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ORIGINAL ARTICLE

Analysis of European hazelnut (*Corylus avellana*) reveals loci for cultivar improvement and the effects of domestication and selection on nut and kernel traits

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Abstract

Turkey is a rich source of European hazelnut (*Corylus avellana*) germplasm with nearly 400 accessions in the national collection. This genetic material encompasses cultivars, landraces and wild genotypes which were characterized for 12 nut and 13 kernel traits over 2 years in the 1990s. Analysis of these attributes revealed both the positive and negative impacts that human selection and breeding have had on hazelnut. Thus, while selection has resulted in larger nuts and kernels, cultivars have fewer nuts per cluster and kernels with larger internal cavities. Breeding has also resulted in a propensity for cultivars to have higher proportions of double kernels and empty nuts, two traits which reduce quality and yield. In addition, it is clear that while selection has successfully increased hazelnut fat content it has not impacted overall flavor, a much more complex trait. The nut and kernel phenotypic data were combined with genotypic data from 406 simple sequence repeat marker alleles for association mapping of the quantitative trait loci (QTL) for the traits. A total of 78 loci were detected in the population with the highest proportions for nut (24%) and kernel (26%) appearance parameters followed by quality (19%), shell thickness (16%) and yield-related (15%) traits. It is hoped that some of the identified QTL will be useful for future breeding of hazelnut for improved nut and kernel yield and quality.

Keywords Filbert · Microsatellites · Simple sequence repeats (SSRs) · Quantitative trait locus (QTL)

Introduction

Corylus avellana, European hazelnut, is a temperate shrub species that has been cultivated since the nineteenth century (Elzebroek and Wind 2008) for its oil-rich nuts. The hazelnut kernel is rich in protein (10–24%) (Pala et al. 1996; Yurttas et al. 2000) and unsaturated fatty acids (50–68%) (Garcia et al. 1994; Koksal et al. 2006), and is especially high in B vitamins and vitamin E (Koksal et al. 2006; Alasalvar et al. 2009). The kernels are consumed whole, ground or in paste form and lend their unique flavor to a variety of confectionery products. Despite currently ranking 6th among tree nuts (behind cashew, walnut, chestnut, almond and pistachio), worldwide production and cultivation area of hazelnut increased by 10% in the decade between 2006 and 2016 (FAOSTAT 2018; GTHB 2018). *C. avellana* requires cool summers and mild winters, and sufficient precipitation (800–1000 mm/year) (Fideghelli and De Salvador 2009). Thus, the crop is predominantly grown in the Black Sea region, around the Mediterranean Sea, and the

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Pacific Northwest. Turkey is the world's largest producer of hazelnuts, responsible for 56% of worldwide production in 2016 (GTHB 2018; FAOSTAT 2018). The next largest producer is Italy (16%) with growers in Georgia, USA, Azerbaijan, China and Iran each accounting for 3–5% of the crop (FAOSTAT 2018).

With a long history of use that stretches back to the Mesolithic (Denison 1995), more than 400 traditional and locally selected hazelnut varieties exist (Gurcan et al. 2010). Of these, only 20 cultivars are currently of commercial significance (Mehlenbacher 2009). Despite its economic value (nearly 3 billion USD in 2014) (FAOSTAT 2018), hazelnut remains largely unimproved by modern plant breeding standards. Breeding *C. avellana* offers several challenges: it is a self-incompatible, wind-pollinated shrub species that requires 3–5 years to reach maturity (Molnar 2011). However, the highly heterozygous nature of the species and the ease of interspecific hybridization within the genus *Corylus* (Molnar 2011) mean that there is a great deal of genetic diversity within existing germplasm collections (Bacchetta et al. 2009) that could be tapped for improvement of the species. In the last decade or so, a concerted effort has been made to develop molecular markers, including random amplified polymorphic DNAs (RAPDs) (Bacchetta et al. 2005; Mohammadzedeh et al. 2014), microsatellite markers (SSRs) (Bassil et al. 2005; Boccacci et al. 2005; Gokirmak et al. 2009; Gurcan et al. 2010; Martins et al. 2015; Ozturk et al. 2018 submitted), amplified fragment length polymorphisms (AFLPs) (Ferrari et al. 2005; Kafkas et al. 2009; Martins et al. 2014) and single nucleotide polymorphisms (SNPs)(Rowley et al. 2009; Pitoni et al. 2013; Torello Marinoni et al. 2018) to characterize this diversity. These markers have also been assembled into molecular linkage maps (Mehlenbacher et al. 2006; Gurcan et al. 2010; Beltramo et al. 2016; Torello Marinoni et al. 2018) that should also facilitate molecular breeding in hazelnut. Thus far, these maps have been used to locate the position of genes for eastern filbert blight resistance (Mehlenbacher et al. 2004; Chen et al. 2005; Sathuvalli et al. 2011a, b) and the self-incompatibility locus (Mehlenbacher et al. 2006; Ives et al. 2014) and, to a very limited extent, for quantitative trait mapping.

