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Research Article

Unravelling seed oil, protein and fiber density in lintless-fuzzless upland cotton (*Gossypium hirsutum*. L) through combining ability estimates and association studies

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Abstract
An investigation was carried out to understand the gene action for seed oil, seed protein, seed gossypol, seed cotton yield and fiber density through intra-*hirsutum* crosses. Combining ability and gene action were studied in eighteen hybrids involving six elite varieties as lines (females) and three fuzzless *G. hirsutum* accessions as testers (males) in Line x Tester analysis. This study revealed the preponderance of non-additive gene action on all the traits studied including seed cotton yield. The elite general combiners identified were CO14 for seed oil and seed protein and CO17 and AKH98-81 for fiber per seed and fiber density. The best specific combiner was SVPR4 x AKH98-81 for seed oil, fiber density and seed cotton yield. A correlation study revealed a strong positive association of seed oil content, lint index, micronaire value, fiber per seed and seed fiber density traits with seed cotton yield. This study indicated the possibility of developing high-yielding hybrids with high seed oil, seed protein content and better fiber quality traits through heterosis breeding.

Keywords: Combining ability, correlation, fuzzless cotton, seed oil, seed protein, seed gossypol, fiber density, seed surface area

INTRODUCTION

Cotton is one of the most significant crops cultivated extensively around the globe, providing raw material for the textile industry. India is one of the world's top cotton growers, contributing about 23 per cent of the total production (COCPC, 2022). In comparison to other countries, the Indian textile industry primarily relies on cotton fibers, accounting for 60 per cent of its usage, while non-cotton fibers make up 40 per cent, in contrast to their 30 per cent usage in other parts of the world (Cotton sector, 2021). Lint fibers are more appreciated than fuzz fibers because they are lengthier and further uniform, which makes them easier to spin into strong and smooth high-quality yarns. (Desrochers and Szurmak, 2017). Fuzzless cotton refers to a type of cotton that has undergone spontaneous genetic changes aiming to decrease or eliminate the occurrence of fuzzy fibers on cotton seeds following the ginning process. Apart from being a vital source of fiber, cotton also provides a nutrient-rich by-product in the form of cotton seed, also recognised as "*World's Richest Seed*". Its oil content ranges from 14-24 per cent and it contains 15-26 per cent protein, depending on the species (Chakraborty and Mayee, 2010). Cotton seed is a significant source of edible oil and is the world's second-largest vegetable oil source. Upon reaching maturity, the cotton seed kernel contains relatively low levels of starch as an energy source. However, its remarkable ability to accumulate protein and oil makes it highly valuable. Unfortunately, there are physical barriers that impede the utilization of the seed, particularly the undesirable presence of cotton seed fuzz, also known as linters. The process of ginning, used to separate the cotton fibers from the seeds, is unable to effectively remove most of the fuzz from upland cotton seeds, thereby complicating their processing and further utilization (Maiti *et al*., 2012). According to chemical analysis, cotton seed oil and groundnut oil share similar physiochemical properties, except that cotton seed oil has a lower amount of free fatty acids, indicating better keeping quality (Ashokkumar and Ravikesavan, 2008). High levels of antioxidants, such as Vitamin E, contribute to the long shelf life of cotton seed oil (Niemiec, 2013). Another challenge associated with utilizing cotton seed as a protein and fat-rich food source for human and livestock consumption revolves around the presence of gossypol, a chemical compound containing terpenoid aldehydes that can be toxic when consumed in larger quantities (Sunilkumar *et al*., 2006). Reducing the amount of anti-nutritional factors like gossypol content in seeds helps to improve the acceptance of cotton seed oil and protein content. Thus, the present investigation is utilizing the scope of developing fuzzless varieties/ hybrids with high lint yield and also possessing desirable seed oil/ protein quality with minimal gossypol content.

