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Investigation of Agro-morphological Characters between 34 Accessions of *Trifolium resupinatum* L

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Agro-morphological characters in 34 populations of Iranian *Trifolium resupinatum* L. germplasm, were investigated. 24 traits for each accession were evaluated according to white clover and forage legume descriptors. Data were analyzed by SPSS and Excel softwares. Statistical tendency parameters for agro-morphological characters were calculated. A cluster analysis based on morphological traits separate populations in two different groups that in one cluster there was an only accession belongs to Kurdestan locality. Factor analysis have shown that 61/24% of collection variations were expressed by six factors. Leaflet shape, calyx trichome, the length of leaflet and inflorescence diameter were the most important morphological characters and the weight of 1000 seeds, number of days until to flowering were significant agricultural traits. . Based on these results, it seems that variation in germplasms is independent to geographical distribution. This research showed that there is a high biodiversity for this species in Iran due to the diverse environmental conditions, so that be useful for breeding programs and marketing.

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Keywords: Persian clover; cline; breeding; Iran.

1. INTRODUCTION

Trifolium L. is one of the most important fodder genus of the *Fabaceae* family in temperate and humid regions, which has great value as fodder and pasture and plays a significant role in feeding livestock. The number of clover species in the world is about 300 species, of which 25 species are agriculturally important, of which only 9 species, including underground clover, are cultivated commercially [1,2]. The largest distribution of clovers is in the Mediterranean region, so that 150-160 species are reported in Eurasia, 60-65 species in America, and 25-30 species in Africa. According to the research of the plant gene bank and genetic reserves of the research institute in Iran, the number of clover species known so far in Iran is about 54 species, of which *Trifolium resupinatum* L. (Persian clover) is cultivated in many areas [3]. This plant is a Mediterranean element and is traditionally cultivated in the natural pastures of Iran, Turkey and Afghanistan [4]. Genetic variation of this species was studied in South-West of Turkey and it was shown that the diversity of qualitative traits in this species is high [5]. Abbasi in 2008, investigated the Persian clover gene pools in National Plant Gene Bank of Iran [3]. He showed that days of flowering and growth habit area are important traits in genetic diversity of this species. Genetic variation between ten *Trifolium* species analyzed by ISSR marker. Results showed that the classification of sections proposed by internal transcribed spacer sequence data opposed to morphological characterization [6]. Also, use of agricultural and morphological traits in grouping, determination of genetic affinity and also determination of identifying the genetic treasury using molecular markers, statistical methods and multivariate analysis in the research of other researchers on Clover and other crops have been used [7-10].

On the other hand, the MBA in Agribusiness Management program specially in forage legume marketing, has an important role. Considering that the *T. resupinatum* species has a high fodder value, so that studying the genetic diversity of Iranian populations of this species can be important in breeding programs and managing the sale and development of germplasms of this species.

Due to the very diverse environmental conditions in Iran, high biodiversity is expected for different ecotypes of this species. The aim of this study is genetic variation in agro-morphological traits of *T. resupinatum* to identify the best genotypes for plant breeding and marketing in our country.

2. MATHERIALS AND METHODS

2.1 Plant Material

34 accessions of *T. resupinatum* were collected from the natural habitats in the two stages containing flowering and seed formation (Table 1).

Table 1. Populations and their localities

Population	Locality
T. res11	Tehran: Karaj, 1520 m
T. res109	Arak:Mahalat, 1740 m
T. res106	Tehran: Karaj, 1500 m
T. res105	Mazandaran: Savadkoh, 480 m
T. res114	Gilan: Asalem to khalkal, 1500 m
T. res118	Mazandaran: Savadkoh, 1150 m
T. res68	Mazandaran: Amol, 2050 m
T. res70	Arak: Komain, 2130 m
T. res97	Golestan: Toskastan, 400 m
T. res98	Mazandaran: Shirgah, 340 m:
T. res100	Esphahan: Lenjan, 1605 m
T. res128	Hamadan: Malayer, 1880 m
T. res129	Gilan: Gonbadkavis, 70m
T. res133	Mazandaran: Amol, 2040 m
T. res135	Hamedan: Nahavand, 1620 m
T. res141	Tehran: Damavand, 1940 m
T. res153	Kermanshah: Gasreshirin, 350 m
T. res159	Hamedan: Nahavand, 1497 m
T. res163	Arak: Ashtiyan, 2110 m
T. res190	Lorestan: Koramabad, 1730 m
T. res191	Lorestan: Borojerd, 1470 m
T. res195	Gilan: Asalem to khalkal, 1510 m
T. res224	Kermanshah: kerned gharb, 350 m
T. res231	Goleatan: Gonbadkavis, 80 m
T. res262	Gilan: Asalem to Khalkal, 1500 m
T. res245	Gilan: : Asalem to khalkal, 1400 m
T.res231	Arak: Sarband, 1858 m
T.res233	Mazandaran: Ghaemshahr, 350 m
T.res234	Golestan: Gonbadkavis, 60m
T.res217	Arak: Tafresh, 1730 m
T.res210	Esphahan: Samirom, 2100 m
T.res211	Arak: Sarband, 2075 m
T.res257	Kurdestan: sanandaj, 1780 m
T.226	Arak: Arak, 2020 m

