J. Agr. Sci. Tech. (2016) Vol. 18: 1105-1115

The *rbcL* Gene Sequence Variations among and within *Prunus* Species

S. Sarhan[1*], F. Hamed[2], and W. Al-Youssef [3]

ABSTRACT

The objective of this study was to detect the level of SNP variations of *rbcL* gene sequences among and within *Prunus* species including 17 locally cultivated and wild relatives of *Prunus,* and two species of the subfamily *Maloideae* (*Malus domestica* and *Pyrus communis*), as out groups. The *rbc*L sequences were amplified, sequenced, and aligned to determine Single Nucleotide Polymorphisms (SNPs). The *rbcL* gene tree of the samples showed two main clusters. The first included the outgroup taxa (*M. domestica* and *P. communis*); and all *Prunus* samples in the second cluster including *Prunus armeniaca*, which separated in a subcluster. Our results indicate that *rbcL* gene sequence analysis provides a well-defined tool to study relationships within and among *Prunus* species, and can be successfully used in constructing reliable phylogenetic tree for *Prunus* accessions.

Keywords: Genetic relationship, *Prunus*, *rbcL*, Sequencing.

INTRODCTION

The *Rosaceae* family contains about 100 genera and 3,000 species (Judd *et al*., 1999), *Prunus* is the largest genus in the subfamily *Prunoideae* (*Amygdaloideae*) including *about* 250 species (Lee and Wen, 2001), Peach (*Prunus persica* L.) and almond (*Prunus dulcis* Mill.; syn. *P. amygdalus* Batsch) are two commercially grown species that belong to the *Prunus* genus (Rehder, 1940). The two species have originated in Southeast and Central Asia, respectively (Watkins, 1979), while the wild relatives of *Prunus* were found growing from eastern China to the Mediterranean Sea (Browicz and Zohary, 1996). According to Rehder (1940), genus *Prunus* is divided into sub-genera of *Prunophora* (*Prunus*), *Amygdalus, Padus, Cerasus* and *Laurocerasus*. Other authors consider them as a separate genus (McVaugh, 1951). The classification of *Prunus* species is complicated, since closely related taxa often differ by only a single morphological character (Shi *et al*., 2013). In addition, typical identification requires reproductively mature material that may be available for only a short period of the year (Julian *et al*., 2009).

Therefore, molecular markers became necessity to study phylogenetic relations of *Prunus* genus. The Single Nucleotide Polymorphism (SNP) and small Insertion and Deletion (InDels), are the most forms of genetic variations in natural populations. They reflect the results of evolution and adaptation (Yamanaka *et al*., 2004; Wright *et al*., 2005). They are frequently used in modern genetics for reverse genetics, linkage analysis, genome-wide association study, genotyping and markers assisted selection. In addition, SNP markers were developed to identify plant diseases resistance (Bakooie *et al.,* 2015).

[1] Department of Essential Sciences, Faculty of Agriculture, Damascus University, Damascus, Syria.
[*]Corresponding author; e-mail:engseba1978@gmail.com
[2] Department of Biotechnology, General Commission for Scientific Agricultural Researches, Syria.
[3] Department of Horticultural Science, Faculty of Agriculture, Damascus university, Damascus, Syria.

[DOR: 20.1001.1.16807073.2016.18.4.4.8]

Several investigations were carried out to assess genetic variation and phylogenetic relationship among and within *Prunus* species using isozyme, RFLPs, RAPDs, SSRs and AFLP (Martı´nez-Go´mez *et al*., 2003; Mowrey and Werner, 1990). The use of SNPs for genetic fingerprinting, parentage verification, and gene mapping was recommended to be applied in the study of genetic diversity of sweet cherry (*Prunus avium*) and was also proved to be useful in other related species within *Amygdaloideae* (Marti *et al*., 2012). At present, techniques for studying the molecular phylogeny and taxonomy of plants rely heavily on chloroplast genome sequence data. This is because the chloroplast genome is haploid with simple and stable genetic structure, where no or very rare recombinations take place, and universal primers can be used to amplify target sequences. In addition, the ease of PCR amplification and sequencing of chloroplast genes using universal primers facilitates phylogeny projects. The chloroplast DNA restriction sites were used to construct the phylogeny of eight cultivated members of *Prunus* (Badenes and Parfitt, 1995). On the other hand, sequences of chloroplast regions of *rbcL, matK*, *trnL/F*, *18S rDNA*, and *ITS* have been used in studies of *Rosaceae* and *Prunus* phylogeny (Morgan *et al*. 1994; Potter *et al*., 2001,2003, 2007; Bortiri *et al*., 2001, 2006). Some of these regions were also shown to be informative within subfamilies such as *Amygdaleae* (Lee and Wen, 2001; Potter *et al*., 2002), and *Maloideae* (Potter *et al.,* 2007).

The Ribulose-Bisphosphate Carboxylase/Oxygenase (RuBisCO) is the enzyme that facilitates the primary CO_2 fixation step in the Calvin cycle. The quaternary structure of the enzyme consists of 8 large and 8 small subunits. Sequences of the *rbcL* large subunit have been used to elucidate higher taxonomic relationships in the angiosperms (Olmstead *et al*., 1992; Chase *et al*., 1993; Qiu *et al*., 1993). The large size (more than 1,400 bp) of *rbcL* provides many characters that can be utilized in phylogenetic analysis. Additionally, the availability of conserved primers allow for rapid amplification and sequencing. The slow synonymous nucleotide substitution rate in chloroplast DNA (cpDNA) compared to nuclear genes, is another reason for the utility of *rbcL* in angiosperm phylogeny studies.

