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RESEARCH ARTICLE

Microsatellite Markers Reveal the Genetic Integrity of Stored Accessions of Cotton (*Gossypium arboreum* L.) in the Indian National Genebank

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Abstract

The regeneration of germplasm from gene banks is a critical step in conserving plant genetic resources. However, it might introduce changes in allele frequencies that can impact genetic diversity. Microsatellite markers have emerged as essential tools for tracking these changes, allowing genebank managers to make informed decisions about their conservation strategies. By using microsatellite markers, genebank managers can ensure the long-term availability and viability of plant genetic resources, contributing to the sustainability of agriculture and biodiversity conservation. The genetic integrity of six cotton accessions (*Gossypium arboreum* L.) conserved in the Indian National Genebank was investigated using microsatellite markers. These accessions were initially conserved in the genebank and subsequently regenerated during the years 2000, 2007 and 2015. The selected five microsatellite marker loci revealed the genetic integrity of these accessions with minor genetic changes at rare alleles. In three accessions, new bands at very low frequencies were observed after regeneration, whereas in two, few bands were lost after regeneration. No significant changes were observed after the regeneration of cotton accessions with respect to Nei's expected heterozygosity and Shannon's Information Index. There were no significant changes in genetic integrity and structure, indicating that the protocol being followed for regeneration was quite efficient and effective.

Keywords: Cotton, *Gossypium arboreum*, Genebank, Genetic integrity, Regeneration, SSR markers.

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Introduction

Cotton belongs to the family Malvaceae and has been known to mankind ever since human civilization started. Its economic importance as a fibre crop stands unparalleled to any other, and has been rightly called as white gold. Cotton is considered to be the backbone of the textile industry in India. The genus *Gossypium* comprises more than 50 species under eight major genome groups. Of these 50 species, four are more widely cultivated worldwide: *Gossypium arboreum* L., *G. herbaceum* L., *G. hirsutum* L., and *G. barbadense* L. India is the only country in the world where all four of these cultivated species are grown on a commercial scale.

Genebanks across the globe have amassed a total of 104780 accessions of cotton (FAO, 2019). The National Genebank at ICAR-NBPGR, New Delhi houses more than 10000 accessions of different species of cotton. A major challenge faced by the genebank curators is maintaining the genetic integrity of the conserved accessions over several years, particularly during regeneration when the viability of stored accessions starts deteriorating. Some of the factors affecting the genetic integrity of the accessions held in *ex-situ* collections include foreign contamination by pollen grains, admixtures, improper handling during regeneration, genetic drift, mislabeling, etc.

Microsatellite markers have been prominently associated with low-copy regions of plant genomes and is a powerful tool for genetic diversity analysis, population structure analysis, gene flow among populations, DNA fingerprinting, association mapping and linkage mapping in crop plants (Zalapa, J. E. *et al.* 2012, Sugita, T. *et al.* 2013, Gyawali, S. *et al.* 2016, Zhao, J. *et al.* 2017). These have been widely used in cotton also for analyzing genetic diversity (Kumar *et al.* 2022, Lu *et al.* 2022), DNA fingerprinting (Santosh *et al.* 2022), hybrid purity testing (Dongre and Parkhi 2005, Rana *et al.* 2006), QTL mapping of various economic traits like fibre quality (Shen *et al.* 2005) and population structure analysis (Tyagi *et al.* 2014, Zhu *et al.* 2019). However, microsatellite markers have not been employed much in analyzing the genetic integrity of the stored accessions except in few crops like wheat (Börner *et al.* 2000) open, pollinated species of rye (Chebotar and Börner 2002), tobacco (PAN *et al.* 2013) pearl millet (Dan *et al.* 2020) etc. To the best of our knowledge, this study is the first to report using simple sequence repeats (SSR) markers for genetic integrity in cotton.

