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GENETIC DIVERSITY ASSESSMENT IN LENTIL (*LENS CULINARIS* MEDIKUS) GENOTYPES THROUGH ISSR MARKER

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ABSTRACT
The genetic diversity study was conducted in 24 lentil genotypes at Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. In the present investigation 20 ISSR (Inter Simple Sequence Repeat) primers were taken, out of these only seven primers gave amplification. These primers gave a total of 44 loci, out of which 36 were polymorphic. ISSR primers 8161-054 at 500 bp, 8161-055 at 700bp and 8161-058 at 1200 bp gave unique bands for different genotypes clearly indicating that these three primers can be used to identify different lentil genotypes. Genetic distances were calculated using Jaccard's Similarity Coefficient, displayed in a dendrogram (UPGMA method). Dendrogram generated by cluster analysis derived from ISSR markers divided 24 lentil genotypes into two clusters A and B at 41% similarity. Major cluster A comprised of 23 and B of one genotype. On the basis of Jaccard's similarity coefficient genotypes PL-098 and L-4147 (0.41) were found genetically most distant suggesting that these varieties can be used in future hybridization programmes to generate desirable segregants. The study Indicates ISSR as a useful tool in determining the genetic diversity among genotypes in lentil as it is not influenced by environmental conditions.

INTRODUCTION

Lentil (*Lens culnaris* Medikus) is one of the most important *Rabi* pulse crop in India. To achieve high level of productivity breeder needs to maintain a pool of desirable donor parents. Lack of sufficient genetic variability for improvement in yield and contributing characters and susceptibility to different biotic and abiotic stresses is the main cause of concern, therefore identification of genetically most distant parents for different traits in any lentil breeding program is of utmost importance for the introgression of desirable genes. Until now most of the estimates of genetic diversity are based on morphological data generated in a specific environment, which are likely to be changed if the genotypes are grown in a changed environment. Hence the estimates based on morphology are not very reliable, on the other hand molecular markers are considered to provide the best estimates of available genetic diversity since these are independent of environmental factors (Tanksley *et al.,* 1989). Inter-simple sequence repeat (ISSR) is a novel PCR technique that uses repeat-anchored or non-anchored primers to amplify DNA sequences between two inverted SSR. These markers do not require a prior knowledge of the SSR targets sequences and are highly reproducible due to their primer length, high stringency achieved by the annealing temperature and were found to provide highly polymorphic fingerprints (Bornet and Branchard, 2001). ISSRs have been used in many crop species including legumes (Ratnaparkhe *et al.,* 1998, Bornet and Branchard., 2001, Iruela *et al.,* 2002, Rajesh *et al.,* 2003, Tahir *et al.,* 2011, Bhareti *et al.*, 2012, Gupta *et al.*, 2012 and Wang *et al.,* 2012). The objective of the present investigation was to evaluate different released lentil genotypes using ISSR markers and to identify most distant genotypes so that these high yielding genetically diverse lentil varieties can be used as parents in breeding programmes.

MATERIALS AND METHODS

The present investigation was carried out during *Rabi* 2011-2012 at Norman E. Borlaug Crop Research Center, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India. The experimental material comprised of 24 lentil genotypes viz. L-4076, K-75, PL-4, PL-8, PL-083, DPL-62, PL-017, DPL-58, PL-5, LL-1024, PL-234, DPL-15, PL-639, LL-864, PL-406, PL-069, L-4188, FLIP-96-51, PL-084, PL-098, L-4147, PL-097, PL-7 and PL-6. Fresh leaf samples from 10-15 days old seedlings were used for DNA extraction. CTAB procedure was used for isolation of DNA (Doyle *et al*., 1987). Quantification of the DNA was done through electrophoresis on a 1% agarose gel. Twenty ISSR primers were tested for their ability to amplify scorable and reproducible DNA fragments. Primers resulting in faint or irreproducible bands were excluded from subsequent analysis. Finally seven primers were selected for this study are given in Table 1. Cluster analysis was done with NTSYSpc using Jaccard's coefficient and unweighted paired grouped method using arithmetic average (UPGMA).

