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MOLECULAR AND MORPHOLOGICAL DISCRIMINATION OF SELECTED PLUM SEEDLINGS FOR ROOTSTOCK BREEDING

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A B S T R A C T

Plum seedlings are extensively used as rootstocks for stone fruits except *P. cerasus*. Genetic diversity in seedlings used for rootstocks might result in variation of scion performance and subsequently non-uniform orchards. This study aimed on investigating variation among several Iranian plum genotypes sampled from seedlings grown for grafting. Thirty-five 3- to 4-year-old seedlings were characterized using 28 quantitative and qualitative traits, as well as 22 RAPD markers. Results of the morphological study revealed remarkable variability in studied traits. Genotypes were separated into three different groups according to their height using cluster analysis performed by Ward's clustering method based on morphological data. Twenty two RAPD primers from 120 screened produced 195 polymorphic reproducible bands (86.75% polymorphism). According to the similarity matrix, the lowest similarity was obtained between the genotype Tansgol (control) and S4-13 and the highest similarity between S11-25 and S11-26. According to the cluster analysis based on Jaccard's similarity coefficients and the UPGMA method at a similarity level of 0.56, the genotypes were divided into six sub-clusters. Significant association between eight morphological traits with RAPD markers was found by marker association analysis done using multiple linear regressions. The application of the methodology in screening elite genotypes is discussed.

Key words: biodiversity characterization, *Prunus domestica*, RAPD markers, seedling

INTRODUCTION

Plums are a diverse group of plants with many species adapted to a broad range of ecogeographic conditions and have been cultivated for centuries (Ayanoglu et al., 2007). This group contains 20-40 species (Okie and Hancock, 2008) that are distributed in different parts of the world. This genus has a wide range of ploidy levels; the Japanese plum (*Prunus salicina* Lindl.), the Myrobalan plum (*P. cerasifera* Ehrh.) and the American plum (*P. americana* Marsh., *P. munsoniana* Wight and *P. angustifolia* Marsh) are diploid (2n=2x=16), compared to the tetraploid plum *P. spinosa* L. (2n=4x=32) and the hexaploid European species (2n=6x=48) (Shimada et al., 1999). Geographically, plums originated in three regions, European plums belong to *Prunus domestica* L., *P. cerasifera* Ehrh., *P. spinosa* L. and *P. insititia* L. species, while Japanese type plums are commonly known as *P. salicina* Lindl. species. American plums arose from numerous species such as *P. americana* Marsh., *P. munsoniana* Wight and *P. angustifolia* Marsh (Okie and Weinberger, 1996).

The European plum has been reported to originate from Asia Minor as a diploid hybrid between *P. cerasifera* (Myrobalan plum) and the tetraploid *P. spinosa* L., which was then doubled to produce a fertile hexaploid (Okie and Weinberger, 1996). Although this hypothesis was heretofore widely accepted, recent cytological studies have suggested that *P. spinosa* itself carries the genomic material from *P. cerasifera* as well as an unknown ancestor (Reynders-Aloisi and Grellet, 1994). Thus, hexaploid plums may have originated from polyploid forms of *P. cerasifera* (Reynders and Salesses, 1991). Among *P. domestica*, several types are recognized such as Green Gage (or Reine Claude) and prunes. The *P. insititia* includes bullaces, damsons, mirabelles and St. Julien types. European plum, *P. domestica*, is the most commonly grown species in cooler regions (Okie and Hancock, 2008).

Japanese and hexaploid plums are mainly grown for their fruits but many also are used as rootstocks for almost all *Prunus* species, except sweet and sour cherries (Salesses et al., 1994). Most of the plum cultivars grown in the world are propagated on rootstocks that are selected for local soil type and vigour requirements (Okie, 1987; Ramming and Cociu, 1991).

