

Development of Potato Varieties Resistant to Late Blight

Through Biotechnology

Strategy

Potato production in sub-Saharan Africa (SSA) has more than doubled since 1994, with 70% of that growth concentrated in Eastern Africa. The most devastating disease is late blight (Figure 1), caused by the water mold *Phytophthora infestans* (Pi) and is still responsible for significant losses which may reach 30% to 75% depending on the varieties in SSA. Global economic losses are estimated at 2,750 million US dollars a year. CIP estimates that a better control of late blight could save US\$ 530 per hectare every year (FAO, 2006).

One of our current strategies to control this devastating disease include stacking broad-spectrum resistance genes isolated from wild potato relatives through transgenesis. The *RB*, *Rpi-blb2* (isolated from *Solanum bulbocastanum*) and the *Rpi-vnt1.1* (isolated from *S. venturii*) *R* genes (Song et al. 2003; Vossen et al., 2005) can be transferred by genetic engineering into susceptible farmer-preferred varieties. These *R* genes were cloned into a triple *R* gene construct (pCIP99) to transform two susceptible potato varieties grown in SSA. 'Desiree' was chosen essentially because of its high transformation efficiency and for testing efficacy with single and the stacked *R* genes whereas 'Victoria/Asante' for its wide adoption as stable variety in Kenya and Uganda. The later will only be transformed with the 3*R* gene stack.



Figure 1: Damage caused by *P. infestans* in the field (left), on potato leaf (center) and tuber (right).

Results

Out of 62 transgenic events produced with *RB* from 'Desiree', we identified 7 that display high level of resistance to Pi using a whole-plant infection (Tab. 1, Fig. 2). Resistance was confirmed and related to number of copies of the *RB* gene (2 to 4). These transgenic events have been transferred to BecA in Kenya. Other transgenic events from 'Desiree' (62, 115, 327 for *Rpi-vnt1.1*, *Rpi-blb2* and the 3*R* genes, respectively).

14 putative transgenic events from Asante / Victoria containing the 3-*R* (Fig. 3) gene stack were analyzed by Southern blotting using transgene specific probes. Only one event presented the expected banding pattern. This result illustrates the difficulty in transforming this variety which appears to be particularly susceptible to kanamycin. Lowering km selection has increased the selection of "escapes" (false positive to the km selection). This event is being screened for gene expression and LB resistance.

New candidate varieties for SSA have been identified, with priority given to three: Shangi (rapidly increasing adoption in Kenya), Tigoni (well established variety in Kenya), and Cruza 148 (grown in the Lake Kivu region, especially in Burundi and Rwanda, and which has been transformed previously at CIP ABL in Peru). This is important experiment because of the difficulty in transforming Asante/Victoria.

The proposed research will involve the identification of high expressers, the assessment of their resistance level, the characterization of the pathogen diversity and virulence, and an innovative seed system that will support a deployment of multi lines of the same variety in time and space. This research is anticipated to be conducted with NARS partners, NARO-Uganda and KARI-Kenya.

