The pepper germplasm resistance screening to bacterial wilt and association mapping

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Abstract

Pepper is an important plant worldwide for its economic benefits and nutrition values. Pepper bacterial wilt (PBW) is one of the most destructive diseases, when plant is diagnosed bacterial wilt, it could be observed browning stem, wilting leaves and lethal characteristic. *Ralstonia solanacearum* is a gram-negative pathogen which cause bacterial wilt in many plant species. It could infect host by getting into root, block the vessel of xylem, eventually obstruct water sent to the top of the plant and cause death. PBW is difficult to control in the field, resistance source selection has been regarded as one of the most effective methods to prevent it. Cotyledon seedling screening method is a rapid way to screen resistant materials against PBW during the cotyledon seedling stage. The objective of this research is assessment of the pepper germplasm resistance to bacterial wilt using cotyledon seedling screening method and identify candidate loci that regulate the resistance. In this research, we analyzed the association between the phenotypes and the genotypes of the pepper collection, and significant SNPs have been found. Whether the significant loci can regulate the resistance of Capsicum species will require further investigation.

Introduction

Pepper (*Capsicum* spp.) belongs to the family Solanaceae, and there are five recognized cultivated species, which are *Capsicum* chinense, *C. annuum, C. pubescens, C. baccatum*, and *C. frutescens*. Pepper can be cultivated in different kind of regions, such as tropical, subtropical, and temperate regions. Pepper is planted throughout the world because of its significant economic benefits and nutritious value. Pepper has rich bioactive compounds which are regarded can prevent disease, against malnutrition, diabetes, and oxidative stress[1, 2]. The content of its bioactive compounds can be an important source of the recommended daily allowance (RDA) of per person and improve overall health of human beings[3].

Ralstonia solanacearum is a Gram negative pathogen which cause bacterial wilt in numerous plant species[4, 5]. R. solanacearum is a soil borne bacterial pathogen, it can survive in the soil for several years and retain the ability to infect the host when its favorite condition comes[6]. R. solanacearum is transmitted, it can be spread through long distance by contaminate soil, water, and weeds[7]. R. solanacearum invades the plant through the root, and the xylem vessels will be blocked by the secreted exopolysaccharides, eventually obstruct water sent to the top of the host plant[6, 8]. If the plant is infected, leaves will show wilting symptoms. When placed a freshly cut stem in clear water, it can be observed a white, milky ooze of microorganism comes out from the cut of the stem[9]. Because of the lethal characteristic of bacterial wilt, it has caused severe economic loss around the world[10].

In cultivating pepper, bacterial wilt is also a most destructive disease and cost significant loss worldwide[11]. Since it is very difficult to prevent bacterial wilt in the field[9], the usual practices of controlling bacterial wilt are host-plant resistance and biological control[12], and resistant cultivar selection is regarded as one of the most effective way to control bacterial wilt[13]. It is reported a rapid way to screen resistant cultivar against R. solanacearum bacterial wilt during the cotyledon seedling stage[4], and it is considered saving time, cost, and space[14]. The research on verify the credibility of the seedling screening method has been done before by the bacteriology unit in World Vegetable Center (unpublished).

The objective of this research is assessment of the pepper germplasm resistance to bacterial wilt using cotyledon seedling screening method and association mapping.

Materials and methods

Plant materials

There are 207 accessions of pepper germplasm, including six different species of *Capsicum* spp., are used in the research (Table 1). The seeds were provided by Gene bank, World Vegetable Center. The pepper seeds first be sterilized by treating with 1% sodium hypochlorite for 5 min and three washes of sterilized water for 5 min. After the sterilization, the seeds were place on a sterile, wet cotton and tissue paper bed in culture container, then incubated in a growth chamber at 28℃ in the dark for 5 to 7 days. After the germination, the growth chamber was set to a 12h photoperiod within the conditions of light intensity in 4891 Lux, 90 μmol m^{-2} s^{-1}. After 10 to 14 days, the pepper seedlings were inoculated. The experiment was conducted in a randomized complete block design (RCBD) with 3 replicates. Each replicate included 10 seedlings. Pepper cultivar PBC066 (resistance) and PBC1367 (susceptible) were used as control plants.