Plum growers are interested in less vigorous, dwarfing plum rootstocks, which are more productive and bear good fruit quality, easier to harvest, precocious and lower costs for cultural practices (e.g., prune, spray and harvest). Therefore, one of the main objectives of stone fruit breeding programs focuses on introducing dwarf rootstocks (Janes and Pae, 2003).

Cultivar identification of plums has traditionally been based on morphological traits, such as leaf and fruit shape, colour, size, seed associated characters, plant growth habits, etc. (Ilgin et al., 2009). Although morphological traits are valuable and

useful in differentiation of genotypes, not all are reliable enough because these traits are influenced by environmental factors, plant age, phenological stage and cultivation conditions (Struss et al., 2001). In recent decades, molecular markers have become useful tools for plant fingerprinting, phylogeny studies, gene tagging, linkage mapping and identifying plant genetic relationships (Ilgin et al., 2009).

The ability to distinguish cultivars could be greatly enhanced by using appropriate molecular markers (Iezzoni and Brettin, 1998). These techniques directly measure variations at the DNA level, and are not affected by environmental factors (Gerlach and Stosser, 1998). Evaluation of plant diversity by both molecular markers and morphological traits can lead to informative judgment of plant individuals. Several PCR - based methods such as Inter-simple sequence repeats (ISSRs) (Roh et al., 2007; Li et al., 2009; Papp et al., 2009; Lisek and Rozpara, 2009), Simple Sequence Repeats (SSRs) (Struss et al., 2003; Wunsch et al., 2004; Ohta et al., 2005), and Amplified fragment length polymorphism (AFLPs) (Ayanoglu et al., 2007; Boritzki et al., 2000; Struss et al., 2001, 2003) have been developed for identifying genetic diversity. Also Randomly Amplified Polymorphic DNA (RAPD) is a useful technique as genetic marker (Williams et al., 1990), which is a fast, inexpensive, and suitable method for producing genetic profiles. RAPD markers were used in *Prunus* genus to reveal genetic relatedness and variability in plant populations, and also to measure the influence of breeding systems on diversity and in plant taxonomy (Shimada et al., 2001; Lisek et al., 2006; Cai et al., 2007; Khadivi-Khub et al., 2008). RAPD markers are extensively adopted due to their experimental simplicity and to analyze the diversity of a wide range of species and samples. Successful application of RAPDs in biosystematics and evolutionary studies has been widely documented for numerous fruit crops including plum, almond, apricot, cherry, and other *Prunus* species (Shimada et al., 2001; Martins et al., 2003; Lisek et al., 2006; Cai et al., 2007; Khadivi-Khub et al., 2008; Kumar et al., 2009). Comparison of RAPDs and ISSRs in determining genetic similarity among apricot (*Prunus armeniaca*) genotypes revealed that RAPD markers were highly efficient regarding polymorphism detection, as they accounted for 97.84% of polymorphism compared with 96.5% for ISSR markers. Also, the number and percent of polymorphic loci, diversity index, effective multiplex ratio, and marker index were higher for RAPD than for ISSR markers (Kumar et al., 2009).

Many studies have been carried on the plum cultivars, and important results have been obtained using RAPD markers (Shimada et al., 1999; Casas et al., 1999; Liu et al., 2007 and Lisek et al., 2007). Hend et al. (2009) studied 27 plum cultivars with a combination of morphological, pomological and RAPD markers and found considerable ge-

netic diversity among them. Plum seedlings are one of the most important rootstocks for stone fruits in Iran. Due to high genetic diversity in seedlings, these seedlings contribute to differences in scion performance and non-uniform orchards.

The aim of this study was to investigate molecular and morphological variation among selected individuals from 2 200 plum seedlings in order to investigate the diversity among the seedlings and to evaluate them for use in future plum breeding programs, especially for rootstock improvements.

