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Molecular Characterization and Determination of Morphological Traits of the Button Medic (*Medicago orbicularis* L.)

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ABSTRACT
The aim of this study is to analyze the morphological traits of button medic genotypes and determine their molecular characterization using ISSR methods. Vegetative traits were assessed at 50% anthesis period, however, generative trait was evaluated at physiological period. Molecular diversity was determined by using 14 ISSR primers. To assess the genetic diversity and the genetic structure of wild button medic (*Medicago orbicularis* L.) collected from seventeen ecologically and geographically different locations of Southeastern Anatolian Region were analyzed by using ISSR at the Biotechnology Laboratory of Çukurova University. According to Jaccard's similarity index, similarity between genotypes ranged from 0.63-1, with an average 0.70. According to dendrogram obtained from the similarity index data, the 17 genotypes clustered into two distinct groups, A and B. Group A was divided in to two (A1 and A2) sub-groups. Kulp, Diyarbakır, Eğil-1, Eğil-2, İdil and Ovabağ fell in to the sub-group of A1. In A2 group, one group was Silvan, Hilvan, Adıyaman, Derik, Malabadi and Erdurağı. Gaziantep, Çermik, Ergani and Palanlı samples categorized in to sub-group A2. The results obtained from this study will be helpful for button medic breeders in Turkey to gain information about genetic diversity and will enable them to make a future strategy for broadening the genetic basis of these crops.

Key words: Button medic, ISSR, *Medicago orbicularis* L.

INTRODUCTION

Button medic is a cool-season annual legume that originated in the Mediterranean region and spreads naturally throughout most of Turkey. Annual *Medicago* spp. has not been recommended as a pasture and forage varieties for Turkey and hence they have not been cultivated.

DNA markers have been used to determine molecular characterization of varieties in recent years in addition to the morphological and biochemical traits. Recent advances in molecular biology have revealed that DNA molecular marker systems can be used to study genetic diversity and to understand better the genetic background of new medic selections before their introduction into the production system and cultivation (Paplauskiene and Dabkeviciene, 2008). The PCR-based techniques have been extensively used in genetic analysis and to identification of molecular markers in plants. Restriction fragment length polymorphisms, RFLPs, are the first used DNA markers in order to reveal genetic relationship in plants (Tanksley *et al*., 1989). However, the high cost and slow disadvantage of RFLPs caused development of PCR-based molecular markers which were RAPD, AFLP, SSR and ISSR. ISSR analysis has successfully been applied in gene mapping (Ammiraju *et al*., 2001; Sica *et al*., 2005; Shi *et al*., 2010), fingerprinting or genetic diversity analysis (Archak *et al*., 2003; Bornet and Branchard, 2004). The ISSR DNA markers have been extensively used in the characterization of crop plants in recent years (Gupta *et al*., 1994; Zietkiewicz *et al*., 1994; Gillings and Holley, 1997; Gilbert *et al*., 1999; Ajibade *et al*., 2000; Arcade *et al*., 2000; Gyulai *et al*., 2000; Liu and Wendel, 2001; Dangi *et al*., 2004; Talhinhas *et al*., 2006).

This study aimed to determine the genetic similarity between 17 genotypes collected from the wild area in the southeastern regions of Turkey. Furthermore, morphological and molecular traits of the genotypes of button medic were determined.

MATERIALS AND METHODS

Material: In the current study, 17 genotypes of button medic collected from the wild were evaluated (Table 1).

Method

Morphologic studies: Experiments were carried with pot grown plants. Experimental design was complete randomized block with ten replications. Morphological traits were determined by means of ten plants.

Molecular data analyses: DNA extracted from fresh leaves taken by 10 g from the 4-5 leave-stage plants according to the method described by Doyle and Doyle (1987).

The images DNA quality and concentration used in PCR analysis were represented in Fig. 1a and b, respectively.