RESULTS AND DISCUSSION

Twenty ISSR primers were initially screened to characterize genetic diversity present among 24 genotypes. Out of twenty primers, only seven showed amplification. A total of 44 loci were detected, out of which, 36 loci were polymorphic and 8

Table 1: Primers Selected for study

Primer code	Primer sequence	Total no. of amplified loci	Total no of polymorphic loci	Total no. of monomorphic loci	Percent Polymorphism
8161-044	AGAGAGAGAGAGAGAGTT	6	5	1	83.33
8161-049	ACACACACACACACACT	9	7	2	77.88
8161-053	GAGAGAGAGAGAGAGAA	6	5	1	83.33
8161-054	GAGAGAGAGAGAGAGACT	8	7	1	87.50
8161-055	AGAGAGAGAGAGAGAGTT	4	3	1	75.00
8161-057	CACACACACACACACAT	7	6	1	85.71
8161-058	CACACACACACACACAGT	4	3	1	75.00

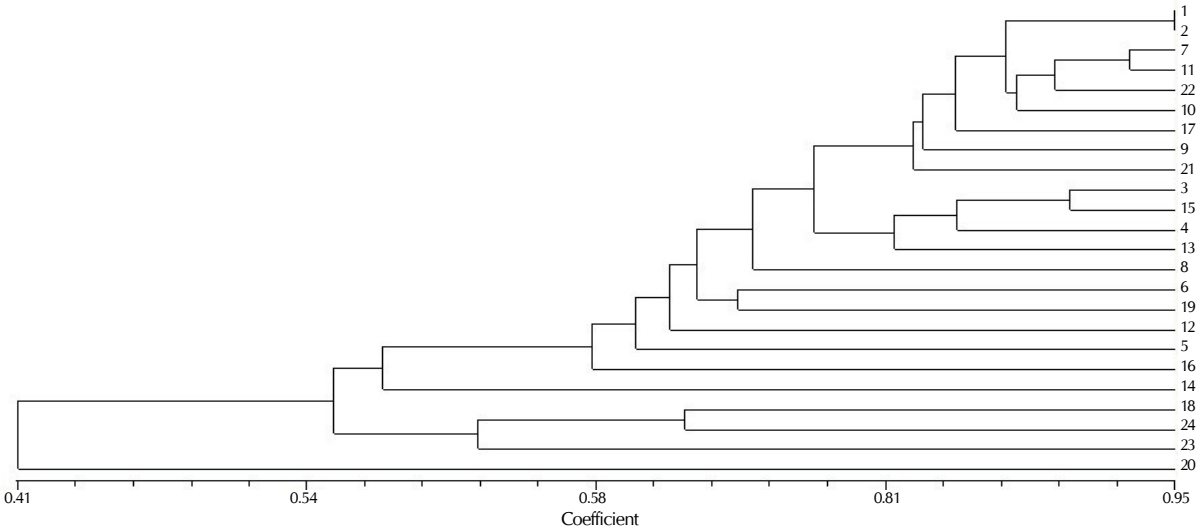

Figure 1: Dendrogram based on UPGMA analysis

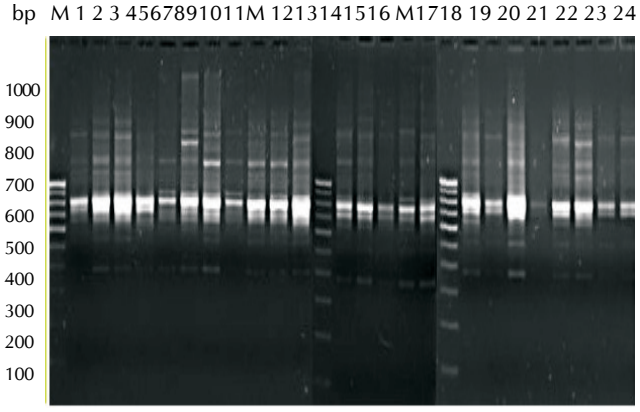

Figure 2: Amplification profile of primer 8161-054

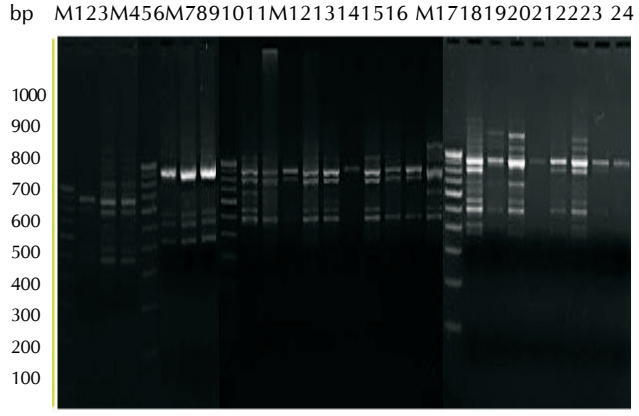

Figure 3: Amplification profile of primer 8161-058

were monomorphic (Table 1). The seven selected primers produced comparatively the maximum number of high intensity bands with minimal smearing, good technical resolution and sufficient variation among different cultivars. The number of amplified loci varied from four in primer 8161-055 and 8161-058 to a maximum of nine in primer 8161-049 with an average of 6.29 loci per primer. Lowest polymorphism was observed for primer 8167-058 (figure-3) and 8167-55 (75%) (Fig. 4) while the primer 8161-054 gave maximum (87.50%) (Fig. 2) polymorphism. A dendrogram based on UPGMA analysis with ISSR data is shown in Fig. 1. Jaccard's similarity coefficient ranged from 0.41 to 0.95. Based on estimated genetic similarity matrix, the highest genetic similarity value was observed between K-75 and L-4076 (0.95) followed by between K-75 and PL-234 (0.93) and between PL-8 and PL-4 (0.90). Based on similarity coefficients it is evident that these genotypes were more similar genetically and hence the hybridization between these genotypes may not be useful in getting desirable segregants. The least similarity value was observed between genotype L-4147 and PL-098 (0.41), followed by between PL-098 and L-4188, PL-7 and PL-097, PL-5 and PL-7 (0.43), between PL-098 and K-75, PL-098 and

