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MODULATION IN GROWTH, SOME PHYSIOLOGICAL ATTRIBUTES AND FIBRE QUALITY IN UPLAND COTTON (*GOSSYPIUM HIRSUTUM* L.) DUE TO FREGO BRACT MUTATION

SAMINA MALIK[1*], MUHAMMAD ASHRAF[1], MUHAMMAD SHAHBAZ[1] AND TANWIR AHMAD MALIK[2]

[1]*Department of Botany, University of Agriculture, Faisalabad, Pakistan.*
[2]*Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan.*
[*]*Corresponding author*: saminatmalik@yahoo.com

Abstract

Frego bract is a mutant type of floral bract in upland cotton. It is an important insect resistant trait; however, some reports in the literature show that frego bract gene has some negative effects on growth and fibre quality of cotton. In order to assess the role of frego bract trait on growth and photosynthesis, a pot experiment was carried out under glasshouse conditions to compare frego bract and normal bract recombinant cotton lines. The characteristics examined were: gas exchange, number of days from flower to boll opening and the traits related to fibre yield and fibre quality. Random Amplified Polymorphic DNA (RAPD) analysis was also conducted. The results revealed that the frego bract and normal bract lines had different genetic combinations. Bract type had a significant positive correlation with fibre strength. Frego bract gene had no negative effect on net photosynthesis, number of bolls per plant, boll weight, number of days from flower to boll opening, plant height, ginning out-turn and fibre quality. This shows that the frego bract gene segregates independently of the traits of agronomic importance, so this trait may easily be incorporated into commercial cotton varieties by simple breeding techniques.

Introduction

Cotton is very susceptible to insect pests such as white flies, aphids, jassids and bollworms (Younas *et al.,* 1980). Since a large sum of money is spent on saving the crop from these pests through widespread use of pesticides, there is a consensus of researchers and progressive farmers that insect resistant cotton varieties should be produced which require less number of pesticide applications or no pesticide applications at all. There are many naturally occurring pest resistant morphological traits in cotton such as frego bract, nectariless, okra leaf, trichomes and gossypol glands etc., (Maxwell & Jennings, 1980; Bhat & Jayaswal, 1989). These traits make the cotton plant a less preferable host for insect pests, hence, limiting pest population (Rahman *et al.,* 2006). Insect resistant cotton varieties can be tailored by manipulating genes for the traits like frego bract, nectariless, high gossypol secretions, okra leaf type and hairiness (Maxwell & Jennings, 1980; Bhat & Basu, 1984; Bhat & Jaysawal, 1989). Rahman (2007) identified DNA markers for frego bract, nectariless, hairiness and reported that these traits are simple in inheritance and can easily be manipulated. The incorporation of these morphological characters related to insect resistance would help reduce pesticide load without affecting the desirable genetic combinations (Rehman *et al.,* 2008).

Frego is a mutant type of floral bract that rolls inward and curls away from the flower bud or developing boll (Maxwell & Jennings, 1980; Rahman *et al.,* 2006). It is known that a single recessive gene located on chromosome 3 is responsible for

controlling the frego bract trait (Smith & Cothren, 1999). In frego bract, the bracteole surface is greatly reduced, hence, such bracts have less boll rot incidence (Jones & Andries, 1969; Rahman, 2007), do not provide shelter to bollworm larvae (Bhat & Basu (1984) and generate relatively less trash in hand picked cotton (Maxwell & Jennings, 1980). A number of studies have shown that frego bract is very useful and provides resistance to important insect pests of cotton. Nyambo (1985) reported the lowest number of *Heliothus armigera* larvae on frego bract cotton. Similarly, Kadapa (1988) reported that frego bract character reduces incidence of bollworm attack in cotton. Recently, Neto *et al*., (2005) also reported that the frego bract trait causes a considerable decrease in boll weevil damage to cotton plants.