In the first report of QTL mapping in hazelnut, Beltramo et al. (2016) used 275 F1 individuals from a cross between two European varieties to determine the locations of genes controlling quantitative morphological traits (vigor, suckering, time of bud burst). These 275 hazelnut progeny were also used to detect 28 QTL for leaf bud burst (Torello Marinoni et al. 2018) While these vegetative traits are undoubtedly critical determinants of yield, knowledge of the loci-controlling nut and kernel size and shape is arguably more useful for hazelnut breeders. To date, only one study has described QTL for nut and kernel yield and quality parameters (Ozturk et al. 2017a). Those QTL were identified by association mapping of ten nut and seven kernel traits in a germplasm panel comprised of 102 wild and cultivated accessions from Slovenia. Our current study examines data for 13 nut and 12 kernel traits assessed in the Turkish national hazelnut collection in the early 1990s. The collection contains 390 accessions (16 cultivars, 232 landraces and 142 wild) which were collected in several Black Sea provinces in Turkey (primarily Ordu, Giresun, Trabzon). We have compared the performance of each group of accessions (wild vs. landraces vs. cultivars) over two seasons, looked for correlations between the traits and identified SSR markers associated with the traits. This represents the most comprehensive analysis of nut and kernel attributes in hazelnut to date.

Materials and methods

Plant material

Nut and kernel trait data and leaf/catkin samples (for DNA isolation) were obtained in situ from a total of 390 *C. avellana* accessions growing in nine provinces in the Black Sea region of Turkey: Giresun (252 accessions) Trabzon (49 accessions), Ordu (46 accessions), Samsun (4 accessions), Rize (3 accessions), Sinop (2 accessions); Artvin, Duzce, Kastamonu (1 accession each) (Suppl. Figure 1). The provenance of 31 of the accessions is unknown. Sixteen of these accessions were standard Turkish cultivars growing at the Hazelnut Research Institute in Giresun. Locally growing landraces and wild materials from the Hazelnut Research Institute represented the majority of sampled hazelnuts: 232 and 142 accessions, respectively, from across the three main locations.

Morphological evaluation

The hazelnut association panel was characterized for 25 nut and kernel traits over two consecutive years (1991, 1992) by Caliskan and Cetiner (1992) using 30 samples per accession. Traits were measured in accordance with UPOV guidelines which provide scales and reference cultivars for scoring each trait (UPOV 1979). The 13 nut traits included measures of nut size and abundance (number of nuts per cluster), appearance (color, number of stripes, shape of top, apex, size of pistil and basal scars, hairiness of the top, involucre adherence after nut fall), and shape [calculated as width + thickness/(2 × length)]. The proportion of empty nuts (in a sample of 30 nuts) was calculated. In addition, shell thickness was determined on hand-cracked nuts using calipers to measure the convex side of each half of the shell. Twelve kernel traits were assessed including kernel size and measures of kernel appearance (shape, shape of top, shape of base,

lateral groove, skin appearance). Percentage was calculated as (kernel weight/nut weight) × 100. The proportion of nuts containing twin kernels (in a 1 kg sample) was also determined. The size of the internal cavity was scored as were kernel blanching (ease of pellicle removal), fat content and flavor. Means and coefficients of variation for cultivars, landraces and wild accessions were calculated separately for comparison. The 2-year data were averaged for each trait. Basic statistics such as correlation analysis between traits and ANOVA were done using PASW software (Norusis 2010).