The first suggestions that *rbcL* gene sequence was appropriate in phylogenetic studies were from Ritland and Clegg (1987) and Zurawski and Clegg (1987), and small scale phylogenetic studies based on *rbcL* sequences were followed (Doebley, 1990; Kim *et al.,* 1992). However, the first collaborative large-scale phylogenetic analysis using *rbcL* sequence data for a broad sampling of seed plants was conducted by Chase *et al*., (1993). The *rbcL* gene was also widely used in phylogeny studies of *Prunus* species (Morgan *et al.,* 1994; Potter *et al*., 2007; Quan and Zhou, 2011), and was found to be useful for identifying variations among and within the genera and species.

The purpose of this study was to detect the level of *rbcL* sequence variations among *Prunus* species and to produce a phylogeny of genetic tree relationships among *Prunus* species based on *rbcL* gene sequence.

MATERIALS AND METHODS

Plant Material

Plant samples investigated in this study (Table 1) were provided by Cergaya and Hott Agricultural Stations which belong to the General Commission for Scientific Agricultural Researches in Syria. The samples comprised five local varieties of almond (*Prunus dulcis*), seven varieties of peach (*Prunus persica*), two genotypes of the wild relatives of *Prunus orientalis*, *Prunus korshinskyi*, Hybrid GF-677 (*Prunus dulcis*♀×*Prunus persica*♂), one local sample of *Prunus armeniaca,* which belongs to the subgenus *Amygdalus*, one variety of each of *Malus domestica* and *Pyrus*

[DOR: 20.1001.1.16807073.2016.18.4.4.8]

Table 1. Investigated *Prunus* species and varieties and out group samples and their codes in this study.

Species	Varieties name	Origin	Code
Almonds	Shami Furk	Local	P.d _sh
Prunus dulcis	Hama34	Local	P.d_34
	Hama47	Local	P.d_47
	Babnis	Local	P.d_bb
	Oja	Local	P.d_oja
P. persica (L.) Batsch	Gioia	Italy	P.p_gi
Peach and Nectarine	Venus	Italy	P.p_ve
	Caldesi2000	Italy	P.p_ca
	Nectaross	Italy	P.p_ne
	Laure	Italy	P.p_la
	May Crest	Italy	P.p_ma
	Pontina	Italy	P.p_po
Prunus armeniaca. L	Klabe	local	P.a_kl
Prunus korshinskyi	(Rootstock)	local	P.kor
Prunus orientals. Mill	(Rootstock)	Local	P.ori1
Prunus orientals	(Rootstock)	Local	P.ori2
Hybrid GF-677	(Rootstock)	Italy	GF-677
Malus domestica	Starking		M.d_st
Pyrus communis	Koshi		P.c_ko

communis (from the sub-family *Maloideae)* as out groups.

DNA Extraction, PCR Amplification, Electrophoresis and Sequencing

Young half folded leaves were used for total genomic DNA extraction based on the modified CTAB method (Doyle and Doyle, 1987). Primer pair used in PCR amplification were described by (Shokralla *et al*., 2010) to amplify about 0.6 Kbp of *rbcL* gene were as follow:

rbcLaF:
$^{5'}$ATGTCACCACAAACAGAGACTAAAG C$^{3'}$

rbcLaR:
$^{5'}$GTAAAATCAAGTCCACCRCG$^{3'}$.

A total volume of 15 µL of PCR reaction mixture contained the following: 0.15 µL of 5 U μL^{-1} Hot start *Taq* DNA Polymerase (Takara-bio, Japan), 0.25 µL of 10 mM dNTPs and 0.5 µL of 50 mM $MgCl_2$, 0.5 µL of each primer (10 pmol) (Vienna Biotech, Austria), 1.5 µL of 10X PCR buffer, 5 µL of 1 ng μL^{-1} DNA sample, and sterile distilled water to adjust to final volume. PCR amplification was performed with a 96 well Veriti thermal cycler (Applied Biosystems, USA) as follows: 95°C for 5 minutes, followed by 40 cycles of 95°C for 1 minute, 60°C for 1 minute and 72°C for 1 minute, and a last elongation step at 72°C for 10 minutes. One microliter of each of the amplified PCR products was tested on 1.5% agarose gel stained with Ethidium Bromide, and visualized under UV for detecting the amplification efficiency.

PCR products were purified with the QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced using the same primers by ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA).

Data Analysis

The obtained sequences were subjected to BLASTn analysis by NCBI server (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to detect homology of the target gene and species. Editing and assembling of

[DOR: 20.1001.1.16807073.2016.18.4.4.8]

sequences were conducted using BioEdit Sequence Alignment editor (Hall, 1999). Phylogenetic and molecular evolutionary analyses were investigated using MEGA version 5 (Tamura *et al*., 2011). Bootstrap analyses were used to assess the robustness of the tree with 1,000 replicates (Felsenstein, 1985).

RESULTS

The primers were used on the DNAs of 19 samples, which showed complete amplifications with a clear band 600 bp which matched the amplified amplicon size, in all samples (Figure 1).

