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GENETIC RELATEDNESS OF LENTIL (*Lens culinaris* L.) GERMPLASM BY USING SSR MARKERS

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Abstract

Ninety six lentil accessions from different origins were collected from National Grain Legume Research Program, Rampur; Regional Agriculture Research Station, Nepalgunj and National Agriculture Genetic Resource Center, Khumaltar, Lalitpur. Among them; four lines were Nepal Local, forty two lines were Nepal Cross; forty seven lines were ICARDA Line and finally three lines were Indian Line. All ninety six accessions were analysed by DNA fingerprinting using thirty three selected polymorphic SSR markers. The characterization was performed in Biotechnology Unit, Nepal Agricultural Research Council, Khumaltar, Lalitpur by using standard protocols. Molecular variance analysis showed that 14 % genetic variation was found between population and 86 % genetic variation was found within population with estimated variance 0.23 between population and 1.35 within population. Highest genetic distance (9) was found between landrace ILL-7979 and RL-20. In the same way, highest Nei genetic distance (0.03) between population was shown by population 1 and population 4; and lowest genetic distance were observed within the same population accessions. The heterozygosity was probably due to the introgression of genes or duplication of microsatellite motif during the breeding and or the course of lentil line evolution. All the accessions included in this study displayed significant amount of genetic variability and genetic relatedness due to different center of origin and different genetic constitutions. The diversity detected in this study may constitute the new materials for future systematic lentil breeding programs.

Keywords: lentil, germplasm, characterization, genotypes, gene

Introduction

The knowledge of genetic diversity and association of characters with yield is of great importance to the breeder for making an improvement of quantitative characters. Molecular marker is used for estimating genetic variation at population level and among closely related species (Nienhuis *et al.*, 1995). Several classes of molecular markers have been developed showing that lentil has relatively low levels of genetic variation (Eujay *et al.*, 1997; Sonnante and Pignone, 2001). Plant descriptors coupled with molecular markers provide a valid evidence of diversity as these are least affected by environmental fluctuations (Ahmad *et al.*, 1997; Jha and Ohri, 1996; Margale *et al.*, 1995).

*Lentil* (*Lens culinaris* Medik.subsp. *culinaris*) is an important principal cool season pulse crop of the Indian Subcontinent, the Middle East, North America, North Africa and West Asia (Erskine, 1996). Nepal has area 1,87,437 ha altogether of lentil 1,51,758 with d per hectare kg yield 810 metric ton productivity and M)°AD, 2011 ,The crop has developed into a range of varieties adapted to diverse growing areas and cultural preferences, and containing unique nutritional compositions, colors, shapes and tastes. A lot of lentil land races, primitive races, indigenous races and wild races are still available in Nepal but they have not been studied properly. The genetic relatedness of lentil based on molecular level has not been studied yet in Nepal. Thus the yield attributing traits, disease resistance traits, insect pest resistance traits, abiotic stress tolerance traits and quality traits have not been identified and, cause delay in breeding for developing elite lines. Now a day the importance of lentil in Nepal is increasing due to its high nutritive value, important components of Nepalese diet, increased internal consumption and exportable commodity to foreign countries. Thus, there is an urgent need to increase the overall production and productivity of this crop through varietal improvement and suitable agronomic practices under rice-maize cropping systems in Nepal. Before initiation of lentil breeding activities there is urgent need to characterize, evaluate lentil germplasm available to us. Therefore present study was conducted
UKS Kushwaha et al. (2013). Int J Appl Sci Biotechnol, Vol. 1(3): 132-136

with an objective of selecting divergent parents based on genetic distance for future lentil breeding programme.

**Materials and Methods**

Diverse lentil germplasm were collected from National Grain Legume Research Program (NGLRP), Rampur; Regional Agricultural Research Station (RARS), Nepalgunj and National Agriculture Genetic Resource Center (NAGRC), Khumaltar. Collected accessions comprised four local line (Nepal Local): pop1; forty two NGLRP, Rampur crossed (Nepal Cross):pop2; forty seven ICARDA (ICARDA Line): pop3; and three from India (Indian Line): pop4.

