PPO – SPECIAL REPORT NO 17 – 2015, 57-62

Assessment of genetic hotspots for *Phytophthora* resistance and their use as molecular markers in potato breeding

KAREN SIEBER[1], GEORG M. FORSTER[1], ANDREAS BERGER[1], THILO HAMMANN[2], ADOLF KELLERMANN[1] AND ANDREA SCHWARZFISCHER[1]

[1] Bayerische Landesanstalt für Landwirtschaft (LfL)
[2] Julius Kühn–Institut (JKI)

SUMMARY
At present, only a few molecular markers are successfully used in potato breeding to select for quality and pathogen resistance (e.g. Song *et al.* 2008, Saderzeh *et al.*, 2006). Especially in resistance breeding the availability of reliable markers would help to accelerate the accumulation of different resistance loci in the potato genome (Schwarzfischer *et al.*, 2010). In our study we assessed published markers within six genetic hotspots for resistance against *Phytophthora infestans*. A variety trial with more than 150 cultivars and breeding clones was used to obtain resistance values (rAUDPC) for each cultivar. Results from genetic analyses showed that six markers produced distinct amplification products from resistance genes derived from wild *Solanum* species in all 150 clones. We then associated marker results with field resistance values. We observed that cultivars which were marker positive for S. *demissum* resistance genes R1 and R3b showed no higher resistance values than cultivars without *S. demissum* background. This result is consistent with observations, that resistance obtained from *S. demissum* R-genes have been overcome by *Phytophthora* pathotypes. However, all 13 clones in the trial set that showed positive marker results for the *S. bulbocastanum* gene *Rpi blb3* proved to be highly resistant against late blight. Interestingly, the majority of the clones, which were found marker positive for *Rpi-blb* genes, have neither *S*. *bulbocastanum* nor *S. stoloniferum* in their pedigree.

KEYWORDS
Potato resistance breeding, *Phytophthora infestans*, R-Genes, Molecular Markers

INTRODUCTION

In potato farming late blight caused by *Phytophthora infestans* poses one of the highest production risks. In conventional farming the problem is met by application of a range of potent pesticides. However, under organic farming conditions only copper based products are presently licensed on the market. Although these chemicals reduce late blight infection they rarely prevent infestation of susceptible cultivars completely. Consequentially, varieties grown under organic

farming conditions need to thrive under reduced pest management, increased mechanical stress and generally lower nitrogen availability than on conventionally managed fields.

To meet the demands of organic potato farmers the German Federal Ministry of Food and Agriculture has since 2012 promoted a joint project which is funded through the "Federal Organic Farming Scheme and other forms of sustainable agriculture" (BÖLN). The project brings together the German expertise in potato breeding: the Julius Kühn–Institut (JKI), the Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), the Bayerische Landesanstalt für Landwirtschaft (LfL), and German potato breeding companies. The aim is to develop new suitable varieties for organic farming which show higher resistance to potato diseases and help reduce accumulation of copper on organically managed lands.

To tackle this task a variety of modern and historic cultivars from potato breeders and the IPK gene bank, respectively, are used to combine quality traits with the late blight resistance of JKI pre-breeding clones and other selected clones. Phenotypic results from a variety trial are used to choose suitable breeding partners. Breeding activities are carried out at the JKI and the LfL to produce a large number of potato seed. Where possible, the progeny is preselected using genetic markers for virus, nematode and late blight resistance.

A central aspect of the project is to select suitable clones in field trials under organic farming conditions. In a participative approach scientists and organic farmers have been choosing from more than 2,000 individuals per year to establish a late blight breeding pool for organic farming since the project started in 2012.

METHODS

Field trials

To assess late blight resistance a set of 150 cultivars and breeding clones was set up in a field trial. Material included LfL breeding clones, pre-breeding material from the JKI, historic cultivars from the IPK gene bank, cultivars from German potato breeders and selected varieties from other European potato breeders. Between 2012 and 2014 the trials were set up each year on three organically managed trial fields, two in Southern and one in Northern Germany as shown in Figure 1. The trials were arranged applying a randomized block design with 10 plants per plot and two replications per field. One trial was set up with fungicide protection to prevent late blight infection and determine the maturity of the cultivars. The five cultivars Anuschka, Ditta, Jelly, Lolita and Princess were used as reference. Phenotypic assessment of *Phytophthora* resistance was carried out under natural infection on the organically grown potatoes. During the epidemic late blight was documented using the percentage of disease-affected foliage (including dead leaves) once or twice a week. Resistance values were obtained calculating the relative area under disease progress curve (rAUDPC) and corrected for maturity using standard methods described in Truberg *et al.* (2009).

