Breeding Science 67: 416–420 (2017)
doi:10.1270/jsbbs.17035

Note

Early selection of potato clones with resistance genes: the relationship between combined resistance and agronomical characteristics

Dorota Milczarek*, Jarosław Plich, Beata Tatarowska and Bogdan Flis

Plant Breeding and Acclimatization Institute – National Research Institute, Młochów Research Centre, Platanowa 19, 05-831 Młochów, Poland

Cultivating resistant varieties of potato is the most effective and environmentally safe method of protecting against pests and diseases that affect potato crops. Therefore, potato breeding is focused on developing more resistant varieties so that the use of plant health products can be reduced during the cultivation cycle. Resistance to late blight, viruses and nematodes is the most important agricultural requirement. The use of molecular markers allows for the effective selection of resistant genotypes at early stages of breeding. However, the impact of early selection for resistance on the agronomic value of the final selected clones is a cause of concern for breeders. This study investigates the relationship between the presence of the combined resistance genes *H1*, *Ry-f*$_{sto}$ and *Rpi-phu1*, which confer resistance to nematodes, potato virus Y and late blight, respectively, and certain agricultural traits. The agronomic performance of most clones with and without the identified resistance genes was similar in terms of tuber yield, tuber size, tuber shape regularity, eye depth and tuber defect intensity. Some combinations with *Ry-f*$_{sto}$ may produce higher yields but may also be associated with more tuber defects. No negative relationships were observed between the combined resistance genes *H1* + *Ry-f*$_{sto}$ + *Rpi-phu1* and potato quality.

Key Words: *Solanum tuberosum*, breeding, MAS, combined resistance, quality.

Introduction

Potato (*Solanum tuberosum* L.) is among the most important food crops worldwide, which also include wheat, maize and rice. Potatoes are consumed in many different forms, including as chips, French fries and other food products. Potatoes are also used for the industrial production of starch and alcohol and the production of animal feed. Unfortunately, potatoes are also the target of many pests and diseases caused by bacteria, fungi, viruses or mycoplasmas. Among the biotic stresses of potato, late blight, viruses and nematodes are the most devastating. In addition to causing yield losses, these pathogens can also cause tuber defects, which can render the tubers unsalable. Late blight, which is caused by the oomycete *Phytophthora infestans*, is generally the most important disease wherever potatoes are grown and may suppress up to 40 to 50% of the attainable yield (Shtienberg *et al.* 1990). *P. infestans* can also cause necrosis on tubers, which promotes the growth of other pathogens and leads to rotting of tubers in storage. Among the many viruses that infect potatoes, potato virus Y (PVY) is currently the most concerning virus, and it causes losses of up to 80% (Van der Zaag 1987). Moreover, isolates of PVY induce severe necrosis on tubers. In the case of nematodes, *Globodera* spp. causes a yield losses of up to 50% (Nicol *et al.* 2011).

Many resistance genes have been obtained from wild *Solanum* species and have been introduced into subsequent breeding programs. Among these genes, the *Ry-f*$_{sto}$ gene confers extreme resistance to PVY and originates from the wild potato species *Solanum stoloniferum* (Flis *et al.* 2005). The most frequently used source of resistance against nematodes is *S. tuberosum* ssp. *andigena*, in which the *H1* gene for resistance to pathotypes Ro1 and Ro4 of *Globodera rostochiensis* was identified (Ellenby 1952). The sources of resistance to late blight have been identified in many *Solanum* species, and *S. demissum* is the most common source. However, resistance controlled by the part of R genes from this species is easily overcome by new pathotypes of *P. infestans* (Allefs *et al.* 2005). A new promising source of resistance to late blight has been recently identified in *S. phureja*, in which the *Rpi-phu1* gene was identified (Śliwka *et al.* 2006).

