Modern approaches to the breeding of potato (*Solanum tuberosum* L.)

E. Tacke
H. Uhrig
W. Rohde
C. Gebhardt
F. Salamini
Max-Planck-Institut für Züchtungsforschung
Abteilung Pflanzenzüchtung und Ertragsphysiologie
Köln, Germany

1. Selection and use of somatic hybrids

In the genus *Solanum*, somatic hybrid combinations have been achieved between *S. tuberosum* and *S. brevidens* (Austin et al., 1985a; Fish et al.; 1988; Pehu et al., 1989; Preiszner et al., 1991), *S. nigrum* (Binding et al., 1982), *S. chacoense* (Butenko and Kuchko, 1980), *S. phureja* (Matteij et al., 1992; Pijnacker et al., 1987; Puite et al., 1986), *S. berthaultii* (Seraaf et al., 1991), *S. circaeifolium* (Matteij and Puite, 1992) and *S. bulbocastanum* (Austin et al., 1993; Helgeson et al., 1986). The application of this technology has been limited, because methods of identifying and selecting hybrid cells are still inefficient. In a few cases biochemical or morphological markers have enabled direct selection of hybrids (Cocking et al., 1977; Lo Schiavo et al., 1983; Masson et al., 1989). The identification of hybrid plants carried out without biochemical selection is time consuming because of the need to screen large populations. Selection at the cellular level circumvents the problem: for example, somatic hybrids have been regenerated from cells chosen by a micromanipulator (Waara et al., 1989), in some cases based on a dual fluorescent labelling of the heterokaryons (Waara et al., 1989). Flow cytometry has also facilitated direct identification of the somatic cells (Afonso et al., 1985; Hammatt et al., 1990).

We have employed a magnetic cell sorter (Miltenyi et al., 1990) (MACS) designed for animal cells to enrich a specific elution fraction with protoplasts derived from fusions between genetically distinct potato strains (Dörr et al., 1994). Magnetic cell sorting is based on the binding of an antibody to the cell membrane, to which superparamagnetic microbeads can be attached. We

substituted the antibody with biotinylated lectins, which bind microbeads through the mediation of streptavidin. Glycosyl groups of the plasmalemma of a fusion partner are first labelled with a lectin and subsequently with magnetic microbeads. These marked protoplasts are recovered in the magnetic fraction of the MACS. The second partner possesses the dominant selectable marker neomycinphosphotransferase II (NPT II) conferring kanamycin resistance. When the protoplasts from both partners are mixed and a fusion treatment is applied, hybrid cells should be lectin labelled and resistant to kanamycin (Fig. 1). Three selected lectins were used as protoplast binders at a concentration of 100 µg/ml in all experiments. ConA and the *Pisum sativum* lectin were found to be capable of binding *S. tuberosum* protoplasts without altering protoplast vitality or leading to agglutination. For *S. bulbocastanum*, only *Wisteria floribunda* lectin was effective. The best medium used was based on VKM-salts (Binding and Nehls, 1977) and contained sorbitol up to an osmolality of 700 mOsm. The mean frequency of regeneration for calli derived from MACS-sorted protoplasts was 82%; the value for the control protoplasts was 72%.

