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Development of a Genetic Linkage Map in Hazelnut (*Corylus avellana*) for the Detection of QTLs

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Abstract

'Tonda Gentile delle Langhe' (TGdL) is the dominant hazelnut cultivar in Piedmont due to the unquestioned technological and quality characteristics of its nuts. The improvement of TGdL by breeding is one of the objectives of the projects of the University of Torino. A F_1 progeny (295 plants) of 'Tonda Gentile delle Langhe' X 'Hall's Giant' was obtained in 2009 to study the segregation of traits of agronomic and technological interest and develop marker assisted selection. Parents differ for big bud mite and nut weevil tolerance, tree vigour, time of budburst, flowering and ripening time, and nut characteristics. A first set of 142 plants of the progeny was analyzed at 92 SSR loci. In 2011, tree vigour, suckering habit, and time of budburst were observed in 295 plants. The preliminary data showed a large variability of the traits with a normal distribution for vigour and time of budburst. Molecular analysis and field observations were statistically analyzed to obtain a first linkage map consisting of 10 LGs covering 452 cM. In the next years, the map will be saturated using further molecular markers, including AFLP and SNPs; more field and lab data will be collected for getting reliable results and detecting the QTL regions in the map.

INTRODUCTION

A few breeding programs are underway in the world to obtain new hazelnut (*Corylus avellana* L.) cultivars that are high yielding and able to resist to particular pathogens or environmental conditions.

In hazelnut the application of molecular techniques for these purposes is recent. The Dipartimento di Colture arboree of the University of Torino (UNITO) isolated and characterized SSR (Simple Sequence Repeat - microsatellite) markers for hazelnut, in collaboration with the Oregon State University (OSU) in Corvallis (Bassil et al., 2005a, b; Boccacci et al., 2005). The SSR markers have been widely used for germplasm characterization, phylogenetic studies and the construction of genetic maps. Recently the first genetic map for this species was published, with the purpose to identify and isolate markers linked to the gene of resistance to Eastern Filbert Blight disease (agent *Anisogramma anomala* (Peck) E. Müller) and to the S-locus of incompatibility

(Mehlenbacher et al., 2006). Molecular markers are currently used at OSU for marker assisted selection aimed at the early detection of the resistant genotypes.

'Tonda Gentile delle Langhe' (TGdL) is the dominant hazelnut cultivar in Piedmont (North-West Italy) due to the unquestioned technological and quality characteristics of its nuts. Yet, the improvement of the cultivar, lacking in yield and susceptible to big bud mite, by breeding is one of the objectives of the projects carried out at the University of Torino. In particular, a progeny (295 individuals) of TGdL x 'Hall's Giant' (HG) was obtained and is being analyzed using molecular markers and studying the segregation of phenotypic traits. The aim of the research is the construction of a genetic linkage map as the basis for the identification of quantitative trait loci (QTL) for traits of agronomic and industrial interest. In addition, this work could yield selections with pest tolerance and adaptability that maintain the excellent quality of TGdL nuts.

MATERIALS AND METHODS

In 2008 a progeny of 295 individuals from a controlled cross TGdL x HG was obtained at UNITO. This progeny segregates for several agronomical and technological traits, such as vigour, sucker habit, big bud mite (*Phytoptus avellanae* Nal.) and nut weevil (*Curculio nucum* L.) tolerance, time of budburst, flowering and ripening time, chemical and physical traits of the nut.