Molecular markers linked to the loci of interest can be used in potato breeding for the selection of resistant

Communicated by Hiroyoshi Iwata
Received April 5, 2017. Accepted June 18, 2017.
First Published Online in J-STAGE on August 8, 2017.
*Corresponding author (e-mail: d.milczarek@ihar.edu.pl)

genotypes. Markers 57R, GP122 and phu6 have been demonstrated as suitable for the selection of clones with genes *H1*, *Ry-f$_{sto}$* and *Rpi-phu1*, respectively (Flis *et al.* 2005, Milczarek *et al.* 2014, Śliwka *et al.* 2013). Instead of phenotypic evaluation, DNA markers can be used in the selection of resistant clones in early generations. Screening for resistance in early clonal generations is a cost-effective and efficient method of reducing the time required to create a new variety (Slater *et al.* 2013). However, this approach raises question about the impact of early selection for resistance on the agronomic value of the final resistant individuals. The use of resistance genes from wild species is typically accompanied by the introduction of unfavorable traits or low-quality traits. Although such adverse features are typically removed during pre-breeding and breeding, unfavorable correlations still occur in the potato germplasm. For example, the resistance to late blight derived from *S. demissum* is associated with lateness (Beketova *et al.* 2006).

The cultivation of resistant cultivars is considered the most economically effective and environmentally safe method of potato crop protection, and understanding the relationships between the resistance that is introduced into varieties and the quality of the observed traits is crucial for breeding new outstanding cultivars. This relationship is especially important as a guide for selection in early generations via the use of molecular markers linked to resistance genes. Furthermore, if the cultivar is bred to include combined resistance, then the relationship between the accumulated genes for various resistances and the agronomical characteristics must be assessed. The aim of this work was to determine the relationship between the presence of *Rpi-phu1*, *Ry-f$_{sto}$* and *H1* genes and the level of potato agronomical characteristics.

Materials and Methods

Plant materials

A total of 208 selections from 3 crosses of the breeding program performed at the Młochów Research Centre were screened using markers of the resistance genes present in their parents (**Table 1**). Clones were also evaluated for agronomic traits (yield, tuber appearance and general plant health) in field experiments.

Diagnostic PCR marker assays

The tested clones were evaluated for the presence of markers 57R, GP122 and phu6, which are linked to *H1*, *Ry-f$_{sto}$ and Rpi-phu1*, respectively. Total genomic DNA was extracted from leaves using the GenElute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO, USA). The PCR amplification of markers 57R, GP122 and phu6 was performed according to the methods of Finkers-Tomczak *et al.* (2011), Witek *et al.* (2006) and Śliwka *et al.* (2013), respectively.

Table 1. Crosses and progenitors, including their resistance genes and the number of evaluated progeny genotypes

Progeny	Female parent	Resistance genes	Male parent	Resistance genes	Number of progeny clones
I	Batja	*H1*	04-IX-4	*Rpi-phu1*	51
II	PS 1761	*Ry-f$_{sto}$*	04-IX-21	*Rpi-phu1*	83
III	TG 97-403	*Rpi-phu1*	PS 1763	*H1*, *Ry-f$_{sto}$*	74
				Total	208

Screening for agronomic traits

The plant material was grown in the field experiment to the 2nd and 3rd clonal generations in 2014 and 2015 (progeny III) or to the 2nd, 3rd and 4th clonal generations in 2012, 2013 and 2014 (progeny I and II). The experimental fields were located in a central region of Poland in Młochów. The experimental fields were free from *Globodera* spp. Full chemical protection against *P. infestans* and potato beetle was used. Chemical protection against aphids was not applied, but infestation of plants with viruses was not observed. The clones were grown in 7-hill plots that were planted at the end of April and harvested in mid-September. Each clone was planted in duplicate (2 × 7-hill plots). The following agronomic traits were evaluated: tuber yield (kg per plant), tuber size, tuber shape regularity and eye depth, which were assessed according to a 9-grade scale (9 = the largest size, the most regular shape or the shallowest eyes), and tuber defects, which were assessed using a 4-grade scale (1 = high intensity of important defects, such as sprouting, stolons or secondary growth; 2 = low intensity of important defects or less important defects, such as cracked skin and/or fat skin and/or skin with symptoms of diseases (i.e., scab or black speck); 3 = few minor defects; 4 = no defects).