The effective resistance of frego bract against boll worms and boll weevils shows that it could be useful in the development of commercial lines (Jenkins & Parrot, 1971; Rahman, 2007). Although literature shows usefulness of the frego bract trait in relation to insect pests, it has not been widely incorporated into the cultivated varieties due to certain misconceptions associated with this trait. For example, according to a few reports frego genotypes are less productive due to shy boll bearing, longer period of flower to boll opening, lower net photosynthesis and poor fibre quality (Jones, 1972; Thaxton *et al.,* 1985; Thomson *et al.,* 1987; Singh, 2004). Only a few cotton cultivars in the world possessing this useful trait have been developed. A commercial cultivar Sicot-3 was developed which contained frego bract genes along with glabrous genes (Reid & Thomson, 1984). Frego bract cultivars BRS-3, BRS-22 and BRS-23 possessing the combination of traits such as frego bract, okra leaf, tough boll rind and moderately glabrous (hairless) have also been developed (Surulivelu, 1996). A germplasm line TAM91-104FG having frego bract trait was developed by the Department of Soil and Crop Science at the College Station Texas (Smith, 2001) and compared with a popular commercial cultivar Stoneville. Frego bract line produced stronger fibre than the check, whereas it equalled the check in fibre fineness, staple length and yield. These reports show that frego bract mutation has no negative effect on the performance of the plant. In view of the above contrasting reports, there is a need to investigate the impact of frego bract trait on growth and important plant physiological phenomena such as gas exchange characteristics and growth characteristics such as plant height, number of bolls per plant, boll weight, number of days from flower to boll opening and fibre characteristics such as ginning out-turn, fibre fineness, fibre strength and fibre length. PCR studies using the RAPD (Random Amplified Polymorphic DNA) analysis technique were conducted to assess whether the frego bract and normal bract recombinant lines had different genetic combinations and if so how far they could influence various growth and physiological attributes mentioned earlier of the cotton plant.

Materials and Methods

Studies were conducted in the Department of Botany and the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad. Seeds of frego bract ((3722LA-566) and normal bract (CIM-70) genotypes and two recombinant lines, frego bract (Recomb-F) and normal bract (Recomb-N) were obtained from the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad and grown in pots each filled with 10 kg loamy soil. Sufficient amount of compost was also mixed in the soil to make it well airy and suitable for cotton growth. The experiment comprised 12 pots for each of the parents and 16 for each of the recombinant lines with a single plant in

each pot. The experiment was arranged in a completely randomized design with four replications. The pots were placed in the wire house of the Botanic Garden, Department of Botany. The planting was done in the normal crop growing season (May, 2006). Optimum irrigation was supplied according to the need of plants.

Gas exchange parameters: A portable, open Infra Red Gas Analyser (IRGA), Model LCA-4 ADC, Analytical Development Company, Hoddeson, England) was used to determine Net CO_2 assimilation rate (*Pn*), Transpiration rate (*E*), Stomatal conductance (g_s) and Sub-stomatal or intercellular CO_2 (*Ci*).

Measurements were conducted under glasshouse conditions. To ensure uniform light and temperature conditions for all the plants, the measurements were made in November, 2006 during 11:00 a.m. to 1:00 p.m. The relative humidity was 47% and the average maximum and min temperatures were 27.2°C and 14.6°C, respectively.

Growth parameters: Data for the growth characteristics like plant height, number of bolls per plant, boll weight and number of days from flower to boll opening were recorded at maturity.

Fibre characteristics: Fibre length, fibre strength and fibre fineness were determined with the help of Spin lab HVI-900 in the Department of Fibre Technology, University of Agriculture Faisalabad. Ginning percentage was calculated using the following formula:

$$\text{GOT (\%)} = \frac{\text{Weight of lint in the sample}}{\text{Weight of seed cotton in the sample}} \times 100$$

Bract type: The frego bract type was taken as 1 and normal bract type was taken as 2 in the data recording.

PCR studies: The RAPD analysis technique was used to study genetic polymorphism of the recombinant lines. DNA was extracted from young leaves of the normal bract and frego bract recombinant lines using CTAB method. The DNA was amplified by Polymerase Chain Reaction (PCR) using 100 oligonucleotide decamer primers from Operon Technologies, USA and Genosys Biotechnology, UK. After PCR, the samples were analyzed by 1% agarose gel electrophoresis running in 0.5% TAE buffer. The purity of genomic DNA was tested with the help of a UV spectrophotometer and the data of amplifications were recorded by taking gel photographs with the help of a gel documentation system.

Statistical analysis

Data of morphological and gas exchange parameters: The data for morphological and gas exchange characteristics were subjected to analysis of variance. In the parent lines, 3 plants were considered as a single replicate and in the recombinant lines 4 plants were considered as one replicate. For comparing the genotypes, Least significant difference (LSD) was calculated following the method described by Steel & Torrey (1980). For a pair of characters correlation coefficients (r) were also calculated with the help of computer software using the data of individual plants.