In cotton breeding programs, it is of utmost importance to enhance the density of fibers and the number of fibers per seed, as these factors significantly impact the yield of lint. The weight of an individual fiber is determined by considering its length, uniformity ratio, and micronaire value (Clement *et al*., 2014). The density of fibers is determined by dividing the number of fibers per seed by the surface area of the seed. The ratio of fuzz to lint fibers per seed also plays a significant role in determining the commercial value of a cultivar. Hence, it is crucial to have a deep understanding of the genetic aspects related to the development of lint and fuzz, in order to breed varieties that yield high quantities of lint and with fuzzless. Such varieties would not only reduce ginning time and energy but also have desirable commercial attributes. The objective of this study is to explore the potential of naked seeded varieties in terms of their seed and fiber quality. Hybridization techniques were employed to gain insights into gene behaviour and combining ability, aiming to identify a suitable breeding approach for developing varieties with desirable traits such as high lint yield and fuzzless seeds. Additionally, the study also aims to enhance characteristics such as high seed index, seed oil or seed protein content, and reduced gossypol content.

MATERIALS AND METHODS

Parents phenotypically distinct for the occurrence lint and fuzz fibers were employed in the study (**Plate 1**), which included the utilization of nine genotypes of *G. hirsutum*, consisting of three fuzzless seed cultures *viz*., TCH1646, MCU5 Lintless and AKH98-81 obtained from Central Institute for Cotton Research, Coimbatore and six elite cultivars namely SVPR4, SVPR6, MCU5, MCU7, CO14 and CO17 obtained from various research stations of Tamil Nadu Agricultural University (TNAU).

Plate 1. Fiber initiation in early stages (10 Days after post anthesis) of boll development in linted fuzzy (MCU7) and lintless fuzzless (TCH1646) parents used in the study

The experiment was conducted at the Department of Cotton, Tamil Nadu Agricultural University, Coimbatore. A crossing block was established and a Line x Tester mating design was employed, where the fuzzless genotypes served as testers and the six cultivated varieties were used as lines. As a result, a total of 18 hybrids were generated during *Summer* 2022. These hybrids, along with the parents were evaluated during the *Rabi* 2022 in Randomized Block Design (RBD) with two replications with a spacing of 90 x 60 cm. Standard agronomic practices and plant protection measures was implemented throughout the crop growth period. The observations on seed and fiber traits including seed cotton yield were recorded in five randomly selected plants in each replication. The traits *viz*., cotton seed oil, cotton seed protein, seed gossypol content, seed index, lint index, seed cotton yield (g), micronaire value, fibers per seed, seed surface area (mm^2) and seed fiber density were recorded.

Representative samples of seed from parental genotypes and F_1 hybrids were collected and acid delinted for analysing the seed oil content by employing Near InfraRed Spectroscopy Analyser (Spectraalyzer Zeutec GMBH NIR Spectroscopy). Seed protein was estimated through total nitrogen content using the Microkjeldhal method suggested by Humphries (1956). Seed gossypol content was estimated using the spectrometric method suggested by Admasu and Chandravanshi (1984). The absorbance was taken at 550 nm and results were calculated and expressed as a percentage. Fiber quality trait, micronaire value was determined using HVI (High Volume Instrument) at the Department of Cotton, TNAU, Coimbatore. The Fibre per seed is calculated by dividing the product of the Lint index and 10,000 by the product of Length, uniformity index, and Micronaire.

Fiber Density was obtained by dividing Fiber Per Seed by Seed Surface Area, whereas seed surface area was calculated as follows, Seed Surface Area (SSA)= 35.74 + 6.59 x seed index (Clement *et al*., 2014). Analysis of variance for RBD and combining ability were calculated at 5% significance level. The gene action for seed cotton yield along with seed and fiber traits and general and specific combining ability effects of parents and hybrids were computed by Line x Tester analysis (Kempthorne, 1957). TNAU STAT software was used for Line x Tester analysis to compute gene action and combining ability. R (4.0.2) software was employed to carry out the correlation between the traits of interest using the packages “corrplot” and “RColorBrewer”.

