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Genotyping by sequencing for SNP discovery and genetic mapping of resistance to race 1 of *Fusarium oxysporum* in watermelon

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ABSTRACT

Management of fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* (*Fon*) (E.F. Sm.) W.C. Snyder & H.N. Han. in watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is largely dependent on cultivation of resistant cultivars. Application of marker-assisted selection (MAS) in conventional breeding programs can accelerate the release of new watermelon cultivars resistant to fusarium wilt. Towards developing tools for MAS, physical (1024 SNPs) and genetic (389 SNPs) maps were developed in the current study for an F_2 population (n = 89; Calhoun Gray x Sugar Baby) segregating for resistance against *Fon* race 1 using the genotyping by sequencing platform. A modified tray-dip method was established for high-throughput phenotyping of the segregating F_3 population. A major quantitative trait locus (QTL) accounting for up to 38.4% of the phenotypic variation in the F_3 population was identified on chromosome 1 on both the physical and genetic maps in a region previously associated with *Fon* race 1 resistance. This resistance locus was consistently detected over five different time points and three different phenotypic screens, showing the reliability of the screening method in discriminating susceptible and resistant genotypes. Eight resistance genes were found within the confidence interval of the identified QTL. SNPs close to this QTL may be exploited in MAS for fusarium wilt resistance in breeding programs. This study confirms the resistance locus on chromosome 1 and demonstrates the use of a physical map for QTL detection in watermelon. The SNPs reported here will be useful for future genetic studies in watermelon.

1. Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is an important crop widely grown for its sweet flesh (Robinson and Decker-Walters, 1997) and edible seeds (Achigan-dako et al., 2008; Edelstein and Nerson, 2002). The United States has over 133,700 acres dedicated to watermelon production with an annual fresh market value of approximately half a billion dollars (United States Department of Agriculture, 2014). The domestication of watermelon through selection for desirable traits has led to narrow genetic diversity in the current elite watermelon cultivars (Levi et al., 2001) and has resulted in the loss of alleles conferring resistance to important bacterial, viral and fungal diseases (Guo et al., 2013; Hawkins et al., 2001). Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* (*Fon*) (E.F. Sm.) W.C. Snyder & H.N. Hans is a production-limiting disease in watermelon growing regions of the world (Boyhan et al., 2001, 2003; Egel and Martyn, 2007; Guner and Wehner, 2004; Hawkins et al., 2001; Martyn and McLaughlin, 1983; Zhou et al., 2010). Four *Fon* races (0–3) have been described in watermelon based on their aggressiveness or their ability to overcome specific resistance in a set of differential cultivars (Bruton, 1998; Egel and Martyn, 2007; Wehner 2008; Zhou et al., 2010).

The persistence of the pathogen in the soil and the evolution of new races make management of fusarium wilt difficult (Bernett, 1936; Bruton, 1998; Egel and Martyn, 2007; Lin et al., 2009; Martyn and Netzer, 1991; Yetisir et al., 2003). Current measures for managing the disease include avoiding infested fields, a 5–7 year crop rotation system, chemical and biological fumigation (Bruton 1998; Egel and Martyn, 2007; Everts and Himmelstein, 2015), the use of resistant root-stocks (Kuniyasu, 1980) and growing watermelon genotypes resistant to the disease (Bruton 1998; Martyn, 2014). Cultivating resistant cultivars is regarded as the best method for managing fusarium wilt (Bruton, 1998; Hopkins et al., 1992; Lin et al., 2009, 2010; Martyn and McLaughlin, 1983; Martyn and Netzer, 1991; Zhou and Everts, 2004). For this reason, many cul-

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tivars resistant to *Fon* races 0 and 1 and a few resistant to race 2 have been developed through breeding programs (Bruton, 1998; Lambel et al., 2014; Wehner, 2008).

