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QTLs and candidate genes for downy mildew resistance conferred by interspecific grape (*V. vinifera* L. × *V. amurensis* Rupr.) crossing

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ABSTRACT

Considering the economic importance of grape (*Vitis* spp.), breeding for downy mildew (*P. viticola*) resistance has made great progress in the last two decades. However, the genetic and genomic information of resistance against grape downy mildew remain largely unknown. In this study, we described the quantitative trait loci (QTL) and candidate genes for downy mildew resistance in susceptible *V. vinifera* cv. 'Red Globe' and *V. amurensis* Rupr. cv. 'Shuangyou'. Two novel QTLs for downy mildew resistance were mapped on linkage group (LG) 15, which have been named *Rpv25* and *Rpv26*, respectively. Candidate gene analysis found that three cysteine-rich receptor protein kinase (CRK) near to two *N* genes in the *Rpv25* locus, and a gene encoding LRR (leucine-rich repeat)-RLK (receptor-like protein kinases) family protein in *Rpv26* locus may be associated to downy mildew resistance in current climatic conditions. The results of the present study provide useful information for grape downy mildew disease resistance breeding.

1. Introduction

It is well known that *V. vinifera* has many preeminent quality traits, whereas does not carry natural resistance to downy mildew (*P. viticola*) infection. Notwithstanding, the grape is threatened by downy mildew, a major goal to grape breeding programs is development of new fungus-resistant cultivars with important agronomic characteristics under current conditions (Van Heerden et al., 2014). The fungus-resistant cultivars can be planted under low protective treatments with a more environmentally friendly and cost-efficient viticulture (Fischer et al., 2004).

Resistance to downy mildew is a typical quantitative trait in grape of the genus *Vitis* (Bellin et al., 2009). To our knowledge, several American *Vitis* species, e.g., *M. rotundifolia, V. rupestris* and Asian species like *V. amurensis* have genetic resistance to downy mildew, which could be used for quantitative trait loci (QTLs) identification and major resistance genes mapping (Merdinoglu et al., 2003; Bellin et al., 2009; Blasi et al., 2011; Schwander et al., 2012; Divilov et al., 2018). *Vitis amurensis* is an ideal grape to resist cold and make ice wine, which thrives naturally in cool climates of Northeast Asia (Liu and Li, 2013). Due to the fungal disease resistance and extensive cold hardiness of this specie, a few hybrid grape cultivars such as 'Shuangyou', 'Shuanghong', 'Bei Mei', 'Gong Zhu Bai' have been developed with a strong emphasis on combining wine quality, disease resistance and cold tolerance, especially in Northern China.

During the last two decades, use of genetic materials with a wide range of backgrounds (e.g., *V. vinifera*, *M. rotundifolia*, *V. amurensis*, *V. riparia* and *V. rupestris*), numerous QTLs for resistance against grape downy mildew have been detected on various linkage groups, except for LG 3 (Merdinoglu et al., 2003; Fischer et al., 2004; Wiedemann-Merdinoglu et al., 2006; Welter et al., 2007; Bellin et al., 2009; Marguerit et al., 2009; Blasi et al., 2011; Moreira et al., 2011; Schwander et al., 2012; Venuti et al., 2013; Zyprian et al., 2016; Ochssner et al., 2016; Divilov et al., 2018). These main QTLs have been named from *Rpv1* and *Rpv24* (VIVC, www.vivc.de). Even though these progresses, only one resistance gene named *MrRPV1*, was cloned from *Muscadinia rotundifolia* at the *Rpv1* locus (Feechan et al., 2013). In addition, the markers developed by QTL analysis cannot be directly used with different genetic backgrounds or for unknown origin population analysis (Ban et al., 2016). Furthermore, a growing body of evidence suggests that resistance against grape downy mildew is affected by the varying climatic conditions. Some major QTLs for downy mildew showed only a small effect or could not be detected in different years (Welter et al., 2007). Taken together, the resistance levels should be

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checked in different months and years.

The earlier genetic maps for grape downy mildew resistance QTL detection were mainly based on SSR (Single sequence repeats), RGA (Resistance gene analogs)-STS (Sequence-tagged sites), SNP (Single-nucleotide polymorphism), RAPD (Random amplified polymorphism DNA), AFLP (Amplified fragment-length polymorphism) and SCAR (Sequence characterized amplified regions) markers (Fischer et al., 2004; Welter et al., 2007; Moreira et al., 2011; Van Heerden et al., 2014; Divilov et al., 2018). In the present study, we report on two novel resistance QTLs conferred by interspecific grape (*V. vinifera* L. × *V. amurensis* Rupr.) crossing, using a high density SLAF (Specific-length amplified fragment)-marker genetic maps (Guo et al., 2015).