PCR data: The data obtained from PCR were scored from good quality photographs of each amplification reaction. All visible and unambiguously scorable fragments amplified by the primers were scored. The fragments that were present in DNA of one genotype and absent in the other were scored as polymorphic fragments.

Results: Significant differences ($p \leq 0.05$) were observed among the four genotypes for the traits such as fibre fineness, ginning out-turn, number of days from flower to boll opening (boll maturity) and the gas exchange characteristics, net CO_2 assimilation rate and stomatal conductance. The traits like fibre strength, fibre length, number of bolls per plant, transpiration rate and sub-stomatal conductance also differed markedly ($p \leq 0.01$), while no significant difference was observed among the genotypes for boll weight and plant height.

In the present studies, the fibre fineness of frego bract genotype (3722LA-566) was lower than that of the other genotypes. Micronaire is the unit of fibre fineness. Higher the micronaire, lower will be the fineness. The frego bract genotype had higher micronaire (lower fineness) than that of the normal bract genotype (Fig. 1A). However, the recombinant frego bract genotype (Recomb-F) had almost the same micronaire value as that of the normal bract variety. The normal bract variety (CIM-70) had the strongest fibre while the frego bract genotype (3722LA-566) the weakest fibre (Fig. 1B). However, the recombinant frego bract genotype (Recomb-F) was almost similar to the normal bract variety in fibre strength. The normal bract variety produced the highest (29 mm) while the frego bract genotype the shortest (24 mm) fibre length (Fig. 1C). From these data it is evident that frego bract mutation causes a slight reduction in fibre length.

The normal bract variety had highest GOT percentage. However, the recombinant frego bract and normal bract genotypes showed nearly the same GOT values (Fig. 1D). The recombinant normal bract genotype (Recomb-N) produced the highest number of bolls per plant (9.8) of all genotypes (Fig. 1E). The frego bract genotype and the normal bract genotype were almost similar in number of days for boll maturity, however, the recombinant normal bract genotype was the most late in maturity (Fig. 1F). No significant difference among the four genotypes was observed for plant height and boll weight. It means plants possessing frego bract trait do not suffer from plant vigour or cotton yield loss.

All four genotypes showed the same trend for gas exchange characteristics. The frego bract genotype had the highest net CO_2 assimilation rate, transpiration rate and stomatal conductance while the normal bract genotype showed the lowest values for these parameters (Fig. 2 A-D). Both recombinant lines (frego and normal bract) had almost the similar values of net CO_2 assimilation rate. The highest value of net CO_2 assimilation rate of the frego bract genotype could be beneficial for its growth and yield, but this is not always so in the same cases as has been observed from yield parameters of the four genotypes.

The correlations among various gas exchange traits revealed that the net CO_2 assimilation rate was positively correlated with transpiration rate and stomatal conductance but negatively correlated with sub-stomatal CO_2 (Table 1). Normal bract type had a positive correlation with fibre strength. Fibre fineness had a negative correlation with fibre length but a positive correlation with GOT. This shows that the alleles for high fibre fineness and the alleles for lower fibre length maybe present on the same chromosome. A positive correlation was also found between fibre strength and fibre length (Table 2).

