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Disease resistance gene transcription in transgenic potato is unaltered by temperature extremes and plant physiological age

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Abstract To date, several dozen plant disease resistance genes have been cloned from a variety of species. Despite successful efforts to isolate functional disease resistance genes and understand their roles in defence responses, surprisingly little is known about their transcriptional regulation. Global climate changes are expected to impact crop plant production, plant physiology and plant-microbe interactions. Understanding the impact of varying environmental and physiological factors on disease resistance gene transcript levels may be key to successful deployment. In this study we analyzed the expression patterns of the *Phytophthora infestans* resistance gene *RB* in transgenic potato plants across a broad range of temperatures and key physiological stages. Our results demonstrate that while temperature extremes have an obvious effect on plant morphology and development, *RB* is transcribed at all ages and temperatures in all genetic backgrounds. Quantitative analyses demonstrate that the *RB* transcript accumulates comparably throughout plant development, including at the tuber initiation stage. Implications for successful deployment of *RB* are discussed.

Keywords Plant age · *RB* · R gene · Real time PCR · Transcript level

Abbreviations

NBS-LRR	Nucleotide binding site-leucine rich repeat
R gene	Resistance gene
RT-PCR	Reverse transcription-polymerase chain reaction

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Introduction

To date, several dozen plant disease resistance (R) genes have been cloned from a wide variety of species (Martin et al. 2003), paving the way for transgenic deployment. The genus *Solanum* includes important crops such as potato (*S. tuberosum* L.), tomato (*S. lycopersicum* L.), and eggplant (*S. melongena* L.). It also includes approximately

1,500 wild species, many of which are rich sources of R genes for crop improvement. Accordingly, in the genus *Solanum* alone, more than a dozen R genes have already been cloned (reviewed in Bradeen et al. 2008; Martin et al. 2003). This number is likely to increase rapidly with the recent release of draft genome sequences of potato (http://www.potatogenome.net/) and tomato (http://solgenomics.net/). The vast majority of cloned R genes, approximately 75%, encode R proteins containing a nucleotide-binding site (NBS) and a leucine rich repeat (LRR) region. Research in various plant species has taught us that the NBS-LRR proteins act as cellular sentinels and switches. In particular, the LRR is involved in direct or indirect protein interactions that detect the presence of a pathogen, while the NBS is involved primarily in signal transduction resulting in activation of defence responses (Innes 2004; Martin et al. 2003). Thus, within the NBS-LRR super-family of R genes, defence against pathogens seems to entail variations on a common theme.

Consistent with the role of cellular sentinel, R proteins are thought to be present in the plant cell prior to pathogen attack (Schornack et al. 2005). Accordingly, R genes are presumed to be constitutively transcribed. In potato, using RT-PCR, Huang et al. (2005) observed transcription of the *R3a* gene, an R gene imparting resistance against the late blight pathogen (*Phytophthora infestans* de Bary), even in uninfected plants. Similarly, constitutive expression has been observed for the tomato *I-2* fungal R gene (Mes et al. 2000), the rice *Xa21* bacterial blight R gene (Century et al. 1999) and the tomato *Mi-1.2* root-knot nematode R gene (Goggin et al. 2004). In response to pathogen attack, some R genes have been shown to be up-regulated (Scheideler et al. 2002; Silvar et al. 2007) while others remain transcribed at steady state levels (Century et al. 1999; Schornack et al. 2005).

Of particular note for the genetic improvement of potato is a suite of three R genes [*RB* (syn. *Rpi-blb1*), *Rpi-blb2,* and *Rpi-blb3*] from *Solanum bulbocastanum* Dunal., a Mexican diploid relative of the potato. These genes confer resistance to foliar late blight disease and are of broad effect, lacking pathogen race specificity associated with other late blight resistance genes (Park et al. 2005; Song et al. 2003; van der Vossen et al. 2003, 2005). All three genes have been cloned (Lokossou et al. 2009; Song et al. 2003; van der Vossen et al. 2003, 2005). Each encodes an NBS-LRR R protein and each holds significant promise for the transgenic control of late blight disease. Previously, we developed an RT-PCR assay specific for the *RB* transgene (Millett and Bradeen 2007). Capitalizing upon single nucleotide polymorphisms introduced during the cloning process and unique to the transgene, our assay is so precise as to differentiate between the *RB* transgene and the native, endogenous *S. bulbocastanum* gene from which it was cloned. The availability of this assay has enabled research aimed at understanding the transcriptional behavior of the *RB* transgene, an important step towards deployment (Bradeen et al. 2009, Kramer et al. 2009; Millett et al. 2009). Importantly, in our previous work, *RB* transcript levels were not surveyed at flowering, a physiological marker for the initiation of tuberization and the development of a major energy sink. To our knowledge, nothing is known about the transcription of *RB* under varying environmental conditions.

