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Introgression of *G. barbadense* Genes into *G. hirsutum* through DNA-mediated Embryo Transformation Approach

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Abstract
Attempts were made to incorporate intraploid interpsecific gene(s) from *Gossyium barbadense* into *G. hirsutum* through DNA-mediated embryo transformation approach. *G. barbadense* DNA was injected into the styles/ovaries of *G. hirsutum*, 24 hours after self pollination. Two phenotypic types of plants i.e. transformed and *G. hirsutum* like were observed in D population. The transformed plants had clearcut variations in boll weight, yield and other quality traits confirmed to recipient parent. D transformations were also heritable and were transmitted to D generation. The transformed plants had better boll weight (4.9 gm), better fibre fineness (3.9 µg/in) and better fibre strength (101.0 TPPSI) compared to the boll weight (3.0 gms), fibre fineness (4.5 µg/in) and fibre strength (91.0 TPPSI) of *G. hirsutum*. The transformation efficiency for quantitative traits was about 20 % where as low for qualitative traits like flower petals with red spot (0.08 %). In the present studies transformed genotypes illustrated higher yield potential and better fibre quality than the recipient (*G. hirsutum*) parent.

Key words: *G. barbadense genes, G. hirstum,* Embryo *transformation, in vivo,* DNA macroinjection, Heritable

Introduction

The two cultivated tetraploid species i.e. *G. hirsutum* (2 (AD) = 52) and *G. barbadense* (2 (AD) = 52) of cotton can be crossed and F hybrid produced is apparently fully fertile. However F progenies confront with a considerable depression in vigour and the net affect of inbreeding is the outcome of types which are practically indistinguishable from the parent species. All intermediate types are at a great selective elimination of donor parent character. Moreover, there is also a considerable selective elimination of donor parent genotype following backcrossing between *hirsutum* and *barbadense* (Stephens, 1949). However, Pima S-1 was developed through selection from complex series of crosses, involving Sea Iland, Pima, Tanguis and Stoneville. The first three were *G. Barbadense* while later was *G. hirsutum.* which probably contributed to cultivar development through limited introgression (Bryan, 1955). The interspecific (*G. hirsutum* x *G. barbadense*) hybridization followed by irradiation of F seed produced stable long staple germplasm (Zhuaib *et al.,* 1981). Recently gene transfer in higher plants through Agrobacterium mediated transformation (Potrykus, 1991) and through the introduction of exogenous DNA solution into the recipient has been reported (Hess, 1969; Holl, 1975; Korohoda and Strzalka, 1979; Soyfer, 1980). Zhou *et al.* (1982) obtained wilt resistance, by injecting DNA, from a resistant species of upland cotton into styles/ovaries of sensitive one, through the transformation of embryo. Zhou *et al.* (1983) also introduced Sea Iland cotton DNA solution into the glandless upland cotton embryos and found transformed progeny. Aslam *et al.* (1995) reported the rapid *in vivo* incorporation of intraploid intraspecific genes in *G. hirsutum* through DNA-mediated embryo transformation technique. The objectives of the study were to incorporate *G. barbadense* gene(s) into *G. hirsutum* through DNA-mediated embryo transformation via DNA macroinjection in lesser time and to overcome the problems confronted, when crossing or backcrossing techniques are applied. The DNA macroinjection method involves the injection of exogenous DNA solution into plants reproductive structure. As a result the developing embryo is transformed *in vivo* and the resultant transformed progeny carry heritable, hereditary change.

Materials and Methods

Two cultivated cotton species, *G. hirsutum* and *G. barbadense*, having the same chromosome number i.e. 2n = 4x = 52 were used in these studies (Table 1). The donor parent *G. barbadense*; has tall monopodial plant, larger yellow flowers having petals with red spot and yellow pollen colour. Moreover, it has conical 3-4 locules bolls, having longer, finer and stronger fibre and late in maturity under Pakistan's climatic conditions. Whereas the recipient parent, *G. hirsutum* (NIAB-78), a locally adapted, sympodial type plant, for cream colour petals/pollen, round and medium bolls with 4-5 locules, having medium fibre quality characters and early in maturity. Both, the donor and recipient parents are clearly distinguishable from each other due to their genetic identity. About 200 plants of the recipient were raised from selfed seeds in the field. Donor parent DNA was extracted from the young leaves following the DNA extraction method, reported by Leonard *et al.* (1986). Injection solutions comprised of DNA mixed with protamine and protamine alone or control solution or check solution (blank solution). Fifty healthy plants of recipient were selected at flowering. Thirty ovaries of selfed flowers were injected with DNA solution, i.e. 24

