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ESTIMATION OF ALLELIC LOSS IN SESAME (*SESAMUM INDICUM* L.) VARIETIES

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Abstract

Thirty four confirmed polymorphic ISSR markers were used to study genetic erosion in 35 sesame (*Sesamum indicum* L.) varieties developed over a period of 45 years (1959 - 2004) in India. Each ISSR band was scored as unique genetic locus as either a heterozygote or dominant homozygote whereas, the absence was considered as a fixed recessive homozygote. A higher proportion of fixed recessive loci meant lower genetic variation for the accession. A total of 235 polymorphic bands were amplified of which three, were present in all the varieties. Nine bands were present in one cultivar each. Comparisons of ISSR variation were made among accessions representing three breeding periods of 15 years each. The level of fixed recessive alleles was observed to be almost the same (38.9, 41.6 and 40.2%) over one time period to the next. The regression of the proportions of fixed recessive ISSR loci over the year of cultivar release generated a linear regression coefficient which was not statistically significant from zero suggesting no significant loss of genetic variation as a consequence of varietal improvement in sesame over a period of 45 years.

Introduction

Sesame (*Sesamum indicum* L. 2n = 26) is an ancient oil seed crop. Its plant is a tropical annual herb grown in tropical to the temperate zones from about 40^0N to 40^0S latitude (Ashri 1998). Sesame is cultivated in many in countries of the African and Asian continent. In India, it is the fifth important oilseed crop. It is mainly cultivated as a rainy season crop although in some parts of the country it can be grown successfully throughout the year. There occurs tremendous phenotypic variability in sesame owing to its cultivation in diverse climatic and geographic conditions. Modern plant breeding has led to reduction in genetic diversity due to the replacement of land race varieties with improved ones. The reduction in genetic diversity is generally known as genetic erosion (Frankel and Bennett 1970) and is defined as "the loss of genetic diversity in a particular location and over a particular period of time. This also includes the loss of individual genes and the loss of particular combinations of genes, such as those manifested in landraces or varieties (FAO/IPGRI 2002). The traditional perception of genetic erosion meant the loss of stable and diverse set of locally adapted landraces resulting from the adoption of a small number of modern varieties (Hawkes 1983). The effect of modern varieties on local diversity is far from obvious. Diversity could even increase if improved germplasm is genetically more heterogeneous than local varieties or if it offers traits that are not present in traditional landraces (Louette and Smale 2000). Fears have often been expressed that modern intensive plant breeding is leading to genetic erosion (Vellve 1993) which if correct, can have serious consequences both for the genetic vulnerability of crops and for their plasticity to respond to changes in the production environment. It is vital for plant breeding programs to maintain sufficient diversity to allow for the production of new varieties able to with stand attack from new races and pathovars of continuously evolving pathogenic microorganisms (Tripp 1996). DNA-marker techniques have been used as tools for measuring genetic diversity for genetic erosion (Fu *et al.* 2003, Huang *et al.* 2007, Heerwaarden *et al.* 2009). The present study was planned to study the loss of allelic variability, if any, in Indian sesame varieties developed over a period of 45 years as a consequence of sesame breeding.

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Materials and Methods

Thirty five Indian sesame varieties were used to study the genetic changes in sesame breeding program from polymorphism data of ISSR markers. The varieties were selected based on the year of their identification. These varieties were commercialized during 1959 to 2004. The 45 years' period of release of varieties was divided into three breeding periods, each of 15 years duration. Thus the period I included varieties released during 1959 to 1974, period II from 1975 to 1989, and period III included varieties released from 1990 to 2004.

DNA extraction and polymorphism for ISSR markers: For ISSR analysis, genomic DNA was extracted from bulk leaf sample of five plants (20 - 25 days old) of each genotype using modified CTAB extraction procedure of Doyle and Doyle (1990). In this procedure, CTAB extraction buffer had high concentration of NaCl and polyvinyl pyrrolidone (PVP) to remove polysaccharides and polyphenoles, respectively. A set of 100 ISSR primers were designed, based on the sequence information published by University of British Columbia (UBC), Biotechnology laboratory, Vancouver, Canada. Initially, the 100 primers were evaluated against four diverse genotypes, namely PI-254707, PI-246386, T-13 and RT-54, to identify the polymorphic primers. Thirty four primers which gave clear polymorphic patterns were selected to assess the polymorphism of selected 35 varieties.

The pattern and extent of ISSR variation were examined with respect to primer, polymorphism and cultivar. Each ISSR band was assumed to represent a unique genetic locus. The presence of a ISSR band was interpreted as either a heterozygote or dominant homozygote and the absence of a ISSR band as a recessive homozygote. A higher proportion of fixed recessive loci obtained would mean lower genetic variation for the accession, when compared with another accession. Thus this measurement provided a simple means of comparing ISSR variations and hence allelic frequencies among various accessions. Comparisons of ISSR variation were made among various groups of accessions representing three breeding periods. Later, a linear regression was made of the proportions of fixed recessive ISSR loci over the years of cultivar release to measure the change in genetic diversity in the sesame cultivars released over the last 45 years.