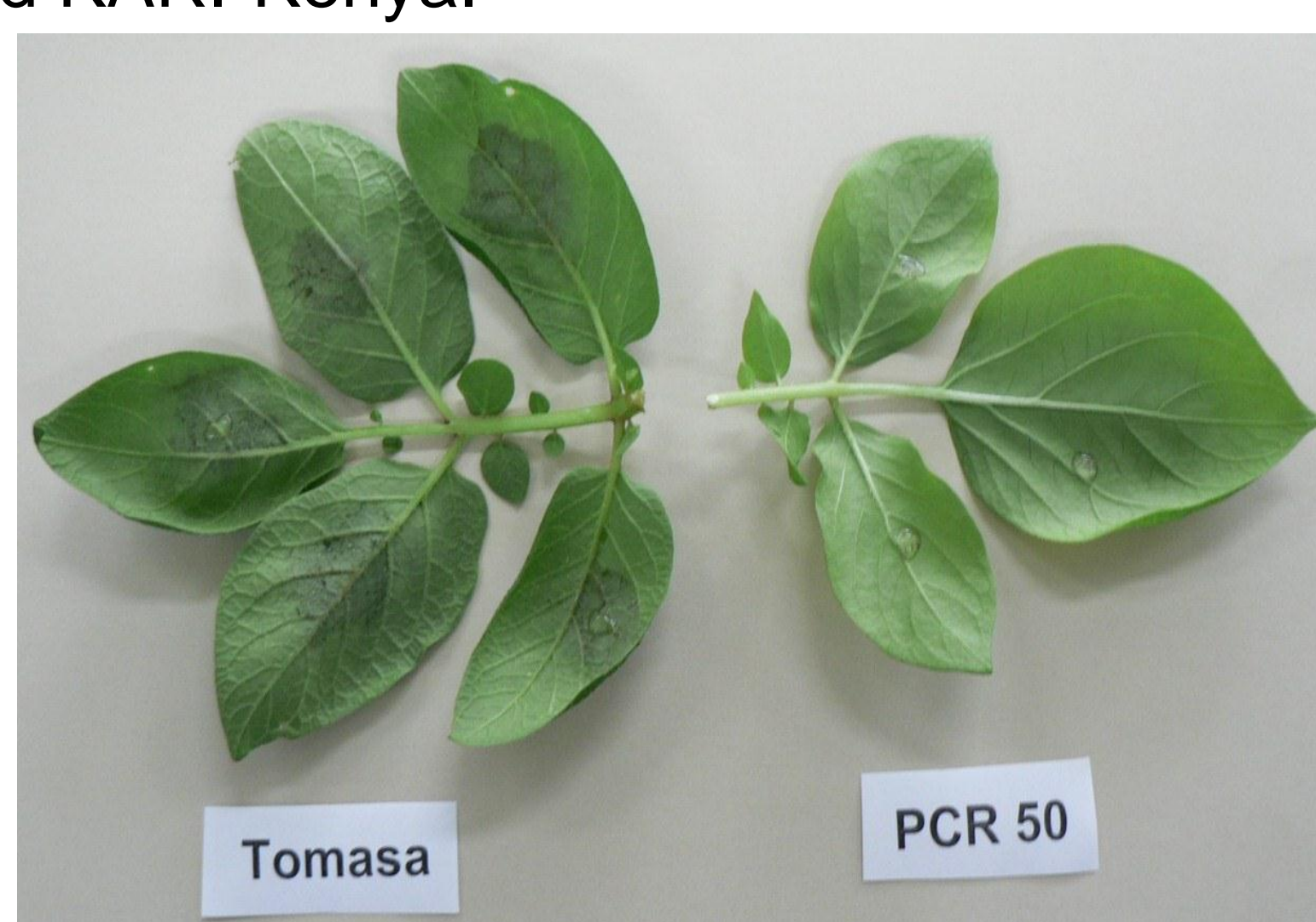


Figure 2: Detached leaf and whole plant assays using the Pi isolate POX067 on two transgenic events showing hypersensitive reaction.

Table 1: Average percentage of plants covered by lesions of late blight at 6 days post inoculation with 5 different *P. infestans* isolates. HR=hypersensitive response

Event/Genotype	PPI57	PHU8	PPI64	POX67	PSR19
Pimpernel	95	100	100	100	100
Tomas Condemayta	100	100	100	100	100
Desiree	85	100	95	100	100
Desiree [RB]28	75	85	100	100	100
Desiree [RB]43	90	90	100	100	100
Desiree [RB]53	80	100	100	100	100
Desiree [RB]9	13	25	40	100	100
Desiree [RB]66	HR	HR	40	100	100
Desiree [RB]67	HR	HR	10	100	100
Desiree [RB]27	HR	10	HR	100	100
Desiree [RB]50	HR	HR	HR	HR	100
Desiree [RB]62	HR	HR	HR	HR	100
Monserrate	15	25	55	100	100
Atzimba	85	30	10	100	1
CIP 387164.4 (LBr 40)	8	5	33	0	10