RESULTS AND DISCUSSION

The mean sum of squares obtained was significant for all the traits studied indicating the existence of potential variability in genotypes employed in this study. Analysis of variance depicting the mean sum of squares for seed and fiber traits along with seed cotton yield is presented in **Table 1**. The genotypic variance was partitioned into hybrids, line, testers and line x tester interaction and was significant for all the traits under investigation. The estimates of GCA and SCA variances along with their ratio are given in **Table 2**. The variance components and GCA/SCA ratio highlighted the occurrence of non-additive gene action for seed and fiber traits studied including seed cotton yield. For all the studied traits, the GCA/SCA variance ratio obtained was less than unity indicating the predominance of dominance gene action. The GCA/SCA ratio manifesting less than unity was also reported by Gnanasekaran *et al*. (2019), Manonmani *et al*. (2020), Max *et al*. (2021) and Chakholoma *et al*. (2022) for seed index, lint index seed cotton yield and micronaire value whereas Ashokkumar and Ravikesavan (2008), Chaudhari *et al*. (2017) and Vaid *et al*. (2022) reported similar results for seed quality traits and seed cotton yield. In contrast, Varghese and Patel, (2020) reported additive gene action for seed oil content.

The proportional contribution of lines, testers, and line x tester interactions were evaluated for all the traits (**Table 2**). Results revealed that contributions from lines *ie*., fuzzy elite cultivars were higher for traits like seed oil content, lint index, seed cotton yield, and seed fiber density whereas no trait recorded upper hand contribution

Table 1. Analysis of variance for combining ability for cotton seed and fiber traits

Source	df	S.OIL	S.PRO	S.GYP	SI	LI	SCY	MIC	FPS	SSA	SFD
REPLICATION	1	0.0367	0.3600	0.0011	0.22	0.0001	1.11	0.0009	3304.6499	9.6514	10.9451
HYBRID	17	8.6779**	3.4717**	0.0145**	4.50**	0.91**	792.41**	0.50**	5420173.60**	195.4891**	603.0683**
LINES	5	19.7244**	2.0456**	0.0185**	7.01**	1.73**	2256.46**	0.33**	8138082.4279**	304.3976**	1028.2236**
TESTERS	2	15.095**	1.4304**	0.0055**	1.02**	0.36**	379.24**	1.01**	4518192.3042**	44.2656**	502.1987**
L X T INTERACTION	10	4.5883**	4.5929**	0.0143**	3.94**	0.61**	143.01**	0.48**	4241615.4452**	171.2795**	410.6645**
ERROR	17	0.2239	0.0952	0.0016	0.12	0.019	3.69	0.01	257094.7537	5.0084	30.6544

(S.OIL – seed oil content, S.PRO- seed protein content, S.GYP- seed gossypol content SCY- Seed cotton yield, SI- Seed index, LI- Lint index, MIC-Micronaire value, FPS- Fiber per seed, SSA- seed surface area, SFD- seed fiber density * and ** indicates significance at 5 % and 1 % level)

Table 2 Genetic variance and proportional contribution for cotton seed and fiber traits

	S.OIL	S.PRO	S.GYP	SI	LI	SCY	MIC	FPS	SSA	SFD
Variance of GCA	0.1839	-0.050	0.00	0.025	0.013	29.206	0.001	53003.94	1.088	8.65
Variance of SCA	2.1822	2.2488	0.0064	1.914	0.298	69.662	0.237	19992260.34	83.13	190.005
GCA/SCA	0.0842	0.0224	0.00	0.013	0.045	0.419	0.003	0.0266	0.013	0.045
Contribution of Lines	66.85	17.33	37.48	45.79	55.74	83.75	19.13	44.16	45.80	50.15
Contribution of Testers	2.05	4.85	4.44	2.67	4.60	5.63	23.91	9.81	2.66	9.80
Contribution of Line x Tester interaction	31.10	77.82	58.07	51.55	39.65	10.62	56.96	46.03	51.54	40.06

(S.OIL – seed oil content, S.PRO- seed protein content, S.GYP- seed gossypol content SCY- Seed cotton yield, SI- Seed index, LI- Lint index, MIC-Micronaire value, FPS- Fiber per seed, SSA- seed surface area, SFD- seed fiber density)

from the fuzzless testers. However, traits *viz*., seed protein content, seed gossypol content, seed index, microniare value, fiber per seed and seed surface area marked with higher contribution from line x tester interaction. Similar results were reported by Monicashree *et al*. (2017), Sulthan *et al*. (2018), Makhdoom *et al*. (2019), Thiyagu *et al*. (2019), Chapara *et al*. (2020), Gnanasekaran and Thiyagu(2021) and Max *et al*. (2021).

