Paper ID: 274
Theme 4. Biodiversity, conservation and genetic improvement of range and forage species
Sub-theme 4.1. Plant genetic resources and crop improvement

Genetic diversity analysis of mutant lines of oat (*Avena sativa* L.) based on RAPD and ISSR analysis

Anoop Kumar Mehta[1]*, M. H. Basha[1], V. K. Gour[1], M .Neeta[2], S. K. Biliaya[1], Satish Kachare[1]
[1]Jawaharlal Nehru Krishi Vishwadiyalaya, Jabalpur, India
[2]Kelkar Institute of Science & Arts, Kelkar Education Trust, Mumbai, India
*Corresponding author e-mail: anoop.mehta@yahoo.co.in

Keywords: Genetic diversity, ISSR, Mutation breeding, Oat, RAPD,

Introduction

The genus *Avena* belongs to the grass family Poaceae and has ploidy levels of diploid, tetraploid and hexaploid with basic chromosome number of 7 (n=7). Oat (*Avena sativa* L.) is one of the most important forage and feed crops of the world. Oat is used as green fodder, straw, hay or silage. Oat grain makes a good balanced concentrate in the rations for poultry, cattle, sheep and other animals. Green fodder contains about 10 to 13% protein and 30 to 35% dry matter. Despite being high fed fodder crop, it is now gaining importance as food due to its unique and important quality characteristics, particularly the lipid and protein in grains (Ruwali *et al.,* 2013)**.** The existing genetic variability for the traits of agronomic importance, such as plant vegetative cycle, is considered restricted. The narrow of the genetic base in cultivated oat varieties can be a constraint on the efficacy of genotype selection in segregating generations (Carvalho and Federizzi, 1989). Genetic variability in existing oat cultivars is not high enough; it hampers the selection of superior genotypes for breeding. Modifications in the genetic structure of plants and an organisms occurs naturally, though at low frequency, but can be increased through physical or chemical mutagens.

Advances in molecular biology have introduced an alternative for variety/genotype identification. The genetic characterization of germplasm helps in their effective conservation and reveals the extent of relationship among the accessions and the estimates of genetic diversity (Singh *et al*., 2012). The selection of RAPD and ISSR were based on their relative technical simplicity, level of polymorphism they detect, cost effective, easily applicable to any plant species and target those sequence which are abundant throughout the eukaryotic genome and are rapidly evolved. A series of studies have indicated that ISSR could be able to produce more reliable and reproducible bands because of the higher annealing temperature and longer sequence of ISSR primers considered superior than RAPD (Bornet *et al*., 2001). ISSR has proved to be useful to study of population genetic studies gene mapping germplasm identification and characterize gene bank accessions as well as to identify closely related cultivars (Cortesi *et al*., 2004). The present research had the following objectives: Assessment of diversity of mutant lines of oat (*Avena sativa* L.) based on RAPD and ISSR analysis.

Materials and Methods

Two oat varieties *i.e*. JO-1 and JHO-851 seeds were treated with three concentrations of ethyl methane sulphonate (20, 25 and 30mM). A set of seeds were pre-soaked in distilled water for 6 h to ensure complete hydration of the seeds before EMS treatment. The treated seeds were planted in the field according to Complete Randomized Block Design with three replications. Few desirable mutant lines were found in M_3 segregating generation *i.e*. broad leaf and early types. Two types of variations, awned and awnless were observed in early mutant.

Isolation of DNA: DNA was isolated using a modified cetyltrimethylammonium bromide (CTAB) method Saghai *et al*. (1984). For each accession, about 5 g of bulked leaf tissue collected from five plants each was grinded to a fine powder using liquid nitrogen and then suspended in 20 mL of extraction buffer (20 mM EDTA (pH 8.0), 100 mM Tris–HCl (pH 8.0), 1.4 M NaCl, 2% CTAB, and 1% β-mercaptoethanol). The suspension was mixed well, incubated at 60°C for 45 min, followed by chloroform–isoamyl alcohol (24:1) extraction and precipitation with 2/3 of the volume of isopropanol at −20°C for 1 h. The DNA was pelleted down by centrifugation at 12,000 rpm for 10 min and suspended in TE buffer (10 mM Tris–HCl, 1 mM EDTA (pH 8.0)). The DNA was purified from RNA and proteins by standard procedures and DNA concentration was estimated by agarose gel electrophoresis and staining with ethidium bromide.

