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# Molecular and Morphological Markers for the Evaluation of Diversity Between *Plantago ovata* in Iran

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**Abstract:** In this study, molecular and morphological variation of 22 population of *Plantago ovata* assessed using RAPD, ISJ and agro-botanical markers. Field experiment was conducted in completely randomized block design whit four replicates. Principal component analysis and clustering based on distance between means of 8 morphological traits were used to detect relationship between accessions. Thirty five RAPD primers produced 142 polymorphic bonds, average 4.05 for each primer. Clustering analysis technique based on RAPD using Unweighted Pair Group Method shown that a closely association exist among morphological and RAPD dendrograms while didn't exist any accordance between ISJ-GS with RAPD and morphological variation. In RAPD–based clustering, all population that was belonged to near area formed closely groups. The ISJ system marker produced 95 DNA fragment with 2.55 polymorphic bands for each semi-random primers. The dendrogram based on ISJ marker did not have accordance with geographical, morphological and RAPD variation. The result of this research verified possibility of use of RAPD and ISJ markers for estimation of genetic diversity, management of genetic resources and determination of repetitive accessions in *Plantago ovata*.

**Key words:** Morphological traits, RAPD, ISJ, *Plantago ovata*

## INTRODUCTION

The genus *Plantago*, of family Plantaginaceae, includes some 200 species (Rahn, 1996). Although its centre of diversity is believed to lie in central Asia, some species have now become dispersed widely, with maximum concentration in the temperate regions. Species of *Plantago* are small herbs, mostly growing as weeds, while some are of medicinal value. *Plantago ovata* is the only cultivated species. The seed husk, called Isfarzeh in Persian, isabgol in Hindi and psyllium in English, is not only a highly effective laxative but is also used in lowering blood cholesterol levels, ice cream making and cosmetics (Dhar *et al*., 2005). In India and Iran, mucilage from (*Plantago ovata*) is obtained by grinding off the husk. The mucilage sold as Isabgol, a laxative which is used to control irregular bowel syndrome and constipation. It is also used in cereals as a treatment of mild to moderate hypercholesterolemia and for reducing blood glucose. It has been used as an indigenous Ayurvedic and Unani medicine for a whole range of bowel problems including chronic constipation, amoebic dysentry and diarrhoea. In Romania and Bulgaria, leaves from *Plantago* major are used as a folk remedy to preventing infection on cuts and scratches because of its antiseptic properties (Dagar *et al*., 2006; Cho *et al*., 2004). Many works based on morphological characters, cytology and enzyme electrophoresis have been used to study the diversity and phylogeny of the *Plantago ovata* (Dhar *et al*., 2006; Bannayan *et al*., 2008; Ronsted *et al*., 2002). Evaluation and characterization to landrace *Plantago ovata* should form an important constituent of collection efforts because of their enormous in-built genetic diversity due to several generations of growing and selection by breeders and farmers. Landraces also constitute a good source of unique genes for stress tolerance, height stability, adaptability to the environments and genetic dynamics (Frankel *et al*., 1995). Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. It has been shown that different markers might reveal different classes of variation (Powell *et al*., 1996; Russell *et al*., 1997). RAPD has several advantages, such as simplicity of use, low cost and the use of small amount of plant material, etc. The RAPD system, which is useful for many crops, is not suitable for the genetic analysis of the large and complex genomes of such cereals as wheat and triticale (Devos and Gale, 1992). The main reason for this is the low rate of polymorphisms detected by RAPD primers, along with the difficulties with the reproducibility of the results. Some approaches aimed at improving the RAPD analysis, such as the selection of a large number of primers or changes in detection techniques, markedly increase

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the time and cost of PCR analysis. Therefore, the development of alternative PCR procedures that use single primers would seem to be an important step. Another PCR based system with semi-random primers targeting the Intron-exon Splice Junction (ISJ), proposed by Weining and Langridge (1991) and developed by Rafalski *et al*. (2001). The sequences of primers were based on the consensus sequences of the intron-exon junction, 7 and 9 bases in length, common for plants and necessary for effective splicing (Brown, 1986). The additional bases were added at random to extend the length of the primers (Rafalski *et al*., 1997).