Results and Discussion

The pattern and extent of ISSR variation were examined with respect to variety specific polymorphism for each primer. Identified 34 ISSR primers amplified 235 polymorphic bands in 35 sesame varieties. The number of polymorphic bands per primer ranged from 2 to 15 with an average of 7 bands (Fig. 1). The primer UBC 859 generated 2 polymorphic bands whereas the primers UBC 890, UBC 835, UBC 850 and UBC 887 generated 15, 14, 12 and 11 polymorphic bands, respectively.

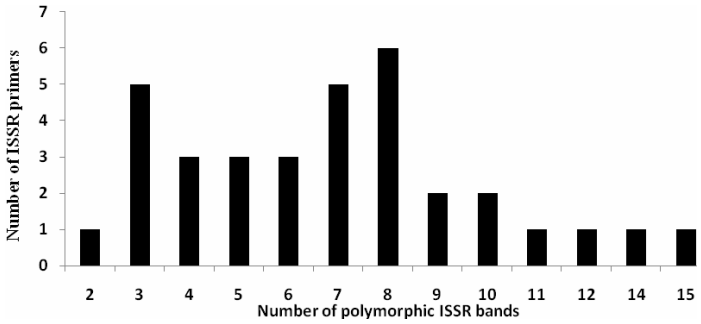

Fig. 1. Extent of polymorphism of evaluated 34 ISSR primers.

The 235 polymorphic bands appeared to be either frequently or rarely (unique) present in the varieties. Three polymorphic bands were present in all the 35 cultivars (Fig. 2) whereas nine polymorphic bands were present only in one cultivar each. In all 62 polymorphic bands (26.4%) were present in most of the 35 cultivars with an occurrence frequency of 0.9 or higher (Fig. 2) whereas 33 polymorphic bands (14%) were present only in a few cultivars with an occurrence frequency of 0.11 or lower. These patterns of ISSR variation are expected for the cultivars, as selection generally forces fixation of both dominant and recessive alleles.

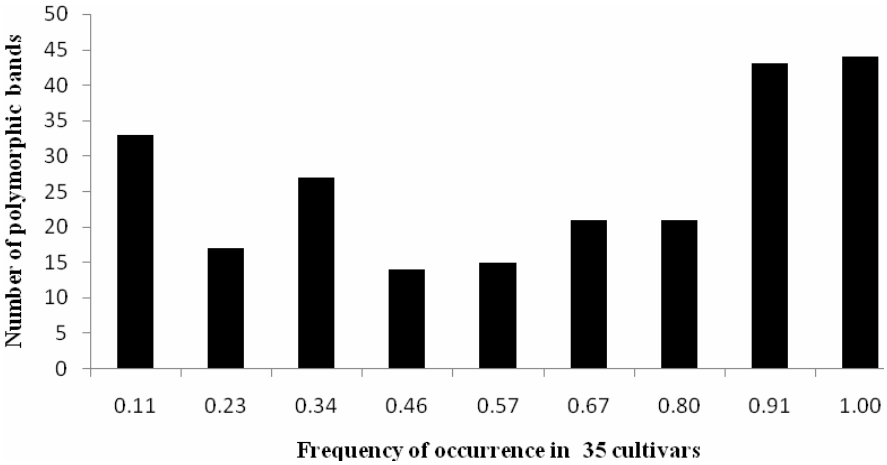

Fig. 2. Frequency of occurrence of polymorphic bands in 35 cultivars of sesame (1959-2004).

There were 10 unique bands in 9 cultivars. Cultivar T-4 had two unique bands whereas, cultivars, G-Til-10, TSS-6 (SVPR-1), MT-75 (Pragati), TMV-6, HT- 1, Thilathera, Padma (JLT-26), and RT-54 had one unique allele each. Two unique bands in T-4 variety had one for primer UBC 851 and another for primer UBC 880. The other primers with unique bands are UBC 815 (G-Til-10), UBC 835 (SVPR-1), UBC 835 (Pragati), UBC 844 (TMV-6), UBC 857 (HT-1), UBC 859 (Thilathara), UBC 887 (JLT-26), and UBC 887 (RT-54). The unique band in HT-1 with UBC 857 ISSR primer is presented as a representative picture. Rarely present bands can be used as unique fingerprint to identify cultivars.

