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Evaluation of Genetic Diversity of Morphological Characteristics Genotypes of *Rubus* species on the slopes of the Alborz

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Abstract: *Rubus* is growing in forests regenerate mainly in a vegetative way; however, detailed studies have shown substantial variability in performance of *Rubus* individuals. The aim of this study was to evaluate the genetic diversity and genetic grouping of *Rubus* in Alborz mountains. First year, the regions of *Rubus* identified, the next year, by referring to pre-determined locations, readings taken. Using the "ward" cluster analysis method based on morphological characteristics were 0.8; genotypes divided in three main groups. The first three components consisted of more than 90% of the total variation. The first component was 0.75, the second component 0.96 and the third component 0.97 variance. The genotype distribution based on factor analysis showed that *R. antalicus*, *R. sanactus* in the positive (+1) pc and genetic variants of *R. caesius*, *R. hyrcanus*, *R. procerus* were negative (+2) pc. The *R. procerus* were negative (-4) for the first factor at the zero-first distance, but in the second factor in the range of +3 to +4 and in the third factor at zero to +1 distance, which indicates that the genotype was weak factor. Based on the results, genotypes of de plot were in a group with more common characteristics, such as *R. caesius*, *R. hyrcanus* and *R. procerus* closed together. The most similarity observed among *R. hyrcanus*, *R. caesius* and *R. sanactus* species. The cluster analysis. *R. caesius* observed in different areas of the mountains and foothills of the Alborz slopes.

Keywords: Cluster analysis, Correlation, PCA, *Rubus*

INTRODUCTION

The genus *Rubus* is composed of a highly heterozygous series of comprises 900-1000 species and has long been deemed taxonomically challenging due to its complexity for apomixes, with a range of ploidy levels from diploid to duodecaploid (Meng and Finn, 2002). Hundreds of species are divided botanically under 15 subgenera, many of which have been used in breeding (Finn *et al.*, 2002a, b). Members of the genus can be difficult to classify into distinct species for a number of reasons, including hybridization between species and apomixes (Dickinson *et al.*, 2007; Evans *et al.*, 2007). *Rubus* is believed to have low genetic variability at the local scale. Despite numerous studies on taxonomy and biology of *Rubus* (Taylor, 2005). Cytological data are being generated and used in an attempt to gain insight into the relationships of the genus (Wang *et al.*, 2008). The subgenus *Rubus* is divided into 12 sections with most of the cultivated blackberries being derived from the

Allegheniensis, *Arguti*, *Flagellares*, *Rubus*, *Ursini*, and/or *Verotrivialis* (Finn, 2008). The fruit is composed of a large number of one-seeded drupelets set together on a small conical core (Jennings 1988). Commercial blackberries are classified into three categories based on cane type: trailing, semi-erect, and erect (Strik, 1992). Trailing types and semi-erect habit blackberries are crown forming and the primocanes trail on the ground surface until lifted and staked. Erect blackberries grow upright, but less vigorously than the semi-erect types, and instead of being crown forming, they sucker beneath the soil line. The genomic number of *Rubus* is seven and species representing all ploidies from diploid to duodecaploid are found in nature. The range in size is from 1- 4 μm (Jennings 1988). The diploid genome has been estimated to be 275 Mbp. Self-incompatibility systems occur in some *Rosaceous* species and it is common among many of the diploid *Rubus* species (Keep, 1968). In most countries, fruit from *Rubus* species is produced for the fresh market. Fruit for processing is usually used in the food and beverage industries where it is used to produce soft drinks, preserves and desserts. Fruits may also be frozen or canned (Clark *et al.*, 2007; Strik *et al.*, 2007). This research was carried out with the aims to identify, collections and morphological study of *Rubus* species in the foothills of the Alborz and Tehran province.

Materials and Methods

The genus *Rubus* contain a large number of ubiquitous environment tax, which some species of *Rubus* are important. In this research, six species of *Rubus* were studied in the Alborz slopes. Identification of samples was referring to the sources of flora Iranica, Flora of Turkey, Flora of Russia. In this regard, the research was carried out in areas of *Rubus* growth in the province of Alborz and Tehran province. In the first year, 10 samples of each species of *Rubus* were randomly selected, numbered and the collected with GPS was determined. In the second stage by measuring characteristics of flowers per cluster, on second half of June for two weeks was performed. For this purpose from different parts of each bush of twenty-four clusters were tagged, in the third stage, on the spring that *Rubus* bushes grown matured thoroughly, investigated the vegetative and reproductive characteristics. Finally, to evaluate the attributes associated with the fruit the genotype of 24 fruits was randomly selected in three replicates traits were recorded for them. The measurement of *Rubus* fruit and flower diameter, fruit length, petiole length, leaf width, leaflet length, width leaf, leaf length by Caliper. The measure the weight of the fruit by balance.