## MATERIALS AND METHODS

Twenty-two *plantago* accessions including Landraces and wild accessions originated from various region of Iran obtained from the agricultural research of Zabol were evaluated in this study. The field experiment was carried out in Agricultural Center of Zabol University at 2006-07 seasons (Table 1, Fig. 1). The experimental design was a randomized complete block with four replications. Each experimental plot was 4 m long and 2 m wide with total area of 6 $m^2$. Irrigation furrows with uniform slopes were constructed in each experimental plot and rows were 25 cm apart. Fertilizer (N:P at a rate of 40:50 kg $ha^{-1}$) was applied before sowing of plant. Due to the differences in maturity, there were three harvest dates for black cumin in both years of the study and three harvest dates in 2003 and two harvest dates in 2004. Harvesting was done manually by pulling the dry plant out of the soil and removing the roots. Final seed yield and yield components were measured from 1 and 0.1 $m^2$ of each plot, respectively. Characters consisted of follicle length, number of follicles per plant, 1000 seed weight, number of seeds per follicle, spikes weight, plant height and straw and seed yield as quantitative traits and Mucilage percentage of seeds and inflation factor as quality traits. Mucilage percentage was measured according to Sharma and Koul (1986).

The data were analyzed by one-way ANOVA using the Statistical Analysis System (SAS) (2001) and means were compared by Duncan's multiple range test at the 5% probability level. Cluster analysis based on similarity matrices was also employed on agro-botanical data using the Un-Weighted Pair Group Method with Arithmetic mean (UPGMA) to obtain a dendrogram. The molecular experiment was carried out at Biotechnology institute of University of Zabol. Genomic DNA was prepared according to the procedure of Davis *et al*. (1986). DNA concentration and quality were measured in 1.2% concentration agarose gel using phage λ DNA as a standard. The same DNA samples used in the RAPD analysis were used as templates for ISJ reaction. The polymerase chain reactions were carried out using a gradient Corbet Research thermocycler. Each amplification for RAPD was performed in a reaction volume of 25 µL containing 10 mM Tris-HCl pH 9.0, 30 mM KCl, 2 mM $MgCl_2$, Triton x100 0.1%, 0.1 mM of each dATP, dCTP, dGTP and dTTP (Sinagene), 15 ng of random primer, 50 ng of genomic DNA and 1 unit of Taq polymerase (Sinagene). The amplification protocol for RAPD was 94°C for 4 min to pre-denature, followed by 45 cycles of 94°C for 1 min, 36-45°C (toward each primer) for 1 min and 72°C

Table 1: City of origin and other distinguishing characteristics for *Plantago ovata* accessions used in this genetic diversity studies

| Name | Origin (city) | Type | Morphological cluster |
|---|---|---|---|
| LP039 | Birjand | Landrace | 3 |
| LP014 | Mashhad | Uncertain | 4 |
| LP017 | Sabzevar | Landrace | 1 |
| LP023 | Save | Landrace | 2 |
| LP085 | Semirom | Landrace | 4 |
| LP032 | Esfahan | Landrace | 2 |
| LP067 | Kerman | Uncertain | 4 |
| LP081 | Shiraz | Landrace | 2 |
| LP011 | Bandarabbas | Uncertain | 1 |
| LP072 | Karaj | Landrace | 4 |
| WP101 | Zabol | Wild | 3 |
| WP102 | Zabol | Wild | 3 |
| WP103 | Ilam | Wild | 1 |
| WP104 | Ilam | Wild | 1 |
| WP105 | Isfahan | Wild | 4 |
| WP106 | Naghade | Wild | 1 |
| WP107 | Ardebil | Wild | 5 |
| WP108 | Ahvaz | Wild | 2 |
| WP109 | Abadan | Wild | 2 |
| WP110 | Naein | Wild | 1 |
| WP111 | Yazd | Wild | 2 |
| WP112 | Sabzevar | Wild | 1 |