Materials and Methods

Plant Material and DNA Preparation

The cotton database was scrutinized for accessions that had been regenerated over the years, and six accessions that were regenerated and conserved consecutively during the years 2000, 2007 and 2015 in the National Genebank at ICAR-NBPGR, New Delhi were finally selected. The seeds of these accessions from the voucher specimen were procured and sown in paper towels in the laboratory. Each accession contained a variable number of seeds and the average number of seeds varied from 35.0 to 72.7 seeds per accession (Table 1). The selected six accessions, regenerated in three cycles, gave 24 combinations of accessions x years.

The DNA from individual seeds of each accession was extracted by following the protocol given by Gupta *et al.* (2012). This modified method used an extraction buffer containing 200 mM tris-HCl, pH 8.0, 200 mM $NaCl_2$, 25 mM EDTA, 0.5% SDS and a CTAB solution with 2% PVP for extracting DNA from cotton seeds. The extracted DNA obtained was dissolved in 50 µL of tris-EDTA (TE) buffer. The quality of the DNA obtained was compared with standard polymerase chain reaction (PCR) products and was found enough to run the PCR. A total of 989 individual seed DNA extractions were completed from the voucher accessions and were used for analyzing the genetic integrity.

PCR Gel Electrophoresis and Data Analysis

Based on earlier studies and initial screening of over 50 microsatellite primers, a total of eight microsatellite loci (BNL3545, DPL0702, NAU2190, JESPR204, NAU1043, NAU5013, NAU6966 and NAU4926) (Table 2) that were well distributed across the various chromosomes of cotton and those having good polymorphism information content (PIC) were used in this study. Five primers in each accession were used for integrity investigation. PCR included 1X PCR buffer, 3.0 mM $MgCl_2$, 0.2 mM dNTP, 0.5U *Taq* polymerase (all reagents from M/S Sigma), 0.1 µM of each forward and reverse primer and 20ng of sample DNA in a single PCR reaction.

After PCR amplification, the PCR products were separated in a gel containing 3% metaphor agarose which was stained using ethidium bromide. PCR products were mixed with loading dye for visualization of the bands. The gel electrophoresis was run in a gel chamber containing tris-acetate EDTA (TAE) buffer at 90 volts against a standard DNA ladder of 100 bp until the proper separation of the bands was obtained, usually from 2 to 3 hours. The gels were visualized under a gel documentation system using UV light and the resulting images were stored. The gel bands were converted to binary data as presence (1) or absence (0) in a scoring sheet and later fed into an Excel sheet for analysis. A number of alleles, allele frequency, gene diversity, observed heterozygosity, expected heterozygosity and fixation index values were calculated using software like Powermarker (Liu and Muse, 2005) and GenAlex (Peakall and Smouse (2012).

Results and Discussion

Germplasm stored in genebanks is subject to various risks, including genetic drift, loss of viability and contamination. To overcome these risks and maintain the genetic diversity of plant species, genebanks periodically regenerate their collections. Regeneration involves growing a subset of the germplasm, harvesting the seeds or tissues, and then restoring these new samples in the genebank. This process ensures the genetic integrity of the stored accessions and helps maintain the long-term availability of these genetic resources for research, breeding and conservation. The genetic integrity of six cotton accessions (*G. arboreum* L.) conserved in the Indian National Genebank was investigated using microsatellite markers. The number of alleles amplified by the microsatellite markers with each *G. arboreum* accession over the three years in which the accessions were regenerated is given in Table 3. Table 4

Table 1: Year wise seed number of cotton (*G. arboreum* L.) accessions used for genetic integrity analysis

S. No.	*Accession*	*Source*	*2000*	*2007*	*2015*
1	IC440885	Meghalaya	69	77	72
2	IC440886	Meghalaya	40	69	60
3	IC440887	Meghalaya	60	46	47
4	IC440888	Meghalaya	70	48	44
5	IC440889	Meghalaya	63	59	60
6	IC440890	Meghalaya	34	33	38
Average			56	55	53

Table 2: List of SSR primers used for the genetic integrity analysis of cotton (*G. arboreum* L) accessions.