The list of the collected germplasm is given in Table 1. Thirty three polymorphic microsatellites marker were used for PCR based on the results of previous report (Hamwieh et al., 2005, 2009). The list of polymorphic markers, their name, sequence information, annealing temperature and amplification size are given in Table 2.

| DNA SN | Variety name         | Source of origin | DNA SN | Variety name         | Source of origin |
|--------|----------------------|------------------|--------|----------------------|------------------|
| 1      | LN-0135              | Nepal Local      | 13     | ILL-10071            | ICARDA           |
| 25     | LN-0136              | Nepal Local      | 14     | ILL-9924             | ICARDA           |
| 91     | Arial                | Nepal Local      | 15     | ILL-6465             | ICARDA           |
| 95     | Khajura Masuro-2     | Nepal Local      | 16     | ILL-9926             | ICARDA           |
| 2      | RL-45                | Nepal Cross      | 17     | ILL-6458             | ICARDA           |
| 3      | RL-67                | Nepal Cross      | 18     | ILL-1020             | ICARDA           |
| 4      | RL-49                | Nepal Cross      | 19     | ILL-6811             | ICARDA           |
| 5      | RL-79                | Nepal Cross      | 20     | HUL-57               | ICARDA           |
| 7      | RL-56                | Nepal Cross      | 21     | Sagun                | ICARDA           |
| 8      | RL-68                | Nepal Cross      | 22     | M.Bharati            | ICARDA           |
| 9      | RL-8                 | Nepal Cross      | 23     | ILL-7162             | ICARDA           |
| 10     | X94S-48              | Nepal Cross      | 24     | ILL-7723             | ICARDA           |
| 34     | RL-4                 | Nepal Cross      | 26     | ILL-3768             | ICARDA           |
| 44     | RL-60                | Nepal Cross      | 28     | ILL-8006             | ICARDA (BM-4)    |
| 47     | RL-70                | Nepal Cross      | 29     | ILL-7537             | ICARDA           |
| 48     | RL-73                | Nepal Cross      | 31     | IL-1                 | ICARDA           |
| 53     | RL-71                | Nepal Cross      | 32     | ILL-7979             | ICARDA           |
| 54     | NR 2001-72-3         | Nepal Cross      | 33     | ILL-7715             | ICARDA           |
| 57     | RL-75                | Nepal Cross      | 35     | ILL-6467             | ICARDA           |
| 58     | RL-35                | Nepal Cross      | 36     | ILL-7164             | ICARDA           |
| 59     | RL-43                | Nepal Cross      | 37     | ILL-3490             | ICARDA           |
| 60     | RL-69                | Nepal Cross      | 38     | ILL-6419             | ICARDA           |
| 61     | RL-44                | Nepal Cross      | 40     | ILL-3111             | ICARDA           |
| 62     | RL-42                | Nepal Cross      | 41     | ILL-2527             | ICARDA           |
| 63     | RL-76                | Nepal Cross      | 42     | FLIP 2006-99L        | ICARDA           |
| 64     | RL-26                | Nepal Cross      | 43     | FLIP 95-1L           | ICARDA           |
| 65     | RL-41                | Nepal Cross      | 45     | FLIP 2009-60L        | ICARDA           |
| 66     | RL-39                | Nepal Cross      | 46     | FLIP 04-60L (ILL-10013) | ICARDA          |
| 67     | RL-58                | Nepal Cross      | 6      | ILL-3338             | ICARDA           |
| 68     | RL-62                | Nepal Cross      | 50     | ILL-6021             | ICARDA           |
| 69     | RL-47                | Nepal Cross      | 51     | FLIP 05-24L (ILL-10045) | ICARDA          |
| 70     | RL-80                | Nepal Cross      | 52     | FLIP 05-24L (ILL-10065) | ICARDA         |
| 71     | RL-21                | Nepal Cross      | 55     | FLIP 2008-7L         | ICARDA           |
| 72     | RL-23                | Nepal Cross      | 56     | FLIP 2009-54L        | ICARDA           |
| 75     | RL-94                | Nepal Cross      | 73     | FLIP 05-52L (ILL-10073) | ICARDA         |
| 78     | NR 2001-71-4         | Nepal Cross      | 74     | ILL-6260             | ICARDA           |
| 79     | RL-74                | Nepal Cross      | 76     | X39S-66L             | ICARDA           |
| 80     | RL-20                | Nepal Cross      | 77     | ILL-10134            | ICARDA           |
| 81     | RL-25                | Nepal Cross      | 83     | ILL-10068            | ICARDA           |
| 82     | RL-95                | Nepal Cross      | 87     | ILL-7664             | ICARDA           |
| 84     | RL-22                | Nepal Cross      | 88     | Digger               | ICARDA           |
| 85     | RL-38                | Nepal Cross      | 89     | Bari Musuro-4        | ICARDA           |
| 86     | RL-5                 | Nepal Cross      | 92     | ILL-6458             | ICARDA           |
| 90     | NX-9901 – 1          | Nepal Cross      | 93     | X 95583              | ICARDA           |
| 96     | RL-28                | Nepal Cross      | 94     | FLIP 2009 – 59L ( ILL 10716) | ICARDA   |
| 97     | RL-78                | Nepal Cross      | 27     | DPL-62               | India            |
| 11     | ILL-2712             | ICARDA           | 30     | WBL-77               | India            |
| 12     | ILL-1970             | ICARDA           | 39     | LG-12                | India            |