To confirm results and identify the SNPs, sequencing of *rbcL* was conducted in all samples. The SNPs found in our work were both transversion, the substitution of a (two ring) purine for a (one ring) pyrimidine (A/T) and (T/G), and transition, a point mutation that changes a purine nucleotide to another purine (A ↔ G). Among *P. armeniaca* and all other samples there was one transition (A/G), whereas *P.dulcis* samples showed a transversion SNP (A/T)

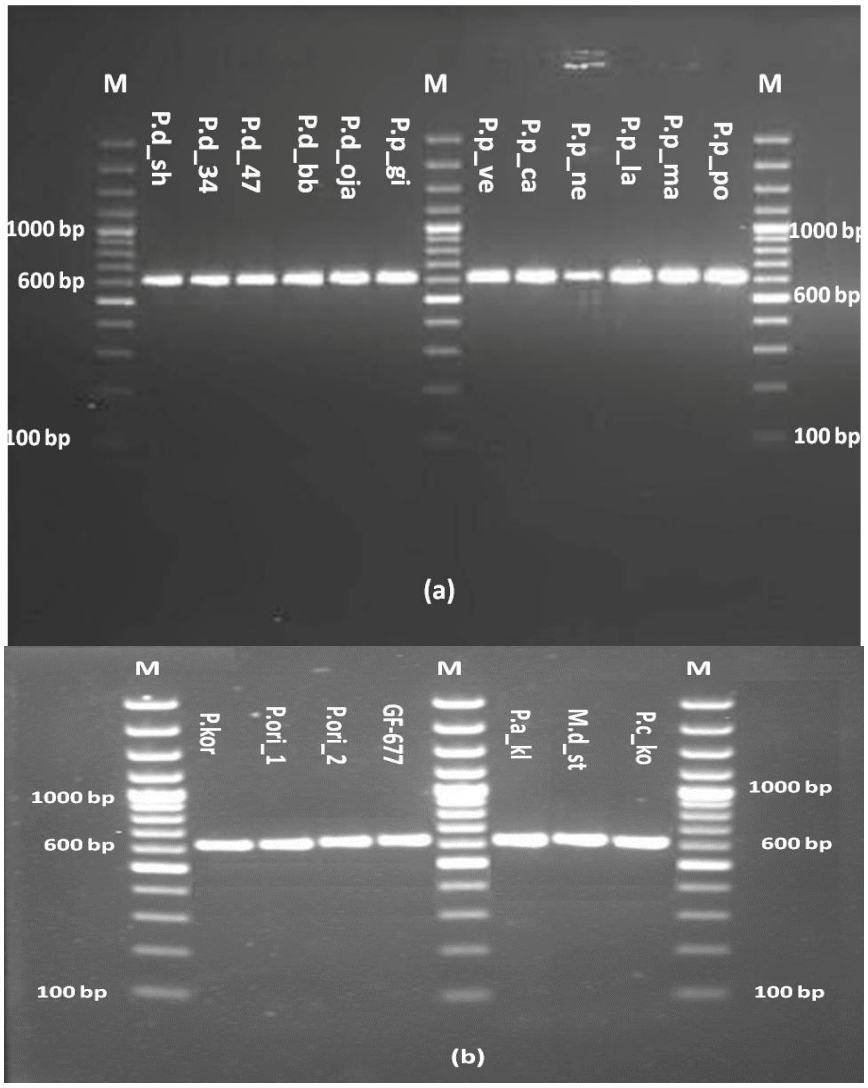

Figure 1. An example for PCR amplification using primer pair of *rbcL* F and *rbcL* R. (M) Molecular weight markers; (a) Samples from *P. dulcis* and *P. persica, and* (b): *Prunus* sp. and out group samples.

[DOR: 20.1001.1.16807073.2016.18.4.4.8]

compared with the rest of the samples, and another clear transversion (T/G) appeared between all *Prunus* sp. and outgroup samples (Figure 2).

The computed nucleotide pair frequencies among all samples showed the presence of three SNPs two transversions and one transition. This data analysis also showed the highest nucleotide changes in *P. armeniaca* when compared to the rest of *Prunus* input sequences. However, the analysis showed no nucleotide substitution within each of *P. persica* and *P. dulcis* (Table 2).

The average of the pairwise distance was 0.002, pairwise distance values within each of the group *of P. Persica* and the group of *P. dulcis* and the wild relatives including hybrid GF-677 was zero, whereas the value 0.002 was between *P. persica* and all other samples of *P. dulcis* and wild relatives, including hybrid GF-677. The pairwise distance between *P. armeniaca* and *P. persica* samples was 0.005. The