S. No.	*Primer*	*Linkage group/Chromosomal Position*	*Forward*	*Reverse*
1	BNL3545	Chr02	AGTCAGTTTTTTGTTAGCAATATGC	AACCATTAATTCCCTATTTAACCG
2	DPL0702	Chr06	GATCTCTCTATCAACGACCAGGTT	CAACCGTCCGTCATTAGTGTAATA
3	NAU2190	Chr14	CAACCAACATATTCCAAACA	TTATTTCGGCCTTGTTTTTC
4	JESPR204	Chr13	CTCCAGGTTCAATGGTCTG	GCCATGTTGGACAAGTAGTC
5	NAU1043	AO7	GTATCCGCCCACAAATAAAG	GCATCGTGAGAGAAAGTGAA
6	NAU5013	Chr20	GTCGGGTCATTACAAGGAAG	CTTCTGCTTTTGGTTTTGGT
7	NAU6966	Chr05.2	GTCATCATTATCGTCAAGTC	AAAGTGAGTTAAGAAAGGCT
8	NAU4926	Chr26	CGCCTCTGTATTCGATTCTC	GCGTAAATAAAGCGAAAACC

Table 3: Alleles amplified in the cotton (*G. arboreum* L.) accessions conserved in the National Genebank after three years of regeneration using microsatellite markers

Accession identity	*Year of regeneration*	*BNL3545*	*DPL0702*	*NAU2190*	*JESPR204*	*NAU1043*	*NAU5013*	*NAU6966*	*NAU4926*
IC440885	2000	-	-	200	190,180	210	200,180	150,100	-
	2007	-	-	190,200	190,180	210	200,180	150,100	-
	2015	-	-	200	190,180	210	200,180	150,100	-
IC440886	2000	200,180	200,150	200	190,180	210			
	2007	200,180	200,150	200	190,180	210,180			
	2015	200,180	200,150	200	190,180	210,180			
IC440887	2000	-	200,150	200	190,	210	-	150,100	-
	2007	-	200,150	200	190,150	210	-	150,100	-
	2015	-	200,150	200	190	210	-	150,100	-
IC440888	2000	200,150	200,150	210,200	-	210	-		150
	2007	200,150	200,150	210,200	-	210	-		150
	2015	200,150	200,150	200	-	210	-		150
IC440889	2000	200	200,180	200	-	210	-		150, 80
	2007	200	200,180	200	-	210	-		150, 80
	2015	200	200,180	200	-	210	-		150, 80
IC440890	2000	200,150	200,150	210	190,180	-	-	-	150
	2007	200,150	200,150	210	190	-	-	-	150
	2015	200,150	200,150	210	190	-	-	-	150

presents a comprehensive overview of the genetic diversity indices, including the Number of alleles (N), number of effective alleles (Ne), Shannon's Information Index (I), average observed heterozygosity (Ho), average expected heterozygosity (He), Nei's Expected heterozygosity (Nei), and pairwise Fst (Fst) values calculated using GenALEx for each individual year. No major changes were observed regarding the number of bands amplified; however, some minor changes were observed which are described below accession-wise.

IC440885

The genetic integrity of the accession IC440885 was tested using five primers, namely NAU2190, JESPR204, NAU1043, NAU5013 and NAU6966 (Table 3). No major changes were observed with four primers, but with one primer, i.e., NAU 2190, in addition to amplification of a 200 bp band in all of the three regeneration years, i.e., 2000, 2007 and 2015, there was also amplification of an additional band of 190 bp which was observed during the year 2007. Possibly due to contamination, this additional band's frequency was quite low (0.029). Change in allele frequency was not found to be significant in case of the NAU2190 locus. A significant change in allele frequency was observed at the locus JESPR204 when compared for the years 2007 and 2015 and for the years 2000 and 2015. The Shannon's Information Index value decreased from 0.41 in 2000 to 0.32 in 2015; however, this difference was not found to be significant. The average gene diversity decreased from 0.29 in 2000 to 0.21 in 2015. This reduction in gene diversity might signal loss of alleles after regeneration which directly affects the genetic integrity of the accession. However, the changes in gene