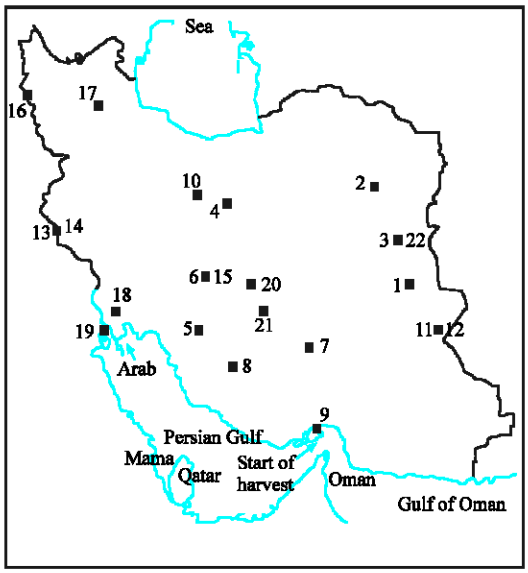


Fig. 1: Map of Iran showing population location

for 1 min, with a final extension at 72°C for 10 min. In ISJ system, Reactions were carried out in 20 μL final volume solution containing 1 x buffer, 0.2 mM of each dNTP, 0.8 μM ISJ primer (IT or ET), 1.5 mM of $MgCl_2$, 2 μL of genomic DNA (20 ng $\mu L^{-1}$) and 0.7 units of Platinum Taq Polymerase (Sinagene). Reactions were run using a touchdown PCR program: denaturation period of 4 min at 95°C, followed by eight cycles of 30 sec at 95°C, 30 sec at 50-62°C and 30 sec at 72°C in which the annealing temperature was decreased by 1°C every cycle, followed by 28 cycles of 30 sec at 95°C, 60 sec at 55°C, 30 sec at 72°C and a final extension for 8 min at 72°C. For each primer, the consistent amplified products were recorded. Each RAPD and ISJ markers was assumed to correspond to a locus with two alleles (presence and absence of the band).

## RESULTS

Accession LP011 originated from Bandarabbas recorded lowest Seed yield (165.15 kg $ha^{-1}$) while LP032 from Isfahan province recorded maximum seed yield (494.15 kg $ha^{-1}$) (Table 2). There was significant difference among average seed yield in landraces and wild accession. There was no significant replication effect for all traits in experiment design (Table 3). The population in each replicate differed significantly to all trait except 1000 seed weight. No. of Pod per plant recorded the highest coefficient of variation among all traits (Table 3).

The highest and lowest of the plant height were belonged to LP072 originated from Karaj and WP111 originated from Yazd, respectively. Path analysis results shown that plant height had most significant positive direct effect on grain yield. WP106 (Naghade) and WP101 (Zabol) recorded maximum and minimum follicles per plant, respectively. Significant block effect was observed for all traits except follicle weight (Table 3). Results of analysis of variance shown that there were significant differences between all traits in 22 accessions except 1000 seeds weight and quality traits (Table 3).

The accessions belonged to neighbor area have more morphologically association. For example accession WP108 (Ahvaz) and WP109 (Abadan) obtained from Khozestan province had closely associated (0.63). LP011 originated from Hormozgan province (South of Iran) and WP107 from Ardebil (North of Iran) antiseptically formed one group. The correlation matrix showed that grain yield was significantly and positively associated with follicle weight, plant height and No. seed per follicle. There was no negative and significant associate among grain yield and all traits (Table 4). The two principal components accounted for about 7 6.78% of total variance with the first principal component taking 50.37% (Table 5). The relative discriminating power of the principal axes as indicated by the eigen values was 6.72 for axis 1 and 3.92 for axis 2. The first principal component that accounted for the highest proportion (50.37%) of total variation was mostly correlated with follicle length, seed per follicle and

