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Effect of GammaRay in the Progeny of Trispecific Hybrid [(*Gossypium hirsutum x G. raimondii*)^2 x *G. sturtianum*]

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Abstract

The objective of this study is to analyse the possibilities of fixing in the progeny of the [(*Gossypium hirsutum* x *G. raimondii*)^2 x *G. sturtianum*] (HRS) trispecies hybrid, the glandless-seed and glanded-plant trait. The expression of the character was analysed in progenies obtained by selfing the HRS BC2S5/9/6/1/51/15 genetic stock. This character is controlled by one or more genes located on introgressed chromosome fragments of *G. sturtianum*. These foreign DNA fragments seem also carriers of gametes terminators genes on the same chromosome fragments and there would be a possible existence of negative interactions between some of the introgressed *G. sturtianum* alleles and the *G. hirsutum* genetic background inducing post-zygotic mortality. In order to break existing lethal links, some of the studied seeds were treated with 15 krad of gamma radiation. The transfer of *G. sturtianum* chromosome fragments introgressed stocks was assessed using ten mapped SSR markers, carried out on 78 plants HRS 'BC2S6'. Gamma irradiation used to overcome lethality in HRS hybrid did not give expected results in M_2 because of high rate of abortion observed in HRS 'BC2S6' seeds. However, gamma ray did not induce the appearance of chimeric tissues in the HRS 'BC2S5/9/6/1/51/15' in M_1 plants. Moreover, the notable fertility improvement observed for some plants issued from irradiated seeds constitutes a clue of the achievement of favourables recombinations due to the gamma ray treatment. The perspectives opened by the results obtained for the stabilization of the glandless-seed and glanded-plant trait in a *G. hirsutum* commercial variety are discussed.

Keywords: cotton, interspecific hybridization, introgression, gossypol, SSR, gamma ray

Introduction

The cotton genus *Gossypium* contains 49 diploid and tetraploid species distributed worldwide (Fryxell, 1992). Cultivated types have spinnable fibers derived from only four species: two diploids (2n=2x=26), *Gossypium arboreum* and *Gossypium herbaceum*, and two tetraploids (2n=4x=52), *Gossympium barbadense* and *Gossypium hirsutum* (Brubaker *et al.,* 1999; Gao *et al.,* 2006). Of these, *G. hirsutum* is economically the most important species in the world. It alone provides nearly 95% of world cotton fiber production. Genome classifications are correlated with the fertility and frequency of chromosome recombination in interspecific hybrids. In general, interspecific hybrids within genomes are fertile; recombining readily, whereas intergenomic hybrids are infertile and exhibit limited bivalence during meiosis (Stewart, 1995).

Mainly grown for its fiber cotton is the second most important potential source of plant proteins and the fifth best oil-producing plant (Lee, 1984). The ability to use this nutrient-rich source of food is hampered by the presence of pigment glands containing toxic terpenoid aldehydes throughout the plant, in nearly all species of the *Gossypium* genus. Ideally, cultivated cotton plants should have glandless-seed for complete use in food and feed, and show glands on all plant parts to resist to pests. The *glandless-seed* and *glanded-plant* trait exist only in some Australian wild diploid species belonging to *Sturtia* and *Hibiscoidea* sections (Brubaker *et al.,* 1996).

In order to introgress the *low-gossypol seed* and *high-gossypol plant* trait from *G. sturtianum* Willis diploid, ($2C_1$) into *G. hirsutum* tetraploid, $2(AD)_h$, the triple hybrid [(*G. hirsutum* x *G. raimondii*)^2 x *G. sturtianum*] (HRS) was created (Mergeai *et al.,* 1997; Vroh Bi *et al.,* 1998). After more than six generations of backcross and selfing, a high segregation of the gossypol content was still observed in the seeds produced by the selected HRS derivatives probably because of the heterozygous state of the *G. sturtianum* genes controlling the researched trait. Two hundred and fourteen mapped SSR markers evenly distributed on the 13 homeologous chromosome pairs of *Gossypium hirsutum* L. were used to monitor the introgression and conservation of SSR loci (alleles) coming from both wild species involved in the creation of HRS hybrid (Benbouza *et al.,* 2010). Among the 93 *G. sturtianum* specific SSR revealed in the triple hybrid, only ten mapped on c2-c14,

c3-c17, and c6-c25 linkage groups are still present in most advanced generations obtained ('BC2S6'). Furthermore a combination of high mortality rate, empty seeds, malformed seeds and few low-gossypol seeds (between 1 to 8%) has been always observed in experiments. Lethality of gametes and/or zygotes can be caused by the presence of lethal gene (s) introgressed from alien species. Their expression depends on the background in which they act. Thus, differential viability of spores, gametes or zygotes provides distorted transmission frequencies.