Table 4: Number of alleles (N), number of effective alleles (Ne), Shannon's Information Index (I), average observed heterozygosity (Ho), average expected heterozygosity (He), Nei's Expected heterozygosity (Nei) and pairwise Fst values in comparison to original population (year 2000), values in Mean±SD in cotton (*Gossypium arboreum* L.) accessions conserved in the National Genebank

Accession identity	*Year*	*N*	N_e	*I*	H_o	H_E	*Nei*	F_{st}
IC440885	2000	1.60 ± 0.54	1.59 ± 0.54	0.41 ± 0.37	0.55 ± 0.51	0.30 ± 0.27	0.29 ± 0.27	0.000
	2007	1.80 ± 0.44	1.43 ± 0.41	0.37 ± 0.29	0.36 ± 0.38	0.25 ± 0.21	0.25 ± 0.21	0.039
	2015	1.60 ± 0.54	1.34 ± 0.36	0.32 ± 0.30	0.29 ± 0.30	0.21 ± 0.20	0.21 ± 0.20	0.066
IC440886	2000	1.60 ± 0.54	1.59 ± 0.54	0.41 ± 0.37	0.58 ± 0.53	0.30 ± 0.27	0.29 ± 0.27	0.000
	2007	1.80 ± 0.44	1.58 ± 0.51	0.43 ± 0.34	0.55 ± 0.49	0.30 ± 0.25	0.30 ± 0.25	0.005
	2015	1.80 ± 0.44	1.52 ± 0.47	0.40 ± 0.33	0.47 ± 0.43	0.28 ± 0.24	0.28 ± 0.24	0.014
IC440887	2000	1.40 ± 0.54	1.35 ± 0.49	0.26 ± 0.36	0.32 ± 0.45	0.18 ± 0.25	0.18 ± 0.25	0.000
	2007	1.60 ± 0.54	1.30 ± 0.43	0.26 ± 0.31	0.28 ± 0.42	0.17 ± 0.22	0.17 ± 0.22	0.011
	2015	1.40 ± 0.54	1.28 ± 0.42	0.23 ± 0.32	0.25 ± 0.38	0.16 ± 0.23	0.16 ± 0.22	0.011
IC440888	2000	1.60 ± 0.54	1.59 ± 0.54	0.41 ± 0.37	0.37 ± 0.51	0.30 ± 0.27	0.29 ± 0.27	0.000
	2007	1.60 ± 0.54	1.50 ± 0.49	0.38 ± 0.35	0.20 ± 0.44	0.27 ± 0.25	0.26 ± 0.25	0.024
	2015	1.40 ± 0.54	1.40 ± 0.54	0.27 ± 0.37	0.39 ± 0.54	0.20 ± 0.27	0.19 ± 0.27	0.105
IC440889	2000	1.40 ± 0.54	1.34 ± 0.47	0.26 ± 0.35	0.22 ± 0.31	0.18 ± 0.25	0.18 ± 0.25	0.000
	2007	1.40 ± 0.54	1.39 ± 0.54	0.27 ± 0.37	0.37 ± 0.51	0.20 ± 0.27	0.19 ± 0.27	0.019
	2015	1.40 ± 0.54	1.39 ± 0.54	0.27 ± 0.37	0.37 ± 0.51	0.20 ± 0.27	0.19 ± 0.27	0.017
IC440890	2000	1.60 ± 0.54	1.59 ± 0.54	0.41 ± 0.37	0.57 ± 0.52	0.30 ± 0.27	0.29 ± 0.27	0.000
	2007	1.40 ± 0.54	1.39 ± 0.54	0.27 ± 0.37	0.38 ± 0.52	0.20 ± 0.27	0.19 ± 0.27	0.107
	2015	1.40 ± 0.54	1.39 ± 0.54	0.27 ± 0.37	0.38 ± 0.53	0.20 ± 0.27	0.19 ± 0.27	0.107

diversity observed in any of the years were not found to be significant. For the sake of comparison, the individual years were assumed to be separate populations and a pairwise FST comparison was performed. The pairwise FST comparison between the various years revealed very low differentiation levels. Analysis of molecular variance (AMOVA) revealed that the total variance in the accession could be partitioned into 70% within individuals, 17% among individuals and 12% among the various regeneration years.