DNA from 210 individuals was extracted using the method reported by Thomas et al. (1993) and analyzed at 7 SSR loci (Boccacci et al., 2005; Bassil et al., 2005a), in order to check the hybridity. PCR reactions were performed in a volume of 15 µl containing 1X PCR buffer, 2.25 mM $MgCl_2$, 200 µM dNTPs, 0.5 µM of each primer, 0.1 U of *Taq* polymerase (Bioline, USA) and 40 ng of template DNA. The thermalcycler program was: 3 min at 95°C; followed by 28 cycles of 30 sec at 95°C, 45 sec at 55°C, 1 min 30 sec at 72°C; a final elongation step was carried out for 30 min at 72°C. Amplification products were analyzed on a capillary sequencer 3130 Genetic Analyzer (Applied Biosystems, USA). Following parentage check, a first set of 142 samples was analyzed at further 85 SSR loci (Bassil et al., 2005a,b; Boccacci et al., 2005; Gürcan et al., 2010; Gürcan and Mehlenbacher, 2010a,b). PCR reactions and analyses were performed as previously described, with annealing temperature ranging from 50°C to 58°C, depending on the primer pair.

All data were processed with JoinMap 4 software, to generate a consensus map, based on a two-way pseudo-testcross strategy. The goodness-of fit between observed and expected segregation data was assessed using the chi-square (χ^2) test. Linkage groups (LGs) were accepted at LOD threshold 4.0; to determine marker order within a linkage group, the following JoinMap parameter settings were used: Rec = 0.40, LOD =1.0. Map distances were converted to centiMorgans using the Kosambi mapping function (Kosambi, 1944). LGs were numbered serially in descending order of genetic length.

Phenotypic observations were carried out in 2011 on the 3 years old progeny; vigour, sucker habit and time of budburst were considered. In early March, just before budburst, the trunk diameter at 20 cm from soil was measured and used as vigour indicator. Time of budburst (third leaf exposition in over 50% of plant buds) of each plant was recorded afterwards. Sucker habit was described by number of suckers produced by each plant in early June. Distribution of phenotypic data for all traits was represented by histograms.

RESULTS AND DISCUSSION

Of the 92 SSR markers that were analysed, 79 segregated in the F_1 population for both parent: 69 loci showed a 1:1:1:1 ratio and 10 had a 1:2:1 ratio. Further 13 loci segregated within only one of the parents (1:1 ratio). Seven loci among the 92 suffered from segregation distortion. The obtained consensus map is reported in fig. 1. The map consists of 10 LGs covering 452 cM with a mean marker density of one marker every 5.5 cM. There were 6 gaps > 10 cM, 5 gaps > 15 cM and no unlinked loci. Other features are reported in table 1. All developed LGs can be aligned, using the markers in common, with the LGs of the map developed by Gürcan et al. (2010), except LG1 and LG8, each derived from the merging of two LGs (LG9-10 and LG2-7, respectively) of the map by Gürcan et al (2010).

Sixteen SSR loci, not previously mapped, segregated in TGdL x HG progeny and were assigned to LGs in the present map. In particular 4 of them (B608, CaT-C001, CaC-B101, B508) were placed in LG1. Since LG1 probably derived from the fusion of two LGs, we tried to analyze separately SSR loci belonging to LG9 and LG10 in Gürcan's map, by adding the 4 SSR loci not previously mapped. Software analyses showed that the new SSRs are very likely linked to LG9. By increasing the number of markers and of analyzed samples we will be able to define a more precise map framework.

In 2011 vigour, sucker habit and time of budburst were examined in 295 individuals of the progeny. High phenotypic variation was observed for these characteristics, as shown by histograms of frequency distribution (fig. 2), with identification of interesting plants. For vigour and sucker habit, parental values were close to each other. Most of the progeny showed a trunk diameter of 2.00-5.99 cm, but 7 plants resulted more vigorous (over 6.00 cm) of the others and of the parents (TGdL = 4 cm, HG = 5 cm). About sucker habit, more than 50% of the progeny developed 1-7 suckers/plant, but in 9 plants suckers were absent.

Time of budburst was very different between the parent cultivars: TGdL showed an early budburst, while HG showed a late one. The progeny showed a time of budburst mostly included between the parental values: only 17 and 41 individuals presented either an earlier or a later budburst, respectively. The study of phenological traits can be functional to understand the mechanisms of hazelnut adaptation in different climatic areas and to facilitate orchard management. Yet, further observations will be necessary to have reliable data and elaborate a map that includes these phenotypic traits.

