UDC 575
https://doi.org/10.2298/GENSR1802395U
Original scientific paper

GENETIC DIVERSITY OF TURKISH *Lathyrus* L. LANDRACES USING ISSR MARKERS

Hüseyin UYSAL [1*], Zeki ACAR[2], İlknur AYAN[2], Orhan KURT[2]

[1]Adnan Menderes University, Agricultural Faculty, Department of Agricultural Biotechnology, Aydin, Turkey
[2]Ondokuz Mayis University, Agricultural Faculty, Department of Field Crops, Samsun, Turkey

Uysal H., Z. Acar, İ. Ayan, O. Kurt (2018): *Genetic diversity of Turkish lathyrus L. landraces using ISSR markers*.- Genetika, Vol 50, No.2, 395-402.

Fifty-one *Lathyrus sativus* L. landraces and one *L. clymenum* L. landrace collected from Turkey and one *L. sativus* cultivar, Gürbüz, were evaluated with ISSR markers in this study to molecular characterization. Three ISSR primers were used and 45 DNA fragment were evaluated, of which 44 bands were polymorphic. The frequencies of scored bands ranged from 0.009 to 0.888 and averaged 0.363. The genotypes MLT04 and NEV02 had high similarity with 0.825 according to pairwise grouping. The furthest pairwise group was Gürbüz and MLT02 with 0.244. The nearest genotype to Gürbüz was CNR03 with 0.577. The pairwise genetic distance between *L. clymenum* and *L. sativus* ranged from 0.353 (accession NEV01) to 0.637 (accession DEN04) and pairwise genetic distance to the cultivar Gürbüz was 0.375. Assessment of genetic relationships among *Lathyrus* genotypes made two main groups. One of them covered only Gürbüz variety and the other covered 52 *Lathyrus* landrace. *L. sativus* and *L. clymenum* separated prominently in the second group.

Keywords: Fodder crop, Grass pea, ISSR, *Lathyrus*, genetic diversity

INTRODUCTION

The Fabaceae is the third largest family of flowering plants; the genus *Lathyrus*, which is a member of the Viciae tribe of Fabaceae family, is one of the largest genera in the family with about 160 annual and perennial species (CHTOUROU-GHORBEL *et al*., 2001; LEWIS *et al*., 2005; EMRE, 2009; CONTI, 2010). The *Lathyrus* L. genus is represented by 76 taxa at the level of species, subspecies and varieties and is separated into 10 sections in Turkey (DAVIS, 1970; ERTEKIN and SAYA, 1990). Agriculturally important *Lathyrus* species are *L.sativus, L. cicera, L.*

Corresponding author: Hüseyin Uysal, Adnan Menderes University, Agricultural Faculty, Department of Agricultural Biotechnology, Aydin, Turkey, e-mail: hüseyin.uysal@adu.edu.tr

ochrus, L. hirsutus, L. tigitanus, L. latifolius, L. sylvestris, L. clymenum. These have been used as forage and grain, principally for animal feed, expecting *L. sativus* which is used particularly as food for human.

The most cultivated *Lathyrus* species is *L. sativus* (Grass pea) which has many agricultural advantages. Grass pea has an amazing capability to survive under harsh environmental conditions (BASARAN *et al*., 2016). This drought tolerant annual leguminous crop is not only tolerant to poor soil conditions and to extremes in moisture content but also resistant to insect attacks (CAMPBELL, 1997; ONAR *et al.,* 2014). Also, *L. sativus* is preferred as a low input rotation crop after rice, as it is hardily tolerant to water logging.

Since Grass pea can withstand extreme environmental conditions and has been realized that can serve as a survival foodstuff in difficult situations. These abilities make it an interesting pulse crop and encourage for a thorough and extensive characterization of its germplasm (PARIHAR *et al.,* 2015). It is known in Turkish as "murdumuk" remained a marginal crop and is grown as forage, feed and rarely as food in Turkey to meet household consumption (BASARAN *et al*., 2016).