Statistical analyses

The mean values of groups of clones with and without one, two or three markers of resistance genes were compared using an ANOVA and Tukey's multiple range test or the Kruskal–Wallis test (in the case of nonparametric data for tuber defects). All of the statistical analyses were performed with the STATISTICA data analysis software system, version 10 (www.statsoft.com).

Results

Gene identification

The number of clones with the identified resistance genes, confirmed by the amplification of the linked markers in the tested progenies is presented in **Table 2**. Marker 57R was amplified for 64 out of 125 tested genotypes. Marker GP122 was amplified for 79 out of 157 tested genotypes. A total of 208 progeny genotypes were evaluated for the presence of the marker phu6, which is linked to *Rpi-phu1*. This marker was amplified for 102 tested genotypes.

Agronomic trait assessment

The mean values of the agronomic traits of evaluated

Table 2. Number of clones with the identified resistance genes *H1*, *Ry-f$_{sto}$* and *Rpi-phu1*

Identified resistance genes	Batja × 04-IX-4	PS 1761 × 04-IX-21	TG 97-403 × PS 1763	Σ
H1	11	–	10	21
Ry-f$_{sto}$	–	20	8	28
Rpi-phu1	14	24	7	45
H1 + *Ry-f$_{sto}$*	–	–	13	13
H1 + *Rpi-phu1*	12	–	7	19
Ry-f$_{sto}$ + *Rpi-phu1*	–	21	6	27
H1 + *Ry-f$_{sto}$* + *Rpi-phu1*	–	–	11	11
none	14	18	12	44

progenies are presented in **Table 3**. The mean values and ranges for the evaluated traits of the clones with and without the identified resistance genes are presented in **Tables 4–6**.

Clones from the progeny II that only included gene *Ry-f$_{sto}$* or with the combinations of genes *Ry-f$_{sto}$* and *Rpi-phu1* and the clones with the combination of genes *H1* and *Ry-f$_{sto}$* from progeny III had higher yield than clones without identified resistance genes (**Tables 5**, **6**). All other clones with identified resistance genes presented yields similar to those of the clones without identified resistant genes (**Tables 4–6**).

Table 3. Mean values and standard deviations of the agronomic traits in the evaluated progenies

Progeny	Cross	Total tuber yield (kg/plant)	Tuber size[a]	Regularity of tuber shape[a]	Eye depth[a]	Defects of tubers[b]
I	Batja × 04-IX-4	1.0 ± 0.5	4.7 ± 1.9	6.0 ± 0.6	5.2 ± 1.9	3.2 ± 1.0
II	PS 1761 × 04-IX-21	1.2 ± 0.6	4.5 ± 1.6	6.1 ± 0.6	5.5 ± 1.3	2.8 ± 1.1
III	TG 97-403 × PS 1763	1.1 ± 0.4	5.2 ± 1.2	6.3 ± 0.7	6.3 ± 0.7	3.9 ± 0.4

[a] Nine-grade scale (9 = the largest size, the most regular shape, the shallowest eyes);
[b] Four-grade scale (1 = high intensity of serious defects, 4 = no defects).

Table 4. Mean values and standard deviations of agronomic traits of clones with and without the identified resistance genes (progeny I: Batja × 04-IX-4)

Presence of resistance genes	Total tuber yield (kg/plant)	Tuber size[a]	Regularity of tuber shape[a]	Eye depth[a]	Defects of tubers[b]
H1	0.92 ± 0.41 A[c]	4.7 ± 1.8 A	6.0 ± 0.5 A	5.3 ± 1.8 A	3.3 ± 1.0 AB
Rpi-phu1	1.04 ± 0.42 A	4.6 ± 1.9 A	6.0 ± 0.6 A	5.2 ± 1.8 A	3.5 ± 0.8 A
H1 + Rpi-*phu1*	0.97 ± 0.49 A	4.9 ± 1.7 A	6.0 ± 0.6 A	5.0 ± 2.0 a	3.1 ± 1.1 AB
none	1.04 ± 0.48 A	4.8 ± 2.0 A	5.9 ± 0.7 A	5.2 ± 1.8 A	2.9 ± 1.0 B

[a,b] See the footnotes to **Table 3**.
[c] Mean values with the same letter do not differ at p = 0.05.