MATERIAL AND METHODS

Plant materials and DNA extraction

A set of 35 plum genotypes with different growth habits, was collected from among 2200 seedlings from different nurseries along with 'Tansgol' for this study. Genomic DNA was extracted from fresh young leaves sampled from each genotype according to Murray and Thompson (1980). The purity and quantity of genomic DNA was determined spectrophotometrically at 260 and 280 nm. Total DNA was diluted to a working concentration of approximately10 ng $\mu l^{-1.}$

Morphological evaluation

Phenotypic characterization of each genotype was performed according to the descriptor provided by the International Plant Genetic Resources Institute (IPGRI-CEC, 1984). Twenty-eight phenotypic characteristics related to vegetative and reproductive characterization were recorded in the third and fourth year of seedling growth during 2008 and 2009, respectively (Tab. 1).

RAPD analysis

From 120 decamer oligonucleotides (TIBMOLBIOL Co., Germany), 22 primers were selected (Tab. 2). Polymerase chain reaction was performed in a total volume of 15 µl containing 20 ng template DNA (2 µl), 7.5 µl PCR 2X Kit (CinaGen Co., Iran), (containing dNTPs, PCR buffer, Taq DNA Polymerase and $MgCl_2$) and 1 µM RAPD primer (1.5 µl).

The reactions were performed in a thermocycler (iCycler, BioRad, USA) programmed as follows: 94 °C for 3 min as an initial denaturation step, followed by 5 cycles of 92 °C for 1 min, 39.5 °C for 1 min, 37 cycles of 92 °C for 30 sec, 37.5 °C for 45 sec, 72 for 2 min; and a final extension at 72 °C for 7 min. PCR products were separated in 1.2% agarose gel at 70 V for 2 hours in 1X TBE buffer; and visualized by ethidium bromide staining and photographed under UV light, by a GelDoc system (UVP, USA).

Data analysis

Analysis of variance was performed for all morphological traits with SPSS software. The cluster analysis of morphological traits was performed using the Ward method with SPSS software. For RAPD data, each gel was analyzed by scoring the presence (1) or absence (0) of polymorphic bands in individual lanes.

Table 1. Morphological traits recorded for 35 plum genotypes

Trait	Unit	Minim	Max	Mean	SD	CV [%]
Growth habit	rank (1-5)	1	5	4.71	0.86	18.24
Height 2008	cm	125	300	222.89	44.81	20.10
Lateral Branch number	number	8	65	24.43	9.81	40.16
Branch length mean	cm	23	195	128	0.39	30.55
Branch position	rank (1-3)	1	3	1.86	1	54.07
Leaf colour	rank (1-3)	1	3	1.34	0.76	56.95
Trunk diameter 2008	mm	17	59.80	42.64	11.05	25.92
Branch angle	rank (1-5)	1	5	2.43	1.50	61.79
Longest branch	cm	44	251	170	0.48	28.53
Spine	rank (0-1)	0	1	0.23	0.43	-
Sucker number	number	0	22	6.17	5.27	85.42
Growth vigour	rank (1-7)	1	7	4.94	1.78	36.04
Yield 2008	rank (1-5)	0	5	1.86	2.05	-
Height 2009	cm	145	430	273.09	59.69	21.86
Trunk diameter 2009	mm	27.50	90	55.86	15.45	27.66
Leaf length	mm	31	61.3	44.8	0.58	13.03
Leaf width	mm	7.8	34.5	24.9	0.53	21.44
Petiole length	mm	7.5	17.9	10.7	0.21	19.37
Leaf length / Leaf width	-	1.17	5.49	1.90	0.67	35.08
Increase height	cm	10	141	50.20	30.20	60.16
Increase Trunk diameter	mm	2	32.60	13.23	7.20	54.44
Start of flowering 2009	day	1	5	2.89	1.323	45.77
Full bloom 2009	day	5	9	6.49	1.011	15.57
End of flowering 2009	day	15	23	18.86	2.07	10.99
Leafing time	day	1	18	6.54	3.407	52.09
Flowering period	day	12	20	15.97	1.932	12.09
Leaf fall time	rank (1-7)	1	7	4.89	1.93	39.5

CV% = (SD/Mean) *100

The NTSYS-pc software ver. 2.02 (Roholf, 1998) was employed to estimate genetic similarities with the Jaccard's coefficient. The matrix of

generated similarities was analyzed by the unweighted pair group method with arithmetic average (UPGMA), using the SAHN clustering module. The stepwise regression analysis of the 8 morphological characters and RAPD data was performed by SPSS software.

