Characterization of Five Microsatellite Markers for Genetic Diversity Structure Analysis of Walnut *(Juglans regia L)* in Five Village in Duhok Province

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Abstract:

Five microsatellites markers (WGA202, WGA009, WGA332, WGA225 and WGA069) were used to characterize Persian walnuts *(Juglans regia* L.) populations in five villages (Sharanesh, Bedohe, Kanizarke (Akre), Kashane and Kuzo) of Duhok province. The microsatellites amplified (PCR products) a total of 186 alleles across all populations. The number of alleles per locus ranged from 4 alleles in Sharanesh and Kashane populations to 10 alleles also in Sharanesh and Kashane populations, with an average of 7.4. The molecular sizes of the amplified bands ranged from 158 bp to 289 bp in all populations. The observed heterozygosity (Ho) within populations ranged from 0.59 at WGA225 to 0.65 in the locus WGA202 with an average of 0.61. The PIC value (0.88) indicated that all markers were highly informative and useful for genetic diversity studies in these populations. The proportion of genetic variation presented among populations accounted for 8.4% of the total genetic diversity so it indicated a moderate level of genetic diversity between populations. The Fis average 0.24 indicated that, there was a regular tendency toward heterozygote deficiency and indicated the presence of inbreeding within the populations. The phylogenetic analysis or unrooted neighbor-joining tree highlighted the genetic distance among those five populations and separated them into two main groups placing each population according to its genetic background. The first group consists of populations Bedohe and Sharanesh in one subgroup and Kashane in second subgroup. The second group consists of populations Akre and Kuzo .

Keywords*: genetic diversity, Duhok, microsatellites ,walnut and population.*

Introduction

The genus *Juglans L*. is made up of 21 species of longlived deciduous trees that produce large woody-shelled nuts. Juglans has been divided into four taxonomic sections as described by Manning (1978): (i) section Cardiocaryon Dode (the east Asian heartnuts) with three species, *J. ailantifolia Carr*., *J. mandshurica Maxim*., and *J. cathayensis Dode*; (ii) section Rhysocaryon Dode (black walnuts) of North, Central, and South America and the West Indies with 16 species; (iii) section Dioscaryon Dode with one species, *J. regia L*. (Persian walnut) distributed from southeastern Europe to the Himalayan mountains; and (iv) section Trachycaryon Dode ex Mann. with one species, *J. cinerea L*. (butternut) in eastern North America. *Juglans regia L*., has an exceptionally wide natural distribution, it occurs from the Carpathian Mountains of Eastern Europe, all through Western Asia, the Himalayan regions of Pakistan, India, Nepal, Bhutan and east into China. Most of walnut populations in Kurdistan including Duhok province have had very little attention from scientists, therefore they are not known if they are native trees from open-pollinated seedlings or new varieties introduced to the area by the local people for many years.

Unfortunately, the traditional methods for characterization and assessment of genetic variability in perennial fruit crop species, based on morphological, physiological and biochemical studies are both time consuming and affected by the environment. The introduction of molecular biology techniques, such as DNA based markers, provides an opportunity for genetic characterization that allows direct comparison of different genetic material independent of environmental influences (Weising *et al.* 1995). Previously, in addition to morphological identification (Zenelli *et al.* 2005), various biochemical and molecular markers have been used for genetic characterization of walnut genotypes. These included isozymes (Ninot and Aleta 2003; Vyas *et al.* 2003; Fornari *et al.* 2001; Busov *et al.* 2002), restriction fragment length polymorphisms (RFLPs) (Fjellstrom and Parfitt 1995), randomly amplified polymorphic DNAs (RAPDs) (Nicese *et al.*1998; Yan-Min *et al.* 2000; Li *et al.* 2007), intersimple sequence repeats ISSRs) (Potter *et al.* 2002), simple sequence repeats (SSRs) (Woeste *et al.* 2002; Dangl *et al.* 2005; Foroni *et al.* 2005; Victory *et al.* 2006; Robichaud *et al* 2006; Karimi *et al.* 2010), amplified fragment length

polymorphisms (Kafkas *et al.* 2005; Bayazit *et al*. 2007) and SNPs (Ciarmiello *et al.* 2011).

Simple sequence repeat (SSR), an array of short motifs of 1– 6 bp in length, are hypervariable and widely spread in both coding and non-coding regions of plant and animal genomes (Kota *et al.*, 2003). The reproducibility, multiallelicism, codominance, relatively abundance, and good genome coverage of SSR markers have made them one of the most useful tools for integration of the genetic, physiological, and sequence-based physical maps in plant species (Powell *et al.* 1996 and Kota *et al.* 2003).