IC440886

The accession IC440886 was analyzed using five microsatellite markers BNL3545, DPL0702, NAU2190, JESPR204 and NAU1043. A minor change was observed in the case of NAU1043 locus. NAU1043 amplified a single band of 210 bp during all the years. However, in the years 2007 and 2015 a new band was also observed at 180 bp. The new band was present at relatively low frequencies of 0.022 and 0.017 in the year 2007 and 2015, respectively. No significant change in allele frequency was observed in any of the loci analyzed. The gene diversity values were more or less similar as these were 0.29 ± 0.27 and 0.28 during the years 2000 and 2015, respectively. Pairwise F_{ST} values revealed very low genetic differentiation among different years. AMOVA revealed that the total variance in the accession could be portioned into 96% within individuals and 4% among various years. No significant difference was observed in Shannon's information index values after the two regenerations.

IC440887

Five primers namely, DPL0702, NAU2190, JESPR204, NAU1043 and NAU6966 were used to analyze the genetic integrity of this accession. All of the primers produced same-sized fragments using PCR amplification of loci except one, i.e., JESPR204. This primer amplified a single band of the size 190 bp in all three years of regeneration. In addition, this primer produced a band of 150 bp during the year 2007 with a very low frequency of 0.025. No significant change in allele frequency was observed at all the loci analyzed. The gene diversity values were more or less similar at 0.18 and 0.16 during the years 2007 and 2015, respectively. There were no statistically significant differences in Shannon's information index after regenerations. Pairwise F_{ST} values ranged from 0.005 to 0.012, indicating low genetic differentiation after regeneration. AMOVA revealed that most of the variance in the accession was within individuals at 98% and only 2% could be attributed to among years.

IC440888

The accession was analyzed for genetic integrity changes using five microsatellite primers, namely BNL3545, DPL0702, NAU2190, NAU1043 and NAU4926. All these five primers produced consistent band across all three years except for the primer NAU2190 which produced 210 bp band during the years 2000 and 2007 while this was found missing during the year 2015. The frequency of the 210 bp band amplified

by NAU2190 steadily decreased from 0.47 in 2000 to 0.22 in 2007 and to 0 in 2015. Out of all the loci analyzed for change in allele frequency, NAU2190 showed a significant change when years 2000 and 2015 were compared. No significant change in Shannon's information Index was observed. A reduction in gene diversity was also observed in 2007, from 0.29 to 0.19 in 2015. Treating the three cycles of regeneration as three populations, pairwise F_{ST} values were found to be low indicating less genetic differentiation between the various years before and after regeneration. The AMOVA revealed that the highest share of the total variance was due to within individuals at 82% while both among individuals and among years had an equal share of 9% of the total variance.

IC440889

The five primers used for testing the genetic integrity of the accession IC440889 included BNL3545, DPL0702, NAU2190, NAU1043 and NAU4926. No changes were observed in this accession with respect to the number of bands amplified across the various regeneration cycles. No significant change in allele frequency was observed in any of the loci. The same was true for Shannon's information Index as well. The average gene diversity was 0.19 and pairwise F_{ST} values were very low indicating less differentiation between the various years after regeneration in the accession. The AMOVA revealed that 89% of the total molecular variance is from within individuals, followed by 9% from among individuals and 2% among the various years.