CONCLUSIONS

This work represents the first efforts to develop a genetic map of hazelnut for the identification of QTLs. The establishment of linkage relationships between molecular markers and traits is the initial step in the identification of the chromosomal regions carrying genes relevant for marker assisted breeding applications. To reach these results it is essential the obtainment of a saturated genetic map. With this purpose, the progeny will be analyzed with other types of molecular markers such as AFLPs (amplified fragment length polymorphisms) and SNPs (single nucleotide polymorphisms) on an increased number of plants. Other traits that will become detectable in the future, such as flowering and ripening time, tolerance to big bud mite and nut weevil, nut chemical and physical characteristics, will be recorded and mapped.

ACKNOWLEDGEMENTS

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Literature Cited

Bassil, N.V., Botta, R. and Mehlenbacher, S.A. 2005a. Microsatellite markers in hazelnut: isolation, characterization, and cross-species amplification. J. Am. Soc. Hortic. Sci. 130:543-549.

Bassil, N.V., Botta, R. and Mehlenbacher, S.A. 2005b. Additional microsatellite markers of the European hazelnut. Acta Hort. 686:105-110.

Boccacci, P., Akkak, A., Bassil, N.V., Mehlenbacher, S.A. and Botta, R. 2005. Characterization and evaluation of microsatellite loci in European hazelnut (*Corylus avellana* L.) and their transferability to other *Corylus* species. Mol. Ecol. Notes 5:934-937.

Gürcan, K., Mehlenbacher, S.A., Botta, R. and Boccacci, P. 2010. Development, characterization, segregation, and mapping of microsatellite markers for European hazelnut (*Corylus avellana* L.) from enriched genomic libraries and usefulness in genetic diversity studies. Tree Genet. Genomes 6:513-531.

Gürcan, K. and Mehlenbacher, S.A. 2010a. Development of microsatellite loci for European hazelnut (*Corylus avellana* L.) from ISSR fragments. Mol. Breeding 26:551-559.

Gürcan, K. and Mehlenbacher, S.A. 2010b. Transferability of microsatellite markers in the Betulaceae. J. Am. Soc. Hortic. Sci. 135(2):159-173.

Kosambi, D.D. 1944. The estimation of map distances from recombination values. Ann. Eugen. 12:172-175.

Mehlenbacher, S.A., Brown, R.N., Nouhra, E.R., Gökirmak, T., Bassil N.V. and Kubisiak T.L. 2006. A genetic linkage map for hazelnut (*Corylus avellana* L.) based on RAPD and SSR markers. Genome 49:122-133.

Thomas, M.R., Matsumoto, S., Cain, P. and Scott, N.S. 1993. Repetitive DNA of grapevine: classes present and sequences suitable for cultivar identification. Theor. Appl. Genet. 86:173-180.

Tables

Table 1. Main features (length, number of markers, density, gaps) of the consensus map.

Hazelnut LG	LG1	LG2	LG3	LG4	LG5	LG6	LG7	LG8	LG9	LG10	Total
Length (cM)	74	64	56	53	53	51	47	34	15	5	452
N° of markers	22	6	5	10	7	9	9	14	7	3	92
Average density	3.4	10.7	11.2	5.3	7.6	5.7	5.2	2.4	2.1	1.7	5.5
Gaps (>10 cM)	0	1	0	2	0	1	2	0	0	0	6
Gaps (>15 cM)	0	2	2	0	1	1	0	0	0	0	5

