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Resistance in watermelon rootstocks to crown rot caused by *Phytophthora capsici*

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ABSTRACT

Crown rot caused by *Phytophthora capsici* is becoming an increasingly important disease of vegetable crops in southeastern U.S. In recent years, the practice of grafting watermelon onto rootstocks belonging to other Cucurbitaceae genera has also been slowly gaining adoption in the U.S. However, it is not known how these rootstocks, developed mainly in Asia, will respond to diseases prevalent in local production areas. We evaluated the available commercial watermelon rootstocks for resistance to Phytophthora crown rot by inoculating them with a zoospore suspension of *P. capsici* in four different trials. Disease development on rootstocks was rated on a 1–9 scale (1 = no symptoms, 9 = plant dead). Based on all the four trials, the commercial *Lagenaria siceraria* (bottle gourd) rootstocks, FR-Strong, Emphasis, Macis and WMXP-3938 were resistant (Mean rating $\leq$3) to crown rot when compared to susceptible watermelon checks (mean rating >8). All *Cucurbita* inter-specific hybrid rootstocks and a watermelon rootstock Ojakkyo, were highly susceptible to crown rot (mean rating >8). Real-time quantitative polymerase chain reaction (qPCR) using two different *P. capsici* specific primers (ITS and β-tubulin) indicated the presence of significantly ($P < 0.0001$) greater amounts of *P. capsici* DNA g^{-1} plant tissue in susceptible *Cucurbita* inter-specific hybrid rootstocks (β-tubulin, mean = 2895 ng) and watermelon (2665 ng) compared to the *L. siceraria* rootstocks (357 ng). Crown rot resistant bottle gourd rootstocks may be useful in areas where *P. capsici* is a recurring problem. The present study identified several commercial bottle gourd rootstocks with resistance to Phytophthora crown rot, and confirmed their levels of resistance using qPCR.

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1. Introduction

Crown rot caused by *Phytophthora capsici* Leonian is a major limiting factor in vegetable production in the Southeastern United States. *P. capsici* is very widely distributed throughout the world and is now known to attack a wide variety of vegetable crops in the family Solanaceae (tomato, pepper, eggplant), Cucurbitaceae (watermelon, squash, pumpkin, melon), and Fabaceae (Gubler and Davis, 1996; Hausbeck and Lamour, 2004; Babadoost, 2004). It also has been reported as pathogenic on several different weed species (French-Monar et al., 2006), and on *Frasier fir* (Quesada-Ocampo et al., 2009). *P. capsici* can infect most parts of the plant and cause a wide variety of symptoms, such as fruit and crown rots and foliar blight on cucurbits, Phytophthora blight and crown rot on pepper, and buckeye fruit rot on tomatoes etc (Gubler and Davis, 1996; Babadoost, 2004; Hausbeck and Lamour, 2004; Keinath, 2007). Managing diseases caused by *P. capsici* has been difficult, especially since the loss of methyl bromide as a soil fumigant. It was expected that with the loss of methyl bromide, soil-borne plant pathogens such as *P. capsici* will become more prevalent forcing the growers to use more fungicides to manage the disease. The loss of methyl bromide for pre-plant soil fumigation was estimated to result in annual yield losses of 15–20% for watermelon in Georgia and Florida (Lynch and Carpenter, 1999). Other reports estimated losses of up to $235 million for strawberries, tomato and other vegetable crops (Osteen, 2003). The loss of methyl bromide has prompted the search for other means to manage diseases caused by soil-borne pathogens.

The most common strategy used to manage *P. capsici* is the regular application of fungicides. Presently several previously registered and new fungicides have been reported to be effective against *P. capsici* on vegetable crops (Matheron and Porchas, 2000; Babadoost and Islam, 2003; Jester et al., 2006; Kousik et al., 2011). However, as with any chemical control, there is always the potential danger for development of resistance in the pathogen population. For example, resistance in *P. capsici* to mefenoxam has been very well documented from most states in the U.S. (Parra and Ristaino, 2001; Hausbeck and Lamour, 2004; French-Monar et al., 2006; Keinath, 2007). Similarly, resistance in *P. capsici* to cyazofamid has also been reported (Kousik and Keinath, 2008). Therefore new

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alternatives for managing *P. capsici* on vegetable crops are constantly needed.