SSR amplification

Total genomic DNA was extracted from the plant tissue (leaves or catkins) by a microprep method (Fulton et al. 1990). A total of 30 SSR markers from Gurcan et al. (2010) were then assessed in the 390 accessions. PCR amplification was done in 20 µl reaction volumes containing 20 ng of DNA, 10 pmol of each primer, 20 uM dNTPs, 2 µl 10X Taq polymerase buffer and 0.6 Unit Taq polymerase. PCR amplifications were performed in a GeneAmp PCR System 9700 (Perkin Elmer Applied Biosystems). The reaction conditions used for all primers consisted of an initial denaturation step at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s and concluded with an extension step at 72 °C for 5 min. PCR products were separated by capillary gel electrophoresis using a Fragment Analyzer (Applied Biosystems) with the DNF-900 dsDNA Reagent Kit (Advanced Analytical) according to the manufacturer's instructions. Because many of the SSR markers yielded more than two fragments and allelism could not be determined, PCR fragments were scored binomially (presence 1, absence 0).

Association mapping

The binary data generated for the SSR markers were associated with the nut and kernel trait data using the GLM and MLM models of TASSEL v.2.1 (Trait Analysis by aSSociation, Evolution and Linkage) software (Bradbury et al. 2007). Linkage disequilibrium (LD) values (r^2 and P values) between SSR markers were determined with the same software. To determine the model with the best fit for association mapping, several models were tested. These included the GLM model without correction, GLM corrected with the Q-matrix of population structure [GLM (Q)], MLM without correction and MLM corrected with the Q-matrix of population structure [MLM (Q)]. The Q-matrix generated at $K = 2$ (subgroup number = 2) was used as covariate (Ozturk et al. 2017b). To determine the best model, the P values generated by each model were analyzed with QVALUE software (Storey 2002) using a false discovery rate (FDR) of 0.05 (Storey and Tibshirani 2003). The probabilities of truly null ($\pi 0$) and significant ($\pi 1$) results were determined through bootstrap analysis. The model with the highest $\pi 1$ value was deemed the best fit and its results are reported. Marker trait associations with $-\text{Log}$ (P value) > 2.3 (equivalent to $P < 0.005$) were considered significant.

Results

Nut and kernel trait diversity

Twenty-five nut and kernel traits were assessed in the 390 hazelnut accessions. Cultivars, landraces and wild material were analyzed as separate groups to compare their performance and breeding potential (Table 1, Suppl. Table 2). Although landraces produced significantly ($P < 0.05$) more nuts per cluster than cultivars, kernel percentage was significantly higher in cultivars. Kernel percentage was also higher in landraces than the wild material which had significantly thicker shells. Cultivars showed a greater propensity toward empty kernels than the other accessions and double kernels were more common in cultivars and landraces than wild material. Cultivars and landraces tended to produce larger and rounder nuts with larger basal scars for the landraces compared to the wild accessions. Both cultivars and landraces outperformed wild accessions in terms of kernel size; these kernels also tended to have larger inside cavities. The kernels of landraces were also more globular than those produced by wild accessions. The fat content of the kernels produced by cultivars was significantly higher than that of the landraces.

Trait correlations

Because many of the trait correlations were significant ($P < 0.01$) (Suppl. Table 3), we have adopted the scale used by De Souza et al. (1998) in judging the strength of these correlations. Very strong ($r^2 > 0.65$) positive correlations were observed between nut and kernel size as well as shape. A strong negative correlation was found between the shapes of the nut top and apex such that nuts with more acute tops had more prominent apices. The remaining correlations were weak ($r^2 < 0.30$) or moderately weak ($0.30 < r^2 < 0.49$) in nature. Among the latter category were positive relationships between nut and kernel sizes, and inside cavity (larger nuts/kernels had larger cavities). Percent kernel was positively correlated with kernel size and negatively with nutshell thickness. Kernels produced in nuts with thicker shells tended to have corkier skin. Finally, flatter topped nuts contained kernels with flatter bases.