The estimation of the resolving power (Rp) (Prevost and Wilkinson, 1999) allowed the evaluation of the ability of the most informative primers to differentiate genotypes.

The regression association was carried out between the morphological data of plum (growth habit, leaf colour, height in 2008, height in 2009, height increase, leaf width, leaf fall time and presence or absence of spines), and the available RAPD profiles of 22 polymorphic primers.

RESULTS

Morphological analysis

Analysis of variance showed significant differences between studied genotypes. The mean and CV percent of different traits are presented in Table 1.

Maximum (300 cm) and minimum (125 cm) height in 2008 and also maximum (430 cm) and minimum (145 cm) height in 2009 were recorded among genotypes. Most traits such as sucker count and height increase had high CV. Results of simple correlation analysis revealed significant correlations among some important characters such as height, growth vigour, colour of leaf, and trunk diameter. Seedling height in years 2008 and 2009 had significant and positive correlations with growth type, trunk diameter, and growth vigour characters. Principle component analysis seedlings revealed eight independent factors contributed to over 79.5% of total variation with 41.5% of the total variation explained by the first three principal components, which accounted for 23.2%, 9.5% and 8.8%, of total variation, respectively. Height in 2008 and 2009, growth vigour, trunk diameter in 2008 and 2009, branch mean length and branch highest length had high loadings on, and were associated with the first component.

The dendrogram for morphological traits grouped the 35 genotypes into three major clusters (I), (II) and (III) (Fig. 1). The main difference between these three main clusters was the height values in 2008 and 2009. The tertiary main cluster (III) contained S11-23, S11-25, and S11-26 genotypes. Genotypes in this group in 2008 and 2009 were less than 160cm and 180 cm tall, respectively.

The second main cluster (II) contained genotypes with very strong growth; with height between 265-430 cm in 2009. The first main cluster (I) contained the remaining genotypes. This group is also divided into two subgroups (I-a, I-b). The first subgroup (I-a) was containing genotypes with the strong growth (height between 253-280 cm in 2009).

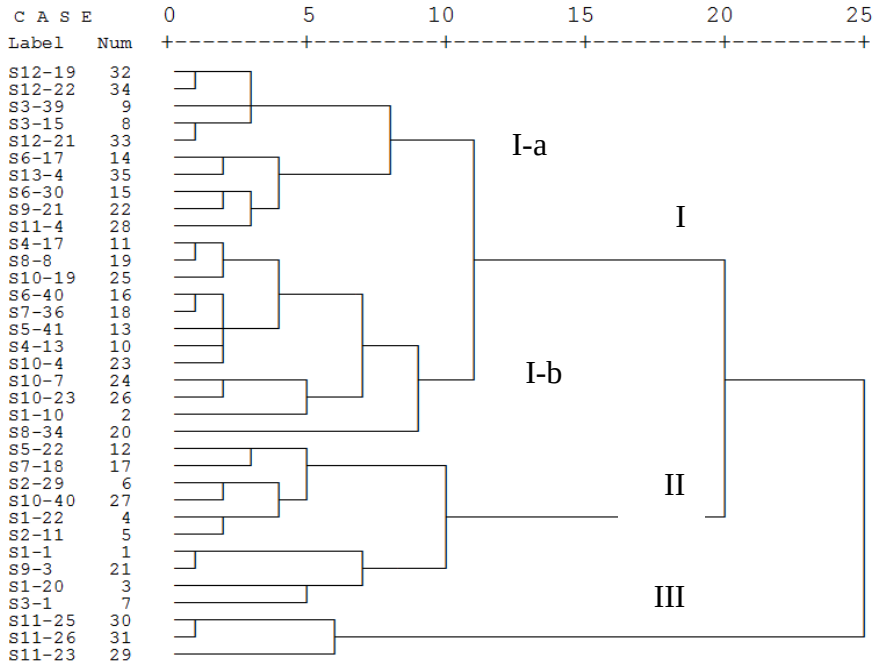

Figure 1. Cluster analysis for 35 plum genotypes based on morphological data

The second subgroup (I-b) was composed of the remaining genotypes with average growth (height between 190-300 cm in 2009).