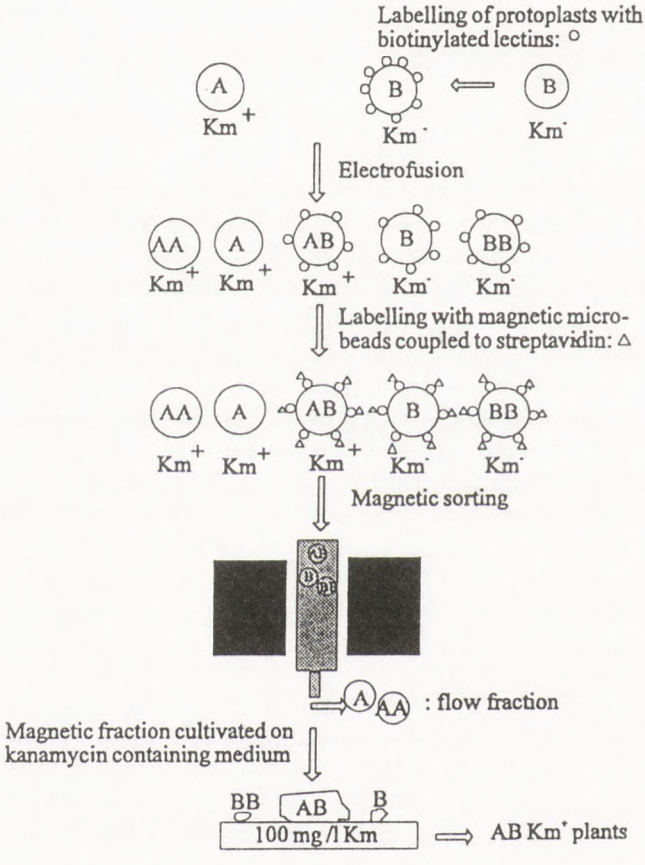

Fig. 1. Scheme illustrating the magnetic separation of lectin-labelled protoplasts bound to magnetic microbeads and the selection of hybrid heterokaryons on kanamycin-containing medium.

Two types of somatic fusions were tested to evaluate the efficiency of MACS. The first fusion combination was intraspecific and based on protoplasts from the two *S. tuberosum* clones. The second fusion was interspecific with protoplasts from the *S. tuberosum* and from *S. bulbocastanum*. Shoots were regenerated from the calli on a medium containing kanamycin to eliminate the non-hybrid shoots derived from the parents susceptible to kanamycin. One shoot from each callus was fingerprinted to establish whether the callus was hybrid or derived from the kanamycin-resistant parent.

Table 1 summarizes the experiment carried out on the intraspecific and interspecific combinations. The percentage of hybrid plants increased from 8.0 to 35.8% in the intraspecific combination and from 27.9 to 81.8% in the interspecific one. Parent plants and randomly chosen hybrid regenerants were transferred to the greenhouse. The chromosome number of parents and regenerants was evaluated in shoot meristems. For the intraspecific combination eight of 15 hybrid plants had a chromosome number of 48, as expected when two cells with 24 chromosomes are fused; six plants had a slightly increased chromosome number and one plant a decreased number. In the case of the interspecific fusion, 16 out of 24 plants had 48 chromosomes, two a slightly decreased number, five an increased number and one was hexaploid. The interspecific hybrids revealed a clear and distinguishable morphology when compared with the parents. All hybrids flowered and produced tubers.

TABLE 1

FREQUENCY IN THE MAGNETIC FRACTION OF SOMATIC HYBRIDS REGENERATED FROM MACS SORTED PROTOPLASTS COMPARED WITH THE SAME FREQUENCY OF UNSORTED CONTROL PROTOPLASTS. THE PROTOPLASTS WERE PREVIOUSLY FUSED BY THE APPLICATION OF AN ELECTRIC FIELD. TWO COMBINATIONS OF GENOTYPES WERE TESTED, ONE INTRASPECIFIC AND ONE INTERSPECIFIC

Type of fusion	Treatment	No. of			
		No. of experiments	Plants analyzed	Hybrid plants	
				No.	Percent
Intraspecific:					
tub A89.2478/39	magnetic fraction	4	145	52	35.8
x tub 1506/60	control untreated	3	199	16	8.0
Interspecific:					
tub PCG1 x blb	magnetic fraction	6	265	217	81.8
BGRC 008006	control untreated	3	136	38	27.9

tub = *S. tuberosum*; blb = *S. bulbocastanum* BGRC 008006

The suitability of the method for obtaining a high number of fusion hybrids is illustrated by the case of the fusion between *S. tubersoum* and *S. bulbocastanum*. This cross cannot be obtained sexually (Novy and Hanneman, 1991; Ramana and Hermsen, 1979) but is nevertheless of interest because

of the resistance of *S. bulbocastanum* to potato virus X, potato virus Y and late blight. Recently, Austin et al. (1993) reported obtaining this fusion hybrid using dual fluorescence labelling and micromanipulation. They were able to obtain seven hybrid calli, while in our experiments we easily regenerated 217 hybrid plants from 265 calli. In the case of this combination only a small fraction of the hybrids developed berries containing viable seeds after pollination, indicating that they were able to reproduce. We emphasize the advantage of our selection scheme: protoplasts sorted by MACS allowed the isolation of a large number of regenerated plants, which in turn made possible the choice of hybrids with the correct chromosome number, morphology, fertility and seed set.