In this study, we examine the impact of growing temperature and plant physiological age, including flowering, on transgene *RB* transcription and transcript levels in three genetic backgrounds of transgenic potato. Our results demonstrate that while temperature extremes have obvious effect on plant morphology and development, *RB* is transcribed at all ages at all temperatures in all genetic backgrounds. Quantitative analysis of *RB* transcript levels in one genetic background demonstrates that the *RB* transcript accumulates at comparable levels throughout plant development, including at and immediately following flowering. Implications for deployment of *RB* are discussed.

Materials and methods

Plant materials

The transformation of potato cultivars Dark Red Norland, Katahdin, and Russet Burbank with the *RB* transgene has been previously described (Bradeen et al. 2009). Transgenic lines SP918 (‘Katahdin’), SP2577 (‘Dark Red Norland’), SP2193 (‘Russet Burbank’), and untransformed ‘Katahdin’, ‘Dark Red Norland’, and ‘Russet Burbank’ were examined in this study. All plants were grown from

greenhouse-grown mini-tubers originating from disease-free in vitro plantlets as described previously (Bradeen et al. 2009).

Experimental design

Mini-tubers of each line were planted in 10 cm square plastic pots in soilless mix and grown to maturity in environmental growth chambers at 10°C, 20°C, or 30°C constant temperature and 16 h artificial light (6×400 Watts, Metal Halide bulbs) per day. Planting dates were staggered by 2 weeks over a 1 month period to allow simultaneous sampling of plants on the first day of flowering and 2 and 4 weeks after the initiation of flowering (i.e., three distinct physiological ages). This ensured that plants of each physiological age were grown under identical environmental conditions. An experimental unit consisted of one plant per line and each line was replicated four times per age per experiment. Pots were arranged in the growth chambers using a complete randomized design. Each experiment was repeated three times. Phenotypic data were collected from all three experiments, while molecular data were generated for two of three experiments. Morphological data collected included days to flowering, plant height, and tuber set.

Transcription assays

Both qualitative (agarose gel-based) and quantitative (real time) RT-PCRs were conducted to evaluate transgene *RB* transcription. For template RNA preparations, the youngest, fully expanded leaf was collected, immediately frozen in liquid nitrogen, and stored at −80°C. RNA was extracted using the SV Total RNA Isolation kit (Promega, Madison, WI) according to manufacturer's recommendations. RNA was quantified using a NanoDrop ND-1000 (NanoDrop Technologies, LLC, Wilmington, DE). Qualitative RT-PCRs were performed as described by Millett and Bradeen (2007). Quantitative RT-PCRs were performed as described by Bradeen et al. (2009), and standardized against *Elongation Factor 1-*α, an appropriate standard for transcriptional study of the potato-*P. infestans* pathosystem (Nicot et al. 2005). Data were compiled into a Microsoft® Excel® for Mac 2004 (v11.3.7, Microsoft Corporation, Redmond, WA) spreadsheet. Excel was used to calculate summary statistics (e.g., averages, standard deviations) and to construct graphs. Differences in average transcript levels were explored using Tukey's test.

Results

Growing temperature had a clear impact on plant development and morphology. The range of temperatures surveyed in this study, 10°C, 20°C, and 30°C, encompass low (10°C) and high (30°C) extremes that are outside of typical potato production conditions. Our results demonstrate that at 10°C plant growth is visibly stunted and plants of all genetic backgrounds failed to flower within the course of our experiment (Fig. 1a). These plants did, however, produce many small tubers (Fig. 1b). As with plants grown at 10°C, all plants grown at 30°C, regardless of genetic background, failed to flower (Fig. 1a). These plants produced spindly stems (Fig. 1a) and failed to set tubers altogether, instead producing a mass of stolons without any visible swelling indicating tuber initiation (Fig. 1b). Plants grown at 20°C morphologically most closely represented field-grown potatoes, although these plants too were somewhat spindly (Fig. 1a), possibly due to a lack of diurnal temperature fluctuation. Plants grown at 20°C produced both flowers and tubers. While tuber yield was highest for plants grown at 20°C, average tuber number per plant was highest at 10°C (Fig. 1b).

