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Association mapping of agro-morphological traits in European hazelnut (*Corylus avellana*)

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Abstract More than half of European hazelnut (*Corylus avellana*) production occurs in Turkey. Despite this dominance, the yield of Turkish cultivars has remained stagnant over the past 10 years with Italian yield nearly double that in Turkey. This difference is due to Turkey's unique cultivation system; hazelnuts are grown in bushy clusters ("ocak" system), not as single trees. Current hazelnut breeding efforts are shifting toward the development of materials for single plant orchards which are much higher yielding; thus, there is a need to explore germplasm for relevant agro-morphological traits and to determine their genetic control. The objectives of this study were to examine data for 44 such traits in 390 hazelnut accessions: 16 cultivars, 232 landraces and 142 wild individuals from nine provinces in Turkey and to map the loci associated with these characteristics using simple sequence repeat markers. Comparison of cultivars, landraces and wild hazelnut accessions revealed the effects of domestication and selection on the crop and indicated that useful alleles for traits such as cropping and reduced alternate bearing may exist in the wild germplasm. A total of 145 quantitative trait loci (QTL) were detected with the largest proportions identified for involucre (26%) and inflorescence (14%) morphology. Several markers co-localized with more than one trait including markers for male catkin abundance which were shared with plant vigor and height. Similarly, markers for female flower abundance co-localized with suckering and alternate bearing. Such markers and their linked QTL should be studied in more detail as they might help breeders select for plant vigor, decreased suckering and increased flower production: traits which will be extremely useful for Turkey's transition to single plant orchards.

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

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Introduction

European hazelnut (*Corylus avellana*) is a deciduous understory shrub species that tends to spread by suckers and form thickets. The vegetative buds are green to reddish in color and range in shape from globular to conical. The oval leaves are typically 5–10 cm long, serrated and pubescent. Taking 3–5 years to reach maturity, hazelnut then produces unisexual inflorescences (catkins), with male and female flowers on a single monoecious plant. The male catkins are generally around 10 cm in length and each contains 150–200 flowers that are capable of producing millions of pollen grains in late winter (Beyhan 2000). The much smaller female flowers have protruding stigmas, are sporophytically self-incompatible, wind-pollinated and produce hard-shelled nuts in clusters of one to eight (Thompson 1979). Each of the globose to ovoid nuts is surrounded by two deeply lobed and serrated involucres that have given the genus its name (from the Greek 'korys' meaning helmet) (Elzebroek and Wind 2008). Hazelnut provides shelter and food to wildlife, and nutshells found at Mesolithic sites indicate that hazelnut was also an important food source for hunter-gatherer societies (Holst 2010).

Hazelnut's native distribution extends through temperate regions of Europe. Requiring cool summers and mild winters, hazelnut was the first woody species to expand its range after the late glacial periods (Nyssen et al. 2016). Pollen and fossil nut records indicate that *C. avellana* had spread north of 50°N latitude by 9000 B.P., suggesting that decreasing winter temperatures have subsequently constrained hazel's range (Zagwijn 1994; Nyssen et al. 2016). Today, hazelnut is cultivated in the coastal climates of the Mediterranean, Black and Caspian Sea regions and the Pacific Northwest. As an alternate bearing species (which produces larger than average yields 1 year and smaller yields the next), hazelnut production varies from year to year. Commercial production of hazelnut is highest in Turkey (56% of 2016 production) (FAOSTAT 2018). The remainder of the crop is produced in a number of countries including Italy, Georgia, USA, Azerbaijan, China, Iran and Spain (FAOSTAT 2018).

The amount of land under hazelnut production in Turkey increased by 7% in the 10 years between 2007 and 2016 with no gain in production (GTHB 2018) indicating that higher yielding cultivars have not been adopted. Indeed, yield has averaged 81 kg per decare (da) over the same time period with very little variation (GTHB 2018). In comparison, over the same 10 year period, Italy's hazelnut production area increased by 4% and yield averaged 154 kg per da (FAOSTAT 2018). The discrepancy between Turkish and Italian hazelnut yield can be attributed to the different production systems in place in the two countries. Both countries use traditional local varieties, however, in Turkey, hazelnuts are grown in an "ocak," a cluster of bushy plants and frequently on substandard soils. In Italy, single trees, often on rootstocks, are grown in orchards. In the US, the amount of land under hazelnut production increased 29% between 2007 and 2016 and yield averaged 277 kg per da (FAOSTAT 2018). While US hazelnut cultivation is similar in many ways to that in Italy (use of rootstocks and single plant orchards), high yielding cultivars have been bred specifically for the fertile soils of Oregon's Willamette Valley (which produces 99% of the US crop) (Oregon Hazelnuts 2018).