The systematic methodology especially based on morphology has been improved mainly by the incorporation of physiology, ecology or biochemical characters (EMRE, 2009). At first, interesting morphological and physiological characters were used in order to find genetic variations. Later on, chemical markers were used to find genetic variations, but in recent years the molecular markers have been used. It is generally believed that the use of molecular markers is more reliable and repeatable as compared to other methods. Molecular markers provide highly discriminatory information and, therefore, are frequently used for genetic studies (CEVIK *et al*., 2015). Some molecular characterization studies of grass pea has been made by using various molecular techniques which are RAPD (CROFT *et al.,* 1999; HANADA and HIRAI, 2000), RFLP and RAPD (CHTOUROU-GHORBEL *et al.,* 2001), AFLP (BADR *et al.,* 2002), ISSR (BELAID *et al.,* 2006), AFLP and SSR (LIOI *et al.,* 2011) to access genetic diversity of *Lathyrus* genus.

The objective of this study is to assess genetic diversity of 53 grass pea genotypes originating from Turkey. The grass pea genotypes, 51 *L. sativus* landraces cultivated in Turkey, one *L. sativus* cultivar and one *L. clymenum* landraces cultivar, were compared in terms of genetic variation to understand their relationship. Since repeated sequences are abundant throughout the plant genome, SSR primers anneal in several regions, typically giving a complex amplification pattern in which fragments are often polymorphic between different individuals (ZIETKIEWICZ *et al*., 1994; UYSAL *et al.,* 2010). ISSR markers have been widely applied to characterize plant germplasm and have demonstrated effectiveness in assessments of plant genetic diversity (UYSAL *et al*., 2010). ISSRs are dominant markers but they have the advantage of analyzing multiple loci in a single reaction (BARDAK and BOLEK, 2012).

MATERIALS AND METHODS

Plant Materials

A germplasm collection was made a total of 52 locations in 12 provinces, and the cultivar Gürbüz was added, so that a total of 53 *Lathyrus* genotypes were used in this study. A list of the collected germplasms used for this study is on Table 1. Germplasm collection was performed as seeds, and the seeds were sowed in Agricultural Faculty of Ondokuz Mayıs University's experimental area to observe morphological, phonotypical and agricultural characters. For

molecular analysis the seeds were germinated in controlled places to avoid insects and funguses. After the germinated plants were about 10 to 15 cm, five plants were harvested for each accession, frozen in liquid nitrogen, and stored at – 80°C until they were used.

Table 1. A list of collected Lathyrus landraces and cultivar used in this study

Sample No	Province	Sample codes	Species	Number of Sample
1	Elazıg	ELZ	*L. sativus*	3
2	Malatya	MLT	*L. sativus*	4
3	Adıyaman	ADI	*L. sativus*	2
4	Nevsehir	NEV	*L. sativus*	6
5	Burdur	BRD	*L. sativus*	3
6	Mugla	MUG	*L. clymenum*	1
7	Denizli	DEN	*L. sativus*	8
8	Usak	USK	*L. sativus*	16
9	Kutahya	KUT	*L. sativus*	1
10	Bursa	BUR	*L. sativus*	4
11	Cankırı	CNR	*L. sativus*	3
12	Samsun	SAM	*L. sativus*	1
13	-	GURBUZ	*L. sativus*	1

DNA Extractions

DNA extractions were done from harvested and frozen plants' young leaves. 100 mg young leaf samples were crushed in liquid nitrogen and transferred to 2 ml Eppendorf tubes. The DNA extraction was done by using QIAGEN DNA extraction kits according to its protocol. The DNA quantity and quality were checked with Nano Drop (Thermo 2000 UV Vis Spectrophotometer) followed by dilution to 5 $ng.\mu l^{-1}$. Extracted DNA samples were stored at + 4°C.

ISSR Analysis

DNA amplification was performed in a thermal cycler (Thermo Electron Cooperation Px2) in a 20 µl total volume containing 10 ng genomic DNA, 2.0 µl 1× PCR Buffer (Sigma), 1.5 mmol l^{-1} $MgCl_2$ (Sigma), 0.1 mmol l^{-1} dNTP mix (Sigma), 1U Taq polymerase (Sigma), 2% deionized formamide (Sigma) and 250 nmol l^{-1} UBC-ISSR primer. The following cycling program was applied: at 94°C denaturation for 5 min. 40 cycles at 94°C for 45 s, at 52°C annealing for 45 s and 72°C for 1 min; and a final elongation step at 72°C for 10 min and holding at 20°C. PCR products were separated in 2% (w/v) agarose gels plus 2 µl/100 ml ethidium bromide (Merck) in 1× TAE by electrophoresis at 100 V for 3 h and the gel digitally photographed.

