Construction of a high-density genetic linkage map and QTL analysis for hazelnut breeding

fruit quality are controlled by many genes; genomic regions containing these genes are known as Quantitative Trait Loci (QTL) (Collard et al. 2005).

Hazelnut is a diploid species (2n=2x=22) with an estimated genome size of 378 Mb (http://www.cavellanagenomeportal.com). Observations carried out during meiosis indicate the presence of reciprocal translocations in some cultivars, including 'Tonda Gentile delle Langhe', 'Barcelona' and 'Tonda di Giffoni' (Salesses 1973; Salesses and Bonnet 1988).

Objective of the study was the construction of high-density genetic maps and the detection of QTL (Quantitative Trait Loci) related to phenological, vegetative and productive traits.

The F1 progeny obtained by crossing 'Tonda Gentile delle Langhe' (female parent, hereafter TGdL) with 'Merveille de Bollwiller' (syn. 'Hall's Giant' male parent, MB) were obtained by controlled cross in February 2008 (Beltramo et al. 2016). The progeny segregated for several phenotypic traits including phenological, vegetative and productive traits. The following traits were evaluated over the 2011-2016 period: time of bud burst, male and female flowering, dichogamy, nut maturity; nut and kernel size (weight and calibre), nut shape (roundness index, RI), shell thickness, percent kernel, ease of pellicle removal after roasting (blanching).

The 275 seedlings and three individuals, obtained from rooted suckers of each of the two parents were planted in November 2010 at the campus of University of Torino, Department of Agricultural, Forest and Food Sciences (45°07'N; 7°58'E; 293 m a.s.l.). The seedlings and parents were planted at a spacing of 4 x 4 m and trained in an open vase system.

The Genotyping-by-Sequencing (GBS) approach (Elshire et al. 2011) was used to discover single nucleotide polymorphism (SNP) markers. The mapping population was a set of 213 (of the 275) F_1 individuals of the progeny TGdL x MB, and included 50 new individuals that were added to the 163 plants already analysed with SSR markers by Beltramo et al. (2016). In October 2014, approximately 3 µg of genomic DNA from each individual and the two parents were sent to the Genomic Diversity Facility at Cornell University - Institute of Biotechnology (USA) (http://www.biotech.cornell.edu/brc/genomic-diversity-facility) for GBS. Raw reads were analyzed for filtering out contaminant substrings and removing reads with poor quality ends (Q<30). SNP mining was conducted by adopting a Samtools-based pipeline. Independent framework linkage maps were constructed for each parent using the double pseudo-testcross mapping strategy (Weeden 1994) trough JoinMap v4.0 (Van Ooijen 2006). A set of 24 microsatellite primer pairs were used to facilitate the identification of the linkage groups (LG).

The two separate parental maps were used to assign putative QTL locations by performing both the simple interval mapping (Lander and Botstein 1989) and multiple QTL mapping (Jansen and Stam 1994), procedures implemented within MapQTL v4.0 software (Van Ooijen et al. 2002).

The GBS approach generated a total of 46.2 Gb of DNA sequences. Single nucleotide substitutions were identified, with a frequency of one SNP every 206 nucleotides. Of the total segregating SNP markers discovered, 34% SNP markers were heterozygous in both parents, while the 32% and the 34% were heterozygous only in TGdL and MB, respectively. Only SNP markers segregating in only one of the parents were retained for map construction. In order to produce more accurate linkage maps, a further stringent selection was applied, considering only markers grouped at threshold logarithm of odds (LOD) ratio of 12.0.