Because our RT-PCR assay is specific to the *RB* trangene, untransformed lines lacked *RB* amplicons in all tests (Fig. 2). In contrast, regardless of genetic background or growing temperature, *RB* transcript was detected in all replicates of each transgenic line. Thus, our results confirm that *RB* is transcribed constitutively, even in the absence of the late blight pathogen, and that growing temperature does not impact whether or not *RB* is transcribed.

To evaluate the effect that physiological age (e.g. flowering and post-flowering stages) has on *RB* transcription at 20 C, leaves of transgenic line SP918 and untransformed 'Katahdin' were sampled at flowering and at 2 and 4 weeks post flowering. Leaves from each of four replicate plants for each line at each physiological age and from each of two experiments were collected. RNA from these leaves was assayed using qRT-PCR. As expected, no *RB* transcript was detected in untransformed 'Katahdin'

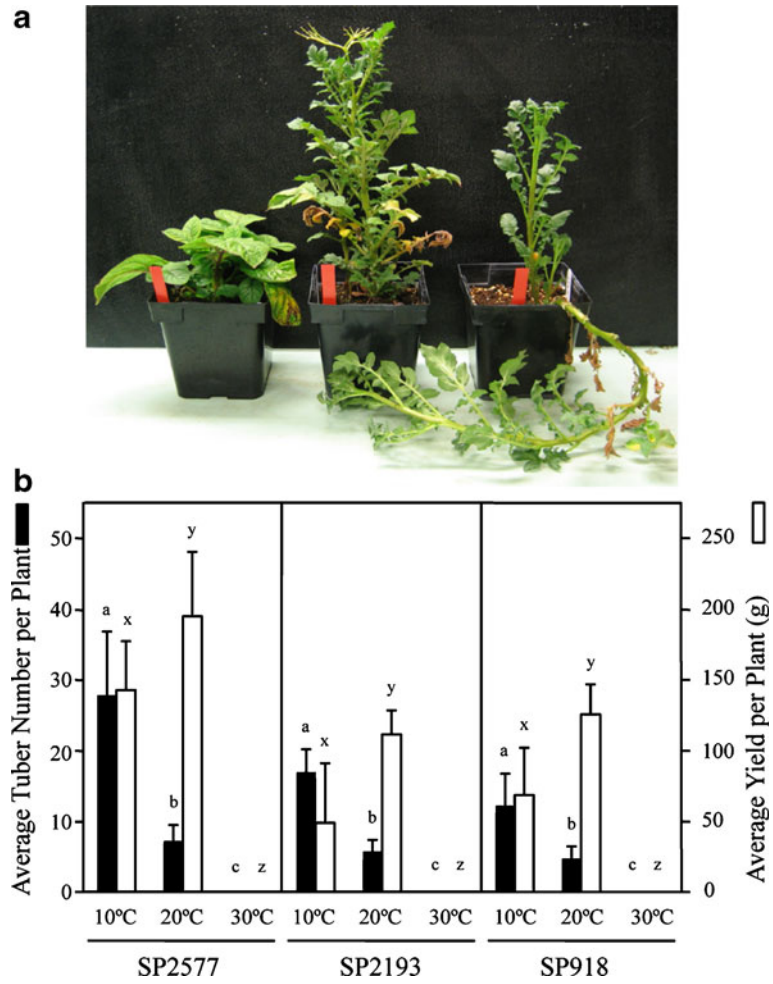

Fig. 1 Growing temperature impacts plant development and tuberization. **a** Three plants of line SP2577 are shown 64 days after planting from greenhouse-grown tubers. Plants were grown in growth chambers at constant temperatures (left to right: 10°C, 20°C, and 30°C). Plants grown at 20°C are post flowering while plants grown at 10°C and 30°C have not yet flowered—evidence that growing temperature impacts plant development. Temperature also impacts plant morphology. Note the stunted appearance of plants grown at 10°C and the spindly appearance of plants grown at 30°C. **b** Growing temperature impacts plant yield and number of tubers produced. Average tuber yields *(white bars)* and average number of tubers per plant (*black bars*) of three transgenic lines (SP2577, SP2193 and SP918) are shown. Error bars indicate standard deviations among replicate plants. Letters above error bars reflect Tukey groupings within a genotype ($P=0.05$). Plants grown at 30°C did not produce tubers. The yield of plants grown at 20°C was higher than that of plants grown at 10°C. By contrast, average number of tubers produced per plant at 20°C was lower than for plants grown at 10°C