IC440890

Five microsatellite markers BNL3545, DPL0702, NAU2190, JESPR204 and NAU4926 were used to analyse the genetic integrity of this accession. There was no change in the bands amplified with respect to four primers, however, one primer, i.e., JESPR204 amplified two bands of the sizes 190 and 180 bp. One band of 190 bp was found consistent in all three years but an additional band of 180 bp which was there during first regeneration in the year 2000, was found to be lost during subsequent regeneration cycles during 2007 and 2015. Change in allele frequency was only found to be statistically significant in the case of JESPR204 loci, between 2000 and 2007 and between 2000 and 2015. No statistically significant changes were observed in Shannon's information index. The gene diversity also decreased from 0.29 in 2007 to 0.19 in 2015. The average gene diversity in this accession was 0.23. Pairwise F_{ST} revealed less genetic differentiation between the three cycles of regeneration in the accession when each individual year was considered as a population. AMOVA results revealed that 93% of the total variance could be attributed to within individuals and 7% due to among years.

Year-wise AMOVA analysis for the six accessions under study indicated that most of the variance was sourced from individuals (55.8%) and within individuals (43.6%) and only 0.4% among the various years. The mean allelic pattern of the combined analysis of all the accessions year-wise are given in Figure 1.

Based on our observations, the genetic integrity of the accessions analyzed has not been altered but some minor changes have been observed. We found the appearance of additional bands, e.g. 190 bp in accession IC440885 and 150 bp in IC440887. However, these were not found to be stable as they were not observed in the subsequent regeneration cycle. Similarly, loss of bands has also been observed in one accession. In the genebank, all regeneration cycles are carried out from the base collection and not from the residual seed left from the previous cycle of regeneration. This practice is to avoid compounding negative effects, if any, on the genetic integrity of the accessions after various cycles of regeneration. These additional bands might not have been sampled from the base collection during regeneration years, where these were found to be missing and might not have been observed using microsatellite markers in the present study. Some other reasons might be that somatic mutations occurring during regeneration cycles might not be stable and revert to the original state in the next cycle of regeneration. Epigenetic modification, PCR artifacts and errors in handling the plant material could also lead to such apparent discrepancies in the presence of absence of a band. The minor fluctuations in the frequencies of bands of the rare alleles observed in the present study might be due to genetic drift. The initial representation of the accession in the gene bank might not be the representation of the entire genetic variation in the accession. Subsequent regenerations may involve different accession subsets, leading to observed frequency fluctuations.

The breeding mechanism in cotton is predominantly self-pollinating; however, cross-pollination has been reported to an extent of 10 to 50%. In the case of *G. arboreum* L., the anthers are in direct contact with the stigma, and the pollen is shed as soon as the flower opens, which makes them predominantly self-pollinated (Kottur, 1921). The natural

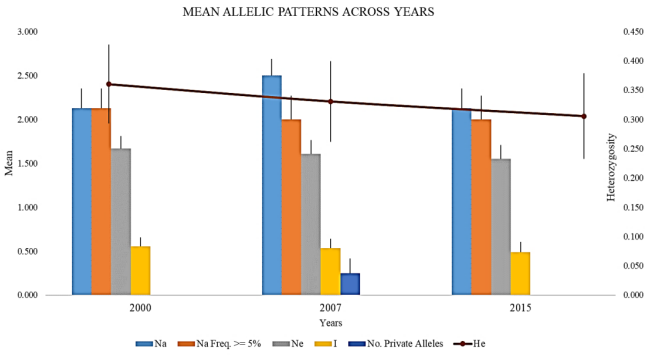

Figure 1: Mean allelic patterns across three cycles of regeneration of cotton (*G. arboreum* L.) accessions conserved in the National Genebank of India

rate of outcrossing in *G. arboreum* L. is about 2% (Afzal *et al*., 1950). The major pollinating agents in cotton are insects; therefore, during the regeneration of cotton, bagging and insect-proofing are done to prevent inadvertent outcrossing. The accessions used in this study have been regenerated in controlled conditions in the field plot at ICAR-CICR, Nagpur. During the regeneration of these accessions, insect-proof nets are used to prevent any inadvertent introduction of foreign pollen or contamination from other accessions. Based on our observations in the genetic integrity of cotton accessions in the genebank, it can be emphasized that the regeneration protocol in place for cotton is quite efficient and effective.