Marker assisted selection

To establish genetic markers for *Phytophthora* resistance breeding we analysed gene regions on six potato chromosomes (see Gebhardt *et al.*, 2006 and references within). Specific amplificates were obtained for markers shown in Table 1. Here we applied standard PCR procedures as described in the particular publications (Table 1) using Thermoprime Taq DNA Polymerase (Thermofisher). All amplificates were checked by sequencing through the LMU sequencing service (http://www.gi.bio.lmu.de/sequencing) and sequence analysis.

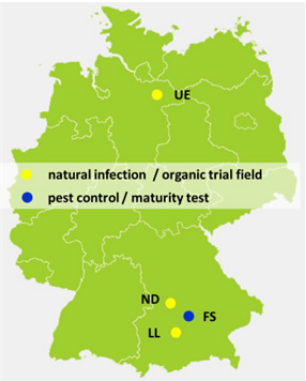

Figure 1. *Position of field trials. Yellow dots show the position of organically managed trials under natural infection.* ***Legend: UE*** *- Uelzen,* ***ND –*** *Neuburg/Donau,* ***FS*** *- Freising,* ***LL*** *– Landsberg/ Lech*

Table 1. *Overview over markers and gene regions assessed in this study*

Gene	Marker	Origin	Chromosome	Reference
Rpi-blb3	Blb3	*S. bulbocastanum*	4	Zhu *et al.*, 2012
Rpi-abpt	Abpt1	*Solanum* spec.	4	Kim *et al.*, 2012
R1	R1	*S. demissum*	5	Ballvora *et al.*, 2002
Rpi-blb1	Blb1	*S. bulbocastanum*	8	Fadina *et al.*, 2013
Rpi-sto1	Sto1	*S. stoloniferum*	8	Zhu *et al.*, 2012,
R3b	R3b	*S. demissum*	11	Rietman *et al.*, 2011

RESULTS AND DISCUSSION

Field trial

Between 2012 and 2014 six out of nine organically managed environments were used for evaluating late blight resistance of cultivars. Because of unfavourable weather conditions in 2013 and 2014 three trials were not infected by *P. infestans*. Estimation of resistance values showed between 83 % and 93 % identity with $R^2 = 0.67$ ($\alpha<0.99$) and $R^2 = 0.89$ ($\alpha<0.99$). Determination of maturity on the fungicide treated trial fields confirmed that material from all maturity indices were present. Late maturity and late blight resistance values could be shown to be positively correlated ($R^2 = 0.,12$; $\alpha<0.95$).

As shown in Figure 2 high resistance could be observed in all JKI pre-breeding clones as well as in a few selected cultivars i.e. Biogold, Vitabella, Bionica, Toluca, Carolus, Tübinger Circe, Saka 6, Axona, Kuras and Sarpo Mira. From the five cultivars Anuschka, Princess, Lolita, Ditta und Jelly which were used as internal standards, only Jelly displayed slightly better resistance values.