Data analysis

After collecting data, descriptive statistics were extracted and exploited. Regarding the data obtained from the measurements (quantitative data), analysis of variance with the hypothetical genotype of the regions as treatment and the regions and individuals within each region was repeated as nesting. The comparison of mean quantitative traits was done by LSD method. The principal component analysis was performed using correlation matrix and cluster analysis using Minimum Variance (Moghadam & Associates, 1994).

Result and Discussion

Comparison Mean

Compare mean characteristics indicates a significant difference examined between the measured genotype of *Rubus*. Comparison of mean of traits showed that there was a significant difference between measured traits of raspberry genotypes. The *R.hirtus* genotype had the largest flower diameter of 1.85mm, the leaflet length was 6.98mm, leaf let width was 4.68mm, Leaf width is 4.69mm, the longest petiole length is 1.77mm. The most number of drupetum in a *Rubus* fruit was 48.70 and fruit diameter was 11.77 mm. In *R.caessius* the smallest leaflet height is 6.23 mm, the

minimum leaf width was 3.70 mm, the minimum fruit length was 8.20 mm, the lowest fruit weight was 0.47 grams, the smallest diameter of the fruit was 9.95 mm and the lowest number of drupetum was 32.20. In *R.hyrceanus* genotype, the lowest leaf width was 10.45 mm. The *R.sanctus* genotype has the lowest diameter of 13.5 mm and the minimum leaf length was 38.5 mm. The color of the flowers of *R.caesius*, *R.sanctus* were white, and on leaves of the genotypes was observed with anthocyanine, leaflets in both genotypes were narrow, oval shaped, and both genotypes leaves have been serrated. Nevertheless, in *R.sanctus*, genotype had leaflets sometimes have petioles and the margin had double serrated. The mean leaflet length in the *R.caesius* genotype was 6.23 and the width was 3.70 mm. The mean leaflet length was 5.88 in *R.sanctus* genotype and 3.85 mm in width. There was also no special order at the time of ripening the fruit. Which was coincident with (Graham and Woodhead 2011). Pearson correlation coefficients between characters indicate a positive or negative correlation between some characteristics. There was a significant positive correlation between leaf length and flower diameter ($r=+0.76^*$), and negative correlated leaf width with flower diameter ($r = -0.91^{**}$), leaflet length with flower diameter ($r = -0.78^*$) and leaflet length with leaf width ($r = +0.89^*$). Between fruit weight with flower diameter was ($r = +0.73^*$). The Negative correlation was significant ($r= -0.85^*$) between number of fruit aggregates and leaflet width. A significant positive correlation was ($r=0.78^*$) between the number and length of fruits. Productivity in *Rubus* is mainly a function of the number of laterals produced per cane, and the number and weight of fruits per lateral. The patterns we observed generalize across species, irrespective of overall plant morphology or pollination system. These correlations between flower size, moisture availability, and suggest that water loss from flowers can influence leaf responses to the environment, which in turn may indirectly mediate an effect on flower size (Lambrecht, 2007). Flower size and subsequently water loss, increase, leaf level control of water loss is expected to be enhanced. Principal component analysis diversity, using the correlation matrix, the first three components were more than 80% and the first two components were more than half diversity (Table 2). *R.antalicus*, *R.sanctus* genotypes had positive values on both components. The *R.caesius*, *R.hyrceanus*, *R.procerus* were negative and the second component was placed in the fourth quarter. The *R.procerus* was placed in the fourth quarter only. The main components of the first and second two-dimensional indicating more similarity Genotypes in the first group with each other. Cluster analysis by UPGMA method divided into three main groups in the range of 0.7 (Fig. 2). Principal component analysis diversity, Using the correlation matrix method, the first three components were more than 80% and the total first two components justified more than half of the total variation (Table 2). Based on the coefficients of the main components for each genotype, two-dimensional was applied scatter plot (Figure 1). The *R.antalicus*, *R.sanctus* were positive on both components. The *R.procerus* genotypes were alone in the fourth quarter. The main components of the first and second two-dimensional charts indicating more similarity genotype in the first group with each other. Therefore, in order to determine genotype used to differentiate between the populations of the principal components analysis PCA. Table 1 shows the results of factor analysis. The factor analysis can describe the evaluated traits as three main factors. The first component was 0.75, the second component 0.96 and the third component accounted for 0.99 variances, and the special values were more than one, the first and second components justified more than 100% of the total variance. Genetic distribution of genotypes based on factor analysis showed that the *R.antalicus*, *R.sanctus* in the positive (+ 1) pc and the *R.caesius*, *R.hyrceanus*, *R.procerus* were negative (-2) pc. This indicates that the Geneva has been the Brigade of the other genotype and has the highest value for the first and second major factors. The *R.procerus* were negative (-4) for the first factor in the range of 0 to 1, but for the second factor in the range of +3 to +4 were positive and for the third factor was the distance from zero to +1, indicates that The genotype characteristics of the component is weak (table 2). Based on the results of cluster