Three ISSR primer pairs were used for the study (Table 2). Before the primers were selected, the ISSR primers were screened on four randomly selected DNA samples and the three primers generated the large number of polymorphic bands used in this study. The gels were scored for each band as presence (1), absence (0), or uncertain (9) in each sample. A few gels were scored twice by two individuals to verify and to minimize possible scoring errors.

Data Analysis

The presence/absence ISSR data were analyzed for the level of polymorphism by counting the total number of bands and the number of polymorphic bands (NPB), calculating the proportion of polymorphic bands (PPB), and generating mean band frequencies (MBF) with respect to primer and accession. To determine the genetic relationship of 53 *Lathyrus* genotypes accessions, the inter-accession genetic distance matrices were analyzed using NTSYS-pc 2.02 (ROHLF, 1998) and clustered with the algorithm of unweighted pair-group method using the arithmetic average (UPGMA).

RESULTS AND DISCUSSIONS

The 3 primer pairs applied in this study generated a total of 45 DNA fragments across 265 plant samples, ranging between 0.23 and 1.28 kilo bases (kb), of which 44 bands were polymorphic (Table 2). The number of polymorphic bands for one primer pair was 13 for UBC848, 15 for UBC835, and 16 for UBC841, averaging 14.7. The mean frequencies of scored bands detected by one primer pair were 0.284 for UBC835, 0.374 for UBC841, and 0.441 for UBC848, averaging 0.363; the range of band frequencies was 0.009 to 0.888.

Table 2. Variation of ISSR markers observed in 265 Lathyrus genotypes for 3 ISSR primer pairs

Primers	Seq. of the primers	NPB	DNA size (kb)		Frequency of the scored bands		
			Min.	Max.	Mean	Min.	Max.
UBC835	$(AG)_8YC$	15	0.30	1.20	0.284	0.009	0.858
UBC841	$(GA)_8YC$	16	0.23	1.10	0.374	0.043	0.839
UBC848	$(CA)_8RG$	13	0.30	1.28	0.441	0.039	0.888
All		44	0.23	1.28	0.363	0.009	0.888

BELAID *et al*. (2006) made a molecular characterization in 5 populations of *Lathyrus* species by using 10 plants each accession and using 4 ISSR primers. The primers generated 60 polymorphic bands. GEDIK (2007) made a molecular characterization in 10 *Lathyrus* genotypes by using 2 plants for each accession and used 10 ISSR primers. The primers generated in total 55 bands and of which 49 were polymorphic. We gained more polymorphic bands than those made by Gedik. This result indicated that to increase variations we must increase the number of accessions and the number of plants for each accession in the molecular study. Gedik also used cultivar Gürbüz in her study and she determined clear differences between Gürbüz and the other accessions, similar to our study.

Genotype-specific variation was also substantial (Table 3). The mean band frequency ranged from 0.180 (Gürbüz) to 0.621 (USK10) and averaged 0.380. The genotype specific proportional variation ranged from 0.063 (DEN05) to 0.769 (USK05).

Pairwise genetic distances among *Lathyrus* genotypes ranged from 0.244 (between Gürbüz and MLT02) to 0.825 (between MLT04 and NEV02) and averaged 0.592 (detailed result not shown). According to these results the closest genotypes were MLT04 and NEV02. The closest genotype to Gürbüz was CNR03 with 0.577 and the farthest genotype to Gürbüz was MLT02 with 0.244. The pairwise genetic distances among landraces and cultivar (Gürbüz) of *L. sativus* averaged 0.432, if it is analyzed excluding *L. clymenum* (MUG01). *L. clymenum*

genotype (MUG01) was also far from the other *L. sativus* genotypes. Their pairwise genetic distance ranged from 0.353 (NEV01) to 0.637 (DEN04). Therefore the closest *L. sativus* genotype to *L. clymenum* was DEN04. If we analyzed the data excluding Gürbüz, the pairwise genetic distances among landraces of *L. sativus* and *L. clymenum* averaged 0.458. The pairwise genetic distance the cultivar (Gürbüz) and *L. clymenum* was 0.375.

