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Potato Pathology and Genomics

TRANSCRIPTIONAL STUDIES OF THE LATE BLIGHT RESISTANCE GENE *RB* IN FOLIAGE OF TRANSGENIC POTATO

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Introduction

Late blight disease of potato (*Solanum tuberosum* L.), caused by *Phytophthora infestans*, is one of the most damaging diseases in any crop. The single late blight resistance gene *RB* was cloned from the wild potato species *S. bulbocastanum* genotype PT29 (Fig. 1; Song, et al., 2003) and transformed into four different potato varieties (Katahdin, Superior, Russet Burbank and Dark Red Norland) (S. Austin-Phillips, unpublished). We evaluated 57 *RB* transgenic (+*RB*) lines in replicated field tests in Minnesota for foliar blight resistance to *P. infestans* US8. Based on resistance phenotypes, these transgenic lines were classified as susceptible (S), moderately susceptible (MS), moderately resistant (MR), or resistant (R) (Fig. 1). Previously we developed an *RB* transgene-specific RT-PCR assay (Millett and Bradeen, 2007) and have adapted it for real time quantification.



Fig. 1. University of Minnesota late blight nursery. (A) resistant *Solanum bulbocastanum* genotype PT29. (B) resistant *RB* transgenic lines (yellow line) and untransformed susceptible lines (red line).

Objectives:

- Expt 1: Explore the relationship between transgene *RB* transcription and resistance
- Expt 2: Evaluate the effects of transgene *RB* dosage on resistance and transcription
- Expt 3: Evaluate the effects of plant age on transgene *RB* transcription and resistance
- Expt 4: Evaluate the effects of varying temperature and plant age on transgene transcription

Materials & Methods

Plant materials

Expt 1 and 2: *RB* transcription, disease resistance, and gene dosage

Leaves from plants grown under standard greenhouse conditions were collected and stored at -80°C.

- 'Katahdin': untransformed and eight *RB* transgenic lines (KAT+*RB*);
- 'Russet Burbank': untransformed and nine *RB* transgenic lines (RusB+*RB*);
- 'Dark Red Norland': untransformed and 23 *RB* transgenic lines (DRN+*RB*);
- 'Superior': untransformed and five *RB* transgenic lines (SUP+*RB*).

Expt 3: *RB* transcription, disease resistance, and plant age

Plants were grown for four, eight, and twelve weeks, and each line was represented by four plants at each age. For molecular assays, plant tissue was excised from plants immediately prior to inoculation, frozen in liquid nitrogen, and stored at -80°C.

Expt 4: *RB* transcription, temperature, and plant age

Four clones of three different *RB* transgenic lines and untransformed varieties 'Dark Red Norland', 'Katahdin', and 'Russet Burbank' were grown in growth chambers at 10, 20 and 30°C.

RNA and DNA extraction:

RNA: SV Total RNA Isolation System (Promega)
DNA: DNeasy Plant Mini kit (Qiagen)

RT-PCR:

Super Script One Step RT-PCR (Invitrogen);

RB specific primers: 2Mama31 and 53 (Millett and Bradeen, 2007);
RNA polymerase II (*RP2*) primers (positive control; Millett and Bradeen, 2007);
PCR conditions detailed in Millett and Bradeen (2007).

Real time qRT-PCR

Super Script III Platinum SYBR Green One Step (Invitrogen);
e1 α primer (endogenous control) (Nicot et al., 2005);
RB specific primers: 2Mama31 and 53 (Millett and Bradeen, 2007).

Real time qPCR

ITaq SYBR Green Supermix (BIO-RAD);
e1 α primer (endogenous control) (Nicot et al., 2005);
RB specific primers: 2Mama31 and 53 (Millett and Bradeen, 2007).

Data Analysis

Data generated by real time PCR were analyzed using Sequence Detection Software v. 1.4.0.25 (Applied Biosystems). The threshold value was adjusted against e1 α reactions for consistent Ct values across multiple experimental plates. Ct values for the *RB* transgene and e1 α were processed with the Visual Basic software Q-Gen (Muller et al. 2002) in Microsoft Excel.

References:

- Song, J., J.M. Bradeen, S.K. Naess, J.A. Raasch, S.M. Weidgas, G.T. Haberisch, J. Liu, H. Kuang, S. Austin-Phillips, C.R. Buell, J.P. Helgeson, J. Jiang. (2003) Gene *RB* cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. Proc. Natl. Acad. Sci. USA 100:9728-9733.
- Millett B. P., J. M. Bradeen (2007) Development of allele-specific PCR and RT-PCR assays for clustered resistance genes using a potato late blight resistance transgene as a model. Theor. Appl. Genet. 114:501-513.
- Millett, B.P., D.S. Mollov, and J.M. Bradeen. Impact of physiological age and plant organ on transcription and function of foliar blight resistance transgene *RB*. Solanaceae Genome Workshop Meeting Madison, WI July 2006.
- Nicot, N., Haubert, J.F., Hoffmann, L., Lewis, O. (2005) Histochemical gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. J. Exp. Bot. 56:2907-2914.
- Muller, P.V., Jansová, H., Mianisz, A.R., Dobbi, Z. (2002) Processing of gene expression data generated by quantitative real-time RT-PCR. Bioinformatics 18:1372-1379.

