Saatgut Österreich

59. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs 2008, 59 – 62
ISBN: 978-3-902559-28-9

Late blight resistance breeding with the potato MT progeny

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Abstract

The rapidly increasing genetic sequence information for Solanum provides a practical means for the design of potato breeding methods that make use of molecular markers. The tetraploid, heterozygous, and inbreeding-susceptible potato requires appropriate efforts to this end. The MF-II x TPS67 cross progeny carrying novel late blight resistance was phenotyped and genotyped to develop molecular markers for selection for its inherent resistance and to facilitate its utilisation in advanced breeding programmes.

The clonal cultivars MF-II and TPS67 were developed at the International Potato Centre. Trialing them in Austria revealed they produce high yield and possess good late blight resistance (LBR). Therefore, we develop genetic maps and markers to efficiently select for the resistance genes inherent of these genetic resources in breeding. Over 200 markers including SSR, CAPS, RFLP, and several other PCR-based types were screened for informativeness, and framework genetic linkage maps were constructed to locate the resistance. TPS67 appears to carry a single major R locus on chromosome IV and MF-II also segregates for a single locus on chromosome XI. It was not known whether these resistance genes can be overcome by extant strains of *Phytophthora infestans*, the causal agent of late blight, how frequently the genes are overcome and whether they would display residual resistance once they are broken down. Besides investigating the resistance phenotype within the MT cross progenies, we have commenced the directed search for markers linked to the resistance genes that could be employed in breeding for blight resistance using these parents. PCR primers for syntenic marker loci already mapped in Solanum were applied and the amplicons directly subjected to sequencing (when 1 or 2 alleles were present) or treated with restrictases. Allele-specific PCR primers were designed on SNP information. Cleaved amplified polymorphic sequence- (CAPS) markers were developed when restrictases yielded segregating products. With this approach we have developed so far molecular markers linked to the LB resistance of MF-II, the search for markers of close linkage to the resistance gene of TPS67 continues. Closely linked crude markers will then be converted to easy-to-use markers for selection in breeding.

Keywords:
Genetic mapping, Molecular marker, *Phytophtora infestans*, potato, *Solanum tuberosum*

Introduction

Late blight is of large economical importance in potato production and efforts are undertaken to increase the plant resistance by widening the genetic base and investigating the molecular bases of the resistance. Current potato cultivars represent a wide range of resistance in the field that covers intermediate phenotypes between the immunity by some „strong“, intact, race-specific, i.e. „vertical“ R proteins, such as those encoded by Black‘s R genes (BLACK et al. 1953) and race-nonspecific „horizontal“ levels of reduced susceptibility under field conditions. While breeders dislike the use of R genes due to their vulnerability to the rapidly adapting pathogen populations, it has been clear that today there is no horizontal resistance known that is strong enough to protect a crop.

Modern molecular genetic research has revealed that R genes are in fact involved in all kinds of true resistance, side by side with a large group of genetic elements contributing to pathogen recognition, signaling, and response. The dynamics of pathogen and host and the flexible tactics used by both of these players parallel with some respect a soccer game with attacking and defending teams and many balls and goals. Therefore it is appropriate to continue research on all elements required for plant resistance, including the R genes, and to identify appropriate means of their sustainable use in the breeding of modern potato cultivars.

Materials and Methods

The tetraploid parental clones MF-II (group tuberosum selection of Indian descent, M. Upadhya, International Potato Centre, unpublished) and TPS67 (group andigena, described in TROGNITZ 1998) were crossed at CIP, Lima, Peru, and at ARC, Austria, to produce offspring segregating for blight resistance. Plants were maintained pathogen-free in vitro and clonal copies were grown in pots and in the field for several trials. Blight resistance was phenotyped by subjecting the individuals of the MT crosses to detached leaflet tests as was described in TROGNITZ (1998), using, in separate, several *P. infestans* isolates varying for the known avirulences and with simple up to highly complex pathotypes. By analysing the patterns of phenotype response to these isolates of every progeny individual in separate, in

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Table 1: **Phenotyping and segregation analysis of late blight resistance in the MT population**

Genotype group	# expected (Lima)	# observed (Lima)	# expected (Austria)	# observed (A)
total MT plants		861		171
RM RT S(RT)	1	131	2	83
RM RT	1	104	-	
RM + RM S(RT)	2	193	1	53
RT S(RT)	1	100	-	
RT	1	94	-	
r1 rT S + r1 rT s	2	217	1	35
unknown		22		
P (Chi-square test)		n.s.		n.s.
inferred parental genotypes	MF-II	R1 r r r SRT s s s	TPS67	RT r r r

combination with segregation analysis for the entire progeny the characteristics and genetic states of inherent factors controlling the blight resistance were discerned. Observed segregation of resistance was fitted to genetic models by applying chi-square tests to contingency tables.

