Chestnut Gall Wasp and Chestnut

Subjects: Plant Sciences

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Castanea sativa is an important multipurpose species in Europe for nut and timber production as well as for its role in the landscape and in the forest ecosystem. This species has low tolerance to chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu), which is a pest that was accidentally introduced into Europe in early 2000 and devastated forest and orchard trees. Resistance to the gall wasp was found in the hybrid cultivar ‘Bouche de Bétizac’ (*C. sativa* × *C. crenata*) and studied by developing genetic linkage maps using a population derived from a cross between ‘Bouche de Bétizac’ and the susceptible cultivar ‘Madonna’ (*C. sativa*). The high-density genetic maps were constructed using double-digest restriction site-associated DNA-seq and simple sequence repeat markers. The map of ‘Bouche de Bétizac’ consisted of 1459 loci and spanned 809.6 cM; the map of ‘Madonna’ consisted of 1089 loci and spanned 753.3 cM. In both maps, 12 linkage groups were identified. A single major QTL was recognized on the ‘Bouche de Bétizac’ map, explaining up to 67–69% of the phenotypic variance of the resistance trait (*Rdk1*). The *Rdk1* quantitative trait loci (QTL) region included 11 scaffolds and two candidate genes putatively involved in the resistance response were identified. This study will contribute to *C. sativa* breeding programs and to the study of *Rdk1* genes.

breeding chestnut ddRAD-seq Dryocosmus kuriphilus Yasumatsu SSR

1. Introduction

Chestnut belongs to the genus *Castanea*, in the Fagaceae family, which includes *Quercus, Fagus,* and *Castanopsis.* There are four major species in the genus *Castanea:* European chestnut (*C. sativa* Mill.), Japanese chestnut (*C. crenata* Sieb. et Zucc.), Chinese chestnut (*C. mollissima* Bl.), and American chestnut (*C. dentata* Borkh.). *C. sativa* is distributed along the Mediterranean basin and Asia Minor, and it is a multipurpose species not only used for nut and wood production, but also for its contribution to the landscape in mountainous areas. This species has very good nut quality, especially the ‘Marrone’ type, which is known for the fine taste and the easy-to-remove pellicle [1]. However, this species is susceptible to two main diseases, ink disease (*Phytophthora cinnamomi* Rands) and canker blight (*Cryphonectria parasitica* Murr.) [2]. In addition, most of the *C. sativa* cultivars are susceptible to chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu).

Interspecific hybridizations have been carried out to overcome the weak points of each chestnut species. In Europe, interspecific crosses between *C. sativa* and *C. crenata* were carried out to introduce resistance genes to ink disease, canker blight, and chestnut gall wasp [2][3][4][5]. In the USA, backcross breeding was aimed at introducing blight resistance from *C. mollissima* into *C. dentata* [6]. Moreover, *C. mollissima* accessions were

introduced in Japanese chestnut breeding programs to improve the ease of pellicle removal [7]. As these papers show, interspecific hybridization is important for chestnut breeding strategies. Therefore, constructing genetic linkage maps and accumulating genetic information among chestnut species is essential for chestnut breeding programs.

2. Chestnut Gall Wasp

The chestnut gall wasp was first introduced from China into Japan in the 1940s and spread throughout Japan in the 1960s. *C. crenata* resistant cultivars, 'Tanzawa', 'Tsukuba', and 'Ishizuchi' were released by a public breeding program in 1959–1968. Initially, these cultivars showed total resistance to gall wasp. However, eventually, the presence of galls was found also in these cultivars, due to the appearance of new ecotypes of the insect [8]. In 1982, the parasitoid wasp *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) was released, and a rapid decrease of the infestation was obtained [9]. To date, the control of *D. kuriphilus* by *T. sinensis* has been successful in Japan.

The chestnut gall wasp was accidentally introduced into Italy and first reported in 2002. It quickly spread to all Italian regions and later into the surrounding countries [10], causing a remarkable decrease of production (−60% in 2014 in Italy). Studies on biological control aimed at introducing the parasitoid wasp *T. sinensis* and at the genetic improvement for resistance to the cynipid were promptly started to solve the problem. The susceptibility to the chestnut gall wasp was evaluated in *C. sativa* and hybrid cultivars [11]. Out of 62 cultivars, 2 *C. sativa*, 1 *C. crenata*, and 4 hybrids between *C. sativa* and *C. crenata* showed total resistance. The resistance of the hybrid cultivar 'Bouche de Bétizac' was extensively studied and was found to have a simple Mendelian inheritance [3]. It was hypothesized that the mechanism of resistance involves a hypersensitive reaction in the buds [12]. The presence of H_2O_2 and the expression of a germin-like protein gene involved in the production of reactive oxygen compounds were revealed in infested buds of 'Bouche de Bétizac' at budburst.