Table 2: Descriptive statistical data of morphological traits for twenty-two accession of *Plantago*

| | Traits | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Statistical parameter | 1000 seeds weight | Seeds per follicle | Follicles per plant | Follicles length | Plant height (cm) | Follicles weight | Straw yield | Seed yield | Mucilage (%) | Inflation factor |
| Mean | 1.90 | 83.90 | 5.67 | 2.21 | 17.31 | 75.90 | 59.10 | 170.60 | 0.08 | 21.00 |
| Minimum | 1.31 | 27.00 | 3.20 | 1.00 | 13.20 | 2.60 | 46.11 | 165.15 | 0.05 | 18.00 |
| Maximum | 4.40 | 122.00 | 10.40 | 3.10 | 22.60 | 198.65 | 149.27 | 494.15 | 0.10 | 22.00 |
| CV | 0.18 | 0.21 | 0.20 | 0.10 | 0.10 | 0.17 | 0.17 | 0.18 | 0.12 | 0.11 |

Table 3: Mean square from analysis of variance for complete block design for 13 traits measured in 22 *Plantago ovata* landrace

| Source | df | Seeds per follicle | Follicles per plant | Follicles length | Plant height (cm) | Follicles weight | Straw yield | Grain yield | 1000 seeds weight | Mucilage (%) | Inflation factor |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Replication | 3 | 679.98 | 9.55 | 0.65 | 14.51 | 4625.37 | 2986.30 | 1730.52 | 3.52 | 0.21 | 235.45 |
| Treatment | 21 | 1459.57* | 25.28* | 0.85* | 2.68* | 8272.12* | 2167.80* | 1451.02* | 1.82 | 0.17 | 437.26 |
| Error | 63 | 336.46 | 3.43 | 0.21 | 3.22 | 2375.12 | 875.69 | 533.28 | 0.72 | 0.28 | 1232.01 |

*Significant in 0.05%

Table 4: Correlation coefficient of 8 traits used in characterizing 22 *Plantago* population

| Traits | Grain yield | Follicles weight | Plant height (cm) | Follicles length | Follicles per plant | Seeds per follicle | 1000 seeds weight | Straw yield |
|---|---|---|---|---|---|---|---|---|
| Grain yield | 1 | 0.90** | 0.40* | 0.21 | -0.18 | 0.30 | 0.15 | 0.91** |
| Follicles weight | | 1.00 | 0.35* | 0.19 | -0.29 | 0.25 | 0.20 | 0.86** |
| Plant height (cm) | | | 1.00 | 0.63** | -0.22 | 0.28 | -0.06 | 0.34* |
| Follicles length | | | | 1.00 | -0.16 | 0.12 | 0.06 | 0.15 |
| Follicles per plant | | | | | 1.00 | -0.08 | 0.08 | -0.14 |
| Seeds per follicle | | | | | | 1.00 | 0.65** | 0.30* |
| 1000 seeds weight | | | | | | | 1.00 | 0.27 |
| Straw yield | | | | | | | | 1.00 |

*Significant in 0.05%

plant height. Characters that were mostly correlated with the second principal component were follicle per plant, follicle length and No. seed per follicle (Table 5). The statistics of cluster analysis based on morphological traits showed 22 landrace clustered to 5 groups when dendrogram cutting in 0.7 similarity coefficient in which LP023 originated from Save and WP111 obtained from Yazd individually formed separated cluster.

According to this analysis, the highest similarity belongs to LP017 and WP112 originated from Sabzevar with 0.75 similarity coefficient and the least similarity belonged to WP106 originated from Naghade and WP110 obtained from Naein with 0.12 similarity coefficients (Fig. 2).

Among 60 RAPD 10-mer primers (Sinagene), 35 were selected that generated PCR products with a clear pattern for all populations studied and showed a repeatable pattern in separate amplification experiments. These primers generated 142 polymorphic PCR products (average 4.05 bonds for each primer) whose size varied from 200 to 2000 bp (Table 6). The degree of polymorphism between populations varied depending on the primer tested from 1-5. A band (locus) was considered as polymorphic if the band differentiates at least any 2 of