So, the high frequencies of heterozygote in HRS hybrid after several generations of crossing and selfing may be due to a preferential transmission system and /or the presence of lethality factor (s) conserved on alien chromosome fragments introgressed from *G. sturtianum*. Post zygotic lethality can be due to the interaction of *G. sturtianum* alleles with *G. hirsutum* genetic background and/or to the expression of the recessive lethality factor (s) when they become homozygote. Mutagenesis can be used to overcome the problem of lethality. The most popular method employed for creating genetic variability is induced mutagenesis through gamma irradiation (Reddy, 1977). Soybean provides an interesting example where this process has been used with success. In the USA, soybean is a major crop and an important source for edible oil production. The lx locus determines shelf life and quality of soybean oil. Two groups of researchers in Japan used gamma irradiation to break the repulsion linkage between the loci lx1 and lx2 in one case and to induce a change in LX1 in another case (Ahloowalia, 2004). Besides gamma irradiation, chemical mutagens like ethyl-nitroso-urea, methylnitroso-urea, ethyl-methane-sulphonate (EMS) and sodium azide (SA) are also used for mutation-assisted breeding. But application of physical mutagenesis like gamma irradiation techniques allows breakage of linkage (s) between useful and lethal gene (s), while chemical mutagenesis causes changes in base pairs. Approaches such as the exposure of seed to ionizing radiations (Micke *et al.,* 1987; Iqbal *et al.,* 1994) resulted in creating genetic variability in different crop species. The objective of this study is to evaluate in M_1 and M_2 generations of an HRS derivative the effect of 15 krad of gamma radiation. Microsatellites markers were used to monitor the introgression of DNA fragments coming from the Australian species *G. sturtianum* Willis in a population of derivatives obtained from the HRS trispecific hybrid.

Materials and methods

Plant materials

The plant materials used in this study were obtained in the framework of a program aiming at improving the nutritional quality of the seed, by trying to develop upland cotton commercial varieties presenting the *glanded-plant* and *glandless-see*d trait of *G. sturtianum* Willis.

Two cultivars of *G. hirsutum* L. $2(A_hD_h)_1$ (NC8 and C2), one accession of *G. raimondii* Ulbr. ($2D_5$) and one accession of *G. sturtianum* Willis ($2C_1$) were used for the creation of the trispecific hybrid HRS (*G. hirsutum* x *G. raimondii* x *G. sturtianum* [$A_hD_hD_5C_1$]) according to the pseudophyletic introgression method (Stewart, 1995). The scheme to create the trispecific hybrid is detailed in Vroh Bi *et al.* (1998). The selected plants were euploid (2n=4x=52) and showed high frequency of chromosome pairing and chiasmata (Mergeai *et al.,* 1997; Vroh Bi *et al.*, 1999).

HRS 'BC2S6' and HRS 'BC2S7' hybrids were produced by selfing in the HRS 'BC2S5/9/6/1/51/15' genetic stock, this plant was chosen for its ability to give segregating progenies for low gossypol content in the seed. The HRS 'BC2S5/9/6/1/51/15' genotype was issued from a seed with 0.36% of seed gossypol content and exhibited the *low-gossypol* seed and *high-gossypol* plant trait. This genotype was also selected because of its fertility and its balanced morphology.

The HRS 'BC2S5/9/6/1/51/15' seeds were first scarified to select the well formed seeds, and half of them were treated with 15 krad of gamma rays at the institute of radio-elements in Fleurus/Belgium.

