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Full Length Research Paper

Inheritance of okra leaf type in different genetic backgrounds and its effects on fibre and agronomic traits in cotton

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Okra leaf (L^0L^0) cottons confer resistance/non-preference against insect pests. The inheritance of this trait was studied in three cross combinations involving a common okra leaf parent (HRVO-1) with three normal leaf (l^0l^0) parents (FH-1000, CIM-446 and Acala 63-74). The non-significant χ^2 in F_2 for leaf shapes in all the crosses fit well against the theoretical monohybrid ratio of 1:2:1 showing incomplete dominance. Observations of 1 okra leaf (L^0L^0) : 2 an intermediate class of sub-okra (L^0l^0) : 1 normal leaf (l^0l^0) were observed in the F_2 populations of the three crosses. In the backcross generations with parent-I, ratios of 1 okra leaf : 1 sub-okra, leaf morphologies were obtained. Similarly, in the backcrosses with parent-II, ratios of 1 normal leaf : 1 sub-okra, an intermediate class of leaf shape were observed. The discontinuous variation for leaf shape in F_2 generations of three crosses observed in the frequency distribution confirmed the qualitative inheritance for this trait. The incorporation of the gene for okra leaf type had no significant effect on the major fibre quality attributes like fibre length, fibre strength, lint percentage, fibre uniformity ratio and fibre fineness. Number of sympodial branches/plant, number of bolls/plant, boll weight and seed cotton yield/plant principal yield assuring traits showed improvement, with the incorporation of the gene for okra leaf type. The gene for okra leaf type can be incorporated in a genotype that covers the insect non-preference and yield enhancements without increasing number of monopodial branches. The justification for the increase in yield and its components is due to the okra leaf morphology conferring resistance/non-preference against insect pests with reduced leaf area allowing better air flow and maximum sunlight penetration through the leaves of the plant.

Key words: *Gossypium hirsutum* L, trichomes, inheritance, fibre traits, agronomic traits.

INTRODUCTION

Insect pests constitute a major factor in hampering the cotton production all over the cotton growing areas of the world. In recent times, insect control has been mostly based on the use of chemical insecticides. During the 1970's and 80's the use of insecticides increased tremendously in almost all cotton producing countries of the world including Pakistan. According to an estimate, about 122.3 million US dollars were spent on the import of insecticides during 2010 to 2011 (GOP, 2010 to 2011).

Extensive use of pesticides is causing damage to soil quality and fertility as well (Chowdhury et al., 2008). Moreover, continuous prevalence of cotton insect pests during the season reduces yield and impairs the fibre quality of the crop (Arshad et al., 2001). Genetic

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resistance in the form of resistant varieties is an effective mean to minimize yield losses caused by insect pests and also leads to reduce the use of insecticides (Morse et al., 2005). The environmental concerns demand cotton production free from insecticides.

Nature has provided cotton with certain insect non-preference traits. Among these morphological traits, okra leaf trait is characterized by deeply cleft and narrowly lobed leaves with less leaf surface area than normal cotton leaf. Okra leaf types were proposed as modified leaf types suppressing whiteflies (Chu et al., 2002). According to Wilson et al. (1991) the okra leaf isolines had 76% as much damage as that of normal leaf strains. There was 41% reduction in the insecticide usage against the pink bollworm attack in the genotypes with okra leaf trait. The damage to cotton plants by the insect populations of whitefly, jassid and thrips was also found limiting as confirmed by Bhatnagar and Sharma (1991).

