The role of soil fertility and soil cations on disease resistance and the severity of Fusarium wilt of watermelon (*Fusarium oxysporum* f. sp. *niveum*)

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Research Title: The role of soil fertility and soil cations on disease resistance and the severity of Fusarium wilt of watermelon (*Fusarium oxysporum* f. sp. *niveum*) (second year results)

Introduction: Fusarium wilt of watermelon, caused by the fungal pathogen *Fusarium oxysporum* f. sp. *niveum* (FON), has been a serious threat for the watermelon production in the United States. The pathogen causes pre- or post-emergence damping-off of young seedlings and wilting of mature plants in the field. A unilateral stem necrosis is a characteristic symptom of Fusarium wilt, which can be easily, visualized when runners or stems are sectioned. Under favorable conditions, yield losses more than 80% can be experienced. Several management strategies like seed treatment, crop rotation, weed control, and chemical management options are available but in most cases, they are inadequate to manage FON epidemic. Apart from these strategies, a different approach to the integrated disease management of FON is required. Being a soil borne fungus, soil fertility and soil mineral nutrition may play an important role in FON infection and severity of symptoms. However, very little information is available on this aspect. Hence, the overall goal of the proposed research is to determine the role of soil fertility on the severity of Fusarium wilt of watermelon.

Materials and methods: Watermelon transplants (cv. Sugarbaby) were established in hydroponics set up under controlled greenhouse conditions. Both high (2X) and low levels (0.5X) of micronutrients (Cu, Fe, Mn and Zn) (X= recommended dose in field) were fed for 7-days. After 7-days of feeding, the plants were evaluated for the expression of disease resistance genes (PR1, PR5, NPR1). Later, sub-set of plants were inoculated with FON (10^5 conidia/ml) and watermelon plants were re-evaluated for expression of disease resistance genes.

Induction of resistance genes in watermelon: Samples (foliage) as stated above were excised with a sterile scalpel and used for RNA extraction using the manufacturer's protocol (RNeasy Plant Mini Kit, St. Louis, MI). Two microliters of RNA were converted to cDNA using the iScript cDNA synthesis kit (Bio-Rad and qPCR was conducted on a Smart Cycler System (Cepheid, Sunnyvale, CA) using the iQ SYBR Green Supermix (Bio-Rad) and specific primer pairs for the marker genes (Table 2). For real-time PCR, 5µl of cDNA were amplified in 25 µl of PCR master-mix containing 12.5µl of SsoFast™ EvaGreen Supermix (Bio-Rad Laboratories Inc., Hercules, CA) and 1µl each of 25 µM primers per sample. Real-time PCR was conducted in a Cepheid Smart Cycler with specific thermal conditions depending on the set of primers used. The reference gene was used as a control, and was assessed. The cycle threshold (Ct) values thus obtained were converted into relative fold differences of marker genes in treated samples

compared with the untreated samples and relative to the endogenous control gene using the 2-ΔΔCt method. This was done after verifying the stability of the endogenous control genes and that the primer pairs had high, comparable PCR efficiencies. Relative fold changes of target genes were calculated and compared for each treatment by Fisher's LSD at $P<0.05$ level or by student's t-test.

Results and discussion:

As observed in year 1 study, watermelon seedlings fed with low Mn resulted in significantly higher PR1 gene expression compared to seedlings fed with high Mn. Theses observations were true for the same treatments both before and after FON inoculation (Table 1). NPR1 was also highly induced with low Mn compared to high Mn treated seedlings both before and after FON inoculation. Again as observed in year 1, Mn feeding did not have significant effect on the expression of PR5 gene both before and after FON inoculation (Table 1). These observations indicate that low Mn feeding (0.5X) can induce PR1 and NPR1 genes prior to FON infection. However, increased application of Mn can reduce the induction of SAR-based resistance genes. Significant effects of PR1 and PR5 induction were also observed with high- and low-Zn feeding, respectively prior to FON inoculation (Table 1). High Zn feeding did not influence expression levels of PR5 and NPR1 genes both before and after FON inoculation (Table 1). However, after FON inoculation, high Zn feeding only resulted in increased PR1 expression compared to NPR1 or PR5 (Table 1). Prior to FON inoculation, high Fe feeding only resulted PR1 expression whereas PR5 and NPR1 were not induced. However, expression levels of three genes were not affected after FON inoculation. Feeding with low Fe resulted in significantly higher NPR1 and PR5 expressions compared to PR1, prior to FON inoculation. Induction of resistance genes were not observed after FON treatments with other micronutrients applied at different rates (Table 1). FON inoculation resulted in down-regulation of NPR1, PR1 and PR5 genes in watermelon in plants control (NPK feeding) (Table 1). Overall, it was observed that micronutrient feeding for 7-days can induce disease resistance genes in watermelon seedlings.

Table 1: Relative expression of disease resistance genes (PR1, PR5 and NPR1) after controlled feeding of high (2X) and low (0.5 X) levels than the recommended dose of Zn, Fe, and Mn. Asterisk (*) denotes significance level at $P<0.05$.

Treatments	Resistant genes	Relative expression	
		Before FON inoculation	48-h post-FON inoculation
High Zn	PR1	2.25*	2.86*
High Zn	PR5	0.88	0.95
High Zn	NPR1	1.02	0.98
Low Zn	PR1	0.92	1.05
Low Zn	PR5	2.78*	1.30
Low Zn	NPR1	0.85	0.92
High Fe	PR1	2.65*	1.02
High Fe	PR5	0.78	0.85
High Fe	NPR1	0.92	1.05
Low Fe	PR1	0.67	1.15
Low Fe	PR5	1.28	0.67
Low Fe	NPR1	3.15*	1.25
High Mn	PR1	0.92	1.02
High Mn	PR5	1.24	1.15
High Mn	NPR1	0.85	0.62
Low Mn	PR1	2.24*	1.65
Low Mn	PR5	1.02	0.92
Low Mn	NPR1	3.38*	2.20
Standard	PR1	2.56*	1.0
Standard	PR5	1.08	1.0
Standard	NPR1	1.78*	1.0