analysis, genotypes in the same group (such as *R.caesius*, *R.hyrceanus*, *R.procerus*) have the least variance in terms of traits, thus have the most similarity, and close to put together. *R.caesius* and *R.sanctus* grouped together. Based on the analysis of major components grouped by two main components genotypes divided mainly into three groups. Cluster analysis with "Ward" method carried out to determine the genetic diversity pattern, classify the genotypes and specify the genetic distance between them. Genotypes were categorized in 9 different clusters with dendrogram cutting at a Euclidian distance of 0.7. The groups formed based on this method were as follows: Group 1: In this group, our genotype *R.hyrceanus*, *R.caesius* and *R.sanctus* had similar characteristics among the measured traits and grouped into one group. Group II: *R.caesius* and *R.sanctus* genotypes were similar in nature to each other. The Genotypes 2 and 3 of *R.sanctus* and *R.hyrceanus*, genotypes 4 and 3 of *R.hyrceanus* and *R.procerus*, and genotypes 2 and 5 of the *R.caesius* and *R.sanctus* populations also have the least distance and they were most similar to each other. According to the results, specimens with genetic distances can used as parental crosses in breeding programs. *Rubus* are free of any harmful effects on the environmental and have high economic potential and their cultivation does not require chemical use to control pests and diseases. Morphological differences in the shape and size of thorns, fruit color and fruit size can considered new production devices developed in breeding programs by reaching the demands of new cultivars. There has been a growing demand from growers processors and consumers for important in fruit quality attributes, to the point where these traits are now quality important for cultivars and indeed even affect decisions regarding commercial release (Graham and Jennings, 2009). Geographical separation, natural *Rubus*, pollen transfer by insects, hybridization and polyploidy are important factors to make and maintain genetic variability in *Rubus* species. In dendrogram figure 2, species that have the closest relationship and were in the same species and the most similarity in the results of cluster analysis. Thorny samples also have a high quality and less damage caused by the attack of pests and diseases, its probable caused the positive effect of epidermal thorns on increasing plant photosynthesis and increasing the amount of primary and secondary metabolites of the plant. Therefore, there can be a positive correlation between the amount of light access and the number of thorns. One of the most valuable roles we can play as a *Rubus* community is to develop cultivars that taste better, so that they are more desirable to eat, and growers can grow that economically so that, in turn, the crop is available at an affordable price for the consuming public.

Conclusion

1. This restricted genetic diversity is a serious concern for the future of *Rubus* breeding, especially when seeking durable host resistance to intractable pests and diseases for which the repeated use of pesticides in some regions is ineffective unsustainable or unacceptable for certain selected markets, such as 'organic production'.
2. Plant habit is important for plantation management and has a major effect on yield Potential. In summer fruiting types, the most important characteristics include the number and height of young canes, consistency of bud break, internode length, lateral length and position of laterals. In primocane fruiting types (where fruit is produced on first year canes) the amount of branching and extent of lateral development on the primocanes are major yield components. In both types, erect, spineless canes are desirable.
3. The incorporation of novel resistance/tolerance to pests and diseases is regarded as essential for the development of cultivars suitable for culture under integrated pest management (IPM) systems. Sources of resistance in diverse *Rubus* sp. to many pests and diseases have been identified and exploited in conventional crossbreeding.

4. germplasm bearing single resistance genes, when planted over extensive areas, can eventually overcome by the rapid evolution of new biotypes of pests, so that new types of host resistance are required to sustain plant protection.
5. Geographical separation, natural *Rubus*, pollen transfer by insects, hybridization and polyploidy are important factors to make and maintain genetic variability in *Rubus* species. –
6. Morphological differences in the shape and size of thorns, fruit color and fruit size can considered new production devices developed in breeding programs by reaching the demands of new cultivars.
7. The local environment seems quite constant, the length of *Rubus* canes changes from year to year; certainly, there is a general pattern of rapid increase in cane length after gap creation, and then a gradual decline in mean plant size.