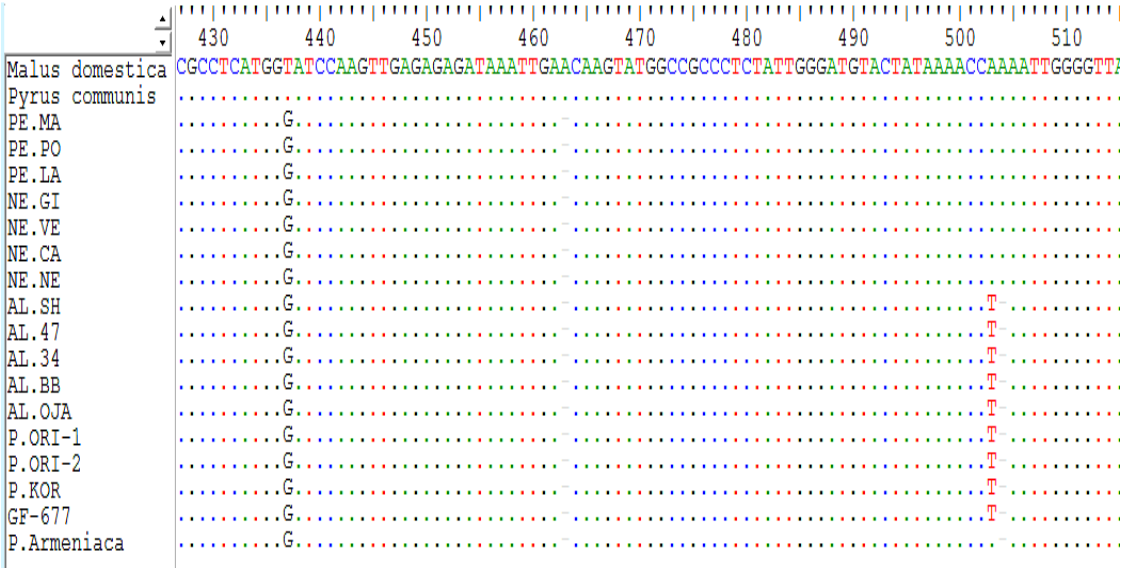

Figure 2. Partial samples of multiple sequence alignment of *rbcL* gene sequences of the samples sequenced (SNPs are indicated).

Table 2. Nucleotide pair frequencies among the studied sequences of the samples.[a]

Samples	II	SI	SV	R	Total
Within all samples	8.00	1.00	1.00	0.61	10.00
P. armeniaca and the rest of *Prunus* sp.	3.00	1.00	0.00	0.36	4.00
Wild relatives and *P. persica*	0.00	0.00	1.00	0.00	1.00
Wild relatives and *P. dulcis*	0.00	0.00	0.00	nc	0.00
Hybrid GF-677 and *P. dulcis*	0.00	0.00	0.00	nc	0.00
Hybrid GF-677 and *P. persica*	1.00	0.00	0.00	0.00	1.00
Within *P. persica*	0.00	0.00	0.00	nc	0.00
Within *P. dulcis*	0.00	0.00	0.00	nc	0.00
Within wild relatives	0.00	0.00	0.00	nc	0.00
P. dulcis and *P. persica*	0.00	0.00	1.00	0.00	1.00

[a] II= Identical DNA nucleotide pairs; SI: Transitional Pairs; SV= Transversional Pairs, R= SI/SV. All frequencies are averages (rounded) over all samples. Analysis conducted in MEGA5 (Tamura *et al*., 2011).

[DOR: 20.1001.1.16807073.2016.18.4.4.8]

corresponding value between *P. armeniaca* and all samples of *P. dulcis,* and wild relatives, including the hybrid GF-677, was 0.007. The highest value of 0.017 was recorded between *Malus domestica* and the samples of *P. dulcis*, and wild relatives, including the hybrid GF-677 (Table 3).

The aligned sequences were used to build a phylogenetic tree using the UPGMA method (Figure 3). Results indicated the presence of two main clusters. The smaller cluster contained plant species *Malus domestica* and *Pyrus communis*, species of subfamily *Pomoideae,* the other samples of *Prunus* genus (subfamily *Prunoideae*) were joined in the bigger cluster. The second main cluster was divided into two further subclusters of *P. armeniaca (*subgenus *Prunus)* alone, and all other samples of *P. persica, P. dulcis.* Wild relatives (subgenus *Amygdalus*) including Hybrid GF-677 joined together in another sub cluster at a high bootstrap value of 95. The subcluster of subgenus *Amygdalus,* was divided into two branches, the first one contained all *P. dulcis* samples and wild relatives with hybrid GF-677, the second clad contained *P. persica* samples (Figure 3).

Table 3. Pairwise distance obtained using the maximum composite likelihood mode.[a]

	M.d_st	P.c_ko	P.p_ma	P.p_p	P.p_la	P.p_g	P.p_ve	P.p_ca	P.p_ne	P.d_s	P.d_47	P.d_34	P.d_bb	P.d_oja	P.ori1	P.ori2	P.kor	Gf-677	P.a_kl
M.d_st	0.000																		
P.c_ko	0.004	0.000																	
P.p_ma	0.015	0.013	0.000																
P.p_p	0.015	0.013	0.000	0.000															
P.p_la	0.015	0.013	0.000	0.000	0.000														
P.p_g	0.015	0.013	0.000	0.000	0.000	0.000													
P.p_ve	0.015	0.013	0.000	0.000	0.000	0.000	0.000												
P.p_ca	0.015	0.013	0.000	0.000	0.000	0.000	0.000	0.000											
P.p_ne	0.015	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000										
P.d_s	0.017	0.015	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.000									
P.d_47	0.017	0.015	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.000								
P.d_34	0.017	0.015	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.000	0.000							
P.d_bb	0.017	0.015	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.000	0.000	0.000						
P.d_oja	0.017	0.015	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.000	0.000	0.000	0.000					
P.ori1	0.017	0.015	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.000	0.000	0.000	0.000	0.000				
P.ori2	0.017	0.015	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
P.kor	0.017	0.015	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
Gf-677	0.017	0.015	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
P.a_kl	0.013	0.011	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.000

[a] All positions with less than 95 % site coverage were eliminated, analyses were conducted in MEGA5 (Tamura *et al*., 2011).