Materials and Methods

Sample collection: Samples (fresh leaves) from five populations of *J. regia L* in the Duhok province were collected from Bedohe (Kanimase), kanizarke (Akre), kuzo (Zawita) , Kashane (Batifa) and Sharaneshe (Darkare). Figure (1). Samples were collected according to procedure described by Karimi *et al.* (2010). Plants within 10 km from each other were considered as belonging to the same population, we limited our sampling to 8 trees per population and each tree separated by a distance of 100m from the other one. The leaves were sampled in the early morning hours and stored at –80 C^0 until their use.

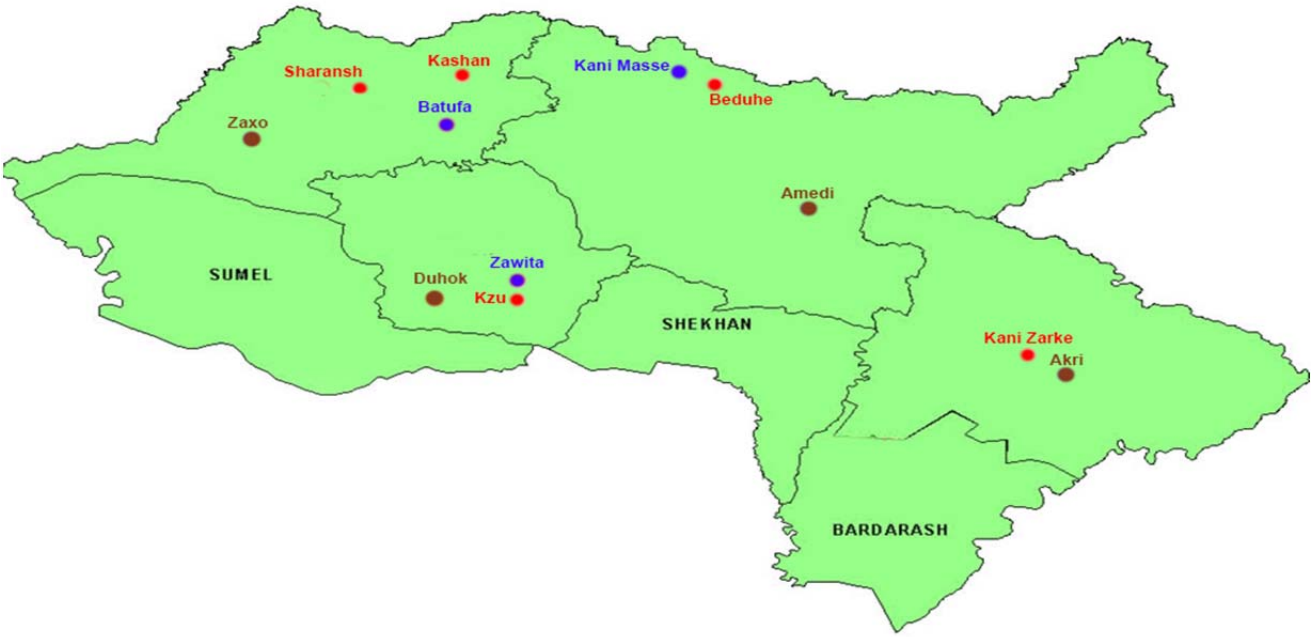

Figure (1): Names and location of five populations of *J. regia L* in the Duhok province. The red circle represents location of population.

Isolation of plant genomic DNA: The DNA was isolated from the plant (leaves) according to the method Dolye and Dolye (1987) with some modification. Three grams of fresh tissue (leaves) were homogenized to powder and added to 10 ml of Extraction buffer (2x CTAB preheated at 65 C^0 for 15 min) [2% cetyl trimethyl ammonium bromide, 50 mM 1,4-dithiothreitol, 0.3% b-mercaptoethanol,1.4 M NaCl, 100mM Tris ,20 mM ethylenediaminetetraaceticacid (EDTA), pH 8.0] and incubated at 65 C^0 for40 min. The aqueous solution was extracted with 10 mL (24:1) of chloroform–isoamyl alcohol and centrifuged at 5 C^0 for 10min at 10,000 r/min, and the aqueous layer retained; 2/3 volumes of the aqueous layer of 95% ethanol were added (at –20 C^0) to precipitate the nucleic acids. The precipitate was washed with 0.2 M ammonium acetate in 75% ethanol and air-dried for 5 min. The DNA was precipitated, washed, dried, and dissolved in TE buffer.

Polymerase chain reaction amplification and electrophoresis. Samples (leaves) were obtained from five walnut populations of Duhok province. Each population contained eight individuals walnut tree leaves. Five primer pairs introduced by Woeste *et al*., 2002 were used to amplify genomic

DNA.(Table 1) The samples were prepared for PCR amplification in the form of single-individual genotypes for detection genetic diversity within populations.

