CIP INTERNATIONAL POTATO CENTER
A CGIAR RESEARCH CENTER
CGIAR

14TH Symposium of the International Society of Tropical Root Crops Africa Branch (ISTRC-AB)

[1] Magembe E., [1] Makoko I., [1] Irukani Q., [2] Arinaitwe A., [2] Barekye A., [2]Baguma, G., [2] Mutebi, D., [2] Wasukira, A., [3] Zhou S., [3]Hui L., and Ghislain M.

[1] International Potato Center (CIP), Nairobi, Kenya
[2] National Agricultural Research Organization (NARO), Uganda
[3] Tennessee State University (TSU), USA

Late blight resistance biotech potato

Funding:
United States Agency for International Development (USAID)
2Blades foundation
United States Department of Agriculture - Foreign Agricultural Services (USDA-FAS)
CGIAR Research Program on Roots, Tubers and Bananas (RTB)

Abstract

Late blight, caused by *Phytophthora infestans*, remains the most devastating disease of potato (*Solanum tuberosum* L.) with about 15-30% annual yield loss in sub-Saharan Africa, affecting mainly smallholder farmers. We show here that the transfer of three resistance (R) genes from wild relatives [RB, Rpi-blb2 from Solanum bulbocastanum and Rpi-vnt1.1 from S. venturii] into potato varieties ‘Desiree’, ‘Victoria’, ‘Tigoni’ and ‘Shangi’ provided complete resistance in the glasshouse and field over several seasons. We observed that the stacking of the three R genes produced a high frequency of transgenic events with resistance to late blight. In the field, 16 resistant transgenic events with the 3R-gene stack from the potato varieties ‘Desiree’ and ‘Victoria’ grew normally without showing pathogen damage and without any fungicide spray, whereas their non-transgenic equivalent varieties were rapidly killed. Yields of two transgenic events from ‘Desiree’ and ‘Victoria’ grown without fungicide to reflect small-scale farm holders were estimated to be 29 t/ha and 45 t/ha, respectively. This represents a three to four-fold increase over the national average. T-DNA insertion characterization by next generation sequencing led us to identify 4 lead transgenic events with ideal recombination sites. Lead Event Vic.172 was assessed in 2 multi-location confined field trials for regulatory studies where we didn’t observe any significant differences with its non-transgenic Victoria. A field detection tool was also developed for future tracking of Vic.172 once released. Thus, these late blight resistant potato varieties, which are the farmers’ preferred, could be rapidly adopted and bring significant income to smallholder farmers in sub-Saharan Africa.

Introduction

Potato production in sub-Saharan Africa (SSA) has doubled since 1994. This increase is being threatened by the oomycete pathogen *Phytophthora infestans* (Pi) which is responsible for significant losses amounting globally to 2.750 million US dollars a year. Host plant resistance and fungicide sprays (up to 20 per season) are neither durable nor complete. Current strategies to control this devastating disease include stacking broad-spectrum resistance genes isolated from wild potato relatives through genetic engineering.

Materials and Methods

3*R* gene construct

The three *R* genes were cloned as genomic fragments (no changes from the original wild species sequence) into a plant transformation vector including the selectable marker gene conferring resistance to kanamycin (pCIP99).

Figure 1: pCIP99 ***3R*** gene stack construct bearing the three *R* genes (*Rpi-blb2*, *RB*, and *Rpi-vnt1.1*) and the selectable marker gene (*nptII*) used for transformation of potato.

Genetic transformation

*Agro*bacterium-mediated transformation of four susceptible potato varieties grown in SSA: ‘Desiree’ chosen essentially because of its high transformation efficiency and for testing efficacy with single and the stacked *R* genes whereas ‘Victoria/Asante’, ‘Shangi’ and ‘Tigoni’ chosen for their wide adoption as stable varieties in the east African region.

LB resistance assay

Detached leaf assays (DLA) and whole–plant assays (WPA) were done to the transgenic events However, WPA were labor intensive, time-consuming (First generation tuber), space constrained by one cubicle in biosafety greenhouse.

Field trails

Over a dozen confined field trials (CFTS) were done in partnership with NARO Uganda at Kachwekano, Agricultural Research and Development Institutes (KaZARDI) to test the level of LB resistance under natural *Pi* exposure. A subsequently multi-location CFTs (ML-CFTs) were done in 3 locations at KaZARDI, RwebitabaZARDI and BuginyanyaZARDI for regularory studies.

Results

Genetic transformation, Bioassays and Molecular Characterization

331 ‘Desiree’, 77 ‘Victoria/Asante’, 102 ‘Tigoni’ and 86 Shangi were transformed with the 3*R* gene construct. Detached leaf and whole–plant assays showed high levels of late blight resistance on 23, 24, 29 and 22 events respectively. Molecular characterization was done to identify those with single T-DNA copy, no backbone vector fragments, presence of the 3*R* genes and *R* gene expression. T-DNA insertion characterization by targeted sequencing identified **Vic.172** as the best lead transgenic event.

Figure 2: LB resistance of events with 3*R* genes by WPA (Left) and field trial for event Vic.172 (Right) under natural exposure 77 days after planting

Field trials

In the CFTs and ML-CFTs, all non-transgenic plants of Desiree and Victoria died and there were no diseased leaves of transgenic plants. Regulatory ML-CFTs show that the transgenic event Vic.172 compared to the variety Victoria it derived from, presented few differences which were not statistically significant across locations and seasons.

