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Morphological variability in some persian clover (*Trifolium resupinatum* L.) populations from Ordu province (Turkey)

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Abstract. The characterization of native populations is of extreme importance for the identification of elite genotypes to be considered for breeding programs. For that reason, the objective of this study was to determine the variability of some morphological characteristics of 15 local Persian clover (*Trifolium resupinatum* L.) populations from the 5 coastal counties of Ordu (Turkey). These populations were classified according to plant height, crown diameter, stem diameter, number of stems/plant, leaflet length, leaflet width and dry weight. For this purpose, data were analyzed by Principal Component Analysis (PCA) and Hierarchical Cluster analysis (HCA). According to HCA, the local populations of Persian clover were grouped in 4 clusters and resulted similar in rates ranging from 54.11% to 90.46%.

Keywords. *Trifolium resupinatum* L. – Principal component analysis – Cluster analysis – Similarity.

Variabilité morphologique de certaines populations de trèfle de Perse (*Trifolium resupinatum *L.), recueillies dans la province d'Ordu (Turquie)

Résumé. *La caractérisation des populations indigènes de plantes est extrêmement importante pour l'identification des génotypes d'élite à inclure dans les programmes de sélection. Pour cette raison, l'objectif de cette étude était de déterminer la variabilité des caractéristiques morphologiques de 15 populations de trèfle de Perse recueillies dans la province d'Ordu (Turquie). Ces populations ont été classées en fonction de la hauteur des plantes, diamètre de la couronne, diamètre de la tige, nombre de tiges/plante, longueur de la foliole, largeur de la foliole et poids sec. À cet effet, les données ont été analysées par analyse en composantes principales (ACP) et analyse de classification hiérarchique (ACH). Selon l'ACP, les populations locales de trèfle de Perse ont été regroupées en quatre clusters et étaient semblables selon des taux allant de 54,11% à 90,46%.*

Mots-clés. *Trifolium resupinatum* L. *– Analyse en composantes principales – Analyse de classification – Similitude.*

I – Introduction

Persian clover is tolerant to waterlogging (Anonymous, 2012), adapted to loam to clay loam soils with ph 6-8, but its cold tolerant is fair (Philipp *et al.,* 2016). It has high regrowth capability after grazing and cutting so it has high feeding value as a pasture or hay (Celen, 2009). Coastal regions of Turkey has Mediterranean climate, and has some problems such as drainage, drought, salinity etc. An annual Persian clover is useable for hay and pasture in this area. However, the cultivation of persian clover is not common in our country. There is only one native registered cultivar in Turkey. New cultivars are required for the increasing of its cultivation and pasture improving, starting from collection of native populations, as essential gene pool for plant breeding.

The assessment of genetic diversity among populations is potentially an important tool for plant breeding purpose. This diversity gives plant breeders the opportunity to select more diverse germplasm to include within their breeding programme (Aitken and McNeil, 2010).

Phenotypic observations in the real farm conditions have importance for the plant breeding programs. PCA and HCA is a useful guide to evaluation of different populations. Aim of the study was to evaluate by relationship among important characteristic of phenotypic observations of some local Persian Clover populations.

II – Materials and methods

Fifteen local population of Persian clover were collected from the 5 coastal counties (Ünye, Fatsa, Perşembe, Altınordu, Gülyalı) of Ordu in Turkey. The counties where local populations were collected are demonstrated on map of Ordu province (P1-P15) (Fig. 1). There are 77 km between Ünye and Gülyalı. While soil pH changes between 5.8 and 7.4, soil texture is clay, clay-loam and sandy. Ten plants from each of the populations were picked up randomly at flowering period in 2011.

Morphology of plants was assessed directly on the picked up plants by measuring the following traits: plant height (PH, cm), number of stems/plant (SN), stem diameter (SD, mm), leaflet width (LW, mm), leaflet length (LL, mm), crown diameter (CD, mm), dry weight/plant (DW, gr). In order to find the main variation trends among important morphological characters (variables) in the local Persian clover populations, the data were analysed by Pearson's Correlation analysis, Principal Component Analysis (PCA) and Hierarchical Component Analysis (HCA).