In a study conducted earlier in rye, an open-pollinating species maintained in the gene bank for 45 years, significant changes in allele frequency was noted using microsatellite markers (Chebotar *et al*., 2003). Significant allele frequency changes were also seen in landraces of barley which were analysed with isozyme markers (Parzies *et al*., 2000). Very few studies have been carried out in the case of self-pollinated or often cross-pollinated species of plants. A genetic integrity study using eight accessions of wheat with microsatellite markers found that only a single accession showed some genetic drift, and no significant changes in genetic integrity were observed (Börner *et al*., 2000). Allelic frequency changes in maize studied using isozyme markers revealed no major changes in allele frequency during regeneration, but minor changes due to genetic drift were observed (Reedy *et al*., 1995).

Our study can be compared to that of Börner *et al*., 2000, who analyzed the genetic integrity of the eight accessions of wheat accessions using microsatellite markers. They observed some minor changes due to genetic drift but no significant changes in genetic integrity after regeneration. The minor changes they observed in the genetic integrity were attributed to genetic drift, unintentional selection, pollen contamination, seed contamination, or mislabeling in self-pollinating crops. The voucher accessions used in our study have uneven sample sizes for different accessions used for genetic integrity analysis that may have hindered ascertaining the accessions' complete genetic structure. Conducting more extensive genetic and environmental tests for the material under investigation would be important. Deeper marker analysis, high-density DNP chips and DNA sequencing might clarify the underlying reasons for the observed variations. Maintaining careful records and quality control procedures like increasing area of plots for higher seed production, more stringent collection and labeling practices after regeneration, use of insect-proof cages to prevent cross-pollination and proper selection of site for regeneration and minimizing biotic and abiotic stresses may help in preserving the genetic integrity of the accessions for more period of time.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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References

Abdurakhmonov IY, RJ Kohel, JZ Yu, AE Pepper, AA Abdullaev, FN Kushanov, IB Salakhutdinov, ZT Buriev, S Saha, BE Scheffler, JN Jenkins and A Abdukarimov (2008) Molecular diversity and association mapping of fiber quality traits in exotic *G. hirsutum* L. germplasm. *Genom*. 92(6): 478-487.

Adhikari J, S Das, Z Wang, S Khanal, R Chandnani, JD Patel, VH Goff, S Auckland, LK Rainville, D Jones and AH Paterson (2017) Targeted identification of association between cotton fiber quality traits and microsatellite markers. *Euphytica.* 213(3): 1-15.

Afzal M and AH Khan (1950) Natural crossing in cotton in western Punjab. Natural crossing under field conditions. *Agron J*. 42 (2): 89-93.

Börner A, S Chebotar and V Korzun (2000) Molecular characterization of the genetic integrity of wheat (*Triticum aestivum* L.) germplasm after long-term maintenance. *Theor. Appl. Genet*. 100(3-4): 494-497.

Brenan JPM and JB Hutchinson (1960) The application of genetics to cotton improvement. *Kew Bull*. 14(2): 249.

Chebotar S, MS Röder, V Korzun, A Börner (2002) Genetic integrity of ex situ genebank collections. *Cell Mol. Biol. Lett*. 7(2A): 437-444.

Chebotar S, MS Röder, V Korzun, B Saal, WE Weber and A Börner (2003) Molecular studies on genetic integrity of open-pollinating species rye (*Secale cereale* L.) after long-term genebank maintenance. *Theor. Appl. Genet*. 107(8): 1469-1476.

Dan X, C Wang, Y Su, A Zhang, R Wang, I Khan and L Huang (2020) Evaluation of genetic integrity of pearl millet seeds during aging by genomic-SSR markers. *Mol. Biol. Rep*. 47(8): 5747-5754.

Dongre A and V Parkhi (2005) Identification of cotton hybrid through the combination of PCR-based RAPD, ISSR and microsatellite markers. *J. Plant Biochem. Biotechnol*. 14(1): 53-55.