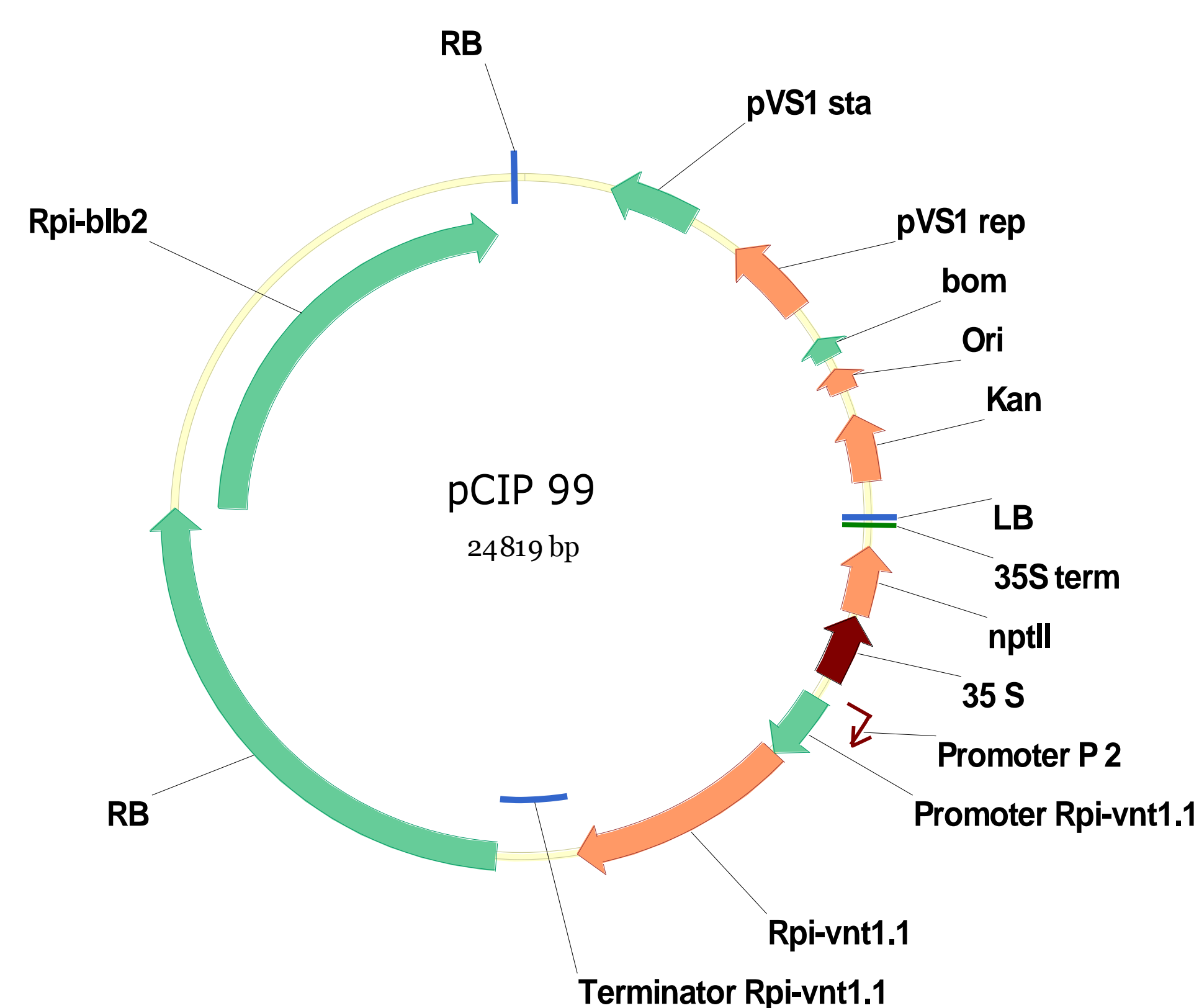


Figure 3: Triple R gene construct: plasmid vector containing the three genes (*RB*, *Rpi-blb2*, *Rpi-vnt1.1*) to be used for transformation.

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