Analysis of some *Capparis* L. accessions from Turkey based on IRAP and seed protein patterns

Mehmet Çelik[1], Tamer Özcan[2]

[1,2]Istanbul University, Faculty of Science, Department of Biology, Division of Botany, Süleymaniye, Fatih-Istanbul/TURKEY

***Abstract*—** *15 accessions from 10 different grid square of Turkey were analysed based on IRAP and seed protein patterns in order to observe the genetic diversity in the gene pool of Capparis. High levels of polymorphisms were detected with IRAP primers (93%) and seed protein electrophoresis (55.5%). Specific delineation between C. spinosa and C. ovata, and segregations of the accessions related to infraspecific status and eco-geographical distributions were presented in the dendrograms and PCA analysis. Significantly correlation between IRAP markers and seed protein profiles of the specimens was detected ($p < 0.0001$). Combination of genomic/proteomic marker systems may be useful approach for determining the broad genetic diversity in gene pool of Capparis, identification of the germplasms and ecologically tolerant genotypes in breeding programs.*

***Keywords*— Capparis, IRAP, seed protein, variation, genetic resources.**

I. INTRODUCTION

Genus *Capparis* as evergreen shrubs, small trees and lianas in the family Capparaceae occur over a wide range of habitat in the tropical and subtropical regions of the world comprising 350 species approximately. In the unrevised APG II system, it is included in Brassicaceace. *Capparis spinosa L., C. ovata Desf., C.leucophylla DC., C. mucronifolia Boiss., C. cartillaginea Decne, C. decidua* (Fosk) Edgew. are the common species in Mediterranean, Balkans and West Asian countries. Five species are native for Mediterranean region (Inocencio, 2006). The plant as a straggling shrubs has simple entire leaves, with or without stipular spines. Flowers showy, with 4 sepals, 4 petals, very many free stamens and an ovary borne on a stipe becoming much elongated in fruit. Fruit is a berry-like, manyseeded capsule (Coode,1965). *C. ovata* and*C. spinosa* as native species have large distributional patterns in Turkey with three accepted varieties for each species. *Capparis* has been used since the ancient times as medicinal plant and food. Many species have recorded uses in herbalism and folk medicine.The number of papers published on this topic so far.The fruits rich in micronutrients and flower buds are widely used pickled as a vegetable condiment (Aktan *et al.* 1981). Alkaloids, lipids, flavonoids, glucosinolates, cancer preventing agents and biopesticides were detected in biochemical analysis on *Capparis*. Antitumoral activity was detected in the extracts from the flower buds containing antioxidants compounds in some species (Venugopal *et al.* 2011). Antiproliferative, antifungal and HIV-1 reverse transcriptase inhibitory activities of a protein in the seeds of *Capparis spinosa*(Lam & Ng 2009), anti-inflammatory and anti-thrombotic activities in the buds and fruits of *Capparis ovata* (Bektaş *et al.* 2012), and antidiabetic and antioxidant activities in *Capparis decidua* (Zia-Ul-Haq*et al.* 2011) were also reported. Many studies on the flavanoid contents (Ferheen*et al.* 2013), proximate compositions and fatty acid contents (Özcan & Akgül 1998; Vyas *et al.* 2009), mineral compositions (Özcan, 2005) and organic acids of the fruits (Ren *et al.* 2012), glucosinolates, fatty acid, sterol, and tocopherol composition of seed oils from *Capparis* species (Matthaus & Özcan 2005) were previously reported. The extracts obtained from the leaves, roots and dry offshoots are used in cosmetic preparates, additives in perfumery and as regenerative agent in hair loss. On the other hand, *Capparis* from arid regions are highly useful in reforestation and landscape gardening in addition to preserve agricultural land and prevent soil erosion with large extensive root systems that penetrate deepley into the ground.*Capparis* as a salt tolerant plant flourishing also in saline hard planes can adapt harsh environmental and climatic conditions and are cultivated in some mariginal fields such as arid and semi-arid regions as alternative crop plants. Some species and varieties as economic plants have been cultivated in Mediterranean countries including Spain, Morocco, Italy, Turkey and Greece. For efficient use of the genetic resources of *Capparis*, molecular marker assisted breeding programs which select the eligible genes distributing among the populations growing in various habitat conditions are needed to improve the cultivars with high product potential. This genus is also important from taxonomical point of view, because the existence of six varieties from two species in Turkey reflect high intraspecific variability. Therefore, observation within and among populational variations, delimitation at infraspecific level, and determination of taxonomical and phylogeographical relations much strictly are fundamental for understanding evolutionary process in addition to development of conservation

strategies. Apart from many published works using morphological descriptors, limited number of studies for estimating the genetic diversity of *Capparis* species were published using molecular markers including RAPD (Vyas *et al.* 2009; Abdel-Mawgood *et al.* 2010; Kumar *et al.* 2013), AFLP (Inocencio *et al.* 2005) and ISSRs (Saifi *et al.* 2011; Bhoyar *et al.* 2012) which are very promising genetic markers for caper identification and population genetic studies. Genomic sequences of novel 18S ribosomal RNA (Banaras *et al.* 2012) and newly isolated beta-tubulin genes (Aman *et al.* 2013) have been also used in the phylogenetic relations of some species including *Capparis*.In a genetic diversity observations of some Turkish *Capparis* populations based on RAPD markers, genetic distances among the populations were very low and greater intraspecific variation in *C. spinosa* L. than *C. ovata* Desf. was recently reported from Turkey (Özbek & Kara 2013). Inter-Retrotransposons Amplified Polymorphisms (IRAPs) as an alternative valuable retrotransposon-based markers are also used to detect genotypes, measure diversity or reconstruct phylogeny (Flavell *et al.* 1999; Kalendar *et al.* 1999; Kumar & Hirochika 2001). As mobile genetic elements, the copy number of retrotransposons was reported to vary even among closely related plant taxa (Leigh *et al.* 2003; Tenaillon *et al.* 2011). Retrotransposons are divided into two groups depending on the presence/absence of long terminal repeats (LTRs). Within the LTR retrotransposons, two subclasses, Ty1-copia and the Ty3-gypsy, are particularly abundant and well analyzed in plants at different taxonomical categories (Park *et al.* 2007; Ma *et al.* 2008). Retrotransposon based molecular markers are excellent tools for detecting genetic diversity within a species (Kolano *et al.* 2013) and genomic changes associated with retrotransposon activity in abiotic stress conditions (Fan *et al.* 2014). There are many applications of multiple retrotransposon families for genetic analysis such as mapping, fingerprinting and marker-assisted selection and evolutionary studies. But no any study was reported on the usage of retrotransposons for detecting of genetic diversity of *Capparis*. On the other hand, protein electrophoresis has been widely used in germplasm discrimination (Iqbal *et al.* 2003) and evaluation of agronomic traits (Ghafoor & Ahmad 2005)based on genetic differences in seed storage protein comparison. Variations of quantitative traits are significantly associated with protein sub-units expressed by individual genes or gene clusters scattered throughout the genome. Observation on the correlation of DNA-based markers and protein patterns provide useful and reliable tools in the comparative genomics/proteomics as expressional fingerprinting of the germplasms. There is a need to use some reliable and reproducible marker combinations to understand the relations and circumscriptions of *Capparis* taxa much strictly, which have probably great variability in Anatolian gene pool. In the framework of this study, it was aimed to determine infrageneric variations and geographical relations within *Capparis* accessions collected from its native range in Turkey based on IRAP and seed protein band patterns, in addition to contribute molecular identification of the germplasm collection representing different habitat conditions.

II. MATERIAL AND METHOD

2.1 Plant material

Capparis L. specimens including two species and 4 varieties were collected from 4–5 individual plant from each native populations distributed in A1, A4, A6, B1, B4, C1, C2, C3, C6, C7 according to the grid system of Turkey in the period of between June and August (Table 1).