DISCUSSION

Our study took the advantage of using *rbcL* gene, taking into consideration that the coding *rbcL* gene is easily amplified and sequenced in most land plants and has an impact in phylogeny investigations by providing a reliable placement of a taxon into a plant family and genus (Kress and Erickson, 2007; Gyulai *et al*., 2012).

Sequences of *rbcL* marker showed several genetic differences among samples. The interspecific genetic diversity was lower than the intraspecific one, that was in contrary with Mattia *et al.* (2011) in their previous research on *Lamiaceae* member. Two SNPs in the *rbcL* region were detected between the three commercial oregano and the other analyzed samples. Whereas, the sequencing of the chloroplast gene *rbcL* of

[DOR: 20.1001.1.16807073.2016.18.4.4.8]

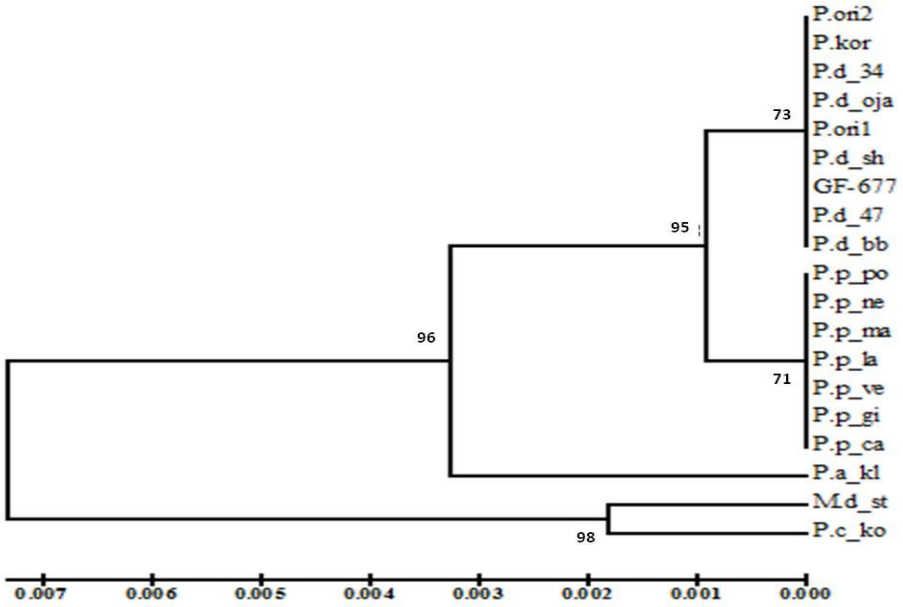

Figure 3. UPGMA dendrogram of all samples (*Prunus, Pyrus* and *Malus*) using the Maximum Composite Likelihood method. Analyses were conducted in MEGA5 (Tamura *et al*., 2011). Values presented bootstraps with 1,000 replicates.

Eryngium spp. showed the presence of three SNPs between studied species (Jawdat *et al*., 2013).This result agrees, to some extent, with our results in the ability of *rbcL* to differentiate between species.

The results of UPGMA cluster analysis were close and agree with some other studies (Morgan *et al*., 1994; Shaw and Small, 2004; Potter *et al*., 2002, 2003, 2007; Lee and Wen, 2001) and showed that all samples gathered into groups which matched their taxonomic classification. This reflects the ability of *rbcL* gene to provide high discrimination of taxa at the level of subfamilies, genera, and species.

In our study, *rbcL* sequence variations were high between the outgroup *Malus domestica* and *Pyrus communis* (sub family *Pomoideae*) when compared with other taxa of *Prunus* (subfamily of *Prunoideae*), where the average pairwise distance was 0.002. The sample of *P. armeniaca* (sub genus *Prunus*) has been considerably differentiated by having four variation sites (Table 2) when compared with all samples of *Prunus* and with wild relatives (subgenus *Amygdalus*). Our results support Watkins (1979), Potter *et al*. (2003) and Mallikarjuna *et al*. (2004) studies.

Two groups of taxa including the wild relatives within subgenus *Amygdalus* formed two separate subclusters, the first one contained *P. dulcis* samples, the wild relatives and the hybrid GF-677. The second sub cluster contained only *Prunus persica* samples. Similar diversification was presented in the result of authors sharing some samples with our study (*P. dulcis, P. armeniaca , P. persica* and some other hybrids) using *matk* and SSRs, and by using CAPS (Cleavage Amplified Polymorphic Sequence) (Bouhadida *et al*., 2007).

The complete matching between the wild relatives *P. korshenskyi* and *P. orientalis* and the *P. dulcis* samples (domesticated almond), with the average of pairwise distance of 0.00, may be due to that *Prunus* wild relatives have been used for a long time as a source of genetic pool in almond breeding programs. However, *rbcL* sequence variations between *Prunus dulcis* samples and their wild relatives were not informative

[DOR: 20.1001.1.16807073.2016.18.4.4.8]

for their identification. Also, this result supports the results of Bortiri *et al*. (2001) and Shaw and Small (2004) in grouping *Prunus dulcis* samples with *P. korshinskyi* and *P. orientalis*.