Table 5: Principal component analysis showing the contribution (factor scores) of each character among the twenty-two *Plantago* accessions

| Characters | Prin 1 | Prin 2 |
|---|---|---|
| Grain yield | 0.27 | 0.10 |
| Follicles weight | -0.17 | 0.11 |
| Plant height | 0.34 | 0.19 |
| Follicles length | 0.37 | 0.11 |
| Follicles per plant | 0.32 | 0.39 |
| Seeds per follicle | -0.11 | 0.29 |
| 1000 seeds weight | 0.15 | 0.16 |
| Straw yield | 0.16 | 0.19 |
| Eigen value | 6.72 | 3.92 |
| Variance (%) | 50.37 | 21.43 |
| Cumulative | 50.37 | 76.80 |

Table 6: Primer sequences, amplified bands, polymorphic in RAPD analysis

| Primer | Sequence (5'-3') | Amplified bands | Polymorphic bands | Primer | Primer sequence (5'-3') | Amplified bands | Polymorphic bands |
|---|---|---|---|---|---|---|---|
| OPP-5 | CCCCGGTAAC | 4 | 2 | OPP-16 | CCAAGCTGCC | 5 | 2 |
| OPP-3 | CTGATACGCC | 3 | 3 | OPP-14 | CCAGCCGAAC | 5 | 2 |
| OPH-19 | CTGACCAGCC | 6 | 3 | OPP-12 | AAGGGCGAGT | 4 | 2 |
| OPH-18 | AATCGGGCCA | 5 | 3 | OPP-11 | AACGCGTCGG | 4 | 2 |
| OPH-13 | GACGCCACAC | 3 | 2 | OPP-9 | GTGGTCCGCA | 5 | 2 |
| OPH-12 | ACGCGCATGT | 3 | 2 | OPH-19 | CTGACCAGCC | 6 | 1 |
| OPH-7 | CTGCATCGTG | 3 | 2 | OPP-8 | ACATCGCCCA | 3 | 2 |
| OPB-20 | GGACCCTTAC | 8 | 8 | OPG11 | GACCGCTTGT | 1 | 1 |
| OPB-18 | CCACAGCAGT | 5 | 3 | OPG08 | GTGACGTAGG | 4 | 2 |
| OPA-1 | CAGGCCCTTC | 4 | 3 | OPG13 | CAAACGTCGG | 4 | 2 |
| OPA-4 | AATCGGGCTG | 4 | 3 | OPG10 | TCCGCTCTGG | 3 | 2 |
| OPA-11 | CAATCGCCGT | 3 | 3 | OPG07 | CCGCATCTAC | 5 | 2 |
| OPA-13 | CAGCACCCAC | 3 | 3 | OPG04 | TGCCTAACC | 2 | 2 |
| OPA-18 | AGGTGACCGT | 8 | 8 | OPG03 | TGCCCGTCGT | 2 | 1 |
| OPB-1 | GTTTCGCTCC | 3 | 3 | OPG02 | CTCTCCGCCA | 3 | 1 |
| OPB-2 | TGATCCCTGG | 2 | 1 | OPG14 | GGAAGTCGCC | 5 | 1 |
| OPB-3 | CATCCCCCTG | 5 | 3 | OPG17 | CTGCATCGTG | 2 | 1 |
| OPP-17 | TGACCCGCCT | 5 | 2 | | | | |