Our interest in the use of somatic fusion to widen the genetic variability of potato, has recently considered the possibility of obtaining other hybrids, particularly using species which cannot be crossed sexually. Somatic hybrids were obtained between *S. tuberosum* and the two species *S. circaeifolium* and *S. raphanifolium*. For these hybrids, as well as for the cross *S. tuberosum* x *S. bulbocastanum*, the backcross generations to *S. tuberosum* were produced. These concerns thirteen independent hybrids for *S. circaeifolium*, three for *S. bulbocastanum* and three for *S. Raphanifolium*. These backcross families are being tested for resistance to *Phytophthora infestans* and *Globodera pallida*.

A similar approach was recently applied to the production of intergeneric fusions within the family *Solanaceae*. These were done using *S. bulbocastanum* and *S. tuberosum* as one of the parents, and *Lycopersicon esculentum* as the second. In Tab. 2 are reported the results obtained: hybrid plants are available which, in spite of their high sterility, will be used in backcross programs.

TABLE 2
NUMBER OF SOMATIC HYBRIDS OBTAINED FROM THE CROSSES
S. BULBOCASTANUM X L. *ESCULENTUM* AND *S. TUBEROSUM* X L. *ESCULENTUM*

Combination		No. of somatic hybrids
Parent 1	Parent 2	
S. bulbocastanum (2x)	*Lycopersicon esculentum* (2x)	10
S. tuberosum (2x)	dto	55
S. tuberosum (2x)	dto	54
S. tuberosum (1x)	dto	150

2. Transgenic potato lines expressing mutant alleles of the gene for the potato leafroll virus 17 kDa protein, the putative viral movement protein, are protected against virus infection

The potato leafroll luteovirus (PLRV) is used as a model system for the study of both basic and applied aspects of its molecular biology. Noncanonical translation strategies used by the protein synthesis apparatus of the plant cell in the translation of PLRV genes operate at the three basic steps of initiation (internal initiation), elongation (frameshifting) and termination (stop codon suppression) (Rohde et al., 1994).

One of the proteins produced by internal initiation and accumulating to high amounts in PLRV-infected as well as in transgenic potato plants, is ORF4 encoding a 17 kDa protein (pr17). This protein is located within the region for the capsid protein (ORF3) and translated from a subgenomic RNA (Tacke et al., 1990). Biochemical characterization of this protein has revealed at least two domains: The basic C-proximal half of pr17 is a single-stranded nucleic acid-binding region (Tacke et al., 1991), while mutational analysis of the pr17 acidic N-proximal region identified an amphipathic α-helix as the domain for protein/protein interactions, leading to the formation of homodimers and higher oligomers (Tacke et al., 1993). In addition, pr17 is subject to posttranslational modification by protein kinase(s). On the basis of these biochemical characteristics, pr17 may act as the putative movement protein of the virus. Such virus-coded functions have been described now for several plant viruses to mediate cell-to-cell movement (short distance movement; Fig.