(data not shown), confirming the specificity of our molecular assay. Figure 3 summarizes quantitative *RB* transcript measurements for SP918. Previously, Millett et al. (2009) noted significant replicate plant-to-replicate plant variation in *RB* transcript levels for two of three surveyed transgenic potato lines, concluding that genome context at the site of transgene insertion may induce variability in *RB* transcript accumulation. Based on these observations, Millett et al. (2009) suggested that researchers interested in studying *RB* transcript levels should average their results across multiple experimental units. Consistent with this advice, in the current study, *RB* transcript levels are averaged across four observations in each of two experiments. Despite this precaution, the use of seed tubers of identical physiological age and environmental history, and use of an environmentally controlled growth chamber in this study, differences among treatments in replicate plant-to-replicate plant variation were still observed, as evidenced by large standard

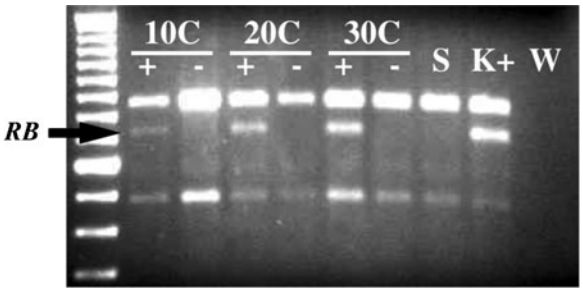

Fig. 2 Growing temperature has no qualitative effect on *RB* transcription. A photograph of an agarose gel documenting transgene *RB* transcription. Our previously published RT-PCR assay (Millett and Bradeen 2007) was employed to test for *RB* transcription in transgenic plants of line SP2577 grown at 10°C, 20°C, and 30°C. Lanes are (left to right): DNA size standard, RT-PCRs of SP2577 (+) and nontransformed 'Dark Red Norland' (−) grown at each temperature, and RT-PCR technical controls [S—nontransformed 'Superior' (negative control), K+—transgenic 'Katahdin' (positive control), W—water; no RNA template (negative control)]. The upper fragment in each RT-PCR (except water) originates from *RNA Polymerase II* and serves as an internal control for each reaction. The fragment, labeled "*RB*" is an amplicon specific to the *RB* transgene and is present in all transgenic plants, regardless of growing temperature, and is absent in all nontransgenic plants. The lower fragment, present in all RT-PCRs (except water) originates from a template unrelated to *RB* and serves as an additional internal control (Millett and Bradeen 2007)

deviations (Fig. 3). These results confirm the observations of Millett et al. (2009) that *RB* transcript level, in at least some transgenic potato lines, is variable. Despite this variation, however, no significant change in transcript levels between flowering, 2 weeks post-flowering and 4 weeks post-flowering was detected for experiment 1, experiment 2, or averaged across experiments (Fig. 3). Thus, our results indicate that plant physiological changes associated with flowering do not impact *RB* transcript levels.

Discussion

Research in multiple plant species (e.g., Caicedo and Schaal 2004; Cannon et al. 2002; Couch et al. 2006; Meyers et al. 2003; Pan et al. 2000; Zhou et al. 2004) has yielded an understanding of genome-wide R gene organization and models for R gene evolution (recent reviews provided by McDowell and Simon 2006; Meyers et al. 2005). However, despite successful efforts to isolate functional R genes, to understand their roles in defence responses, and to understand their evolutionary histories, little research has been expended towards understanding the transcriptional regulation of R genes.

In recent decades, global potato production has shifted, with production increases in tropical and subtropical regions and concomitant decreases in temperate regions (Bradeen et al. 2008; Hijmans 2003; Levy and Veilleux 2007). Continued global climate change is likely to impact agriculture (including potato production), plant physiology, and plant-microbe interactions in largely unpredictable ways (Coakley et al. 1999; Garrett et al. 2006). With these factors in mind, understanding the impact of varying environmental and physiological factors on R gene transcript levels may be key to successful R gene deployment. In this study we analyzed expression patterns of the *P. infestans* resistance gene *RB* across various temperatures and physiological stages.