Dongre AB, DM Ramteke, MR Bhandarkar, MP Raut and KJ Meshram (2011) Genetic diversity analysis of *Gossypium arboreum* (diploid cotton) cultivars revealed by PCR based molecular markers. *J. Cotton Res. Dev*. 25(1): 9-13.

Food and Agriculture Organization (2015) *The Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture*. Commission on Genetic Resources for Food and Agriculture Assessments.

Gupta R, US Chandrashekar, SK Chakrabarty and M Dadlani (2012) A simple modified method of DNA extraction from seeds for PCR amplifications. *Indian J. Agri. Sci*. 82(1): 75-77.

Gutiérrez OA, S Basu, S Saha, JN Jenkins, DB Shoemaker, CL Cheatham and JC McCarty (2002) Genetic distance among

selected cotton genotypes and its relationship with F2 performance. *Crop Sci*. 42(6): 1841-1847.

Hamblin MT, ML Warburton and ES Buckler (2007) Empirical Comparison of Simple Sequence Repeats and Single Nucleotide Polymorphisms in assessment of Maize diversity and relatedness. *PLOS ONE*. 2(12): e1367.

Liu S, RG Cantrell, JC McCarty and JM Stewart (2000) Simple Sequence Repeat–Based Assessment of Genetic Diversity in Cotton Race Stock Accessions. *Crop Sci*. 40(5): 1459-1469.

Pan Y, Y Liu, M Ren, XW Zhang, JY Mou, XY Chen, X Cong and ZD Wang (2013) Analysis on tobacco Genetic Integrity of different populations by SSR Markers. *J. Plant Genet. Resour.* 14(5): 978-984.

Park YH, MS Alabady, M Ulloa, B Sickler, TA Wilkins, J Yu, DM Stelly, RJ Kohel, OM El-Shihy and RG Cantrell (2005) Genetic mapping of new cotton fiber loci using EST-derived microsatellites in an interspecific recombinant inbred line cotton population. *Mol. Genet. Genom*. 274(4): 428-441.

Parzies HK, W Spoor and RA Ennos (2000) Genetic diversity of barley landrace accessions (*Hordeum vulgare* ssp *vulgare*) conserved for different lengths of time in ex-situ genebanks. *Heredity*. 84(4): 476-486.

Peakall R and PE Smouse (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*. 28(19): 2537-2539.

Rao NK, EH Roberts and RH Ellis (1988) A comparison of the quantitative effects of seed moisture content and temperature on the accumulation of chromosome damage and loss of seed viability in lettuce. *Ann Bot*. 62(3): 245-248.

Reedy ME, AD Knapp and KR Lamkey (1995) Isozyme allelic frequency changes following maize (*Zea mays* L.) germplasm regeneration. *Maydica*. 40(3): 269-273.

Shen X, W Guo, X Zhu, Y Yuan, JZ Yu, RJ Kohel and T Zhang (2005) Molecular mapping of QTLs for fiber qualities in three diverse lines in upland cotton using SSR markers. *Mol. Breed*. 15(2): 169-181.

Thompson PA, RD Smith, JB Dickie, RH Sanderson and RJ Probert (1981) Collection and regeneration of populations of wild plants from seed. *Biol. Conserv.* 20(3): 229-245.

Tyagi P, MA Gore, DT Bowman, BT Campbell, JA Udall and V Kuraparthy (2014) Genetic diversity and population structure in the US upland cotton (*Gossypium hirsutum* L.). *Theor. Appl. Genet*. 127(2): 283-295.

Ulloa M, IY Abdurakhmonov, M Perez, R Percy and JM Stewart (2013) Genetic diversity and population structure of cotton (*Gossypium* spp) of the New World assessed by SSR markers. *Botany*. 91(4): 251-259.

Yeh FC (1997) Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belg J. Bot*. 129: 157.

Yu J, Z Zhang, DA Zhu C Tabanao, G Pressoir, MR Tuinstra, S Kresovich, RJ Todhunter and ES Buckler (2009) Simulation appraisal of the adequacy of number of background markers for relationship estimation in association mapping. *Plant Genom*. 2(1): 63-77.