The SSR reaction protocol was based on the protocol of Dangle *et al.* (2005). Amplification reactions were performed in a volume of 15 mL containing 1 x polymerase chain reaction (PCR) buffer, 25 ng genomic DNA, 200 mMdNTPs, 0.2 mM of each primer, 2 mM MgCl2, and 0.5 unit of *Taq* DNA polymerase For DNA amplifications, an Eppendorf thermocycler was programmed according to the following profile: 94 C^0 for 5min, 30 cycles at 94 C^0 for 30 sec, the appropriate primer annealing temperature (Table 1) for 1 min and 72 C^0 for 40 sec; and a final elongation at 72 C^0 for 2 min, one cycle. After amplification, 5 mL of each sample was loaded and electrophoresed on a 2% horizontal agarose gel to control for positive amplification and to determine the approximate amount of product. Then, 3 µl of each sample was electrophoresed on a 6% polyacrylamide gel containing 1x TBE buffer. After electrophoresis, the gel was silver stained using the procedure of Bassam *et al*. (1991). In all cases, PCR reactions were performed at least twice to ensure that allele sizes were consistent. Allele sizing and scoring was done using a 100 bp DNA ladder (MBI-Fermentas, Vilnius, Lithuania) as the length reference.

Table (1) Five walnut microsatellite primers

	Primers name	Annealing temperatures	Amplicon size range (bp)	Primer sequence
1	WGA202	58 C^0	246-289 bp	F. CCCATCTACCGTTGCACTTT R. GCTGGTGGTTCTATCATGGG
2	WGA009	50 C^0	242-258 bp	F. CATCAAAGCAAGCAATGGG R. CCATTGCTCTGTGATTGGG
3	WGA332	59 C^0	212-230 bp	F. ACGTCGTTCTGCACTCCTCT R. GCCACAGGAACGAGTGCT
4	WGA225	59C^0	198-206 bp	F. AATCCCTCTCCTGGGCAG R. TGTTCCACTGACCACTTCCA
5	WGA069	45 C^0	158-182 bp	F- TTAGTTAGCAAACCCACCCG R- AGATGCACAGACCAACCCTC

Data analysis. The allele's data were entered in the form of single-individual genotypes. The following parameters of genetic variation were assessed for each population: number of observed alleles (No), observed heterozygosity (Ho), and expected heterozygosity (He) (Nei, 1987), inbreeding coefficient (F-statistic) F_{st} and F_{is} (Nei, 1987) were calculated at each locus and over all loci using POPGENE version 1.32 software **.** Polymorphic information content (PIC), availability (A), and genetic distance (Nei, 1972) were calculated by using Power Marker version 3.25 software.Finally, in order to visualize the relationships between provenances, and obtained tree with neighbor-joining method based on (Nei, 1972). The phylogenetic tree was constructed by using Power Marker version 3.25 software. The tree was then viewed by using the TREEVEIW version 1.66 software.

Results and Discussion

The total number of alleles scored in five populations was 186 alleles with the sizes between 158 bp to 289 bp. The number of alleles per locus varied from 4 alleles in both Sharanesh and Kashane populations at locus (WGA225) to 10 alleles in Sharanesh population at locus (WGA202) and Kashane population at locus (WGA069), with an average of 7.4 alleles per locus. Table (2)

The average number of alleles per locus obtained (7.4) showed to be much higher than some other reported studies on *Juglans regia* using different DNA markers. In a very recent report using SSR markers this value were found to be 5.5 and 6.2 in (Foroni *et al.,* 2005 and Pollegioni *et al.,* 2011) respectively. In other studies, however, the average of allelic variation was higher than the average obtained in this study. For example: Victory *et al* (2006) using SSR marker reported the allelic variation value as 9.13, whereas in another study using SSR marker, the average of allelic variation was 12.9 (Pollegioni *et al.,* 2009).

Table (2): A summary of the number of alleles per each population revealed by five *Juglans* microsatellite loci

Primers	Number of observed alleles (No) in each population					Average number of allele
	Sharanesh	Bedohe	Akre	Kashane	Kuzo	
WGA202	**10**	**6**	**9**	**9**	**7**	41
WGA009	**7**	**8**	**8**	**6**	**8**	37
WGA332	**8**	**7**	**6**	**7**	**9**	37
WGA225	**4**	**6**	**6**	**4**	**6**	26
WGA069	**9**	**8**	**9**	**10**	**9**	45
Sub total	**38**	**35**	**38**	**36**	**39**	37.2
Average	**7.6**	**7.0**	**7.6**	**7.2**	**7.8**	**7.4**
Total				**186**		

In order to obtain reliable data analysis, the value of availability (A, number of observed alleles per number of individuals sampled) was determined. This value was found to be high in all populations with an average 0.91. This average indicated that the number of null allele (not amplified) was low in all populations with an average 0.19 Table (3).