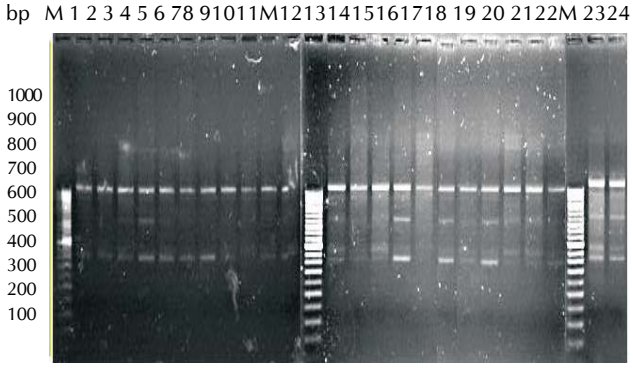

Figure 4: Amplification profile of primer 8161-055

PL-083, PL-098 and PL-017, PL-098 and LL-1024, PL-097 and PL-098 (0.45), between PL-098 and PL-4, PL-098 and DPL-58, PL-098 and PL-234, PL-098 and DPL-15, PL-098 and PL-406, PL-7 and DPL-58, PL-7 and PL-639 (0.47), between PL-098 and L-4076, PL-7 and DPL-58 (0.50), between PL-098 and PL-5, PL-7 and K-75, PL-7 and PL-017, PL-7 and PL-069, PL-017 and PL-084, PL-6 and PL-406 (0.52). It is clear that the genotypes L-4147 and PL-098 are genetically most distant indicating if these two varieties are used in hybridization programme by the breeder a wide range of genetic variability will be observed in F_2 and subsequent segregating generations thereby providing scope of selecting desirable genotypes. Dendrogram represented the two major clusters A and B at 41% similarity. Cluster B has only one genotype *ie*. PL-098.The major cluster 'A' can be sub divided into two minor sub clusters A1 and A2. The sub cluster A1 includes 3 genotypes *viz.,* PL-7, PL-6 and FLIP 96-51. Sub cluster A2 includes L-4076, K-75, PL-017, PL-234, LL-1024, PL-097, L-4188, PL-5, PL-4, PL-8, PL-406, PL-639, DPL-58, DPL-15, L-4147, DPL-62, PL-084, PL-083, PL-069, LL-864. On the basis of dendrogram generated it is further evident that the genotype PL-098 and L-4147 are most distant genetically and hence these can be used in the hybridization programme.

In the present study ISSR primers 8161-054 at 500 bp, 8161-055 at 700bp and 8161-058 at 1200 bp gave unique bands for different genotypes which clearly indicates that these primers can be used to identify different lentil genotypes. In different crop species different workers (Ajibade *et al.,* 2000, Souframanien *et al*., 2002, Lohithaswa *et al*., 2003, Chattopadhyay *et al*., 2005, Chowdhury *et al.,* 2006, George *et al*., 2006, Fikiru *et al.,* 2007, Rao *et al*., 2007, Datta *et al*., 2010, Datta and Lal., 2011 and Ruwali *et al*., 2013) suggested that ISSR markers can be successfully used in the identification of genotypes.

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