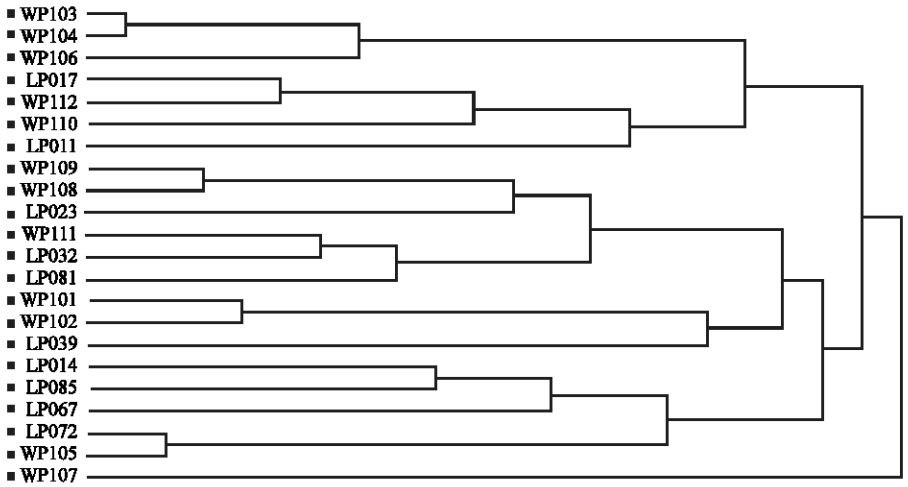


Fig. 2: Genetic similarity among *Plantago* genotypes revealed by UPGMA cluster analysis based on morphological data between 22 accessions

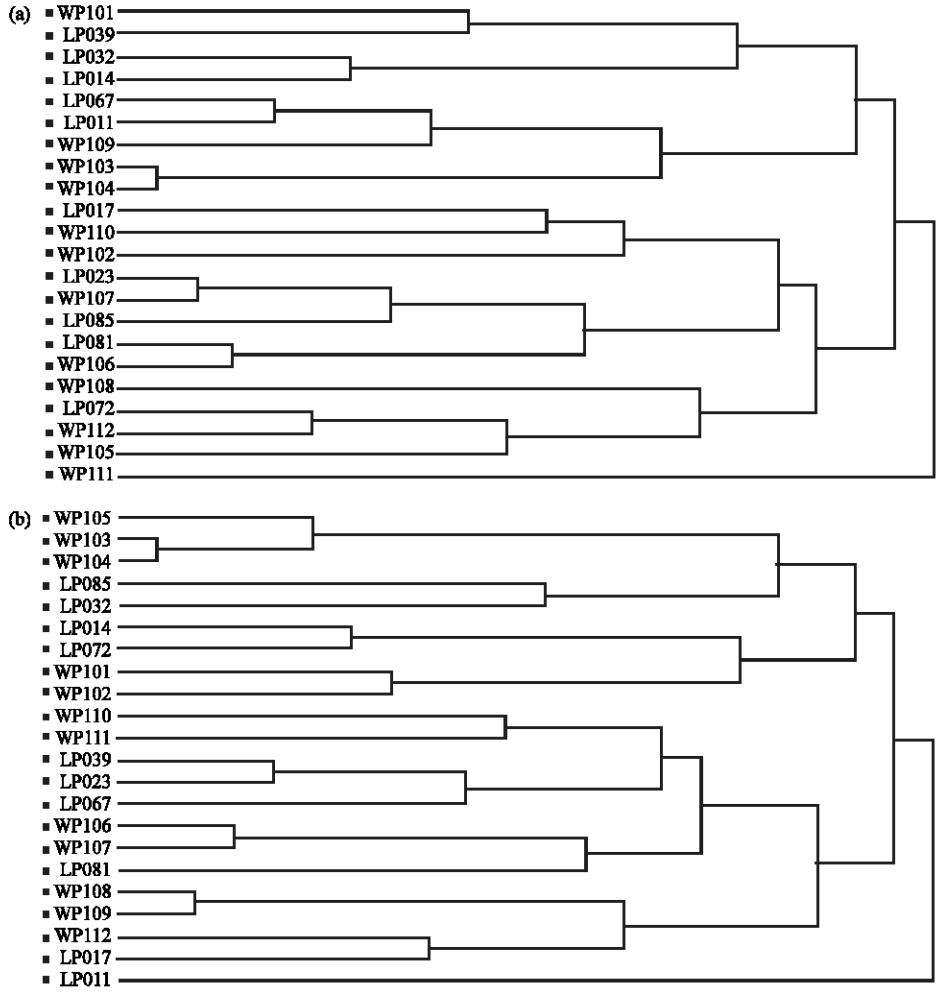


Fig. 3: Dendrograms of 22 *Plantago ovata* accessions constructed from RAPD-GS (a) and ISJ- GS (b) matrices