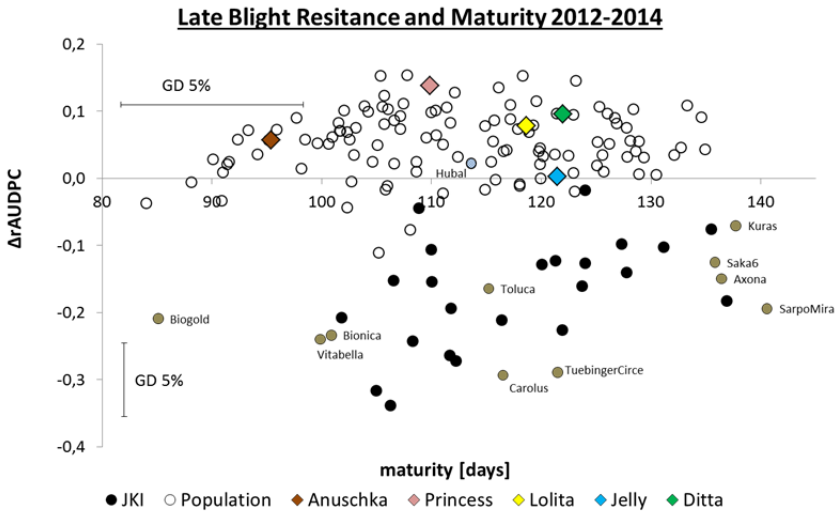

Figure 2. *Maturity corrected late blight resistance (ΔrAUDPC) vs. maturity obtained in field trials between 2012 and 2014 of JKI-prebreeding clones, populations, and standard varieties*

Marker-assisted selection

To find resistance donors in the breeding material which can be used by marker assisted selection (MAS), six potentially useful genome regions were analysed as shown in Figure 3. Distinct amplificates were obtained in all clones for markers Blb3, Abpt1, R1, Blb1, Sto1 and R3b.

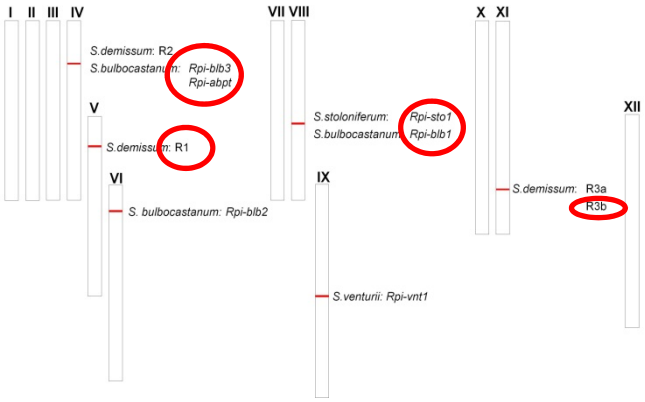

Figure 3. *Regions in the potato genome investigated as molecular markers. Rectangular bars depict chromoses, roman numerals indicates chromosome number. Markers with distinct sequence results are highlighted with red circles*

By correlating positive marker signals and high field resistance, cultivars carrying *S. demissum* derived markers showed no field resistance against *P. infestans* (see Fig. 4). Positive correlations were detected for markers derived from *S. bulbocastanum* and *S. stoloniferum* from two genome regions: Blb3 and Abpt1 (chromosome IV) and Blb1, Sto1 (chromosome VIII). Only one JKI pre-breeding clone was found marker positive for this gene region. As this clone also carries markers for other gene regions, no conclusions can be drawn.
Blb3 and ABPT were amplified from Biogold, Vitabella, Bionica, Hubal, Saka 6, Kuras and nine JKI pre-breeding clones. Interestingly, none of these marker positive JKI clones had *S. bulbocastanum* in their pedigree. Two of the Blb3/Abpt marker positive clones had a *S. stoloniferum* background. The remaining clones came from a mixed breeding background with wildtype genes from *S. andigena*, *S. phureja* and *S. demissum*. While there are no nucleotide differences between all obtained sequences from this marker, therefore *blb3* gene homologues seem to be present in some individuals of these wild species.

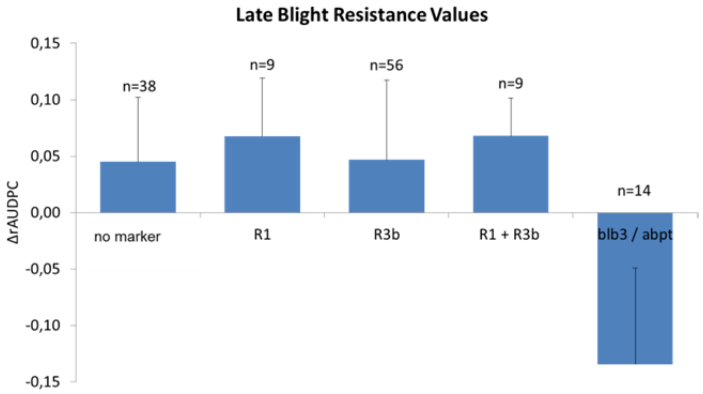

Figure 4. *Correlation between marker results and late blight resistance values. Results show that varieties carrying S. demissum derived genes R1 and R3b show no field resistance to present-day's population of P. infestans in these field trials. Breeding clones carrying BLB3 and ABPT, however, were highly resistant*