Table 1 Nut and kernel traits for hazelnut accessions

Traits	Cultivar ($n=16$)			Landraces ($n=232$)			Wild ($n=142$)		
	Mean ± SE	CV %	Range	Mean ± SE	CV %	Range	Mean ± SE	CV %	Range
Nut									
Adherence of involucre on fruits (1–9)	1.1 ± 0.1a	44	1–3	1.4 ± 0.1a	104	1–9	1.6 ± 0.1a	99	1–9
Apex (3–7)	4.6 ± 0.4a	32	3–7	4.3 ± 0.1a	34	3–7	4.3 ± 0.1a	36	3–7
Average number (1–9)	5.8 ± 0.4a	25	3–9	6.8 ± 0.1b	23	3–9	6.7 ± 0.1ab	24	3–9
Color (1–4)	2.6 ± 0.2a	31	1–4	2.7 ± 0a	20	1–4	2.7 ± 0a	19	2–4
Empty (1–9)	6.9 ± 0.5a	25	5–9	4.5 ± 0.1b	48	1–9	4.7 ± 0.2b	50	1–9
Hairiness of top (3–7)	4.5 ± 0.4a	30	3–7	4.8 ± 0.1a	33	3–7	4.7 ± 0.1a	34	3–7
Number of stripes on shell (3–7)	4.4 ± 0.4a	36	3–7	4.6 ± 0.1a	32	3–7	4.5 ± 0.1a	33	3–7
Shape (1–7)	2.8 ± 0.5ab	61	1–7	2.7 ± 0.1a	53	1–7	3.4 ± 0.1b	47	1–7
Shape of top (1–7)	2.4 ± 0.3a	47	1–4	2.7 ± 0.1a	41	1–4	2.6 ± 0.1a	42	1–4
Shell thickness (1–9)	4.0 ± 0.7ab	63	1–9	4.4 ± 0.2a	59	1–9	5.4 ± 0.2b	50	1–9
Size (1–9)	5.8 ± 0.3ab	21	4–9	5.7 ± 0.1a	15	4–9	5.5 ± 0.1b	13	3–7
Size of basal scar (3–7)	3.9 ± 0.3ab	32	3–7	4.4 ± 0.1a	24	3–7	4.1 ± 0.1b	28	3–7
Size of pistil scar (3–7)	4.4 ± 0.4a	32	3–7	4.0 ± 0.1a	36	3–7	4.2 ± 0.1a	36	3–7
Kernel									
Appearance of skin (1–9)	2.4 ± 0.4a	67	1–7	2.7 ± 0.2a	86	1–9	2.8 ± 0.2a	83	1–9
Blanching (1–9)	7.3 ± 0.5a	26	3–9	7.3 ± 0.2a	32	1–9	7.4 ± 0.2a	30	1–9
Double kernel (1–9)	2.1 ± 0.6ab	103	1–7	1.9 ± 0.1a	105	1–9	1.4 ± 0.1b	86	1–9
Fat content (3–7)	4.5 ± 0.4a	30	3–7	3.7 ± 0.1b	29	3–7	3.8 ± 0.1ab	31	3–7
Flavor (3–7)	4.8 ± 0.3a	26	3–7	5.1 ± 0a	13	3–7	5.1 ± 0a	9	3–7
Inside cavity (1–9)	5.6 ± 0.5a	36	3–9	4.5 ± 0.1a	48	1–9	3.8 ± 0.2b	57	1–9
Lateral groove (1–9)	5.0 ± 1.1a	83	1–9	4.2 ± 0.3a	93	1–9	4.1 ± 0.3a	95	1–9
Percentage of kernel (1–9)	4.8 ± 0.4a	34	3–7	3.5 ± 0.1b	56	1–9	2.8 ± 0.2c	65	1–9
Shape (1–8)	3.3 ± 0.6ab	67	1–8	2.9 ± 0.1a	59	1–8	3.8 ± 0.2b	53	1–8
Shape of base (1–3)	2.3 ± 0.1a	20	2–3	2.3 ± 0a	21	1–3	2.3 ± 0a	21	1–3
Shape of top (1–3)	1.2 ± 0.1a	46	1–3	1.2 ± 0a	41	1–3	1.2 ± 0a	40	1–3
Size (1–9)	5.6 ± 0.3a	19	4–8	5.0 ± 0.1a	20	2–8	4.7 ± 0.1b	22	2–7

Means with different letters within a row are significantly different according to analysis of variance (ANOVA) and least significant difference (LSD) test ($P<0.05$)