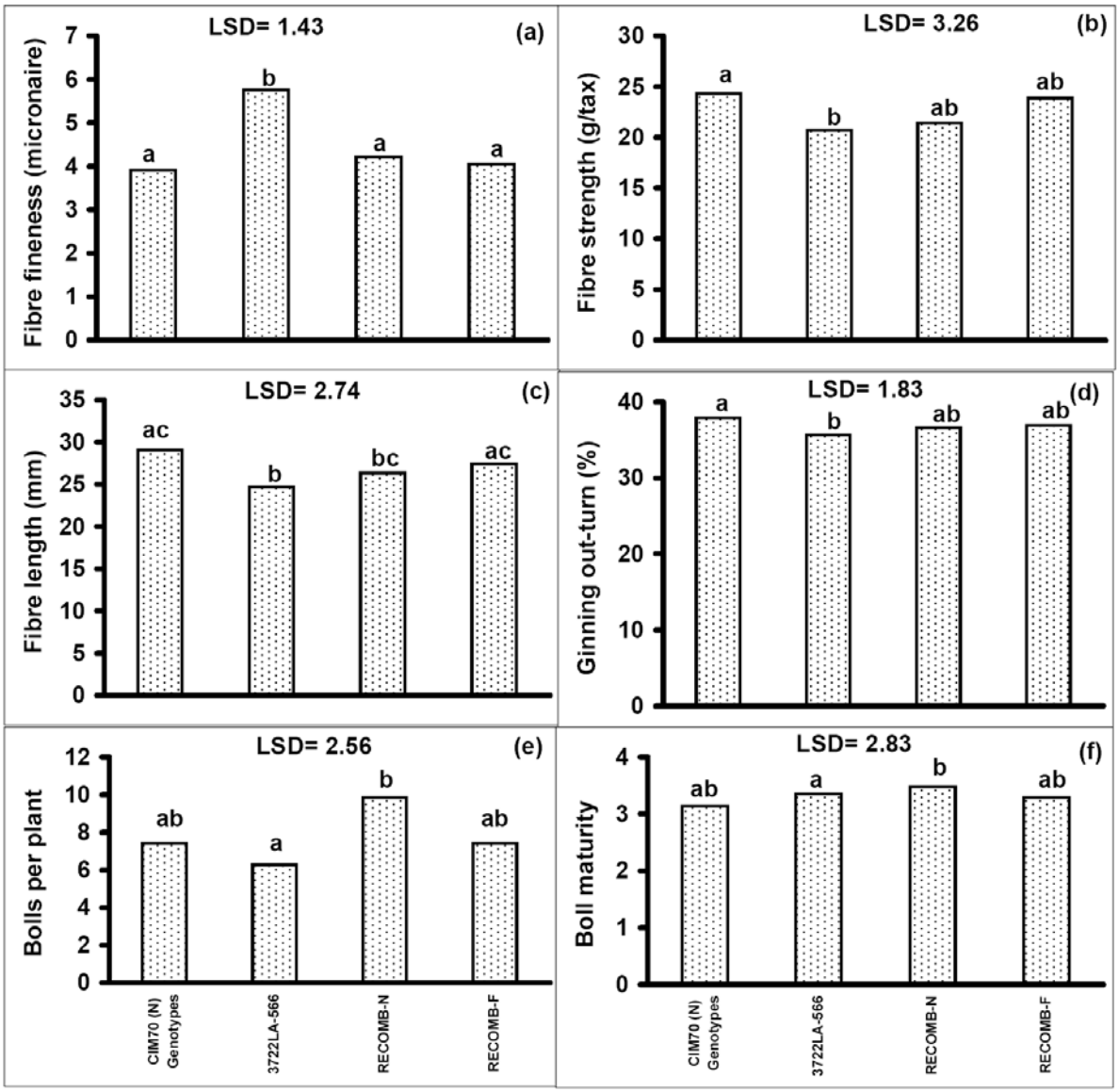

Fig. 1. Fibre fineness (a), fibre strength (b), fibre length (c), ginning out-turn (d), bolls per plant (e), and boll maturity (f) in four cotton genotypes CIM-70(N), 3722LA-566(F), RECOMB-N and RECOMB-F (n= 4). Bars sharing same letters do not differ significantly at $p \leq 0.05$.

The absorbance of genomic DNA tested by a spectrophotometer showed that it had a ratio of 1.78 at 260 nm and 280 nm which suggested that the DNA was of good quality. Of the 100 primers used in RAPD analysis, 98 primers amplified the DNA molecules. Of the total DNA molecules amplified in PCR, 34 % were polymorphic.

RAPD-PCR amplification photograph with five primer combinations is shown in Fig. 3. The RAPD analysis revealed that the frego bract and normal bract recombinant lines had different genetic combinations.

Primers	**Sequences**
GLE-1	CCCAAGGTCC
GLE-2	CGTGCGGGAA
GLE-3	CCAGATGCAC
GLE-4	GTGACATGCC
GLE-5	TCAGGGAGGT

Table 1. Correlation Matrix for values of net CO_2 assimilation rate (*Pn*), transpiration rate (*E*), stomatal conductance (g_s) and sub- stomatal conductance (*Ci*).