Host resistance to *P. capsici* has been identified in pepper and several resistant commercial hybrids are available (Babadoost et al., 2003; Louws and Driver, 2005; Foster and Hausbeck, 2010). However, most cucurbits including watermelons are highly susceptible to crown rot (Henz and Lima, 1998) and foliar blights (Holmes et al., 2002; Jester et al., 2006) caused by *P. capsici* (Gubler and Davis, 1996; Babadoost, 2005; Gevens et al., 2008) and the availability of resistance in cucurbits is relatively scarce. A Korean pumpkin cultivar was reported to have high levels of resistance to crown rot caused by *P. capsici* (Lee et al., 2001). Recently resistance to crown rot was reported in few squash accessions (Padley et al., 2008).

In the U.S., bottle gourd and *Cucurbita* inter-specific hybrids (*Cucurbita maxima* × *Cucurbita moschata*) are currently being evaluated as rootstocks for commercial watermelon production (Taylor et al., 2006; Hassell et al., 2008; Davis et al., 2008; King et al., 2010) and the interest has increased as an alternative disease prevention strategy to the use of methyl bromide fumigation (Thies et al., 2010). Watermelons are grafted on diverse rootstocks in many parts of the world primarily for managing soil-borne diseases (Oda, 2002; Lee and Oda, 2003; Cohen et al., 2007; Davis et al., 2008; Bruton et al., 2009). The practice of grafting watermelon onto gourd rootstock for commercial production first began in Japan and Korea in the late 1920's (Lee, 1994). In Japan, most (>90%) of the commercially cultivated watermelon is grafted on bottle gourd rootstocks (Oda, 1993, 2002; King et al., 2010). About 60–70% of the watermelon grown in Israel, are grafted (Koren and Edelstein, 2004; Cohen et al., 2007). The acreage of grafted watermelons is rapidly increasing in Turkey which is the second largest producer of watermelons in the world (Yetisir and Sari, 2007).

Although the process of grafting for managing diseases appears to be fairly straight forward, many factors play a role in selecting suitable rootstocks that will ultimately be successful. Some of these factors include the adaptation of grafted plants to the local environment, resistance to prevailing diseases, and the appropriate rootstock–scion combination (Yetisir and Sari, 2003, 2007; Yetisir et al., 2003; Cohen et al., 2007). One of the primary benefits of using rootstocks of a different genera is to manage soil-borne diseases particularly Fusarium wilt of watermelon. Fusarium wilt (*Fusarium oxysporum* f. sp. *niveum*) is the most commonly reported disease worldwide on watermelon and it can be successfully managed with grafted transplants (Miguel et al., 2004; Oda, 2002; Yetisir et al., 2003; Cohen et al., 2007). Most of the rootstocks especially the bottle gourd (*Lagenaria siceraria*) and Cucurbita inter-specific hybrid rootstocks being used in Asia and Europe are resistant to Fusarium wilt of watermelon as these two types of rootstocks are non-hosts to *F. oxysporum* f. sp. *niveum*, the pathogen that causes Fusarium wilt of watermelon (Cohen et al., 2007; Bruton et al., 2009).

Currently several commercial rootstocks that are resistant (non-host resistance) to Fusarium wilt of watermelon are available in the U.S.A. Grafting is also being done in Mexico using these rootstocks to grow watermelon especially to manage soil-borne diseases. Most available rootstocks are relatively old releases from about 15 years ago (King et al., 2010). Many of these rootstocks are currently being evaluated in the U.S. for use in commercial production. However, it is possible that these rootstocks may be susceptible to other diseases prevalent in the U.S. and commercial Cucurbit cultivars with genetic resistance to *P. capsici* have not been reported (Babadoost, 2005; Davis et al., 2008). Because of the increasing interest and adoption of grafting watermelon in the U.S., we examined the commercially available rootstocks for resistance to crown rot caused by *P. capsici* which is prevalent on vegetable crops in the southeastern United States. To our knowledge these rootstocks have not been evaluated for resistance to crown rot caused by *P. capsici*. In addition we used real-time quantitative polymerase chain reaction (qPCR) as a tool to confirm resistance in these commercial rootstocks.