2. Materials and methods

2.1. Plant material

An F1 population used in this study (n = 149) was derived from the cross of 'Red Globe' (*V. vinifera* L.) and 'Shuangyou' (*V. amurensis* Rupr.). Hybrid seeds were collected in September 2009 and sown in March 2010 (Guo et al., 2015). The male parent 'Shuangyou' is an ideal grape cultivar to resist cold with quantitative resistance against downy mildew (Yu et al., 2012), while the female parent 'Red Globe' is susceptible to downy mildew cultivar (Van Heerden et al., 2014). Parents and offspring were grown in the greenhouse for controlled artificial inoculations on leaf discs from detached leave at Shenyang Agricultural University (Liaoning, China, 41° 50' N, 123° 24' E, 55 m asl). In addition, the susceptible cultivar 'Centennial Seedless' (*V. vinifera* L.) grown outdoors were selected for strain of *P. viticola* collection. For evaluation of resistance to *P. viticola*, artificial inoculations were performed in three years (2015, 2016 and 2017). In 2015 and 2016, artificial inoculation was performed one time in June. In 2017, artificial inoculation was performed three times, in June, July and August, respectively.

2.2. Downy mildew resistance analysis on artificially inoculated leaf discs

The *P. viticola* sporangia was prepared according to previous studies (Bellin et al., 2009) with some modifications. Infected *V. vinifera* cv. 'Centennial Seedless' leaves at the oil spot stage were obtained from vineyard and then sub-cultured in the laboratory at 20 ℃, 100% RH with a photoperiod of 16/8 h (light/dark, respectively). After five to six days incubation, *P. viticola* sporangia were brushed from the incubation of detached leaves (Fig. 1A) into centrifuge tubes and re-suspended in distilled water for leaf discs test.

The leaf discs test was preformed following a protocol modified from Boso and Kassemeyer (2008). The fifth or sixth leaf from the apex of 1-year-old branches were detached and rinsed with distilled water. Leaf discs of 15 mm diameter were excised with a cork borer. Every 10 leaf discs were distributed in one petri dishes with the abaxial surface up on water agar (0.8% w/v). Each leaf disc was inoculated with a 50 μL droplet of sporangial suspension at 100 sporangia/μL and incubated in a growing chamber at 20 ℃, 100% RH with a photoperiod of 16/8 h (light/dark, respectively). The droplets on leaf discs were removed with sterile filter paper after 24 h. Thirty leaf discs of each genotype were inoculated for the degree of downy mildew resistance evaluation.

The symptoms development was investigated 7 days post-inoculation (dpi) as described by Zhao et al. (2014). Sporulation density (visual rating) was scored with a rating of 0, 1, 3, 5, 7 or 9, denoting the estimated percentage of lesion over the whole leaf disc; Grade 0 = no symptoms, 1 = 0.1–5.0%, 3 = 6.0–25.0%, 5 = 26.0–50.0%, 7 = 51.0–75.0%, 9 = 75.1–100% (Fig. 1B). The grades were then converted into a susceptibility index (SI) according to the following equation (Wang et al., 1998):

$$SI = \frac{\sum (Grade\ value \times infected\ leaf\ disc\ numbers\ in\ that\ grade)}{Total\ of\ leaf\ disc\ numbers \times the\ highest\ grade} \times 100$$

According to the SI value, the resistance level of each genotype was ranked in five categories, including extremely resistant (ER), highly resistant (HR), resistant (R), susceptible (S) and highly susceptible (HS), denoting the SI values of 0.0, 0.1–5.0, 5.1–25.0, 25.1–50.0 and 50.1–100.0, respectively.

The normality of downy mildew resistance distribution was evaluated by the Shapiro-Wilk test. Phenotypic correlations between years were determined with the non-parametric Spearman correlation coefficient. Broad-sense heritability (H^2) was calculated as previously described by Duchêne et al. (2009). Statistical analyses were performed using the *R* 3.3.3 package (R Development Core Team, 2014).

2.3. Detached whole leaf test

Twenty-five whole leaves of parents were inoculated with *P. viticola* sporangia as described previously for the leaf discs test and incubated in the same conditional. Except for the whole leaves, they were inoculated with 250 μL droplet of sporangial suspension. The inoculated leaves were sampled at 0, 1, 3, 5, 7 and 9 dpi. Five replications of paternal or maternal leaves were sampled at each time point. After rinsing with distilled water, the leaves were frozen by liquid nitrogen and stored at −80 ℃ until use.

2.4. Genetic mapping

The high-density genetic map employed in this study has been constructed using specific length amplified fragment sequencing (SLAF-seq) and published by Guo et al. (2015). The genetic map consists of 19 Linkage groups (LG) with 7199 of polymorphic markers, the total genetic distance of the map was 1929.13 cM, with an average distance of 0.28 cM between each maker. Most of the markers showed a good collinearity which was consistent with those in the reference genome (Jaillon et al., 2007).

Fig. 1. The *P. viticola* infected grape leaves. (A) *P. viticola* inoculated 'Centennial Seedless' leaves at 6 dpi. (B) The ordinal visual scale for the grades to infected leaf disc.