Hazelnut breeding programs were established in the US in the 1960s while Turkey's first efforts date to the 1980s (Thompson et al. 1996). While over 400 Turkish varieties exist, most growers still rely on a relatively small number of traditional and locally selected cultivars which are suited to "ocak" cultivation (Fideghelli and De Salvador 2009). Indeed there are only 18 registered cultivars in the country (Balik et al. 2016). Wild species in the genus *Corylus* offer a number of desirable traits (early maturation, drought tolerance, disease resistance and non-suckering growth) (Molnar 2011) but remain largely untapped despite their sexual compatibility with *C. avellana* (Erdogan and Mehlenbacher 2000). Similarly, the rich genetic diversity within the species has only recently been explored (Boccacci et al. 2008; Gokirmak et al. 2009; Pop et al. 2010; Martins et al. 2013; Ozturk et al. 2017a, b). Molecular markers have been developed for diversity analysis and have also been used to construct molecular linkage maps (Mehlenbacher et al. 2006; Gurcan et al. 2010; Beltramo et al. 2016; Torello Marinoni et al. 2018), making it possible for the first time to map quantitative trait loci (QTL) in hazelnut (Beltramo et al. 2016; Ozturk et al. 2017a; Torello Marinoni et al. 2018). Such mapping should allow more efficient breeding using marker-assisted selection (MAS).

The first report of QTL mapping in hazelnut described the detection of loci for plant vigor, suckering and time of bud burst in 275 F1 progeny from a cross between two European varieties (Beltramo et al. 2016). More recently, Torello Marinoni et al. (2018) identified 28 QTL regions for leaf bud burst in the same F1 progeny. The Turkish national hazelnut collection was characterized for a much wider array of agro-morphological traits, including plant, twig, bud, leaf, flower and involucre attributes as well as phenology in the early 1990s (Caliskan and Cetiner 1992). The collection consists of 390 hazelnut accessions: 16 cultivars, 232 landraces and 142 wild individuals from nine provinces in Turkey (Artvin, Duzce, Kastomonu, Rize, Samsun, Sinop, Trabzon, Ordu, Giresun). The existing data for these traits, collected over two growing seasons, allowed us to compare the performance of each group of accessions (wild vs. landraces vs. cultivars), detect correlations between traits and, most significantly, detect QTL via association mapping using simple sequence repeat (SSR) markers. This work describes association mapping of 44 agro-morphological traits. These results should prove useful for hazelnut breeding programs worldwide as well as Turkish programs which are currently shifting toward the development of cultivars and rootstocks for single plant orchards.

Materials and methods

Plant material

Agro-morphological 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 accessions), Artvin, Duzce, Kastamonu (1 accession each). 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 locations.

Morphological evaluation

The hazelnut association panel was characterized for 44 agro-morphological 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). Plant traits included vigor, habit, height, area (m^2), shoot density, suckering and off-shooting. The following twig characteristics were evaluated: 1-year old shoot length, thickness, hairiness and lenticel density. The color and shape of leaf buds were also scored. Leaf blade size, shape and the hairiness and stomatal density of the abaxial surface were assessed as were petiole length and hairiness. The amount and length of male inflorescences were determined as well as the pollen germination ratio. The amount of female inflorescences was scored as were stigma color and number. A number of involucre traits were examined including: constriction, length (as compared to nut length), indentation, serration of indentation, hairiness, hair density and thickness of callus at the base. Phenological measures included the times of leaf bud burst and leaf fall, the timing of male and female flowering (50%), and the comparative timing of male and female inflorescence development. The pollen shedding and pollen receiving periods of male and female inflorescences, respectively, were scored. To analyze nut traits, yield-related traits, namely cropping efficiency (nut yield per unit trunk cross-sectional area (g/cm^2) and tendency toward alternate bearing (estimated from percentage of inflorescence bud drop in 'on-years') were also evaluated. Finally, the time of nut cluster formation (50%), the rate of nut cluster formation compared to female flowers (%) and the time of ripening were determined. 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 was 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 mM 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 to the agro-morphological 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 $-$ Log (P value) > 2.3 (equivalent to $P < 0.005$) were considered significant.

