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USE OF SOMATIC HYBRIDISATION TO TRANSFER RESISTANCE TO LATE BLIGHT AND *POTATO VIRUS Y* (PVY) INTO CULTIVATED POTATO

ABSTRACT

Protoplast fusion was used to produce more than 500 symmetric interspecific somatic hybrids between wild *Solanum* species, which belong to the series *Pinnatisecta*, *Etuberosa* and *S. tuberosum* L. cultivars or potato breeding clones. The used genebank accessions of *S. cardiophyllum* and *S. tarnii* were resistant to *Phytophthora infestans,* while the accessions of *S. cardiophyllum*, *S. tarnii*, as well as of *S. etuberosum*, were also highly resistant to PVY (strains: PVY^{O}, PVY^{N}, PVY^{NTN}, PVY^{C}, PVY^{N} Wilga). In most fusion combinations vigorous and genetically stable hybrid material has been selected. A number of interspecific somatic hybrids were fertile and could be backcrossed with cultivated potato. The use of detached leaf and tuber tests and mechani–cal inoculation, grafting and virus transmission by vectors, revealed that several somatic hybrids and backcross (BC) clones were resistant to foliage and tuber blight, as well as to PVY.

Key words: Phytophthora infestans, protoplast fusion, PVY, resistance, wild *Solanum* species

INTRODUCTION

Wild potato species provide potato breeders with a valuable resource of resistance to pests and diseases (Hawkes 1990). The use of exotic germplasm could be important for increasing the resistance gene pool of cultivated potato. Genetic barriers, such as partial sexual incompatibil–ity, embryo abortion and hybrid sterility, restrict the introduction of foreign germplasm into the gene pool of potato. The symmetric somatic hybridisation, *via* cell fusion, can be used to combine the genomes of two

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parental plants of the same or different plant species without meiotic segregation. Therefore, the method is a useful tool for overcoming sexual barriers. After division of the fusion products and callus formation, plants are regenerated. These are then evaluated for resistance to the major potato diseases and pests, as well as for agronomic traits (Millam *et al.* 1995).

Solanum cardiophyllum (*cph*) Lindl., *S. tarnii* (*trn*) Hawkes et Hjerting, and *S. etuberosum* (*etb*) Lindl., of the series *Pinnatisecta* and *Etuberosa* (Hawkes 1990), respectively, are an important source of resistance to biotic and abiotic stresses. *S. etuberosum* shows resistance to important viral diseases (Valkonen *et al.* 1992b, Thieme *et al.* 2000), aphids (Valkonen *et al.* 1992a, Thieme and Thieme 1998) and bacterial diseases, and *S. cardiophyllum* to bacterial diseases (black leg) caused by *Erwinia* spp., fungal diseases (late blight) caused by *P. infestans* (Hanneman and Bamberg 1986) and nematodes (Hawkes 1990). Because of its high resistance to late blight and PVY, *S. tarnii* should also be of interest to potato breeders (Thieme *et al.* 2003). The described wild species are diploid (2n=2x=24), 1 endosperme balance number (EBN) species, which are extremely difficult to be directly crossed with potato. We have been using them for several years for breeding purposes by employing somatic hybridisation and other biotechnological methods.

MATERIALS AND METHODS

Plants of accessions of *S. cardiophyllum* (*cph*), GLKS 108 and *S. tarnii* (*trn*), GLKS 2870 from IPK Genebank External Branch "North", Groß Lüsewitz, Germany, and of *S. etuberosum* (*etb*), k–9141 from VIR, St. Petersburg, Russia, were propagated *in vitro* on MS medium. Potato cvs Agave and Delikat, as well as dihaploid clone 67 and breeding clone 158, which have important agronomic traits, were also cultivated *in vitro*. Clone 158 is highly resistant to late blight. Pollen of field–grown plants of breeding clone 90N and cvs Delikat and Sonate was used in backcrosses of selected somatic hybrids under greenhouse conditions.

Symmetric protoplast fusion was used to produce somatic hybrids between wild species and potato cultivars or breeding clones, according to the methodology described previously (Thieme *et al.* 1997). The hybrids were confirmed by estimating their ploidy level using flow cytometry and SSR analysis of the regenerants (Dinu and Thieme 2001).