Then, gossypol content in irradiated seed and not irradiated seed was evaluated by the visual method developed by Benbouza *et al.* (2002). This method of indirect quantification of the seed gossypol content is based on the relation between gossypol content (in% of seed kernel mass) and the number of glands per seed section, following the model: %G=b x (N/S); where %G is the content of gossypol in %, N is the number of gossypol glands per seed section, S is the area of the seed section expressed in mm^2, and b is the regression coefficient calculated for the progeny of a particular genotype.

In M_1, 61 irradiated seeds and 61 not irradiated seeds of HRS BC2S5/9/6/1/51/15 (0.36%G) were followed from germination to harvest. Growth in M_1 was weekly monitored regarding height, diameter, and number of vegetative and fruiting branches.

In the next step, progenies of the most fertile 'BC2S6' plant from irradiated and non-irradiated seeds, with low gossypol content (< 0,2%G) were chosen to assess stability of HRS hybrid and expression of low gossypol seed in M_2. So, in M_2, the harvested seeds from the non irradiated plant HRS 'BC2S6/9/6/1/51/15/7' (0.16%G) and from the irradiated plant HRS 'BC2S6/9/6/1/51/1519' (0.15%G) were monitored. The gossypol content of 49 and 46 seeds respectively from irradiated and non irradiated seeds was evaluated.

Microsatellite marker analysis

In order to monitor the transmission of alleles coming from the Australian wild species the *G. Sturtianum*, ten specifics SSRs markers conserved on c2-c14, c3-c17, and

c6-c25 linkage groups in HRS progeny (Benbouza *et al.,* 2010) were tested. Microsatellites markers in cotton are chromosome-specific and evenly distributed along chromosomes (Liu *et al.,* 2000; Nguyen *et al.,* 2004). Such markers reveal a higher level of polymorphism than others.

Total DNA was extracted from the young leaves of each plant with the CTAB method following Benbouza *et al.* (2006a). SSR analyses were carried out as described in Benbouza *et al.* (2006b) and Liu *et al.* (2000). Amplification was performed with PTC 100 and 200 thermal cyclers. After the addition of 20µl of loading buffer (98% formamide, 10 mM EDTA, bromophenol blue, xylene cyanol), the mixes were denaturated at 92°C for 3 min, and 5 µl of each sample were loaded onto a 6% polyacrylamide gel with 7,5 M urea and electrophoresed in 0.5% TBE buffer at 110-120 W. A silver staining technique was used to reveal amplified SSR products as described in Benbouza *et al.* (2006b).

Results and discussions

Comparison between plants from irradiated and non-irradiated seeds in M_1 showed differences in early plant growth (at germination). Tab. 1 and 2 present the results for germination, emergence, survival of selected seed, and fertility of surviving plant respectively.

Globally, non-irradiated seed gives better results. The irradiated seed showed lower rate of germination (81.97%) and lower rate of plant survival (57.38%). While, non-irradiated seed showed respectively 100% and 63.93%. The same tendency was found regarding HRS 'BC2S6' growth, and plant fertility. Indeed, a morphology analysis followed with weekly measure showed that non-irradiated plants grow slightly much faster than irradiated plants. But these results are not statically significant. Furthermore, Tab. 2 shows that plants from non-irradiated seeds are more fertile (73.24 seeds per harvested plant) and the number of sterile plants is lower (1 plant; 3%), while plants from irradiated seeds showed respectively 51.62 seeds per harvested plant and 18 (46%) sterile plants. These results suggest that gamma radiation in M_1 increases the problems of lethality and sterility already observed in HRS hybrid. In fact, the starting plant material consisted of seeds generally stunted and malformed, which had very low germination. These observations are the consequence of interspecific hybridization. The transfer of desired genes or gene clusters from alien species to superior cultivars is often accompanied by unacceptable wild traits due to redhibitory genes also present in the transferred chromosome segment (Endo, 1990; Marais *et al.,* 2010). The Australian diploid species *G. sturtianum* (genome C) possesses valuable traits such as gossypol free seeds. However this specie belongs to the tertiary germplasm pool which is the most difficult group to use to introgress genes into domesticated cotton (Vroh Bi *et al.,* 1998; Becerra *et al.,* 2007). In some populations derived from interspecific crosses it is difficult to obtain stable progeny lines (Plieske *et al.,* 1998). Similar transmission problems have been encountered with other species involving interspecific hybridizations (Heijbroek *et al.,* 1988; Tonguç *et al.,* 2003).