TABLE 1
ORIGINS OF CAPPARIS ACCESSIONS

No. of specimen	*Location / Grid square*	*Taxa*
1	*Ankara to Beypazarı / A4*	*C. ovata Desf. herbacea Willd.*
2	*Konya to Aksaray / B4*	*C .ovata Desf.var.canescens (coss.) Heywood.*
3	*Amasya /A6*	*C. ovata Desf. herbacea Willd.*
4	*Şanlıurfa to Bozova / C7*	*C .ovata Desf.var. palaestina Zoh.*
5	*Antalya to Muratpaşa / C3*	*C. spinosa L.var. spinosa Zoh.*
6	*İzmir to Balçova / B1*	*C. ovata Desf. herbacea Willd.*
7	*Marmara Ereğlisi to Kınalı / A1*	*C .ovata Desf.var.canescens (coss.) Heywood.*
8	*Burdur / C3*	*C. ovata Desf. herbacea Willd.*
9	*Bodrum to Aydın **(S3)** / C1*	*C. spinosa L.var. spinosa Zoh.*
10	*Antalya to Kemer **(S6)** / C3*	*C. spinosa L.var. spinosa Zoh.*
11	*Antalya to Isparta **(S5)** / C3*	*C. spinosa L.var. spinosa Zoh.*
12	*Kahramanmaraş / C6*	*C. spinosa L.var. spinosa Zoh.*
13	*Balıkesir to Sarımsaklı **(S2)** / B1*	*C .ovata Desf.var.canescens (coss.) Heywood.*
14	*Marmaris to Muğla **(S4)** / C2*	*C. spinosa L.var. spinosa Zoh.*
15	*Balıkesir to Burhaniye / B1*	*C .ovata Desf.var.canescens (coss.) Heywood.*

S2-6 represent the localities from which seed samples also collected. S1 (C. spinosa var.spinosa) was collected additional locality from B1

Herbarium materials were prepared from the specimens and determined using Flora of Turkey. Leaves and some fruit samples of the accessions also transported to the laboratory in polypropylene bags and kept in deep -freezer (-18 ℃) until the analysis performed.

2.2 DNA analysis

Genomic DNA isolation was carried out on the leaf samples of 15 *Capparis* accessions by using CTAB method (Doyle andDoyle 1987). DNA concentrations of the samples were detected in nanodrop spectrophotometer (BioSpec-nano; Shimadzu-Biotech). 1 µl DNA samples run on polyacrylamide gel (1.5 %), died with ethidium bromide and visualized under UV (BIORAD, Molecular Imager®, ChemiDoc™ XRS+ with Image Lab™ Software). PCR reaction was firstly carried out using 13 IRAP primers on the DNA sample of a *Capparis* accession. 5 primers (LTR 1; LTR 4; LTR 5; LTR 6 ve LTR 7) showing high polymorphism were selected and applied on the genomic DNA of 15 *Capparis* accessions (Table 2). Amplification reaction was carried out in 25 µl volume including 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.4 mM dNTP mixture (dATP, dGTP, dCTP ve dTTP), 0,4mM for each IRAP primer, 50 ng genomic DNA and 2U Taq DNA polimerase (Invitrogen). An optimized program (Kalender *et al.* 2010) was used in PCR reaction (Thermocycler Model-9700, Perkin-Elmer, Boston, MA, USA). After denaturation of genomic DNA at 95˚C for 3 min., PCR reaction comprise of 35 cycle at 95 ˚C for 15 sec., 55˚C for 30 sec., 72˚C for 3 min. and 72˚C for 10 min. as last extension reaction. Amplification products obtained from each primer run on polyacrylamide gel (1.5 %) at 80 v., died with ethidium bromide and visualized under UV at gel documentation and image analyse system (BIORAD, Molecular Imager®, ChemiDoc™ XRS+ with Image Lab™ Software).

TABLE 2
THE LIST OF IRAP PRIMERS SELECTED, THEIR NUCLEOTIDE SEQUENCES, THE NUMBER OF LOCI, RANGE OF BASE PAIRS, NUMBER AND PERCENT OF POLYMORPHIC BANDS

No	Primer	Nucleotide sequences (5'-3')	Position and orientation	Range of amplified bands per sample	Number of total loci	Size (bp) of fragment	Number of Polymorphic loci	Polymorphic loci (%)
1	LTR 1	ACCCCTTGAGCTAACTTTTGGGGTAAG	1282 →1308	1-5	6	500-1150	5	83.3
2	LTR 4	AGCCTGAAAGTGTTGGGTTGTCG	1111 ←1133	4-12	18	170-1250	18	100
3	LTR 5	CTGGCATTTCCATTGTCGTCGATGC	971 ← 995	3-14	17	300-2250	17	100
4	LTR 6	GCATCAGCCTGGACCAGTCCTCGTCC	586 ← 611	3-8	15	800-5100	15	100
5	LTR 7	CACTCAAATTTTGGCAGCAGCGGATC	460 →486	5-11	15	270-1500	11	73.3

2.3 Isolation and determination of total protein from seed samples

Specimens were pulverised in liquid nitrogen and homogenised with 1 ml extraction buffer at +4 ˚C (1 M Sükroz, 10 mM HEPES pH 7.0, 5 mM $MgCl_2$, 1 mM EDTA, 10 mM 2 -merkaptoetanol, 0.1 mM PMSF ve 5 mM Benzamidin) and protease inhibitor (Sigma) for each 1 g samples. Homogenate including all protein fraction in 1.5 ml eppendorf were santrifuged in cooled microfuge (at +4 ˚C) at 13 000 rpm for 5 min. All supernatants were taken into eppendorfs. Bradford method was applied for determination of total protein amounts. After preparing BSA standart (Catalog Number P0914), samples in microplates were measured at ELISA spectrophotometer at 595 nm wavelength. Total protein concentrations were calculated based on standart curve.

2.4 SDS Page Gel Electrophoresis

BioRad protean 2 gel system was used. After preparing of resolving and stacking gel according to the protocol, resolving gel was poured into instrument with using pipette. N-butanol was added with 2 mm high onto the gel for occuring smooth polymerisation surface. N-butanol removed after polymerisation and glasses washed with H_2O. Stacking gel are added and leaved for polymerisation. Equal amounts of protein samples mixed with 2 x sample stacking buffer (0.125 M Tris, %4 SDS, % 20 gliserol, %10 2-merkaptoetanol, pH 6.8), and denaturated at 95 ˚C'de 5 min. The plates are washed with running

buffer for removing the acrilamide residues before the loading of denaturated protein samples into the plates. The samples are loaded into the plates and connected to power with 16 mA (80 V) at the start, and raised to 30 mA (160 V). The system were fixed at 40 mA for 6 hours for separating the protein bands. After the running of marker bant stained with brome phenol blue edge to 0.5 cm of the gel, electrophoresis are terminated. The gels are taken into Coomassie Brillant Blue for at least two hours in the shaker. Dying solution are removed and destaining solution replaced continually until the dye removed from the gel for making apparent the bant patterns. The size of the protein band products of the specimen was estimated from 10 to 170 kDa protein ladder (Thermo Scientific Cat no: 26616 PageRuler Prestained Protein Ladder).

2.5 Statistical analysis

Homologous fragments in the gel patterns obtained with each IRAP primer were scored for the presence (1) or absence (0) to create binary matrices. Dendrogram based on obtained data for genetic relationships between genotypes was constructed using the unweighted pair-group method with arithmetic average (UPGMA) and Jaccard coefficient of similarity was employed in calculation of distance based on the markers resulting from IRAP analysis though the software XLSTAT 2013.5 and MVSP 3.22 (Multivariate Statistics Package). Principle component analysis based on IRAP data was conducted with statistiXL. Correlation analysis (Pearson) of the accessions based on IRAP data was carried out. Hierarchical cluster analysis using average linkage (Between Groups) were carried out based on protein band patterns of the seeds with a statistical package program (SSPS 11.5). Pearson correlation analysis was also performed between matrices (IRAPs/Seed protein) based on Mantel test to obtain the significance of the correlation coefficient. The p value has been calculated using the distribution of r(AB) estimated from 10,000 permutations.

