Does the Foliar Blight Resistance R Gene *RB* Function for Tuber Blight Control?

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This poster can be found at http://ppg.coafes.umn.edu/

Importance

- Potato is the fourth most important food crop in the world and is the single most important dicot food crop (http://apps.fao.org)
- Late blight (caused by *Phytophthora infestans*) is responsible for multibillion dollar losses worldwide annually
- *P. infestans* attacks both foliage (Fig. 1b, c) & tuber (Fig. 5b, d)
- *RB*, recently cloned from the wild potato species *Solanum bulbocastanum* (genotype PT29; Fig. 1a), imparts broad-spectrum foliar blight resistance to all known races of *P. infestans*, the causal organism of late blight (PNAS, 100: 9128)
- NBS-LRR genes, like *RB*, are presumed to be constitutively expressed in all plant tissues so that pathogens are readily detected
- A functional *RB* in tubers might provide hope for controlling losses to late blight during storage

Main goal

Does the foliar blight resistance R gene *RB* function for tuber blight control?

Use:
- RT-PCR (*RB* expression)
- Inoculation assays (function)

to compare:
- Wild species (*S. bulbocastanum*)
- Transgenic cultivated potato (*S. tuberosum* cultivar Katahdin +*RB* (Kat+*RB*))

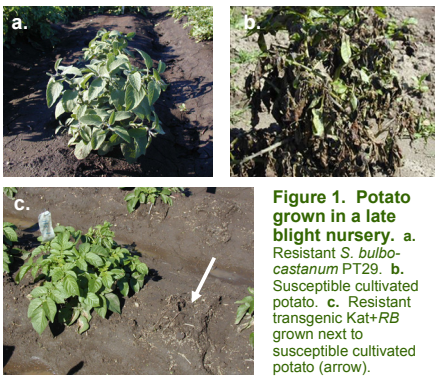

Figure 1. Potato grown in a late blight nursery. **a.** Resistant *S. bulbocastanum* PT29. **b.** Susceptible cultivated potato. **c.** Resistant transgenic Kat+*RB* grown next to susceptible cultivated potato (arrow).

Materials & methods

Plant material

- *S. bulbocastanum* leaves & tubers (PT29)
- *S. tuberosum* cultivar Katahdin leaves & tubers (Kat and Kat+*RB* SP922, SP951, & SP966)

RT-PCR

- Primer design challenges
  - Alleles at *RB* locus (functional *RB* vs non-functional *rb*)
  - Homologues (Within gene cluster and different genome locations)
- RNA extractions
  - Leaves- 3rd leaf from top
  - Tubers- 1 cm^3 near outer surface 2 cm from center lengthwise towards stolon end
  - Extractions with SV Total RNA Isolation kit by Promega (Madison, WI)
- Reactions run with SuperScript™ One-Step RT-PCR (Invitrogen, Carlsbad, CA)

Inoculation assay

- Tubers assayed at 1 week and 6 weeks post-harvest (wph)
- Whole tuber (APS Biol. & Cult. Tests, 17: PT03)
  - Wound tuber & add 10 µL *P. infestans* inoculum (7000 sporangia/mL, cold treated at 4°C for 1 hr, RT for 30 min)
  - Store at RT, >90% RH, dark for 34 hrs followed by 15°C, >90%RH, dark for 11 days
  - Measure depth and radius of infection

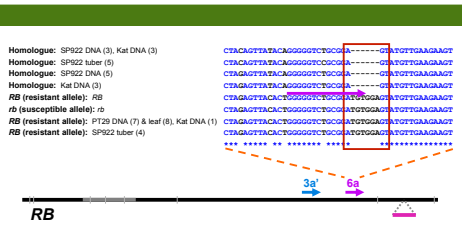

Figure 2. 5' primer (6a) based on 6 bp indel present in *RB* differentiates *RB* from homologues. Specificity for *RB* locus is achieved by using the indel that is absent in the non-functional *RB* homologues.

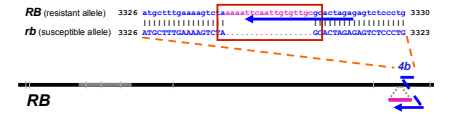

Figure 3. 3' primer (4b) based on 18 bp indel present in *RB* differentiates for resistance allele. Specificity for *RB* (resistance allele) is achieved by using the indel that is absent in the susceptible *rb* allele.