Table 1: Characteristics of locations where button medics are collected

Location	Elevation (m)	Latitude (°N)	Longitude (°E)
Zeyrek/Kulp/Diyarbakır	854	38°28’11.0”	40°51’39.1”
Silvan/Diyarbakır	620	38°8’31.4”	41°00’29.2”
Gaziantep	891	37°7’39.8”	37°23’26.9”
İdil/Mardin	773	37°34’00.2”	41°90’00.0”
Derik/Mardin	780	37°22’00.1”	40°16’00.0”
Petekkaya/Çermik/Diyabakır	710	38°13’99.0”	39°45’99.0”
Agricultural faculty/Diyarbakır	650	37°54’52.4”	40°16’21.2”
Malabadi/Silvan/Diyarbakır	613	38°09’13.5”	41°12’12.6”
Alatosun (Karacadağ)/Diyarbakır	1252	37°37’00.5”	40°04’00”
Erdurağı/Kurtalan/Siirt	630	37°90’25.5”	41°57’61”
Eğil-1/Diyarbakır	900	38°15’24.4”	40°5’00.2”
Ovabağ/Diyarbakır	670	37°43’00.8”	39°59’00.3”
Hilvan/Şanlıurfa	600	37°35’21.2”	38°57’13.2”
Eğil-2/Diyarbakır	900	38°15’24.4”	40°5’00.2”
Esence/Adıyaman	850	37°75’24.4”	38°25’22.4”
Mildağı/Ergani/Diyarbakır	957	38°15’21.5”	39°41’43.8”
Palanlı/Adıyaman	750	37°49’55.4”	38°18’36.2”

Table 2: Characteristics of primer used in study

Primer Name	DNA sequencing (3’-5’)	Temperature bonding (°C)
UBC810	GAG AGA GAG AGA GAG AT	50
UBC812	GAG AGA GAG AGA GAG AA	50
UBC813	CTC TCT CTC TCT CTC TT	50
UBC817	CAC ACA CAC ACA CAC AA	50
UBC825	ACA CAC ACA CAC ACA CT	50
UBC826	ACA CAC ACA CAC ACA CC	52
UBC834	AGA GAG AGA GAG AGA GYT	52
UBC836	AGA GAG AGA GAG AGA GYA	52
UBC840	GAG AGA GAG AGA GAG AYT	52
UBC847	CAC ACA CAC ACA CAC ARC	52
UBC849	GTG TGT GTG TGT GTG TYA	52
UBC851	GTG TGT GTG TGT GTG TYG	54
UBC855	ACA CAC ACA CAC ACA CYT	52
UBC856	ACA CAC ACA CAC ACA CYA	52

Primer names and properties are obtained from the University of British Columbia are shown in Table 2.