The GF-677 rootstock is a vegetatively propagated hybrid between the Spanish almonds 'Garfi' [*P. dulcis* (Mill.) D. A., Webb] as the female parent and the North American peach 'Nemared' [*P. persica* (L.) Batsch] as the pollen donor. This hybrid has joined the group of *P. dulcis* and wild relatives in one major sub-cluster. This can be supported by the fact that *rbcL* gene is maternally inherited. Furthermore, a partial sequence may lead to a high match percentage that may not reflect an accurate identification of the query sequence (Schori and Showalter, 2011).

Reliable identification of variation below species level would provide valuable insight into subspecies ranges, and habitat differentiation, additionally, it would help in defining the important variation within species. (Kane *et al*., 2012).

Peach samples included in this study were vegetatively propagated. Therefore, it is not expected to obtain high variation level in a single gene locus such as *rbcL*. In addition, the fact is that *rbcL* gene is maternally inherited which makes it highly conserved within cultivars belonging to the same species and sharing the same origin of maternally chloroplast genome. The reason along the low evolutionary rate of this chloroplast gene suggests that cultivars from both species (*P. persica and P. dulcis*) included in this study seem to be one taxa in each group.

Finally, a good level of discrimination based on *rbcL* marker was observed between studied species and less or absence of variation within species. The analysis of genetic variations among groups of *Prunus* samples and wild relatives using *rbcL* gene allowed us to cluster successfully all samples supporting their morphological characteristics, and their botanical classification. Although *rbcL* gene sequence was not able to identify cultivars, which belonged to the same species, it is effectively variable at species level and much more detailed work is needed on the complete gene sequence. In addition, using other chloroplast genes such as *matK* or PCR based markers like microsatellites should also be investigated to further clarify genetic diversity of *Prunus*.

REFERENCES

1. Badenes, M. L. and Parfitt, D. E. 1995. Phylogenetic Relationships of Cultivated *Prunus* Species from an Analysis of Chloroplast DNA Variation. *Theor. Appl. Genet.,* **90:** 1035–1041.
2. Bakooie. M., Pourjam, E., Mahmoudi, S. B., Safaie N. and Naderpour, M. 2015. Development of an SNP Marker for Sugar Beet Resistance/Susceptible Genotyping to Root-Knot Nematode. *J. Agr. Sci. Tech.,* **17:**443-453.
3. Bortiri, E., Oh, S. –H., Jianguo, J., Baggett, S., Granger, A., Weeks, C., Buckingham, M., Potter, D. and Parfitt, D. E. 2001. Phylogeny and Systematic of *Prunus* (Rosaceae) as Determined by Sequence Analysis of ITS and the Chloroplast trnL-trnF Spacer DNA. *Sys. Bot.,* **26:** 797-807.
4. Bortiri, E., Vanden, H. and Potter, D. 2006. Phylogenetic Analysis of the Morphology in *Prunus* Reveals Extensive Homoplasy. *Plant. Syst. Evol.,* **259**: 53-71.
5. Bouhadida, M. M., Juan, P. E., Pinochet, G., Sánchez, J. M., Ángeles, M. and Yolanda, G. A. 2007. Chloroplast DNA Diversity in *Prunus* and Its Implication on Genetic Relationships. *J. Amer. Soc. Hor. Sci.,* **132(5):** 670-679.
6. Browicz, K. and Zohary, D. 1996. The Genus *Amygdalus* L. (Rosaceae): Species Relationships, Distribution and Evolution under Domestication. *Genet. Resour. Crop. Evol.,* **43:** 229-247.
7. Chase, M. W., Morgan, D., Soltis, D. E., Olmstead, R. G. Ses, D. H., Duvall, M. R., Price, R. A., Hills, H. G., Qiu, Y. -L., Kron, K. A., Chaeis, J. H., Kress, W. J., Donoghue, M. J. Clark, W. D., Hedren, M., Gaut, B. S., Jansen, R. K., Kim, K. J., Wimpee, C. F., Smith, J. G., Furnier, G. R., Straus, S. H. Xiang, Q. -Y., Plunkett, G. M., Soltis, P. S., Swensen, S., Eguiarte, L. E.,

[DOR: 20.1001.1.16807073.2016.18.4.4.8]

Learn, G. H., Barrett, S. C. H., Graham, S. and Libert, V. A. 1993. Phylogenetics of Seed Plant: An Analysis of Nucleotide Sequences from the Plastid Gene *rbcL. Ann. Missouri Bot. Gard.,* **80:** 528-580.