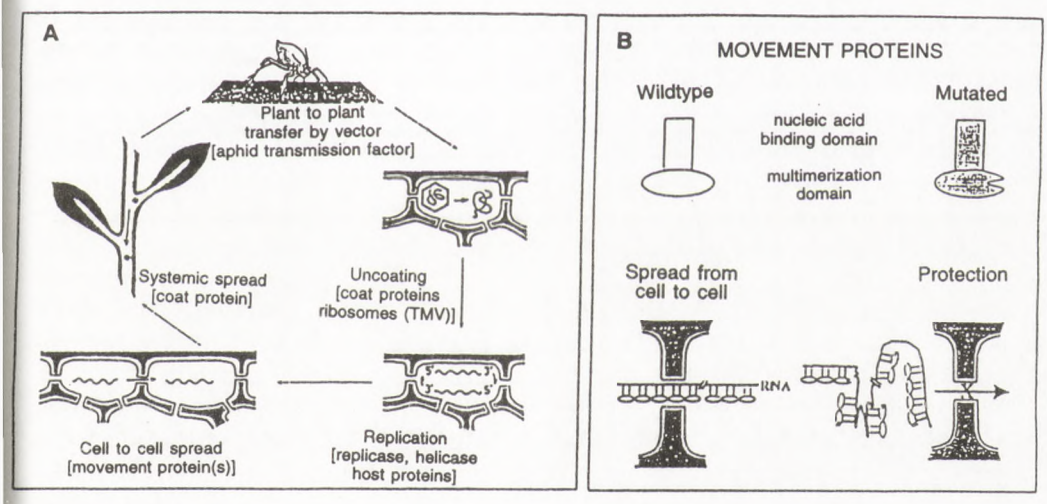

Fig. 2. (A) Life cycle of PLRV. (B) Proposed mechanism for pr17, the putative PLRV movement protein.

2A). Thus PLRV pr17 may act as a chaperone molecule shaping PLRV RNA into complexes that are protected from nuclease digestion and capable of cell-to-cell transfer through the plasmodesmata of cells in the phloem tissue where the virus replicates.

Based on the concept of pathogen-derived protection, pr17 was mutated in the identified domains and mutant alleles with N- or C-terminal extensions were constructed. The variant proteins can still form heterodimer with wild-type pr17 and their binding to PLRV RNA is not affected. According to our proposed protection strategy (Fig. 2B) it is envisioned, that the resulting complexes with PLRV RNA assume a conformation that is not capable to cell-to-cell transport through the plasmodesmata.

The wildtype pr17 gene and mutant alleles under the transcriptional control of the 35S CaMV promoter were stably transformed into potato, and the resulting lines were tested in grafting experiments for the protection against PLRV infection. These experiments provided evidence that the RNA itself rather than the protein contributes to PLRV infection. As viral movement proteins bind to nucleic acids in a sequence unspecific manner, the above described protection strategy is supposed to confer resistance also to other viruses. Results of resistance experiments with the potato virus X and Y suggest that several of the mutant alleles provide a broad range protection against potato viruses that are due to high yield losses in potato.

3. RFLP markers in potato breeding

As potatoes are vegetatively propagated via tubers, there was no need to select for highly fertile genotypes during potato breeding. This fact, together with the tetrasomic inheritance of the crop, prevented the development of genetic linkage maps. Reduction of the ploidy from the tetraploid to the diploid level is possible either by pollination of tetraploid genotypes with certain diploid strains of *Solanum phureja*, which induced the parthenogenetic development of diploid gametes into plants (Hermsen and Verdenius, 1973; Hougas et al., 1964), or by regenerating plants from diploid male gametes via anther or microspore culture of tetraploid parents (Dunwell and Sunderland, 1973; Powell and Uhrig, 1987). Diploid patatoes are, however, largely self-invompatible. This fact, and the high genetic load present in the species, makes the construction of pure lines in most cases impractical. The RFLP map of potato is based, therefore, on segregating progeny of highly heterozygous diploid parents (Gebhardt et al., 1994; Ritter et al., 1990).

Two diploid heterozygous *S. tuberosum* subsp. *tuberosum* breeding lines were crossed to give a F1. Pollinating an individual F1 plant with one of the parents yielded a backcross progeny of 67 lines (Gebhardt et al., 1991; Gebhardt et al., 1989). Total genomic DNA was isolated from freeze-dried leaves and shoots of plants grown in the greenhouse under normal daylight conditions. The DNA was digested with the four base cutter restriction enzymes

*Taq*I, *Rsa*I and *Alu*I, respectively. Restriction fragments were separated on 4% polyacrylamide gels under denaturing conditions and transferred to nylon membranes by electroblotting. The separation range was between 250 and 2000 bases resolving minimum length differences of ca. 5 bases. The RFLP map as derived from this particular intraspecific cross is ca. 1050 centimorgan long and covers approximately 80 to 90% of the potato genome. Two hundred and ninety-nine DNA marker probes and one morphological marker (color of the tuber skin) identified 384 loci (Gebhardt et al., 1994) (Fig. 3).

Several dominant resistance alleles acting against the potato cyst nematode *Globodera rostochiensis*, potato virus X and Y, and the fungus *Phytophthora infestans* have been introduced into the cultivated potato from wild *Solanum* species (Ross, 1986). They are inherited as single dominant factors (Cockerham, 1970; Mastenbroek, 1953; Toxopeus and Huijsman, 1953). They were also analysed genotypically with sets of RFLP markers selected according to their position on the RFLP map in a search for linkage between RFLP alleles and the resistance allele.