Genetic mapping of the resistance genes was performed using PCR-based markers of various types according to standard protocols. The mapping calculations were done in TetraploidMap (HACKETT and LUO 2003).

The *S. demissum* R1 gene conferring resistance to late blight was searched for with the PCR primers 76-2sf2 and 76-2SR developed by BALLVORA et al. (2002).

Results and Discussion

Determination of resistance factors by segregation analysis

Phenotyping of 861 MT cross segregants at Lima allowed for the distinction of two major R factors, RM from MF-II and RT from TPS67, and an unknown factor from MF-II that suppressed the resistance conferred by RT only in presence of specific *P. infestans* isolates (*Table 1*).

Attempts to detect these genetic elements of blight resistance on a population of 171 individuals phenotyped in Austria led to the tentative identification of plants carrying RM and RT, whereas the suppressor of RT (SRT) could not be observed due to absence of *P. infestans* isolates that invoked its action (*Table 1*, bottom).

In Austria, 80 progenies of the MT cross (by their inferred resistance genotype; 30 RT., 30 RM rT., 20 rM rT) were

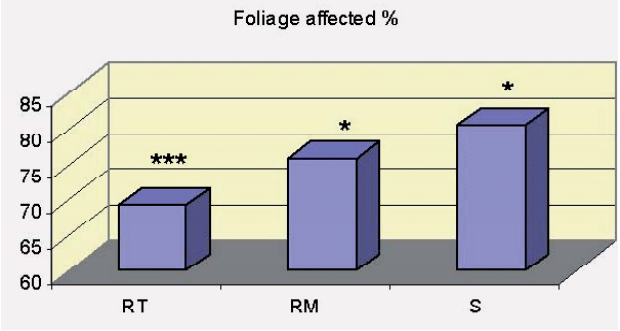

Figure 1: **Effect of R phenotype on LB resistance in the field. Field trial 2005, Meires, Austria. Population MF-II x TPS67 80 progeny (30 RT, 30 RM, 20 S) no fungicide, natural infestation, average of 3 disease readings, percent foliage area affected on 10-plant-plot. * = P<0.05, *** = P<0.001 (t-test and analysis of variance).**

trialed in the field, at Meires, Waldviertel. In a trial in 2003, blight did not develop due to the dry and exonerably warm climate. In a second trial in 2005, using a complete block design with two replications of plots of 10 plants, foliar resistance was determined by the inherent resistance. Although *P. infestans* occurring at the trial site was able to overcome both R genes, both genes developed considerable residual effects that contributed significantly to the resistance observed (*Figure 1*).

A small albeit highly significant residual resistance effect of these two R genes was also measured in the detached leaflet assays using various *P. infestans* isolates as described above. Therefore, the usefulness of the two R genes even when they are overcome by the pathogen, is evident.

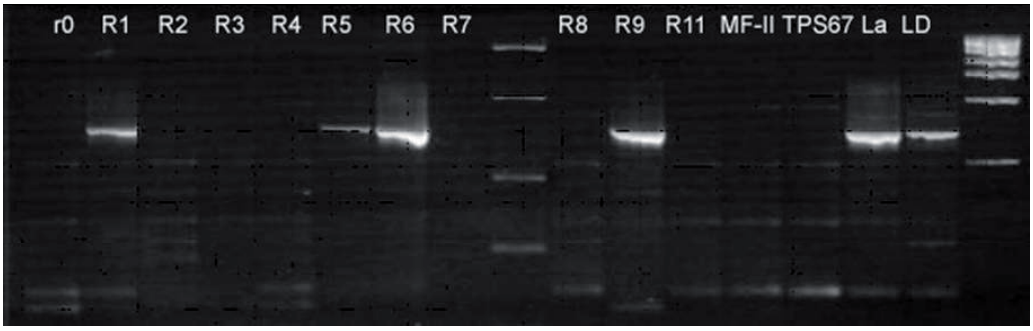

Figure 2: **Amplification of the potato (*S. demissum*) R1 allele conferring resistance to *P. infestans* carrying Avr1, with allele-specific primers 76-2sf2 and 76-2SR (BALLVORA et al. 2002). The R1-fragment is absent in MF-II and TPS67, but present in R1, R5, R6, R9, La (Laura) and LD (Linzer Delikatess).**

Experiments to determine the identity of RM and RT with known R genes

PCR amplification on total DNA from the parental clones MF-II and TPS67 with primers specific for the potato R1 gene (Ballvora et al. 2002) did not produce a product (*Figure 2*) indicating that none of the R genes in the MT population is R1. From the isolates of *P. infestans* used and their virulences we had already inferred that other known R genes were not identical with RM and RT. Therefore, it is likely that both genes are additional to those of the Black's series of R genes.