Table 3. ISSR frequencies of the genotypes

Genotype	Frequency			Genotype	Frequency		
	Mean	Min.	Max.		Mean	Min.	Max.
ELZ01	0.355	0.227	0.477	USK01	0.292	0.097	0.452
ELZ02	0.329	0.214	0.455	USK02	0.415	0.379	0.462
ELZ03	0.315	0.172	0.386	USK03	0.428	0.355	0.517
MLT01	0.391	0.364	0.429	USK04	0.273	0.226	0.321
MLT02	0.303	0.207	0.393	USK05	0.416	0.258	0.769
MLT03	0.395	0.341	0.500	USK06	0.350	0.273	0.393
MLT04	0.409	0.318	0.477	USK07	0.399	0.318	0.615
ADI01	0.356	0.227	0.432	USK08	0.321	0.241	0.538
ADI02	0.463	0.387	0.568	USK09	0.310	0.250	0.393
NEV01	0.367	0.194	0.568	USK10	0.621	0.409	0.714
NEV02	0.383	0.188	0.477	USK11	0.523	0.477	0.545
NEV03	0.355	0.318	0.386	USK12	0.499	0.290	0.682
NEV04	0.260	0.069	0.568	USK13	0.618	0.545	0.682
NEV05	0.555	0.455	0.614	USK14	0.421	0.295	0.568
NEV06	0.450	0.250	0.682	USK15	0.376	0.172	0.591
BRD01	0.430	0.379	0.500	USK16	0.333	0.188	0.432
BRD02	0.473	0.386	0.571	KUT01	0.306	0.143	0.523
BRD03	0.348	0.295	0.432	BUR01	0.438	0.386	0.500
MUG01	0.391	0.250	0.523	BUR02	0.344	0.250	0.386
DEN01	0.450	0.341	0.568	BUR03	0.505	0.455	0.538
DEN02	0.459	0.386	0.523	BUR04	0.310	0.205	0.409
DEN03	0.421	0.227	0.545	CNR01	0.218	0.161	0.318
DEN04	0.492	0.432	0.552	CNR02	0.315	0.258	0.448
DEN05	0.270	0.063	0.523	CNR03	0.205	0.136	0.273
DEN06	0.302	0.103	0.387	SAM01	0.290	0.214	0.379
DEN07	0.333	0.227	0.432	Gürbüz	0.180	0.114	0.241
DEN08	0.378	0.207	0.538	**All**	**0.380**	**0.063**	**0.769**

Pairwise mean genetic distances among *Lathyrus* genotypes showed that the Gürbüz cultivar were closer to *L. sativus* genotypes than to the genotype of *L. clymenum*. However, some genotypes of *L. sativus* were farther from cultivar Gürbüz than genotypes *L. clymenum*. This result may be due to geographical differences. We can say that with some exceptions there is a relation between similarity of genotypes and geographical origin. For this reason, genotypes of *L. sativus* grouped according to geographic range in the dendrogram (Figure 1) in this study.

According to these results, we can easily say that probably there has been seed exchange among the farmers living in the same region, nearby, or in some cases, in regions some distance away.

Assessment of genetic relationships among *Lathyrus* genotypes grouped them according to species (Figure 1). Cultivar Gürbüz was one group (G1) and *L. clymenum* (MUG01) was another group (G2), and the others were included within the largest group. The largest group comprised a total of seven sub-groups. The sub-groups were largely grouped according to geographic origins of landraces. For example the largest sub-group G7 consisted of the materials from Aegean Regions, the second largest sub-group G10 was the materials from Turkey's Southeast Region.

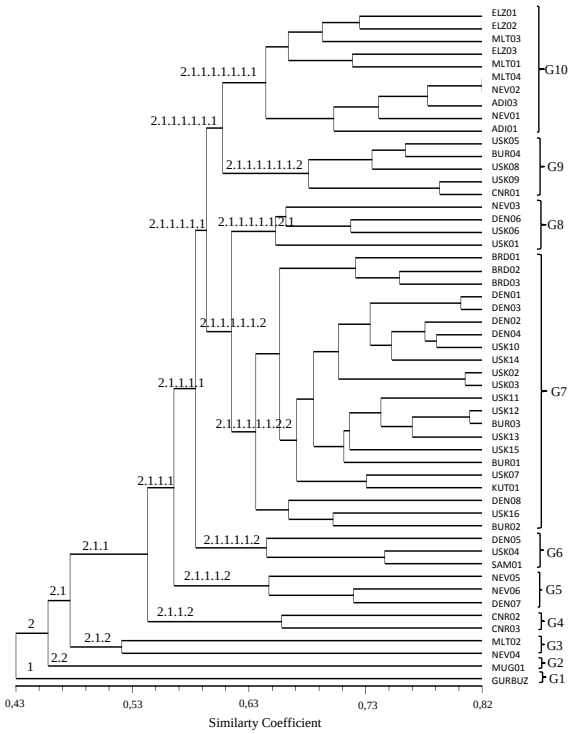

Figure 1. Genetic relationship of 53 *Lathyrus* genotypes based on the genetic distances obtained from the analysis of molecular variance for 44 polymorphic ISSR bands (G: Group)