III. RESULTS

In the PCR amplification based on 5 IRAP primer having higher number of band scored, 71 band totally were identified between 170-5100 bp in *Capparis* accessions collected from 15 different locations represented in 10 grid squares of Anatolia. 66 band of total product are polymorphic (approx. 93 %), while 5 out of total band only are monomorphic. All bands produced by LTR4, LTR5 and LTR6 are polymorphic (100 %). Gel profile of LTR1 was represented with the lower number of band scored. Percentage of polymorphic bands for LTR7 calculated at the lower level (73.3 %) (Figs 1,2).

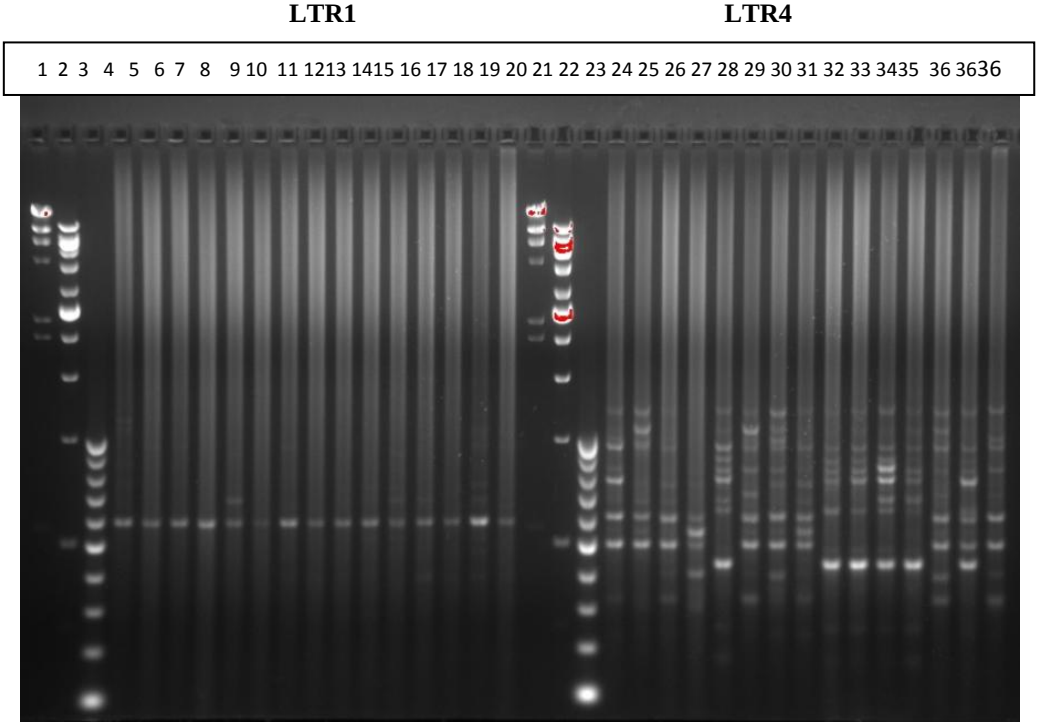

FIGURE 1 GEL IMAGES OF THE GENOMIC DNA OF 15 *CAPPARIS* ACCESSIONS BASED ON THE PRIMERS LTR1 AND LTR4. LANE 1, λ / HIND III; LANE 2, 1KB MARKER; LANE 3, 100 BP; LANE 4-18, LTR1 *CAPPARIS* 1-15; LANE 19, λ / HIND III; LANE 20, 1 KB MARKER; LANE 21, 100 BP; LANE 21-36, LTR4 *CAPPARIS* 1-15.

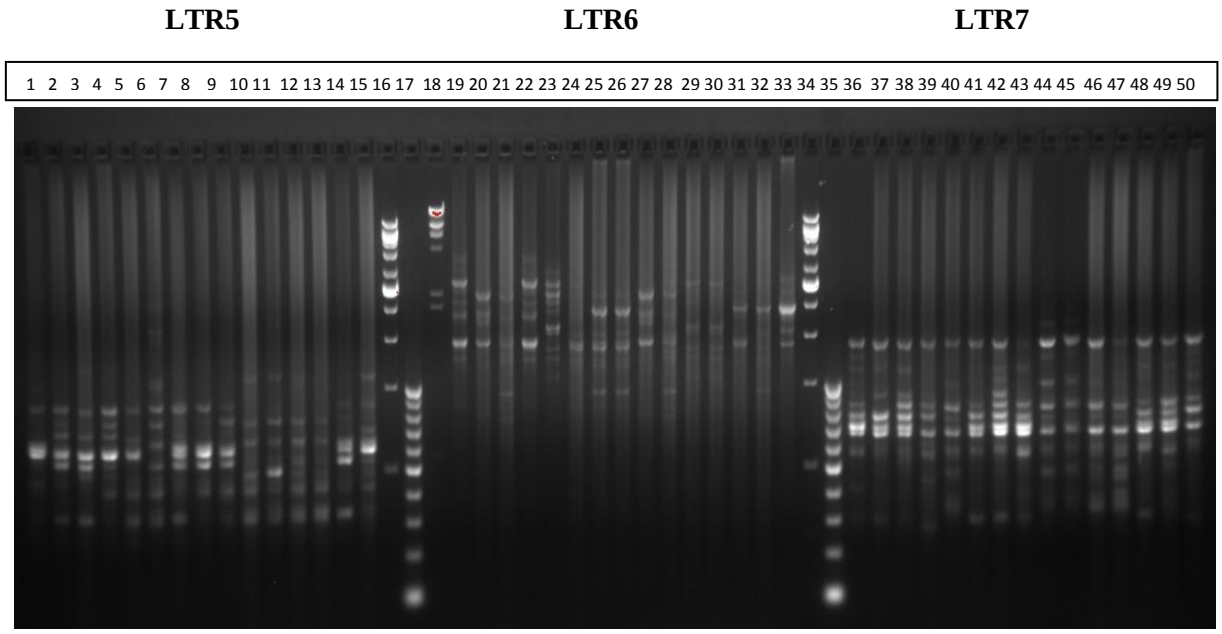

FIGURE 2 GEL IMAGES OF THE GENOMIC DNA OF 15 CAPPARIS ACCESSIONS BASED ON THE PRIMERS LTR5, LTR6 AND LTR7. LANE 1-15, LTR5 CAPPARIS 1-15; LANE 16, 1KB MARKER; LANE 17, 100 BP; LANE 18, λ / HIND III; LANE 19-33, LTR6 CAPPARIS 1-15; LANE 34, 1KB MARKER; LANE 35; 100 BP; LANE 36-50 LTR7 CAPPARIS 1-15.

The ranges of the sizes of the band scored are between 500 and 1150 for LTR1, 170-1250 for LTR4, 300-2250 for LTR5, 800-5100 for LTR6 and 270-1500 for LTR7 (Table 2). In the UPGMA dendrogram based on IRAPs pattern, the accessions of *C. spinosa* and *C. ovata* were located separately in two main clads. In the first clad, geographically related accessions of *C. spinosa* var. *spinosa* were clustered into two subclads, as Irano-Turanian and Mediterranean populations. Only one accessions of this taxon was located in the separate clad. The second clad was also divided into two subclade including *C. ovata* var. *palaestina* from C7 grid square in southeastern part of Anatolia and the other accessions of *C. ovata*. Last subclade comprising of 8 accessions separated into an A4 population of *C. ovata* var. *herbacea* and the other populations of this species collected from broad range of the distributions including (B1, B1), A6, B1, (C3, A1) and B4 grid squares as distinct indivudial branches generally, showing broad genetical diversity of this stock. An accession of *C. spinosa var*. *spinosa* from C2 population placed additionally in this group (Figure 3).