In upland cotton, okra and normal leaves are two major types. Okra leaf is a deeply lobed leaf shape which is a monogenic trait governed by incompletely dominant (L^0) to normal leaf gene (l^0) in the upland cotton. The hybrid of normal × okra leaves was intermediate leaf shape between the two phenotypic extremes, which indicated the incomplete pattern of inheritance. In the Acala types of *G. hirsutum*, the action of three allelic genes for leaf shape was compared as normal (broad), okra (narrow), and super okra. The leaf of the hybrid of normal × okra was intermediate (Niles, 1980). The gene for narrow okra leaf is controlled by L_0 (Endrizzi et al., 1984). Whereas, okra leaf type trait belongs to an allelic series having a minimum of five members: L^0 (okra), L^s (super okra), L^e (sea island), L^u (sub okra) and l (normal) (Andries et al., 1969). The expressivity of L^0 for okra leaf type was studied by Rahman and Khan (1998) in F_1 and F_2 generations of different genetic backgrounds, by involving HR-Velvet okra with other broad leafed varieties. There are some traits for which the quantitative method of measurement can not be applied. Instead, this including leaf shape can be measured on phenotypic basis by using the visual rating system (Rahman and Khan, 1998; Frelichowski et al., 2005).

Okra leaf type cottons are not commercially grown extensively. Ulloa (2006) has also advocated the genetic potential for improvement in agronomic traits in the populations with the okra leaf morphology. From industrial point of view, agronomic and fibre quality traits hold a key position. Breeding through conventional tools has not lost its significance even in the presence of modern tools of genetic engineering. Keeping in view, the extensive use and consumption of cotton and its products, there is a need to incorporate the gene for okra leaf type into promising cotton genotypes, which in turn will not only minimize the possible insect pest attack but also reduce insecticide usage. This will contribute towards better yield and fibre quality attributes. Limited studies have been conducted to understand the effect of okra leaf type on yield and fibre quality improvement in upland cotton.

Okra leaf morphology can be utilized to control the insect population. In view of the merit of okra leaf over the normal leaf with respect to its insect non-preference, the study was designed to transfer the gene of okra leaf (L^0) into three normal leaf upland cotton cultivars through conventional means in order to get information on the pattern of inheritance for this trait and to evaluate its effect on different agronomic and fibre traits of cotton.

MATERIALS AND METHODS

Three normal leaf cotton genotypes (Acala 63-74, FH-1000, CIM-446) and one okra leaf type genotype (HRVO-1) were selfed for four generations by growing two generation in a year to maintain purity in a glasshouse and field during 2003 to 2004. The parents were planted in 30 × 30 cm earthen pots, in a glasshouse during November, 2004. In the glasshouse temperature was maintained at 30 ± 2°C during the day and 25 ± 2°C at night by using built in steam heaters. The plants were exposed to natural sunlight supplemented with artificial lighting, for a photoperiod of 16 h (ICAC, 2007). Three normal leaf cotton genotypes, mentioned earlier were crossed to okra leafed parent (HRVO-1) during February through March, 2005 to obtain fresh seed for planting F_1. The F_1 and their parents were sown during the normal crop season of 2005 to 2006. The seed of the F_1, F_2, BC_1 and BC_2 generations was produced for each of the four combinations through manual selfing and crossing. The F_1 plants of each cross were divided in three groups for developing BC_1, BC_2 and F_2 for each combination. The experiment in the field was laid out in a randomized complete block design with three replications for each set of the six generations of the three crosses. A single plot (4.5 × 0.75 m) was assigned to each of the parents and their respective F_1 in each replication while, four rows in each replication were assigned to each of the backcrosses and eight rows were assigned to raise the F_2 population of each cross. The seeds of each of the six generations of the three crosses was dibbled, maintaining 15 plants in a row spaced 30 cm within the row and 75 cm between the rows during the normal crop season of the year 2006. The research was conducted at the experimental area of the University of Agriculture, Faisalabad Paksistan. During the crop season, a total of 346.3 mm precipitation was received and the rest of the water requirement was met through canal irrigation. The experimental area has sandy loam soil profile with pH level of 8.5. Prior to planting of cotton, chickpea trials were harvested from the same field. The experimental field was fertilized with N-P-K at 100-75-00 Kg/ha. Irrigation both by canal and turbine water was applied to the experimental material with an interval of 7 to 10 days. All other agronomic and cultural practices were kept uniform to minimize the experimental error. Ten plants were selected randomly from the parents and their F_1 while, fifty and thirty plants in each replication were selected in F_2 and backcrosses to record the data during 2006 to 2007.