Bacterial strain and inoculum preparation

Pss71 (Phylotype I, Biovar 3, Race 1) is a Ralstonia solanacearum species complex isolated from pepper collected from Linnei, Yunlin District, Taiwan in 1991. Streak the selected strain, Pss71 on TTC plate and incubated at 30℃ for 2 to 3 days. Then multiplication of the culture on 523 plate at 30℃ overnight. Suspend the overnight cultured bacterial mass in sterile water. Adjust the concentration at approximately $1x10^8$ CFU/mL (OD_{600nm}= 0.3-0.32) for inoculation.

Cotyledon seedling screening method

Consider the route of R. solanacearum infected plants, the roots of the plants or seedlings might be a proper way to make sample get infected[8]. The root tip of the pepper seedling was cut by sterilized scissors before inoculation. The pepper seedling was inoculation by root-dipping for 1 min in the inoculum. Each inoculated seedling was transferred to an empty petri dish for air exposure for 5 min, then transferred to a

2 mL of microfuge tube with 2.0 mL of sterilized water and kept in a growth chamber at 28℃ with 12h photoperiod within the conditions of light intensity in 4891 Lux, 90 μmol m^{-2} s^{-1}. The demonstration of experimental operation as shown in figure 1. Disease severity (disease scale 0 to 3) and disease incidence were recorded 10 days after inoculation. For disease severity, 0 and 1 were resistant (negative), 2 and 3 show different disease severity of wilt symptoms (positive). And for disease incidence, indicated to the previous research done by the bacteriology unit in World Vegetable Center, wilting percentage (W%) below 40% was regarded resistant (unpublished). The formula for calculating W% is present below.

$$W\% = \frac{wilting\ \ seedling\ \ number}{Tested\ \ seedling\ \ number} \times 100$$

Association mapping

To investigate the genetic structure in the collection, we performed a PCA in TASSEL v5.0. The plot chart was performed using R Studio. GWAS with two corrections was conducted by TASSEL v5.0 (A citation is needed for TASSEL), and the model chosen eventually was model MLM. After the correction, the Manhattan plot was plotted in TASSEL v5.0.

Data analysis

The analyses were performed using R Studio (A citation is needed for R) and TASSEL v5.0.

Result

Pepper germplasm resistant reaction

The distribution of pepper germplasm resistant reaction shows in Figure 2. There were 30 resistant pepper accessions in total, and the percentage of total resistance was 14.49% (Table 1). The induvial resistance of different *Capsicum* species also showed in Table 1. *Capsicum* chinense Jacq. has the highest resistance percentage. The

quantify of collected data was acceptable for conducting association map in the following experiment.

Association mapping

For the principal Component Analysis (PCA), the x-y scatter shows the clustering of *Capsicum* species (Figure 3). Every *Capsicum* specie is marked by different colors. The PCA indicates different genetic clusters in the collection, suggesting models with different population correction should be checked to reduce the false discovery rate.

Figure 4 shows the two models conducting the calibration. On the left is model GLM, and on the right is model MLM. The model that is close to the expected distribution could lower the false positive rate. Therefore, MLM was used to represent the GWAS result.

The Manhattan plot shows the distribution of SNP signals (Figure 5). Every point is one SNP. The *p* values were plotted along y-axis with -log10 transformation; hence, the higher value means more significant. The definition of significance is when the value of one point is higher than 3. Based on the threshold, we could target some significant SNPs to screen for our candidate genes regulating the resistance of *Capsicum* species.

Discussion

Cotyledon seedling screening method

The limit of poor germination rate and contamination could affect the final quantity of data. There were 245 accessions be conducted in the experiment, but there were only 207 data were collected. If more data could be collected, it would be better to the statistical analysis.

Association mapping

There were only two model were run in this research. If more models would be conducted in the research, the signal that show up repeatedly in different models might means it is the real signal we would like to analyze in the future.

