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Genetic variation within the purple carrot population grown in Ereğli District in Turkey

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Abstract: Although the majority of carrots grown in Turkey and around the world are orange, the production of anthocyanin-containing purple carrots has recently increased in Turkey due to the extraction of anthocyanin from these carrots for use as a natural food colorant. Purple carrot production for this purpose is concentrated in Ereğli District in Konya Province, and open-pollinated local purple carrot cultivars or landraces have been used for this production. Genetic variation within the local purple carrot populations in this region is of interest to plant breeders worldwide for the development of new purple carrot cultivars. Therefore, in this study, genetic variation within the purple carrot population grown in Ereğli District was assessed using SSR markers. Purple carrot samples were collected by visiting 14 purple carrot growing fields in this region. A total of 20 SSR markers were used. Two SSR markers were monomorphic and the remaining 18 SSR markers amplified 106 SSR alleles. The number of polymorphic alleles per SSR marker ranged from 1 to 14. The polymorphism information content varied from 0.29 to 0.85, and the expected heterozygosity ranged from 0.32 to 0.87. STRUCTURE analysis indicated the presence of two distinct populations within the purple carrot genotypes used in carrot production in Ereğli District. The genetic similarity of the genotypes ranged from 0.20 to 0.70. These results indicate that the genetic variation within these carrot populations in Ereğli District is high, and the purple carrot populations in this region are valuable genetic resources for the development of new purple carrot cultivars.

Key words: *Daucus carota* L, genetic variation, purple carrot, SSR marker, STRUCTURE analysis

1. Introduction

Carrot (*Daucus carota* L.) is an important vegetable crop grown worldwide. According to the Food and Agriculture Organization of the United Nations (FAO), world carrot production in 2013 was approximately 37.2 million metric tons. The great majority of this carrot production consists of orange carrots due to the accumulation of carotenoid in their roots. Although yellow carrots with xanthophyll, red carrots with lycopene, purple carrots with anthocyanin, and white carrots with no pigmentation all exist, limited land has been dedicated to their production.

Orange carrots are preferred for their high content of carotenoid, which is the precursor of vitamin A. Unlike orange carrots, purple carrots contain anthocyanin pigments. Anthocyanin pigments are water-soluble colored flavonoids that can give plant organs a red, blue, or purple color (Harborne and Williams, 2000). Due to the various health-related benefits of anthocyanins, such as protection against oxidative stress, coronary heart disease, inflammation, some types of cancer, and other age-related diseases (Ross and Kasum, 2002), the popularity of consuming an anthocyanin-rich diet has been increasing recently. In addition, depending on the pH, the color of anthocyanin can change from red to purple, and, for this reason, anthocyanin pigments have gained recent attention as a natural food colorant for use in the ready-to-eat food industry. Purple carrots are rich in cyanidin glycoside-type anthocyanin pigments and can accumulate up to 17–18 mg/100 g fresh weight in their storage roots (Montilla et al., 2011).

According to the Turkish Statistical Institute (TÜİK), approximately 558,000 t of carrots were produced on 10,500 ha in Turkey in 2014. More than 93% of this production was in Konya (331,593 t), Ankara (131,400 t), and Hatay (60,483 t) provinces, and the majority of this carrot production consisted of orange carrots. Historically, purple carrots with high anthocyanin content have been used for the production of a local drink called 'şalgam' in the Mediterranean region of Turkey. However, the amount of purple carrot production for making şalgam is currently very limited. On the other hand, purple carrot production in Turkey has been increasing dramatically in recent years

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due to the use of purple carrots for anthocyanin extraction by the pigment industry. Currently, more than 90% of the purple carrots produced in Turkey are being used by the pigment industry, and all of the anthocyanin pigment extracted in Turkey is being exported. Purple carrots are produced in Konya and Hatay provinces, but purple carrot production is most concentrated in Ereğli District in Konya Province, which produces more than 95% of the purple carrots in Turkey.