Results

Agro-morphological trait diversity

A total of 390 hazelnut accessions were evaluated for 44 agro-morphological traits describing plant, twig, bud, leaf, flower and involucre attributes as well as phenology. These data have been previously described by Caliskan and Cetiner (1992). Cultivars, landraces and wild material were analyzed as separate groups to compare their performance and breeding potential (Table 1, Suppl. Table 1). The wild material significantly ($P < 0.05$) outperformed the landraces for several plant traits including plant area, height and vigor. While cultivars tended to be shorter than the wild material, they had greater variation for height. However this difference may be due to the small sample size for cultivars. The density of shoots was greater in cultivars than landraces although both landraces and wild accessions showed more off-shooting than the cultivars. Landraces had larger leaves than wild lines. The abaxial leaf surfaces of both landraces and wild material tended to be hairier and have fewer stomata than those of cultivars which tended to show less variation for these traits. Both cultivars and landraces demonstrated significantly earlier leaf bud burst and leaf fall than the wild accessions. Involucres were longer in cultivars and landraces, and the involucres of cultivars tended to be more serrate and hairier than those of the other accessions. The quantities of male and female inflorescences tended to be lower in landraces than cultivars and wild material with less variability in the cultivars. Flowering (both male and female) was earlier in cultivars than in the other material and cultivars also produced nut clusters earlier than landraces (which were ahead of wild accessions for this trait). The nuts of both cultivars and landraces ripened earlier than those of wild accessions. Cropping efficiency was higher in cultivars and wild material than landraces. Cultivars also showed a greater tendency toward alternate bearing than either landraces or wild accessions.

Trait correlations

We used the scale of De Souza et al. (1998) to evaluate the strength of the many significant ($P < 0.01$) correlations we observed between the 44 traits (Suppl.

Table 1 Agro-morphological traits for hazelnut accessions

Traits	Cultivar (n = 16)			Landraces (n = 232)			Wild (n = 142)		
	Mean ± SE	Range	CV %	Mean ± SE	Range	CV %	Mean ± SE	Range	CV %
Plant									
Area (m^2) (1–9)	6.1 ± 0.4^{ab}	3–9	29	5.8 ± 0.1^{a}	1–9	22	6.5 ± 0.1^{b}	3–9	21
Habit (1–9)	5.9 ± 0.3^{a}	5–7	17	6.0 ± 0.1^{a}	3–7	23	6.0 ± 0.1^{a}	3–9	23
Height (1–9)	3.9 ± 0.5^{ab}	2–9	54	4.1 ± 0.1^{a}	1–9	34	4.5 ± 0.1^{b}	2–8	29
Off shooting (1–9)	5.9 ± 0.3^{a}	5–7	17	7.1 ± 0.1^{b}	3–9	17	7.4 ± 0.1^{b}	3–9	14
Suckering (1–9)	6.8 ± 0.2^{a}	5–7	10	6.7 ± 0.1^{a}	3–9	21	7.0 ± 0.1^{a}	3–9	16
Vigor (3–7)	4.6 ± 0.4^{ab}	3–7	32	4.6 ± 0.1^{a}	3–7	30	5.3 ± 0.1^{b}	3–7	27
Twig									
Density of lenticels (3–7)	5.5 ± 0.3^{a}	3–7	21	5.2 ± 0.1^{a}	3–7	19	5.2 ± 0.1^{a}	3–7	17
Density of shoots (3–7)	6.0 ± 0.3^{a}	5–7	17	5.0 ± 0.1^{b}	3–7	27	5.3 ± 0.1^{ab}	3–7	25
Hairiness (3–7)	5.0 ± 0.3^{a}	3–7	21	4.9 ± 0.1^{a}	3–7	27	4.9 ± 0.1^{a}	3–7	23
Length (3–7)	4.4 ± 0.4^{a}	3–7	32	4.5 ± 0.1^{a}	3–7	30	4.7 ± 0.1^{a}	3–7	30
Thickness (3–7)	4.9 ± 0.3^{a}	3–7	28	4.8 ± 0.1^{a}	3–7	30	4.8 ± 0.1^{a}	3–7	27
Leaf									
Blade hairiness (3–7)	3.1 ± 0.1^{a}	3–5	16	3.8 ± 0.1^{b}	3–7	27	4.0 ± 0.1^{b}	3–7	26
Blade shape (1–3)	2.1 ± 0.2^{a}	1–3	37	1.9^{a}	1–3	39	1.7 ± 0.1^{a}	1–3	38
Blade size (3–7)	4.8 ± 0.3^{ab}	3–7	21	5.4 ± 0.1^{a}	3–7	24	5.1 ± 0.1^{b}	3–7	23
Bud color (1–3)	1.3 ± 0.1^{a}	1–2	36	1.1^{a}	1–2	31	1.2^{a}	1–3	34
Bud shape (1–3)	1.9 ± 0.1^{a}	1–2	18	2.0^{a}	1–3	21	2.0^{a}	1–3	24
Density of stomata (blade) (3–7)	5.5 ± 0.3^{a}	3–7	25	4.2 ± 0.1^{b}	3–7	32	4.5 ± 0.1^{b}	3–7	31
Petiole hairiness (3–7)	4.9 ± 0.3^{a}	3–7	24	4.7 ± 0.1^{a}	3–7	33	4.8 ± 0.1^{a}	3–7	33
Petiole length (3–7)	4.4 ± 0.4^{a}	3–7	32	4.6 ± 0.1^{a}	3–7	34	4.5 ± 0.1^{a}	3–7	32
Time of leaf bud burst (1–9)	5.3 ± 0.2^{a}	4–7	18	5.8 ± 0.1^{a}	2–8	18	6.1 ± 0.1^{b}	3–9	15
Time of leaf fall (1–9)	3.1 ± 0.3^{a}	1–5	37	4.1 ± 0.1^{a}	1–9	51	4.7 ± 0.2^{b}	1–9	44
Involucre									
Construction (1–9)	4.5 ± 1.0^{ab}	1–9	91	5.4 ± 0.3^{a}	1–9	73	3.9 ± 0.3^{b}	1–9	98
Density of hairiness (3–7)	4.9 ± 0.4^{a}	3–7	32	3.9 ± 0.1^{b}	3–7	32	3.6 ± 0.1^{b}	3–7	30
Hairiness (1–9)	8.5 ± 0.5^{ab}	1–9	24	8.0 ± 0.2^{a}	1–9	34	6.8 ± 0.3^{b}	1–9	53
Indentation (3–7)	5.8 ± 0.3^{a}	3–7	22	5.8 ± 0.1^{a}	3–7	21	5.9 ± 0.1^{a}	3–7	20
Length (compared to nut length) (1–9)	5.9 ± 0.3^{a}	5–7	17	5.7 ± 0.1^{a}	3–9	24	4.7 ± 0.1^{b}	1–7	29
Serration of indentation (1–9)	7.4 ± 0.3^{a}	5–9	18	6.2 ± 0.1^{b}	3–9	25	6.3 ± 0.1^{b}	3–9	23
Thickness of callus at base (3–7)	6.5 ± 0.3^{a}	3–7	18	6.5 ± 0.1^{a}	0–7	16	6.3 ± 0.1^{a}	3–7	17
Inflorescences									
Amount of female inflorescences (1–9)	4.9 ± 0.3^{ab}	3–7	28	4.2 ± 0.1^{a}	1–9	40	4.7 ± 0.1^{b}	1–9	36
Amount of male inflorescences (1–9)	4.9 ± 0.3^{ab}	3–7	25	4.0 ± 0.1^{a}	1–9	45	4.6 ± 0.1^{b}	1–9	37
Comparative time of male and female inflorescences (1–3)	2.0 ± 0.3^{a}	1–3	52	2.0 ± 0.1^{a}	1–3	46	2.1 ± 0.1^{a}	1–3	43
Male inflorescences length (3–7)	4.9 ± 0.3^{a}	3–7	24	4.4 ± 0.1^{a}	3–7	28	4.6 ± 0.1^{a}	3–7	23
Number of stigma in a bud (3–7)	5.5 ± 0.2^{a}	5–7	16	5.2 ± 0.1^{a}	3–7	17	5.4 ± 0.1^{a}	3–7	17
Pollen germination ratio (%) (1–9)	7.3 ± 0.4^{a}	5–9	24	6.3 ± 0.2^{a}	1–9	43	6.4 ± 0.2^{a}	1–9	39