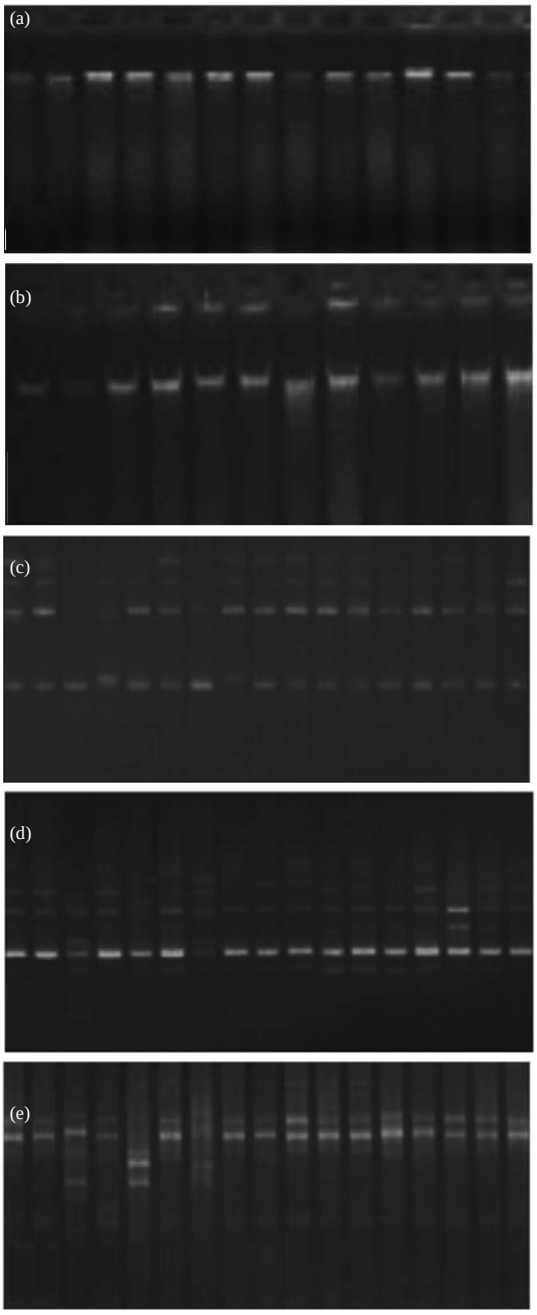

Fig. 1(a-e): (a) Quality, concentration, DNA amount obtained from some plants, (b) DNA samples determined 5 ng uL^{-1} for using PCR, (c) Result of PCR obtained by using ISSR801 primers in ecotype of 17 button medic, (d) Result of PCR obtained by using ISSR857 primers in ecotype of 17 button medic and (e) Result of PCR obtained by using ISSR865 primers in ecotype of 17 button medic

The ISSR protocols were as proposed by Zietkiewicz *et al*. (1994). As DNA temperature bounding of used ISSR DNA primers based on values reported from Kafkas *et al*. (2006). Polymorphic bands displayed in the gel were calculated by classifying depending on whether 1 (there) or 0 (not) and forming a matrix genetic similarity coefficient according to Jaccard (1912). Cluster analysis were obtained from NTSYSpc2.01software package.

RESULTS AND DISCUSSION

Dry matter and seed yield: Dry matter weight per plant was found to vary between 1.49 and 3.76 g. The highest weight of dry matter (3.76 g plant^{-1}) was obtained from Eğil 1. The lowest weight (1.26 g) was from Diyarbakir sample (Table 3). Seed yield per plant ranged between 0.39 and 1.42 g. The highest seed weight was obtained from Mildağı. The lowest weight was recorded from the Silvan samples. Interrante and Muir (2004) reported that dry matter yield change between 0.43 and 2.27 g plant^{-1}, seed yield between 0.22 and 2.78 g plant^{-1}.

Genetic variability: Present study has 14 primers total for the ISSR, where the primer name, DNA sequencing primers, a total number of bands and the scored ratio polymorphism are given in Table 4. Polymorphism of ISSR801, ISSR857, ISSR865 primers have shown of 17 button medic, respectively in Fig. 1c-e. Rate of polymorphism changed between 33% and 100%. High polymorphism reported that *Medicago sativa* L. (Ertus *et al*., 2014), *M. truncatula*, *M. lupulina* and *M. ruthenica* (Yan *et al*., 2009; Zitouna *et al*., 2013) maintained high level of polymorphism in the *Medicago* genus.

Jaccard similarity coefficient values are given in Table 5. Average Jaccard similarity coefficient was found 0.70. The closest genetic relationship was 0.87 between the 83 and 79 while the most distant genetic relationship was found between 95 and 84. By using ISSR data the dendrogram is drawn according to Jaccard genetic similarity coefficients with package software NTSYS-pc2.1 according to the method of UPGMA (Unweighted Pair Group with Arithmetic Average) is shown in Fig. 2. Based on result of the analysis two main groups A and B were composed (Fig. 2). Mainly they divided into two groups. The first was group A1, Diyarbakir, Eğil-1, Eğil-2, İdil and Ovabağ. Second group A2 has two subgroups as Silvan, Hilvan, Adıyaman, Derik, Malabadi and Erdurağı in group 1 and Gaziantep, Çermik, Ergani and Palanlı were in group 2. Jaccard similarity coefficients calculated by using the results of ISSR DNA analysis and dendogram created by using the data. Button medic collected from different regions did not create a group according to the region they were collected. It showed that button medic, especially collected from different regions have a high genetic diversity in their own. Jaccard genetic proximity values clearly shows these findings. However the average value of genetic closeness was 0.70, this proximity reduces to 0.50-0.60 among the different ecotypes of button medic.

