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Polygenic response of potato to late blight following exposure to long-day or short-day by monitoring of gene expression with a cDNA microarray

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Abstract

Potato was exposed in a factorial-design experiment to short-day and to long-day for 4 weeks. Following the photoperiod treatment, the plants were inoculated with the late blight agent, *P. infestans*. Long-day had a strong resistance-enhancing effect. The transcriptome at 24 h post inoculation was analysed by hybridisation to the RLP microarray representing 4000 genes involved in resistance, light, and photoperiod perception. The complex alterations of the transcriptome are under study.

Keywords: Microarray, *Phytophtora infestans*, potato, resistance, *Solanum tuberosum*

Introduction

It is a well known phenomenon that late maturing cultivars are highly sensitive to light and photoperiod and tuberise under the inductive short days at the end of the European season, whereas early cultivars form tubers even in long-day. Both, tuberisation and flowering have been demonstrated to be controlled by the same set of genetic loci (RODRIGUEZ-FALCON et al. 2006). We constructed and used a cDNA microarray as a research tool to study the genetic inter-relationships of maturity and late blight resistance in potato.

Materials and Methods

Plants and trials tackling photoperiod and resistance response interactions

Three potato cultivars differing in their maturity and late blight resistance were involved. MF-II is the male-sterile female parent of Indian descent (M. UPADHYA, International Potato Center, Lima, Peru, 1999, pers. comm.) of a true potato seed (TPS) variety developed at the International Potato Centre. MF-II possesses a single R gene for resistance to blight (B. TROGNITZ et al., unpublished). TPS67 is the pollinator parent of the same TPS variety, it also carries a major late blight resistance gene (B. TROGNITZ et al. unpublished). Both clones are late maturing in Europe and present elevated levels of blight resistance, even when their R genes are broken down by *Phytophthora infestans* populations occurring in the area. In contrast, the Austrian cultivar Linzer Delikatess is early-maturing and highly susceptible to blight.

Pot plants (2 replications/treatment, 4 pots/rep) were pre-treated for 4 wk with either long-day (LD; 15 h) or short-day (SD; 8 h light) and then kept under natural >14-h-light. Immediately following the photoperiod treatment part of the plants were inoculated with water to serve as a control („C"), and all other plants were inoculated with *P. infestans* („P") to invoke the inherent genomic response to blight. Leaf samples from the upper part of the plants were shock-frozen in liquid nitrogen 24 h post inoculation, for analysis of the transcriptome. Late blight disease was evaluated at 4 and 6 dpi and reported as the average percent foliage blighted, for all cultivars and photoperiod pre-treatments. In addition, flowering intensity, increase of total stem length from start to completion of the photoperiod treatments, and tuber development were recorded.

Analyses of variance and appropriate comparisons of means were applied for data analysis.

RLP Microarray and hybridisation of cDNA samples

The thematic Resistance-Light-Photoperiod response (RLP) microarray was built using a normalised cDNA library of the short-day adapted Peruvian cultivar Yungay (field resistant, no R genes as evaluated by its susceptibility to the 0-race of *P. infestans*) challenged by late blight (3475 probes, produced by VERTIS GmbH, Freising, Germany) and cDNA clones selected from resources held at The Institute of Genomic Research (1177 probes purchased from the Arizona Genomics Institute, Arizona, USA.), 19 probes for pathogen defense-related genes of *S. caripense* (F. TROGNITZ 2004), and several housekeeping genes for control purposes.

Extraction of RNA, generation of cDNA, fluorescent dye labeling, and two-color hybridisation on the RLP array were performed following standard procedures. Information on these procedures is available upon request from the authors.