	Pn	*E*	g_s	*Ci*
Pn		0.53**	0.76**	-0.43**
E	0.35**		0.61	0.12
g_s	0.43**	0.38**		0.21
Ci	-0.40**	0.18	0.22	

Table 2. Correlation matrix for the traits, bract (Brt), net CO_2 assimilation rate values (*Pn*), fiber fineness (FF), fibre strength (FS), fiber length (FL), ginning out-turn (GOT) and number of bolls per plant (B/P) for the genotypes CIM-70 (normal parent), 37221A-566 (frego parent), RECOMB-N (recombinant normal bract line) and RECOMB-F (recombinant frego bract line).

	Brt	*Pn*	FF	FS	FL	GOT	B/P
Pn	0.08						
FF	0.05	0.16					
FS	0.27*	0.22	0.07				
FL	0.17	0.08	-0.38**	0.41**			
GOT	0.23	0.14	0.28*	0.04	0.07		
B/P	0.06	0.24	0.21	0.16	0.23	0.25	
BM	0.15	0.23	0.14	0.04	0.22	0.08	0.13

* = p≤0.05, ** = p≤0.01

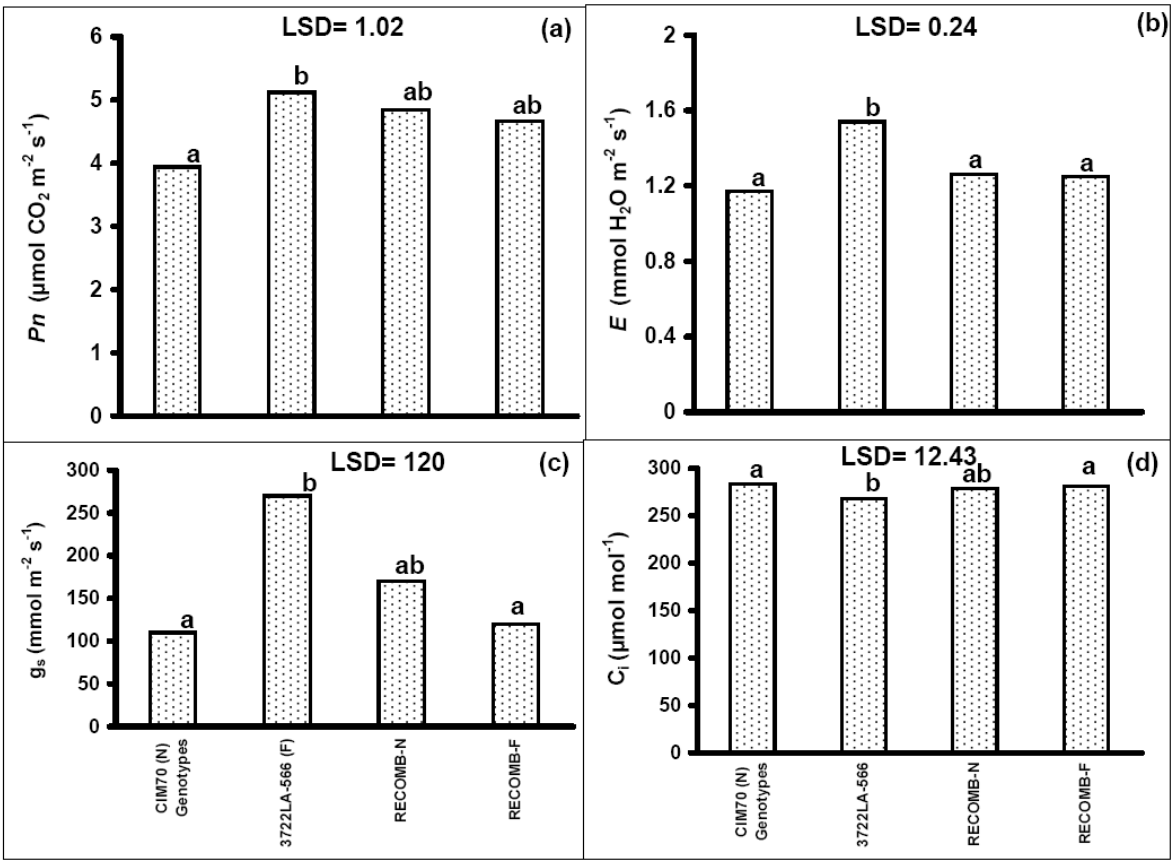

Fig. 2. Net CO_2 assimilation rate (*Pn*) (a), transpiration rate (*E*) (b), stomatal conductance (g_s) (c) and sub-stomatal CO_2 concentration (C_i) (d) of four cotton genotypes CIM-70(N), 3722LA-566(F), RECOMB-N and RECOMB-F (n= 4). Bars sharing same letters do not differ significantly at p≤0.05.