Table 1 continued

Traits	Cultivar (n = 16)			Landraces (n = 232)			Wild (n = 142)		
	Mean ± SE	Range	CV %	Mean ± SE	Range	CV %	Mean ± SE	Range	CV %
Pollen receiving period (female) (1–9)	7.8 ± 0.3[a]	5–9	16	8.1 ± 0.1[a]	3–9	18	8.5 ± 0.1[a]	4–9	12
Pollen shedding period (1–9)	5.8 ± 0.5[a]	3–9	35	6.8 ± 0.1[a]	1–9	30	6.9 ± 0.2[a]	2–9	28
Stigma color (1–3)	2.1 ± 0.1[a]	1–3	28	1.8[a]	1–3	42	1.9 ± 0.1[a]	1–3	37
Time of female flowering (1–9)	5.5 ± 0.4[a]	3–8	28	6.8 ± 0.1[b]	3–9	23	6.7 ± 0.1[b]	4–9	22
Time of male flowering (%) (1–9)	5.3 ± 0.3[a]	3–7	23	6.6 ± 0.1[b]	2–9	26	6.6 ± 0.2[b]	2–9	28
Nut									
Cropping (1–9)	4.6 ± 0.5[a]	1–9	45	3.3 ± 0.1[b]	1–9	56	3.8 ± 0.2[a]	1–9	49
Rate of nut cluster formation compared to female flowers (3–7)	6.5 ± 0.2[a]	5–7	14	6.3 ± 0.1[a]	3–7	18	6.1 ± 0.1[a]	3–7	19
Tendency toward alter bearing (1–9)	5.4 ± 0.6[a]	1–9	46	3.8 ± 0.1[b]	1–7	44	3.8 ± 0.1[b]	1–7	45
Time of nut cluster formation (1–9)	5.6 ± 0.4[a]	3–7	28	6.9 ± 0.1[b]	1–9	26	7.9 ± 0.1c	3–9	19
Time of ripening (1–9)	4.5 ± 0.3[a]	2–7	27	5.3 ± 0.1[a]	1–9	33	5.9 ± 0.1[b]	2–9	26