Besides this, we know that all mutagenesis have deleterious effects (Sudhakaran, 1971; Kowyama *et al.,* 1994; Viccini, 2002). In many instances Sheidai *et al.* (2002) report that mutation breeding using ionizing radiation (including gamma rays) has led to phenotypic and cytogenetic abnormalities. So, the coupling of deleterious effects of mutagenesis and deleterious effects of interspecific hybridization leads logically to increased stability problem in the plant produced from the HRS irradiated derivatives. Koornneef, (2002) found an increment of abortion and sterility accordingly to a gamma ray treatment. These abnormalities are due to the accumulation of chromosome rearrangements during meiosis, consequently to change, inversion and deletion caused by a gamma ray treatment. Nevertheless, the notable fertility improvements observed between some irradiated seed with low gossypol content (104.5 seed per harvested plant), while non-irradiated seeds produced 66 seed per harvested plant, constitutes a clue of the achievement of favourable recombinations due to the gamma ray treatment.

Secondly, SSR analysis with the ten conserved markers (Tab. 3 and 4) showed no genotypic difference between irradiated and non-irradiated plants. They are all 100% heterozygote. Except the primer BNL 226b which has 1 plant in non-irradiated progeny and 4 plants in irradiated progeny which lost *G. sturtianum* allele, they are homozygote *G. hirsutum* at 100% in this locus.

These results show that the use of gamma ray to induce deletion in the targeted linkage group hasn't yet given the desired results. These findings are consistent with Chopra (2005), working on barley following mutagenesis treatment, who showed that no mutants were recovered in the M_1 or M_2 generations of autotetraploids. Furthermore, the same author found that results of mutation experiments with diploid (*Gossypium arboreum*) and tetraploid (*G. hirsutum*) cotton were totally different from those of wheat and other cereals (Brock, 1980; Chopra, 2005). Cotton was found to be relatively resistant to mutagenic treatments and very few mutants could be recovered in M_2

Tab. 1. Germination, emergence and plant survival

Genotype	Total number of seeds	Germinated seed (%)	Seedlings (%)	Adult plants (%)
'BC2S5 9/6/1/51/15' irradieted	61	50(82)	39(64)	35(57)
'BC2S5 9/6/1/51/15' not irradieted	61	61(100)	40(66)	39(64)

Tab. 2. Fertility analysis of plants HRS 'BC2S6' irradiated and non-irradiated

'BC2S6' irradiated					
Gossypol content of analysis plants	Number of survival plants	Number of harvest plants (%)	Number of sterile plants (%)	Total of harvested seed	Number of seed per harvest plant
0,001-0,2%	5	2(40)	3(60)	209	104.50
0,21-0,50%	21	9(43)	12(57)	319	35.44
0,51-1,50%	13	10(76)	3(24)	556	55.60
Total or average	39	21 (54%)	18(46%)	1084	51.62
'BC2S6' non-irradiated					
Gossypol content of analysis plants	Number of survival plants	Number of harvest plants (%)	Number of sterile plants (%)	total of harvested seed	Number of seed per harvest plant
0,001-0,2%	6	5(83)	1(17)	330	66.00
0,21-0,50%	22	22(100)	0	1940	88.18
0,51-1,50%	11	11(100)	0	513	4664
Total or average	39	38(97%)	1(3%)	2783	73,24

and M_3 generations. It was observed that diplontic selection was highly operative in cotton which ensured that only cells with normal complement of chromosomes were functional to produce gametes. Indeed, in maize, 15 krad of gamma irradiation caused a reduction of 55% germination while 82% of germination was obtained in HRS 'BC2S6 ' from irradiated seeds at the same dose. In wheat, 10 Krad of gamma irradiation is the optimal dose to induce mutation (Sear, 1993). This relative resistance of cotton to ionizing radiation could also explain that, contrary to what has been observed in other species, the treatment with gamma ray (15 krad) on the HRS 'BC2S6' hybrid seeds did not induce the appearance of chimeric tissues in the M_1. Therefore, the critical or useful dose of mutagenesis varied among species and also ranged from genotype to genotype within species (Chopra, 2005).