RAPD-PCR Amplification: PCR amplifications were carried out using a Bio-Rad 3.03 version thermo-cycler. 15 primers were used. Out of these 10 primers did not give satisfactory results. Amplifications were performed for 35 cycles with denaturation at 94˚C for 1 min, annealing at 37˚C for 1 minute and extension at 72˚C for 2 minutes. The reaction mixture (20 µl) contained 20 ng of DNA template, 10 mM of Tris-Hcl (pH 9.0), 2 mM MgCl2, 50 mM KCl, 200 mM dNTP mix, 0.25 mM primer, 5 mM of spermidine and 0.8 units of Taq DNA polymerase.

ISSR-PCR Amplification: 18 ISSR primers were used for screening; only 10 primers gave the polymorphic bands. PCR amplification was done using a Bio-Rad 3.03 version thermo-cycler. Amplification was performed for 35 cycles with denaturation at 94˚C for 1 min, annealing at 56˚C for 1 min. and extension at 72˚C for 2 minutes. Initial denaturation was done at 94˚C for 5 min and a final extension step of 5 min. at 72˚C was also included. The reaction mixture (25 µl) contained 20 ng of DNA template, 10 mM Tris-HCl pH 9.0; 50 mM KCl; 0.1% Triton X-100; 1.5 mM MgCl2; 0.1 mM dNTP; 2 mM primer; 0.5 unit of Taq DNA polymerase.

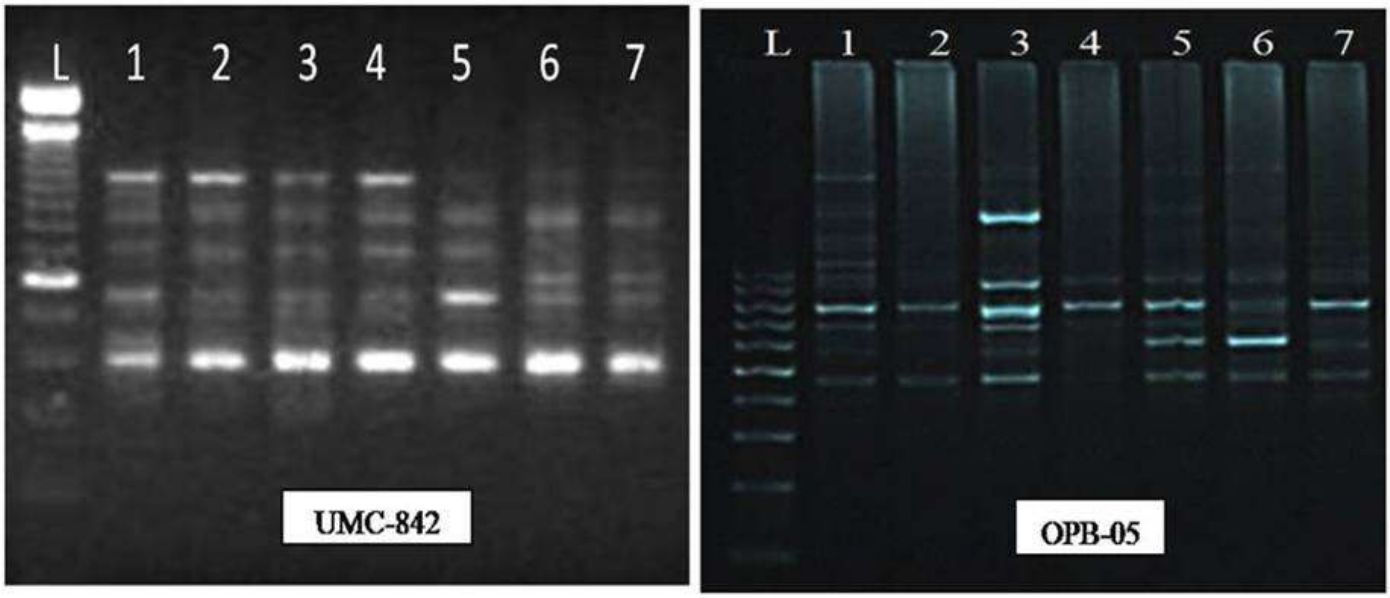

Fig. 1: ISSR (UMC-842) and RAPD (OPB-05)-PCR amplification of genomic DNA of mutant lines of oat. L: Ladear, Lanes 1. Jo-1, 2. M-8, 3. M-30, 4. M-40, 5. JHO-851, 6. A-7, 7. A-9.