CONCLUSION

The origin of *L. sativus* is unknown (SAMMOUR, 2007); however its presumed center of origin is South and Central Asia (SMART, 1990), Mediterranean and Central Asia (DUKE, 1981) or Central Asia and Abyssinia (VAVILOV, 1951). Our data is based on molecular analysis reported for *Lathyrus* species originating in Turkey. This study provides some ideas for researchers in making molecular characterizations. Additional populations from *L. sativus* and other *Lathyrus* sections from Turkey and other Asian countries must be represent, and other

molecular markers must be used in future studies to determine *Lathyrus* origin. In addition, morphological and phonotypical characterization must be done.

ACKNOWLEDGMENTS

The authors would like to thank Charles E. Fiero for editing this article. This research was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) (Grant no: TOVAG-110O316).

Received, April 04th, 2017
Accepted February18th, 2018

REFERENCES

BADR, A., H. EL SHAZLY, H. EL RABEY, L.E. WATSON (2002): Systematic relationships in *Lathyrus* sect. *Lathyrus* (Fabaceae) based on amplified fragment length polymorphism (AFLP) data. Can. J. Bot., *80*:962-969.

BASARAN, U., H. MUT, E. GULUMSER, M. COPUR DOGRUSÖZ (2016): Evaluation of Turkish grass pea (*Lathyrus sativus* L.) collections for its agronomic characters with a special reference to ODAP content. Legume Research, *39*(6):876-882.

BARDAK, A., Y. BOLEK (2012): Genetic diversity of diploid and tetraploid cottons determined by SSR and ISSR markers. Turk. J. Field Crops, *17*(2):139-144.

BELAID, Y., N. CHTOUROU-GHORBEL, M. MARRAKCHI, N. TRIFIIFARAH (2006): Genetic diversity within and between populations of *Lathyrus* genus (*Fabaceae*) revealed by ISSR markers. Gen. Res. Crop. Evol., *53*: 1413-1418.

CAMPBELL, C.G. (1997): Grass pea, *Lathyrus sativus* L. promoting the conservation and use of underutilized and neglected crops. Nr 18. Rome, Italy: Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany/International Plant Genetic Resources Institute, p. 92.

CHTOUROU-GHORBEL, N., B. LAUGA, D. COMBES, M. MARRAKCHI (2001): Comparative genetic diversity studies in the genus *Lathyru*s using RFLP and RAPD markers. Lathyrus and Lathyrism Newsletter, *2*: 1-7.

CONTI, F. (2010): A new species of *Lathyrus* L. (*Fabaceae*) from Central Apennine (Italy), Plant Biosystems- An International Journal Dealing with all Aspects of Plant Biology: Official Journal of the Societa Botanica Italiana, *144*:4, 814-818.

CROFT, A.M., E.C.K. PANG, P.W.J. TAYLOR (1999): Molecular analysis of *Lathyrus sativus* L. (grasspea) and related *Lathyrus* species. Euphytica, *107*:167-176.

CEVIK, S., S. UNYAYAR, A. ERGUL (2015): Genetic relationships between cultivars of *Cicer arietinum* and its progenitor grown in Turkey determined by using the SSR markers. Turk. J. Field Crops, *20*(1):109-114.

DAVIS, P.H. (1970): Flora of Turkey and East Aegean Islands. Edinburgh, Vol. *3*: 328-369.

DUKE, J.A. (1981): Handbook of legumes of world economic importance. Plenum, New York.

EMRE, I. (2009): Electrophoretic analysis of some *Lathyrus* L. species based on seed storage protein. Gen. Res. Crop. Evol., *56*:31-38.

ERTEKIN, S.A., O. SAYA (1990): A new record for the flora of Turkey. Turk. J. Bot., *15*(1):75-77

GEDIK, A. (2007): Research on the determination of morphologıcal and molecular dıversity among some varieties, lines and cultivar of grasspea (*Lathyrus sativus* L.). M. Sc. Thesis, Çukurova University, Institute of Natural and Applied Sciences, Adana.