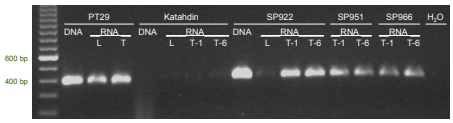

Figure 4. RT-PCR with primers 6a & 4b. After 100 bp ladder, the lanes contain the indicated templates. L = leaf, T = tuber, 1 = 1 week post-harvest (wph), 6 = 6 wph. Expected size = 408 bp.

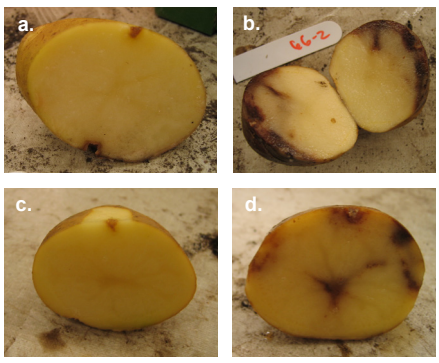

Figure 5. Whole tuber assays. **a, c: Untransformed Kat water-inoculated.** These controls are representative of non-diseased tubers (uninoculated and inoculated) from 1 & 6 wph assays, respectively. **b, d: Kat+*RB* *P. infestans*-inoculated.** These are representative of diseased tubers (inoculated) from 1 & 6 wph assays, respectively.

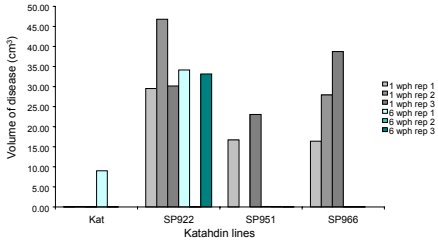

Figure 6. Whole tuber assays. Untransformed Kat & Kat+*RB* lines tested at 1 & 6 wph. Tubers were stored at ~4°C after harvest.

Results

RT-PCR (*RB* expression)

- 5' primer design
  - 5' primer 3a' was suggested by Primer3 www.cgi v 0.2 (http://frodo.wi.mit.edu/)
  - We identified a 6 bp indel present in PT29 and Kat+*RB* (Fig. 2)
  - 5' primer specific to *RB* (6a) designed based on 6 bp indel (Fig. 2)
  - 6a differentiates *RB* from its homologues
- 3' primer design
  - 18 bp indel found in functional *RB* allele (Fig. 3; PNAS, 100: 9128)
  - 3' primer specific to *RB* (4b) designed based on most of 18 bp indel designed (Fig. 3)
  - 4b differentiates the resistance allele *RB*
- Primer set 6a-4b showed amplification in only those plants samples that contain a functional *RB* (i.e., PT29 and Kat+*RB*; Fig. 4)
- *RB* was expressed in
  - PT29 leaf & tuber (Fig. 4)
  - Kat+*RB* SP922 leaf & tuber (Fig. 4)
  - Kat+*RB* tubers at both 1 & 6 wph (Fig. 4)

Inoculation assay (*RB* function)

- Whole tuber
  - There is a substantial amount of variability within replicates during each trial, though the overall amount of disease is substantially different between lines at each time point (Fig. 6)
  - Kat+*RB* lines appear to gain resistance between 1 & 6 wph, SP951 & SP966 more so than SP922 (Fig. 6)
  - Kat is more resistant at 1 wph than
    - Kat+*RB* lines at 1 wph
    - Kat at 6 wph

    but this could be attributed to a different physiological age of Kat than the Kat+*RB* plants (Fig. 6).
  - The Kat+*RB* lines vary in the mean amount of resistance, with SP922 being the least resistant (Fig. 6)
  - Further replication, including other cultivars carrying *RB*, will substantiate these data

Conclusions

- Primer design is critical for detecting *RB*
  - Primer set 6a-4b results in allele- and homologue-specific amplification of *RB*
- *RB* is a good model for NBS-LRR gene studies. Consistent with NBS-LRR data, *RB* is constitutively expressed
  - in different tissues which are both natural host targets of *P. infestans*
  - in tubers at varying time points
- Further analysis is required to fully explore *RB* function in tuber
- *RB* may not be responsible for tuber blight resistance
  - Resistance differences could result from changes in activation of down-stream signals

Next steps

- Test additional plant tissues in *S. bulbocastanum*, Kat+*RB* lines, and other cultivated potato +*RB* lines for *RB* expression
- Complete additional tuber and foliar assays for *RB* function
- Correlation of *RB* gene expression and foliar and tuber blight resistance

Acknowledgment

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