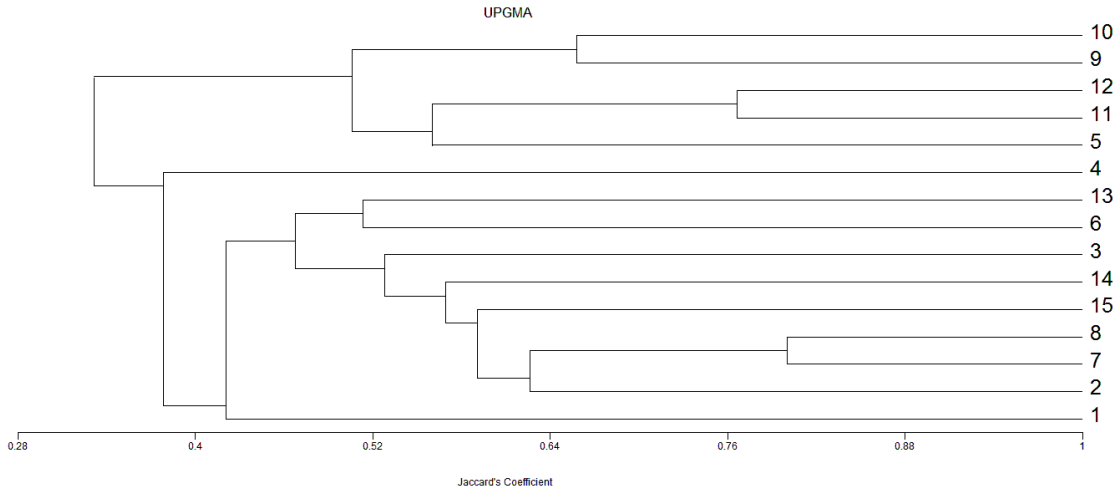

FIGURE 3 DENDROGRAM TREE OF 15 CAPPARIS GENOTYPES OBTAINED FROM UPGMA CLUSTER ANALYSIS USING IRAP LOCI BASED ON JACCARD SIMILARITY COEFFICIENTS

In the principle component analysis based on five primers producing high percentage of polymorphism show that the first two axes of this analysis accounted for 53.18 % of the variation in the data. Principle components 1 and 2 explain 34.84 and 18.33 % of total variation, respectively. This analysis defines these relatively distinct groups. Taxonomical and eco-geographical relations of the accessions were observed in the scatter plot (Figure 4.).

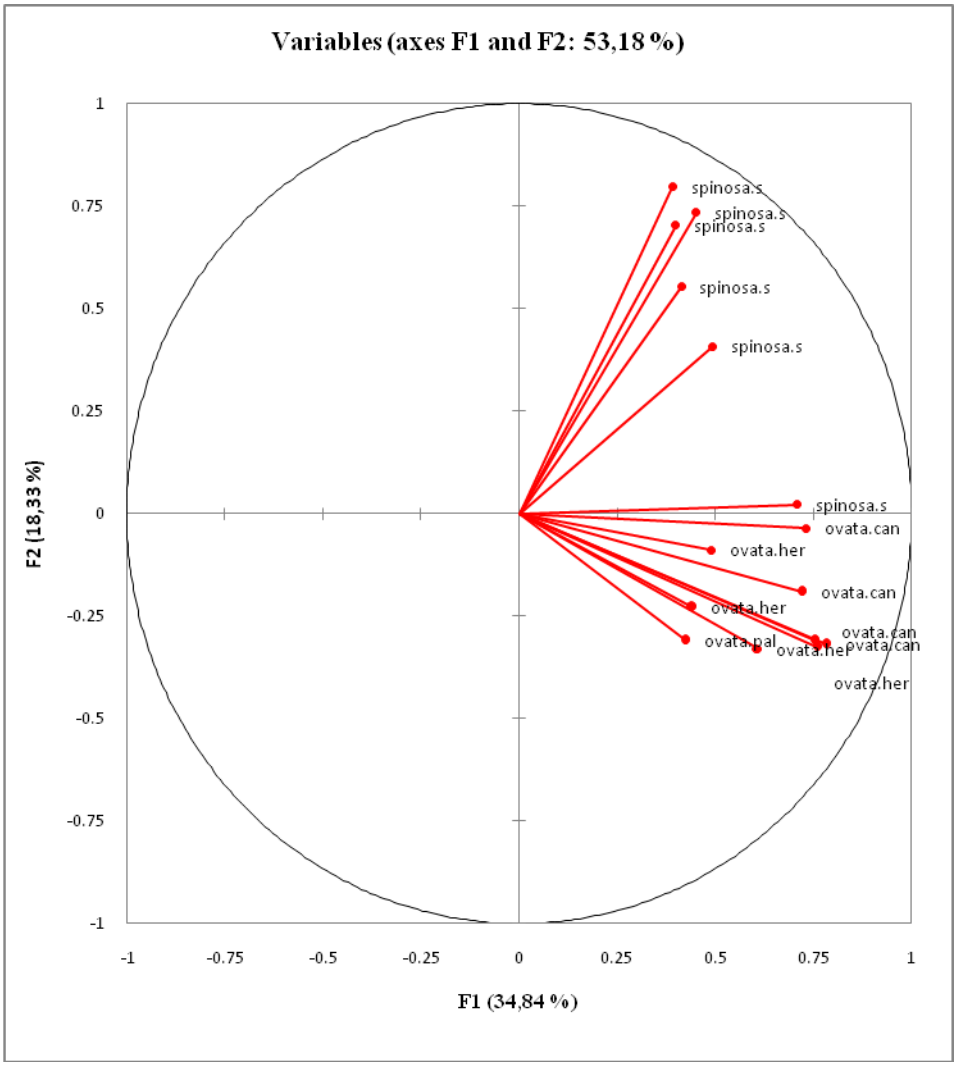

FIGURE 4 PRINCIPLE COMPONENT ANALYSES OF THE ACCESSIONS BASED ON IRAP DATA

Accessions of *C. spinosa* and *C. ovata* are clearly segregated in the diagram. One accession of *C. spinosa* var. *spinosa* from C2 population located close to *C. ovata* accessions.Genetic similarity matrix of the populations using data from the primers producing highest percentage of polymorphism was demonstrated in Table 3.Corelation coefficient (Pearson) was detected between 0.113 and 0.961, and significant at $p<0.005$ and $p<0.001$ levels in taxonomically and geographically related accessions. Six accessions of the seeds of *Capparis* was also analysed for total protein contents and their electrophoretic band patterns in order to observe taxonomical and geographical relations of the accessions, and to test the correspondance with the molecular marker results. Total protein amounts were detected between 12.35 % (*C.ovata* var. *canescens*) and 16.28 % (*C. spinosa* var. *spinosa*) in the specimens (Table 4). Total number of loci scored is 18 between 15-170 kDa, and 10 of the total number is polymorphic corresponding to 55.5 % genetic diversity (Figure 5). Monomorphic bands were observed in the ranges of 65-105 kDa generally. The bands in the 4 accessions from *C. spinosa* var. *spinosa* were detected at 35 kDa level except for two accessions from northern Aegean region of Turkey including *C. spinosa* var. *spinosa* and *C .ovata* var. *canescens*. The last both samples produced much condense band patterns at 20 kDa grade compare to other samples. Extra bands with 17 kDa in *C .ovata* var. *canescens* as a difference from *C. spinosa* var. *spinosa* were detected in the same cluster.