Although more than 90% of the orange carrots in Turkey are being produced using hybrid cultivars, purple carrot production still uses local open-pollinated purple carrot cultivars or landraces. These local open-pollinated purple carrot cultivars or landraces were developed and maintained by farmers by selecting carrot storage roots with intense purple color (locally called "black carrots"). Therefore, these local purple carrot cultivars or landraces still present great phenotypic diversity for purple carrot breeding programs. For example, the tissue distribution of root purple pigmentation among plants in these landraces or local cultivars ranges from thin pigmented cell layers in the periderm to intensively colored xylem, cambium, and phloem tissues of the root. Similarly, a high level of phenotypic variation is also present in the leaves and flowers of the purple carrot plants from this region. Although the phenotypic variation within these local purple carrot cultivars or landraces is very valuable for breeding programs worldwide, the genetic variation in these populations has not been analyzed previously. Therefore, the purpose of this study was to assess the genetic variation among the local cultivars used in purple carrot production in Ereğli District using simple sequence repeat (SSR) markers.

2. Materials and methods

2.1. Plant materials and DNA extraction

Plant materials were collected by visiting 14 local carrot growing fields in Kuzukuyu and Akören villages in Ereğli District during the harvest season (Figure 1). Two purple carrot genotypes donated by Prof Philipp W Simon (USDA, University of Wisconsin-Madison, WI, USA) were included in the analysis for comparison. Purple carrot roots were collected from the field and brought to the laboratory for DNA extraction. Young leaves developed from the purple carrot roots were sampled and used for DNA extraction. DNA samples were extracted from lyophilized and powdered 20 mg of leaf tissue using a modified CTAB method described by Futherer

Figure 1. Map of the collection sites in Ereğli District in Konya, Province, Turkey. Shaded areas indicate the location of purple carrot growing fields visited.

et al. (1995). The concentration of each DNA sample was measured using a Qubit fluorometer (Invitrogen, USA) and adjusted to 20 ng/µL for use in SSR analysis, and the samples were stored at –20 °C.

2.2. SSR analysis

Twenty SSR markers developed by Cavagnaro et al. (2011) were used for the amplification of SSR markers among 16 purple carrot genotypes. The forward primers were tailed with the M13 sequence (GACGTTGTAAAACGACGGCC) at the 5' end (Schuelke, 2000). Each 20 µL of PCR mixture for the amplification of SSR markers contained 1.0 U of *Taq* DNA polymerase (Thermo Scientific, USA), 1X reaction buffer, 0.10 µM M13 sequence-tailed forward primer, 0.20 µM reverse primer, 0.20 µM M13 primer labeled with infrared dye either at 700 nm or 800 nm (LICOR, USA), 0.2 mM dNTPs, and 40 ng of DNA. The thermal cycling conditions were as follows: 2 min at 94 °C; 28 cycles of 45 s at 94 °C, 1 min at annealing temperature of each SSR primers, and 1 min and 10 s at 72 °C; 7 cycles of 45 s at 94 °C, 1 min at 54 °C, and 1 min and 10 s at 72 °C; and a final extension step of 5 min at 72 °C. PCR was performed using a GenAmp 2720 Thermal Cycler (Applied Biosystems, USA), and the PCR products were separated on 6% polyacrylamide gels at 30 W for 3 h using a LICOR 4300 automated sequencer system (LICOR).

2.3. Data analysis

A genetic similarity matrix of purple carrot genotypes based on SSR markers was calculated using the Dice coefficient (Dice, 1945). An unweighted pair group method with arithmetic averages (UPGMA) dendrogram based on the Dice similarity matrix was constructed using the NTSYSpc v.2.21 program (Exeter Software, New York, NY, USA). The polymorphism information content (PIC) was calculated using the formula

$$PIC = 1 - \sum_{i=1}^{j} P_i^2 - 2\sum_{i=j+1}^{j}\sum_{j=1}^{i-1} P_i^2 P_j^2,$$

where Pi and Pj are the frequencies of the ith and jth alleles at a locus with l allele in a population, respectively (Botstein et al., 1980). The heterozygosity (He) values for SSR markers were calculated according to Hildebrand et al. (1992):

$$He = 1 - \sum p_i^2,$$

where P_i is the frequency of the ith allele.

The population structure of the purple carrot genotypes used in this study was assessed using model-based Bayesian clustering implemented in STRUCTURE v.2.3.4 (Pritchard et al., 2000). Possible Ks (where K is an assumed fixed number of subpopulations in the entire population) from 1 to 10 were examined with 5 replicates. Each replication run was conducted with a burn-in period of 100,000 steps followed by 20,000 Monte Carlo Markov Chain (MCMC) replications using an admixture model and correlated allele frequencies options. The most likely number of subpopulations (K) was determined using the method described by Evanno et al. (2005).