2.2 Agro-morphological Characters

In morphological study, 24 agro-morphological traits for each accession were evaluated

according to white clover and forage legume descriptors (Table 2).

2.3 Morphological Data Analysis

Multivariate statistical analysis was done based on quantitative and qualitative characters. The statistical parameters for each accession included mean, standard deviation for each attribute was done (Table 3). Factor analysis was done in order to identify the most important sources of variation. Cluster analysis based on Average linkage was done.

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Table 2. Agro-morphological traits in *T. resupinatum* accession

Character	Code
Leaflet shape	1-elliptic, 2- oblanceolate, 3- obovate, 4- rounded, 5-Rombic
Leaflet length	mm
Leaflet width	mm
Petiole length	mm
Petiole width	mm
Height	mm
Stem length	mm
Number of days to flowering	mm
Inflorescence number	mm
petal color	1-white; 2- pink; 3- red
Seed color	1-yellow; 2- brownish, 3 green; 4- redish
Leaf polymorphism	1- +; 2- -
Stem diameter	mm
calyx trichome	mm
Leaflet base shape	1- Runded; 2- wedge
Leaflet tip shape	
Inflorescence diameter in fruit stage	mm
petal length	mm
Seed weight	gr
Leaf/stem	number
Humidity percentage	number
Inflorescence diameter in flowering stage	mm
Inflorescence shape	1-rounded; 2- elliptic
Calyx length	mm

Table 3. Mean, SD, Min, Max and Range of attribute changes of characters

Character	Mean	SD	Min	Max	Range
Leaflet shape	1.84	0.39	1	7	6
Leaflet length	475	1.8	1	9	8
Leaflet width	5.56	1.66	1	9	8
Petiole length	4.07	1.28	1	9	8
Petiole width	5.13	1.6	3	9	6
Height	4.84	3.73	1	9	8
Stem length	3.85	1.42	1	7	6
Number of days to flowering	95.51	13.66	61	111	50
Inflorescence number	3.45	1.23	1	7	6
Petal color	0	0	0	0	0
Petal length	2.39	1	1	7	8
Seed color	1.56	0.21	1	3	6
Leaf polymorphism	3.24	1.66	1	9	2
Stem diameter	2.99	1.68	3	7	8
calyx trichom	3.21	1.09	1	3	4
Leaflet base shape	3.24	1.8	1	7	2
Leaflet tip shape	3.37	1.79	1	9	6
Inflorescence diameter in fruit stage	3.28	1.55	1	9	8

Character	Mean	SD	Min	Max	Range
Seed weight	1.39	0.26	0.8	3.03	2.23
Leaf/stem	1.42	0.2	0.13	2.8	2.67
Humidity percentage	0.61	0.18	0.78	1.82	1.04
Inflorescence diameter in flowering stage	2.48	1.8	1	9	8
Inflorescence shape	2.39	0.79	1	3	2
Calyx length	2.63	1.38	3	7	4