hours after self pollination. Injections were made with a microsyringe and 10 microliter of solution was injected into each treatment. DNA solutions were stored under refrigeration and were kept immersed in ice during the injection process to avoid degradation. Some Ovaries were self pollinated (Cont-II) while others were also injected with blank solution (Cont-II) both were uses as control experiment. Matured bolls were harvested and D seed (D = Zero generation of DNA introduction) were prepared for planting. About two hundred D seeds alongwith the seeds obtained from self and blank treatments i.e. Control-1 and Control-2 were planted in the field at a spacing of 60x75 cm. The germinated D plants were studies for phenotypic alterations and the data on morphological changes were recorded. At maturity phenotypically altered plants of D generation were harvested and evaluated for yield and quality traits. These transformed plants of D generation were studied in D generation.

Results

D_1 population showed two types of plants (recipient like and transformed). Out of 123 F plants; 98 plants were like recipient and 25 plants had phenotypic changes. The transformation efficiency for phenotypic characters came out to be 20 percent. The transformed plants were more faster in growth, had more height, more monopodial branches and longer 10th sympodial than the recipient like plants (Table 2). Moreover the transformed plants had larger leaves with longer petiole and flowers with large calyx and intermediate to normal corolla. However the transformed plants of D generation had no change for flower colour, pollen colour and red petal spot. No phenotypic changes were observed in the population raised from the seed obtained from Cont.I and Cont.II treatments.

The morphology altered D plants showed variations for various economic traits (Table 2). The transformed plants had better boll weight (3.3-4.8 gms) and higher yield per plant (155-431 gms) than the recipient. Similarly fibre qualities were also found disturbed in the transformed plants. More conspicuous changes were observed for fibre fineness and fibre strength. Micranaire value and fibre strength of the transformed plants ranged from 4.1 to 3.8 (µg/in) micronaire value and 90-91 (TPPSI) of fibre strength of the recipient parent parent (*G. hirsutum*). However the results did not show much increase in fibre length over the recipient parent (*G. hirsutum*).

The results of D generation revealed that the transformations either phenotypic or for economic traits were found persistent (Table 3). The transformed progenies maintained their phenotypic changes, preserved better boll weight and higher yield over the recipient parent. Out of 25 transformed progenies, one D progeny (D-104-1) had one plant with red petal spot. Red petal spot, a monogenic (R2R2) and incompletely dominant trait (Stephens, 1974), introgressed from *G. barbadense* into *G. hirsutum* and expressed in D generation. Another progeny (D-101-7), had one plant with complete sterility. The complete sterility trait was confirmed through cross pollinations with *G. hirsutum* pollen. At maturity, the D progenies showed fibre quality characters similar to those found in D generation (Table 3).

Discussion

It is evident from the results reported that intraploid interspecific genes their quantitative or qualitative have been successfully incorporated from *G. barbadense* into *G. hirsutum* through DNA macroinjection. Similarly the incorporation of wilt resistant genes from a resistant upland cotton into a wilt susceptible upland cotton. *G. hirsutum* was reported by Zhou *et al*. (1982) through DNA macroinjections. This method of direct DNA incorporation *in vivo*, has an advantage over other methods currently being used for the transformation of plant species (Potrykus, 1991; Shillito, *et al*., 1985; De la Pena, 1987; Crossway *et al*., 1986, Finer and McMullen, 1990; Fromm *et al.*, 1985; Horsch *et al.*, 1985). In all those methods, first the individual cells are transformed and then the transformed cells are selected and regenerated into a whole plant through callus culture. Since all the cotton genotypes are not amenable through tissue culture method therefore all the transformed cells cannot be cultured to develop plants. It is because of genotype-specificity of cotton towards callus culture (Trolinder and Xhixian, 1989). Genotype-specificity is a genetic trait and is controlled by quantitative genes (Gawel and Robacker, 1990). The efficiency of transfer of intraploid interspecific genes within Gossypoum which is otherwise difficult due to chromosome structural differences (Stephens, 1949), is greater (20%). Whereas the transformation efficiency for qualitatively inherited traits like red petal spot etc. is lower (0.08 %).

