x LRA 5166 and L 604 x MCU 7 exhibited significantly positive heterosis for all the traits including oil content and seed cotton yield.

### **Combining ability and Gene action studies for seed oil and seed protein**

The concept of general combining ability and specific combining ability was put forward by Sprague and Tatum in 1942 who demonstrated this phenomenon in single cross hybrids of Maize. Since then the concept has been extended moreover many crops and also many genetic models.

In a line x tester analysis involving 16 genotypes of *G. hirsutum*, Boghra et al. (1985) noticed predominance of non-additive gene action for oil content. Genetic analysis of seed protein content in cotton by Padmini and Singh (1985) disclosed that both *GCA* and *SCA* variances were significant in F<sub>1</sub> generation. Among the parents, Khandwa-2 registered highest positive *gca* effects and high per se value. Kohel (1987) reported significant specific combining ability for seed oil per cent, seed index and seed oil index in a diallel cross involving glanded and glandless lines. The parent glandless had the highest seed oil per cent. Analysis of combining ability by Rakhmanov et al. (1987) in F<sub>1</sub> intra specific hybrids involving *G. hirsutum* and *G. barbadense* varieties as parents disclosed that *G. barbadense* hybrids were the most promising, exceeding the better parent in oil content. Singh and Narayanan (1988) suggested non-additive gene action operating seed oil content.

In the study of combining ability for oil content fibre yield and quality in parents and hybrids by Dani (1989b) revealed that IC 794 was a good combining parent for oil index per seed and T3-11 was a general combiner for fibre quality. Singh and Narayanan (1991) suggested pedigree breeding for improving seed oil content and seed oil index in upland cotton without affecting the other economic characters. Reciprocal differences for seed oil content among F<sub>1</sub> and F<sub>2</sub> were reported by Dani (1992). He also reported dominance effects to be more important than additive effects for seed index and seed oil index. Dani (1993) further studies revealed significant reciprocal differences besides additive, dominance and epistatic components of variance. Dominance effects were important for seed index and seed oil index.

Preetha (2003) studied 32 F<sub>1</sub> hybrids of *G. hirsutum* for seed oil content. She also recorded six hybrids namely TCH 1569 x TCH 1002, TCH 1599 x KC 2, MCU 5 x Shana, MCU 9 x TCH 1002, TCH 1627 x Sumangala and TCH 1452 x KC 2 displayed significant positive *sca* effects, the values are being 2.472, 1.970, 1.705, 1.508, 1.407, and 1.105 respectively. Rashmi et al. (2004) studied 3 F<sub>1</sub> hybrids of *G. arboreum*. She suggested additive gene action operating seed oil content.

Ashokkumar and Ravikesavan (2008) studied 4 lines, 7 testers and their 28 F<sub>1</sub> hybrids of *G. hirsutum* for seed oil and seed protein content. We have noticed the best general combiners among the parents were F 776 and Surabhi for seed oil and MCU 5 for seed protein and the best specific combiners in the hybrids were Surabhi x TCH 1646 and Surabhi x F 1861 for seed oil and MCU 12 x TCH 1644 for seed protein content. In this study we have observed cotton seed oil and seed protein is predominantly controlled by non-additive gene action. Consequently, we have suggested that seed oil and seed protein was improved by heterosis breeding in upland cotton.

### **Molecular breeding**

#### **Molecular Markers**

The complex genetics and strong environmental effects hinder progress in seed quality trait breeding cotton species. The use of molecular markers can improve an understanding of the genetic factors conditioning seed oil and protein, and is expected to assist in selection of superior genotypes. Genetic mapping provides an essential tool to understand the genetic architecture of quantitative traits at the molecular level. DNA markers linked to quantitative trait loci (QTL) controlling seed protein content have been identified in soybean (Chung et al., 2003; Panthee et al., 2006), rice (Tan et al., 2001), barley (See et al., 2002) and field pea (Tar'an et al., 2004). DNA markers associated with loci controlling seed

oil content or fatty acid composition have been identified in soybean (Kianian et al., 1999), rapeseed (Zhao et al., 2006), sunflower (Bert et al., 2003; Pérez-Vich et al., 2004) and canola (Hu et al., 2006). However, currently only one result has been reported in cotton. Song and Zhang (2007), reported to identify quantitative trait loci (QTL) associated with seed physical and nutrient traits in cotton. They have used a population of 140 BC<sub>1</sub>S<sub>1</sub> lines developed from a cross between 'TM-1' and 'Hai7124' which was evaluated and linkage map consisting of 918 markers from this population was used to identify QTL using QTLNetwork-2.0 software. Eleven single QTL were identified for kernel percentage, kernel oil percentage, kernel protein percentage. He also suggested these QTL detected for seed oil and protein content in cotton is expected to be useful for further breeding programmes targeting development of cotton with improved seed oil and protein content. A recent study using chromosome substitution lines identified chromosome 4 of the 3-79 in a *G. barbadense*, introgressed TM-1 background, to be associated with seed oil, protein and fiber percentage (Wu et al., 2009). Badigannavar, (2010) studied the possibility of screening a broad array of germplasm for molecular marker associations with these traits using association/LD principles. He identified common markers (E4M3\_255, E4M3\_218, E6M2\_595 and E3M3\_60) governing seed oil and protein content in cotton. A significant QTL (qPP-D9-1) for total protein percentage was identified in a BC<sub>1</sub> population involving *G. hirsutum* and *G. barbadense* parents but it did not reflect large variations in protein components (Song and Zhang 2007). Marker assisted introgression and transfer of specific alleles would also undoubtedly increase the efficiency of seed quality focused breeding programs in the future.