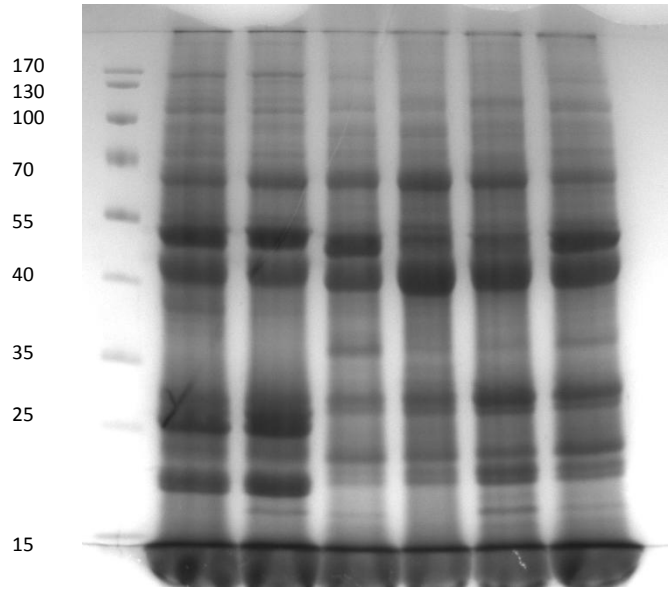

FIGURE 5 PROTEIN BAND PATTERNS OF 6 *CAPPARIS* ACCESSIONS

TABLE 3
GENETIC SIMILARITY MATRIX OF *CAPPARIS* ACCESSIONS BASED ON JACCARD COEFFICIENT

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1														
2	0,41	1													
3	0,375	0,563	1												
4	0,385	0,441	0,324	1											
5	0,319	0,357	0,267	0,302	1										
6	0,37	0,45	0,349	0,326	0,3	1									
7	0,447	0,658	0,537	0,409	0,373	0,479	1								
8	0,422	0,595	0,553	0,415	0,347	0,457	0,8	1							
9	0,271	0,4	0,366	0,31	0,465	0,28	0,438	0,477	1						
10	0,265	0,267	0,295	0,191	0,488	0,327	0,4	0,375	0,658	1					
11	0,267	0,238	0,182	0,186	0,553	0,395	0,3	0,271	0,415	0,639	1				
12	0,302	0,275	0,244	0,22	0,568	0,372	0,306	0,304	0,462	0,568	0,767	1			
13	0,432	0,6	0,371	0,343	0,31	0,514	0,525	0,462	0,286	0,375	0,389	0,4	1		
14	0,455	0,513	0,513	0,349	0,5	0,396	0,6	0,619	0,477	0,404	0,419	0,395	0,425	1	
15	0,455	0,553	0,475	0,415	0,404	0,558	0,6	0,619	0,413	0,347	0,356	0,333	0,541	0,545	1
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

TABLE 4
TOTAL PROTEIN CONTENTS (%) IN THE SEEDS OF SPECIMENS AND THEIR BANDS SCORED IN ELECTROPHORESIS

Sample	Total protein %	Band scored
1	14.93	14
2	12.35	15
3	12.50	13
4	16.28	12
5	14.90	14
6	14.89	15

In the hierarchical cluster analysis, a dendrogram was constructed with two main clad (Figure 6). The accessions of *C. spinosa* var. *spinosa* and *C .ovata* var. *canescens* from northwestern part of Anatolia were located in the same clad of the

dendrogram. The other geographically related accessions of *C. spinosa* var. *spinosa* from the middle and southern part were placed in the second clad. Only one accession from C1 middle part distribution was located in the separate branch of this clad. C2 accession was clustered with C3 populations from southern Anatolia as a distinct clad showing close relations with their locations. Considerable relations between the results obtained from protein band profiles and the geographical distribution patterns of the accessions was observed. The results from IRAP profiles correspond with the protein data. Correlations between the matrices of IRAPs and seed protein data were detected at significant level ($p < 0.0001$; $r(AB) = 0.066$).

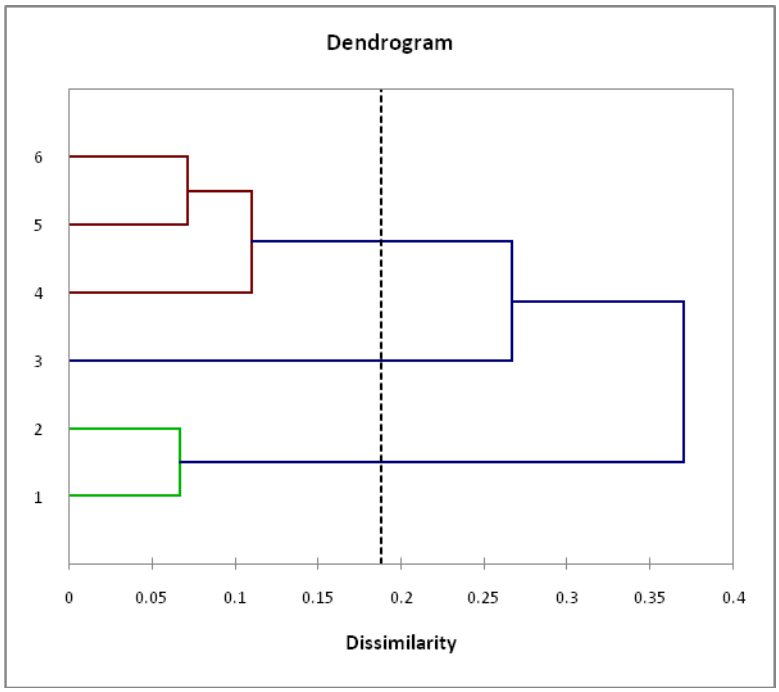

FIGURE 6 UPGMA DENDROGRAM BASED ON TOTAL PROTEIN BAND PATTERNS OF THE SEEDS OF SIX CAPPARIS ACCESSIONS

IV. DISCUSSION

In the present study, populational segregation in the studied gene pool of *Capparis* was achieved based on IRAP and total protein band patterns of the specimens. Investigated traits as a reliable and useful genomic/proteomic marker test which are significantly correlated with each others reflect different aspects of taxonomical concept. Macro-morphological characters effected by environmental conditions may have a wide margin of error in traditional classification of *Capparis*. Some variations in the habitus from distinct localities were described in the flora of Turkey. Therefore, morphological variability in diagnostic features obscure taxonomical limits for identification of the varieties of both species native for Turkey. It is needed much reliable and consistant parameters in order to establish populational and taxonomical relations of this genus. Up to date, this study is the first to survey of IRAP-based assesment of genetic diversity in the *Capparis* genome. Retrotransposon-based molecular markers has great potential in determination and identification of the germplasms, evolutionary history of a genome and the relationships between taxa in different plant groups and agriculturel crops (Gribbon *et al.* 1999). The inter-retrotransposon amplified polymorphism (IRAP) method displays insertional polymorphisms by amplifying the segments of DNA between two retrotransposons. The copy number of retrotransposons may vary even among closely related plant taxa (Tenaillon *et al.* 2011). It is generally accepted that retrotransposons affect genome size, organization and function (Parisod *et al.*2009). Many phylogenetic comparisons of populations of retrotransposons, particularly Ty1-copia-like elements, have been performed within and among related groups of taxa. Large variations in retrotransposon copy number are observed over relatively short evolutionary time scales and their mode of replication significantly increases genome size in higher plants. It was reported that at least 50 % maize genome (Shirasu *et al.* 2000) and 55 % of *Sorghum* (Paterson *et al.* 2009) are composed of retrotransposons. In genetic analysis, usage of multiple retrotransposon families reveal specific differences of each transposition event in the historic activity. Amplified bands derived from the newly inserted retrotransposon elements may be polymorphic, appearing only in plant lines in which the insertions have taken place. Different elements as high-copy-number dispersed sequences may be differently distributed in