Among six lines and three testers, none of the parents were found to be good general combiners for all the traits. However, the line CO14 was found an elite general combiner for majority of the traits *viz*., seed oil content, seed protein content, seed gossypol content, seed index, seed cotton yield and seed surface area in the desired direction (**Table 3**). Among the testers, AKH98-81 recorded significant positive *gca* effects for seed index, lint index, seed cotton yield, fiber per seed and fiber density, whereas the line CO17 can be used as a general combiner in breeding programme as it recorded significant *gca* effects for seed oil content, seed cotton yield, along with fiber per seed and fiber density traits.

The lines namely, MCU5, CO14 and CO17 along with tester TCH1646 were found to be elite general combiners for seed cotton yield combined with one or more seed nutritional traits. For fiber density, CO17 and AKH98-81 were found significant along with seed cotton yield and fiber per seed. Only a few reports were found for traits combining seed and fiber quality such as Ashokkumar and Ravikesavan (2008), Munawar and Malik (2013), Solanke *et al.* (2015), Rehman *et al*. (2020), Varghese and Patel, (2020) and Vaid *et al*. (2022).

On evaluating the hybrids for specific combing ability effects among eighteen hybrids, SVPR4 x AKH98-81 (**Plate 2**) had high positive *sca* effects for traits *viz*., seed oil content, seed cotton yield, fiber per seed and

Table 3. Estimation of *gca* effects for cotton seed and fiber traits

PARENTS	S.OIL	S.PRO	S.GYP	SI	LI	SCY	MIC	FPS	SSA	SFD
					LINES					
SVPR4	-1.77**	-0.10	0.05**	0.95**	0.71**	-21.06**	0.01	1638.85**	6.28**	9.63**
MCU7	-1.67**	-0.13	-0.02	-0.83**	0.11	-13.30**	0.22**	-260.62	-5.48**	1.64
CO17	1.19**	-0.81**	-0.01	-1.40**	-0.08	22.48**	-0.37**	923.18**	-9.20**	19.54**
SVPR6	-1.30**	0.43**	0.03	-0.45**	-0.92**	-14.60**	-0.13**	-1723.85**	-2.95**	-15.37**
CO14	2.53**	0.87**	-0.10**	1.41**	-0.06	23.02**	0.02	-425.28	9.31**	-11.20**
MCU5	1.01**	-0.26	0.04*	0.31*	0.23**	3.46**	0.26**	-152.28	2.03*	-4.24
					TESTERS					
TCH1646	0.21	0.38**	-0.02	0.08	-0.23**	3.32**	-0.41**	-328.51*	2.08**	-5.05**
MCU5 LL	-0.41**	-0.30**	-0.00	-0.63**	0.15**	-5.17**	0.38**	-379.42*	-1.70*	-2.24
AKH98-81	0.20	-0.08	0.02	0.56**	0.08*	1.85**	0.03	707.92**	-0.39	7.29**
SE (gi)	0.29	0.26	0.02	0.21	0.08	1.27	0.07	352.96	1.41	3.77

(S.OIL – seed oil content, S.PRO- seed protein content, S.GYP- seed gossypol content SCY- Seed cotton yield, SI- Seed index, LI- Lint index, MIC-Micronaire value, FPS- Fiber per seed, SSA- seed surface area, SFD- seed fiber density * and ** indicates significance at 5 % and 1 % level)