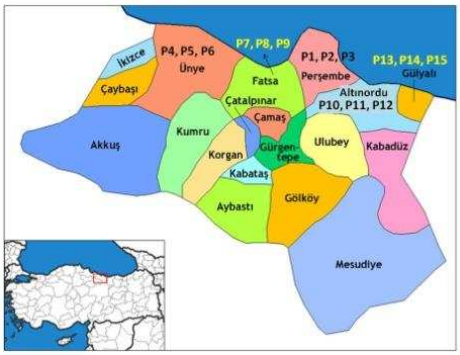

Fig.1. Map of Ordu Province (Turkey) *Numbers on map are demonstrated population number.

First, Pearson's correlation coefficient was computed and then PCA (Promax rotation) was applied to the morphological characters. The suitability of PCA was determined by the Bartlett's test of sphericity and the KMO index to measure sampling adequacy. PCA, a data reduction technique, to construct new, uncorrelated variables or principal components (PCs). The PCs with eigenvalues greater than 1.0 were considered in the analysis. Following PCA, variables were grouped using HCA according to the factor scores derived from PCA. Ward's method used because it joins at each stage the cluster pair whose merger minimizes the increase in the total within-group error sum of squares, based on the Euclidean distance between centroids. All data analyses were performed by using IBM SPSS 23 and Minitab 17 statistical software.

III – Results and discussion

The overall mean values for all morphological characters (variables) measured and their standard deviations were presented in Table 1. While plant height ranged from 15.85 to 41.80 cm, dry weight changed between 0.15 and 9.22 g. PCA requires that it should be some correlations greater than 0.30 between the variables included in the analysis. For this set of variables, all correlations in the matrix were higher than 0.30, satisfying this requirement (Table

2). Furthermore, the KMO measure was 0.832 and Bartlett's test of sphericity was significant (χ^2 =793.804, p<0.001).

Table 1. Means and standard deviations of variables obtained from populations (n=10). P: Populations

P	Plant height	Stem number/plant	Stem diameter	Leaflet width	Leaflet length	Crown diameter	Dry weight/plant
1	29.68± 6.62	8.00±2.26	2.82±0.90	10.48±1.44	15.46±2.01	4.89±1.68	2.19±1.50
2	30.25±9.46	5.40±2.76	1.56±0.37	9.63±1.59	13.35±2.53	3.26±0.91	0.70±0.55
3	17.07±3.55	2.40±1.51	1.22±0.28	6.85±1.13	10.29±1.77	2.27±0.72	0.15±0.12
4	40.78±9.83	6.00±1.83	3.17±0.83	13.04±1.69	20.12±2.54	4.78±1.11	3.86±2.33
5	23.96±10.22	10.00±4.37	3.35±1.39	9.64±2.95	13.48±3.63	5.29±2.72	2.54±2.77
6	34.45±10.83	8.60±5.44	2.88±1.14	12.17±2.71	16.60±4.30	5.77±3.77	4.18±5.39
7	35.12±10.52	10.20±5.35	3.81±1.47	12.08±3.26	17.09±4.03	8.18±3.08	9.22±8.19
8	31.93±13.76	3.90±2.33	2.44±0.70	10.09±1.81	16.33±2.58	3.93±1.84	1.74±2.19
9	15.85±5.25	3.30±1.57	1.55±0.69	6.35±1.414	11.23±2.16	2.60±1.38	0.49±0.53
10	38.72±16.63	8.40±2.50	2.74±1.37	8.28±1.43	12.66±1.84	6.88±2.23	6.13±4.78
11	37.82±9.54	5.90±2.23	2.26±0.94	9.71±1.64	14.46±2.12	5.24±1.76	2.61±1.73
12	31.96±16.95	6.40±2.37	2.60±0.90	10.48±2.08	15.01±3.28	5.00±2.10	2.18±2.32
13	41.80±6.17	5.70±2.00	2.06±0.73	9.99±1.14	14.08±2.34	5.19±0.90	2.78±2.58
14	37.22±11.38	8.00±2.16	2.07±0.52	10.28±2.34	15.08±3.86	6.52±1.50	3.67±4.08
15	37.05±10.41	9.90±4.36	2.17±0.57	10.06±2.17	13.79±2.50	6.54±2.63	4.88±3.15

As the scree plot indicate there are two factors in the model of the PCA. Eigenanalysis of the correlation matrix shows that the first two principal components (PCs) eigenvalues more than 1.0 (PC1, 4.49 and PC2, 1.052). Eigenvalue of the third component (PC3) is 0.540. Although the factor loading of the PC3 influenced the commonalities of each element, it was not extracted because its eigenvalue is less than 1.0. The major characters described by the PC1 and PC2 are presented in Table 3. PC1 was mainly loaded by morphological characters. Clearly the first factor of the initial solution was much more important than the other. PC3-PC7 was discarded because of very low variance contributions.