Resistance to *Fon* race 1 is widely described in Calhoun Gray and is thought to be controlled by a single dominant gene designated *Fo-1* with a few modifier genes (Guner and Wehner, 2004; Martyn and Netzer, 1991; Netzer and Weintall, 1980; Wehner 2008; Zhang and Rhodes, 1993). However, early efforts to identify loci associated with resistance to *Fon* race 1 primarily utilized the resistant PI 296341-FR (var. citroides). Using PI 296341-FR, Xu et al. (1999) identified a randomly amplified polymorphic DNA (RAPD) marker (OP01/700) weakly linked to *Fon* race 1 resistance at a 3 cM distance. This marker was cloned and sequenced by Xu et al. (2000) and converted into a sequence characterized amplified region (SCAR) marker for use in marker-assisted selection (MAS). Hawkins et al. (2001) attempted to identify quantitative trait loci (QTL) associated with resistance to *Fon* race 1 using F_2 and F_3 populations generated from a cross between PI 296341-FR and New Hampshire (susceptible). However, no useful marker-trait associations were found in this study. Harris et al. (2009) used degenerate primers to target resistant genes in PI 296341-FR encoding nucleotide binding site–leucine-rich repeat proteins and identified three expressed sequence tags (EST) disease resistance homologs. However, none of these EST markers mapped closely to RAPD marker (OP01/700) previously described by Xu et al. (1999) in PI 296341-FR. The inability to find useful marker-trait associations in PI 296341-FR in earlier studies was probably due to low genome coverage and high segregation distortion of marker alleles in this PI (Levi et al., 2011). The narrow genetic diversity among cultivated watermelon has previously hindered genetic mapping studies in elite by elite biparental populations due to low genetic diversity. However, the recent advancement in next generation sequencing technology has provided an affordable platform for studying genome-wide variation in watermelon populations leading to the development of genetic maps of sufficient genome coverage (Cheng et al., 2016; Sandlin et al., 2012; Ren et al., 2012), including a consensus map based on 386 SNPs, 698 simple sequence repeats, 219 insertion-deletion and 36 structure variation markers (Ren et al., 2014). However, the marker-density in these maps is low compared to major crops such as maize (*Zea mays*) (1.15 million markers, Liu et al., 2015), soybean (*Glycine max*) (21,478 markers, Song et al., 2016), wheat (*Triticum aestivum*) (30,144 markers, Maccaferri et al., 2015) and rice (*Oryza sativa*) (30,984 markers, Spindel et al., 2013). Genotyping by sequencing (GBS) (Elshire et al., 2011) is a highly multiplexed next-generation sequencing technology that can generate thousands of markers in any plant species and involves sequencing of reduced genomic libraries followed by alignment of the generated reads to identify SNP variations (Barba et al., 2014; Elshire et al., 2011). GBS allows pooling of barcoded samples into a single sequencing lane thus reducing the cost of genotyping per data point (Elshire et al., 2011). Despite the ability of GBS to generate thousands of SNP markers, the low genetic diversity in cultivated watermelon limits the number of polymorphic markers available to study elite by elite populations. Using GBS, Lambel et al. (2014) identified only 266 usable SNPs in elite by elite F_2 population [HMw017 (resistant) × HMw013 (susceptible)] segregating for resistance against *Fon* race 1and detected seven QTL associated with resistance, including a major QTL (R^2 = 59.9%) on chromosome 1. This major QTL was independently confirmed by Ren et al. (2015) in a recombinant inbred population developed from a cross between PI 296341-FR and the cultivar 97103.

There is need to identify more SNPs among elite watermelon cultivars to allow the development of higher density genetic maps which would be useful in estimating the true positions of QTLs underlying agronomically important traits in watermelon. Furthermore, future genome wide association studies (Zhao et al., 2011) and prediction of genomic breeding values in watermelon lines will require dense marker haplotype maps (Denis and Bouvet, 2011).

Breeding for genetic resistance and developing molecular tools for MAS are dependent upon the ability to differentiate resistant and susceptible genotypes. These studies often require screening of large plant populations. However, as with many other crops (Barbedo, 2014; Furbank and Tester, 2011; Montes et al., 2007), advancement in high-throughput phenotyping technologies for robust trait evaluation in watermelon lags behind that of genotyping technologies for genomic applications. Several phenotyping methods have been developed for screening of fusarium wilt resistance in watermelon including the root-dip method, which is the most common in watermelon breeding and genetic studies (Dane et al., 1998; Freeman and Rodriguez, 1993; Hawkins et al., 2001; Lambel et al., 2014; Martyn and McLaughlin, 1983; Martyn and Netzer, 1991; Ren et al., 2015; Yetisir et al., 2003; Zhou and Everts, 2004, 2007; Zhou et al., 2010). In this method, seedlings are uprooted at the first true leaf stage and the roots are washed off under running water, dipped in inoculum and transplanted into pots containing a substrate. Although reliable, this method is tedious and labor intensive, especially when applied to large populations. Several other phenotyping methods have been developed as alternatives to the root-dip method: pipetting, injection and the tray-dip method. The pipetting method delivers inoculum to the substrate surrounding the seedling (Gunter and Egel, 2012; Kurt et al., 2008; Wechter et al., 2012; Zhou et al., 2010), while the injection method delivers the inoculum into the seedling stem using an insulin syringe (Boyhan et al., 2001, 2003). For the tray-dip method, seeds are sown in trays containing a substrate and placed inside a larger plastic flat containing the same substrate. At the first true leaf stage, the seedling tray is lifted off the larger flat and rinsed briefly with water. The roots growing through the cells' drainage holes are trimmed to a uniform length of 2.5–3 cm and the entire tray is dipped into a shallow flat containing 250–300 mL of inoculum for 10–20 min. The tray is then placed back to the larger flat containing the substrate (Martyn and Netzer, 1991; Zhou et al., 2010). Although effective, these methods are also resource intensive for large-scale screening of fusarium wilt resistance.

The aim of the current study was to confirm the genetic locus associated with resistance to *Fon* race 1 and identify SNPs for future genetic studies among elite watermelon cultivars. In addition, we validated a high throughput phenotyping method for large-scale screening of watermelon seedlings in breeding and genetic studies using a modified tray-dip method.

2. Materials and methods

2.1. Plant materials

A cross between Calhoun Gray (resistant) and Sugar Baby (susceptible) cultivars was made in the greenhouse and a single F_1 plant was selfed to yield F_2 (n = 89) seeds. Each individual F_2 plant was selfed to generate F_3 families that were phenotyped for resistance to *Fon* race 1 in the greenhouse. Charleston Gray, which is described as having intermediate resistance to *Fon* race 1, was also included in the study as a control.

2.2. Fungal inoculum preparation

Race 1 of *Fon* [(B05-07), provided by Anthony Keinath, Clemson University], was grown (14 h/10 h dark cycle) on quarter-strength potato dextrose agar (Becton, Dickinson and Company, NJ, USA) for 12 days. After that time, 1 cM^2 agar plugs were transferred into 250 mL erlenmeyer flasks containing 100 mL potato dextrose broth (Becton, Dickinson and Company). The fungal cultures were