It has been reported by Neuhaus *et al*. (1987) that transformation efficiency of plant cells can be significantly increased when the DNA is directly injected into the nucleus. Moreover it has been also reported (Kohler *et al.*, 1989) that the transfer of interspecific genes is enhanced by irradiating the donor DNA with low doses of X-rays. Because the low dose irradiation of plant cells, results in single strand breaks, less base damage and to a lesser extent double strand breaks (Howland *et al*., 1975).

These studies showed that the red petal spot was transferred from *G. barbadense* into *G. hirsutum* and was expressed in D generation. Similar results have been reported by Werner and Cornish (1985) in Nicotiana. One completely sterile plant was also found in one of the progeny (D-101-7) in D generation. Such type of results have also been reported in F progenies of interspecific crosses of *G. hirsutum* x *G. barbadense* by Beasley and Brown (1942). These are the clear and strong genetic markers based evidences, supporting the introgression of *G. barbadense* genes into *G. hirsutum* via DNA macroinjections.

This is a straight forward approach and does not require the

Table 1: Frequency of transformations obtained in D1 generation following the introduction of *G. Barbadense* DNA into *G. hirsutum*

Injection Treatments	Do seeds planted (No.)	Plant studied (No.)	Transformed plants (No.)	Recipient like plants (No.)	Efficiency of transformation (%)
DNA + protamine solution	180	123	25	98	20
Protamine solution along (Cont. II)	40	20	No change	-	-
Self pollination (Cont. I)	40	20	No change	-	-

Table 2: Phenotypic and economic traits (Range) of transformed and recipient like genotypes in D1 population

		D_1 population		
Characteristics		Transformed	Recipient*	Donor**
Plant height (cm)		114.0-158	112.0-122	158.0-200
Monopodial (No.)		0.0-3	0.0-1	2.0-4
Sympodial (No.)		20.0-33	24.0-312	18.0-30
10th sympodial	a) Length (cm)	32.0-82	26.0-61	60.0-105
	b) Nodes (No.)	6.0-14	6.0-14	60.0-105
Boll weight (gms)		3.3-4.8	2.0-3.6	3.5-4.5
Yield/Plant (gms)		155.0-431	152.0-224	52.0-165
G.O.T. (%)		33.3-37.7	34.0-36	36.0-38
Fibre length (mm)		26.0-28	25.0-26	33.0-34
Fibre fineness (MV)		3.6-3.8	4.4-4.47	3.5-3.6
Fibre strength (TPPSI)		85.0-101	90.0-91	101.0-105
Fibre maturity (%)		77.0-81	76.0-79	80.0-81

*G. hirsutum; **G. barbadense

Note: The transformed plants. I. Were more vigorous/faster in growth than the recipient; II. Had larger leaves with intermediate to long petiole; III. Had medium to normal flowers and with bigger calyx.

Table 3: Phenotypec and economic traits (Range) of transformed and reciepient like genotypes in D_1 population

		D_2 population		
Characteristics		Transformed	Recipient*	Donor**
Plant height (cm)		108.0-133	97.0-113	150.0-185
Monopodial (No.)	0.0-4	0.0-1	2.0-4	
Sympodial (No.)		19.9-30.4	21.0-24	18.0-27
10th sympodial	a) Length (cm)	19.4-45.8	23.3-285.3	58.0-95
	b) Nodes (No.)	5.0-14.6	6.2-12.9	6.0-9
Boll weight (gms)	3.9-4.9	3.0-3.4	4.5	
Yield/Plant (gms)		2961.0-3701.9	222.3	50.0160**
G.O.T. (%)		33.3-37.7	34.0-36.0	39.0
Fibre length (mm)	27.0-28.5	27.0-27.4	34.0	
Fibre fineness (MV)		3.7-4.0	4.4-4.5	3.8
Fibre strength (TPPSI)		86.0-101	80.0-91	105.0
Fibre maturity (%)		76.7-90.1	78.5-79.5	81.0

G. hirsutum*; **G. barbadense*; * = gm/plant

Note: 1. One transformed progeny (D-101-1) and one plant with flowers having red petal spot. II. One transformed progeny (D-101-7) had one plant with complete sterility. Were more vigorous/faster in growth than the recipient; II. Had larger leaves with intermediate to long petiole; III. Had medium to normal flowers and with bigger calyx.

regeneration of the transformed cell into whole plant through callus culture. Therefore this approach could potentially be used for the incorporation of foreign genes into *G. hirsutum* in order to develop imporved germplasm of cotton.

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