3. Results and discussion

A total of 20 SSR markers were used to assess genetic diversity in the purple carrot population grown in Ereğli District in Konya Province. While 18 of these SSR markers were polymorphic, the remaining two were monomorphic with a single allele. The number of polymorphic alleles per SSR marker varied from 1 to 14 (Table 1). BSSR22 and BSSR03 were amplified only in the genotypes from the USA, but BSSR03 was also amplified in another genotype

Table 1. Number of alleles, expected heterozygosity (He) and polymorphism information content (PIC) of SSR markers.

SSR markers	Number of alleles	He	PIC
BSSR03*	1	-	-
BSSR04	6	0.69	0.65
BSSR12	8	0.78	0.75
BSSR126	5	0.64	0.60
BSSR14	4	0.59	0.53
BSSR14	3	0.32	0.29
BSSR22*	1	-	-
BSSR43	3	0.58	0.49
BSSR52	8	0.80	0.78
BSSR89	8	0.83	0.81
BSSR128	monomorphic	-	-
GSSR03	7	0.61	0.58
GSSR04	14	0.87	0.85
GSSR05	3	0.42	0.35
GSSR06	4	0.67	0.61
GSSR11	7	0.79	0.76
GSSR14	10	0.81	0.79
GSSR16	5	0.67	0.62
GSSR24	7	0.80	0.77
GSSR69	monomorphic	-	-
Mean	5.78	0.68	0.64

* indicates SSR markers that were not amplified in all purple carrot genotypes.

from Ereğli District, probably due to the nucleotide variation within the primer sites of these markers. Among the analyzed genotypes, 106 SSR alleles were amplified, and the average number of alleles per SSR marker was 5.78. Heterozygosity expected (He) of each SSR marker ranged from 0.32 to 0.87 with an average of 0.68, while the polymorphism information content (PIC) ranged from 0.29 to 0.85 with a mean of 0.64. Baraski et al. (2012) found that the mean number of alleles per SSR marker in carrot was 7.6, and the average He and PIC were 0.67 and 0.63, respectively. In another study assessing genetic variation within the cultivated carrot populations, the average number of SSR alleles per SSR marker was 9.37, and PIC and He were 0.56 and 0.61, respectively (Maksylewicz and Baranski, 2013). These results suggested that the variation of SSR markers within the purple carrot population grown in Ereğli District is very high.

The number of gene pools indicated by the STRUCTURE analysis in this study was estimated to be K = 3 with the highest ΔK value (22.25) (Figure 2), followed by K = 4 and K = 2 with ΔKs of 2.25 and 1.75, respectively. Based on the STRUCTURE analysis, one of the populations consisted of the two genotypes from the USA (red bars in Figure 2). The purple carrot genotypes collected from Ereğli District were assigned to two different populations. Although purple carrot genotypes were collected from two different villages, the populations were not based on the collection sites. On the other hand, during the visits to carrot production fields, farmers indicated that a new purple carrot cultivar was recently introduced into the region from an unknown origin. One of the populations consisted of the genotypes native to the Ereğli region (green bars in Figure 2), while the other population contained genotypes from the recently introduced purple carrot cultivar (blue bars in Figure 2). Purple carrot genotypes native to the Ereğli region taste better because they were originally selected for the production of şalgam. Genotypes from the recently introduced purple carrot cultivar did not taste good but had dark purple roots, indicating a high concentration of anthocyanin (Figure 3). The genotypes of these two groups can be distinguished phenotypically because plants of the recently introduced cultivar have hairy leaves and petioles and a bluish-green leaf color. In previous studies using SSR markers, carrot germplasms were divided into two gene pools, Western and Asian (Baraski et al., 2012; Maksylewicz and Baranski, 2013). In this study, we identified three gene pools. One contained purple carrots from the USA, probably representing the Western gene pool, while the others are two different gene pools composed of purple carrots of Asian origin.