Table 7: Primer sequences, amplified bands, polymorphic in ISJ analysis

| Code | Sequence 5' to 3' | No. of amplified products | No. of poly-morphic bands |
|---|---|---|---|
| ET32 | ACTTACCTGGGCACG | 7 | 4 |
| ET34 | ACCTACCTGGGCGAG | 5 | 3 |
| ET35 | ACCTACCTGCCGCAG | 5 | 3 |
| ET38 | ACTTACCTGAGGCGCGAC | 8 | 8 |
| ET39 | ACTTACCTGCTGGCCGGA | 6 | 2 |
| ET40 | ACTTACCTGGCCAGCTGC | 5 | 4 |
| ET41 | ACTTACCTGCCTGCCGAG | 4 | 4 |
| ET42 | ACTTACCTGGCACGCCTC | 6 | 4 |
| IT1 | CCGGCAGGTCAGGTAAGT | 6 | 4 |
| IT3 | GCAGAGGGCCAGGTAAGT | 4 | 4 |
| IT4 | CTGCGGCCACAGGTAAGT | 2 | 1 |
| IT5 | GGCGGAGAGCAGGTAAGT | 2 | 2 |
| IT31 | GAAGCCGCAGGTAAG | 7 | 4 |
| IT33 | GATGCCCCAGGTAAG | 7 | 4 |

22 populations. The highest number of band was obtained with primers OPB-20 and OPB-18 while the lowest number was obtained with primer OPJ11 (Table 6).

In semi specific PCR (ISJ), 14 primers were used belonging to four groups of Intron targeting (IT), Exon Targeting (ET) 15 and 18 bases in length (Table 7). These primers produced 95 DNA fragment (50 polymorphic bands) with 6.78 bands per primer. The majority of primers revealed polymorphism between populations and only three of them generated non-polymorphic band pattern. (Table 7) the highest and lowest of the polymorphic band belonged to IT4 and ET38, respectively. The average number o polymorphic band for each semi random primer was 2.55. Using q square test, number of polymorphic band obtained from 18-mer primer were significantly more than 15-mer primer. The most satisfactory results were obtained using IT primers, 18 bases in length. All the 156 bands, generated from 35 RAPD primers, were subjected to calculate the genetic similarity index (RAPD-GS) among the 22 accessions (Fig. 3). The RAPD-GS value ranged from 0.19 to 0.75 with the mean of 0.45. The highest genetic similarity was found between WP103 and WP104 accessions originated from Ilam province while the lowest was observed between WP105 (Isfahan) and LP011 (Bandarabbas) (Fig. 3a). The ISJ-derived data were

subjected to calculate the genetic similarity (ISJ-GS). The genetic similarity coefficient varied between 0.17 and 0.83, with the average of 0.49. The minimum GS value derived between WP111 and WP101 accessions while the maximum GS value derived between WP103 and WP104 accessions originated from Ilam (Fig. 3b). Results ISJ system could not show the conformity with geography dispersal, for example, LP085, LP032, WP105 and WP110 obtained from Isfahan province were straggled between three groups while WP106 originated from Northwest and LP081 obtained from South that have too distance, closely abut together. The average genetic similarity value based on ISJ markers was lower than of than that of RAPD markers approximately among the all populations. These result suggested that higher genetic diversity could be detected by ISJ markers than of RAPD markers among the 22 *Plantago* population from Iran. Using RAPD data five groups were clustered. LP011 belonged to Hormogan province formed one group, separately. Group one was included WP105 (from Isfahan city), WP103 and WP104 (from Ilam) and LP085 and LP032 (from Isfahan province). Group two was included LP014 and LP072 (belong to center of Iran) and WP101 and WP102 (from East of Iran).

Group three containing WP110, WP111 (obtained from center), LP039 from Birjand in east of Iran, LP023 from Save, LP067 from Kerman, WP106 and WP107 from north and LP081 from Farc province. Another accessions was situated in group for including WP108, WP109 from Khozestan province in Southwest, WP112 and LP017 from Sabzevar. The RAPD cluster analysis have a nice accordance with a geography dispersal except in group three that accessions from center that mingle with another accessions. WP104 and WP105 obtained from Ilam province had most similarity in all clustering systems. This show that this population verily is oneness and LP011 in three systems formed one group separately hence the cytogenetical study needs until dependency of this population to ovata species was revealed. The correlation of pair wise distances between all pairs of groups for RAPD compared to morphological was 0.45. The correlation of pair wise distances among all pairs of *Plantago* for RAPD and ISJ was 0.19. The cluster analysis generated using ISJ markers reveal five group when dendrogram cutting in 0.6 coefficient similarity. In this clustering, populations belong to same province in this clustering were segregated among another population while WP103 and WP104 with maximum similarity formed one groups.