According to Table 3, PC1 included the variables PH, SN, SD, CD and DW; PC2 includes the variables LW and LL (>±0.3). The components explained 79.21% of the total variance in the variables which was included on the components. Therefore, these variables have emerged as important for the selection study.

HCA was utilized to investigate the similarities and dissimilarities among the local populations with respect to morphological characters. The similarity levels (%) showing the relationships between the fifteen populations through the HCA was shown in the cluster dendrogram (Fig. 2). The dendrogram clearly showed that the populations grouped into two major clusters based on similarity indices. There is one minor cluster (Cluster IV) in the first major cluster. Another major cluster again divided into three sub-minor clusters, while the first sub-minor cluster having six populations (Cluster I), four populations include to the second sub-minor cluster (Cluster II) and the third sub-minor cluster is represented by three populations (Cluster III). Maximum number of accession (6 populations) was clustered in to cluster I, whereas minimum number of accession (2 populations) was in cluster IV. The clusters were similar in rates ranging from 54.11 (Cluster III) to 90.46% (Cluster IV). Among the 15 populations, 3 and 9 in the first major cluster (Cluster IV) showed highest similarity indices. Those populations were collected, because of the shortest, thin and small leafed plants in the all populations. Even though they belonged to different districts of Ordu province, their collecting places close to each other. In addition to these, memberships of the clusters and their average distance from centroid obtained from HCA were determined and presented in Table 4. While the highest average distance from centroid of

intra-cluster was found 1.748 in Cluster III, the lowest average distance from centroid of intra-cluster was found 0.408 in Cluster IV.

Table 2. Pearson's correlation coefficients amongst variables.

	PH	SN	SD	LW	LL	CD
SN	0.50**					
SD	0.50**	0.60**				
LW	0.44**	0.43**	0.51**			
LL	0.51**	0.40**	0.53**	0.88**		
CD	0.65**	0.79**	0.69**	0.49**	0.50**	
DW	0.65**	0.68**	0.69**	0.43**	0.50**	0.81**

** Correlation is significant at the 0.01 level.
PH: Plant height (cm), SN: Stem number/plant, SD: Stem diameter (mm), LW: Leaflet width (mm), LL: Leaflet Length (mm), CD: Crown diameter (mm), DW: Dry weight/plant (gr)

Table 3. Eigenanalysis of the rotated correlation matrix of the first two PC.

Characters	PC1	PC2
PH	**0.660**	0.160
SN	**0.907**	-0.097
SD	**0.713**	0.168
LW	-0.018	**0.973**
LL	0.021	**0.956**
CD	**0.947**	-0.022
DW	**0.930**	-0.040
Eigen Value	4.492	1.052
% Total community	64.171	15.035
Cumulative variance (%)	64.171	79.207

Table 4. Membership populations of the clusters and their average distance from centroid according to HCA

Clusters	Membership Populations	Avg. distance from centroid
I	1, 2, 8, 11, 12, 13	1.118
II	5, 10, 14, 15	1.349
III	4, 6, 7	1.748
IV	3, 9	0.408

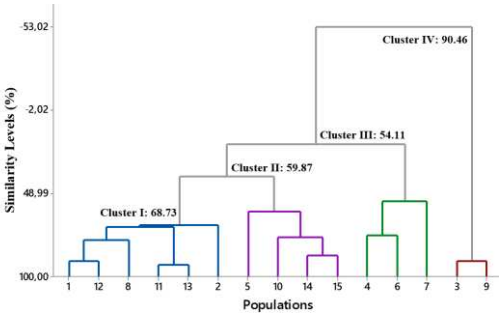

Fig. 2. Similarity levels of populations according to dendogram of HCA.

IV – Conclusion

In this study, a high variability of morphology amongst the some local Persian clover populations selected from Ordu was found. The variables plant height, stem number/plant, stem diameter, crown diameter, dry weight, leaflet width and leaflet length were found as principle components for determine to genetic similarity. The local populations 3 and 9 completely differed from the others with respect to these variables. Those populations were collected, because of the shortest, thin and small leafed plants in the all populations, even though they belonged to different districts of Ordu province. While the highest similarity was found among those populations, the lowest similarity was found among the local populations 4, 6 and 7. These populations have big leaflet. This study can assist geneticists and breeders to identify populations with desirable characteristics for inclusion in Persian clover breeding programs.

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