Rating system for leaf shape

In order to classify leaf shape, a qualitative system of classification including a visual rating of leaf shape was used. Leaf shape was categorized into, broad normal leaf (grade-I), sub-okra (grade-II) and narrow okra (grade-III). Data for leaf classification were recorded in accordance with the given categories (Fig**ure** 1).

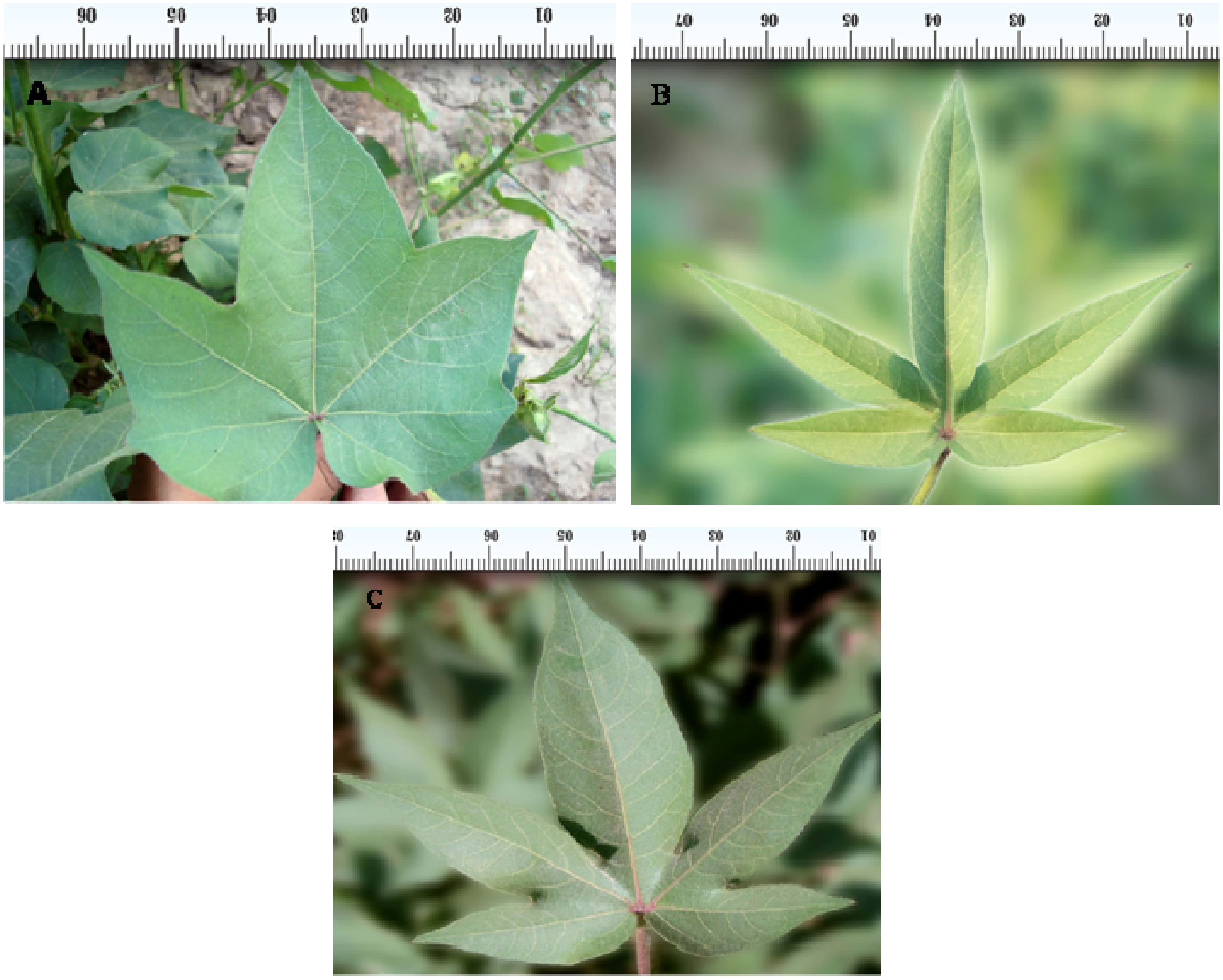

Figure 1. Variable classes in leaf types (a) Normal leaf (b) Okra leaf (c) Sub-okra leaf.