Results and Discussion

Plant experiments

Blight was significantly ($P<0.001$) reduced on LD pre-treated, genetically resistant cultivars, whereas the susceptible Linzer Delikatess developed severe disease symptoms under all conditions (*Figure 1*). For the blight resistant cultivars, MF-II and TPS67, those parts of the plants that had developed during the day length treatments, were less affected

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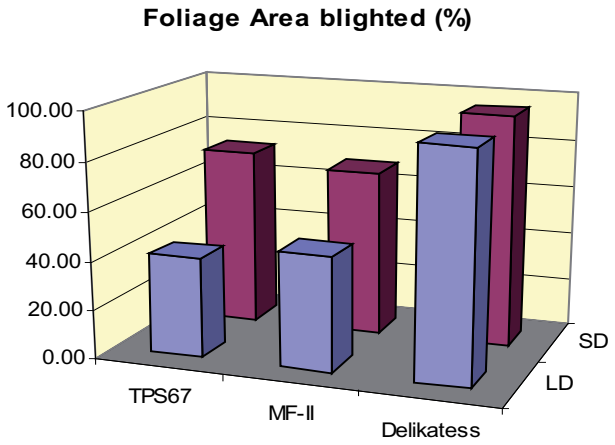

Figure 1: **Effect of photoperiod on late blight resistance of potato. Cultivars TPS67 and MF-II carrying active blight resistance genes developed after 4 wk 15-h-day (LD) higher resistance levels to blight than after 8-h lighting (SD). The susceptible and early cultivar Linzer Delikatess remained equally susceptible under all conditions.**

by blight than the older parts in LD-treatment; SD-treated plants did not show these differences (*Figure 2*).

There was a tendency to increased plant growth under LD ranging from 31 (Linzer Delikatess) to 40 cm (TPS67) increase of total stem length, relative to 30 and 37 cm increase under SD. MF-II and TPS67 flowered only after LD but not after SD, and tuberization was induced by SD in these two cv.s, whereas Linzer Delikatess did not flower under any conditions and produced tubers independent of day length.

The positive correlation of long vegetative periods and blight resistance in the field is a well known phenomenon. Late potatoes generally display lower blight scores than earlier types and LOWE and HARRISON (1995) showed by quantitative PCR that *P. infestans* can produce much more biomass within host tissue under short-, relative to long day length. Despite many and intensive efforts by breeders to break this correlation and to breed early-maturing, blight resistant potato cultivars, this has been of limited success (UMAERUS and UMAERUS 1994).

Potato flowering promotion under long-day was already observed by CLARKE and LOMBARD (1936). The initiation of tubers by accumulating short-day cycles was reviewed by VAN DEN BERG and EWING (1991) and RODRIGUEZ-FALCON et al. (2006) reviewed the inherent genetic mechanisms of these processes. Both flowering and the formation of tubers characterise important stages of the potato's ontogenetic development and their timing seems to have a strong impact on the length of the total vegetative period. Therefore, there is an obvious interrelationship between flowering, tuberisation, senescence, and blight resistance in the field that must have its bases in the underlying genetic mechanisms. We isolated messenger RNA from foliar tissues of the experimental materials following photoperiod treatment and challenge by *P. infestans* and transcribed these into cDNA pools for microarray-mediated monitoring of corresponding gene expression patterns.

Figure 2: **Effect of photoperiod on late blight resistance of potato foliage. One week after inoculation with *P. infestans*, SD-treated samples were killed by blight. LD-pretreated samples were partially resistant and the upper parts of the foliage that had developed during the period of light treatment were much less diseased than older plant parts.**

Microarray experiments

Four hybridisations of two contrasting cDNA populations at a time onto the probes of the array were made for each potato cultivar in separate. We chose a design for these hybridisations that permitted comparative analysis of cDNA pools representative of four experimental treatments; namely the combinations of LD and inoculation with water (LDC), LD and *P. infestans* (LDP), and correspondingly, SDC and SDP. Data sets comprising these four treatments and contrasts of the treatments were generated for each cultivar in the LIMMA module of the R statistical analysis software. Analyses of gene expression patterns included clustering by various parameters and review of potential function as derived from the genes annotations. By drawing the contrast of the contrasts (LDP-LDC) and (SDP-SDC), a total of more than 800 probes/genes on the RLP array were revealed that appeared significantly regulated by the underlying individual treatments ($P<0.001$, *Table 1*).