Plate 2. **Phenotypes of seed depicting fuzz trait in SVPR4 x AKH98-81 cross. A) SVPR4 (female parent) B) F_1 hybrid C) AKH98-81 (male parent)**

fiber density along with high negative *sca* effect for seed gossypol content (**Table 4**). Five hybrids *viz.,* SVPR4 x AKH98-81, MCU7 x TCH1646, CO17 x MCU5LL, SVPR6 x TCH1646 and CO14 x MCU5LL had high *sca* effects for fiber density along with fiber per seed. On considering the seed nutritional traits, three hybrids *viz*., SVPR4 x AKH98-81, MCU7 x MCU5LL and CO17 x TCH1646 recorded significant *sca* effect for seed cotton oil while MCU5 x AKH98-81 recorded significant *sca* effect for seed protein content without compromising seed cotton yield. Similar investigations were also carried out by Ashokkumar and Ravikesavan (2008), Munawar and Malik (2013), Solanke *et al*. (2015), Rehman *et al*. (2020), Varghese and Patel, (2020) and Vaid *et al*. (2022).

The correlation study indicated a highly significant positive correlation between seed cotton yield and traits *viz*., seed oil content, lint index, micronaire value, fiber per seed and seed fiber density (**Fig. 1**). The positive association between seed oil and yield was highlighted in the study of Chaudhari *et al*. (2017) and the positive association of lint index, micronaire values where depicted by Monisha (2018) and Manonmani *et al*. (2019). However, a significant negative correlation with seed gossypol

Table 4. Estimation of s*ca* effects for cotton seed and fiber traits

S. No.	Cross Name	S.OIL	S.PRO	S.GYP	SI	LI	SCY	MIC	FPS	SSA	SFD
1.	SVPR4 x TCH1646	0.48	0.84**	-0.03	-0.77**	-0.14	-5.13**	0.08	-527.48	-5.06**	-0.42
2.	SVPR4 xMCU5LL	-1.93**	0.46	0.14**	0.75**	0.36**	1.25	0.62**	-812.12*	4.95**	-11.05*
3.	SVPR4xAKH98-81	1.44**	-1.29**	-0.11**	0.02	-0.22*	3.88*	-0.7**	1339.61**	0.11	11.47**
4.	MCU7xTCH1646	-2.01**	0.70**	0.09**	-0.50	0.31**	-2.68	-0.15*	917.53*	-3.28	12.01**
5.	MCU7xMCU5LL	1.73**	-1.53**	-0.12**	0.6**	0.08	8.16**	0.2**	-419.66	3.96*	-7.75
6.	MCU7xAKH98-81	0.28	0.83**	0.03	-0.10	-0.40**	-5.48**	-0.05	-497.87	-0.68	-4.26
7.	CO17xTCH1646	1.55**	-0.32	-0.04	1.7**	-0.88**	3.74*	-0.16*	-1422.53**	11.17**	-25.83**
8.	CO17xMCU5LL	-1.14**	-0.93**	0.05	-0.72**	0.62**	2.77	0.19**	1100.76**	-4.75**	15.70**
9.	CO17xAKH98-81	-0.42	1.26**	-0.01	-0.97**	0.25**	-6.51**	-0.03	321.77	-6.42**	10.14*
10	SVPR6xTCH1646	-0.43	0.30	0.01	1.57**	0.52**	9.4**	-0.25**	1624.51**	10.33**	9.11*
11.	SVPR6xMCU5LL	-0.14	0.43	-0.03	-0.79**	-0.51**	-12.87**	-0.01	-960.41*	-5.20**	-6.67
12.	SVPR6x/AKH98-81	0.56	-0.72**	0.02	-0.78**	-0.01	3.47*	0.25**	-664.10	-5.13**	-2.44
13.	CO14xTCH1646	-0.02	-1.98**	-0.02	0.16	0.21*	3.23*	-0.14*	760.67*	1.04	6.79
14.	CO14xMCU5LL	1.61**	2.69**	-0.05	0.25	0.04	2.61	-0.46**	1203.06**	1.62	9.87*
15.	CO14xAKH98-81	-1.59**	-0.71**	0.07*	-0.40	-0.26*	-5.84**	0.61**	-1963.73**	-2.66	-16.66**
16.	MCU5xTCH1646	0.42	0.46*	-0.01	-2.15**	-0.03	-8.56**	0.62**	-1352.69**	-14.19**	-1.66
17.	MCU5xMUC5LL	-0.14	-1.11**	0.02	-0.09	-0.60**	-1.93	-0.54**	-111.63	-0.58	-0.10
18.	MCU5xAKH98-81	-0.27	0.65**	-0.00	2.24**	0.63**	10.49**	-0.08	1464.32**	14.77**	1.76
	SE(ij)	0.3346	0.2182	0.0282	0.24	0.09	1.35	0.06	358.53	1.58	3.91