The persistence of high frequencies of heterozygosity for all conserved *G. sturtianum* SSR markers, after several generations of selfing, and after gamma ray application in M_1, indicates that the cytogenetic/genetic conditions for obtaining homozygote at high frequency are not met. The unequal segregation of alleles is the consequence of self-incompatibility and zygote selection. There are several possible explanations for high transmission of these chromosomes fragments in *G. hirsutum* background. They include (1) the presence of gametocidal gene on aliens fragments, (2) post zygotic lethality due to genetic interaction of *G. sturtianum* recessives alleles with *G. hirsutum* genetic background, and (3) zygotic lethality due to the presence of lethality factor (s) on the conserved alien fragments which are expressed in homozygote state. However, discovering of expected gamma ray effects needed at least two generations of meiosis involving chromosome segregation and recombination.

Preliminary study of M_2 shows that abortion and mortality observed in M_1 still remains (Tab. 5). In fact, only 47% of seed from M_1 irradiated parent are well formed, while 75% from non irradiated seed are well formed (Tab. 5). Analysis of gossypol content (Fig. 1) highlights significantly the increase of the number of seed with high gossypol content. These results are very encouraging, but obviously in M_2 the problem of lethality is always present and is higher in irradiated seeds where 53% of seed are lost, while, only 25% non-irradiated seeds are lost (Tab. 5).

Tab. 3. Quantification of the transfer of SSR markers through auto-pollination of the selected HRS 'BC2S6' from HRS BC2S5/9/6/1/51/15irradiated (39 individuals)

Chromosome	Conserved SSR markers	Homozygote *G. hirsutum* (%)	Heterozygote (%)	Homozygote *G. sturtianum* (%)	Number of individuals without DNA amplification
C2	BNL3590	0	37 (100)	0	2
	BNL3971	0	39 (100)	0	0
	BNL2443b	0	37 (100)	0	2
	BNL226b	4 (12)	34 (88)	0	1
C3	BNL3989	0	37 (100)	0	2
	CIR058	0	31 (100)	0	8
	CIR228a	0	39 (100)	0	0
C6	BNL3359b	0	39 (100)	0	0
C25	BNL3436	0	39 (100)	0	0
	BNL1153	0	39 (100)	0	0

Tab. 4. Quantification of the transfer of SSR markers through auto-pollination of the selected HRS 'BC2S6' from HRS 'BC2S5/9/6/1/51/15' non-irradiated (39 individuals)

Chromosome	Conserved SSR markers	Homozygote *G. hirsutum* (%)	Heterozygote (%)	Homozygote *G. sturtianum* (%)	Number of individuals without DNA amplification
C2	BNL3590	0	39 (100)	0	0
	BNL3971	0	39 (100)	0	0
	BNL2443b	0	37 (100)	0	2
C3	BNL226b	1 (3)	38 (97)	0	0
	BNL3989	0	39 (100)	0	0
	CIR058	0	39 (100)	0	0
	CIR228a	0	35 (100)	0	4
C6	BNL3359b	0	38 (100)	0	1
C25	BNL3436	0	39 (100)	0	0
	BNL1153	0	39 (100)	0	0

Tab. 5. Results of scarification of seeds from some inbred plants HRS 'BC2S6/9/6/1/51/1519' (0.15 %G) plants issued from non irradiated seed and 'BC2S6/ 9/6/1/51/15/7' (0.16%G) issued from irradiated seed

Genotype (gossypol content)	Total number of seeds	Number of well formed seed (%)	Number of malformed seed (%)	Number of empty seed (%)	Rates of lost seed
'BC2S6/9/6/1/5/15/7' (0.16%G)	150	71(47)	41(27.5)	38(25.5)	53%
'BC2S69/6/1/5/15/19' (0.15%G)	96	72(75)	7(7)	17(18)	25%