Data scoring and statistical analysis of RAPD data

RAPD bands were scored using binary matrix '1' for presence and '0' for absence. Data was analyzed using NT-SYS-pc version 2.1. Pair-wise genetic similarities between accessions were estimated using the Jaccard's similarity coefficient. A dendrogram was constructed based on similarity coefficient values by adopting the sequential hierarchical agglomerative non-overlapping (SHAN) clustering technique of unweighted pair group method of arithmetic mean (UPGMA) which is a variant of the average linkage clustering algorithm.

Results and Discussion

Mutation breeding is relatively quicker method for improvement of crops. Many physical and chemical mutagens have been used for induction of useful mutants in number of crops. However, the variability so induced may be in positive or negative direction. Genetic markers can be used to portray diversity within the cultivated germplasm and to identify grouping of cultivars which are adapted to particular regions. Earlier, assessment of genetic diversity has traditionally been made through morphological characters, which are often limited in number, have complex inheritance and vulnerable to environmental conditions. In present study, diversity induced through chemical mutagen (EMS) was studied in M_3 generation using RAPD and ISSR markers.

Based on electrophoretic banding pattern of RAPD and ISSR primers, pair wise genetic similarity among 7 accessions for genetic diversity was estimated and dendogram was generated using Unweighted Pair Group method with "UPGMA" sub programme of "NTSYS"-pc (Fig.2). Total 97 bands were generated among them 68 bands was found polymorphic with an average 4.5 bands per primer. The average percentage of polymorphism across 15 primers was 70.10%. The percentage of polymorphism of Oat genotypes ranged from 42.85% (OPB-05) to 100% (UBC-840). The maximum fragments (10) were generated by primer UMC-05, whereas primer UBC-845amplified minimum (3) fragments.

The cluster analysis indicates that 7 populations of mutant oat formed two major cluster groups. The major cluster I included 2 genotypes *viz.* A-9 and A-7 and major cluster II divided 2 sub clusters i.e. IIA and IIB. Among these 2 sub clusters, IIA contained alone JHO-851, M-40, M-50 and second (IIB) minor cluster contained M-8 and Jo-1 genotypes. Three dimensional scaling of 7 mutant Oat accessions also showed similarity according to cluster analysis. In this, accessions were divided in to two groups, first group contain only two accessions namely A-9 and A-7 were divers from other accessions. Group B contained 5 accessions namely JHO-851, M-40, M50, M-8 and Jo-1.