(S.OIL – seed oil content, S.PRO- seed protein content, S.GYP- seed gossypol content SCY- Seed cotton yield, SI- Seed index, LI-Lint index, MIC-Micronaire value, FPS- Fiber per seed, SSA- seed surface area, SFD- seed fiber density * and ** indicates significance at 5 % and 1 % level)

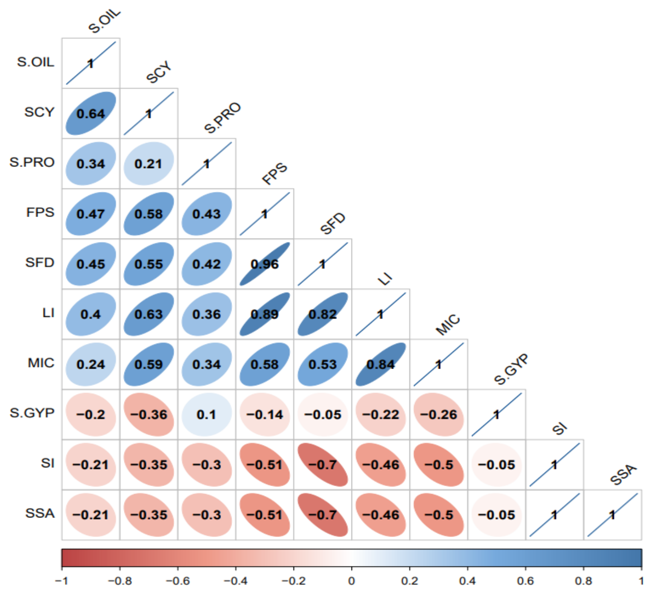

Fig. 1. Genotypic correlation between seed cotton yield with seed and fiber quality traits
(S.OIL – seed oil content, S.PRO- seed protein content, S.GYP- seed gossypol content SCY- Seed cotton yield, SI- Seed index, LI- Lint index, MIC-Micronaire value, FPS- Fiber per seed, SSA- seed surface area, SFD- seed fiber density and Blue and red indicates positive and negative correlation respectively.)

content, seed index and seed surface area was observed. Seed oil content also exhibited a negative correlation with seed gossypol content, seed index and seed surface area (Zeng *et al*., 2015).

This research emphasized the dominant role of non-additive gene action in the control of all the traits examined. Despite the successful development of numerous high-yielding cotton varieties and hybrids, it is now crucial to explore the potential benefits of cotton seed by-products through the concept of 'Mining above the Ground'. Given that cotton seeds account for over 66 per cent of the total weight of the cotton plant, it makes economic sense to efficiently utilize these seeds to maximize the overall output of the cotton crop. Furthermore, there is a significant demand for enhancing the fiber traits of cotton seeds. In light of this, it is recommended to incorporate breeding programs targeting both seed and fiber traits, in addition to seed cotton yield. From this study, the parental varieties CO14, CO17, and AKH98-81 show promise and can be considered for such breeding programs. Notably, the hybrid SVPR4 x AKH98-81 demonstrated favourable performance in terms of the investigated seed and fiber traits, as well as seed cotton yield, making it a suitable candidate for further exploration in heterosis breeding.

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