| S.N. | SSR No. | Forward                  | Reverse                  | Annealing temp. (Tm) used for PCR (°C) | Expected size (bp) |
|------|---------|--------------------------|--------------------------|----------------------------------------|--------------------|
| 1    | SSR 34-2| CGGCCGATGAACTAAAG        | CATTTCTTCACAAACGCAAC     | 58                                     | 185                |
| 2    | SSR 66  | GGTAGTGAGGAGCAAGACG      | GCATCACTGACAGAGCACCC     | 55                                     | 253                |
| 3    | SSR 90  | CATTGTTACACACCCCTAC     | CGATTAAAGAGAAGAGGACAC    | 55                                     | 181                |
| 4    | SSR 132RN| CGGAGACGACACAAGAAG      | CTGCTGCTATGAGAGGACAC     | 32                                     | 330                |
| 5    | SSR 191 | GCAATTTCCTTCTTGCTACAC   | GGGCAGATGGATACAGCAG      | 33                                     | 238                |
| 6    | SSR 197 | CAAGACGATGAGGAGCAACG     | GAGCTGCGATCAGAGGAGAC     | 53                                     | 137                |
| 7    | SSR 207 | GAGGAGATTGCTAGAGTGAC     | GATTTGCTTGCTGGTGAGTGC    | 53                                     | 227                |
| 8    | SSR 230 | GCAACACACAAACTACCATAC   | AAGCTGACGACGCTGGAGC      | 53                                     | 251                |
| 9    | SSR 33  | CAAACAGACGACGACGACG     | CTGCTGCTGCTACAGAGGAC     | 56                                     | 289                |
| 10   | SSR 19  | GACTCATACACACACTGAGAG   | GAACAGGAGGGTTGACTGACG    | 59                                     | 240                |
| 11   | SSR 48  | CTTTCTTCACACACTCAGAC    | CTGCTGCTGCTACAGAGGAC     | 57                                     | 167                |
| 12   | SSR 96  | TTTTCTTCACACACTCAGAC    | GAATACCTACGAGACGAGAG     | 57                                     | 210                |
| 13   | SSR 99  | GGGGATTTTGGAGGAGGAGA    | CTTGAGATGCTGGCTCTGAG     | 57                                     | 161                |
| 14   | SSR 107 | GGAGGAGGCAAAATGAAAT    | GGAGAATGAGAAGGGGAATG     | 56                                     | 161                |
| 15   | SSR 113 | CACATGACGACGAGGAGGAG    | CAAAATAGGAGGAGGAGGAG     | 51                                     | 211                |
| 16   | SSR 119 | GAACTCACTTCTATCCTAGG    | GAACTCACTTCTATCCTAGG     | 49                                     | 266                |
| 17   | SSR 124 | GTATCAGACTGCTGCTTCTATG | GAACTCACTTCTATCCTAGG     | 52                                     | 174                |
| 18   | SSR 130 | CACAGATGACGAGGAAGGAG    | CAAGAAGAGGCCAGAAGGAG     | 52                                     | 196                |
| 19   | SSR 156 | GTTACTTAGTTGACATGAGAG   | CAATAATGGGGGTACAGGAG     | 53                                     | 176                |
| 20   | SSR 167 | CACATAGCTAGAGGAGTCTC    | CTTGACGTCTACACTCAGC      | 54                                     | 160                |
| 21   | SSR 199 | TTGCTGATGCTGCTTCTAGG    | CTTGCTGATGCTGCTTCTAGG    | 51                                     | 182                |
| 22   | SSR 204 | GCACATAGCTACCATCTTGT    | CTGACTTCTGACTTCTTCTTATG  | 53                                     | 186                |
| 23   | SSR 212.1| CTTGCTGCTTCTTCTTCTT    | GCTGAGGGATGAGGAGGAG     | 50                                     | 181                |
| 24   | SSR 213 | CACATGACGACGAGGAGGAG    | GAAATGCTGCTTGAGGAGTCA    | 51                                     | 151                |
| 25   | SSR 309.2| GTATGCTGCTCTGCTTCTTG   | GAGGAGAAGAGTTTCTGAG      | 50                                     | 182                |
| 26   | SSR 317-1| GTTGGGCTGATATTTGTGCAC  | GTAAAGACTTATGGAAGACTAC  | 53                                     | 308                |
| 27   | SSR 317-2| CGCAGTACATCTTGCTTGATG  | GAGACTACTAATGACGAGC      | 57                                     | 120                |
| 28   | SSR 323 | AGGTGGAACAAACACACAGGAGATTG | GCTGACGCTATGCTACAGT    | 51                                     | 250                |
| 29   | SSR 336 | GTGAAACCACTGACTTCTTTAAG | CCGCTGAGGAGGAGGAGGAC     | 54                                     | 253                |
| 30   | SSR 183 | GCTGACGCTGAGCAAGGAC    | CATATAAGACGGAGGAGGAC     | 52                                     | 119                |
| 31   | SSR 202 | GACATCAGCTACATTCTTAC    | GCTCTTATCATCATTACACTAC   | 52                                     | 220                |
| 32   | SSR 28  | GAAGGCAATGAGATACGAGT    | GAGAAGAAGGAGGAGGAGGAG    | 53                                     | 383                |
| 33   | SSR 72  | CAAACAGACGAGCTGGCGAG    | GAGCAGCTGAGGAGGAGGAG     | 55                                     | 253                |
DNA fingerprinting was conducted with SSR markers. This fingerprinting was performed in Biotechnology unit, Nepal Agricultural Research Council, Khumaltar, Lalitpur. Lentil DNA extraction was done by Modified CTAB method (Doyle and Doyle, 1987) using standard protocol followed by DNA quantification, PCR amplification, gel separation and scoring of gel separated bands using standard protocol. The amplified products were scored as bands on visualization on gel on UV illuminator. Only the reliable bands were included in analysis. The presence of bands was scored as “1” and absence of band was scored as “0”. The respective data analysis, data entry and processing was carried out by using Microsoft Excel 2007. Percentage of molecular variance and genetic distance were found out by GenAlEx 6.5b3.xls.