In the potato-*P. infestans* pathosystem, humidity and temperature strongly influence disease development. The effects of temperature on *P. infestans* sporangia germination have been studied by Crosier (1934), Melhus (1915), Ribeiro (1983), and Sato (1994). The optimal temperature for direct sporangium formation is 18–22°C. Temperatures below 12°C favour indirect germination via zoospore formation while temperatures in the range of 21–24°C favour germ tube production from zoospores and direct sporangia germination. All germination pathways require high moisture conditions. In the current study, temperature but not relative humidity was controlled. Because relative humidity varied inversely with temperature in our growth chambers (not shown), we could not test the effect of growing temperature on disease resistance directly. Specifically, were inoculation studies conducted in the growth chambers, the impacts of plant disease resistance gene function would be confounded with environmental effects (variable temperature and relative humidity) on pathogen viability and function. Previously we demonstrated that the transgenic lines surveyed in the current study are resistant (SP2193) or moderately resistant (SP918 and SP2577) to *P. infestans* US8 (Bradeen et al. 2009). Importantly, in the same study, disease resistance phenotypes correlated with *RB* transcript levels. Thus, it is possible to infer that *RB* transcript levels in the current study may be reliable predictors of resistance phenotypes.

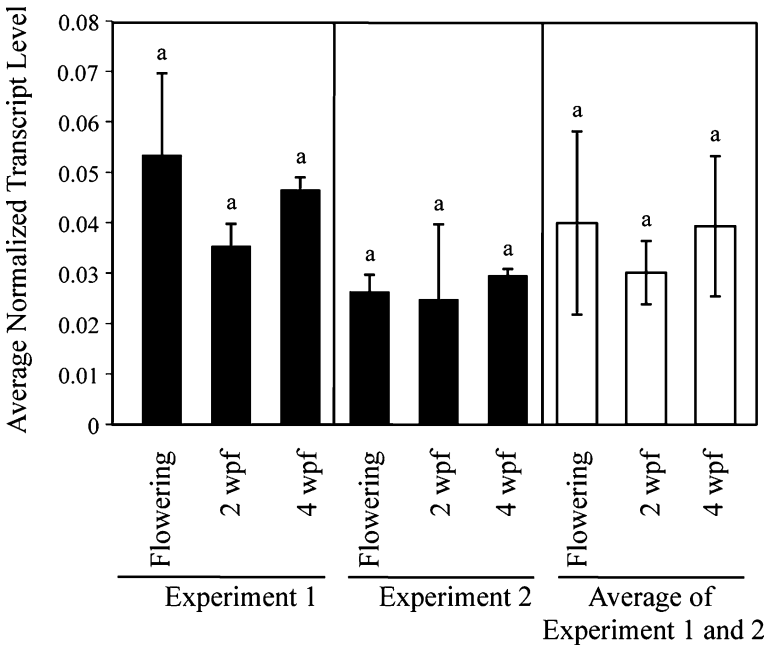

Fig. 3 *RB* transgene transcript levels are statistically constant during plant development. Average *RB* transgene transcript levels, normalized to *Elongation Factor 1-α* (Y axis), were quantified for transgenic line SP918 grown at 20°C in two separate experiments over three plant physiological ages: at flowering, 2 weeks after the initiation of flowering (2 wpf), and 4 weeks after the initiation of flowering (4 wpf) (X axis). Single experiment observations are represented as black bars and the average observation across experiments within a plant age are represented as white bars. Error bars indicate standard deviations among replicate plants. Letters above error bars reflect Tukey groupings within an experiment (P=0.05). *RB* transcript is present at all plant ages and average transcript levels remain statistically constant (α=0.05) at all plant ages surveyed in each experiment and averaged across both experiments

Until now, no study has reported the impact of growing temperature on the transcription of *P. infestans* resistance genes. At a phenotypic level, high temperatures decreased potato resistance to *P. infestans* (Rubio-Covarrubias et al. 2006). Our current results provide evidence that the *RB* transgene is transcribed in independent transgenic lines in three genetic backgrounds [SP918 ('Katahdin'), SP2577 ('Dark Red Norland') and SP2193 ('Russet Burbank')] at extreme (10°C and 30°C) and normal (20°C) growing temperatures. In contrast, temperature-sensitive resistance genes have been described in other host-pathogen systems. In *Arabidopsis,* Yang and Hua (2004) showed that both resistance gene expression and phenotypic resistance to *Peronospora parasitica* was influenced by temperature. Daolin et al. (2009) demonstrated that low temperature influenced wheat resistance to *Puccinia striformis* but did not alter resistance gene transcription. Other examples are reported by Wang et al. (2009), Whitham et al. (1994) and Xiao et al. (2003). Because *RB* transcript levels correlate with resistance phenotypes (Bradeen et al. 2009), our current results may suggest that *RB* will impart foliar late blight resistance under a broad range of environmental conditions. This point warrants further study and direct experimental demonstration.