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

Table 2). Plant vigor was very strongly ($r^2 > 0.65$) correlated with height and area. The correlation between these latter two traits was moderately strong ($0.50 < r^2 < 0.64$). Other traits showing moderately strong relationships were the amount of female inflorescences and cropping efficiency and the timing of both nut cluster formation and ripening. Among the moderately weak ($0.30 < r^2 < 0.49$) correlations were positive relationships between plant vigor and off-shooting, male and female inflorescence production and cropping efficiency. Not surprisingly, suckering and off-shooting were correlated. Plant height was correlated with male inflorescence amount and cropping efficiency. Taller plants also tended to abscise their leaves later. Pubescence of shoots and petioles was correlated as were involucre indentation and serration. Positive relationships were observed between the timing of leaf bud burst and female flowering, nut cluster formation and ripening times. The amount of male inflorescences was related to plant area, the length of male catkins, female inflorescence production, ripening and cropping efficiency. The timing of male and female flowering was correlated and later male flowering tended to be accompanied by a higher pollen germination ratio. Earlier female flowering was correlated with a longer period of pollen receptivity.

Association mapping

To identify QTL controlling the agro-morphological traits, 406 polymorphic fragments generated from 30 SSR primers in 390 hazelnut accessions were analyzed for their association with the various traits. Different association mapping models [GLM, GLM (Q), MLM, MLM (Q)] were used and the proportion of significant results compared. The proportion of significant results ($\pi 1$) was highest with GLM (Q) analysis. A total of 18,722 combinations were tested and 276 (1.4%) 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 (0.9%). 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 145 (0.8%) significant associations were detected between the SSR markers and 41 of the 44 agro-morphological traits (Table 2). The three unmapped traits were stomatal density, pollen germination ratio and time of female flowering. The SSR marker with the greatest number of significant associations was B603, 17 (11.7%) of the associations for 13 traits were linked to this marker including four plant traits (area, height, off-shooting, vigor), one twig trait (density), one leaf character (blade hairiness), three involucre characters (hair density, hairiness, length), one inflorescence measure (male catkin length) and three phenological traits (pollen receiving period and times of leaf fall and ripening). The second most significant marker was B648 (associated with 10 traits). We have tried to point out instances of co-localization of different traits where they have seemed relevant or interesting.

Plant traits Three SSR marker loci were associated with plant area while only one SSR marker was detected for plant habit (A635_297). Five loci were associated with plant height including one (B603_327) which also affected plant area, shoot density and vigor. Two SSR marker loci (including three alleles of SSR B603) were linked to off-shooting with three different loci identified for suckering (two of these were B602 fragments). Seven SSR marker loci were associated with plant vigor.

Twig traits Lenticel density mapped to a number of positions: five different SSR loci. Shoot density was associated with only B603_327 as mentioned above. Six alleles were identified for shoot hairiness, however, these loci were generated by just two SSR markers (A602 and B652). Single loci were identified for 1-year old twig attributes including shoot thickness and length.

Leaf traits Leaf parameters included measures of leaf blade and petiole size and hairiness. The greatest number of significant SSR markers was identified for leaf blade size: six fragments distributed across five SSRs. Of these fragments, one (B606_402) was also linked to petiole length. Another blade size allele (B613_192) co-localized with blade hairiness. Petiole length was associated with two additional loci. Two QTLs were detected for leaf blade shape. No overlap was found between the loci affiliated with leaf blade and petiole hairiness: three QTLs for leaf blade and one QTL for petiole pubescence. Leaf bud characters mapped to single loci with SSR markers B612 and A640 affecting bud color and shape, respectively. The time of leaf bud burst was linked to two SSR loci whereas five QTLs were identified for the time of leaf fall.

Involucre traits One-quarter of the QTLs identified in our analysis (37 of 145) influenced involucre traits. Involucre serration indentation was the trait with the greatest number of marker associations in this study: 11 significant associations distributed across six SSR markers (including three B612 alleles). A611_200 was linked to both involucre indentation serration and hair density. Four additional hair density QTLs were detected. Involucre hairiness mapped to three locations; there was no overlap in the QTLs identified for these involucre pubescence traits. Other involucre traits were linked to between two and four SSR markers each. Thus, involucre length was associated with two SSR markers, constriction with three markers and indentation with four loci. Nine alleles distributed across seven SSR markers were identified for callus thickness. One of these (B606_402) was linked to involucre hair density, petiole length and leaf blade size.