8. Doebley, J., Durbin, M., Golenberg, E. M., Clegg, M. T. and Ma, D. P. 1990. Evolutionary Analysis of the Large Subunit of Carboxylase (rbcL) Nucleotide Sequence among the Grasses (*Gramineae*). *Evol.,* **44:** 1097-1108.
9. Doyle, J. J. and Doyle, J. L. 1987. A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. *Phyto. Chem. Bull.,* **(19):** 11–15.
10. Felsenstein, J. 1985. Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evol.,* **39:** 783-791.
11. Jawdat, D., Elias, R., Al- Faoury, H. and Al-Safadi, B. 2013. Dentification of a Polymorphic Region in the Chloroplast Gene *rbcL* by Ecotilliing. *Inter. J. Bot.,* **9:** 140-146.
12. Judd, W. S., Campbell, Ch. S., Kellogg, E. A. and Stevens, P. F. 1999. "*Plant Systematics*: *A Phylogenetic Approach"* Sinauer Associates, Sunderland, Massachusetts, ISBN: 0–87893–404–9, 464 PP.
13. Gyulai, G., Szabó, Z., Wichmann, B., Bittsánszky, A., Waters Jr, L., Tóth, Z. and Dane, F. 2012. Conservation Genetics: Heat Map Analysis of nuSSRs of aDNA of Archaeological Watermelons (Cucurbitaceae, *Citrullus l. lanatus*) Compared to Current Varieties. *Genes Genomes Genomics*, **6(SI1):** 86-96.
14. Hall, T. A. 1999. Bio Edit: A User-friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucl. Acid. Symp. Ser.,* **41:** 95-98.
15. Julian, R., Star, R., Robert, F., Naczi, C. and Briannan, N. 2009. Plant DNA Barcodes and Species Resolution in Sedges (*Carex*, *Cyperaceae*). *Mol. Ecol. Res.,* **9(Suppl. 1):** 151–163.
16. Kane, N., Svinsson, S., Dempewolf, H., Yong Yang, J. I., Zhang, D., Johannes, M., Engels, M. and Cronk, Q. 2012. Ultra-barcoding in Cacao (the *Obroma* spp.; *Malvaceae*) Using Whole Chloroplast Genomes and Nuclear Ribosomal DNA. *Amer. J. Bot.,* **99(2):** 320–329.
17. Kim, K. J., Jansen, R. K., Wallace, R. S., Michaels, H. J. and Palmer, J. D. 1992. Phylogenetic Implications of *rbcL* Sequence Variation in the *Asteraceae*. *Ann. Missouri Bot. Gard.,* **79:** 428-445.
18. Kress, W. J. and Erickson, D. L. 2007. A Two-locus Global DNA Barcode for Land Plants: The Coding *rbcL* Gene Complements the Non-coding *trnH-psbA* Spacer Region. *PLoS ONE*, **2(6):** e508. doi:10.1371/journal.pone.0000508.
19. Lee, S. and Wen, J. 2001. A Phylogenetic Analysis of *Prunus* and the *Amygdaloideae* (Rosaceae) Using ITS Sequences of Nuclear Ribosomal DNA. *Amer. J. Bot.,* **88**: 150-160.
20. Mallikarjunam, K., Aradhaya, C., Weeks, C. and Simon, J. 2004. Molecular Characterization of Variability and Relationship among Seven Cultivated and Selected Wild Species of *Prunus L.* Using Amplified Fragment Length Polymorphism. *Scientia Hort*., **103:** 131-144.
21. Marti, A. F., Athanson, B., Koepke, T., Forcad, C. F., Dhingra, A. and Oraguzie, N. 2012. Genetic Diversity and Relatedness of Sweet Cherry (*Prunus avium* L.) Cultivars Based on Single Nucleotide Oolymorphic Markers. *Front. Plant Sci.,* **3:** 116. doi: 10.3389/fpls00349.
22. Martı´nez-Go´mez, P., Arulsekar, S., Potter, D. and Gradziel, T. M. 2003. An Extended Interspecific Gene Pool Available to Peach and Almond Breeding as Characterized Using Simple Sequence Repeat (SSR) Markers. *Euphytica*, **131:** 313–322.
23. Mattia, F. D., Bruni, I., Galimberti, A., Cattaneo, F., Casiraghi, M. and Labra, M .2011. A Comparative Study of Different DNA Barcoding Markers for the Identification of Some Members of *Lamiaceae*. J. *Food Res.,* **44**: 693-702.
24. McVaugh, R. 1951. A Revision of North American Black Cherries (*Prunus serotina Ehrh*.), and Relatives. *Brittonia,* **7:** 279-315.
25. Morgan, D. R., Soltis, D. E. and Robertson, K. R. 1994. Systematic and Evolutionary Implications of *rbc*L Sequence Variation in *Rosaceae*. *Amer. J. Bot.,* **81**: 890–903.
26. Mowrey, B. D. and Werner, D. J. 1990. Phylogenetic Relationships among Species of *Prunus* as Inferred by Isozyme Markers. *Theor. Appl. Gen.,* **80:** 129–133.
27. Olmstead, R. G., Michaeis, H. J., Scott, K. M. and Palmer, J. D. 1992. Monophyly of the Asteridae and Identification of Their Major Lineages Inferred from DNA

[DOR: 20.1001.1.16807073.2016.18.4.4.8]

