REDUCED AGRONOMIC PERFORMANCE IN BACTERIAL WILT-(*Ralstonia solanacearum* species complex) TOLERANT PEPPER LINES UNDER GLASSHOUSE CONDITIONS

Herbert Dustin R. Aumentado[1], Derek Barchenger[2], Fe Dela Cueva[1], and Mark Angelo O. Balendres[1*]
[1]Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines Los Baños, Los Baños, Laguna, Philippines, 4031; [2] The World Vegetable Center (WorldVeg), Tainan City, Taiwan
* Corresponding author: mobalendres@up.edu.ph

ABSTRACT

Host resistance is the most efficient and economically viable management approach against bacterial wilt disease caused by *Ralstonia solanacearum* species complex (RSSC), the most destructive soil-borne disease infecting a wide range of economically important crops. This study aims to determine pepper lines that have resistance to two genomic species of bacterial wilt. Fifteen advanced pepper lines were assessed for their resistance to RSSC– *R. pseudosolanacearum (Rp;* also known as Phylotype I [Asiaticum]) and *R. solanacearum* (*Rs;* also called Phylotype II [Americanum]) under glasshouse conditions. The trial was carried out in 15 replicate plants per line, arranged randomly in blocks, and inoculated through soil drenching (without root wounding). Initially, nine lines exhibited high resistance to *Rp,* and ten pepper lines had high resistance to *Rs*. However, during their growing stage, we observed reduced growth performance of the lines in comparison to the resistant check and control plants. With this, RSSC population density was determined, and the presence of the RSSC population was found inhabiting the stems of the initially identified resistant pepper lines, thus categorizing them as tolerant pepper lines. Consequently, evaluation of the plant's agronomic performance (plant height, leaf color, and plant vigor) revealed the significantly diminished growth performance of the tolerant pepper lines in comparison with their control plants. In the absence of wilting symptoms, these tolerant pepper lines served as carriers of the bacterial wilt pathogen. Reduced agronomic performance may be associated with growth trade-off wherein the plants require a substantial demand of resources to activate plant defense, thus resulting in possible growth and yield impacts in hot spot areas of bacterial wilt at field conditions.

INTRODUCTION

Pepper (*Capsicum annuum*) is one of the most important economic crops that belong to the Solanaceae family. Its price can reach as much as Php 1,000 per kilogram (USD 20), making pepper cultivation attractive to small-scale growers as a source of income in the country (Balendres and Dela Cueva, 2020).

Pepper production is continuously affected by diseases such as the bacterial wilt caused by *Ralstonia solanacearum* species complex (RSSC). It is one of the most economically important soil-borne diseases of pepper in the tropics and subtropics. RSSC is considered the most destructive bacterial pathogen that affects over 450 plant species worldwide (Mansfield *et al.*, 2012; Wicker *et al.*, 2007), resulting in heavy yield loss of up to 100%.

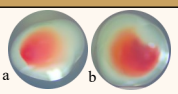
The existence of phylotypes of this pathogen and the inefficient and unsuccessful chemical (bactericides) applications make the disease problematic and difficult to manage. In the Philippines, two species, *R. pseudosolanacearum (Rp; aka* Phylotype I and of Asian origin) and *R. solanacearum* (*Rs;* aka Phylotype II and of American origin) cause bacterial wilt in peppers.

Many efforts have been made to control this disease such as cultural methods (e.g. grafting, soil amendments, crop rotation) and biofumigation (Justo *et al.*, 2012) that aid in mitigating the impact of the disease, but plants succumb to high inoculum levels in the soil. Deploying resistant plants is the most durable and sustainable management approach to combat bacterial wilt disease.

In this study, we utilized advanced pepper lines that have *R. solanacearum* resistance from the World Vegetable Center (WorldVeg), Taiwan conditions to evaluate their resistance to two bacterial wilt genomic species— *R. pseudosolanacearum* and *R. solanacearum* in the Philippines.

METHODOLOGY

1. Sowing and Transplanting of Pepper Lines
2. Single colony isolation and purification of virulent RSSC strains



Typical colonies of (a) *Rp* and (b) *Rs* (*100x* magnification)

3. Absorbance adjustment of the two *Ralstonia* species using Spectrophotometer OD_{600}= 0.3

4. Glasshouse pot inoculation thru soil drenching w/out wounding

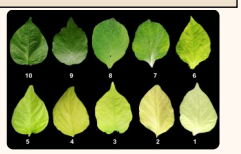
5. Disease Severity (Winstead & Kelman, 1952)

Rating	Reaction observed
0	No wilting
1	> 10% wilted leaves of the plant
2	11-25% wilted leaves of the plant
3	26-50% wilted leaves of the plant
4	51-75% leaves of the plant
5	> 75% leaves of the plant

6. Disease Reaction of Pepper Lines

Disease Index	Reaction
0.00-0.2	Highly Resistant
0.21-0.3	Resistant
0.31-0.4	Moderately Resistant
0.41-0.5	Moderately susceptible
0.51-0.6	Susceptible
0.61-0.9	Highly Susceptible
0.91-1.0	Extremely Susceptible

7. Bacterial Population in Peppers (log CFU/ ml)
8. Assessment of Agronomic Characters (Plant Height, Plant Vigor, Leaf Color)

