Coverletter

Title: **Field trial for Fusarium wilt race 2 resistance in watermelon**

Summary:

In this study we proposed a field trial to (i) confirm resistance, (ii) make further selections in the field, and (iii) collect data for further inheritance studies for a watermelon selection (WS896) that is resistant to *Fusarium oxysporum* f.sp. *niveum* race 2 (FOS 2) in the greenhouse. Our results indicated that field resistance in WS-896 was not fixed, but indicated field resistance in the parental line, WS147. Fourteen lines with high resistance to FOS 2 were identified in the segregating population for further breeding efforts and population-wide data was collected for future inheritance and genomic studies. A number of the selected resistant lines had desirable horticultural traits such as large fruit and red flesh. The selections made here will form the basis for further breeding efforts for resistance to Fusarium wilt race 2 in watermelon.

Grant Amount: $5,000

Time Line:

Start Date: April 2013

End Date: December 2013

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2. Background

Fusarium wilt is a limiting factor in the production of watermelon crop and has been a priority for watermelon growers for a long time. Currently the disease is controlled by avoidance of infested fields, crop rotation systems and the use of soil fumigants. Developing disease resistant cultivars would be the most effective way to control the disease and for races 0 and 1 there are resistant seeded varieties. However, despite the identification of resistance to race 2 in PI 296341 (Martyn and Netzer, 1991) more than 20 years ago, this has not led to the development of any resistant cultivars with edible fruit. This is largely due to the undesirable fruit characteristics of PI 296341, which is a different subspecies (*Citrullus lanatus* var. *citroides*) than cultivated watermelon (*C. lanatus* var. *lanatus*). This causes problems for breeders because the inheritance of some genes in crosses between citroides and cultivated watermelon does not conform to expectations (due to recombination suppression and preferential inheritance of certain chromosomal regions). Other resistant citroides have identified since then, but it remains to be seen whether they will present the same challenges as PI 296341 (Dane et al., 1998; Wechter et al., 2012).

We have identified a *C. lanatus* var. *lanatus* selection (WS896) that shows consistent resistance to Fusarium race 2 in the greenhouse. This selection has red flesh and BRIX of between 8 and 9 and it is anticipated that, compared to citroides, it will be easier to transfer resistance from this line to cultivated watermelon. However, this line has not been tested under field conditions.

In order to test this selection in the field, we proposed to artificially inoculate a field with FOS 2 since artificial inoculation gives more consistent results than counting on occurrence of natural infection (Hunt Sanders, personal communication). We evaluated WS896 field resistance and made further selections from the segregating population.

In addition to testing the WS896 itself, we also evaluated progeny from a population developed by a crossing WS896 with Charleston Gray. This will enable us to use this field data in future to estimate how many genes are involved in resistance. We can also use this information to supplement greenhouse information we are using to determine the chromosomal regions

responsible for the resistance (independently funded). The significance of identifying the chromosomal region involved is that it will accelerate breeding through marker assisted selection.

3. Objectives/goals

(i) Confirm resistance of the selection WS896 to *Fusarium oxsysporum* f.sp. *niveum* race 2
(ii) Make further selections in the field, and
(iii) Collect data that will make it possible in future to determine how many genes are involved in resistance and which chromosomal regions are of important for breeding resistant cultivars.

4. Materials and Methods

WS896, Charleston Gray, F_3 progeny and several controls (including WS147, the parental line of WS896) were sown in the greenhouse and after two weeks, hardened off and transplanted by hand on 4/24/2013 at the UGA Horticulture Farm in Watkinsville, Georgia. Plants were planted in a randomized complete block design with 3 replications (blocks) of 4 plants per line.

Inoculum of FOS 2 isolated in Georgia (Strain 04122 supplied by Bennie Bruton) was prepared by growing the pathogen in QPDA for 10 days and transferring agar plugs into potato dextrose broth on a shaker for two weeks. Field inoculation was carried out by pouring 40 ml of 1×10^6 inoculum onto each of the transplant holes two days after transplanting (4/26/2013). Data on disease severity was taken every 3 days, with the final disease incidence recorded 26 days after inoculation. Plants were score on a 0 to 5 scale with 0 = no symptoms and 5 = dead.

A number of resistant lines (score < 1) lines were selected and self-pollinated by hand, taking care to cover both male and female flowers before they opened to prevent pollen contamination.

5. Results

The selection WS896 did not show high field resistance, however WS147, the parental line of WS896 did show resistance under field conditions (Fig. 1). The F_3 population (118 lines) segregated for resistance, with 14 lines with a score of < 1.0 (Fig 1) and 5 of these lines showed high resistance (Score < 0.5). Resistant lines were selected for further breeding efforts (Figs 1

and 2). In addition to high levels of resistance, some of these lines also had desired horticultural traits such as large fruit, pink/red flesh, thin rind and sweet flesh (brix ~8; Fig 3).