Inflorescence traits Five loci were associated with the quantity of male catkins. This trait co-localized with plant vigor (at B603_373) and with height (at B631_300). Male inflorescence length mapped to eight fragments, three of these generated from SSR B662. One of these, B662_220, had an LD value of 0.07. Two of the B662 fragments associated with catkin length also impacted involucre and stigma characters, namely B662_220 (involucre length and stigma color) and B662_281 (involucre indentation and stigma number). Three significant SSR markers were detected for the abundance of female inflorescences. Of these, B602_349 was also associated with suckering. Stigma color and number mapped to three and two loci, respectively. Different alleles of marker B662 were associated with both traits. The time of male flowering was associated with three fragments from SSR B612, two from B602 and one additional SSR marker. The comparative timing of male and female flowering was associated with SSR marker B651. A single locus was detected for pollen shedding period. Pollen receiving period was associated with four SSR markers. Interestingly, there was no overlap in the SSR markers identified for traits related to flowering time.

Table 2 Hazelnut SSR markers associated with agro-morphological traits0

Trait	SSR locus	− LOG (*P* value)	LD value (r^2)
Plant			
Area	B603_327	2.89	0.03
	B652_245	2.70	0.03
	A605_244	2.44	0.03
Habit	A635_297	2.43	0.03
Height	B648_280	2.70	0.03
	A602_264	2.57	0.03
	B631_300	2.51	0.03
	B603_327	2.49	0.02
	A602_237	2.32	0.03
Off shooting	B603_442	2.89	0.03
	B603_313	2.77	0.03
	B648_302	2.39	0.03
	B603_339	2.37	0.02
Suckering	B602_355	2.85	0.03
	A602_20	2.85	0.03
	B602_349	2.66	0.03
	B635_398	2.42	0.03
Vigor	B603_327	2.92	0.03
	B641B_259	2.89	0.03
	A605_268	2.82	0.03
	B612_218	2.72	0.03
	B628_294	2.39	0.03
	B602_422	2.38	0.03
	B603_373	2.35	0.02
Twig			
Density of lenticels	B640_326	2.80	0.03
	A605_235	2.68	0.03
	A640_367	2.46	0.03
	B641B_436	2.46	0.02
	A606_138	2.35	0.03
Density of shoots	B603_327	2.51	0.02
Hairiness	B652_245	2.89	0.03
	B652_193	2.85	0.03
	B652_292	2.52	0.03
	A602_378	2.52	0.03
	B652_209	2.32	0.03
	B652_236	2.31	0.03
Shoot length	B631_166	2.52	0.03
Thickness	B613_310	2.51	0.03
Involucre			
Construction	B648_266	2.85	0.03
	A611_232	2.72	0.03
	B631_166	2.37	0.03
Density of hairiness	B603_427	2.70	0.02

Table 2 continued

Trait	SSR locus	− LOG (*P* value)	LD value (r^2)
	B606_402	2.59	0.03
	A611_20	2.57	0.02
	B651_331	2.41	0.02
	B648_280	2.39	0.03
Hairiness	B603_268	2.72	0.03
	B635_418	2.54	0.02
	B789_218	2.42	0.03
Indentation	B612_204	2.60	0.03
	B640_436	2.49	0.03
	A613_148	2.37	0.02
	B662_281	2.33	0.02
Length (compared to nut length)	B662_220	2.74	0.02
	B603_404	2.43	0.02
Serration of indentation	B631_306	3.54	0.09
	B612_320	3.0	0.04
	B612_267	2.92	0.03
	B612_346	2.82	0.03
	B625_254	2.80	0.03
	A604_159	2.77	0.03
	A613_153	2.68	0.02
	B631_260	2.68	0.03
	B625_429	2.51	0.03
	B631_218	2.51	0.03
	A611_20	2.44	0.02
Thickness of callus at base	A604_215	2.77	0.03
	A640_463	2.54	0.03
	A640_451	2.49	0.03
	A604_332	2.43	0.02
	B640_326	2.42	0.03
	B625_244	2.40	0.03
	A606_192	2.32	0.02
	B635_470	2.31	0.03
	B606_402	2.31	0.03
Leaf			
Blade hairiness	B662_314	2.48	0.02
	B603_404	2.42	0.02
	B613_192	2.33	0.03
Blade shape	B648_302	2.49	0.03
	B612_294	2.39	0.03
Blade size	B635_398	2.74	0.03
	A606_150	2.62	0.03
	B613_192	2.59	0.03
	B606_402	2.49	0.03
	B631_20	2.48	0.03
	B606_448	2.43	0.03