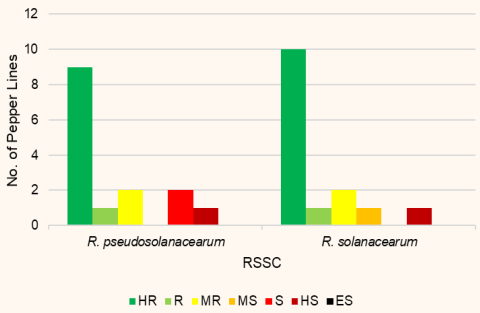


RESULTS AND DISCUSSION

I. Response of pepper lines to RSSC

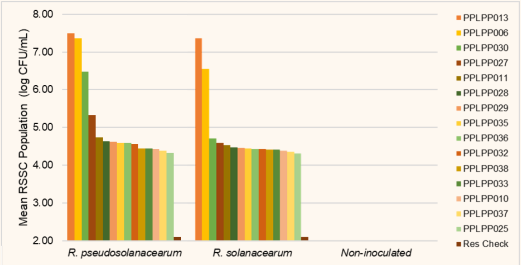
Of the fifteen evaluated pepper lines, in *Rp*-inoculated treatment, nine (9) exhibited high resistance (HR), one line was resistant (R), two pepper lines were moderately resistant (MR), two lines were susceptible (S), and one pepper line was identified as highly susceptible (HS). Whereas in *Rs* inoculated pepper lines, ten exhibited high resistance (HR), one pepper line was resistant (R), two lines were moderately resistant (MR), and one pepper line was identified as highly susceptible (HS). The resistant and susceptible checks responded accordingly throughout the trial.

Frequency distribution of reaction based on disease index (DI) of pepper lines evaluated resistance to two *Ralstonia* species at 4-weeks post-inoculation.



II. RSSC population on pepper lines

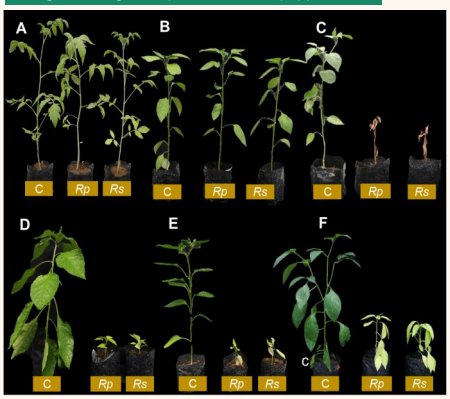
Population of RSSC in inoculated (*Rp* and *Rs*) and non-inoculated pepper lines at 28 days-post inoculation. Viable cells grown on TZCA medium are reported as log CFU per milliliter (CFU/ml).



Highest populations in *Rp*-inoculated pepper lines and *Rs*- inoculated pepper lines were found in PPLPP030 and PPLPP013, respectively which exhibited high susceptibility in RSSC while the **identified resistant lines obtained the lower mean RSSC populations**. The collected plant samples of the non-inoculated (Control) pepper plants of each lines had zero RSSC population.

All of the inoculated pepper lines used in this study were invaded by the bacterial wilt pathogen, RSSC regardless of their degree of resistance or susceptibility to the disease. The majority of the plants of the identified resistant lines did not show any wilt symptoms throughout the conduct of the study, but the pathogen was still present in the plants. **Population of the both *Ralstonia* species in the identified resistant lines was relatively lower compared to the susceptible pepper lines but higher than resistant check** which indicated that the RSSC transmission and multiplication and was not completely inhibited and resisted but was only diminished.

III. Agronomic growth performance of pepper lines



A. Resistant Check- Tomato; B. Resistant Pepper Line; C. Susceptible Check- Pepper; D-F. Tolerant Pepper Lines (Control– C; *R. pseudosolanacearum*– *Rp,* and *R. solanacearum*– *Rs*)

- On **plant height,** the non-inoculated pepper lines had significantly taller plants (*P>0.001*) compared to pepper plants inoculated with both *Rp* and *Rs* which are not significantly different from each other.
- On **plant vigor**, the non-inoculated pepper lines had significantly better crop stand and vigor (*P>0.001*) in compared to pepper plants inoculated with both *Rp* and *Rs* which are not significantly different from each other.
- On **leaf color**, the non-inoculated pepper lines pepper lines had significantly greener leaf color (*P>0.001*) compared to pepper plants inoculated with both *Rp* and *Rs* which are not significantly different from each other.

Although, these pepper lines demonstrated resistant to wilting symptoms, we observed that these pepper lines inoculated with either *Rp* and *Rs* had much shorter plant height, had frail/ poor plant vigor, and paler leaf color compared to non-inoculated pepper plants, and resistant checks. The reduced agronomic performance in *Rp*- and *Rs*-inoculated plants, 28 days-post inoculation, indicated that the surviving plants had not fully been resistant to the bacterial wilt pathogen, only tolerant. Thus, may exhibit latent infection.

CONCLUSION

This study initially identified **9 and 10 pepper lines that had high resistance to *Rp* and *Rs***, respectively. Some pepper lines are resistant in *Rp* but not in *Rs*, vice-versa, thus it is necessary to develop strain-/ species-specific resistance to RSSC.

RSSC population density was found inhabiting the stems of the initially identified resistant pepper lines, thus categorizing them as tolerant pepper lines.

The inoculation and infection of RSSC in pepper lines in glasshouse conditions resulted in **reduced agronomic performance by plant height, plant vigor, and leaf color**. These tolerant pepper lines served as symptomless carriers of the bacterial wilt pathogen.

Reduced agronomic performance may be associated with growth trade-off wherein the plants require a substantial demand of resources to activate plant defense, thus resulting in possible growth and yield impacts in hot spot areas of bacterial wilt at field conditions.

Nevertheless, these pepper lines will still play an essential part in the forthcoming pepper bacterial wilt resistance breeding program.

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