Figures

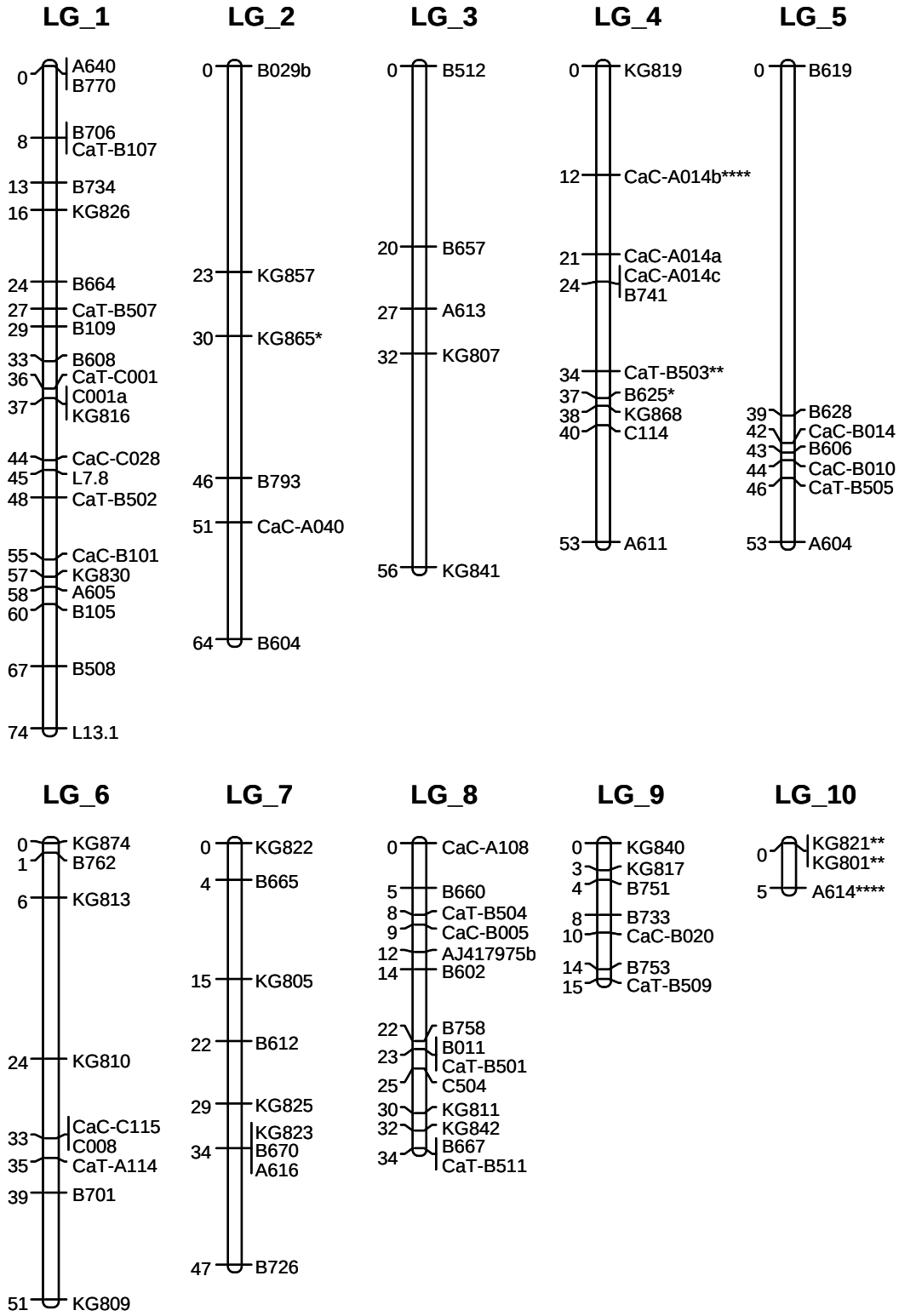

Fig. 1. The SSR consensus map obtained from the F_1 Hazelnut progeny TGdL x HG. Marker names are shown to the right of each LG, with map distances (in cM) to the left. Asterisks indicated markers showing segregation distortion at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.005$ (***), $P < 0.001$ (****).

