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Development of transgenic potato with resistance to bacterial wilt using PFLP and EFR genes

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Abstract

We report here the progress on testing the sweet pepper gene *pflp* in potato. *pflp* protein increases the production of antimicrobial compounds produced during the plant defense reaction. 94 transgenic events with the *pflp* gene and no backbone vector sequences have been produced. Quantitative expression of *pflp* from three biological repeats of 23 greenhouse-grown transgenic events led to the identification of low, medium, and high transgene expressers.. Bioassays using a local strain of *R. solanacearum* revealed that two high transgene expressers were exhibiting higher tolerance to *R. solanacearum* than the moderately resistant variety Cruza-148.

Background

Figure 1: Bacterial wilt disease on potato tubers and plants

- Bacterial wilt (BW) caused by soil-borne *Ralstonia solanacearum* limits production of potato, where yield losses can be 20-70%.
- BW is the second most important cause of yield losses by potato farmers in Kenya after late blight.
- Potato is vegetatively-propagated through tubers, *R.s* easily spreads through planting material resulting in the wide-spread distribution of the pathogen in potato producing countries.
- Chemical, biological and cultural control methods of BW have proven to have limited to no success in controlling the disease.
- Breeding for BW resistance has been unsuccessful, no stable and high-level source of resistance has been found. A few moderately resistant varieties exist like Cruza-148
- Genetic engineering using the plant ferredoxin-like protein (PFLP) from sweet pepper and the elongation factor receptor (EFR) from *Arabidopsis thaliana* have been shown to enhance resistance against bacterial diseases such as banana *Xanthomonas* wilt and tomato BW respectively (Tripathi et al., 2014; Lacombe *et al*., 2010). Here, we test these genes in potato.

Materials and methods

- The Kenyan popular potato variety Shangi was genetically transformed by *Agrobacterium tumefaciens* EHA105 bearing pBI-35s-pflp construct.
- PCR, Southern blot, and real time quantitative PCR (RTqPCR) were done using standard protocols.
- Transgenic events were assessed for tolerance to BW using a local strain of *R, solanacearum* by injuring and inoculating plant roots. Cruza-148 and non-transgenic Shangi were used as the tolerant and susceptible controls, respectively
- Latent infection testing of asymptomatic plants by plating stem extracts on semi-selective media, diagnostic PCR for *Rs* detection and by planting asymptomatic plants for three months.

Results

- 94 transgenic events were successfully transformed with *pflp* gene without the vector backbone sequence
- Southern blot analysis determined the T-DNA copies in the transgenic events. Seven transgenic events had single copies, five had two copies, and the remaining had more than two copies
- 18 transgenic events were selected as either low, medium or high *pflp* gene expressers after RTqCPR. Bioassays were conducted on these to determine tolerance to bacterial wilt in five separate assays.
- ANOVA analysis indicated a significant difference in the tolerance of transgenic events to bacterial wilt p = 0.00 (Figure 2). Tukey's HSD test revealed nine transgenic events with significantly lower average wilt scores

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                Number of obs =       245    R-squared     =  0.2708
                Root MSE      =   1.07849    Adj R-squared =  0.2093

------------------------------------------------------------------------------
      Source | Partial SS      df       MS         F     Prob>F
-------------+----------------------------------------------------------------
       Model |  97.205932      19   5.1161017      4.40   0.0000
       event |  97.205932      19   5.1161017      4.40   0.0000
    Residual |  261.70512     225   1.1631339
-------------+----------------------------------------------------------------
       Total |  358.91105     244   1.4709469
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Figure 2: ANOVA at the alpha value of 0.05 of transgenic events and their controls shows a statistically significant difference in wilting.

- Latent infection tests revealed asymptomatic transgenic plants had low bacterial load compared to the non-transgenic plants

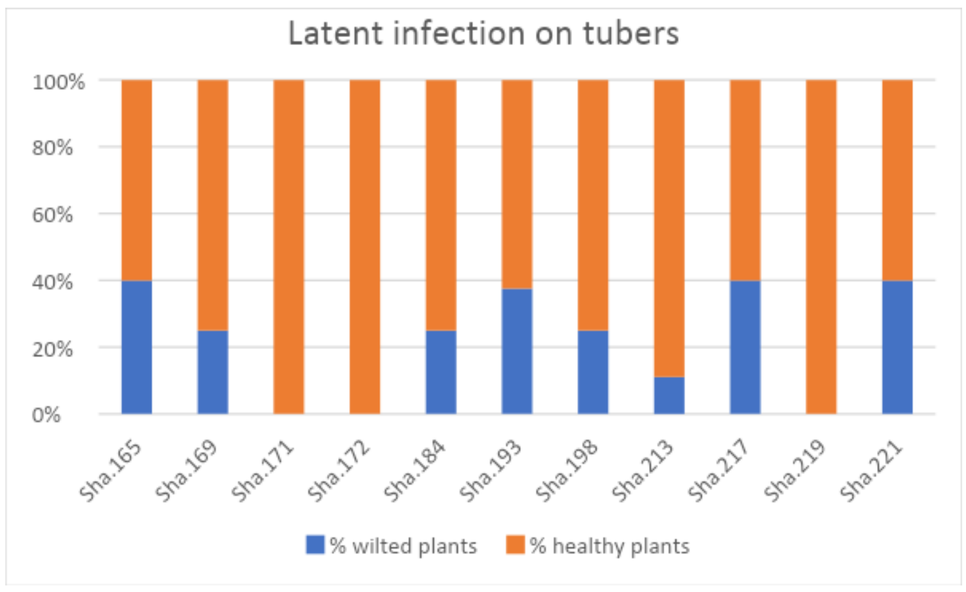

Figure 3: Latent infection in tubers planted from inoculated asymptomatic plants. The orange bars show the percentage of healthy plants after three months, while the blue proportions show the percentage of wilted plants after three months of observation.

Conclusions

Bioassays of transgenic events, and latent infection testing has shown that the *pflp* gene confers delay of BW disease symptoms in potato. Additionally, it has greatly reduced the bacterial load in the infected plants which is an important trait to reduce spread of the disease through tuber seeds. We are currently performing yield analysis of these transgenic events in the greenhouse. We expect that transgenic plants will have better yield under disease stress

Acknowledgement

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