2. Materials and methods

2.1. Seeds, plants and growth conditions

Diploid (seeded) watermelon cultivars (WM), 'Mickey Lee', 'Crimson Sweet', and 'Black Diamond' were used as checks in these experiments and 16 commercial rootstocks that were either *Cucurbita* inter-specific hybrid (ISH, *C. maxima* × *C. moschata*), *L. siceraria* (bottle gourd, LS) or watermelon (WM) were evaluated. All the ISH and bottle gourd rootstocks (LS) evaluated in this study are resistant (non-host resistance) to Fusarium wilt (*F. oxysporum* f. sp. *niveum*) of watermelon. Seeds of the check cultivars were obtained from Willhite seeds (Poolville, TX, Willhiteseeds.com). Seeds of commercial rootstocks, 'Emphasis' (LS), 'Ojakkyo' (WM) and 'Strong Tosa' (ISH) were provided by Syngenta seeds (Boise, ID). Seeds of rootstocks, 'Gladiator' (ISH), 'No-1' (ISH), 'Iron Cap' (ISH), and 'Geo Sprit' (LS) were provided by Sakata Seed America (Morganhill, CA). Seeds of 'Shintosa Camel' (ISH) and 'Macis' (LS) were provided by Nunhems (Parma, ID). Seeds of 'Chilsung Shintosa' (ISH) and 'FR-Strong' (LS) were provided by Seminis (Oxnard, CA). Seeds of 'WMXP-3943' (ISH), 'WMXP-3938' (LS), 'WMXP-3944' (LS) and 'WMXP-3945' (LS) were provided by Harris Moran (Modesto, CA). Seeds of 'WR-15006' (ISH) were provided by Zeraim Gedera (Israel).

In the first two experiments (experiment 1 & 2), seeds were planted in 2.5-cm, 50 cell trays filled with metro-mix 360 (Sun Gro Horticulture, Canada) and placed on germination mats to enable uniform germination. After emergence of seedlings the trays were placed on benches in an air conditioned greenhouse with day time temperatures maintained at 25 ± 2 °C and night time temperatures at 21 ± 2 °C. Each watermelon cultivar, inter-specific hybrid and *Lagenaria* rootstocks were replicated four times with five plants per replication. The experiments were arranged as a randomized complete block design with replications being the block and rootstocks arranged randomly within each replication.

A second set of similar experiments (experiments 3 & 4), were conducted with most of the above mentioned rootstocks and watermelon cultivars. The experimental arrangement was similar to experiments 1 & 2. However, seeds were planted in larger 9-cm square pots filled with metro-mix 360. For these experiments there were four plants of each rootstock or commercial watermelon cultivar per replication and there were a total of four replications. The experiments were arranged as a randomized complete block design with replications being the block and rootstocks arranged randomly within each replication.

2.2. Pathogen cultures and inoculation

Cultures of *P. capsici* were maintained on V8 juice agar (Keinath, 2007; French-Monar et al., 2006). Because the rootstocks evaluated belonged to different genera we used five isolates of *P. capsici* obtained from different crops and fields in South Carolina. Four of the isolates were provided by A.P. Keinath (Clemson University). The *P. capsici* isolates were isolated from watermelon (*Citrullus lanatus*), Zucchini (*Cucurbita pepo*), Squash (*C. moschata*), pepper (*Capsicum annuum*). All the isolates were confirmed as *P. capsici* using specific PCR primers (Ristaino et al., 1998; Zhang et al., 2006) and morphology of the colony and sporangia. Plants were inoculated five weeks after seeding. For preparation of inoculum equal volumes of spores (2.5×10^4 ml^{-1} zoospores) from each