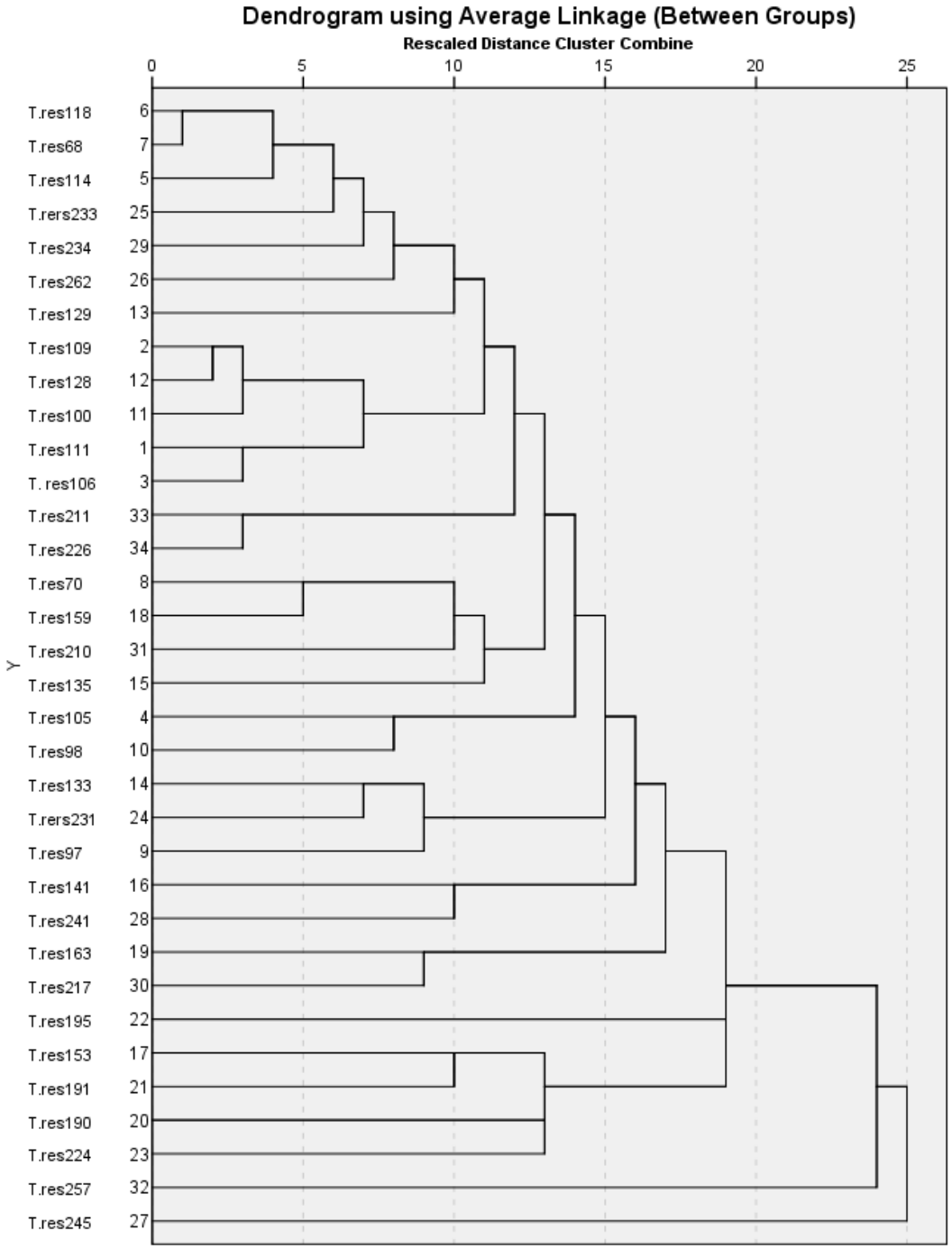

Fig. 1. Cluster analysis of 34 accessions of *T. resupinatum*

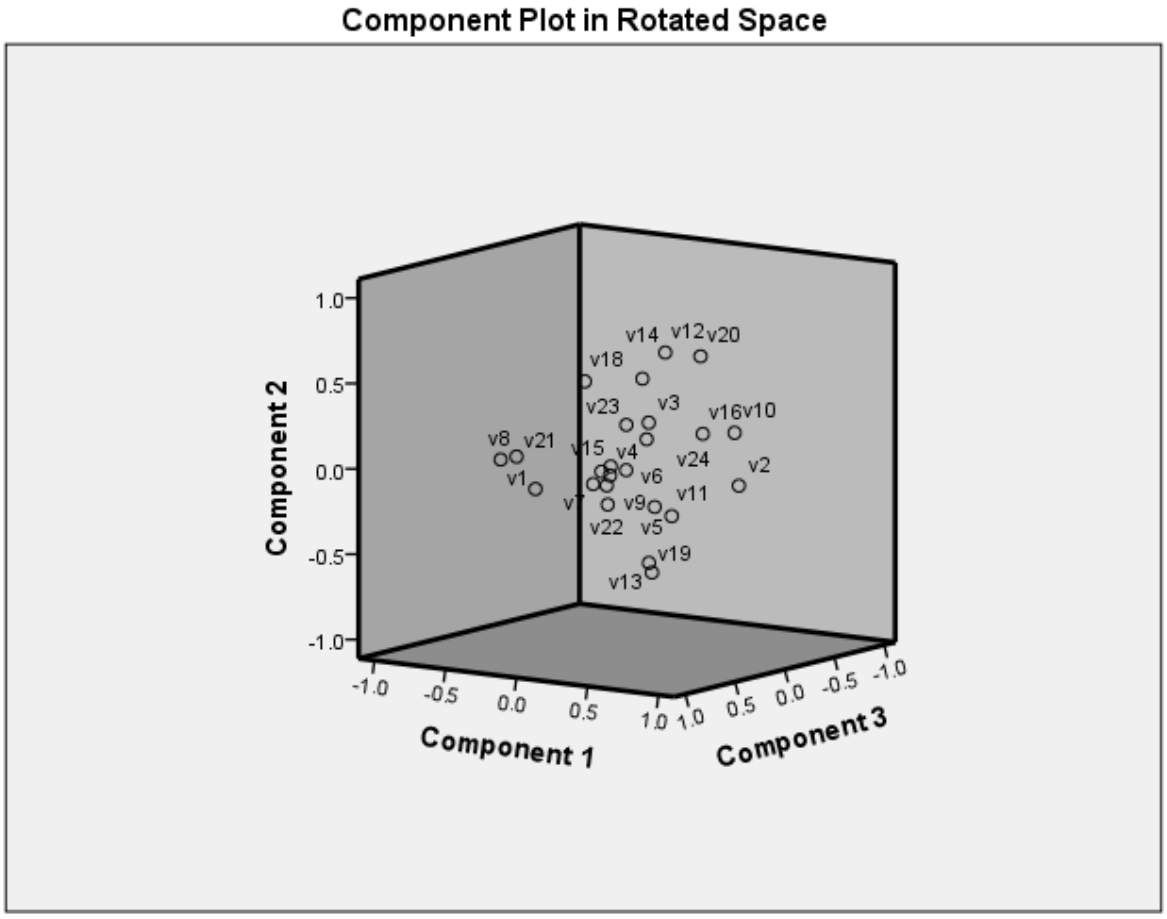

Fig. 2. PCA analysis of 34 accessions of *T. resupinatum*

3. RESULTS AND DISCUSSION

In this research, we examined agro-morphological characters to find the main characters that important for variation.

The results showed that the investigated populations had differences in terms of the studied traits. The day's number until flowering and height of each accession have the highest standard deviation and the petal color has the lowest standard deviation. Height with leaf / stem ratio, seed color with 1000 seed weight and 1000 seed weight with leaf / stem ratio had a positive correlation (>0.05). In all accessions, the height was from 0 meters to more than 2750 meters. Most of the samples have been present in areas where the average rainfall is from 100 to 1000 millimeters per year. The average annual temperature of the samples sites was mainly between 5^{o}-20^{o} c. However, some accessions were located in areas with a temperature of more than 20^{o} c. Based on the soil texture, most of the samples were located in areas where the soil was sand and loam or sandy loam or relatively heavy soils. These accessions were collected from three vegetation regions of Iran. The important point is that most of the samples are in separate sub-groups but in the same cluster according to the climatic conditions. This showed that the climatic conditions were important in the selection of traits, but the geographical region was not very important in the separation of samples.