Association mapping

A total of 406 polymorphic fragments were generated from the thirty SSR primers. The fragment profiles of the 390 hazelnut accessions were scored and analyzed to localize QTL underlying the nut and kernel traits. Different association mapping models [GLM, GLM (Q), MLM, MLM (Q)] were used and the proportion of significant results compared. Based on this analysis, GLM (Q) analysis was chosen because it gave the highest proportion of significant results ($\pi 1$). A total of 11,396 marker pair combinations were tested and, of these, 188 (3.0%) associations were at a significance level $P \leq 0.01$, r^2 (LD level) ≥ 0.01. We present those results here, focusing on markers with a P value less than 0.005 (1.6%). The LD values (r^2) of the significant markers tended to be quite small (typically 0.02 or 0.03). Therefore, we have only mentioned marker LD values in excess of 0.03.

A total of 78 (0.6%) significant marker–trait associations were discovered (Table 2). Thirty-five (45%) of these associations were marker fragments linked to 10 of the 13 nut traits. No SSR markers were identified for color, shape of the top and size of the basal scar. A total of 43 associations were found between the SSR marker alleles and 10 of the 12 kernel traits with no SSR markers detected for kernel shape.

Nut traits

Several of the yield- and quality-related traits such as average number of nuts per cluster, involucre adherence, proportion of empty nuts, and nutshell striping, shape and size mapped to one or two loci each. A large number of marker associations were detected for shell thickness: seven different SSR markers encompassing a total of 12 alleles. The shape of the nut apex was associated with seven separate SSR alleles including three fragments from SSR B648.

Table 2 Hazelnut SSR markers associated with nut and kernel traits

Traits	SSR locus	− LOG (*P* value)[a]	LD value (r^2)
Nut			
Adherence of involucre	A613_178	2.54	0.02
Apex	B631_306	2.85	0.03
	B648_216	2.80	0.03
	B603_404	2.74	0.03
	B648_354	2.47	0.03
	B648_246	2.44	0.03
	A640_367	2.38	0.03
	A635_297	2.33	0.03
Average number	B788_181	3.00	0.04
	B652_266	2.52	0.03
Empty	B640_443	2.80	0.03
Hairiness of top	B606_402	2.89	0.03
	B641A_386	2.72	0.03
	B631_218	2.32	0.03
	B631_300	2.32	0.03
Number of stripes on shell	B662_245	2.51	0.02
	B631_184	2.49	0.03
Shape	A613_200	2.92	0.03
	CAC-B753_234	2.32	0.03
Shell thickness	B603_373	2.92	0.03
	B648_216	2.89	0.03
	B603_301	2.80	0.03
	B651_256	2.77	0.03
	B651_263	2.77	0.03
	B631_260	2.70	0.03
	A635_219	2.60	0.03
	B603_313	2.55	0.02
	B613_310	2.55	0.03
	B648_210	2.51	0.02
	B648_266	2.51	0.03
	A606_182	2.36	0.02
Size	A602_400	2.52	0.03
Size of pistil scar	A640_386	2.74	0.03
	A616_184	2.64	0.03
	B602_387	2.35	0.03
Kernel			
Appearance of skin	B660_300	2.85	0.03
Blanching	B651_320	2.73	0.07
Double kernel	B631_166	4.38	0.09
	A640_431	2.89	0.03
	B625_254	2.62	0.03
	B640_389	2.60	0.03
	B606_448	2.31	0.03
Fat content	B651_291	2.60	0.03
	B641B_259	2.55	0.02
	B612_236	2.43	0.03
	B612_334	2.43	0.03
	A606_182	2.40	0.02
	B612_218	2.39	0.03
	B648_210	2.39	0.02
	B612_301	2.34	0.03
Flavor	B635_383	2.59	0.03
	A606_192	2.51	0.02
	A611_210	2.48	0.02
	B648_280	2.40	0.03
Inside cavity	CAC-B753_303	2.96	0.03
	B631_200	2.70	0.03
Lateral groove	A613_169	2.49	0.02
Shape of base	B631_260	3.00	0.04
	A602_323	2.85	0.03
	B652_376	2.82	0.03
	A602_246	2.70	0.03
	A602_212	2.60	0.03
	A602_264	2.60	0.03
	B641A_455	2.55	0.03
	A602_223	2.46	0.03
	B606_323	2.44	0.03
	A602_256	2.43	0.03
	B651_263	2.40	0.03
	A602_252	2.37	0.03
	A602_237	2.36	0.03
	A602_136	2.35	0.03
Shape of top	B631_260	2.70	0.03
	B660_280	2.60	0.03
	B648_216	2.48	0.02
	B648_210	2.33	0.02
Size	B631_218	2.96	0.04
	B660_219	2.77	0.03
	A606_203	2.51	0.03

[a]Negative log10-transformed *P* values

Attributes of the nut top, including top hairiness and pistil scar size, were associated with four and three SSR markers, respectively.