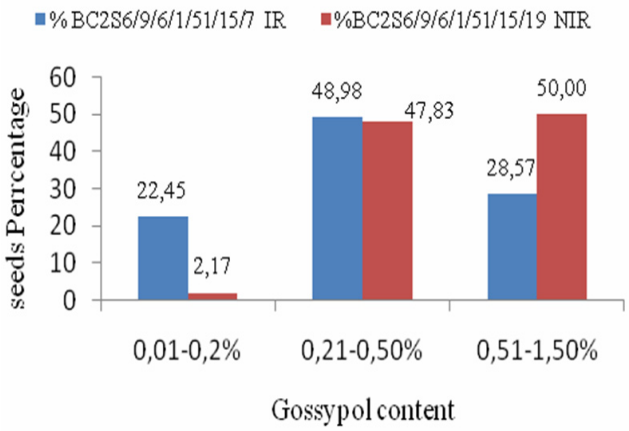

Fig. 1. Analysis of gossypol content of the HRS 'BC2S6' progeny of issued from Irradiated seed (49 seeds) and non-irradiated seed (46 seeds)

Conclusions

In interspecific crosses, segregation distortion and linkage drag may hinder the success of breeding programmes. The segregation distortion of particular loci could cause serious problems in introgression breeding if they are closely linked to agronomically important genes. In fact, preferential loss/recovery of a specific allele, chromosome, or genome between two generations can result from numerous phenomena, e.g., zygotic lethality. Gamma irradiation used to overcome lethality in HRS hybrid did not give expected results in M_2. However, the relative resistantce of cotton to mutagenic treatments observed, and improvement of fertility in M_1 added to the increase of low gossypol seed expression in M_2 due to gamma radiation, shows that monitoring must continue on the next generation. Agro morphological, genetic and cytological analysis of M_2 and M_3 hybrid should give interesting results.

References

Ahloowalia, B. S., M. Maluszynski and K. Nichterlein (2004). Global impact of mutation-derived varieties. Kluwer Academic Publishers Euphytica 135:187-204.

Benbouza, H., J. M. Lacape., J. M. Jacquemin., B. Courtois., F. B. H. Diouf., D. Sarr, N. Konan., J. P. Baudoin and G. Mergeai (2010). Introgression of the low-gossypol seed and high-gossypol plant trait in upland cotton: Analysis of [(*Gossypium hirsutum* 3 *G. raimondii*)2 3 *G. sturtianum*] trispécifique hybrid and selected derivatives using mapped SSRs. Mol Breeding 2:273-286.

Benbouza, H., G. Lognay, R. Palm, J. P. Baudoin and G. Mergeai (2002). Development of a visual method to quantify the gossypol content in cotton seeds. Crop. Sci 42:1937-1942.

Benbouza, H., J. M. Jacquemin, J. P. Baudoin and G. Mergeai (2006b). Optimization of a reliable, fast, cheap and

sensitive silver staining method to detect SSR markers in polyacrylamide gels. Biotechnol. Agron. Soc. Environ 10(2):77-81.

Benbouza, H., J. P. Baudoin and G. Mergeai (2006a). Amélioraton de la méthode d'extraction d'ADN au CTAB appliquée aux feuilles de cotonnier. Biotechnol. Agron. Soc. Environ. 10:73-76.

Brock, R. D. (1980). Mutagenesis and crop improvement. In: Biology of crop productivity. (Carlson P.S., ed.). Academic Press, New York.

Brubaker, C. L., C. G. Benson., C. Miller and N. D. Leach (1996). Occurence of terpenoid aldehydes and lysigenous cavities in the "glandless" seeds of Australian *Gossypium* species. Australian J Bot. 44:601-612.

Brubaker, C. L., F. M. Bourland and J. F. Wendel (1999). The origin and domestication of cotton. *In* Cotton. *Edited by* C.W. Smith and J. T. Cothren. J. Wiley and Sons, New York.

Chee, P. X., C. X. Draye, L. Jiang, T. A. Decanini, R. Delmonte, C. W. Bredhauer, Smith and A. H. Paterson (2005). Molecular dissection of interspecific variation between Gossypium hirsutum and Gossypium barbadense (cotton) by a backcross-self approach: I. Fiber elongation. Theor. Appl. Genet. 111:757-763.

Chopra, V. L. (2005). Mutagenesis: Investigating the process and processing the outcome for crop improvement. Current Science 89(25):353-359.