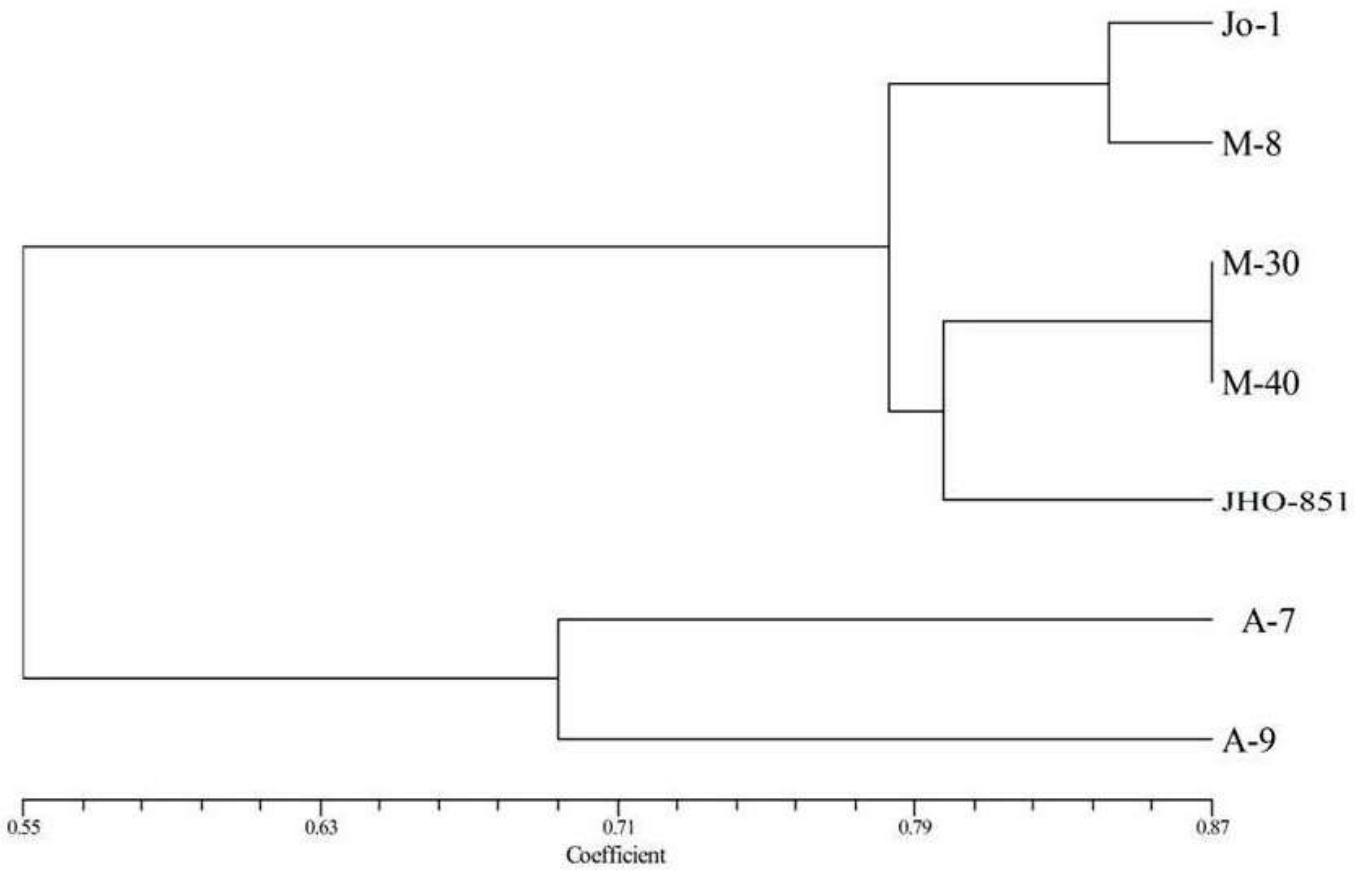

Fig. 2: Dendogram showing clustering of 7 mutant lines of oat constructed using UPGMA based on Jaccard's similarity coefficient obtained from RAPD and ISSR analysis

Conclusion

Based on the current study it was demonstrated that RAPD and ISSR markers could be used for the evaluation of mutant lines of *A. sativa* and differentiation of individual genotypes. These RAPD and ISSR markers are cheap and cost effective on compared to SNP markers. Evidently, the method could be successfully applied for expended analyses of oat accessions or genotype identification. The diverse genotypes can be utilized in hybridization programme.

References

Bornet, B. and M. Branchard. 2001. Nonanchored Inter Simple Sequence Repeat (ISSR) markers: Reproducible and specific tools for genome fingerprinting. *Plant Molecular Biology Reporter*. **19**: 209-215.

Cortesi, P., M. P. Ottaviani and M. G. Milgroom. 2004. Spatial and genetic analysis of a flag shoot subpopulation of *Erysiphe necator* in Italy. *Phytopathology*. 94:544-550.

Ruwali, Y., J. S. Verma and L. Kumar. 2013. Comparative genetic diversity analysis of oat (Avena sativa L.) by microsatellite markers and morphological rainfed expressions. *African Journal of Biotechnology* **12** (22):3414-3424.

Saghai Maroof, M. A., K. M. Soliman, R A. Jorgensen and R W. Allard. 1984. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proc Natl Acad Sci.* 81**:** 8014–8018.

Singh, S. K., G. R. Lavanya, K. V. Bhat, G. S. Babu, L. Arya, M. Verma, Z. Hussain, S. Roy, R. S. Rathi1 and A. K. Misra. 2012. Microsatellite Markers Revealed Genetic Diversity in Mungbean Mutant Lines. *Indian Journal of Hill Farming*. 25**(1):**1-8