Acknowledgements

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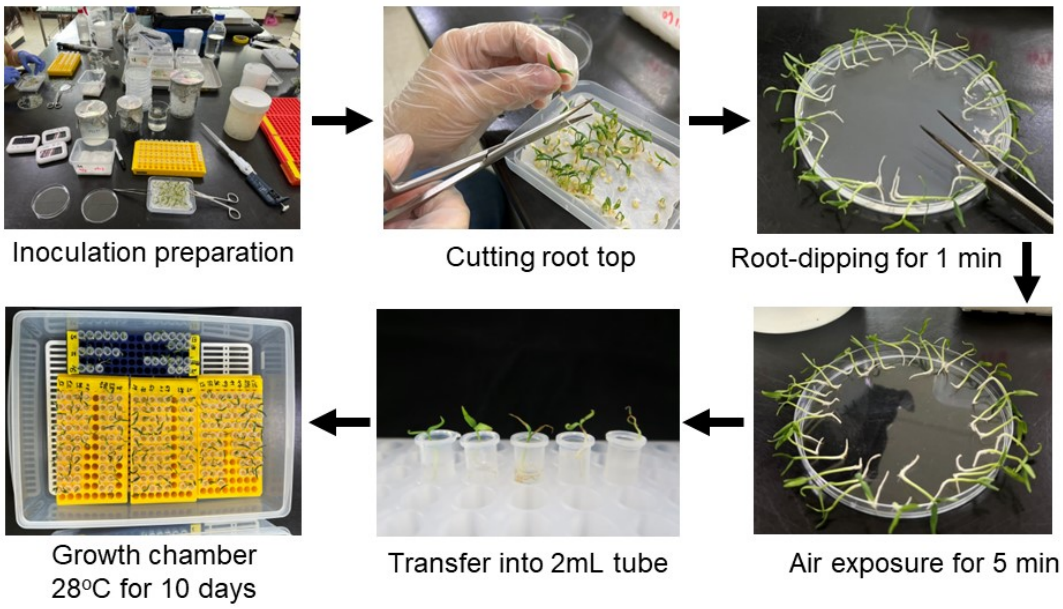

Figure 1. The processes of inoculation of cotyledon seedling screening method.

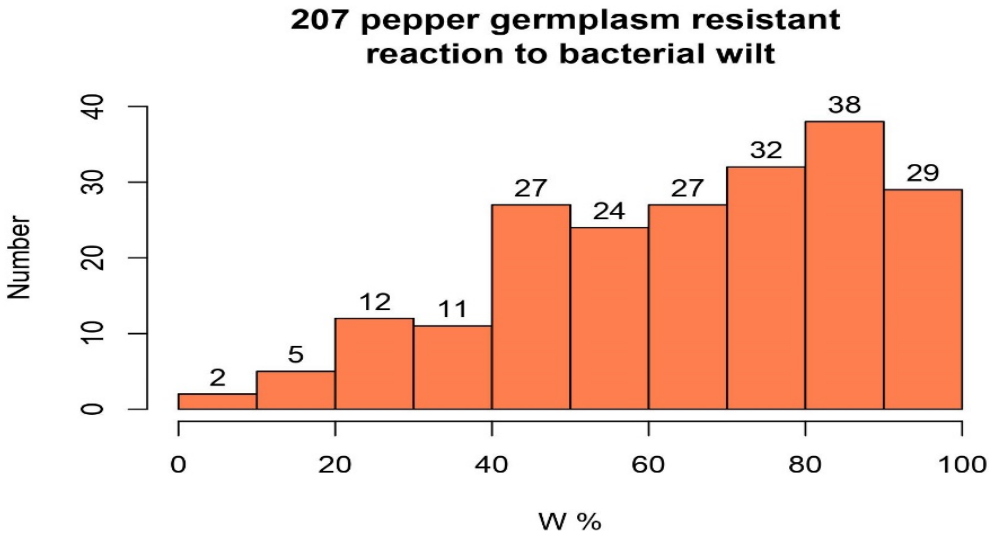

Figure 2. The histogram of pepper germplasm resistant reaction to bacterial wilt. The x-axis shows the accession wilting percentage counted by the W% formula. And the y-axis shows the number of pepper accession