Sequences of *rbcL. Ann. Missouri Bot. Gard.,* **79:** 249-265.
28. Potter, D. and Parfitt, E. 2001. Phylogeny and Systematics of *Prunus* (Rosaceae) as Determined by Sequence Analysis of IT'S and the Chloroplast *trnL-trnF* Spacer DNA. *Sys. Bot.,* **26:** 797-807.
29. Potter, D. F., Gao, P. E., Bortiri, S., Oh, H. and Baggett, S. 2002. Phylogenetic Relationships in *Rosaceae* Inferred from Chloroplast *matK* and *trnL-trnF* Nucleotide Sequence Data. Plant. *Syst. Evol.,* **231:** 77-89.
30. Potter, D. 2003. Molecular phylogenetic studies in Rosaceae. In: "*Plant Genome: Biodiversity and Evolution*", (Eds.): Sharma A. K. and Sharma A., Vol. 1, Part A: Phanerogams, Science Publishers, Inc. Enfield (SA), New Hampshire, Plymouth (UK), pp. 319–351.
31. Potter, D., Eriksson, T. R., Evans, C. S., Oh, J. E., Smedmark, E., Morgan, D. R., Kerr, M., Robertson, K. R., Arsenault, M., Dickinson, T. A. and Campbell, C. S. 2007. Phylogeny and Classification of *Rosaceae*. *Plant. Syst. Evol.,* **266**: 5–43.
32. Qiu, Y. L, Chase, M. W., Les, D. H. and Parks, C. R. 1993. Molecular Phylogenetic of the *Magnoliidae*: Cladistic Analyses of Nucleotide Sequences of the Plastide Gene *rbcL. Ann. Missouri Bot. Gard.,* **80:** 587-606.
33. Quan, X. and Zhou, S. L. 2011. Molecular Identification of Species in *Prunus* sect. *Persica* (Rosaceae), with Emphasis on Evaluation of Candidate Barcodes for Plants. *Plant Sys. Evol.,* **49:** 138–145.
34. 34-Rehder, A. 1940. Manual of Cultivated Trees and Shrubs Hardy in North America. 2nd Edition, MacMillan, New York, USA.
35. Ritland, K.and Clegg, M. T. 1987. Evolutionary Analysis of Plant DNA Sequences. *Amer. Natur.,* **130:** 74–100.
36. Schori, M. and Showalter, A. M. 2011. DNA Barcoding as a Means for Identifying Medicinal Plants of Pakistan. *Pak. J. Bot.,* **43:** 1-4.
37. Shaw, J. and Small, R. L. 2004. Addressing the ''Hardest Puzzle in American Pomology:'' Phylogeny of *Prunus* sect. *Prunocerasus* (Rosaceae) Based on Seven Noncoding Chloroplast DNA Regions. *Amer. J. Bot.,* **91:** 985–996.
38. Shi, S., Li, J., Sun, J., Yu, J. and Zhou, S. 2013. Phylogeny and Classification of *Prunus sensu* Lato (Rosaceae). *JIPB,* **55(11):** 1069-1079.
39. Shokralla, S., Gregory, A. Singer, C. and Hajibabaei, M. 2010. Direct PCR Amplification and Sequencing of Specimens' DNA from Preservative Ethanol. *Biotechniques*, 48: 233-234.
40. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S .2011. MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol. Bio. Evol.,* **28:** 2731-2739.
41. Yamanaka, S., Nakamura, I. Watanabe, K. N. and Sato, Y. 2004. Identification of SNPs in the Waxy Gene among Glutinous Rice Cultivars and Their Evolutionary Significance during the Domestication Process of Rice. *Theor. Appl. Genet.,* **108:** 1200–1204.
42. Watkins, R., 1979. Cherry, Plum, Peach, Apricot, and Almond: Prunus spp. (Rosaceae). *Evol. Crop Plant.,* 339: 242-247.
43. Wright, S. I., Bi, I. V., Schroeder, S. G., Yamasaki, M., Doebley, J. F., McMullen, M. D. and Gaut, B. S. 2005. The Effects of Artificial Selection on the Maize Genome. *Sci.,* **308:** 1310–1314.
44. Zurawski, G. and Clegg, M. T. 1987. Evolution of Higher Plant Chloroplast DNA-coded Genes: Implications for Structure-function and Phylogenetic Studies. *Ann. Re. Plant. Phy.,* **38:** 391–418.

[DOR: 20.1001.1.16807073.2016.18.4.4.8]

تغییرات توالی ژن *rbcL* در درون و در بین گونه های هلوسا *Prunus*

س. سرهان، ف. حامد، و و. الیوسف

چکیده

هدف این پژوهش، بررسی سطح تغییرات SNP توالی ژن *rbcL* در درون و در بین گونه های هلو سا *Prunus* بود و ۱۷ گونه کشت شده محلی و خویشاوندان وحشی هلوسا ها و دو گونه از زیرخانواده *Maloideae (Malus domestica and Pyrus communis)* به عنوان گروه پرت (outgroup) را در بر می گرفت. در انجام پژوهش، توالی های *rbcL* تکثیر، توالی بندی و همردیف شد تا چندشکلی های نوکلئوئید مجرد (SNP) تعیین شود. نتایج درخت ژنی (gene tree) *rbcL* مربوط به نمونه ها، دو خوشه اصلی را نشان می داد. اولین آنها شامل رده گروه پرت (*M. domestica* و *P. communis*) بود ولی نمونه های هلوسا ها شامل *Prunus armeniaca* همگی در خوشه دوم قرار گرفتند که در یک زیرخوشه جدا شدند. نتایج چنین اشاره داشت که برای بررسی روابط درون گونه و روابط بین گونه ها ، تجزیه توالی ژن*rbcL* ابزاری به دست می دهد که به خوبی مشخص است و می تواند با موفقیت در ترسیم درخت تکاملی و تبار زایی برای نمونه های ثبت شده گونه هلو سا به کار رود.

[DOR: 20.1001.1.16807073.2016.18.4.4.8]