the varieties, cultivated forms or wild relatives of *Capparis*. In Turkey, there are many populations of *Capparis* all around Turkey in a wide range of habitat conditions including altitude, climate and regions such as arid, sub-humid and humid areas, fields, limestone slopes, saline soils, wast and disturbed ground near cultivation etc. As a drought and fire resistant plant, the deepest root system and wide ecological amplitude allow it to withstand harsh environments range and suitable for combating desertification and erosion (Sakçalı *et al.* 2008). Eco-geographical factors may have a significant effect on the genetic diversity of this genus. Retrotransposons are also considered to be an important factor in genome plasticity (Kidwell & Lisch 1997). Transcriptional and transpositional activations of transposable elements are mainly induced by abiotic and biotic stresses (Grandbastien,1998), and the activation has been regarded as the mechanism of genotypic remolding.*Capparis* as light loving and salt tolerant plant is well grown under intense light and dry hot conditions on nutrient poor, sandy, drained and gravely soils. Therefore, it is expected to have high retrotransposon activity in its genome. Insertional dynamics of transposable elements could promote morphological and karyotypical changes, some of which might be potentially important for the process of microevolution, allowing species with plastic genomes to survive as new forms or even as new species in times of rapid climatic change (Belyayev *et al.*2010). The dendrogram and PCA analysis demostrated that the accessions of *C. spinosa* and *C. ovata* were clearly segregated. First leaving clad from the cluster of *C. ovata* is var. *palaestina* which is well differentiated with morphological traits from the other varieties of *C. ovata*. Remaining specimens are generally located in separate branches collected from geographically diverse populations. Distances between the sampling locations are at least 150 km for representing much more genetic diversity in Anatolia. In addition to discrimination at variety level, such clustering pattern account for spatial and genotypic divergency reflecting adaptation of the varieties to different habitat conditions. Besides, some intermediate forms in *Capparis* at specific and infraspecific levels were reported in the previous studies (Zohary, 1960; Coode, 1965; Higton & Akeroyd 1991). There are also some examples for intermediate types between *C. ovata* and *C. spinosa* in culture conditions (Barbera & Di Lorenzo 1984). In the Flora of Turkey, it was reported that specimens from C5 population of var. *spinosa* have leaves intermediate in size between var. *spinosa* and var. *aegyptia*. Some specimens from B1 grid square also seems to be intermediate between var. *spinosa* and *C. ovata* Desf. var. *canescens* (Coss.) Heywood (Coode, 1965). Additionally, Zohary reports having seen intermediates between var. *aegyptia* and *C. ovata* var. *canescens*.Some introgressions or collateral cultivations may reveal the existence of local variants. In the PCA analysis based on IRAP data, one accession of C. *spinosa* var. *spinosa* from C2 grid square has occured near *C. ovata* gene pool. This accession that may be a transient form located in the IRAP cluster of *C. ovata*, implying its hybrid origin. In the protein electrophoresis, this specimen has clustered with the other *C. spinosa* accessions collected from Mediterranean populations as a separate clad. Considering distribution range of the varieties of *C. spinosa* and their morphological features, regarding accession may be related with var. *inermis*. Besides, *C. spinosa* var. *aegyptica* recorded in Flora Hellenica and Flora of Turkey in Samos island. It is needed to confirm the existence of this variety from Turkey wih using combined molecular markers. One accession from northern population of var. *spinosa* was also clustered with *C. ovata* var. *canescens* based on protein data. Some intermediate populations having hybrid forms were also reported between *C. spinosa* and *C. ovata* based on RAPD analysis and morphological observations (Özbek & Kara 2013),implying that var. *canescens* may be a variety of C. *spinosa* instead of *C. ovata*. *C. spinosa* was recognised as a single species without infraspecific categories by some authors (Jacobs, 1965; Higton & Akeroyd 1991). This species whichhas long been cultivated for its edible buds exhibite broad range of distribution in Mediterranean basin compare to the other species of *Capparis* (The Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity). The amount of genetic diversity seems to be related to population size (Nosrati *et al.*2012). Therefore, high polymorphism ratios may be expected in this species. Morever, high polymorphisms and eco-geographicalcorelations within the accessions of *C. spinosa* var. *spinosa* were also observed using IRAP and protein data. On the other hand, *C. ovata* var. *herbacea* as the commonest variety in Turkey with high number of polymorphic fragments in IRAP analysis has been reported to have some intermediate forms with var. *palaestina*. An accession of var. *herbacea* from A6 grid square was placed as a separate clade within the group of *C. ovata*. This species was clearly segregated into three subclade including var. *palestina*, var. *herbacea* that may be related with cultivated forms and the others from Mediterranean and Irano-Turanian populations. *C. ovata* var. *palaestina* distributing in Mesopotamia is distinguished from the other 2 varieties by its generally smaller leaves and white-canescent indumentum. This variety which is well differentiated with morphological characters and molecular assesment from the other varieties of *Capparis* are the first separating branch from *C. ovata* gene pool in the dendrogram. Parallel results with our findings on *C. ovata* var. *palaestina* were reported based on RAPD analysis (Özge & Kara 2013). The amount of genetic diversity within the populations are influenced by both the breeding system and the evolutionary history of each population. On the other side, retrotransposons have been demonstrated to provide suitably polymorphic markers for variety identification or breeding purposes (Vicient *et al.* 2001; Boyko *et al.* 2002), and sensitive enough to detect rapid genome changes (Belyayev *et*

*al.*2010).Retrotransposon markers are also sufficiently stable to allow their use in mapping projects (Huo *et al.*2009). Identification of commercial cultivars, landraces and breeding lines based on IRAP method was demostrated in different clusters of *Linum usitatissimum* L. and relatively lower level of polymorphism was reported in breeding lines and the cultivars compare to wild accessions (Smy´kal *et al.* 2011). Obtained results proved the sensitivity of IRAP analysis for discriminating the accessions from diverse populations in addition to specific delineation. In general, PCA analysis of natural *Capparis* populations based on IRAP data displayed evident delimitation of both species with representing total variation of 53.18 %. Although genetic distances among 15 Turkish *Capparis* populations was reported to be very low (Özbek &Kara 2013), high polymorphism detected in the present study may account for the broad genetic base of *Capparis* in Anatolia, indicating also high resolution capacity of the IRAP marker. It was also reported that low level of genetic diversity values within population based on RAPD markers may be explained with the breeding system of *Capparis* represented by self-pollination in extremely habitat conditions (Özbek & Kara 2013). Molecular variance analysis using combined data of RAPD and ISSR showed maxiumum variation within the natural population in *Capparis spinosa* from trans-Himalayas, followed by among population (Bhoyar *et al.* 2012). It will be better to analyse genetic diversity within a population using additional and combined marker systems. IRAP analysis showed that the method are successful to differentiate the accessions including varieties of *C. spinosa* L. and *C. ovata* Desf.. Morover, high polymorphism within the *C. spinosa* var. *spinose* gene pool represented by various ecotypes was observed by overlapping data obtained from IRAP and protein analysis. Although high polymorphism ratios were reported in some populations of *Capparis* from Turkey based on RAPD patterns (74.49 %) (Özbek & Kara 2013) and North Morocco using ISSR markers (75.51 %) (Saifi *et al.* 2011), polymorphic fragments produced by IRAPs primers with 93 % indicate high retrotransposon density within the genome of wild *Capparis* accessions which is useful in analysis of genetical structure, marker-assisted selection, populational segregation, genetic diversity within population and infraspecific delineation. The elucidation of genetic diversityis highly important for genetic improvement as well. Morever, phylogenetic resolution between closely related germplasm accessions benefits from increasing the number of polymorphic markers scored. Replicative mechanism of transposition may resulted in a high degreeof heterogeneity and insertional polymorphism in this genus. It was reported that a retrotransposon that has been active recently in evolutionary terms will be polymorphic between individuals within a species. Contrary, inactive retrotransposons for a long time which are less polymorphic at the intra-specific level may be more informative at the inter-specific or higher taxonomic levels (Leigh *et al.* 2003). Evolutionarily younger transposition events often result in relatively homogeneous groups of retroelements (Hill *et al.*2005). High level of polymorphism within the same varieties may account for dense retrotransposon activity in *Capparis*, indicating high adaptation and speciation potential in addition to utility of IRAP markers in taxonomic and phylogenetic relations of this genus. Obtained results may be applied to all accessions of *Capparis* in Anatolia as reliable marker test in order to establish core collections and structure of the germplasms. Earlier molecular marker analyses by RAPD, AFLP and ISSR showed relatively a low diversity in *Capparis*. Here, it has been taken advantage of ubiquity and abundance of LTR retrotransposons in genomic diversification of this genus. It may be better to observe the relation between seed storage protein patterns and the molecular marker data from taxonomical and phylogenetical points of view as genomic/proteomic approach. Obtained results may also contribute the identification of the germplasm with high product potential, disease resistant and ecologically tolerant genotypes in *Capparis*. A combination of different marker systems can therefore provide a comprehensive evaluation of the evolutionary history of a genome and the relationships between taxa. Although great variability in morphological characters of *Capparis* seeds (Özbek & Kara 2013), reliability of the regarding results were checked with the seed protein data as a constant tool to characterise the genetic structure of the natural populations of *Capparis*. SDS-PAGE of seed storage proteins showed high inter-accession diversity (55.5 %) and some differentiation on the basis of origin or source. Protein data segregated the accessions according to localities, reflecting also actual expression profiles for the adaptation to the growing conditions. Remarkable correlation between the protein profiles and eco-geographical patterns of the germplasms collected from neighbouring regions are also probably due to derived progenies of the same ancestor. High variability in the protein band patterns of the accessions also indicate the broad genetic base of *Capparis* in order to select the germplasm with better quantitative traits in the breeding programmes. Variation at protein fractions in the vicinity of QTLs may be an indication of genetic variation potentially available to breeding programmes for improving yield potential. It was reported that variation in 17 quantitative traits of *Vigna unguiculata* (L.) Walp are related to seed storage protein patterns as a useful tool for discrimination of the germplasm, expressed by individual genes or gene clusters scattered in the genome (Iqbal *et al.* 2003). Some chromosome locations controlling seed storage proteins were also identified, and assumed that distribution pattern of the genes controlling seed storage protein probably exists universally among family specific or even all plants and animals (Li *et al.* 2013). Protein band patterns may be useful for delimitations at specific and variety level of *Capparis* accessions. Obtained band profiles also reflect the adaptation of the