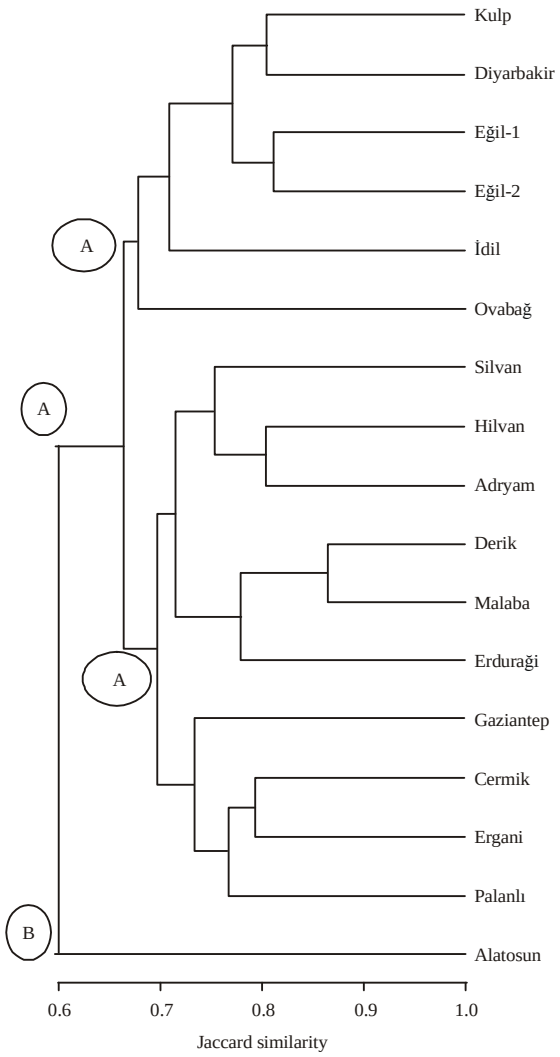

Fig. 2: Dendrogram obtained using ISSR data according to the UPGMA method applying NTSYSpc-2.1 package program

Table 3: Averages of dry matter and seed weight

	DM (g plant^{-1})		Seed yield (g plant^{-1})	
Location	Mean	Std.dev.	Mean	Std.dev.
Zeyrek/Kulp/Diyarbakır	3.25	0.09	1.13	0.08
Silvan/Diyarbakır	1.86	0.11	0.39	0.07
Gaziantep	2.05	0.10	0.69	0.05
İdil/Mardin	2.18	0.11	0.75	0.06
Derik/Mardin	2.73	0.11	1.02	0.14
Petekkaya/Çermik/Diyabakır	1.87	0.11	0.62	0.05
Agricultural faculty/Diyarbakır	1.26	0.12	0.65	0.04
Malabadi/Silvan/Diyarbakır	1.49	0.21	0.55	0.09
Alatosun(Karacadağ)/Diyarbakır	1.76	0.13	0.51	0.08
Erdurağı/Kurtalan	2.34	0.10	0.63	0.05
Eğil-1/Diyarbakır	3.76	0.12	1.15	0.26
Ovabağ/Diyarbakır	2.39	0.09	0.72	0.04
Hilvan/Şanlıurfa	1.85	0.10	0.71	0.05
Eğil-2/Diyarbakır	2.74	0.11	1.14	0.07
Esence/Adıyaman	1.86	0.10	0.81	0.05
Mildağı/Ergani/Diyarbakır	2.22	0.13	1.42	1.72
Palanlı/Adıyaman	2.13	0.19	0.73	0.05
Mean	2.22		0.80	

DM: Dry matter

Table 4: ISSR primary used in research, DNA sequencing and total number of scored bands and polymorphic band number and ratio