Results

Expt 1: Relationship between *RB* transcription and resistance

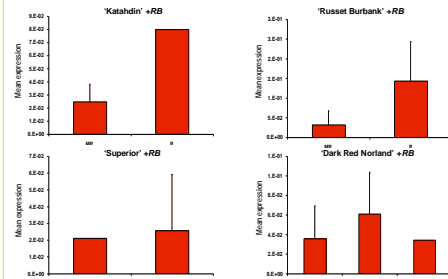


Fig. 2. A direct correlation between transgene copy number and disease resistance for three transformed varieties. Mean transgene transcription levels vs. disease resistance classes. Error bars indicate standard deviation. For 'Dark Red Norland' transgenic lines, higher transgene transcription does not always result in enhanced resistance.

Expt 2: Effects of *RB* dosage on resistance

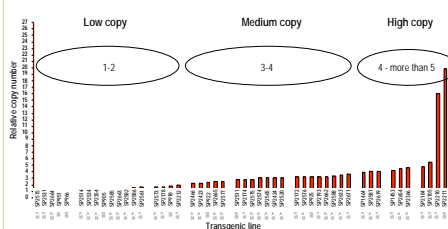


Fig. 3. Transgenic lines vary in relative transgene copy number. Results from qPCR experiments revealed transgenic lines contained between 1 and approximately 25 transgene copies. For discussion purposes, we grouped transgenic lines into three groups: low copy (relative transgene copy number of 1 or 2), medium copy (relative transgene copy number of 3 or 4) and high copy (relative copy number >4).

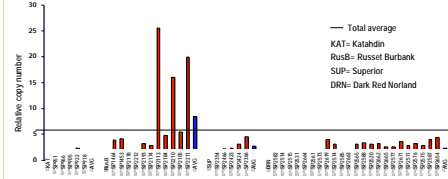


Fig. 4. Individual varieties also vary in *RB* copy number. 'Russet Burbank' +*RB* lines have the highest average transgene dosage (AVG=8.52). In contrast 'Katahdin' +*RB* lines showed the lowest average transgene dosage (AVG=1.90).

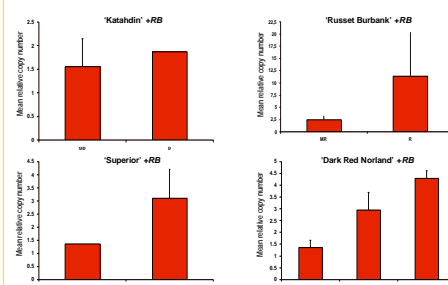
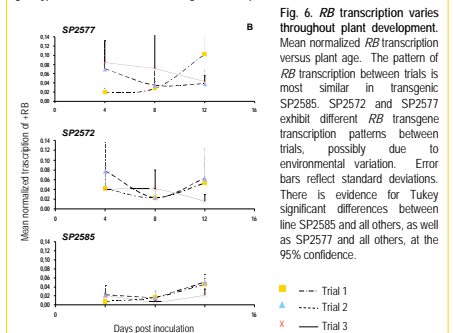


Fig. 5. A direct correlation between transgene dosage and late blight resistance. Mean transgene dosage vs. disease resistance classes. Error bars indicate standard deviation.

Expt 3: Effects of plant age on *RB* transcription and resistance

Effects of plant age on *RB* transgene transcription: *RB* transgene transcript levels were assessed in three independent inoculated plants each of *RB* transgenic lines SP2572, SP2577, and SP2585 at 4, 8, and 12 weeks post planting. RNA from three plants each of PT29 and untransformed 'Dark Red Norland' (DRN) from each age was pooled by genotype and also tested for *RB* transgene transcript levels.

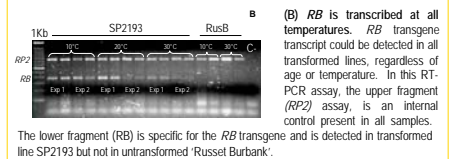
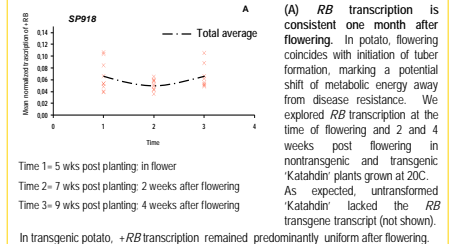


Expt 4: Effects of temperature and plant age on *RB* transcription



Fig. 7. 'Dark Red Norland' line SP2577 64 days old grown at 30, 20, and 10°C (left to right). Temperature affects plant development.

Fig. 8. *RB* transgene transcription in relation to age and temperature.



Conclusions

- Higher transgene copy numbers correlate with enhanced resistance
- In general, observed resistance phenotypes directly correlate with transcript levels; higher gene transcription correlates with enhanced resistance
- The trend of decreasing resistance with plant age previously reported by our laboratory (Millett et al. 2006, SOJ) cannot be a direct effect of transgene transcription
- Transcription of transgene *RB* was nearly constant for one month post flowering and was not affected by temperature