Table 2 continued

Trait	SSR locus	− LOG (*P* value)	LD value (r^2)
Bud color	B612_301	2.41	0.03
Bud shape	A640_405	2.89	0.03
Petiole hairiness	B635_388	2.62	0.03
Petiole length	A611_250	2.85	0.03
	B606_402	2.82	0.03
	B651_268	2.33	0.03
Time of leaf bud burst	A604_143	2.66	0.02
	A606_150	2.32	0.03
Time of leaf fall	A613_20	2.89	0.03
	B603_268	2.70	0.02
	B651_256	2.57	0.03
	B660_219	2.52	0.03
	B660_30	2.34	0.03
Inflorescences			
Amount (female)	B602_349	2.72	0.03
	B641B_444	2.64	0.02
	B651_331	2.54	0.02
Amount (male)	A613_153	2.60	0.02
	B603_373	2.54	0.02
	B641A_338	2.47	0.03
	A601_242	2.39	0.03
	B631_30	2.37	0.03
Comparative time of male and female inflorescences	B651_326	2.89	0.03
Inflorescence length (male)	B648_216	2.96	0.03
	B662_245	2.96	0.03
	A613_225	2.85	0.03
	B662_220	2.67	0.07
	A613_148	2.54	0.02
	A601_157	2.52	0.03
	B662_281	2.36	0.02
	A604_159	2.33	0.02
Number of stigma in bud	A613_178	2.46	0.02
	B662_281	2.43	0.02
Pollen receiving period (female)	B641B_347	2.70	0.02
	B652_266	2.64	0.03
	B603_346	2.54	0.02
	B635_461	2.40	0.03
Pollen shedding period	A601_121	2.51	0.03
Stigma color	B662_220	2.85	0.03
	B648_273	2.51	0.02
	B635_418	2.37	0.02
Time of male flowering	B612_334	2.85	0.03
	B612_340	2.82	0.03
	B612_346	2.62	0.03
	B602_377	2.41	0.03

Table 2 continued

Trait	SSR locus	− LOG (*P* value)	LD value (r^2)
	B602_387	2.40	0.03
	A606_138	2.35	0.03
Nut			
Cropping	B648_228	2.82	0.03
Rate of nut cluster formation compared to female flowers	B648_266	2.34	0.03
Tendency toward alter bearing	B628_296	2.59	0.03
	A605_183	2.47	0.03
	B602_334	2.44	0.03
	B602_363	2.41	0.03
Time of nut cluster formation	B625_265	2.96	0.04
	B648_216	2.89	0.03
	B648_280	2.80	0.03
Time of ripening	B603_313	2.77	0.03
	B652_347	2.62	0.03
	B603_427	2.38	0.02
	A604_143	2.34	0.02

Negative log10-transformed *P* values

Nut traits Two yield-associated traits were analyzed. Cropping efficiency mapped to a single QTL. Tendency toward alternate bearing was associated with three SSR markers, including two fragments from B602. None of these loci were associated with other traits. The time of nut cluster formation and the rate of nut cluster formation compared to female flowering were both associated with B648 alleles. This was the sole SSR linked to the rate trait whereas an additional marker was linked to timing of nut formation. Plant height co-localized with nut cluster timing at one of these alleles (B648_280). Time of ripening was associated with three SSR markers independent of those linked to nut cluster phenology. One of these fragments (B603_313) was also associated with off-shooting and another (A604_143) with time of leaf bud burst. Leaf bud emergence time and leaf blade size co-localized to A606_150.

Discussion

Effects of domestication and breeding on agro-morphological traits

Domestication and human selection have had a number of effects on the agro-morphological characteristics of hazelnut. Plant attributes such as vigor, area and height are reduced in landraces as compared to wild material; and, while shoot density is higher in cultivars than landraces, off-shooting is lower in the commercially grown accessions. The leaves of wild accessions and landraces tend to be hairier and have fewer stomata than cultivars. Both of these attributes can provide greater protection against insects and disease and help to reduce plant transpiration rates. Because hazelnut is sensitive to water stress and yield is heavily dependent on the plants receiving adequate amounts of irrigation (Mingeau et al. 1994; Cristofori et al. 2014), introducing wild and locally adapted material into breeding programs could help improve drought tolerance in the crop. Domestication and breeding appear to have had inadvertent effects on involucre morphology. As compared to wild growing

hazelnuts, these bracts tended to be longer, hairier, and more constricted and serrate in the cultivated materials. Human selection has increased the abundance of male and female inflorescences and deliberate breeding appears to have shifted hazelnut toward earlier flowering, nut set and ripening. The timing of vegetative bud emergence and leaf abscission have been similarly affected. While the superior cropping efficiency of cultivars over landraces was expected, the fact that the wild lines also performed better than landraces suggests that useful alleles for this trait may exist in the wild germplasm. Finally, the greater tendency of cultivars to display year-to-year fluctuations in nut crop (alternate bearing) is unexpected but also indicates that greater consistency in nut production could be achieved.

While many significant correlations were discovered between the agro-morphological traits, the majority were deemed moderately weak or weak. The strongest relationships were between plant vigor (which was scored on a qualitative scale) and plant area as well as plant height, traits that are clearly interdependent. A moderately strong correlation between the abundance of female inflorescences and cropping efficiency suggests that selection at the flowering stage could be an effective means of improving nut yields. Similarly, the associations between nut cluster formation and ripening times suggests that selection for early nut set will lead to accessions that ripen earlier.