Primer Name	DNA sequence (3'-5')	Total No. of scored bands	Number of polymorphic band	
			n	%
UBC810	$(GA)_8T$	9	3	33
UBC812	$(GA)_8A$	5	4	80
UBC813	$(CT)_8T$	6	5	83
UBC817	$(CA)_8A$	8	5	63
UBC825	$(AC)_8T$	7	3	43
UBC826	$(AC)_8C$	6	2	33
UBC834	$(AG)_8YT$	6	4	67
UBC836	$(AG)_8YA$	8	5	63
UBC840	$(GA)_8YT$	3	3	100
UBC847	$(CA)_8RC$	4	3	100
UBC849	$(GT)_8YA$	5	4	80
UBC851	$(GT)_8YG$	4	4	100
UBC855	$(AC)_8YT$	5	4	80
UBC856	$(AC)_8YA$	6	5	83
Total/average		82/5.85	54/3.85	66

Table 5: Similarity coefficient values of 17 button medic ecotypes determined according to the Jaccard (1912) using ISSR DNA analysis

Parameters	73	74	77	78	79	81	82	83	84	85	87	88	90	91	92	94
74	0.71															
77	0.68	0.73														
78	0.70	0.67	0.68													
79	0.78	0.70	0.67	0.65												
81	0.78	0.75	0.76	0.66	0.77											
82	0.82	0.62	0.66	0.73	0.72	0.72										
83	0.75	0.76	0.73	0.63	0.87	0.75	0.69									
84	0.68	0.69	0.58	0.68	0.56	0.68	0.59	0.57								
85	0.75	0.72	0.65	0.63	0.78	0.79	0.62	0.80	0.69							
87	0.81	0.61	0.65	0.72	0.67	0.64	0.79	0.65	0.61	0.65						
88	0.71	0.57	0.65	0.63	0.70	0.67	0.69	0.76	0.57	0.68	0.73					
90	0.79	0.76	0.65	0.71	0.74	0.78	0.70	0.68	0.65	0.72	0.69	0.65				
91	0.76	0.61	0.69	0.76	0.67	0.71	0.78	0.68	0.65	0.65	0.82	0.72	0.69			
92	0.77	0.78	0.75	0.69	0.76	0.72	0.67	0.78	0.62	0.73	0.75	0.65	0.81	0.70		
94	0.65	0.69	0.75	0.65	0.72	0.80	0.67	0.78	0.59	0.73	0.59	0.73	0.66	0.66	0.71	
95	0.77	0.65	0.75	0.65	0.72	0.76	0.80	0.73	0.55	0.65	0.70	0.69	0.70	0.70	0.71	0.80

Average genetic proximity: 0.70

The dendrogram shows two distinguishable cluster regrouping A and B which are recorded by collecting locations. The UPGMA also revealed significant differentiation between two groups. But no relationship was observed between locations by grouping. This result is in line with a phylogenetic study (Ellwood *et al*., 2006) among *M. truncatula*. Zitouna *et al*. (2013) although found correlation between genetic and geographic distances based on the latitude and altitude position different location they found no significance correlation between genetic and geographic distance at the same level. Generally diversity can be affected by ecological factors. These factors can also effect on genetic differentiation (Tilman, 1999). On contrary to Tilman (1999), no relationship was found between locations and variability of button medic landraces. This result suggests that the landraces are well adapted to Turkey.

It shows the genetic diversity of button medic and that can be used in different areas for the purposes. In this study, the genetic diversity of button medic collected from different regions of Southeast Anatolia Region by using ISSR molecular marker techniques has been tried to reveal. It has been concluded that ISSR markers can be used easily to study of genetic diversity of button medic.

These results are in agreement with other *Medicago* species and PCR systems. Ertus *et al*. (2014) have found have found have found high genetic diversity among the *Medicago sativa* L. ecotypes and cultivars using RAPD. According to microsatelitte analysis Yan *et al*. (2009) have indicated differentiation between *M. truncatula* and two related species, *M. lupulina* and *M. ruthenica*.

CONCLUSION

In this study, the genetic diversity of button medic collected from different regions of Southeast Anatolia Region by using ISSR molecular marker techniques has been revealed. It has been concluded that ISSR markers can be used easily to study the genetic diversity of button medic. Landraces which are evaluated in this study are well adapted to climatic conditions of Turkey. These results on button medic landraces of Turkey will be able to develop selection strategies for Turkish germplasm in the face of improving new fodder varieties for dry environmental areas.

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