Kernel traits

Single trait associations were detected for skin appearance, blanching and lateral groove with none for kernel shape. Five SSR markers were detected for double kernel (B631_166 had the highest LD value of 0.09). Fat content linked to eight fragments from five different SSR markers, including four separate B612 alleles (B612_218, B612_301, B612_236, B612_334). Four flavored QTL were detected. The shape

of the kernel base was linked to the greatest number of markers: 14 alleles from six different SSR markers. Seven of these fragments were generated from marker A602: A602_212, A602_223, A602_246, A602_252, A602_256, A602_264, A602_323. Of the 14 significant alleles, B631_260 had the highest LD value: 0.04. Kernel top shape was associated with four SSR marker loci. Kernel size was linked to three SSR loci.

Co-localization

The SSR marker with the greatest number (11) of significant associations was B631. Nine (36%) of the nut and kernel traits mapped to seven different B631 alleles: four nut characters (apex, top hairiness, shell stripes and thickness) and five kernel attributes (double kernel, inside cavity, base and top shapes and size). Co-localization of traits was observed at two of the B631 fragments. Kernel top and base shape, and nutshell thickness were all associated with B631_260. B631_218 was detected for kernel size and nut top hair. The second highest number of associations (10) was observed for B648 and A602. Nine of the associations to A602 involved kernel base shape. An additional fragment generated by this SSR (A602_400) was linked to nut size. A total of five different traits (nut apex and kernel top shape, shell thickness, kernel flavor and fat content) were significantly associated with six alleles of marker B648. Three traits mapped to B648_210: nutshell thickness, kernel top shape and fat content. Nutshell thickness and kernel top shape also mapped together (along with nut apex) at B648_216. Similarly, shell thickness and kernel base shape co-localized to B651_263. Shell thickness also overlapped with fat content at A606_182.

Discussion

Effects of domestication and breeding on nut and kernel traits

Our analysis of nut and kernel traits across cultivars, landraces and wild accessions has revealed some of the impacts that human selection and breeding have had on these attributes in hazelnut. While domesticated accessions typically produce larger kernels than wild material, selection for this important yield variable may have inadvertently led to larger inside cavities, a quality defect. Landraces produce more nuts per cluster than cultivars. However, the kernels within those nuts are smaller as indicated by a significantly lower kernel percentage (the proportion of nut weight that the kernel contributes). Presumably, this is a result of an organism having limited resources to allocate toward reproduction: the so-called principle of allocation (Cody 1966). Indeed studies in a number of herbaceous and woody species (Aarssen and Jordan 2001; Henery and Westoby 2001; Moles et al. 2004) have confirmed the inverse relationship between seed mass and seed number (fecundity) in plants. The wild material, in contrast, has a lower kernel percentage and thicker shells. Here too, artificial selection for larger kernels may have diverted plant resources from nutshell to seed (kernel) development. Selection for larger nuts may have also contributed to the higher incidence of double kernels in landraces than wild material, another quality defect. Our data also suggest that domestication has impacted nut and kernel shape: the landraces analyzed in our study produce rounder nuts containing rounder kernels than the wild accessions. The proportion of empty kernels tends to be a highly variable trait that depends on environmental conditions, including mineral nutrient availability. Boron deficiency in particular is suspected to impede fruit set but there seems to be no consensus on whether or not applied boron reduces the incidence of blank fruits in hazelnut (Shrestha et al. 1987; Ferran et al. 1997; Silva et al. 2011). The fact that this trait was highest in cultivars suggests that mutations conferring parthenocarpy may have arisen in hazelnut as they have in other self-incompatible fruit crops (e.g., apple and pear) (Zohary 2004). Finally, our comparison revealed that breeding has improved kernel nutritional quality, as evidenced by cultivars' higher fat content. Interestingly, however, our results suggest that there have been no significant improvements in flavor. Given the limited and subjective nature of the hazelnut flavor descriptor, it is doubtful that it captures the complexity of this trait which depends on both taste components and aromatic compounds.