The observed heterozygosity (*Ho*) value per each population ranged from 0.58 in Sharanesh population to 0.628 in Akre population with an average 0.61(Table 3). The (*Ho*) of a given locus ranged from 0.59 (WGA225) to 0.65 (WGA202), with an average over all eight loci of 0.61 (Table 4). The average expected heterozygosity (He) was 0.87 per each population and ranged from 0.84 to 0.889 in Sharanesh and Kuzo respectively (Table 3) .The value of (He) per locus also varied from 0.81 at WGA225 to 0.96 at WGA202 with an average 0.90 (Table 4) .

Table (3) Summary of Genic Variation Statistics for five *Juglans* population (Sharanesh, Bedohe, Akre, Kashane and Kuzo). Availability (A), expected heterozygosity (He), observed heterozygosity (Ho), and Polymorphic information content (PIC).

Populations	A	Ho	He	PIC
Sharanesh	0.925	0.5893	0.8463	0.766
Bedohe	0.925	0.6214	0.8862	0.802
Akre	0.900	0.6286	0.8872	0.803
Kashane	0.825	0.6048	0.8722	0.779
Kuzo	0.975	0.6179	0.8897	0.811
Average	0.910	0.6124	0.8763	0.792

The Ho in *J. regia* per each locus in this study was found to be higher than the average Ho (0.54) reported in some population in the Veneto region in Northern Italy (Pollegioni *et al.,* 2009), whereas this value were close to the average Ho (0.69) reported in five population in Dohuk providence of Kurdistan (Abbas and Jubrael., 2013), but lower than those reported (0.72) in the four provinces of Iran populations (Ebrahimi *et al*., 2011). From this result, it may be noticed that, the observed heterozygosity value was lower than expected, this may suggest that, there is a regular tendency toward heterozygote deficiency, and this may also indicate the presence of inbreeding within the populations (Coulson *et al.,*1998).

Table (4) Summary of Genic Variation Statistics for five *Juglans* microsatellite Loci. Availability (A), expected heterozygosity (He), observed heterozygosity (Ho) and Polymorphic information content (PIC).

Marker	A	Ho	He	PIC
WGA202	0.95	0.6579	0.9618	0.946
WGA009	0.85	0.6176	0.8973	0.873
WGA332	0.95	0.6053	0.9098	0.889
WGA225	0.925	0.5946	0.8153	0.779
WGA069	0.875	0.6000	0.9358	0.915
Average	0.91	0.6151	0.9040	0.881

The highest polymorphic information content (PIC) value (0.81) was observed in Kuzo population and the lowest value (0.77) in Sharanesh population with an average 0.79 in each population. Tables (3) The highest PIC value per locus was (0.94) for the primer WGA202 and the lowest value was 0.779 for the primer WGA225 with an average 0.88 Table (4). This may suggest that all markers were highly informative; because the (PIC) value was higher than 0.50 in all loci, thus, they could be considered as useful markers for genetic diversity studies for *J. regia* populations grown in this region (Botstein *et al.,* 1980).

The average PIC value per locus in this study was found to be higher than values (0.68, and 0.57) reported in (Karimi *et al*,. 2010 and Pollegioni *et al*., 2011) respectively.

The F_{st} ranged from 0.05 for locus WGA069 to 0.11 in locus WGA225 with an average value of 0.084 Tables (5). This may suggest that the genetic differentiation in this study was a moderate one because the F_{st} value was higher than 0.05 and lower than 0.15 (Weir and Cockerham, 1984).

Table (5) Summary of (Fst and Fis) for five *Juglans* microsatellite Loci

Locus	F_{st}	F_{is}
WGA202	0.1095	0.2219
WGA009	0.0660	0.2560
WGA332	0.0788	0.2693
WGA225	0.1103	0.1675
WGA069	0.0579	0.3172
Average	0.0842	0.2492

This average value was found to be higher than the genetic differentiation among populations reported in other studies for *J. regia* based on microsatellite markers. For example,(Pollegioni *et al* 2011) reported the value of F_{st} (0.053) in Veneto region at Northern Italy. But this average was lower than F_{st} (0.122) reported in some population in western Iran (Karimi *et al* .,2010) and F_{st} (0.106) reported by Fornari *et al* (2001) who had analyzed different population of *J. regia* from Europe and USA .The average of F_{st} obtained here 0.084, indicated that 8.4% of the total genetic diversity existed among populations and 91.6% within populations.