Genetic mapping of RM and RT

Over 200 PCR primers were tested to obtain markers segregating in the MT population and to construct a raw framework map. The resulting linkage groups were assigned to the consensus Solanum chromosomes by markers derived from consensus sequence (COS II) markers as displayed at the SOL genomics network site; http://www.sgn.cornell.edu/markers/cosii_markers.pl. Resistances RM and RT were applied as qualitative genetic markers on this framework map. RT was mapped to chromosome IV (not shown), whereas RM resides on chromosome XI (*Figure 3*).

Unfortunately, none of the markers found to be linked to the resistance genes segregating in the MT population can be immediately used for marker assisted selection. All markers detected occur in duplex or even triplex and the majority are linked in repulsion to the allele conferring resistance. Therefore, marker fragments are presently cloned and sequenced to develop simplex marker alleles that are linked in coupling to the resistance. When these new marker primers will be available they will be tested in the MT population and descendants thereof as soon as these will be generated by the breeder.

Conclusions

Two novel R genes conferring resistance to late blight have been detected in tetraploid potato accessions and they can be used in breeding and varietal selection. We have demonstrated that these genes even when broken down bring about a residual but significant contribution to resistance in the field. Therefore, these R genes are useful for the enhancement of blight resistance in present time and they can be used in breeding.

Molecular markers for use in selection for these resistance genes are being developed and selection by markers can complement or perhaps even substitute the time-consuming, chance dependent „classical" selection entirely by the phenotype.

Acknowledgements

This work was supported by the BIOEXPLOIT project (6th framework programme, European Commission, FOOD-CT-2005-513959).

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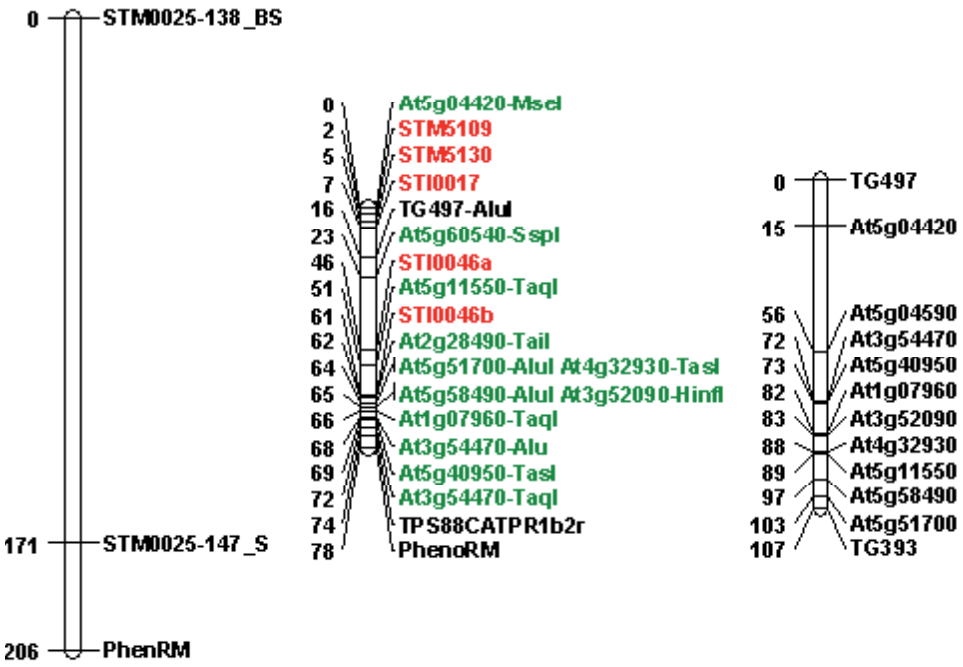

Figure 3: **Map of Chromosome XI for the MT population and localization of the novel gene *Rpi-tbrM1* conferring resistance to late blight. Left; framework map, the resistance marker is indicated as PhenRM. Right; corresponding region of the tomato chromosome XI and location of COS II markers. Centre; planned enrichment of the MT framework map with COS II-derived and other markers.**

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