The correlations observed in our analysis were not surprising. All of them involved traits that are obviously interdependent (for example, nut and kernel size and shape, percent kernel and kernel size). The moderately weak correlation between nut/kernel size and inside cavity supports our hypothesis that selection for larger nuts may unintentionally lead to kernel quality issues.

Loci-controlling nut and kernel traits

The number of traits (25) and accessions (390) evaluated make this one of the largest association mapping studies performed in hazelnut to date. The LD values of the QTL we have detected are quite small, suggesting that these nut and kernel traits are truly quantitative in nature and inheritance (controlled by multiple genes). Each of the loci we have identified can, therefore, be considered a minor QTL, explaining a small amount of the total phenotypic variation in the trait.

A total of twelve QTL (15% of the total number) were for five yield-related traits (kernel and nut size, nut number, emptiness and double kernels). Nineteen loci (24%)

impacted nut external appearance (shape, apex, pistil scar size, top hairiness, stripes and involucre adherence), whereas 20 (26%) influenced kernel appearance (shapes of top and base, lateral groove and skin texture). Quality parameters (fat content, flavor, blanching, inside cavity) were linked to 15 QTL (19%). A striking proportion of QTL (12; 16%) impacted nutshell thickness.

Co-localization of QTL for different traits may indicate pleiotropy of a single genetic locus or, alternatively, the presence of linked genes. Thus, we might imagine that a single kernel-shaped gene is operating in the vicinity of B631_260 to influence the shape of the kernel top and base, two characters that show some correlation ($r^2 = 0.20$). Whether or not this same gene might also influence nut shell thickness seems more speculative, but it is worth noting that shell thickness and kernel base- and top-shaped QTL overlap at three other locations (B648_210, B648_216, B651_263) and thickness and kernel base shape are weakly correlated ($r^2 = 0.26$). A weak negative correlation ($r^2 = -0.23$) exists between nut apex and kernel top shape, two shape traits that co-localize to B648_216. Nutshell thickness and fat content are weakly correlated ($r^2 = 0.23$), and both are linked to B648_210. Of course, this correspondence may be coincidental: possibly a function of low marker density (lacking knowledge of marker position we have no way of judging this) and the sheer number of shell thickness and kernel-shaped QTL. We found no evidence of pleiotropy or genetic linkage of QTL for the most strongly correlated traits (nut and kernel size, nut and kernel shape and nut apex and nut top shape) in our study. This is not surprising given how few QTL were detected for these particular traits (one nut- and three kernel-sized loci, one nut but no kernel-shaped loci, seven apexes but no nut top-shaped loci).

While Ozturk et al. (2017a) used 26 of the 30 SSR markers used in the current work to perform association mapping of nut and kernel traits in Slovenian hazelnut, only seven of the traits they examined overlap with this study: nut shape, emptiness, shell thickness, nut and kernel weight/size, kernel percentage and twin/double kernel. QTL were identified for only three of these traits in the Slovenian hazelnut germplasm, namely, shell thickness and emptiness and kernel weight. For these three parameters, two shared trait–marker linkages were detected. In both studies, shell thickness was associated with SSR B648, with linkages specifically to B648_261 and B648_210/216/226 in the Slovenian and Turkish populations, respectively. In addition, two different alleles of SSR B640 were linked to blank fruits: B640_443 in this study, B640_81 in Ozturk et al. (2017a). Considerable disparity was seen in the numbers of QTL identified in that previous study: only two for shell thickness (compared to 12 here) and 22 for nut emptiness (compared to the one locus revealed in the current study) (Ozturk et al. 2017a). Because very little QTL analysis has been done thus far in hazelnut (Torello Marinoni et al. 2018; Beltramo et al. 2016; Ozturk et al. 2017a), it is our hope that this study forms a foundation for future studies to unravel the genetic basis of yield and quality traits in this important nut crop.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest. The work complies with ethical standards. This article does not contain any studies with human participants or animals performed by the authors.

Data availability Data will be available at http://plantmolgen.iyte.edu.tr/data/ upon publication.

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