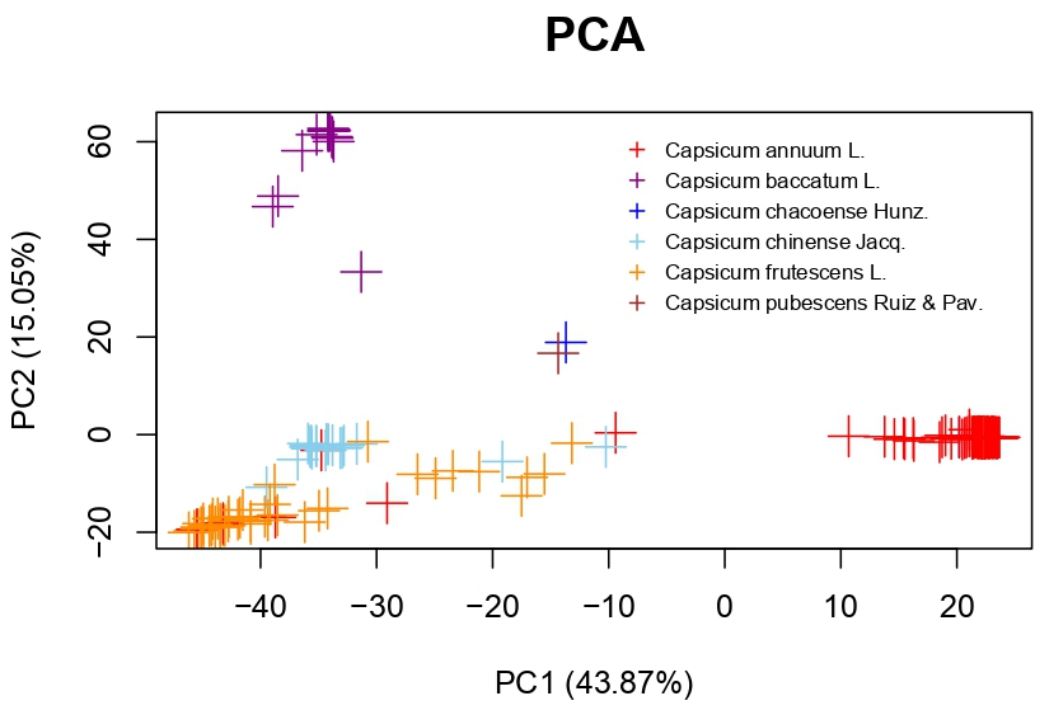

Figure 3. The x-y scatter of PCA structure. The indication of colors represent for different *Capsicum* species was shown in the chart. The x-axis represents PC1, which account for 43.87%. And the y-axis represents PC2, which account for 15.05%.