Table 5. Mean values and standard deviations of the agronomic traits of clones with and without the identified resistance genes (cross II: PS 1761 × 04-IX-21)

Presence of resistance genes	Total tuber yield (kg/plant)	Tuber size[a]	Regularity of tuber shape[a]	Eye depth[a]	Defects of tubers[b]
Ry-f$_{sto}$	1.33 ± 0.64 A[c]	4.6 ± 1.8 a	6.1 ± 0.6 A	5.5 ± 1.2 A	2.6 ± 1.0 B
Rpi-phu1	1.08 ± 0.45 B	4.5 ± 1.5 A	6.2 ± 0.6 A	5.5 ± 1.3 A	2.9 ± 1.1 A
Ry-f$_{sto}$ + *Rpi-phu1*	1.37 ± 0.54 A	4.3 ± 1.4 A	6.0 ± 0.6 A	5.4 ± 1.2 A	2.9 ± 1.1 AB
none	0.98 ± 0.50 B	4.4 ± 1.7 A	6.2 ± 0.5 A	5.7 ± 1.2 A	3.0 ± 1.0 A

[a,b,c] See the footnotes to **Tables 3** and **4**.

Table 6. Mean values and standard deviations of the agronomic traits of clones with and without the identified resistance genes (cross III: TG 97-403 × PS 1763)

Presence of resistance genes	Total tuber yield (kg/plant)	Tuber size[a]	Regularity of tuber shape[a]	Eye depth[a]	Defects of tubers[b]
H1	0.85 ± 0.27 CD[c]	5.0 ± 1.2 a	6.5 ± 0.8 AB	6.5 ± 0.6 A	3.9 ± 0.4 A
Ry-f$_{sto}$	1.06 ± 0.46 BCD	5.2 ± 1.1 A	5.9 ± 0.7 B	6.0 ± 0.8 A	3.8 ± 0.7 A
Rpi-phu1	0.94 ± 0.37 BCD	4.8 ± 1.0 A	6.4 ± 0.5 ab	6.5 ± 0.4 A	3.9 ± 0.5 A
H1 + *Ry-f$_{sto}$*	1.26 ± 0.46 AB	5.2 ± 1.3 A	6.2 ± 0.7 AB	6.1 ± 0.7 A	3.8 ± 0.5 A
H1 + *Rpi-phu1*	0.78 ± 0.33 D	5.0 ± 1.3 A	6.8 ± 0.6 A	6.6 ± 0.5 A	3.9 ± 0.5 A
Ry-f$_{sto}$ + *Rpi-phu1*	1.54 ± 0.40 A	5.5 ± 0.9 A	6.1 ± 0.5 ab	6.1 ± 0.6 A	4.0 ± 0.0 A
H1 + *Ry-f$_{sto}$* + *Rpi-phu1*	1.16 ± 0.32 ABC	5.4 ± 1.3 A	6.3 ± 0.7 ab	6.2 ± 0.7 A	3.9 ± 0.4 A
none	0.88 ± 0.27 CD	5.3 ± 1.3 A	6.4 ± 0.8 AB	6.4 ± 0.6 A	4.0 ± 0.2 A

[a,b,c] See the footnotes to **Tables 3** and **4**.

All of the clones with and without the identified resistance genes had similar tuber sizes, tuber shape regularity and eye depths. Clones from the progeny II that only included gene *Ry-f*$_{sto}$ exhibited more tuber defects than the clones without identified resistant genes (**Table 5**). This relationship was not observed for clones from progeny III (**Table 6**). Clones from the progeny I that only included gene *Rpi-phu1* exhibited less tuber defects than the clones without identified resistant genes (**Table 4**). All other clones with identified resistance genes presented similar tuber defects as the clones without identified resistant genes (**Tables 4–6**).

Discussion

Cultivating resistant varieties of potatoes is the most economically effective and environmentally safe method of protecting against pests and diseases that affect potato crops. However, breeding for resistance is not the primary goal of breeding programs, which is breeding for quality. Although the relationship between high levels of various resistance phenotypes and reduced levels of agronomical characteristics has not been previously reported, there is a belief that the accumulation of resistance genes may result in reduced quality. Moreover, introducing high resistance to viruses may cause a reduction in the frequency of seed potato exchange by potato growers, which will ultimately lead to the spread of soil-borne pathogens. However, reports have indicated that the demand for resistant varieties has increased among ecological and starch potato growers (Plich *et al.* 2015).