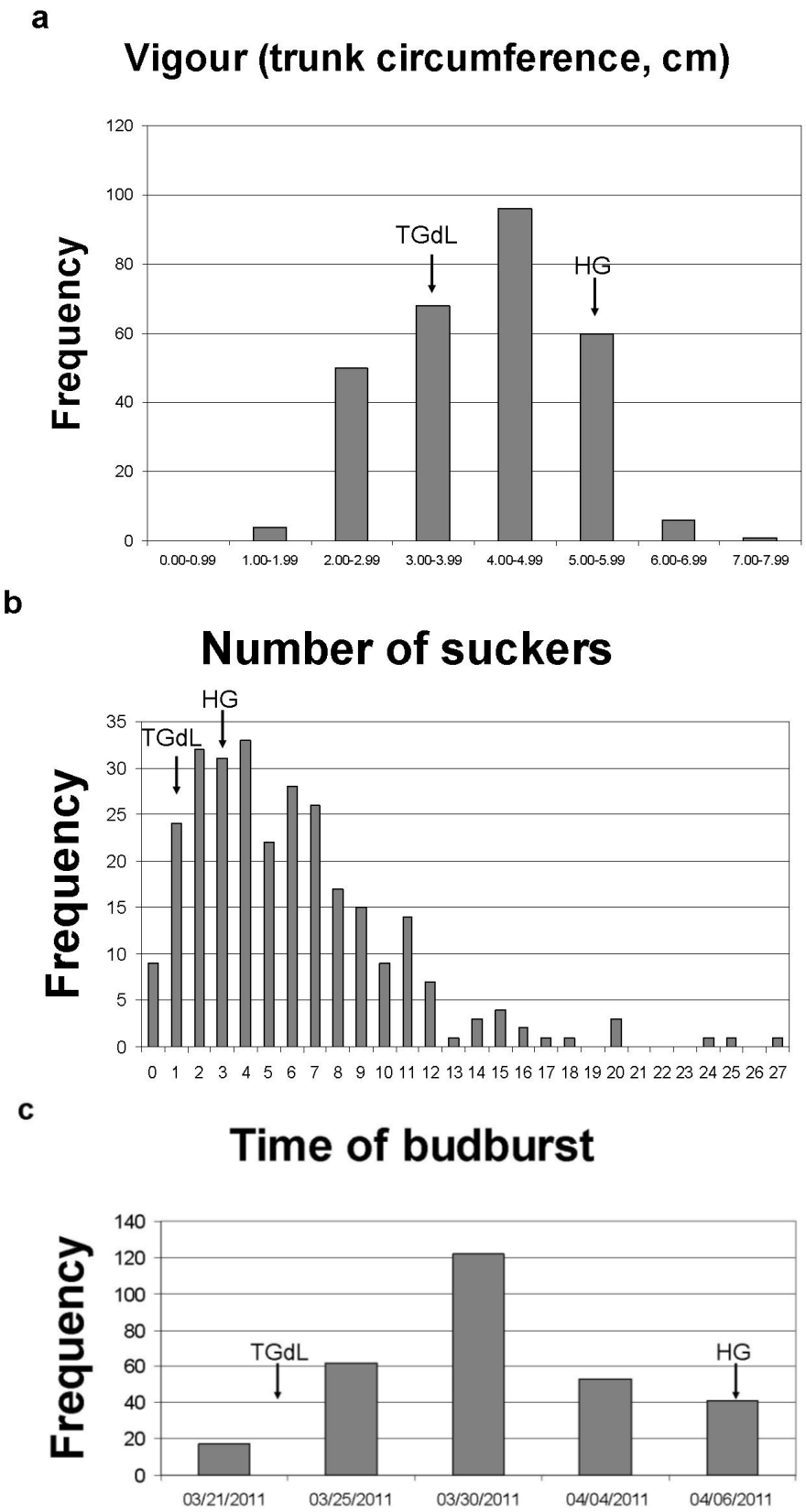

Fig. 2. Frequency distribution observed in the progeny 'Tonda Gentile delle Langhe' x 'Hall's Giant' in 2011 for vigour, number of suckers and time of budburst. Parental mean values are shown by arrows. Frequency: number of individuals of the progeny.