Host developmental stage may also influence R gene transcription and/or function. Cao et al. (2007) demonstrated that rice bacterial blight resistance mediated by *Xa3* increased from the susceptible juvenile stage to the fully resistant later adult stages. In contrast, De Ilarduya and Kaloshian (2001) demonstrated that developmentally-regulated resistance phenotypes correlated poorly with transcription of tomato *Mi1-2*. Similarly, using transgenic potato lines, Millett et al. (2009) found that *RB* was transcribed at uniform levels in pre-flowering, post-flowering, and near-senescing plants despite modulation of resistance levels. Importantly, Millett et al. (2009) did not test *RB* transcript levels at flowering. Potato flowering coincides with tuber initiation, marking a critical metabolic change in the potato life

cycle (Trindade et al. 2003). In the current study, we assessed *RB* transcript levels at flowering, 2 weeks post-flowering and 4 weeks post-flowering. Consistent with Millett et al. (2009) our results confirm that *RB* is constitutively transcribed in uninfected plants and that transcript levels are neither qualitatively nor quantitatively influenced by plant developmental stage.

This work concludes detailed functional and phenotypic characterization of transgenic potato lines carrying the *P. infestans* resistance gene *RB*. We have demonstrated that additional copies of the *RB* transgene enhance *RB* transcript levels and disease resistance, regardless of genetic background (Bradeen et al. 2009). We have also demonstrated that transgenic potato plants at differing physiological stages (pre-flowering, flowering, post-flowering, and near-senescing) accumulate comparable levels of *RB* transcript despite obvious differences in disease resistance phenotypes (Millett et al. 2009; present paper). Finally, in the current study, we have shown that temperature does not qualitatively impact *RB* transcription.

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References

Bradeen, J. M., Carputo, D., & Douches, D. S. (2008). Potato. In C. Kole & T. C. Hall (Eds.), *A compendium of transgenic crop plants: Transgenic sugar, tuber and fiber crops* (pp. 117–156). Oxford: Blackwell.

Bradeen, J. M., Iorizzo, M., Mollov, D. S., Raasch, J., Kramer, L. C., Millett, B. P., et al. (2009). Higher copy numbers of the potato *RB* transgene correspond to enhanced transcript and late blight resistance levels. *Molecular Plant-Microbe Interactions, 22*, 437–446.

Caicedo, A. L., & Schaal, B. A. (2004). Heterogeneous evolutionary processes affect *R* gene diversity in natural populations of *Solanum pimpinellifolium*. *Proceedings of the National Academy of Sciences, 101*, 17444–17449.

Cannon, S. B., Zhu, H., Baumgarten, A. M., Spangler, R., May, G., Cook, D. R., et al. (2002). Diversity, distribution, and ancient taxonomic relationships within the TIR and non-TIR NBS-LRR resistance gene subfamilies. *Journal of Molecular Evolution, 54*, 548–562.

Cao, Y., Ding, X., Cai, M., Zhao, J., Lin, Y., Li, X., et al. (2007). The expression pattern of a rice disease resistance gene *Xa3*/*Xa26* is differentially regulated by the genetic backgrounds and developmental stages that influences its function. *Genetics, 177*, 523–533.

Century, K. S., Lagman, R. A., Adkisson, M., Morlan, J., Tobias, R., Schwartz, K., et al. (1999). Developmental control of *Xa21*-mediated disease resistance in rice. *The Plant Journal, 20*, 231–236.

Coakley, S. M., Scherm, H., & Chakraborty, S. (1999). Climate change and plant disease management. *Annual Review of Phytopathology, 37*, 399–426.

Couch, B. C., Spangler, R., Ramos, C., & May, G. (2006). Pervasive purifying selection characterizes the evolution of *I2* homologs. *Molecular Plant-Microbe Interactions, 19*, 288–303.

Crosier, W. (1934). *Studies in the biology of Phytophthora infestans (Mont) de Bary*. Ithaca: Cornell University Agricultural Experiment Station.

Daolin, F., Uauy, C., Dsistelfeld, A., Blechl, A., Epstein, L., Chen, X., et al. (2009). A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. *Science, 323*, 1357–1360.