Molecular analysis

Twenty-two primers with good and reproducible polymorphic bands were used for analysis among the 36 studied genotypes. Selected primers produced a total of 219 DNA fragments (Tab. 2) from which 24 fragments were monomorphic and 195 fragments showed to be polymorphic. The size of amplified fragments ranged between 300 and 3000 bp for all primers. The highest number of bands was 16 (TibMolBiol-BD04 primer), and the highest polymorphic band ratio was 100% (BB03, BB05, BD04, BD05, BD11, BD13BD17 and BD18 primers). The average percentage of polymorphism among all the primers used was 86.8%. Total resolving power was 92.2 with the mean of 3.8 and maximum of 6.9 (BD04). The similarity matrix showed the lowest similarity (0.21) between the Tansgol and S4-13 and the highest similarity (0.77) between the S11-25 and S11-26.

According to the cluster analysis based on Jaccard's similarity coefficients and UPGMA method (Fig. 2) and at a similarity level of 0.56, the genotypes were divided into six subclusters, containing 30, 1, 1, 1, 2, and 1 samples, respectively. Tanasgol genotype was separated individually from others at similarity of 0.27.

Table 2. The sequence, polymorphism, and resolving power results for RAPD primers used to study 35 plum genotypes

Number	Primer	Sequence	Number of amplified fragments			Rp
			total	polymorphic	% polymorphism	
1	TIBMBA08	5'-CCACAGCCGA-3'	13	10	76.92	3.78
2	TIBMBA16	5'-CCACGCATCA-3'	10	9	90	3.24
3	TIBMBA20	5'-GAGCGCTACC-3'	12	11	91.67	5.08
4	TIBMBB01	5'-ACACTGGCTG-3'	13	12	92.31	6.16
5	TIBMBB03	5'-TCACGTGGCT-3'	11	11	100	5.35
6	TIBMBB04	5'-ACCAGGTCAC-3'	6	4	66.67	1.68
7	TIBMBB05	5'-GGGCCGAACA-3'	9	9	100	3.68
8	TIBMBB07	5'-GAAGGCTGGG-3'	7	6	85.71	1.78
9	TIBMBB08	5'-TCGTCGAAGG-3'	13	12	92.31	4.76
10	TIBMBB09	5'-AGGCCGGTCA-3'	7	5	71.43	2.16
11	TIBMBB11	5'-TGCGGGTTCC-3'	6	4	66.67	2.54
12	TIBMBB14	5'-GTGGGACCTG-3'	8	5	62.50	2.59
13	TIBMBD01	5'-TCACTCGCTC-3'	10	9	90	2.59
14	TIBMBD04	5'-TCGGGTGTTG-3'	16	16	100	6.92
15	TIBMBD05	5'-GTGCGGAGAG-3'	11	11	100	5.08
16	TIBMBD11	5'-CAACCGAGTC-3'	10	10	100	6
17	TIBMBD13	5'-CCTGGAACGG-3'	10	10	100	4.92
18	TIBMBD15	5'-TGTCGTGGTC-3'	8	7	87.50	1.95
19	TIBMBD17	5'-GTTCGCTCCC-3'	10	10	100	4.05
20	TIBMBD18	5'-ACGCACACTC-3'	13	13	100	5.51
21	TIBMBE05	5'-GGAACGCTAC-3'	9	7	77.78	3.84
22	TIBMBE06	5'-AAGCGGCCCT-3'	7	4	57.14	1.68
Total	-	-	219	195	-	85.35
Mean	-	-	9.95	8.86	86.75	3.88