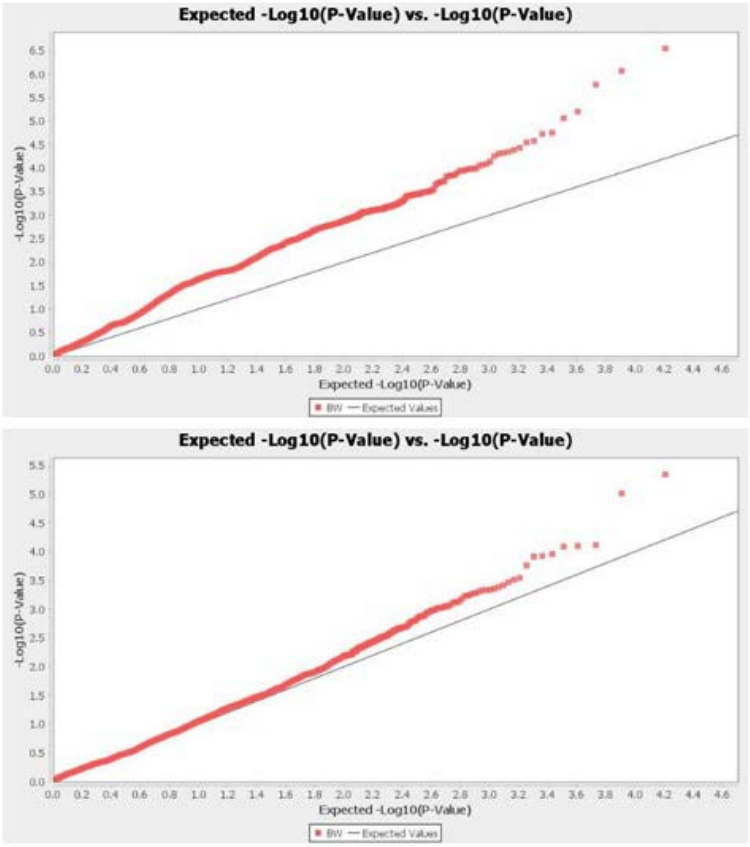

Figure 4. The quantile–quantile plots of s of different genome-wide association study panels. The quantile–quantile plots above is model GLM, and the one below is model MLM. The x-axis represents expected P-Value, and the y-axis represents the observed P-Value. The black line in the chart represents the P-Values, and the red line represents the expected values of the sample.

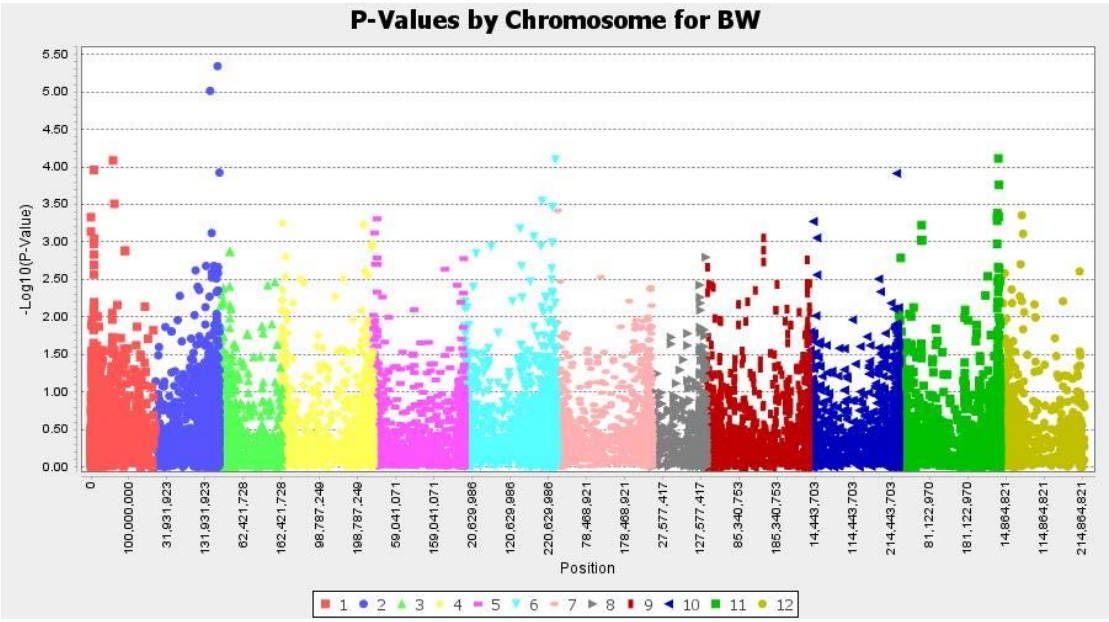

Figure 5. The Manhattan plot of the P-Values by chromosome for BW. The x-axis represents the position of genome, and the y-axis represents the observed P-Value.

Table 1. Pepper germplasm materials in the study

Capsicum spp.	Resistant	Susceptible	Total	Resistant (%)
C. annuum L.	19	110	129	14.73
C. baccatum L.	1	13	14	7.14
C. chinense Jacq.	4	16	20	20.00
C. frutescens L.	6	37	43	13.95
C. pubescens Ruiz & Pav.	0	14	1	0.00
Total	30	177	207	14.49