The estimation of genetic structure in this study was indicated that the moderate level of genetic differentiation was observed among these five populations according to F_{st} values. This may be due to that forest trees generally display high within-population diversity and low differentiation among populations. Deforestation and/or progressive selection of valuable genotypes for nut production and removal of vigorous trees with high wood quality leading to the negatively affecting genetic variability (Hamrick *et al.* 1992 and Müller-Starck *et al.* 1992).

The F_{is} value in this study ranged from (0.167) for locus WGA225 to (0.317) in locus WGA069 with an average (0.249) per each locus. These results indicated that all five loci have the tendency toward heterozygote deficiency.

The average of F_{is} value obtained was higher than (0.199) reported in some populations in Central and Southwestern China (Wang *et al* .,2008) and (0.021) reported by Pollegioni *et al* (2011). The value of F_{is} indicated that, there was a regular tendency toward heterozygosity deficiency, this indicating that the presence of inbreeding within the populations. The genetic structure information that obtained in this study was essential for conservation and management of walnut tree.

Genetic distances were calculated for each pair of populations to estimate the extent of their divergence Table (6). The lowest genetic

distance (0.55) was found between populations Bedohe and Sharanesh and the highest genetic distance (1.02) was found between populations Sharanesh and Kuzo. In the five populations, the average genetic distance among populations equaled 0.87.

The values of genetic distances among populations as well as between individuals are vary for plant breeding programs, for example plant breeders usually selects two varieties with a high genetic distance between them in order to obtain the widest possible crosses. However in other cases if the breeder wishes to introduce a certain character which is controlled by a gene or a group of genes for specific variety without wide variation in the genetic material of this variety which contains desired characters. In this case the breeder selects close variety that has this character within a studied variety because it is very difficult to find genetic variation between varieties depending on morphological characters (Smith, 1992).

Table (6) genetic distance between five *Juglans regia* populations (Sharanesh, Bedohe, Akre, Kashane and Kuzo)

Populations	Sharanesh	Beduhe	Akre	Kashane	Kuzo
Sharanesh	00000				
Beduhe	0.5556	00000			
Akre	0.6178	0.7508	00000		
Kashane	0.5970	0.8007	0.6923	00000	
Kuzo	1.0234	0.7045	0.6652	0.7587	00000

The dendrogram separated the five populations into two main groups. The first group consists of populations Bedohe , Sharanesh and Kashane . The second group consists of populations Akre and Kuzo . The first group divided into two subgroup, Beduhe and Sharanesh were in one subgroup and Kashane in another subgroup. In the first cluster or group the Sharanesh and Bedohe populations had closest genetic relationship together then the second subgroup consist of Kashane population as detected in Figure (2). Also in second group Akre and Kuzo had close genetic relationship together. The phylogenetic analysis highlighted the genetic distance among the populations, placing each according to its genetic background. These results confirmed the power of microsatellites to diversify *Juglans regia* in five populations.

The unrooted tree determined in this study observed that the populations separated one from another according to their genetic differentiation that present among them and not according to the geographical distribution. This may be attributed to the fact that all populations belong to Duhok province and the area or geographical distance between populations is not very wide to produce high amount of genetic differentiation among populations. In addition , the habitat of the populations are approximately similar including climate, temperature, rainfall , landform whereas suitable for growth of walnut tree in this region. (Abbas and Jubrael., 2013)

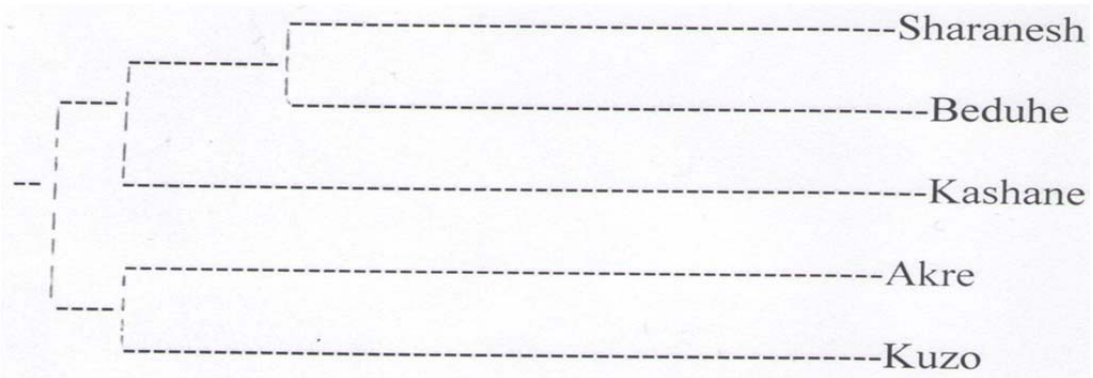

Figure (2): Unrooted neighbor-joining tree representing the genetic relationships between five *Juglans regia* populations (Sharanesh, Bedohe, Akre, Kashane and Kuzo

References

Abbas. A. 2013. Genetic Diversity Structure of Walnut *(Juglans regia L)* populations in Duhok Province Revealed by SSR Markers. M.Sc. thesis: University of Zakho.