Agronomic and fibre traits

At maturity the data on various agronomic traits viz; plant height, number of monopodial branches, number of sympodial branches, number of bolls, seed cotton yield per plant and boll weight were recorded. Seed cotton was hand picked from the mature bolls in two pickings. Fibre quality characteristics like fibre length, fibre fineness, fibre strength, fibre elongation percentage, lint percentage and fibre uniformity ratio of each plant of a generation were measured using spin lab high volume instrument (HVI-900-A). A minimum of 10 g sample of lint from each of the guarded plants in each generation was pre-conditioned to moisture applicability for at least four to five hours prior to testing in the HVI-900-A.

Statistical analysis

Chi-squared values and probabilities of goodness of fit of the segregation ratios of F_2 and backcross generations were tested against theoretical ratio (Harris, 1912). Phenotypic and genotypic correlation coefficients between leaf shape, agronomic and fibre traits were determined using the F_2 data. Phenotypic correlation coefficients were calculated following Dewey and Lu (1959) using minitab, a computer software programme. The genetic correlations (r_g) between two characters X and Y were calculated following Falconer (1981).

RESULTS

Genetic studies

Non-significant chi-squared values were observed for the segregating ratios in F_2 and backcross generations of the three crosses (Table 1). Observations of 1 normal: 2 sub-okra:1 okra, leaf types were noted in the F_2 populations of the three crosses. In the backcrosses with parent-I, ratios of 1 okra: 1 sub-okra, leaf types were obtained. Similarly, in the backcrosses with parent-II, ratios of 1 normal: 1 sub-okra, leaf types were observed (Table 1). The

Table 1. Chi-Squared values and probabilities of goodness of fit of segregation ratios of F_2 and backcross generations in a study of inheritance of okra leaf type trait.

Cross	Generation	Expected ratio	Observed value			Expected value			χ^2 value	Probability
			Normal leaf	Sub-okra	Okra leaf type	Normal Leaf	Sub-okra	Okra leaf type		
HRVO-1 × FH 1000	F_2	1: 2:1	43	76	31	37.5	75	37.5	1.95	0.25-0.10
	BC_1	1:1	-	42	48	-	45	45	0.40	0.75-0.50
	BC_2	1:1	38	52	-	45	45	-	1.88	0.25-0.10
HRVO-1 × CIM 446	F_2	1: 2:1	30	80	40	37.5	75	37.5	2.00	0.50-0.25
	BC_1	1:1	-	50	40	-	45	45	1.11	0.50-0.25
	BC_2	1:1	39	51	-	45	45	-	1.60	0.25-0.10
HRVO-1 × Acala 63-74	F_2	1: 2:1	39	69	42	37.5	75	37.5	1.08	0.75-0.50
	BC_1	1:1	-	44	46	-	45	45	0.04	0.90-0.25
	BC_2	1:1	49	41	-	45	45	-	0.71	0.50-0.25

segregating pattern for leaf type in F_2 populations in three crosses is shown in Figure 2. It is clear from the Figure 1 that the leaf type segregated into three major shape categories. Almost an equal number of plants exhibited okra and normal leaf types, while a large number of plants exhibited intermediate leaf type (sub-okra) in the F_2 generation.