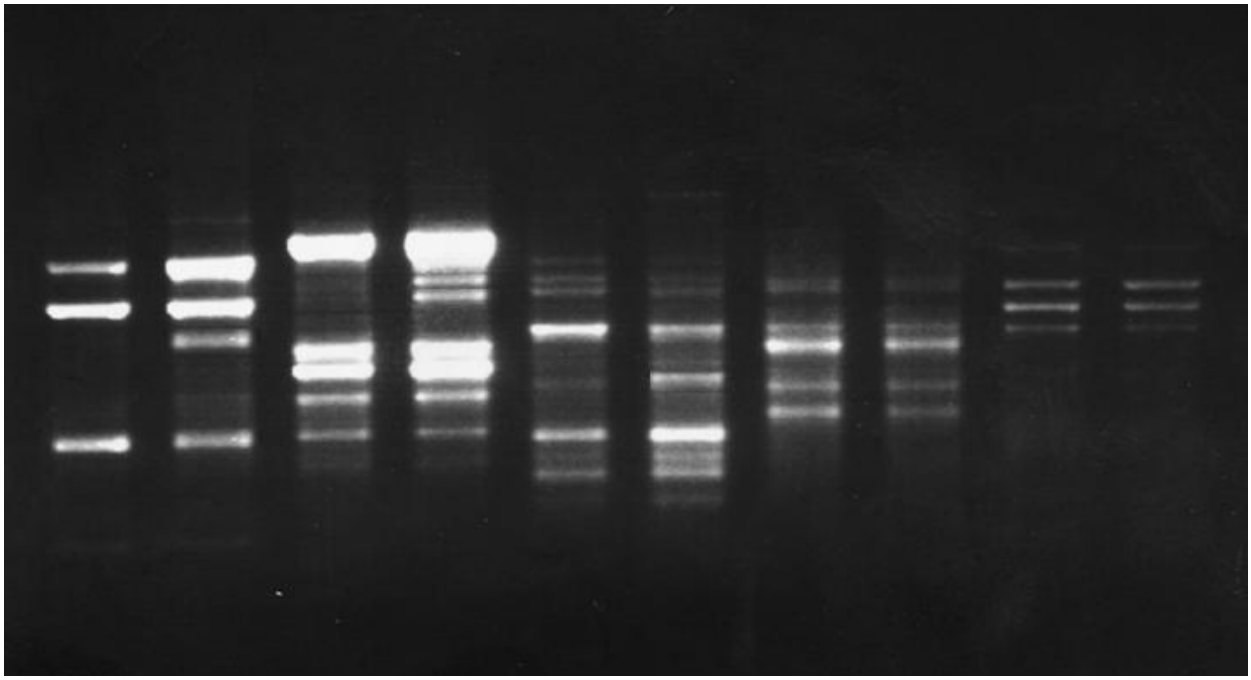

Fig. 3 RAPD amplification of genotypes Recomb-N (1) and Recomb-F (2) by the primers, GLE-1, GLE-2, GLE-3, GLE-4 and GLE-5.

Discussion

The RAPD technique has been widely used for the study of genetic diversity in plants (Cao *et al.,* 1998: Hu & Quirose, 1991; Malik *et al.,* 1996; Dongre & Parkhi, 2005). In the present study, RAPD analysis showed that the two recombinant genotypes (normal and frego bract) were genetically diverse.

Cotton fibre has a global importance, therefore, improvement of fibre properties in cotton is the main objective of plant scientists. Fibre quality refers to fibre fineness, fibre length and fibre strength. In the present study, since the recombinant frego bract genotype had almost similar fibre length as the normal bract variety, it may be suggested that the frego bract gene had no negative effect on fibre quality except a slight effect on fibre length. Smith (2001) also conducted a comparative study of a popular commercial cotton cultivar with frego bract line. In view of his results, frego bract line equaled the commercial variety in fibre fineness, fibre length and yield and it even produced stronger fibre than the normal variety. The results of the present studies showed that the recombinant frego bract genotype had almost the same plant height, number of bolls per plant, boll weight, number of days from flower to boll opening and ginning out-turn as the normal bract variety.