CONCLUSIONS

The field trials set up to evaluate late blight resistance showed that a representative number of German varieties and breeding clones revealed great differences in both, days to maturity and susceptibility against *P. infestans*. Many high-quality cultivars revealed a low degree of late blight resistance. However, pre-breeding material from the JKI and some selected varieties can be used as donors for late blight resistance in potato breeding. Gene regions found to carry resistance genes may be used as molecular markers. However, more markers need to be developed, analysed and evaluated within field trials. These markers need to target distinct wild type genes and produce single products from the potato genome. Markers found in this study which correlate with high field resistance will hopefully be evaluated in a future project using populations derived from breeding partners described in this study.

ACKNOWLEDGEMENTS

The authors like to thank all partners involved in this project: the Groß Lüsewitzer Kartoffelsortimente (GLKS), the organic potato farmers Max Kainz, Martin Huber and Christian Vinnen, and the Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V. (GFP). The project is funded by the Bundesministerium für Ernährung und Landwirtschaft through the „Bundesprogramm Ökologischer Landbau und andere Formen nachhaltiger Landwirtschaft" (BÖLN).

REFERENCES

Ballvora A., M.R. Ercolano, J. Weiû, K. Meksem, C.A: Bormann, P. Oberhagemann, F. Salamini and C. Gebhardt, 2002. The R1 gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. The Plant Journal 30(3), 361-371.

Gebhardt C., D. Bellin, H. Henselewski, W. Lehmann, J. Schwarzfischer, J.P. Valkonen, 2006. Marker-assisted combination of major genes for pathogen resistance in potato. Theor. Appl. Genet. 112, 1458-1464.

Kim H.-J., H.-R. Lee, K.-R. Jo, M. Mortazavian, D.J. Huigen, B. Evenhuis, G. Kessel, R.G. Visser, and E. Jacobsen, 2012. Broad spectrum late blight resistance in potato differential set plants MaR8 and MaR9 is conferred by multiple stacked R genes. Theoretical and Applied Genetics, 124, 5, 923-935.

Rietman H., G. Bijsterbosch, L.M. Cano, H.-R. Lee, J.H. Vossen, E. Jacobsen, R.G.F. Visser, S. Kamoun, and V.G.A. Vleeshouwers, 2011. Qualitative and Quantitative Late Blight Resistance in the Potato Cultivar Sarpo Mira Is Determined by the Perception of Five Distinct RXLR Effectors. MPMI. 25/7, 910–919.

Schwarzfischer A., A. Behn, J. Groth, M. Reichmann, A. Kellermann and Y.S. Song, 2010. Markergestützte Selektion in der praktischen Kartoffelzüchtung -. Erfahrungen und Perspektiven. Bericht über die 60. Arbeitstagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs, 73-76.

Sattarzadeh A., U. Achenbach, J. Lübeck, J. Strahwald, E. Tacke, H.R. Hofferbert, T. Rothsteyn and C. Gebhardt, 2006. Single nucleotide polymorphism (SNP) genotyping as basis for developing a PCR-based marker highly diagnostic for potato varieties with high resistance to *Globodera pallida* pathotype Pa2/3. Mol. Breed. 18, 301-312.

Song Y.S. and A. Schwarzfischer, 2008. Development of STS markers for selection of extreme resistance (*Ry sto*) to PVY and maternal pedigree analysis of extremely resistant cultivars. Am. J. Pot. Res. 85, 159-170.

Zhu S., Y. Li, J.H. Vossen, R.G.F. Visser and E. Jacobsen, 2012: Functional stacking of three resistance genes against *Phytophthora infestans* in potato. Transgenic Res., 21:89–99.

Truberg B., T. Hammann, U. Darsow and H.-P. Piepho, 2009. Empirischer Vergleich verschiedener Methoden zur Reifekorrektur von Daten zum Befall mit Krautfäule (*Phytophthora infestans* (Mont.) de Bary) in Selektionsexperimenten bei der Kartoffel (*Solanum tuberosum* subsp. *tuberosum*). Journal für Kulturpflanzen, 61 (3). 77–81.