accessions into growing conditions. High polymorpism of the proteom between and within varieties of *Capparis* may be related with retrotransposon activity induced with biotic and abiotic stress conditions. Dynamic of retrotransposons in *Capparis* genom may be responsible for producing of some specific additional protein fragments serving well adaptation and the suitable functions in the stress conditions. Populations of several colonizing species across broad geographic and environmental ranges have been confirmed to be consisted of genetically similar populations of highly plastic genotypes (Hermanutz & Weaver 1996). A great evolutionary plasticity is also exist in *Capparaceae* flowers for adaptation to different pollinating agents. Six accepted varieties of both *Capparis* species have very large and interesting distribution patterns in different climatic and edaphic conditions in Turkey which may reflect its tolerance capacity and the distant geographical speciation and progressive radiation potential in its native range. It is essential to enforce the protection of natural populations and to expand the scope of protection of natural populations of *Capparis* in Anatolia. This study demonstrated the utility of high-resolution IRAP markers based on the LTRs of retrotransposons and seed protein electrophoresis for distinguishing *Capparis* accessions, and confirmed that those markers are highly informative for genetic diversity and phylogenetic studies. Obtained results from examined traits revealed high level of polymorphism at infraspecific levels in *Capparis*. This task awaits further large-scale genetic diversity analysis and establish of gene bank collections of *Capparis* populations distributed in the brod range of habitat conditions in Anatolia.

ACKNOWLEDGEMENTS

This study was supported by Research Fund of Istanbul University. Project No:35185. The authors are gratefully acknowledge to Associate Professor Dr. Yelda Özden Çiftçi and Research Assistant Veysel Süzerer from Gebze Institute of Technology for her valuable supports in the laboratory works.

REFERENCES

[1] Abdel-Mawgood A.L., Jakse J., Al-Doss A.Ai & Assaeed A.M. (2010) Genetic structure and diversity within and among six populations of Capparis decidua (forssk.) edgew. From Saudi Arabia. African Journal of Biotechnology 9(38):6256-6263.

[2] Aktan N., Bilgir B & Elgin E. (1981) Pickling product and keeping of capers flower buds. Ege University Agricultural Faculty Journal 18: 259-273.

[3] Aman S.,Iqbal M.,Abbas .S,Banaras S., Awais M., Ahmad I., Shinwari Z.K & Shakeel S.N. (2013) Molecular and comparative analysis of newly isolated beta-tubulin partial gene sequences from selected medicinal plants. Pakistan Journal of Botany 45(2):507-512.

[4] Banaras S., Aman S., Zafar M., Khan M., Abbas S., Shinwari Z.K & Shakeel S.N. (2012)Molecular identıfication and comparative analyss of novel 18s ribosomal RNA genomic sequences of a wide range of medicinal plants. Pakistan Journal of Botany 44(6):2021-2026.

[5] Barbera G & Di Lorenzo R.D. (1984) The caper culture in Italy. Acta Horticulturae 144:167–171.

[6] Bektas N., Arslan R.,Goger F.,Kirimer N & Öztürk Y. (2012) Investigation for anti-inflammatory and anti-thrombotic activities of methanol extract of Capparis ovata buds and fruits. Journal of Ethnopharmacology 142(1):48-52

[7] elyayev A., Kalendar R., Brodsky L., Nevo E., Schulman A.H & Raskina O. (2010) Transposable elements in a marginal plant population: temporal fluctuations provide new insights into genome evolution of wild diploid wheat. Mobile DNA1:6.

[8] Bhoyar M.S.,Mishra G.P.,Naik P.K., Murkute A.A & Srivastava R.B. (2012) Genetic Variability Studies Among Natural Populations of Capparis spinosa from Cold Arid Desert of Trans-Himalayas Using DNA Markers. National Academy Science Letters 35(6):505-515.

[9] Boyko E., Kalendar R., Korzun V., Korol A., Schulman A.H & Gill B.S. (2002) A high-density cytogenetic map of the Aegilops tauschii genome incorporating retrotransposons and defence related genes: insights into cereal chromosome structure and function. Plant Molecular Biology 48:767–790.

[10] Coode M.J.E.(1965) Capparis L..In: Davis P.H. (eds).Flora of Turkey and the East Aegean Islands. Edinburgh University Press, Edinburgh, pp. 496-498.

[11] Doyle J.J & Doyle J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11-15.

[12] Fan F., Bowen C., Zhang T., Ding G & Wen X. (2014) LTR-Retrotransposon activation, IRAP marker development and its potential in genetic diversity assesmentof masson pine (Pinus massoniana). Tree Genetics and Genomes10:213-222.Flavell A.J., Knox M.R., Pearce S.R& Ellis T.H.N.(1999) Retrotransposon-based insertion polymorphisms (RBIP) for high-throughput marker analysis. Plant Journal 16: 643-650.

[13] Ferheen S., Rasool M.A., Imran M., Fareed G., Afza N & Malik A. (2013) Capspinosin, a New Flavonoid from Capparis spinosa. Journal of Chemical Society of Pakistan 35(3):984-986.

[14] Ghafoor A & Ahmad Z. (2005) Diversity of agronomic traits and total seed protein in black gram Vigna mungo (L.) Hepper.Acta Biologica Cracoviensia Series Botanica 47(2):69-75.

[15] Grandbastien M.A. (1998) Activation of plant retrotransposons under stress conditions. Trends in Plant Science 3(5):181–187.

[16] Gribbon B.M., Pearce S.R., Kalendar R., Schulman A.H., Paulin L., Jack P.L., Kumar A & Flavell A.J. (1999) Phylogeny and transpositional activity of Ty1-copia group retrotransposons in cereal genomes. Molecular and General Genetics 261:883–891.

[17] Hermanutz L.A & Weaver S.E. (1996) Agroecotypes or phenotypic plasticity? Comparison of agrestal and ruderal populations of Solanum ptycanthum. Oecologia 105:271–280.