Molecular markers can be used to screen large populations at early stages of breeding, which increases the effectiveness of selecting for resistant genotypes (Barone 2004). Markers for genes that provide resistance to viral diseases and nematodes are recommended for extensive use in potato breeding programs (Asano and Tamiya 2016). Marker-assisted selection (MAS) using PCR-based diagnostic assays was used by Gebhardt *et al.* (2006) to select clones harboring the resistance genes *Ry*$_{adg}$, *Gro1*, *Rx1* and *Sen1*, which provide resistance to PVY, nematodes, PVX and potato wart (*Synchytrium endobioticum*), respectively.

Markers 57R, GP122 and phu6 have been demonstrated as suitable for the selection of clones with genes *H1*, *Ry-f*$_{sto}$ and *Rpi-phu1*, respectively (Flis *et al.* 2005, Milczarek *et al.* 2014, Śliwka *et al.* 2013). Potato clones with various combinations of resistance genes, i.e., *H1* + *Ry-f*$_{sto}$, *H1* + *Rpi-phu1*, *Ry-f*$_{sto}$ + *Rpi-phu1* and *H1* + *Ry-f*$_{sto}$ + *Rpi-phu1*, were selected via the use of these molecular markers. It is unclear how obligatory selection for resistance may influence a decrease in the agronomic value of selected resistant progeny. This can limit the use of markers in the initial phase of selection due to the concern of rejection of valuable genotypes. In this study, the agronomic traits in clones without identified resistance genes were compared with the traits observed in clones selected for the presence of identified resistance genes, and the results indicate that a negative relationship does not occur between the presence of the combined resistance genes and agronomical characteristics within the tested groups of clones. The agronomic performance of almost all of the clones with the identified resistance genes was similar to that of the clones without resistance genes with regard to the tuber yield, tuber size, regularity of tuber shape, eye depth and tuber defect intensity. Clones from the progeny II with the single gene *Ry-f*$_{sto}$ or with the genes *Ry-f*$_{sto}$ + *Rpi-phu1* and the clones with the genes *H1* + *Ry-f*$_{sto}$ from progeny III had higher yields than clones without identified resistance genes. However, this finding was not observed for the clones with the single gene *Ry-f*$_{sto}$ or with the genes *H1* + *Ry-f*$_{sto}$ + *Rpi-phu1* from progeny III. Clones from cross II with the single gene *Ry-f*$_{sto}$ exhibited more tuber defects than the clones without identified resistant genes, but this finding was not observed for the clones with the single gene *Ry-f*$_{sto}$ from cross III or for the clones with combinations of genes *Ry-f*$_{sto}$ + *H1*, *Ry-f*$_{sto}$ + *Rpi-phu1* and *Ry-f*$_{sto}$ + *H1* + *Rpi-phu1* from crosses II and III. It is known that combining ability is an important factor in potato breeding (Gopal 1998); thus, these relationships appear to be related to the influence of the combination of many genes and not the presence of resistance genes. The findings show that some combinations with the gene *Ry-f*$_{sto}$ may produce higher yields but may also be associated with more tuber defects. However, no negative relationship was observed between the presence of the combined resistance genes *H1* + *Ry-f*$_{sto}$ + *Rpi-phu1* and the quality of the potatoes. In conclusion, the early selection for combined resistance to late blight, PVY and nematodes does not adversely impact the agronomic value of final resistant selections.

Acknowledgements

This study was partly supported by the Polish Ministry of Science and Higher Education/National Science Centre (project number 1-3-00-7-02).

Literature Cited

Allefs, J.J.H.M., M.W.M. Muskens and E.A.G. van der Vossen (2005) Breeding for foliage blight resistance in the genomics era. *In*: Haverkort, A.J. (ed.) Potato in progress: science meets practice. Wageningen Academic Publishers, The Netherlands, pp. 255–267.