Results and Discussion

Molecular variance analysis

Molecular variance analysis for genetic diversity of ninety six genotypes of lentil was carried out by GenAlEx6.5b3.xls. 14 % genetic variation was found between population and 86 % genetic variation was found within populations (figure 1). Estimated variance between population was 0.228 with 14.42 % and within population was 1.358 with 85.57 % out of 1.581 with 100% with PhiPT 0.144 (Table 3). This showed that high genetic relatedness were between population and far relatedness were within population.

Fig. 1: Percentage of molecular variance between and within population for 96 lentil accessions.

| Source       | Df | SS      | MS   | Est. Var. | %     |
|--------------|----|---------|------|-----------|-------|
| Between Pops | 3  | 16.430  | 5.477| 0.228     | 14%   |
| Within Pops  | 92 | 124.476 | 1.353| 1.353     | 86%   |
| Total        | 95 | 140.906 | 1.581| 100%      |       |

Stat Value P (rand >= data)

PhiPT = AP / (WP + AP) = AP / TOT Key: AP = Est. Var. Between Pops, WP = Est. Var. Within Pops (PhiPT max=0.918; Phi'PT=0.157 P(rand >= data) 0.010)

Table 4. Pairwise population matrix of nei unbiased genetic distance for 96 lentil accessions with four population

|       | Pop1 | Pop2 | Pop3 | Pop4 |
|-------|------|------|------|------|
| Pop1  | 0.000|      |      |      |
| Pop2  | 0.015| 0.000|      |      |
| Pop3  | 0.032| 0.019| 0.000|      |
| Pop4  | 0.033| 0.029| 0.015| 0.000|

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Genetic distance

The pairwise population matrix showed that highest (0.033) Nei Genetic Distance was found between pop 1 and pop 4 and lowest was found within the same population i.e. pop1, pop2, pop3 and pop4. Similarly, pop3 and pop2 had highest (0.017) Nei Unbiased Genetic Distance and lowest distance was found within the same population (Table 4 and 5). Pop1 (Nepal Local) and pop2 (Indian Line) had highest genetic distance which might be due to different center of origin and different genetic constitutions. Similarly, genetic relatedness were found within the populations which might be due to same center of origin and similar genetic constitutions.

Highest genetic distance (9) was found between landrace 32 (ILL-7979) and 80 (RL-20) calculated from GenAlEx6.5b3.xls. The highest and lowest level of genetic distance was 0.027273 and 0 respectively. The difference between the highest and the lowest inter genotypic distance indicates the moderate variability among the 96 genotypes of lentil.

Conclusion

Highest genetic distance (9) was found between landrace ILL-7979 and RL-20. Similarly, high genetic relatedness were found within the same population which might be due to same center of origin and similar genetic constitutions. In the same way high genetic distance were found between Nepal Line and Indian Line which might be due to different center of origin and different genetic constitutions. The level of genetic relatedness detection largely depends on the type of molecular markers, nature of SSR repeat motif, number of SSR markers and the genetic relatedness of the lentil germplasm to be analysed. All ninety six genotypes involved in the study exhibited wide range of genetic variability due to different center of origin, different genetic constitution. The genetic relatedness detected in this study may constitute the foundation for future systematic lentil breeding programs.

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