Genotypic and Phenotypic Correlations

The correlation of leaf type with all other traits related to fibre (fibre length, fibre strength, fibre uniformity ratio, fibre fineness and lint percentage) showed non-significant association except for fibre elongation where significant association was recorded in all the three crosses (Table 2). While cross HRVO-1 × Acala 63-74 showed positive and significant association of leaf type with fibre elongation both at the genotypic and phenotypic levels. In rest of the two crosses, there was negative correlation between leaf type and fibre elongation.

It is exhibited from Table 2 that plant height had negative association with the leaf type in all the crosses while significant in HRVO-1 × CIM-446. Number of monopodial branches had a non-significant and positive correlation with leaf type in the crosses HRVO-1 × FH-1000 and HRVO-1 × CIM-446 whereas, a negative but significant correlation was noted between leaf type and number of monopodial branches cross of HRVO-1 × Acala 63-74. A significant and positive correlation was recorded for number of sympodial branches and number of bolls with leaf type for genotypic and phenotypic levels was recorded in all the three crosses (Table 2). While positive and significant phenotypic correlation was noted for seed cotton yield and boll weight with leaf type in all crosses.

DISCUSSION

Continuous efforts have been made by the cotton breeders to explore new genotypes maintaining high yield potentials, reduced input costs along with improved fibre traits. A well organized breeding programme initially focused on yield enhancement, but there also existed a need to consider other traits of economic importance. The results of the present study provided evidence that inheritance pattern involved in okra leaf type trait is simple having no drastic effect on seed cotton yield and fibre traits.

Genetic studies

The parents in the three crosses involving okra leaf and normal leaf plants were hybridized to obtain sub-okra (L^0l^0) progeny in F_1 showing incomplete dominance (Table 1). The segregation in the backcrosses with parent-I and parent-II also confirm to the theoretical ratio of 1:1 further confirmed the incomplete pattern of inheritance. The segregation of the leaf shape in F_2 generation into three classes: okra leaf, normal and the intermediate leaf shape (sub-okra) and fitting into the theoretical 1:2:1 a monohybrid ratio of incomplete dominance in the present study corroborated the findings of Rahman and Khan (1998). The non-significant χ^2 in F_2 for leaf shape in all the crosses fit well against the theoretical ratio. However, Rahman and Khan (1998) observed modifying gene effects in the phenotypic expression of sub-okra leaf shape in heterozygous condition but in contrast to this, the present findings did not confirm the presence of epistasis. The segregating pattern in F_2 for leaf shape into three different types or shapes suggested incomplete dominance in the three