Bassam, B. J., G. C. Anollés., P. M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem. 196: 80–83.

Bayazit, S., K. Kazan., S. Gulbitti., V. Çevik., H. Ayanoglu, and A. Ergul. 2007. AFLP analysis of genetic diversity in low chill requiring walnut (*Juglans regia L.*) genotypes from Hatay, Turkey. Sci Hort. 111:394–398.

Botstein, D., R. L. White., M. Skolnick, and R. W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms, American Journal of Human Genetics. 32: 314-331.

Busov, V. B., G. Rink, and K. E. Woeste. 2002. Allozyme variation and mating system of black walnut (*Juglans nigra L.*) in the central hardwood region of the United States. For Genet 9:319–327.

Ciarmiello, L. F., P. Piccirillo., G. Pontecorvo., A. D. Luca., I. Kafantaris, and P. Woodrow. 2011. A PCR based SNPs marker for specific characterization of English walnut (*Juglans regia L.*) cultivars. Mol Biol Rep. 38:1237–1249.

Coulson, T. N., J. M. Pemberton., S. D. Albon., M. Beaumont., T. C. Marshall., J. Slate., F. E. Guinness, and T. H. Clutton-Brock. 1998. Microsatellites reveal heterosis in red deer. Proc. R. Soc. Lond. B. 265:489-495.

Dangl, G. S., K. Woeste., M. K. Aradhya., A. Koehmstedt., C. Simon., D. Potter., C. A. Leslie, and G. McGranahan. 2005. Characterization of 14 microsatellite markers for genetic analysis and cultivar identification of walnut. J Am Soc Hort Sci. 130:348–354.

Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissues. Phytochem. Bull. 19:11–15.

Ebrahimi. A., R. Fatahi, and Z. Zamani. 2011. Analysis of genetic diversity among some Persian walnut genotypes (*Juglans regia L.*) using morphological traits and SSRs markers Scientia. Sci Hortic.130:146–151.

Fjellstrom, R. G, and D. E. Parfitt. 1995. Phylogenetic analysis and evolution of the genus *Juglans* (*Juglandaceae*) as determined from nuclear genome RFLPs. Plant Syst Evol. 197:19–32.

Fornari, B., M. E. Malvolti., D. Taurchini., S. Fineschi., I. Beritognolo., E. McCaglia, and F. Cannata. 2001. Isozyme and organellar DNA analysis of genetic diversity in natural/ naturalized European and Asiatic walnut (*Juglans regia*) populations. Acta Hort. 544:167–178.

Foroni, I., R. Rao., K. Woeste, and M. Gallitelli. 2005. Characterization of *Juglans regia L.* through SSR markers and evaluation of genetic relationships among cultivars and the 'Sorrento' landrace. J Hort Sci Biotech. 80:49–53.

Hamrick, J. L., M. J. W. Godt, and S. L. Sherman-Broyles. 1992. Factors influencing levels of genetic diversity in woody plant species. New Forest. 6:95–124.

Kafkas, S., H. Ozkan, and M. Sutyemez. 2005. DNA polymorphism and assessment of genetic relationships in walnut genotypes based on AFLP and SAMPL markers. J Am Soc Hort Sci. 130:585–590.

Karimi, R., A. Ershadi., K. Vahdati, and K. Woeste. 2010. Molecular characterization of Persian walnut populations in Iran with microsatellite markers. Hort Sci. 45:1403–1406.

Kota, R., S. Rudd., A. Facius., G. Kolesov., T. Thiel., H. Zhang., N. Stein., K. Mayer, and A. Graner. 2003. Snipping polymorphisms from large EST collections in barley (*Hordeum vulgare* L.). Mol Genet Genomics. 270:24–33

Li Z, Z, Lanying and X, Qianwen 2007 Identification of RAPD markers linked to thickness gene of shuck in walnut. Adv Biol Res 1:137–140

Manning, W.E. 1978. The classification within the Juglandaceae. Ann. Mo. Bot. Gard. 65: 1058-1087.