Table 1: **Total numbers of probes/genes on the RLP microarray that appeared significantly regulated within cv. MF-II, TPS67, or Linzer Delikatess following inoculation with P. infestans subsequent to LD and SD (PiSD-PiLD), treatment with opposite daylength (SD-LD), and day length treatment versus *P. infestans* challenge (DL-Pi).**

Contrast	MF-II Resistant	TPS67 Resistant	Linzer Delikatess Susceptible
PiSD-PiLD	817	791	394
SD-LD	1526	370	167
DL-Pi	1456	934	306

Searching among these 800 probes/genes for those that appear differentially regulated by both LD + *P. infestans* and SD + *P. infestans* within the two blight-resistant cultivars, MF-II and TPS67, but that were not or oppositionally regulated in the susceptible cultivar Linzer Delikatess revealed a small group of 20 cDNAs that were highly significantly regulated and that were at the same time significantly differently regulated in SD relative to LD. Nine of these encode proteins of unknown function, whereas 9 cDNAs represent genes of putative functions in ion transport, ethylene, jasmonate, and ABA signaling, protein catabolism and transport, and resistance. Two separate probes of the array that are homologous to the Arabidopsis thaliana gene *NDR*1 (*NONSPECIFIC DISEASE RESISTANCE* 1, CENTURY et al. 1997) were also strongly regulated in the resistant but not in the susceptible cultivar.

Subsequent experiments will be performed to confirm these results and to investigate the specific role of *NDR*1 and the other relevant genes in the context of both photoperiod response and late blight resistance.

Conclusions

Our experiments confirm late blight resistance is strongly compromised by SD pre-treatment of the green plant parts. Photoperiod also affects plant growth, tuber yield, and flowering of sensitive potato genotypes.

The thematic RLP microarray (5000 single genes, 18% of potato genome) is a useful tool to discern gene expression patterns of solanaceous plants upon challenge by stress and of genes related to perception of light and photoperiod.

Already one day after first contact of potato hosts with the *P. infestans* pathogen the expression levels of up to 1000 genes, the equivalent of some 3% of the potato genome, may be altered.

Even between genotypes of comparable resistance level, profound differences in gene expression occur. However, evidence supports the hypothesis that only a few genes may be responsible for the discriminatory expression patterns of resistant vs. susceptible potato phenotypes, represented here the cultivars MF-II and TPS67 (resistant) and Linzer Delikatess (susceptible). Whether some or all of the genes involved in photoperiod-dependent blight resistance also are causally involved in the intitiation of flowering, tuberisation, and thus, in shaping the individual length of a genotype's vegetative period, will require additional data analyses and studies.

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References

LOWE, R. and J.G. HARRISON, 1995: Stability of R-gene resistance to late blight in potato leaves. SCRI Ann Rep 1995: 117-118.

CLARKE, A.E. and P.M. LOMBARD, 1939: Relation of length of day to flower and seed production in potato varieties. Amer Potato J 16: 236-244.

VAN DEN BERG, J.H. and E.E. EWING, 1991: Jasmonates and their role in plant growth and development, with special reference to the control of potato tuberization: a review. Amer Potato J 68: 781-794.

STEWART, H.E., J.E. BRADSHAW and B. PANDE, 2003:The effect of the presence of R-genes for resistance to late blight (*Phytophthora infestans*) of potato (*Solanum tuberosum*) on the underlying level of field resistance. Plant Pathol 52: 193-198.

UMAERUS, V. and M. UMAERUS, 1994: Inheritance of resistance to late blight. in: Mackay GR (ed) Inheritance of resistance to late blight. pp. 365-401. CAB International, Wallingford, UK.

CENTURY, K.S., A.D. SHAPIRO, P.P. REPETTI, D. DAHLBECK, E. HOLUB and B.J. STASKAWICZ, 1997: *NDR*1, a pathogen-induced component required for *Arabidopsis* disease resistance. Science 278: 1963-1965.

RODRIGUEZ-FALCON, M., J. BOU and S. PRAT, 2006: Seasonal Control of Tuberization in Potato: Conserved Elements with the Flowering Response. Annu. Rev. Plant Biol. 57:151-80.

TROGNITZ, F., 2004: Genetic and molecular characterization of late blight resistance in *Solanum caripense*. Ph D thesis, Univ Agric Vienna, Austria, 127 pp. (http://magnum.bibvb.ac.at/ALEPH/-/ext-find?base=ACC01&find=IDN=AC04118996).