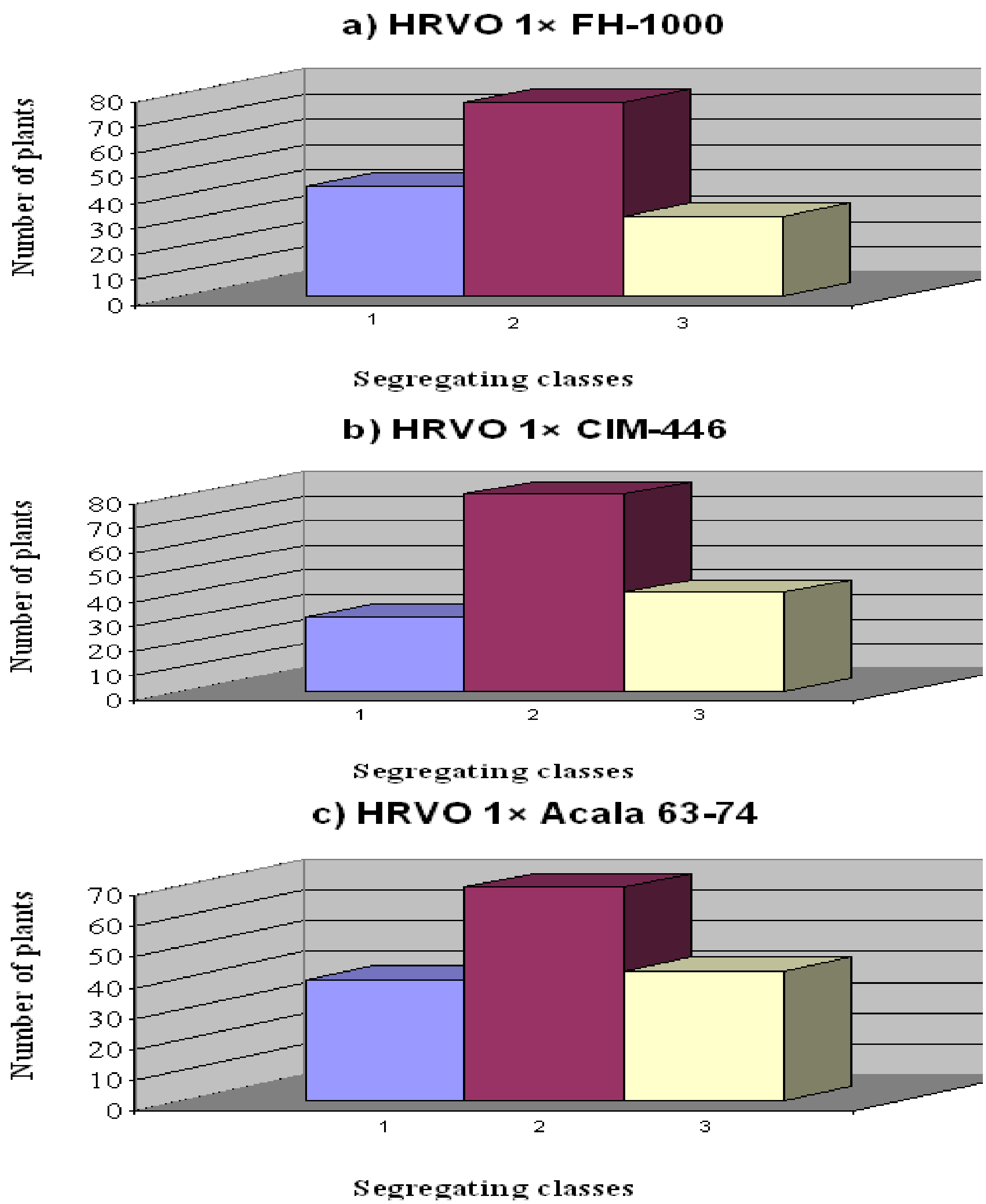

Figure 2. Segregation in F_2 generation for leaf shape in three crosses1 = Normal leaf, 2 = Sub-okra leaf, 3 = Narrow okra leaf.

crosses (Figure 2). The two homozygous extremes for leaf type: okra (L^0L^0) and broad/normal (l^0l^0) were easily distinguishable.

Genotypic and phenotypic correlations

The higher values of genotypic correlation coefficients than the phenotypic correlation coefficients where ever present, indicated that the correlation between the two characters was not only due to genes but environment also played its role in the expression of the character (Nawab et al., 2011). The fibre fineness is recorded in micronaire value, higher the magnitude of micronaire value, lesser will be the fineness of the fibre and vice-versa. In case of leaf type, the data generated for the expression of this trait was on visual ranking system. The maximum value for leaf type on the basis of visual

Table 2. Genotypic (upper value) and phenotypic (lower value) correlations for leaf type and agronomic/fibre traits in three cross combination.

Trait	1	2	3	Trait	1	2	3
	Leaf type				Leaf type		
Lint %	0.48	-0.38	-0.32	PH	-0.39	-0.71**	-0.13
	-0.46	-0.35	-0.28		-0.38	-0.70**	-0.13
FL	-0.42	-0.45	-0.04	NMB	0.39	0.17	-0.78**
	-0.43	-0.38	-0.03		0.36	0.17	-0.76**
FS	-0.39	-0.39	-0.30	NSB	0.77**	0.69**	0.80**
	-0.37	-0.37	-0.28		0.77**	0.62**	0.79**
FE	-0.70**	-0.76**	0.99*	NBP	0.81**	0.64*	0.65**
	-0.68*	-0.75*	0.98**		0.76**	0.60*	0.65**
U%	-0.44	-0.30	0.40	SCY	0.78	0.56	0.83**
	-0.38	-0.21	0.39		0.78**	0.55*	0.83**
FF	-0.40	0.30	-0.40	BWt	0.76	0.74	0.92
	-0.35	0.26	-0.35		0.76**	0.74**	0.93**