Müller-Starck, G., P. Baradat, and F. Bergmann. 1992. Genetic variation within European tree species. New Forest. 6:23–47.

Nei, M. 1972. Genetic distance between populations. American Naturalist. 106: 283-292.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University press, New York.

Nicese, F. P., J. I. Hormaza, and G. H. McGranahan.1998. Molecular characterization and genetic relatedness among walnut (*Juglans regia L.*) genotypes based on RAPD markers. Euphytica. 101:199–206.

Ninot, A, and N. Aleta. 2003. Identification and genetic relationship of Persian walnut genotypes using isozyme markers. J Am Pomol Soc. 57:106–114.

Potter, D., G. Fangyou., G. Aiello., G. Leslie, and G. McGranahan. 2002. Intersimple sequences repeat markers for fingerprinting and determining genetic relationships of walnut (*Juglans regia*) cultivars. J Am Soc Hort Sci. 127:75–81.

Pollegioni, P., K. Woeste., A. Major., G. S. Mugnozza, and M. E. Malvolti. 2009. Characterization of *Juglans nigra L., Juglans regia L*. and *Juglans x intermedia (Carr.)* by SSR markers: a case study in Italy. Silvae Genet. 58:68–78.

Pollegioni, P., K. Woeste., I, Olimpieri., D. Marandola., F. Cannata, and M. E. Malvolti. 2011. Long term human impacts on genetic structure of Italian walnut inferred by SSR markers Genetics and Genomes.7: 707-723.

Powell, W., G. C. Machray, and J. Provan. 1996. Polymorphism revealed by simple sequence repeats. Trends Plant Sci. 1:215–222.

Robichaud, R. L., J. C. Glaubitz., O. E. Rhodes, and K. Woeste. 2006. A robust set of black walnut microsatellites for parentage and clonal identification. New Forests. 32:179–196

Smith, B. D. 1992. River of change. essays on early agriculture in eastern north america.SmithsonianPress,Washington, D.C.

Victory, E. R., J. C. Glaubitz., O. E. Rhodes, and K. E. Woeste. 2006. Genetic homogeneity in *Juglans nigra* (*Juglandaceae*) at nuclear microsatellites. Am J Bot. 93:118–126.

Vyas, D., S. K. Sharma, and D. R. Sharma. 2003. Genetic structure of walnut genotypes using leaf isozymes as variability measure. Sci Hort. 97:141–152.

Wang, H. and D. Pei. 2008. Genetic diversity and structure of walnut populations in Central and Southwestern China revealed by microsatellite markers. J Am Soc Hortic Sci. 133:197–203.

Weir, B. S and C. C. Cockerham. 1984. Estimating F-statistics for theanalysis of population structure. Evolution. 38:1358–1370.

Weising K., H, Nybom., K, Wolff and W, Meyer 1995 DNA fingerprinting in plants and fungi. CRC Press Inc, Boca Raton, Florida

Woeste, K., R. Burns., O. Rhodes, and C. Michler. 2002. Thirty polymorphic nuclear microsatellite loci from black walnut. J Hered. 93:58–60.

Yan-Min, W., L. Ying., D. Feng-Xiang, and X. Sheng-Ke. 2000. Study on different ecological types of Chinese walnut (*Juglans regia*) using RAPD markers. J Beij For Univ. 22:23–27.

Zenelli G., H, Kola and M, Dida 2005 Phenotypic variation in native walnut populations of Northern Albania. Sci Hort 105:91–100

سالو خه تێن پێچ نیشاندە ریێن دیڤچونێن سادە ێین دووبارە کری بۆ دیارکرنا بنیا تا جوراوجوریا ژنێتکیا گیزا (*Juglans regia*) ل پێچ گوندێن پارێز گە ها دهوکێ

پوختە:

د ڤێ ڤەکولینێ دا، ئەڤ 5 نیشاندەرێن دیڤچوونێن سادەیێن دوبارەکری (SSR Marker) (WGA202 ۹..WGA– ۳۳۲ WGA225– WGA069) بکار هاتینە، ئەوژی ب مەرەما دیارکرن و سالۆخدانا پێنج کومەلێن گیزا فارسی (Juglans regial) ل هەر پێنج گوندێن (شەرانش، بێدوهێ، کانی زەرکێ، کەشانێ و کوزۆ) کو هەمی ئەڤ گوندێن ناڤبری سەر ب پارێزگەها دهۆکێ ڤە نە.