Proportion of fixed recessive loci for each genotype was calculated by counting the number of recessive loci for that genotype over the total 235 polymorphic loci. The proportion of fixed recessive ISSR loci over the cultivars ranged from 31.7 to 48.8 per cent with an average of 40.5 per cent (Fig. 3). A higher proportion of fixed recessive loci suggested lower genetic variation for the accession, as compared with accession having lower proportion of fixed recessive loci. Cultivar YLM-17 was observed to have highest number (110) of fixed alleles, whereas cultivar Gujrat Til-10 had the minimum (72) fixed alleles. Comparisons of ISSR variations (Table 1) were made on the basis of fixed recessive alleles among accessions representing three breeding periods i.e. 1959-1974, 1975-1989 and 1990- 2004.

Table 1 shows that cultivars belonging to earliest period (1959 - 1974) had least number of average recessive fixed alleles, whereas there was not much difference among the cultivars belonging to two other periods. The regression of the proportions of fixed recessive ISSR loci over the registration years (Fig. 4) generated a linear regression coefficient (R^2) of 0.0019 which was

not statistically significant from zero (F = 0.23). The results suggest that the level of genetic diversity fluctuated over one period to the next and there appeared to be no significant loss of genetic variation as a consequence of crop improvement efforts.

Table 1. Fixed recessive ISSR loci (%) in three breeding periods.

Breeding period	Fixed recessive loci (%) in three breeding periods		
	Min.	Max.	Mean (± Sd)
1959-1974	34.20	43.83	38.90 ± 4.15
1975-1989	36.32	48.89	41.58 ± 3.81
1990-2003	31.72	47.42	40.15 ± 4.54

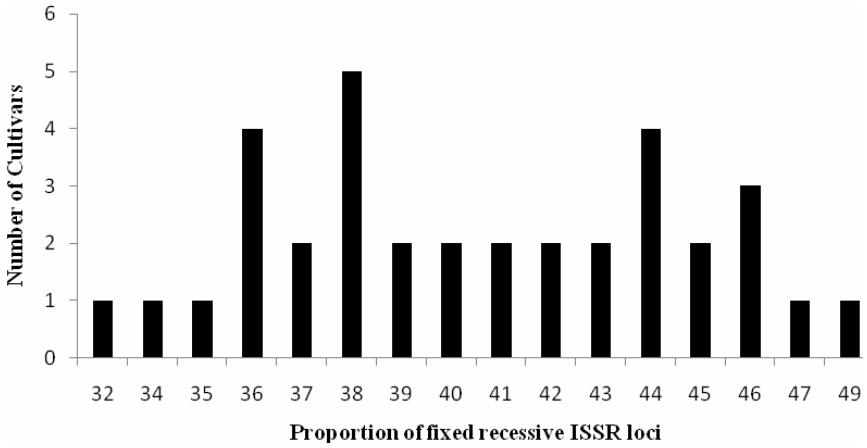

Fig. 3. The proportion of fixed recessive ISSR loci in 35 cultivars.

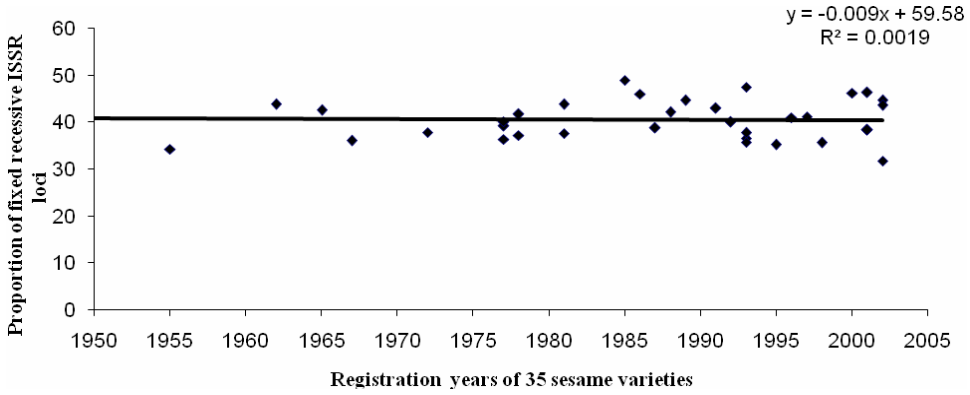

Fig. 4. Relationship between the proportion of fixed recessive ISSR loci and registration year of the sesame varieties released from 1959 to 2004.

The modern plant breeding has resulted in no apparent loss of allele numbers or genetic diversity in linseed (Fu *et al*. 2003), maize (Huang *et al.* 2007) and wheat (Heerwaarden *et al.* 2009).

Khlestkina *et al.* (2004) also did not observe any significant differences in both the total number of alleles per locus and in the polymorphism information content (PIC) values for samples of cultivated wheat collected over an interval of 40 to 50 years in four comparable geographical regions in Europe and Asia.