L% = Lint percentage, FL = Fibre length, FS = Fibre strength, FE = Fibre elongation, U% = Fibre uniformity ratio, FF = Fibre fineness, PH = Plant height, NMB = Number of monopodial branches/plant, NSB = Number of sympodial branches/plant, NBP = Number of bolls/plant, SCY = Seed cotton yield/plant, BWt = Boll weight. 1= HRVO-1 × FH-1000, 2= HRVO-1 × CIM-446, 3= HRVO-1 × Acala 63-74. *P < 0.05 = Significant, **P < 0.01 = Highly significant.

ranking was assigned to the okra leaf, and minimum value to the normal leaf morphologies. Positive correlation value for leaf type, indicated the okra leaf type while, the negative correlation value indicated the normal leaf morphology (Table 2).

The non-significant correlation of leaf type with all fibre traits (fibre length, fibre strength, fibre uniformity ratio, fibre fineness and lint percentage) except for fibre elongation revealed no correlation of okra leaf type with the expression of these traits as shown in Table 2. These results are in agreement to the findings of Percy (2001). In all the three crosses, the significant values showed correlation between fibre elongation and leaf type. The positive and significant correlation of leaf type with fibre elongation in the cross HRVO-1 × Acala 63-74, meant that okra leaf type would result in enhanced fibre elongation whereas, in the other two crosses the negative correlation gave the understanding of negative correlation between these two traits which meant that the decrease in the fibre elongation was correlated with the okra leaf morphology. The negative correlation between the two traits is in agreement to the findings of Meredith et al. (1996).

Negative and significant association of plant height with okra leaf type in HRVO-1 × CIM-446 meant that plant height would decrease with okra leaf morphology which might be due the effect of a unique genetic background of one parent that is CIM-446, as in the other two crosses the association remained non-significant and negative. But on the other hand, this decrease in plant height did not affect the other yield traits. Only in the cross HRVO-1 × Acala 63 to 74 there existed a negative correlation between okra leaf morphology and number of mono-podial branches whereas, a positive correlation between okra leaf type and number of sympodial branches was observed in the three crosses. Number of bolls, boll weight and seed cotton yield per plant had a positive correlation with leaf type which meant that okra leaf type had a direct affect on yield of seed cotton. This indicated that the gene for okra leaf type can be incorporated in a genotype without increasing number of monopodial branches which ultimately covers the insect non-preference and yield enhancements (Rahman et al., 2005).

The reason for increased yield associated with okra leaf shape, was explained as a significant reduction in the incidence of boll rot and its tolerance against white fly (Soomro et al., 2000), whereas, another reason for increased yield was proposed by Pettigrew (2003) due to the reduced leaf area per plant in the okra leaf with open plant canopy in okra leaf plots which allowed better air flow and more sunlight to penetrate to the lower plant zones. These factors had increased the photosynthetic efficiency, resulting in increased yields.

Conclusion

The results of the present study provided the evidence for incomplete dominance of okra leaf trait. The incorporation of the gene for okra leaf type had no significant effect on the major fibre quality attributes like fibre length, fibre strength, lint percentage, fibre uniformity ratio and fibre fineness. Number of sympodial branches/plant, number of bolls/plant, boll weight and seed cotton yield/plant are principal yield assuring traits which showed improvement with the incorporation of the gene for okra leaf type. It is obvious from the present findings that the increase in yield, associated with okra leaf morphology is due to its non-preference for insect pests and reduced leaf area allowing better air flow and maximum sunlight penetration.

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