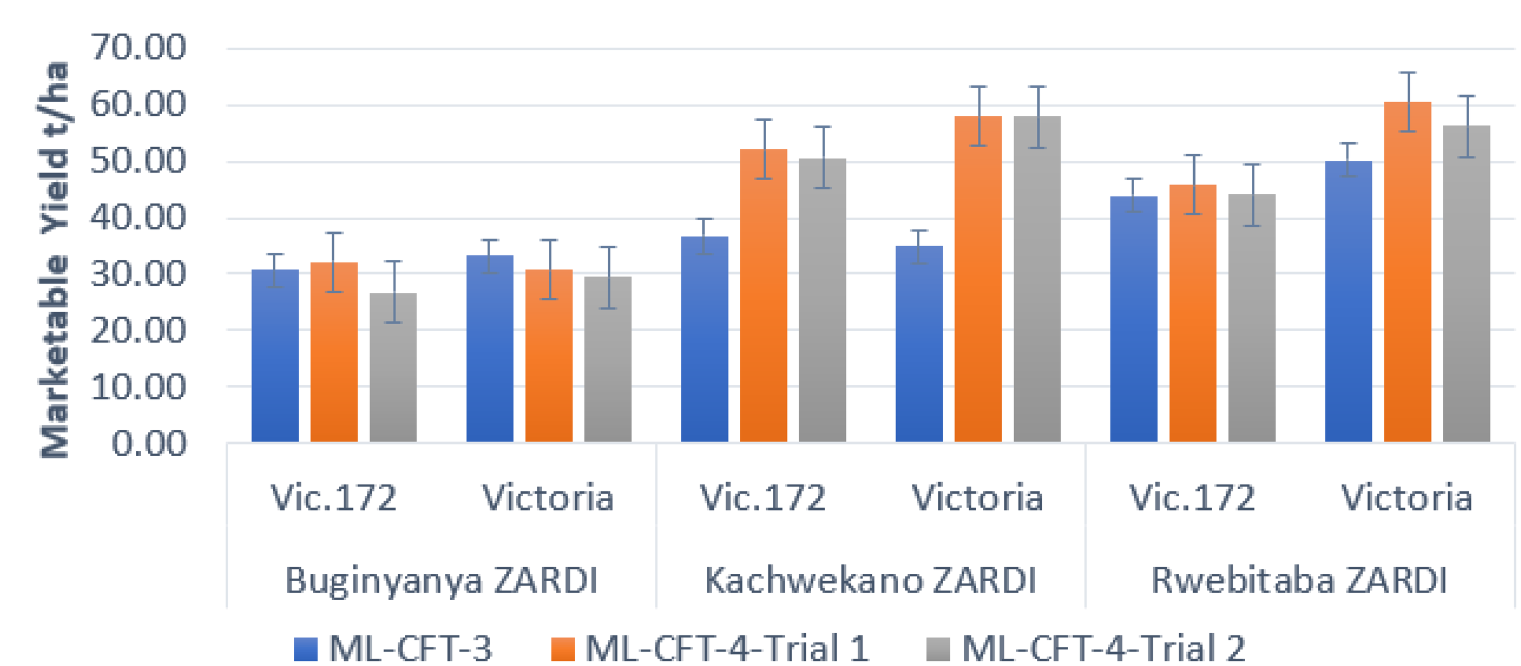

Figure 3: Marketable yield of transgenic event Vic.172 and the variety Victoria at each of the three locations averaged over the three trials. Error bar represents the standard error.

Event specific Detection in the Field

A rapid field detection method for event Vic.172 which takes about 2 and half hours from sampling to result was developed and validated. The method involves field DNA isolation using the MagicTip™, PCR set up using labelled primers with Biotin and DIG and thermocycling using a miniPCR machine. The PCR amplicons are loaded on the sample port of the lateral flow and results on are visible by eye.

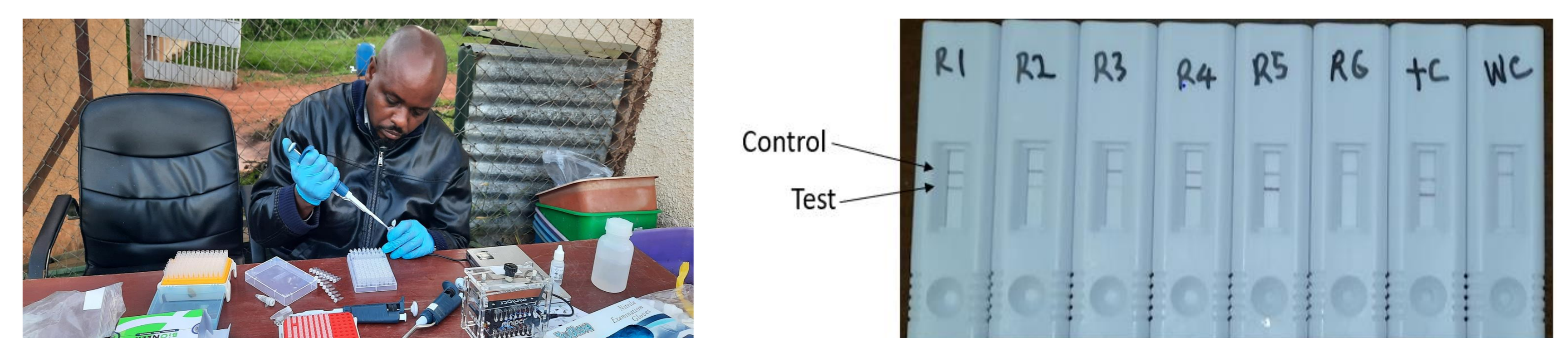

Figure 4: Field DNA extraction and PCR next to the CFT in KaZARDI (left) and lateral flow detection of PCR amplicons. The control line indicates that the test strips have functioned properly while the test line indicates a successful amplification of the amplicon which is specific for event Vic.172