De Ilarduya, O. M., & Kaloshian, I. (2001). *Mi-1.2* transcripts accumulate ubiquitously in resistant *Lycopersicum esculentum*. *Journal of Nematology, 33*, 116–120.

Garrett, K. A., Dendy, S. P., Frank, E. E., Rouse, M. N., & Travers, S. E. (2006). Climate change effect on plant disease: genomes to ecosystems. *Annual Review of Phytopathology, 44*, 489–509.

Goggin, F. L., Shah, G., Williamson, V. M., & Ullman, D. E. (2004). Developmental regulation of *Mi*-mediated aphid resistance is independent of *Mi*-1.2 transcript levels. *Molecular Plant-Microbe Interactions, 17*, 532–536.

Hijmans, R. J. (2003). The effect of climate change on global potato production. *American Journal of Potato Research, 80*, 271–280.

Huang, S., van der Vossen, E. A. G., Kuang, H., Vleeshouwers, V. G. A. A., Zhang, N., Borm, T. J., et al. (2005). Comparative genomics enabled the isolation of the *R3a* late blight resistance gene in potato. *The Plant Journal, 42*, 251–261.

Innes, R. W. (2004). Guarding the goods: new insights into the central alarm system of plants. *Plant Physiology, 135*, 695–701.

Kramer, L. C., Choudoir, M. J., Wielgus, S. M., Bhaskar, P. B., & Jiang, J. (2009). Correlation between transcript abundance of the *RB* gene and the level of the *RB*-mediated late blight resistance in potato. *Molecular Plant-Microbe Interactions, 22*, 447–455.

Levy, D., & Veilleux, R. E. (2007). Adaption of potato to high temperature and salinity-a review. *American Journal of Potato Research, 84*, 487–506.

Lokossou, A. A., Park, T., van Arkel, G., Arens, M., Ruyter-Spira, C., Morales, J., et al. (2009). Exploiting knowledge of R/Avr genes to rapidly clone a new LZ-NBS-LRR family of late blight resistance genes from potato linkage group IV. *Molecular Plant-Microbe Interactions, 22*, 630–641.

Martin, G. B., Bogdanove, A. J., & Sessa, G. (2003). Understanding the functions of plant disease resistance proteins. *Annual Review of Plant Biology, 54*, 23–61.

McDowell, J. M., & Simon, S. A. (2006). Recent insights into *R* gene evolution. *Molecular Plant Pathology, 7*, 437–448.

Melhus, I. E. (1915). *Germination and infection with the fungus of the late blight of potato (Phytophthora infestans)*. Madison: University of Wisconsin Agricultural Experiment Station.

Mes, J. J., van Doorn, A. A., Wijbrandi, J., Simons, G., Cornelissen, B. J. C., & Haring, M. A. (2000). Expression of the *Fusarium* resistance gene *I-2* colocalizes with the site of fungal containment. *The Plant Journal, 23*, 183–193.

Meyers, B. C., Kozik, A., Griego, A., Kuang, H., & Michelmore, R. W. (2003). Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. *The Plant Cell, 15*, 809–834.

Meyers, B. C., Kaushik, S., & Nandety, R. S. (2005). Evolving disease resistance genes. *Current Opinion in Plant Biology, 8*, 129–134.

Millett, B. P., & Bradeen, J. M. (2007). Development of allele-specific PCR and RT-PCR assays for clustered resistance genes using a potato late blight resistance transgene as a model. *Theoretical and Applied Genetics, 114*, 501–513.

Millett, B. P., Mollov, D. S., Iorizzo, M., Carputo, D., & Bradeen, J. M. (2009). Changes in disease resistance phenotypes associated with plant physiological age are not caused by variation in R gene transcript abundance. *Molecular Plant-Microbe Interactions, 22*, 362–368.

Nicot, N., Hausman, J.-F., Hoffmann, L., & Evers, D. (2005). Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. *Journal of Experimental Botany, 56*, 2907–2914.

Pan, Q., Wendel, J., & Fluhr, R. (2000). Divergent evolution of plant NBS-LRR resistance gene homologues in dicot and cereal genomes. *Journal of Molecular Evolution, 50*, 203–213.

Park, T. H., Gros, J., Sikkema, A., Vleeshouwers, V. G. A. A., Muskens, M., Allefs, S., et al. (2005). The late blight resistance locus *Rpi-blb3* from *Solanum bulbocastanum* belongs to a major late blight *R* gene cluster on chromosome 4 of potato. *Molecular Plant-Microbe Interactions, 18*, 722–729.