Factor analysis have shown that 61/24% of collection variations were expressed by six factors. Leaflet shape, calyx trichome, date of flowering, weight of 1000 seeds, the length of leaflet and inflorescence diameter were the most important characters in morphological variation. Cluster analysis based on agro-morphological characters separated the accessions in two clusters. *T. res*245 (Kurdestan accession) separated from the others (Fig. 1). In second cluster, accessions were placed in different

groups based on geographical regions. The northern, central and northwestern accessions are placed in separate sub-clusters, but in the same group. PCA analysis is confirmed these results (Fig. 2). Based on these results, it seems that various ecological conditions are effective on the diversity of traits. However, in the other groups, accessions with different geographical areas can be observed. Benneth (2000) studied the genetic variation of five species of *Trifolium* L. from south-west Turkey and showed that morphology of the outbreeding species was in relation to climate [5]. Similar results have been obtained in other studies [11,12]. It seems that, the diversity of populations has not relation with the geographical pattern. The independence of genetic diversity from geographic diversity can be seen in other reports of this type of research [13,14]. As shown in the figure, the majority of the accessions are located at relatively the same genetic distance, and only a few of them, which are mainly related to the Northwestern parts, are at a relatively far distance.

4. CONCLUSION

In general, it can be said that there are very good materials for breeding in most of the quantitative and qualitative characteristics of this species, which can be used by breeders to produce various cultivars of Persian clover.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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