For assessing resistance to late blight, a leaf test was performed (Darsow *et al.* 1988). Leaflets were detached from greenhouse–grown plants and drop–inoculated with a highly aggressive isolate of *P. infestans* (complex virulence spectrum: v1–11). A tuber slice test was used for determining the tuber resistance to late blight (Darsow 1987, 1991). For electron microscopy, the leaves of cv. Delikat, wild species *trn* and a somatic hybrid between *trn* and cv. Delikat were inoculated with zoospore suspension of *P. infestans*. The inoculated leaves were sampled

1, 2 and 3 days after inoculation and processed as described by Kang (1996).

To evaluate resistance to PVY, greenhouse–grown plants were mechanically inoculated with the virus. For grafting, tobacco plants and *in vitro* plantlets were used as PVY–infected recipients and scions, respectively. The grafted plants were grown for four weeks at 20˚C. The newly developed plant parts were sampled and tested by ELISA. The wild species were tested for PVY resistance using strains of PVY^{O}, PVY^{N}, PVY^{NTN}, PVY^{C} and PVY^{N} Wilga by J. Schubert, Institute of Resistance Research and Pathogen Diagnostics, Aschersleben, Germany.

The BC clones were produced by embryo and seed culture (Thieme 1991).

RESULTS AND DISCUSSION

Plants of selected accessions of *S. cardiophyllum*, *S. tarnii* and *S. etuberosum* were tested to confirm their high resistance to late blight and PVY. All of them displayed high resistance to PVY (strains: PVY^{O}, PVY^{N}, PVY^{NTN}, PVY^{C}, PVY^{N} Wilga). *Cph* and *trn* also exhibited a high level of resistance to *P. infestans,* with scores of between 7 and 9 after single leaf and tuber tests.

Electrofusion of mesophyll protoplasts of wild species (*cph*, *trn* and *etb*) and *S. tuberosum* accessions (cvs Agave and Delikat, breeding clones 67 and 158) yielded more than 500 somatic hybrids (Table 1). The hybrids were confirmed by molecular marker analysis (Fig. 1).

Table 1

Results of somatic hybridisation and backcrossing

Combination	Somatic hybrids (*n*)	Backcross generation (BC)
S. cardiophyllum (+) cv. Agave	73	–
S. cardiophyllum (+) cv. Delikat	77	–
S. tarnii (+) cv. Delikat	68	BC_1
S. etuberosum (+) clone 158	6	BC_1
S. etuberosum (+) clone 67	120	BC_1, BC_2, BC_3
S. etuberosum (+) cv. Agave	54	–
S. etuberosum (+) cv. Delikat	117	BC_1

The tests indicated that 24 *trn* (+) cv. Delikat hybrids were resistant to PVY and foliage blight. Several of these hybrids were fertile and had a better tuber shape when grown under field conditions than had the *etb* (+) potato hybrids.

In order to improve the agronomic traits of the *trn* (+) cv. Delikat and *etb* (+) breeding clone 67 somatic hybrids, the first and third backcross generations, respectively, were produced by seed and embryo culture (Table 1).

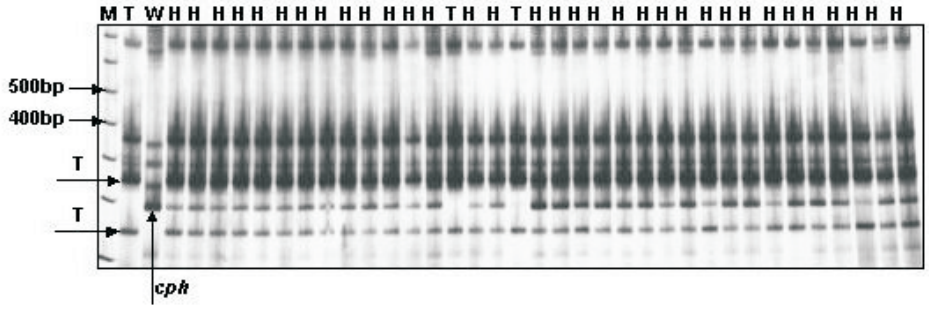

Fig. 1. Use of fragment analysis of the *def4* gene associated SSR marker $(AT)_n$ to select 33 somatic hybrids after protoplast fusion of *S. cardiophyllum* (+) cv. Delikat, Size marker (M), characteristic bands of cv. Delikat (T), *S. cardiophyllum* (W), the somatic hybrids (H)

Somatic hybrids of *etb* (+) clone 158, which showed no infection with PVY after mechanical inoculation, were backcrossed with cv. Sonate. Among the 108 BC_1 clones, 28 displayed resistance to foliage blight with scores of ≥7 (Table 2, Fig. 2). After assessing the resistance to late blight by a tuber slice test, 19 out of 91 clones were classified as resistant (scores: 6.1–8.0) and 5 as highly resistant (scores: 8.1–9.0; Table 2). These preliminary data were confirmed by further greenhouse and field tests.