The genetic similarity of purple carrot genotypes collected from Ereğli District ranged from 0.20 to 0.70, indicating a high amount of genetic variation (Table 2). The genetic similarity of purple carrot genotypes collected from Kuzukuyu village ranged from 0.26 to 0.70, while the genetic similarity ranged between 0.21 and 0.63 among the genotypes from Akören, indicating the presence of high genetic variation among purple carrots collected from each village. An UPGMA dendrogram showing the genetic relationships among purple carrot genotypes was developed using the Dice similarity matrix (Figure 4). The purple carrot genotypes clustered in three distinct groups, representing the three populations identified by STRUCTURE analysis. One of the groups contains genotypes from the USA, while the other two groups consist of the genotypes collected from Ereğli District. Purple carrot genotypes native to Ereğli District clustered into one group, while genotypes from the introduced purple carrot cultivar clustered in another group. Although the genotype Akören-7 has the phenotype of purple carrots native to Ereğli District, it clustered with genotypes from the introduced cultivars. Similarly, this genotype was

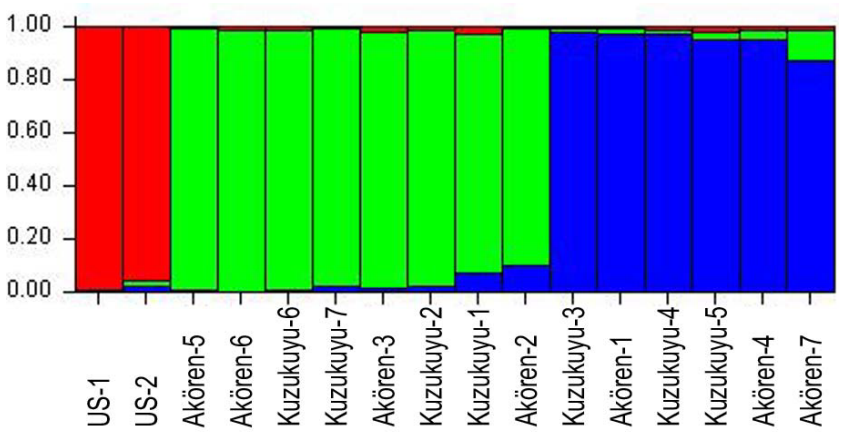

Figure 2. STRUCTURE bar plot based on 16 SSR markers. Different colors represent different gene pools.

Figure 3. Photographs of A) purple carrot genotypes native to the Ereğli region and B) introduced purple carrot genotypes.

Table 2. Genetic similarity between the purple carrot genotypes based on the Dice coefficient.

	USA-1	USA-2	Kuzukuyu-1	Kuzukuyu-2	Kuzukuyu-3	Kuzukuyu-4	Kuzukuyu-5	Kuzukuyu-6	Akören-1	Akören-2	Akören-3	Akören-4	Akören-5	Akören-6	Akören-7
USA-2	0.69														
Kuzukuyu-1	0.24	0.26													
Kuzukuyu-2	0.32	0.27	0.38												
Kuzukuyu-3	0.31	0.33	0.45	0.41											
Kuzukuyu-4	0.33	0.42	0.41	0.43	0.68										
Kuzukuyu-5	0.30	0.41	0.32	0.45	0.70	0.69									
Kuzukuyu-6	0.29	0.31	0.42	0.54	0.38	0.41	0.43								
Akören-1	0.27	0.33	0.45	0.47	0.63	0.64	0.60	0.45							
Akören-2	0.19	0.18	0.55	0.56	0.50	0.50	0.44	0.53	0.50						
Akören-3	0.22	0.25	0.55	0.38	0.43	0.36	0.24	0.59	0.34	0.57					
Akören-4	0.29	0.38	0.44	0.39	0.63	0.50	0.52	0.41	0.63	0.45	0.36				
Akören-5	0.19	0.21	0.40	0.36	0.29	0.24	0.27	0.57	0.36	0.45	0.53	0.27			
Akören-6	0.26	0.24	0.33	0.44	0.20	0.24	0.22	0.59	0.31	0.38	0.22	0.21	0.35		
Akören-7	0.20	0.26	0.55	0.32	0.42	0.53	0.40	0.39	0.50	0.44	0.43	0.54	0.41	0.33	
Kuzukuyu-7	0.08	0.11	0.34	0.51	0.26	0.26	0.28	0.45	0.37	0.50	0.38	0.36	0.43	0.35	0.50