3. Genetic Linkage Maps

Several genetic linkage maps have been assembled for *Castanea* accessions. A map of *C. dentata* × *C. mollissima* was first constructed using random amplified polymorphic DNAs (RAPDs) allowing the detection of molecular markers associated with blight resistance [13]. Subsequently, *C. sativa* maps were built using intraspecific cross [14][15][16]. In 2013, the whole genome sequence of *C. mollissima* was released [17], consisting of 724.0 Mb in 41,260 scaffolds (N50, 39.6 Kb) with 91.2% coverage of estimated genome size (794 Mb). In the same year, a highly informative genetic map of *C. mollissima* was constructed, including 329 simple sequence repeats (SSRs) and 1064 single nucleotide polymorphisms (SNPs) markers using an expressed sequence tag database created by next-generation sequencing [18]. This consensus map consisted of 12 linkage groups ranging from 50.6 to 90.4 cM and encompassed 742.3 cM with an average distance of 0.64 cM between each pair of loci. More recent maps of

C. sativa and *C. crenata* were constructed and anchored to the consensus map by Kubisiak et al. [18] using SNPs and anchor SSRs [4][19].

Some molecular markers associated with important agronomic traits were developed in the genus *Castanea*. The blight resistance genes of *C. mollissima* were mapped and introgressed by backcrossing into *C. dentata* [13][18]. The molecular markers associated with ease of pellicle removal were developed and applied in *C. crenata* breeding programs [19]. The quantitative trait loci (QTL) associated with agronomic traits including nut weight and pericarp splitting were identified from intraspecific crosses of *C. crenata* [20]. QTLs for adaptive traits, such as time of budburst, growth, and carbon isotope discrimination were identified in *C. sativa* [21]. In addition, QTLs for resistance to *P. cinnamomi* were identified in an interspecific cross progeny from *C. sativa* and *C. crenata*. However, molecular markers associated with 'resistance to *D. kuriphilus'* have not been identified yet.

The genotyping by sequencing (GBS) method [22] has illustrated a cost-effective way to identify thousands of polymorphic markers. This method is based on the construction of a library based on reducing genome complexity using restriction enzymes, to ensure sufficient read depth for polymorphism discovery. Double-digest restriction site-associated DNA-Seq (ddRAD-Seq) is a modified GBS approach that involves a two-enzyme double digestion to reduce cost and time to prepare the sequencing libraries. After the double digestion, a precise size selection is applied to exclude too short and too long fragments, resulting in greater flexibility and robustness in region recovery [23]. In silico prediction prior to actual analysis contributes to optimization of the experimental conditions for ddRAD-Seq, e.g., choices of enzymes and plant materials [24]. As the cost of next-generation sequencing (NGS) has dramatically decreased [25], more and more genetic studies involved in genetic mapping, genome-wide association mapping, and population genetics have applied the ddRAD-Seq methods [24][26][27][28][29].

4. Conclusions

Euro-Japanese F1 hybrids cultivars in Europe were obtained by INRA Bordeaux to increase the resistance of cultivated chestnuts to ink disease and canker blight. Recently, some of these cultivars showed the interesting trait of resistance to gall wasp. However, the nut organoleptic quality of the hybrid cultivars is considered much lower than that of *C. sativa* cultivars due to the lower quality of the Japanese chestnuts. Nevertheless, *C. crenata* can be seen as a major source of genes of resistance or tolerance to pests and pathogens. Once these genes are known, the acquired knowledge can be used in breeding programs. A large effect QTL, expressed across two growing seasons, was mapped on the Bouche map linkage group K and explained up to 67–69% of the phenotypic variance of the response to *D. kuriphilus*. A putative gene for a metacaspase-1b proteins was found in one of the scaffolds linked to the *Rdk1* QTL region. The high-density maps developed in this study support further genetic studies, and once a better reference genome will be available, it will allow a more in-depth exploration of the regions flanking the trait. In addition, the obtained BC1 progeny can be used to develop molecular markers for resistance to chestnut blight and ink disease as well as for other agronomic traits, including nut quality. Further

analysis on progenies from different parental lines or genome-wide association (GWAS) approaches could contribute to finding more regions of interest as well as to confirm the newly identified one.

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