### Genetically modified cotton

The selective breeding utilizing natural variants or induced mutations has been used to develop a range of improved oils in the major temperate oilseed crops, including high-stearic (HS) soybean (*Glycine max*; Graef et al., 1985), high-oleic (HO) rapeseed (*Brassica napus*; Auld et al., 1992), HO peanut (*Arachis hypogaea*; Norden et al., 1987), and HS (Osorio et al., 1995). Nevertheless, owing to a lack of any significant genetic variation for fatty acid composition in cottonseed oil and the allotetraploid nature of cultivated cotton, classical breeding techniques and induced mutagenesis have so far been unsuccessful in developing improved cottonseed oil. To overcome the limitations of conventional breeding approaches, genetic engineering techniques have now been successfully employed to modify the fatty acid composition in a number of oilseed crops. In particular, posttranslational gene silencing (PTGS) has been used to down-regulate the activity of the desaturase enzymes that control the synthesis of the major seed oil fatty acids, principally

stearoyl-acyl-carrier protein (ACP)  $\Delta$ 9-desaturase, which converts stearic acid into oleic acid, and oleoyl-phosphatidylcholine (PC)  $\omega$ 6-desaturase, which converts oleic acid into linoleic acid.

Liu et al. (2002) genetically modified the fatty acid composition of cottonseed oil using the recent technique of hairpin RNA-mediated gene silencing to down-regulate the seed expression of two key fatty acid desaturase genes, ghSAD-1- encoding stearoyl-acyl-carrier protein  $\Delta$ 9-desaturase and ghFAD2-1-encoding oleoyl-phosphatidylcholine  $\omega$ 6-desaturase. Hairpin RNA-encoding gene constructs (HP) targeted against either ghSAD-1 or ghFAD2-1 were transformed into cotton (*Gossypium hirsutum* cv Coker 315). His results showed that down-regulation of the ghSAD-1 gene substantially increased stearic acid from the normal levels of 2% to 3% up to as high as 40%, and silencing of the ghFAD2-1 gene resulted in greatly elevated oleic acid content, up to 77% compared with about 15% in seeds of untransformed plants. In addition, palmitic acid was significantly lowered in both high-stearic and high-oleic lines. Similar fatty acid composition phenotypes were also achieved by transformation with conventional antisense constructs targeted against the same genes, but at much lower frequencies than were achieved with the HP constructs. Through intercrossing the high-stearic and high-oleic genotypes, it was possible to simultaneously down-regulate both ghSAD-1 and ghFAD2-1 to the same degree as observed in the individually silenced parental lines. Liu et al. (2000) studied

Inverted-repeat-based gene constructs targeted against two key cotton seed-specific fatty acid desaturase genes, ghSAD-1, encoding stearoylacyl carrier protein  $\Delta$ 9-desaturase and ghFAD2-1, encoding microsomal  $\omega$ 6-desaturase, were transformed into cotton. In his results showed that expression of ghSAD-1 and ghFAD2-1 in the inverted-repeat orientation has increased levels of stearic and oleic acids, respectively and Interestingly, the content of palmitic acid in both high-stearic and high-oleic lines was substantially reduced. Similarly, Ganesan et al., (2006) successfully used RNAi to disrupt gossypol biosynthesis in cottonseed tissue by interfering with the expression of the delta-cadinene synthase gene during seed development. Therefore these materials offer the promise of developing cotton seed oil products with greatly improved nutritional demand to consumers.

### CONCLUSION

Cottonseed oil is an edible oil extracted from the seeds of cotton plant of several species, primarily *Gossypium hirsutum* and *Gossypium herbaceum*. Its fatty acid profile generally consists of 70% unsaturated fatty acids including 18% monounsaturated (oleic), 52% polyunsaturated (linoleic) and 26% saturated (primarily

palmitic and stearic). These fatty acids make it stable frying oil without the need for additional processing. Cotton seed meal is used to principally as a protein concentrate feed for livestock. Currently human population growth is rapidly increasing. In this condition we want to fulfill the consumer edible oil requirement. Therefore, cotton crop is primarily cultivated for fibre production in most of the countries in globally. In addition, cotton seed also have the sufficient amount oil and protein content. Cotton germplasm and cultivated accessions are having higher amount of seed oil and protein content. Hence, the conventional and molecular breeding methods are being important for developing variety or hybrids with high oil and protein content along with high fibre yield.

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