HANADA, H., M. HIRAI (2000): Classification of sweet pea (*Lathyrus odoratus* L.) and everlasting pea (*Lathyrus latifolium* L.) by random amplified polymorphic DNA (RAPD) analysis. J. Jpn. Soc. Hort. Sci., *69*:758-763.

LEWIS, G., B. SCHRIRE, B. MACKINDER, M. LOCK (2005): Legumes of the world. Royal Botanic Gardens, Kew.

LIOI, L., F. SPARVOLI, G. SONANTE, G. LAGHETTI, F. LUPO, M. ZACCARDELLI (2011): Characterization of Italian grasspea

(*Lathyrus sativus* L.) germplasm using agronomic traits, biochemical and molecular markers. Gen. Res. Crop. Evol., *58*:425-437.

ONAR, A.N., B. YAVUZ ERDOĞAN, A. AYAN, Z. ACAR (2014): Homoarginine, b-ODAP, and asparagine contents of grass pea landraces cultivated in Turkey. Food Chemistry, *143*:277-281.

PARIHAR, A.K., G.P. DIXIT, D. SINGH (2015): Genetic variability analysis for quantitative traits in a germplasm set of grasspea (*Lathyrus* spp.). Legume Research, *38*(4):461-464.

ROHLF, F.J. (1998): NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.02e. Setauket: Applied Biostatistics Inc., Exeter Software.

SAMMOUR, R., A.Z. MUSTAFA, S. BADR, W. TAHR (2007): Genetic variations in accession of *Lathyrus sativus* L. Acta Bot. Croat., *66*(1):1-3.

SMARTT, J. (1990): Grain Legumes: Evolution and genetic resources. Cambridge University Press, Cambridge.

UYSAL, H., Y.B. FU, O. KURT, W.G. PETERSON, A. DIEDERICHSEN, P. KUSTERS (2010): Genetic diversity of cultivated flax (*Linum usitatissimum* L.) and pale flax (*Linum bienne* Mill.) as revealed by ISSR markers, Gen. Res. Crop. Evol., *57*(7):1109-1119.

VAVILOV, N.I. (1951): The origin, variation, immunity and breeding of cultivated plants. Chronica Bot., *13*: 13-47.

ZIETKIEWICZ, E., A. RAFALSKI, D. LABUDA (1994): Genome Fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification, Genomics, *20*: 176-183.

GENETIČKI DIVERZITET TURSKIH *Lathyrus* L. LOKALNIH POPULACIJA UPOTREBOM ISSR MARKERA

Hüseyin UYSAL [1*], Zeki ACAR[2], İlknur AYAN[2], Orhan KURT[2]

[1]Adnan Menderes Univerzitet, Poljoprivredni Fakultet, Departman za poljoprivrednu biotehnologiju, Aydin, Turska
[2]Ondokuz Mayis Univerzitet, Poljoprivredni fakultet, Departman za ratarstvo, Samsun, Turska

Izvod

Lokalne populacije *Lathyrus sativus* L. (ukupno 51) i jedna *L. clymenum* L. sakupljene u Turskoj, kao i jedan kultivar *L. sativus*, Gürbüz, ocenjene su ISSR markerima. Korišćena su tri ISSR prajmera za ocenu 45 DNK fragmenata, kod kojih su 44 trake bile polimorfne. Frekvencija ocenjivanih traka bila je u opsegu od 0.009 do 0.888, u proseku 0.363. Genotipovi MLT04 i NEV02 ispoljili su visoku sličnost. Najudaljeniji par bio je Gürbüz i MLT02, sa 0.244. Najbliži genotip Gürbüz-u bio je CNR03 sa 0.577. Genetička distance između *L. clymenum* i *L. sativus* bila je od 0.353 (uzorak NEV01) do 0.637 (uzorak DEN04), a genetička distance sa kultivarom Gürbüz bila je 0.375. Procenom genetičkih relacija između *Lathyrus* genotipova dobijene su dve grupe: jedna predstavlja samo varijetet Gürbüz, dok druga obuhvata 52 *Lathyrus* lokalne populacije. *L. sativus* i *L. clymenum* su se jasno odvojile u drugu grupu.

Primljeno 10.IV.2017.
Odobreno 18. II. 2018.