Table 2

Evaluation of BC_1 clones of the somatic hybrid *etb* (+) clone 158 for resistance to foliage and tuber blight (9 – resistant, 1 – susceptible, *second test)

Genotype	Foliage blight score	Genotype	Tuber blightscore
clone 158	9.0	clone 158	7.6
S. etuberosum	4.4	*S. etuberosum*	non tuber–bearing
cv. Sonate	5.1	cv. Sonate	5.3
BC_1 clones		BC_1 clones	
n=73	<7	n = 1	1.0 – 2.0
n=35	≥7	n = 24	2.1 – 4.0
n=7*	<7	n = 42	4.1 – 6.0
n=28*	≥7	n = 19	6.1 – 8.0
		n = 5	8.1 – 9.0

Electron–microscopical observations of *P. infestans*–infected leaves from the parental genotypes (cv. Delikat, *trn*) and the somatic hybrid revealed differences both in the development of the oomycete and in the host response. There were more hyphae and haustoria in the leaves of

the susceptible potato cultivar than in those of either *trn* or the hybrid. The host cell response to the pathogen was different in the somatic hybrid than in cv. Delikat: the large papilla and encasement were often detected in *trn* and the hybrid.

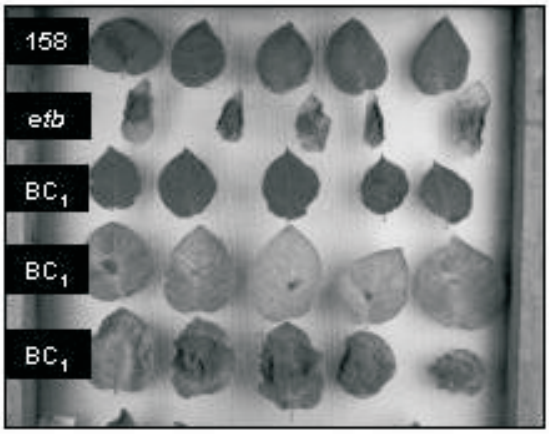

Fig. 2. Different responses of the late blight (LB) resistant potato clone 158, LB susceptible wild species *S. etuberosum* (donor of PVY resistance) and three BC_1 clones of the somatic hybrid *etb* (+) clone 158 to *P. infestans* in a single leaf test

Interspecific somatic hybrids and their progenies could result in the production of new breeding clones expressing a higher resistance to late blight and viruses (Helgeson *et al.* 1998, Novy *et al.* 2002). Furthermore, the use of such material could facilitate the study of the mechanisms and genetic basis of the resistance to pathogens.

CONCLUSIONS

- Genebank accessions of the wild species *S. cardiophyllum* and *S. tarnii* exhibited resistance to *P. infestans*. Besides, *S. cardiophyllum*, *S. tarnii* and *S. etuberosum* displayed high resistance to PVY (strains: PVY^O, PVY^N, PVY^{NTN}, PVY^C, PVY^N Wilga).
- Resistance to foliage blight and PVY was transferred from the wild species and highly resistant breeding clones to somatic hybrids and their progenies.
- Interspecific symmetric protoplast fusion as a routine method is used for the transfer of possibly novel diseases resistance genes from wild species into cultivated potato.
- Further electron–microscopic studies of the ultrastructure of potato genotypes susceptible and resistant to *P. infestans* are needed for a better understanding of the relations between host and pathogen.
- Field trials are important for estimating resistance and agronomic traits of somatic hybrids and their progenies for breeding purposes.

- Somatic hybridisation is a valuable tool for increasing the genetic diversity of potato breeding material and can usefully complement classical breeding programs.
- Interspecific somatic hybrids provide an interesting material for fundamental research on genome interactions, mechanisms of introgression and genetic control of resistance traits.

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