## DISCUSSION

**Morphological analysis:** In this study, we analyzed 22 accessions of *Plantago ovata* and evaluation of eight morphological traits including yields and related traits. The results of ANOVA indicated there were high significantly diversity between 22 accession for all traits except 1000 seed weight. The correlation between traits shown that straw yield, follicles weight and plant height had significantly and positively associated with grain yield. These correlations were similar to those found in the evaluation of *Plantago* by Bannayan *et al.* (2008). The most important factor in grain yield in plantain are green index (plant height and straw yield). several studies have demonstrated the importance of plant height and straw yield as important component in *Plantago* (Ganpat *et al*., 1992; Nadjafi *et al*., 2006). The positive and significant correlation between plant height with follicles length and follicles weight may indicated that taller plants of *Plantago* were prosperous in seed productivity (Ganpat *et al*., 1992). According to cluster analysis based on morphological traits, 5 groups were formed when dendrogram cutting in 0.7 similarity coefficient. Results indicated that group I had population with more plant height, straw yield and grain yield. Accessions belong to this groups had most potential for crop productivity and accessions in group three had most potential for mucilage production.

**Molecular analysis:** The major limitation of morpho-agronomical characterization is that this kind of evaluation often involves a high number of descriptors, many of which influenced by the environment, particularly those conditioned by many genes. The RAPD technique has been found to be a useful and robust tool for detecting genetic diversity and determining genetic relationships within and among plant species. In this study, average number of bonds for each primer was 4.05. The high levels of allelic diversity of RAPD markers observed in this study probably were associated with the extensive rang of genetic diversity represented in the panel of *Plantago* accessions in Iran. Dendrogram based on RAPD marker was accord with the dendrogram based on morphological marker. This result has coordination with some of studies by Chen *et al.* (2000) and Devos and Gale (1992). Other studies using cruciferous species to compare the resolving power RAPD markers in determining genetic relationships have shown RAPDs to be reliable for intraspecific comparisons and among closely related species, but less reliable for higher taxonomic associations (Halldén *et al*., 1994). In RAPD-based clustering, all population that was belonged to near area formed closely groups for example WP106 and WP103 and WP106 and WP107. The ISJ system marker produced 95 DNA fragment with 2.55 polymorphic bands for each semi-random primers while for RAPD it was 4.05. These results are in disagreement with studies in maize inbred lines (Rafalski *et al*., 2001).

The semi random primer that are used in this study may did not have efficiency for showing genetic diversity between populations. The ISJ data also gave a structure with five groups that were not related to evident morphological characteristics nor to RAPD clustering, however some concordance between the morphological, RAPD and ISJ were highlighted, with several cases of similar proximities between accessions. The absence of a relationship between the morphological and genetic similarities was also found for wild populations of other plant (Greene *et al*., 2004; Steiner and Santos, 2001). Several reasons may account for the discordance between the morphological traits and RAPD and ISJ marker. First, the less number of semi-random primers could not cover vast area of *Plantago* genome. Second, in defiance of some studies (Weining and Langridge, 1991; Rafalski *et al*., 1997) these primers don't belong to intron-exon splice junction of all plants. Third, morphological variation is strongly associated with environmental variation; the morphological similarities observed may be due to different combinations of alleles producing similar phenotypes that might result in morphological similarities or differences that are not proportional to the underlying genetic differences. The information found here evidenced the high genetic diversity. It could be valuable to use both variations obtain from molecular and morphological to select parents of improved varieties. A breeding program can be started within any morphological and RAPD cluster found in this study without risk of inbreeding.

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