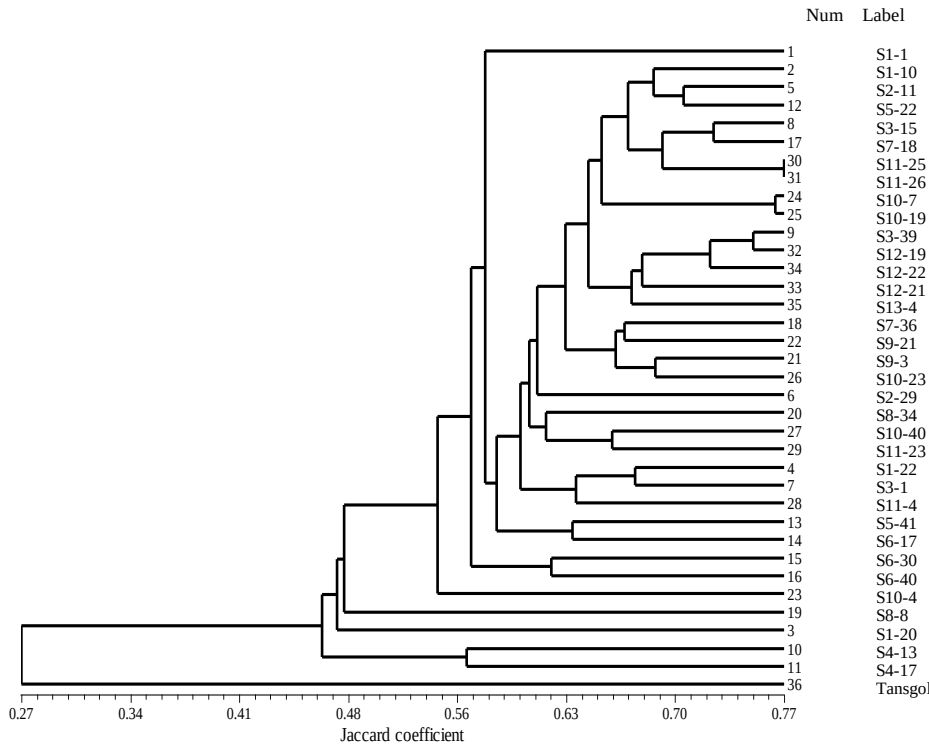

Figure 2. UPGMA dendrogram of 36 plum genotypes based on data of 22 RAPD primers

Regression analysis for association between morphological and molecular data

Correlation between 185 polymorphic RAPD markers in 35 plum seedlings with each of the 8 morphological traits was calculated by the stepwise regression method. RAPD markers showed association with morphological traits of growth habit, leaf colour, 2008 height growth, 2009 height growth, height increase, leaf width, leaf fall time and presence of spines (5, 15, 7, 20, 8, 4, 2 and 14 polymorphic bands, respectively) (Tab. 3). The highest R^2 in regression analysis was related to the 5 and 15 markers associated with growth habit and leaf colour (100%); among which BD05 (at 300 bp) and BB01 (at 700 bp) had maximum R^2, respectively (Fig. 3).

DISCUSSION

The conservation of genetic resources and characterization of local cultivars require knowledge of their genetic potential and the relationship among them. Morphological study showed high variability in studied characters. Using a phenotypic analysis, Hend et al. (2009) showed that pomological and tree growth traits

Table 3. Informative markers resulted by stepwise regression analysis between polymorphic RAPD bands with some important rootstock characters in plum