[18] Hill P., Burford D., Martin D.M.A & Flavell A.J. (2005) Retrotransposon populations of Vicia species with varying genome size. Molecular Genetics and Genomics 273:371–381

[19] Higton R.N & Akeroyd J.R. (1991) Variation in Capparis spinosa L. in Europe. Flora Europaea 106(2):104–112

[20] Huo H., Conner J.A & Ozias-Akins P. (2009) Genetic mapping of the apospory-specific genomic region in Pennisetum squamulatum using retrotransposon-based molecular markers. Theoretical and Applied Genetics 119:199–212.

[21] Iqbal M.S.,Ghafoor A.,Qureshi A.S & Ahmad Z. (2003) Genetic dissimilarities in cowpea (Vigna unguiculata (L.) Walp.) for protein peptides and their significance for quantitative traits loci. Pakistan Journal of Botany 35(3):377-386.

[22] Inocencio C., Cowan R.S., Alcaraz F., Rivera D & Fay M.F. (2005) AFLP fingerprinting in Capparis subgenus capparis related to the commercial sources of capers. Genet Resources and Crop Evolution 52:137–144.

[23] Inocencio C., Rivera D., Obo´n M.C., Alcaraz F & Barren˜a A. (2006) A systematic revision of Capparis section Capparis (Capparaceae). Annals of the Missouri Botanical Garden 93(1):122–149.

[24] Jacobs M. (1965) The genus Capparis (Capparaceae) from the Indus to the Pacific. Blumea 12(3):385–541.

[25] Kalendar R,. Grob T., Regina M., Suomeni A & Schulman A .(1999) IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques. Theoretical and Applied Genetics 98: 704–711.

[26] Kidwell M.G & Lisch D. (1997) Transposable elements as sources of variation in animals and plants. Proceeding ofthe National Academy of Science of the United States of America 94:7704–7711

[27] Kolano B., Bednara E & Weiss-Schneeweiss H .(2013) Isolation and characterization of reverse transcriptase fragments of LTR retrotransposons from the genome of Chenopodium quinoa (Amaranthaceae). Plant Cell Reports 32:1575–1588.

[28] Kumar A& Hirochika H.(2001) Applications of retrotransposons as genetic tools in plant biology. Trends in Plant Science6: 127–134.

[29] Kumar S., Sharma R., Kumar V.,Vyas G.K & Rathore A. (2013) Combining molecular-marker and chemical analysis of Capparis decidua (Capparaceae) in the Thar Desert of Western Rajasthan (India). Revista de Biologia Tropical 61(1):311-320.

[30] Lam S.K & Ng T.B. (2009)A protein with antiproliferative, antifungal and HIV-1 reverse transcriptase inhibitory activities from caper (Capparis spinosa) seeds. Phytomedicine 16(5):444-450

[31] Leigh F., Kalendar E.R., Lea E.V., Lee E.D., Donini E.P & Schulman A.H. (2003) Comparison of the utility of barley retrotransposon families for genetic analysis by molecular marker techniques.Molecular Genetics and Genomics 269: 464–474.

[32] Li X.,Islam S.,Yang H.A.,Ma W.J.&Yan G.J. (2013) Identification of chromosome regions controlling seed storage proteins of narrow-leafed lupin (Lupinus angustifolius). Journal of Plant Research 126(3):395-401.

[33] Ma Y., Sun H.Y., Zhao G.L., Dai H.Y., Gao X.Y., Li H & Zhang Z.H .(2008) Isolation and characterization of genomic retrotransposon sequences from octoploid strawberry (Fragaria ananassa Duch.). Plant Cell Reports 27:499–507

[34] Matthaus B & Özcan M. (2005) Glucosinolates and fatty acid, sterol, and tocopherol composition of seed oils from Capparis spinosa var. spinosa and Capparis ovata Desf. var. canescens (Coss.) Heywood. Journal of Agricultural and Food Chemistry 53:7137-7141.

[35] Nosrati H., Feizi M.A.H., Mazinani M. & Haghighi A.R. (2012) Effect of population size on genetic variation levels in Capparis spinosa (Capparaceae) detected by RAPDs. EurAsian Journal of BioScience 6:70–75.

[36] Özbek Ö & Kara A. (2013) Genetic variation in natural populations of Capparis from Turkey, as revealed by RAPD analysis. Plant Systematics and Evolution 299:1911–1933.

[37] Özcan M & Akgül A. (1998) Influence of species, harvest date and size on composition of capers (Capparis spp.) flower buds. Nahrung 42:102-105.

[38] Özcan M. (2005) Mineral composition of different part of Capparis ovata Desf. var. canescens (Coss.) Heywood growing wild in Turkey. Journal of Medicinal Food 8(3):405-407.

[39] Parisod C., Salmon A., Zerjal T., Tenaillon M., Grandbastien M.A & Ainouche M. (2009) Rapid structural and epigenetic reorganization near transposable elements in hybrid and allopolyploid genomes in Spartina. New Phytologist 184:1003–1015.

[40] Park J.M., Schneeweiss G.M & Weiss-Schneeweiss H. (2007) Diversity and evolution of Ty1-copia and Ty3-gypsy retroelements in the non-photo synthetic flowering plants Orobanche and Phelipanche (Orobanchaceae). Gene 387:75–86

[41] Paterson A.H, Bowers J.E., Bruggmann R.et al. (2009) The Sorghum bicolor genome and the diversification of grasses. Nature 457:551–556.

[42] Ren Q,X.,Chen W.,Zhao H.J.,Wu Z.B & Zhang H. (2012) Organic acids from Capparis spinosa fruit .Chemistry of Natural Compounds 48(5):868-869.

[43] Saifi N., Ibijbijen J & Echchgadda G. (2011) Genetic diversity of caper plant (Capparis ssp.) from North Morocco.Journal of Food Agriculture and Environment 9 (3-4):299-304.

[44] Sakçalı M.S., Bahadır H & Öztürk M. (2008) Eco-Physiology of Capparis spinosa L. : A plant suitable for combating desertification. Pakistan Journal of Botany 40(4):1481-1486.

[45] Shirasu K., Schulman A.S., Lahaye T & Schulze-Lefert P. (2000) A contiguous 66-kb barley DNA sequence provides evidence for reversible genome expansion. Genome Research 10:908–915.

[46] Smy´kal P., Bacˇova´-Kerteszova´ N., Kalendar R., Corander J., Schulman A.H & Pavelek M. (2011) Genetic diversity of cultivated flax (Linum usitatissimum L.) germplasm assessed by retrotransposon-based markers. Theoretical and Applied Genetics122:1385–1397.

[47] Tenaillon M.I., Hufford M.B., Gaut B.S & Ross-Ibarra J. (2011) Genome size and transposable element content as determined by highthroughput sequencing in maize and Zea luxurians. Genome Biology and Evolution 3:219–229.

[48] Venugopal Y., Ravindranth A., Kalpana G & Prabhakar P.R.(2011) Anti-tumor activity of Capparis sepiaria on Ehrlich Ascites Carcinoma in mice. International Journal of Biomedical Research 2:262-71.

[49] Vicient C.M., Kalendar R& Schulman A.H. (2001) Envelope-class retrovirus-like elements are widespread, transcribed and spliced, and insertionally polymorphic in plants. Genome Research 11:2041– 2049.

[50] Vyas G.K., Sharma R., Kumar V., Sharma T.B & Khandelwal V. (2009) Diversity analysis of Capparis decidua (Forssk.) Edgew. using biochemical and molecular parameters. Genetic Resources and Crop Evolution 56:905–911.

[51] Zia-Ul-Haq M., Cavar S., Qayum M., Imran I & de Feo V. (2011) Compositional Studies: Antioxidant and Antidiabetic Activities of Capparis decidua (Forsk.) Edgew. International Journal of Molecular Sciences 12(12):8846-8861.

[52] Zohary M. (1960) The species of Capparis in the Mediterranean and the Near Eastern Countries. Bulletin of the Research Council of Israel 8D: 49-64.