Photosynthsis is genetically controlled and different varieties of the same species may have different net photosynthetic rates (Malik *et al.,* 1999). Singh (2004) reported that the frego bract genotypes were associated with lower CO_2 assimilation rates. However, in the present study, the frego bract genotype had higher net CO_2 assimilation rate than the normal bract genotypes. Similarly, the frego bract genotype had higher transpiration rate and stomatal conductance than those of the other cultivars.

Correlation measures the degree of interdependence between a pair of characters. Knowledge of correlation is required to obtain the expected response of other characters when selection is applied to a particular character of interest in a breeding programme. It is known that stomatal conductance has a positive correlation with net CO_2 assimilation because of increase in CO_2 diffusion into leaves (Taiz & Zeiger, 2002; Ashraf *et al.,* 2003). The present study revealed a positive correlation of net CO_2 assimilation rate with transpiration rate and stomatal conductance which shows that the high rate of transpiration and stomatal conductance might have resulted into higher net photosynthesis. Thus, it can be suggested that an increase in the rate of photosynthesis was due to an increase in stomatal conductance of leaves. Wang *et al.*, (2003) reported a strong positive correlation between net photosynthesis and transpiration rate. A negative correlation between net photosynthesis and sub-stomatal CO_2 in the present study showed that the higher photosynthetic rate depleted the CO_2 concentration in sub-stomatal cavities at a higher rate.

In the present study, no correlation was found between net photosynthesis and any of the agronomic traits studied (fibre fineness, fibre strength, fibre length, GOT, number of bolls per plant, boll weight, plant height and boll maturity). Abdullaev (1997) reported a relationship between net photosynthesis and fibre quality characters in upland cotton. He found a high positive correlation between net photosynthesis and cotton fibre. His results revealed that high net photosynthesis was one of the factors for longer and finer fibre. Cundong Li *et al.*, (2004) studied some physiological characteristics of both source and sink of different boll weight genotypes of cotton. They reported that larger boll weight genotypes had more soluble sugar and starch content compared to smaller boll weight genotypes which indicated better photosynthetic capacity of such genotypes.

In the present study, frego bract type had a significant positive correlation with fibre strength. Similar results have earlier been observed by Rahman (2007) in cotton. In view of some reports, the frego bract gene is located on chromosome 3 (Endrizzi *et al.,* 1984; Smith & Cothren, 1999). The genes for fiber strength are also reported to be located on chromosome 3 in cotton (Shen *et al.,* 2005; Frelichowski. *et al.,* 2006). However, Smith *et al.,* (2001) developed a frego bract strain which produced stronger fibre than normal bract strain showing that the negative linkage of frego bract with fibre strength is not very close. According to Rahman (2007) this negative linkage can be broken by crossing over through intensive hybridization and promising cotton genotypes with improved insect resistance can be tailored by incorporating the frego bract trait. The absence of correlation of bract type with net photosynthesis, plant height, boll weight, number of bolls per plant, boll weight, number of days from flower to boll opening, fibre length or fibre fineness shows that the frego bract gene is not linked with these important agronomic traits and it segregates independently.

In the present study, fibre micronaire (fineness) had a negative correlation with fibre length. Joshi (1976), Lancon *et al.*, (1993) and Rahman (2007) also found a negative association of micronaire with fibre length. The lower the micronaire value, higher was the fineness of fibre, so fineness and fibre length were positively correlated. Micronaire had a positive correlation with ginning out-turn. Ulloa & Meredith (2002) and Rahamn (2007) also found a positive correlation between ginning out-turn and micronaire value. In the present study, fibre length had a positive correlation with fibre strength. These results are in agreement with those of Ying & Jun (2004) and Ulloa (2006). This suggests that the genotypes with high fibre length yield fine and strong fibre.

In view of the results of the present study, it may be concluded that the frego bract trait has no negative effect on the growth, gas exchange and fibre quality of upland cotton and thus it can be successfully incorporated into commercial cultivars using any combination of agronomic traits.

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