RAPD					
Band length	main marker	adjusted R^2	total R^2	marker	character
300bp	$BD05_{300}$	66.6	100	5	growth habit
700bp	$BB01_{750}$	35.9	100	15	leaf colour
300bp	$BE05_{300}$	28.7	84.2	7	height 2008
300bp	$BE05_{300}$	21.4	99.5	20	height 2009
750bp	$BD15_{750}$	25.6	90.4	8	increase height
2300bp	$BB08_{2300}$	37.2	93.9	10	leaf width
1250bp	$BD11_{1250}$	24	43.5	2	leaf abscission time

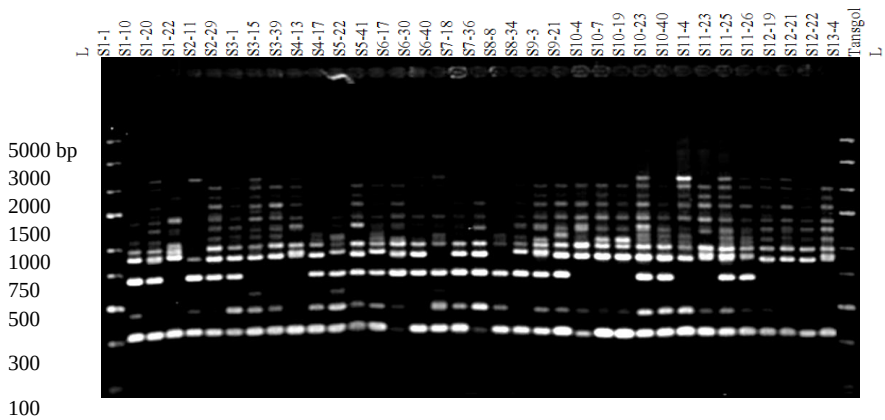

Figure 3. Polymorphism in DNA samples of 35 plum genotypes, amplified by TIBMBB01primer. The arrow indicates the 700 bp fragment, which showed correlation with leaf colour (see text for details)

were able to distinguish morphological variability of plum cultivars. Ganji Moghadam et al. (2006) evaluated morphological characters of 12 sour cherry populations for means of investigation of genetic variation and identity of sour cherry dwarf genotypes for cherry rootstock breeding. In that investigation genotypes were divided into 4 sub-clusters: very dwarf, dwarf, semi-dwarf and standard. The similarity between the morphological grouping approach of the present study and that of Ganji Moghadam et al. (2006) suggests that height is a suitable criterion for grouping the genotypes.

The morphological analysis is suggesting S11-23, S11-25, and S11-26 genotypes are dwarf genotypes, making them valuable resources for rootstock and further breeding projects.

Furthermore, the genotypes S1-20, S1-22, and S3-1 were vigorous with an upright habit growth, and thus, might be valuable for low vigour sites due to undesirable soil conditions as well as be easier to propagate (i.e., grafting) (Gyeviki et al., 2008).

In the present study, 22 primers were tested resulting in 195 polymorphic bands, and genetic similarity ranged between 0.27 to 0.77, indicating high diversity among the studied seedlings. The estimated resolving power (Rp) (85.4) shows the efficiency of the exploited primers. Studying genetic diversity in 27 plum cultivars using 10 RAPD primers, Hend et al. (2009) obtained 97.3% polymorphism, a total Rp of 82 and 0.18-0.80 genetic similarity among genotypes. Shimada et al. (1999) also studied genetic variation of plum cultivars by RAPD markers and reported 24% polymorphism. It is notable that the Shimada study was focused on commercial genotypes, while samples for this study and that of Hend et al. (2009) were focused on indigenous plant materials. The higher diversity among indigenous genotypes compared to that of commercial ones, can be addressed by the genetic drift due to selection within commercial cultivars.

The correlation between phenotypic and molecular classifications suggested the use of two kinds of markers for identification and characterization of local germplasm was feasible. The utility of combined genetic (RAPD) and phenotypic characteristics reveals variation among the plum genotypes; therefore, this can provide a more comprehensive understanding of the diversity in plum germplasm.