Donini *et al.* (1998) concluded from a set of UK RL winter wheat that changes in the composition and occurrence of alleles rather than the number of alleles characterized the flux of genetic diversity over time. While in a study of Nordic spring wheat, Christiansen *et al.* (2002) concluded that genetic diversity was enhanced by plant breeding in the first quarter of the 20th century, fell during the second quarter, but increased again after 1960.

Both allelic reduction and genetic shift have been reported for Canadian hard red spring wheat germplasm released from 1845 to 2004 (Fu *et al.* 2006). Similarly Roussel *et al.* (2004) observed a 25 per cent decrease in allelic richness in French bread wheat accessions by comparing landraces to varieties. A loss of genetic diversity was also reported for CIMMYT and its related modern wheat cultivars in comparison to *Triticum tauschii* and traditional land race cultivars (Reif *et al.* 2005).

DNA marker techniques have emerged as effective tools for directly measuring genetic diversity and hence to test for the occurrence of genetic erosion (Almanza-Pinzon *et al.* 2003). In the present study, although no indications for genetic erosion were uncovered in the evaluated sesame cultivars, the concept of using unadapted and wild germplasm for broadening the genetic base of crop plants (Tanksley and McCouch 1997) will help in the maintenance of genetic diversity.

Present results indicate a low level of genetic diversity and non significant loss of genetic variation was observed as a consequence of Indian sesame breeding programmes over a period of 45 years. The information may be helpful in developing and panning breeding strategy for sesame. Therefore, it is essential for sesame varietal development to identify the diverse genotypes for broadening the germplasm for improving selection and genetic gain.

References

Ashri A. 1998. Sesame breeding. Plant Breed Review **16**:179-228.

Almanza-Pinzon MI, Khairallah M, Fox PN and Warburton ML 2003. Comparison of molecular markers and coefficients of parentage for the analysis of genetic diversity among spring bread wheat accessions. Euphytica **130**:77-86.

Christiansen MJ, Andersen SB and Ortiz R 2002. Diversity changes in an intensively bred wheat germplasm during the 20th century. Molecular Breeding **9**:1-11.

Donini P, Stephenson P, Bryan GJ and Koebner RMD 1998. The potential of microsatellites for high throughput genetic diversity assessment in wheat and barley. Genetic Resources and Crop Evolution **455**:415-421.

Doyle JJ and Doyle JL 1990. Isolation of plant DNA from fresh tissues. Focus **12**:13-15.

FAO/IPGRI 2002. Review and development of indicators for genetic diversity, genetic erosion and genetic vulnerability GDEV: summary report of a joint FAO/IPGRI workshop Rome, 11-14 September, 2002.

Frankel OH and Bennett E 1970. Genetic resources in plants-their exploration and conservation. Blackwell Scientific Publications, Oxford, U.K.

Fu YB, Peterson GW, Yu JK, Gao L, Jia J and Richards KW 2006. Impact of plant breeding on genetic diversity of the Canadian hardred spring wheat germplasm as revealed by EST derived SSR markers. Theoretical and Applied Genetics **112**:1239-1247.

Fu YB, Rowland GG, Duguid SD and Richards KW 2003. RAPD analysis of 54 North American flax Cultivars. Crop Science **43**:510-515.

Hawkes JG 1983. The diversity of crop plants. Harvard University Press, Cambridge, MA.

Heerwaarden JV, Hellin J, Visser RF, and Eeuwijk FAV 2009. Estimating maize genetic erosion in modernized smallholder agriculture. Theoretical and Applied Genetics **119**: 875-888.

Huang XQ, Wolf M, Ganal MW, Orford S, Koebner RMD and Roder MS 2007. Did modern plant breeding lead to genetic erosion in european winter wheat varieties? Crop Science **47**:343-349.

Khlestkina EK, Huang XQ, Quenum FJB, Chebotar S, Roder MS and Borner A 2004. Genetic diversity in cultivated plants-loss or stability? Theoretical and Applied Genetics **108**:1466-1472.

Louette D and Smale M 2000. Farmers' seed selection practices and traditional maize varieties in Cuzalapa, Mexico. Euphytica **113**:25-41.

Reif JC, Zhang P, Dreisigacker S, Warburton ML, Ginkel MV, Hoisington D, Bohn M and Melchinger AE 2005. Wheat genetic diversity trends during domestication and breeding. Theoretical and Applied Genetics **110**:859-864.

Roussel V, Koenig J, Beckert M and Balfourier F 2004. Molecular diversity in French bread wheat accessions related to temporal trends and breeding programmes. Theoretical and Applied Genetics **108**:920-930.

Tanksley SD and McCouch SR 1997. Seed banks and molecular maps: Unlocking genetic potential from the wild. Science **277**: 1063-1066.

Tripp R 1996. Biodiversity and modern crop varieties: Sharpening the debate. Agriculture and Human Values **13**:48-3.

Vellve R 1993. The decline of diversity in European agriculture. Ecology **23**: 64-69.

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