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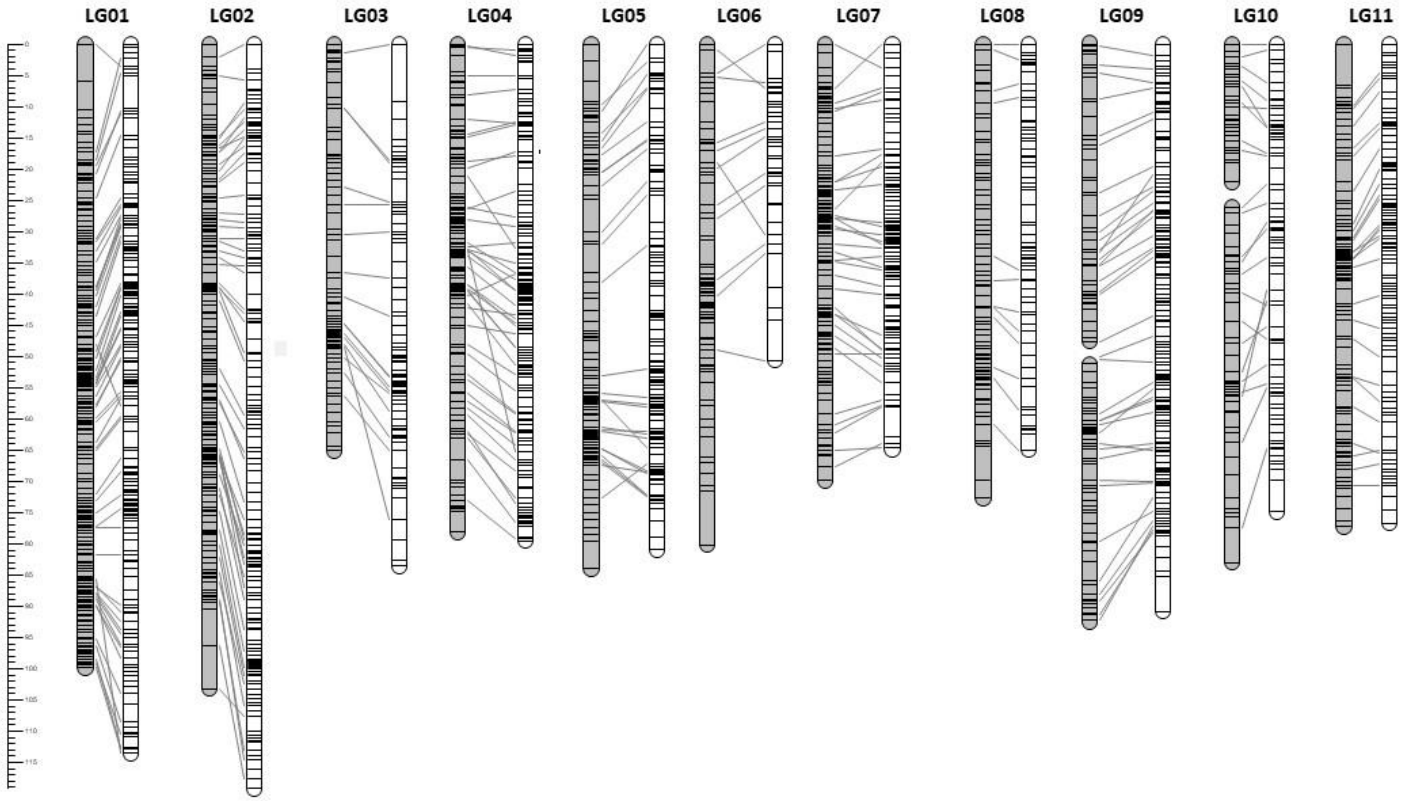

Figure 1. Genetic maps of the *Corylus avellana* cultivars 'Tonda Gentile delle Langhe' (female parent, on the left) and 'Merveille de Bollwiller' (male parent, on the right) aligned on the base of markers developed on common scaffolds.

Table 1. QTLs found in *Corylus avellana* cultivar 'Tonda Gentile delle Langhe' for nut weight, nut and kernel calibre, kernel roundness index and blanching: number of major and minor QTL, and linkage group where they were detected during each season of observation.

TRAIT	SEASON	MAJOR QTL	MINOR QTL	Linkage Group (LG)
Nut Weight	2013		1	LG_07
	2014	1	3	LG_07
	2015		3	LG_07
Nut Calibre	2013		3	LG_02, LG_04, LG_06
	2014		4	LG_01, LG_02, LG_04, LG_06
	2015		4	LG_01, LG_02, LG_04, LG_06
Kernel Calibre	2013		3	LG_05
	2014		2	LG_05
	2015		3	LG_05
RI Kernel	2013		3	LG_04, LG_09a, LG_11
	2014		4	LG_04, LG_08, LG_09a, LG_11
	2015		5	LG_04, LG_08, LG_09a, LG_11
Blanching	2013	1	2	LG_01, LG_02, LG_07
	2014		3	LG_01, LG_02, LG_05
	2015		2	LG_01, LG_10b