It was noteworthy that 'Tansgol' (probably a hybrid between plum and apricot), a phenotypically intermediate genotype between plum and apricot, was genetically separated from other studied genotypes. Similar results were reported when it was compared to apricot samples using RAPD markers (Jannatizadeh et al., 2011). Since this genotype is late-blooming, it may be valuable for breeding late flowering genotypes to avoid late frost damage.

In this study, some cases of similarity were observed between clustering based on morphological and RAPD data. For example, S11-25 and S11-26 genotypes (low height, upright growth, without spines, few suckers) were placed close in the one group based both on morphological and molecular profile. In other cases, there were remarkable differences but the correlation was not significant ($r = 0.14$). Similarly, Khadivi-Khub et al. (2008) found no significant correlation between morphological and RAPD data when studying genetic diversity in sweet cherry. Several studies have compared the use of morphological and molecular data to examine their relationships and most of these studies showed that relationships between two methods were low (e.g. Martinez et al., 2003; Semagn, 2002; Vollmann et al., 2005; Zamani et al., 2007). Two reasons have been suggested by Semagn (2002) for these relationships: (i) molecular markers cover a larger

proportion of the genome, including coding and non-coding regions, than the morphological markers and (ii) molecular markers are not subjected to artificial selection compared to morphological markers. Therefore, these morphological traits could not have been characterized by RAPD markers. It is, however, a district possibility that some correlation could be observed between molecular and morphological data when more in-depth details such as data for leaf, flower, fruit, and additional molecular markers such as ISSR, SSR and AFLP are included.

In this study, significant association was found between some morphological traits and RAPD markers by multiple regression analysis. For example $BB01_{700}$ primer had high R^2 for leaf colour trait. Genotypes having this band exhibited green leaf colour (rank 1) and when absent, the leaf colour was purple (rank 3) (Fig. 3). This might be a candidate marker from coding region close to the locus controlling leaf colour. Khadivi et al. (2008) who studied genetic diversity in some sweet cherry cultivars with RAPD markers, found significant associations between some traits and RAPD markers.

For efficient use of the candidate superior genotypes commercially, it will be essential to evaluate these genotypes in terms of rooting and rootstock-scion compatibility, which is likely to demand a case-by case investigation.

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MOLEKULARNE I MORFOLOGICZNE ZRÓŻNICOWANIE SIEWEK ŚLIWY STANOWIĄCYCH PODKŁADKI DLA DRZEW PESTKOWYCH

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S T R E S Z C Z E N I E

Siewki śliwy znajdują szerokie zastosowanie jako podkładki dla wielu drzew pestkowych, z wyjątkiem wiśni (*P. cerasus*). Zastosowanie odmiennych genetycznie podkładek może skutkować zmianami w rozwoju zrazów i tym samym uzyskaniem niepożądanej zmienności roślin w sadzie. Celem badań była ocena zmienności wybranych genotypów śliwy, stosowanych w Iranie jako podkładki drzew pestkowych. Trzydzieści pięć trzy- i czteroletnich siewek scharakteryzowano na podstawie analizy 28 cech morfologicznych i przy użyciu wybranych starterów RAPD. Na podstawie oceny morfologicznej i analizy wyników metodą Warda genotypy podzielono na trzy grupy. W reakcjach z 22 starterami RAPD uzyskano 195 produktów polimorficznych, stanowiących 86,75% wszystkich wygenerowanych produktów. Najmniej spokrewnione były genotypy Tansgol i S4-13, a najbardziej S11-25 i S11-26. Analiza wyników amplifikacji oparta na teście Jaccarda i metodzie UPGMA wykazała obecność sześciu podgrup w obrębie analizowanych siewek. Stwierdzono także korelacje między ośmioma cechami morfologicznymi i wynikami uzyskanymi w reakcjach RAPD. Możliwość zastosowania opisanych metod do oceny genotypów podkładek elitarnych jest dyskutowana.

Słowa kluczowe: bioróżnorodność, *Prunus domestica*, markery RAPD, siewki