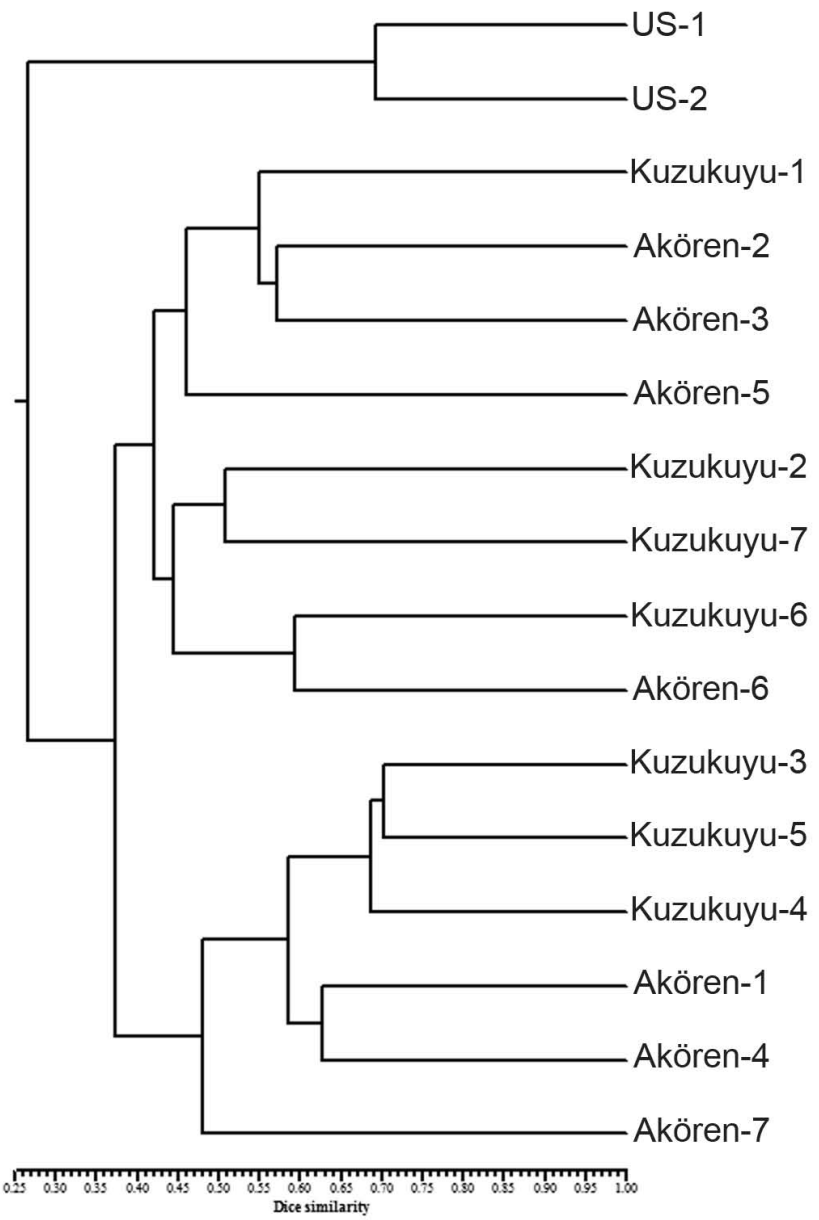

Figure 4. The UPGMA dendrogram demonstrating genetic relationships based on the Dice similarity matrix of 18 SSR markers.

assigned to the population with other genotypes from introduced cultivars by STRUCTURE analysis. In this region, farmers tend to produce their own seeds. During seed production, the farmers have not always given proper attention to isolation distance to prevent cross-pollination, and therefore this genotype could be the result of an uncontrolled cross between a native and an introduced purple carrot genotype.

In conclusion, a high level of genetic variation was detected among the purple carrot genotypes collected from villages in Ereğli District based on SSR markers. This high genetic variation could be useful for a carrot breeding program to develop new purple carrot cultivars. Although purple carrots in the region have been grown for making a local drink called şalgam, the majority of purple carrots produced are currently being used by the pigment industry. Therefore, a new cultivar with high anthocyanin content was introduced into the region, increasing the genetic variation among purple carrots grown in the region. Purple carrot production in the region has previously used open-pollinated purple carrot cultivars. However, hybrid cultivars with high anthocyanin content have been

developed and will probably be used for further purple carrot production. At this point, this high level of genetic variation should be preserved by collecting purple carrot germplasm from the region before local open-pollinated cultivars are replaced by hybrids.

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