Asano, K. and S. Tamiya (2016) Breeding of pest and disease resistant potato cultivars in Japan by using classical and molecular approaches. JARQ 50: 1–6.

Barone, A. (2004) Molecular marker-assisted selection for potato breeding. Am. J. Potato Res. 81: 111–117.

Beketova, M.P., P.E. Drobyazina and E.E. Khavkin (2006) The *R1* gene for late blight resistance in early and late maturing potato cultivars. Russ. J. Plant Physiol. 53: 384–389.

Ellenby, C. (1952) Resistance to the potato root eelworm, *Heterodera rostochiensis* Wollenweber. Nature 170: 1016.

Finkers-Tomczak, A., E. Bakker, J. de Boer, E. van der Vossen, U. Achenbach, T. Golas, S. Suryaningrat, G. Smant, J. Bakker and A. Goverse (2011) Comparative sequence analysis of the potato

cyst nematode resistance locus *H1* reveals a major lack of co-linearity between three haplotypes in potato (*Solanum tuberosum* ssp.). Theor. Appl. Genet. 122: 595–608.

Flis, B., J. Hennig, D. Strzelczyk-Żyta, C. Gebhardt and W. Marczewski (2005) The *Ry-f*$_{sto}$ gene from *Solanum stoloniferum* for extreme resistant to *Potato virus Y* maps to potato chromosome XII and is diagnosed by PCR marker GP122$_{718}$ in PVY resistant potato cultivars. Mol. Breed. 15: 95–101.

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

Gopal, J. (1998) General combining ability and its repeatability in early generations of potato breeding programmes. Potato Res. 41: 21–28.

Milczarek, D., A. Przetakiewicz, P. Kamiński and B. Flis (2014) Early selection of potato clones with the *H1* resistance gene—the relation of nematode resistance to quality characteristics. Czech J. Genet. Plant Breed. 50: 278–284.

Nicol, J.M., S.J. Turner, D.L. Coyne, L. den Nijs, S. Hockland and Z. Tahna Maafi (2011) Current nematode threats to world agriculture. *In*: Jones, J., G. Gheysen and C. Fenoll (eds.) Genomics and molecular genetics of plant-nematode interactions, Springer, Berlin, pp. 21–43.

Plich, J., B. Tatarowska and B. Flis (2015) Evaluation of selected Polish potato cultivars and breeding lines regarding their resistance against *Phytophthora infestans* and usefulness for organic farming. Progress in Plant Protection 55: 452–457.

Shtienberg, D., S.N. Bergeron, A.G. Nicholson, W.E. Fry and E.E. Ewing (1990) Development and evaluation of a general model for yield loss assessment in potatoes. Phytopathology 80: 466–472.

Slater, A.T., N.O.I. Cogan and J.W. Forster (2013) Cost analysis of the application of marker-assisted selection in potato breeding. Mol. Breed. 32: 299–310.

Śliwka, J., H. Jakuczun, R. Lebecka, W. Marczewski, C. Gebhardt and E. Zimnoch-Guzowska (2006) The novel, major locus *Rpi-phu1* for late blight resistance maps to potato chromosome IX and is not correlated with long vegetation period. Theor. Appl. Genet. 113: 685–695.

Śliwka, J., M. Świątek, I. Tomczyńska, E. Stefańczyk, M. Chmielarz and E. Zimnoch-Guzowska (2013) Influence of genetic background and plant age on expression of the potato late blight resistance gene *Rpi-phu1* during incompatible interactions with *Phytophthora infestans*. Plant Pathol. 62: 1072–1080.

Van der Zaag, D.E. (1987) Yield reduction in relation to virus infection. *In*: de Bokx, J.A. and J.P.H. van der Want (eds.) Viruses of Potatoes and Seed-Potato Production, Pudoc, Wageningen, pp. 146–150.

Witek, K., D. Strzelczyk-Żyta, J. Hennig and W. Marczewski (2006) A multiplex PCR approach to simultaneously genotype potato towards the resistance alleles *Ry-f sto* and *Ns.* Mol. Breed. 18: 273–275.