Loci controlling agro-morphological traits

The number of traits (44) and accessions (390) evaluated make this one of the largest association mapping studies performed in hazelnut to date. The LD values of the QTLs we have detected are quite small, suggesting that these agro-morphological traits are controlled by multiple genes, each with a small effect on phenotype. Environmental conditions also undoubtedly play a large role in determining these attributes.

Of the 145 QTLs identified, 19 (13%) related to whole plant characteristics (vigor, area, height, habit, shoot density, off-shooting and suckering). Seventeen QTLs (12%) influenced appearance and size of leaf buds, blades and petioles while a slightly smaller proportion (13 QTLs; 9%) impacted twig morphology (length, thickness, lenticel density and hairiness). Over one-quarter (26%) of the QTLs we identified influenced involucre morphology. A relatively large number of loci (21; 14%) were detected for just five inflorescence traits: abundance of male and female and length of male inflorescences, stigma color and number. Seven QTLs (5%) each influenced aspects of leaf lifespan and male flowering (timing and pollen shedding period). Twelve (8%) of the loci impacted the phenology of fertilization and nut development.

We expect QTLs of interdependent traits to co-localize. Thus, the fact that plant vigor, area, height and shoot density all mapped to a single locus (B603_327) was not surprising. Other explanations for overlapping QTLs are pleiotropy (one gene affecting two or more traits) or genetic linkage (two genes inherited together). Linkage may also simply be an artifact of low marker density. While high-resolution mapping can help discriminate between these hypotheses, our analysis does not allow us to do more than point out and speculate on interesting correspondences in QTLs locations. Nevertheless, it should be noted that genes involved in development (Xiao et al. 2014) and secondary metabolite production (Boycheva et al. 2014) have been found to cluster in plant genomes. Thus, the fact that leaf blade size and petiole length map to the same fragment (B606_402) may indicate that a single gene impacts both leaf parameters. Two involucre attributes (callus thickness and hair density) are also associated with this locus. As bracts, involucres are modified leaves but the disparity of the traits themselves makes it more likely that separate genes are controlling leaf and involucre traits at this position in the hazelnut genome. Similarly, the co-localization of blade size and hairiness to B613_192 may be due to linkage or merely coincidental. More interesting are overlaps seen between male catkin abundance QTL and separate loci for plant vigor (B603_373) and plant height (B631_300). Female inflorescence abundance co-localized with suckering and alternate bearing tendency (B602_349). Because the capacity to produce flowers is contingent on photosynthetic productivity and resource abundance, one can imagine how the aforementioned co-localized traits could be tied to single genes. A606_150 was associated with both the time of leaf emergence and leaf blade size, two leaf development traits. Two phenological traits, vegetative bud burst and ripening time co-localized at A604_143.

Quantitative trait mapping in hazelnut is truly in its infancy (Beltramo et al. 2016; Ozturk et al. 2017a; Torello Marinoni et al. 2018). Beltramo et al. (2016) were the first to report QTLs for agronomic traits in hazelnut: 15 SSR loci associated with trunk circumference, suckering index (number of suckers/trunk circumference) and leaf bud burst in a population of 275 F1 plants from a cross between the European varieties Tonda Gentile delle Langhe and Merveille de Bollwiller. Comparing our results with those of this previous study reveals a single common association: between SSR marker A602 and suckering. Beltramo et al. (2016) detected this SSR marker (which mapped to LG_10 in their population) in each of the 3 years of their analysis and found that it explained an average of 11% of phenotypic variation in the trait, suggesting that this locus might be a good target for MAS to improve sucker habit. More recently, 28 QTL regions were linked to leaf bud burst in the same F1 population using single nucleotide polymorphism (SNP) markers (Torello Marinoni et al. 2018). While we detected two QTLs for leaf bud burst, we cannot compare the position of their QTLs to ours because we did not use a segregating population and our SSR marker loci are unmapped. Despite this shortcoming, our study is the largest scale QTL analysis of hazelnut to date (44 morphological traits examined in 390 accessions that capture the diversity of the species). It is our hope that our results, however preliminary in nature, will contribute toward an increased understanding of and ability to select for agronomically important traits in hazelnut. Among the QTLs that warrant further investigation are those that might help breeders select for plant vigor, decreased suckering, increased flower production, earlier ripening and greater insect and drought stress tolerance.

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Authors' contribution AF analyzed results and drafted manuscript; SCÖ generated genotypic data and performed mapping; HIB, SKB and GK provided plant material and phenotypic data; SD and AF devised experiments; AF obtained funding and revised draft. All authors approved of submitted manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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

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