ئەنجامێ دووهند بوونێ ب رێکا کارلێکا زنجیرەییا ئەنزیمێ پەلرە (PCR ۱۸٦) ئەلیلان ل گشت کومەلان ب ڤی شێوەیە: هژمارا ئەلیلان ل کومەلا شەرانش و کە شانێ ژ چار ئەلیلان دەست پێدکەت هەتا دهە ئەلیلان هەرل وان کومەلان کو سەرجەمێ ئەلیلان ل هەر کومەلەیەکێ (۷.٤) ئەلیلن. کێشا گەردییا سەرجەم شەریتێن دووهند بووی ژ (bp ۱۵۸) دەستپێدکەت و ل سەرجەم کومەلان دگەهیتە (bp ۲۸۹). رێژا دوو گەردییا بەرچاڤ ژ (۰.۵۹) ل جهێ (WGA ۲۲۵) دەستپێدکەت و دگەهیتە (۰.٦۵) ل (WGA ۲۰۲) ، ب ڤی شێوەی تێکرایێ وێ د هەر جهەکیدا دبیتە (۰.٦۱). نرخێ (PIC) کو (۰.۸٦) ە، وەسا دیار دکەت سەرجەمێ نیشاندەرا ب مفانە و پێزانینێن باش د بواری جوراوجورییا ژنتیکێ دناڤبەرا کومەلاندا هەیە.

رێژا جوراوجورییا ژنتیکێ دناڤبەرا کومەلاندا (۸.٤٪) یە. د ڤێ رێژا بەردەست دا دیار دبیت ئاستێ جوراوجوریێ دناڤبەرا کومەلاندا یا ناڤنجییە. تێکرایا (Fis) کو (۰.۲٤)هند، دیار دکەت ئەڤ کومەلە بێ بەرەڤ لاوازییا دوو رەگیێ ڤە دچن. ئەڤ چەندە ژ ئەگەرێ جووت بوونا ناڤخوییە د ناڤا ڤان کومەلاندا.

ژ لایێ دویراتییا ژنتیکێ ڤە، ئەڤ پێنج کومەلە ب سەر دوو گروپان دابەش دبن. ئەڤ دابەش بوونە ل سەر بنەمایێ ژنتیکییا کومەلەییە.چە قە ك ژ گروپێ ئێکێ هەر دوو کومەلێن (شە نش، بێدوهێ) بخوەڤەدگریت وچە قێ دووێ کە شانێ و. (ئاکرێ و کوزۆ) دچنە ژێر خانا گروپێ دووێ .

دراسة خصائص خمسة مؤشرات (microsatellite) لتركيب التنوع الوراثي لمجاميع الجوز *Juglans regia* L في خمسة قرى تابعة لمحافظة دهوك

الخلاصة:

تم استخدام 5علائم صغرية (تكرار **تسلسل** بسيط) (WGA009, WGA202, , WGA069, WGA225, WGA33). لوصف تجمعات الجوز الفارسي(*Juglans regia* L.) في خمس قرى من محافظة دهوك (شرانش ، بيدوهي ، كاني زركي (عقرى) ، كشان وكوزو).

ضوعفت المصغرات (تكرارات تسلسل بسيط) من مجموع ۱۸٦ أليل الى التجمعات. عدد الاليلات لكل موقع رتب من اربعة اليلات في تجمعين شرانش وكشاني الى عشرة أليل في نفس التجمع، بمعدل ۷.٤. رتبت الاوزان الجزيئية للحزم المضاعفة من ۱۵۸ الى ۲۸۹ قاعدة نيتروجينية في كل التجمعات. الزيجات المتغايرة الملاحضة في التجمعات رتبت من ۰.۵۹ في WGA225 .الى ۰,٦۵. في موقع WGA202 بمعدل ۰.٦۱. اشارت قيمةPIC (۰.۸۸) الى ان كل العلائم كانت معلمة بشكل عالي و مفيدة في دراسة التغاير الوراثي في هذه التجمعات.

كانت نسبة التغاير الوراثي بين التجمعات هي ۸.٤٪ من مجموع التغاير الوراثي ولذا فهو يدل على مستوى متعادل من التغاير الوراثي بين المجتمعات. اشار معدل Fis والبالغ ۰.۲٤ الى ان هناك ميل ثابت باتجاه نقص الزيجات المتباينة و اشار ايضا الى وجود تزاوج داخلي (زواج الاقارب) بين التجمعات.

تحليل تطور السلالات (النشوء والتطور) اوالشجرة المتجاورة–المترابطة الغير جذرية بينت المسافة الجينية بين هذه التجمعات الخمسة وفصلتها الى مجموعتين رئيسيتين ، ووضع كل تجمع على اساس الخلفية الوراثية. المجموعة الاولى تتالف من مجموعتين ثانويتين الاولى: بيدهو و شرانش و الثانية: كشاني. المجموعة الثانية تتالف من تجمع أكري و كوزو.