Ribeiro, O. K. (1983). Physiology of asexual sporulation and spore germination in *Phytophthora*. In D. C. Erwin, S. Bartnicki-Garcia, & P. H. Tsao (Eds.), *Phytophthora, its biology, taxonomy, ecology, and pathology*. St. Paul: APS.

Rubio-Covarrubias, O. A., Douches, D. S., Hammerschmidt, R., da Rocha, A., & Kirk, W. W. (2006). Effect of photoperiod and temperature on resistance against *Phytophthora infestans* in susceptible and resistant potato cultivars: effect on deposition of structural phenolics on the cell wall and resistance to penetration. *American Journal of Potato Research, 83*, 325–334.

Sato, N. (1994). Effect of sporulating temperature on the limit temperature of indirect germination of the sporangia of *Phytophthora infestans*. *Annals of the Phytopathological Society of Japan, 60*, 60–65.

Scheideler, M., Schlaich, N. L., Fellenberg, K., Beissbarth, T., Hauser, N. C., Vingron, M., et al. (2002). Monitoring the switch from housekeeping to pathogen defense metabolism in *Arabidopsis thaliana* using cDNA arrays. *The Journal of Biological Chemistry, 277*, 10555–10561.

Schornack, S., Peter, K., Bonas, U., & Lahaye, T. (2005). Expression level of *avrBs3*-like genes affect recognition specificity in tomato *Bs4*- but not in pepper *Bs3*- mediated perception. *Molecular Plant-Microbe Interactions, 18*, 1215–1225.

Silvar, C., Merino, F., & Diaz, J. (2007). Differential activation of defense-related genes in susceptible and resistant pepper cultivars infected with *Phytophthora capsici*. *Journal of Plant Physiology, 165*, 1120–1124.

Song, J., Bradeen, J. M., Naess, S. K., Raasch, J. A., Wielgus, S. M., Haberlach, G. T., et al. (2003). Gene *RB* from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. *Proceedings of the National Academy of Sciences, 100*, 9128–9133.

Trindade, L. M., Horvath, B. M., van Berloo, R., & Visser, R. G. F. (2003). Analysis of genes differentially expressed during potato tuber life cycle and isolation of their promoter regions. *Plant Science, 166*, 423–433.

van der Vossen, E. A. G., Sikkema, A., Hekkert, B. T. L., Gros, J., Stevens, P., Muskens, M., et al. (2003). An ancient R gene from the wild potato species *Solanum bulbocastanum* confers broad-spectrum resistance to *Phytophthora infestans* in cultivated potato and tomato. *The Plant Journal, 36*, 867–882.

van der Vossen, E. A. G., Gros, J., Sikkema, A., Muskens, M., Wouters, D., Wolters, P., et al. (2005). The *Rpi-blb2* gene from *Solanum bulbocastanum* is an *Mi-1* gene homolog conferring broad-spectrum late blight resistance in potato. *The Plant Journal, 44*, 208–222.

Wang, Y., Bao, Z., Zhu, Y., & Hua, J. (2009). Analysis of temperature modulation of plant defense against biotrophic microbes. *Molecular Plant-Microbe Interactions, 22*, 498–506.

Whitham, S., Dinesh-Kumar, S. P., Choi, D., Hehl, R., Corr, C., & Baker, B. (1994). The product of the tobacco mosaic virus resistance gene *N*: similarity to toll and the interleukin-1 receptor. *Cell, 78*, 1101–1115.

Xiao, S., Brown, S., Patrick, E., Brearley, C., & Turner, J. G. (2003). Enhanced transcription of the Arabidopsis disease resistance genes RPW8.1 and RPW8.2 via a salicylic acid-dependent amplification circuit is required for hypersensitive cell death. *The Plant Cell, 15*, 33–45.

Yang, S., & Hua, J. (2004). A haplotype-specific resistance gene regulated by *BONZAI1* mediates temperature-dependent growth control in *Arabidopsis*. *The Plant Cell, 16*, 1060–1071.

Zhou, T., Wang, Y., Chen, J. Q., Araki, H., Jing, Z., Jiang, K., et al. (2004). Genome-wide identification of NBS genes in *japonica* rice reveals significant expansion of divergent non-TIR NBS-LRR genes. *Molecular Genetics and Genomics, 271*, 402–415.