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Table 1. Number, minimum, maximum, average and coefficient of variation in characteristics of *Rubus* genotypes evaluated slopes of the Alborz

Fruit diameter mm	Number of fruitlet	Fruit weight g	Fruit length mm	Petiole length mm	Leaflet width mm	Leaflet length mm	Width leaf mm	Leaf length mm	Flower diameter mm	genotypes	Row
10.32	40.65	0.72	9.84	1.45	4.21	6.45	10.81	11.09	1.66	<i>Rubus antalicus</i>	1
9.95	32.20	0.47	8.20	1.69	3.70	6.23	10.70	14.55	1.87	<i>R.caesius</i>	2
11.57	45.57	0.48	10.90	1.41	4.46	6.39	11.08	11.87	1.61	<i>R.hyrcanus</i>	3
11.76	42.86	0.54	11.04	1.74	4.55	6.80	11.69	11.82	1.60	<i>R.procerus</i>	4
10.40	40.21	0.71	9.86	1.46	3.85	5.88	10.45	11.45	1.35	<i>R.sanactus</i>	5
0.82	4.51	0.10	1.01	0.16	0.39	0.39	0.43	1.23	0.19	Coefficient variance %	6
65.79	23.31	3.52	59.80	9.54	25.48	38.74	65.97	73.19	9.97	Sum	7
9.95	32.20	0.47	8.20	1.41	3.70	5.88	10.45	11.09	1.35	Minimum	8
11.77	45.57	0.72	11.04	1.77	4.69	6.98	11.69	14.55	1.87	Maximum	9

Table 2: eigenvalues, variance percentage and the variance cumulative first principal component characteristics

The main component	Eigen values	Cumulative percentage
1	20.71	0.75
2	3.69	0.96
3	1.41	0.97
4	0.21	0.99
5	0.34	1

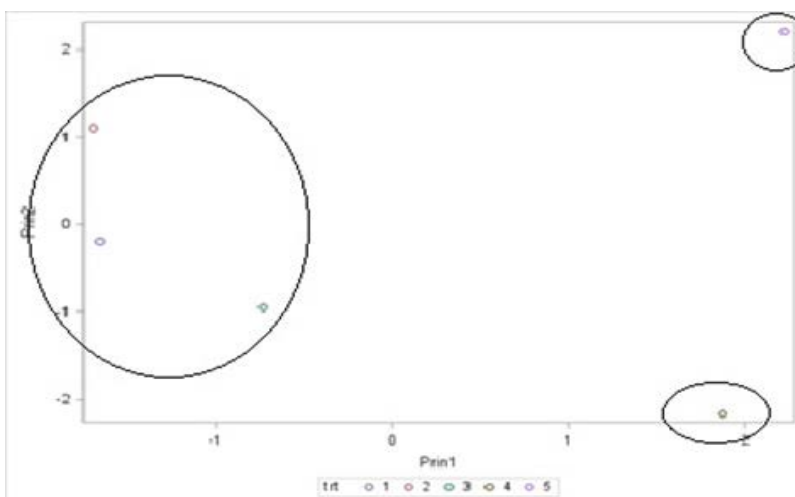


Figure 1: genotype group based on their value in the first and second principal components (1) *R. antalicus*, (2) *R. caesius*, (3) *R. hyrcanus*, (4) *R. procerus*, (5) *R. sanactus*.

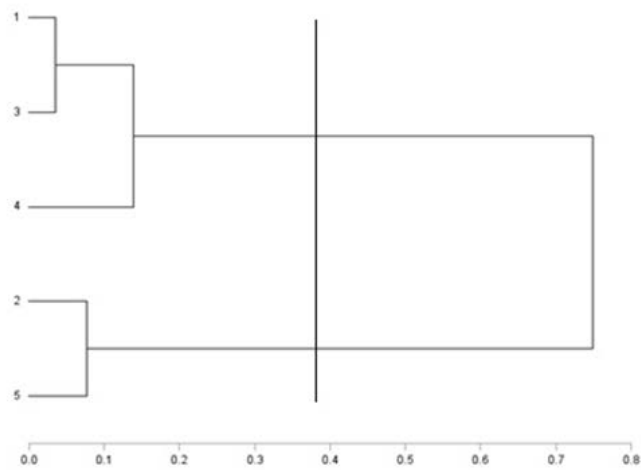


Figure 2: geographic origin of the dendrogram based on genotype collected from Tehran and Alborz, (1) *R. antalicus*, (2) *R. caesius*, (3) *R. hyrcanus*, (4) *R. procerus*, (5) *R. sanactus* species