Fryxell, P. A. (1992). A revised taxonomic interpretation of *Gossypium* L. (Malvaceae). Rheedea 2:91-114

Gao, W., Z. J. Chen, J. Z. Yu, R. J. Kohel, J. E. Womack and D. M. Stelly (2006). Wide-cross whole-genome radiation hybrid mapping of the cotton (*Gossypium barbadense* L.) genome. Mol Gen Genomics 275:105-113.

Iqbal, R. M. S., M. B. Chaudhry, M. Aslam and A. A. Bendasha (1994). Development of a high yielding cotton mutant, NIAB-92 through the use of induced mutations. Pakistan J. Bot 26:99-104.

Jiang, C. X., P. X. Chee, P. Draye, C. Morrell, W. Smith and A. H. Paterson (2000). Multi-locus interactions restrict gene flow in advanced-generation interspecific populations of polyploid *Gossypium* (cotton). Evolution 54:798-814.

Kasha, K. J. (1974). Haploids from somatic cells, p. 67-87. In: haplods in higher plants-advance and potential. Proceedings of the last symposium on haploids in higher plant, 10-14 June 1974. Ontario, Canada, University of Guelph.

Koornneef, M. (2002). Classical mutagenesis in higher plants. Department of Genetics, Wageningen University, Dreijenlaan 2, 6703 HA Wageningen, The Netherlands.

Lee, J. A. (1984). Cotton as a world crop. In: Kohel RJ, Lewis CL (eds) Cotton. Agronomy Monograph. Crop Science Society of America. Madison 24:1-25.

Lee, J. A. (1982). Linkage relationships between *Le* and *Gl* alleles in cotton. Crop. Sci 22:1211-1213.

Liu, S., S. Saha, D. Stelly *et al.* (2000). Chromosomal assignment of microsatellites loci in cotton. Am Genet Assoc 91:326-332.

Mergeai, G., J. P. Baudoin and I. Vroh Bi (1997). Exploitation of trispecies Hybrids to introgress the glandless seed and glanded plant trait of *Gossypium sturtianum* Willis into *G. hirsutum* L. Biotechnol Agron Soc Environ 1:272-277.

Mergeai, G., J. P. Baudoin and I. Vroh Bi (2000). Production of high-gossypol cotton plants with low-gossypol seed from trispecific hybrids including Gossypium sturtianum willis. p. 206-210. In New frontiers in cotton research. Proc. World Cotton Res. Conf-2, Greece.

Micke, A., B. Donini and M. Maluszynski (1987). Induced mutations for crop Improvement-a Review. Trop. Agric. (Trinidad) 64:259-78.

Nguyen, T., M. Giband., P. Brottier *et al.* (2004). Wide coverage of the tetraploid cotton genome using newly developed microsatellites markers. Theor Appl Genet. 109:167-175.

Reddy, P. S., M. V. Reddi, B. T. Raju and S. M. Ali (1977). Creation of genetic variability by recourse to irradiation in groundnut (*Arachis hypogaea* L.). Oleagineux 32 (2):59-62.

Sear, E. R. (1993). Use of Radiation to transfer Alien Chromosome Segments to Wheat. Crop. Sci. 33:897-901.

Sheidai, M., H. Azarani and Z. Hosseininejad (2002). Cytogenetic study of gamma irradiated lines of cotton (*Gossypium hirsutum* L.). J. Sci. I. R. Iran 13(4):311-322.

Stewart, J. McD (1995). Potential for crop improvement with exotic germplasm and genetic engineering, p.313-337. In> G. A. Constable and N. W. Forrester Eds. Challenging the future. Proc. World Cotton Res. Conf.- 1, Brisbane, Australia.

Vroh, Bi I., J. P. Baudoin and G. Mergeai (1998). Cytogenetics of the 'glandless-seed' and 'glanded-plant' trait from *Gossypium sturtianum* Willis introgressed into upland (*Gossypium hirsutum* L.). Plant Breed. 117:235-241.

Vroh, Bi I., A. Maquet, J. P. Baudoin, P. Du Jardin, J. M. Jacquemin and G. Mergeai (1999). Breeding for low gossypol seed and hugh gossypol plants in upland cotton. Analysis of tri species hybrids and backcross progenies using AFLP and mapped RFLPs. Theor. Appl. Genet. 99:1233-1244.