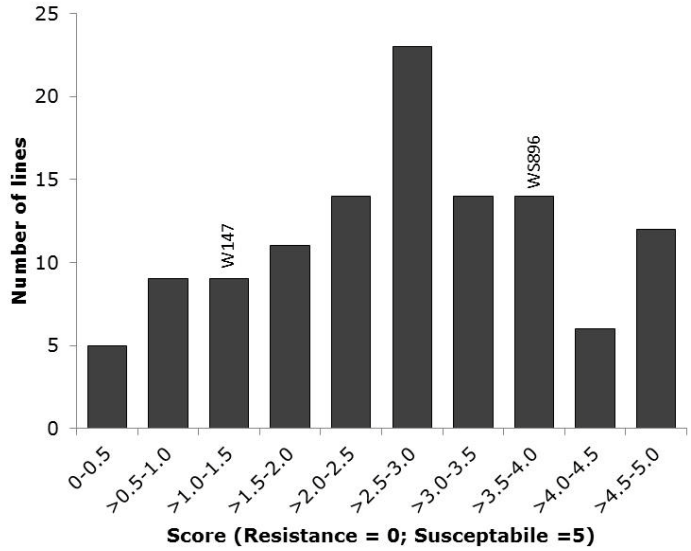

Figure 1. Distribution of Fusarium wilt race 2 field resistance in the F_3 population (26 days after inoculation).

Figure 2. Example of a resistant line selected for future breeding efforts.

Figure 3. Examples of two lines with high field resistance to Fusarium race 2. Lines #146 and #220 had average disease scores of 0.42 and 0.33, respectively.

The data from the segregating population (Fig. 1) is currently being used to identify chromosomal regions associated with resistance under USDA grant #2010-85117-20550.

6. Budget - Spending

Supplies: $5,000

The funds were used to buy potting soil, fertilizer, plastic mulch, irrigation tape, stakes, flags, pesticides/fungicides, and dosatron needed to grow a field trial. Funds were also used for items needed to prepare and inoculate the pathogen, including a doser, hoses and buckets.

7. Parties involved with the project, including the Lead Investigator

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8. Emphasize which NWA priority area your project is based on

PRIORITY AREA: Fusarium wilt

Literature Cited

Dane, F., L.K. Hawkins, J.D. Norton, Y.-S. Kwon, and Y.-H. Om. 1998. New resistance to race 2 of *Fusarium oxysporum* f.sp. *niveum* in watermelon. Cucurbit Genetics Cooperative Report 21:37-39.

Martyn, R.D. and D. Netzer. 1991. Resistance to races 0, 1, and 2 of Fusarium wilt of watermelon in *Citrullis* sp. PI 296341-*FR*. HortScience 26:429-432.

Wechter, W.P., C. Kousik, M. McMillan, and A. Levi. 2012. Identification of resistance to *Fusarium oxysporum* f. sp. *niveum* race 2 in *Citrullus lanatus* var. *citroides* plant introductions. HortScience 47:334-338.

Addendum to report: **Field trial for Fusarium race 2 resistance in watermelon**

Fusarium wilt is a serious disease that affects watermelon production areas throughout the U.S. This pathogen is soil-borne and has 4 races (0, 1, 2, and 3) which cause different levels of symptom severity. The best way to control the disease would be to grow cultivars that are resistant to the disease, however no such cultivars exist for race 2. Some wild citron types with resistance have been identified, but since these have many undesirable fruit characteristics (e.g. small fruit with hard, bitter flesh) it has been difficult to use this resistance to breed high quality commercial watermelon cultivars.

We have identified a watermelon selection (WS-896) that is resistant to Fusarium race 2 in the greenhouse. This selection also has less undesirable fruit characteristics and we therefore hope it will be easier to use this selection to breed resistant cultivars. However, before we can consider using this selection in breeding, we have to confirm that the resistance is effective under field conditions and make further selections if needed. Our results indicated that WS896 did not show high levels of field resistance. However, the parental line WS147 did showed high resistance, as did 14 lines from the segregating population.

We made several selections from the segregating population, including lines #146 and #220 which had high levels of resistance and good horticultural fruit traits (Brix). These lines will form the basis for future breeding effort for Fusarium race 2 resistance in watermelon. In addition, we were also able to collect data that will make it possible to determine the chromosomal regions associated with resistance. This information will speed up selection during the breeding for resistance to Fusarium race 2.