Using this approach, two unlinked loci have been identified on potato chromosomes V and VII that confer resistance to *Globodera rostochiensis*. The locus on chromosome VII (*Gro1*) (Barone et al., 1990) may correspond to the *Fb* gene which was first described by Ross (1962). The second locus on chromosome V corresponds to the *H1* gene discovered by Ellenby (1954) in the *S. tuberosum* ssp *andigena* accession CPC 1673 (Gebhardt et al., 1993). Two unlinked loci for extreme resistance to potato virus X (*Rx*) have also been detected: *Rx1* on chromosome XII and *Rx2* again on chromosome V. *Rx2* was separated by approximately 60 map units from the *H1* locus (Ritter et al., 1991). Race specific resistance alleles to *Phytophthora infestans* have also been mapped. The *R1* allele confers hypersensitive resistance to all races of the fungus except the ones being homozygous for the virulence allele *v1*. The *R1* locus was identified again on chromosome V in the same chromosomal segment harbouring the *Rx2* locus (Leonards-Schippers et al., 1992). The *R3*, *R6* and *R7* alleles mapped to a different locus on chromosome XI (El-Kharbotly et al., 1994; El-Kharbotly et al., in preparation).

As a result of the mapping experiments, markers are known which are linked to the resistance loci, with less than 5% recombination frequency. Our strategy was then targeted on the development of allele specific marker assays for nematode resistance alleles based on the polymerase chain reaction (PCR) (Niewöhner et al., 1995). DNA sequence information was obtained from marker clones and used for designing PCR primers. Small amounts of genomic DNA extracted from small samples of leave tissue were sufficient to amplify the alleles at a marker locus which are detected on ethidium bromide stained agarose gels. The alleles amplified by PCR were polymorphic in some cases and not in others. Amplified alleles which are not polymorphic were distinguished after digestion with restriction enzymes. Additional sequence information was obtained from amplified alleles and sequence differences between marker alleles were identified. The sequence differences were used

I
II
III
IV
V
VI
R1
Rx2
H1
CP46
TG24
CP45
GP206(a)
Sr1 CP108
GP184
βGluc
GP90
CP11
CP69
GP196
GP208
GP33(c)
CP111
GP187
GP166
GP124
GP235
GP28(a)
CP62
CP63
TG53
GP128(a)
GP1(d)
CP100
GP206(b)
GP232(b)
GP93
CP64
GP175
GP207
CP19
TG59
GP212
GP192
GP88
GP167(a)
GP194
GP228
CP13
CP132
GP1(c)
TG5
GP74(a)
GP201
TG21
CP15(b)
CP48(a)
GP236
GP176(a)
CP158(b)
GP33(a)
GP26
GP98
GP136(a)
GP199
GP128(b)
GP205
GP23
GP35(c)
rbcS-c
CP70(b)
GP131
GP86
GP214
GP209
CP157
GP216
GP176(b)
CP109
rbcS-2
CP70(a)
CP15(a)
CP112(a)
TG102
GP80
CP6
GP226
4CL(a)
rbcS-1
GP1(a)
GP25
IG74
CP133
IG134
GP180(a)
PAT(b)
CP137(d)
GP221
GP180(b)
GP172
IG62
GP35(d)
PC116
GP220(a)
GP128(c)
CP55(a)
IG123
GP162(b)
GP75
TG22
GP83
GP82
GP165
GP35(h)
GP35(g)
CP57
GP186
GP21
GP198(c)
GP213
GP11(b)
GP104(g)
IG23
Actin
TG69
GP78
GP22
CP31
GP28(b)
GP179
GP234
GP17(a)
GP188
GP85(a)
CP113
GP215(b)
GP89
GP24
CP12
CP50
GP233
WUN2
TG115
CP61
GP79
GP73(c)
WUN1
GP102
GP164
GP202
GP136(b)
TG118
GP96(a)
CP18
4CL(b)
GP30
GP35(f)
GP211
CP104(d)
GP76
GP36(b)

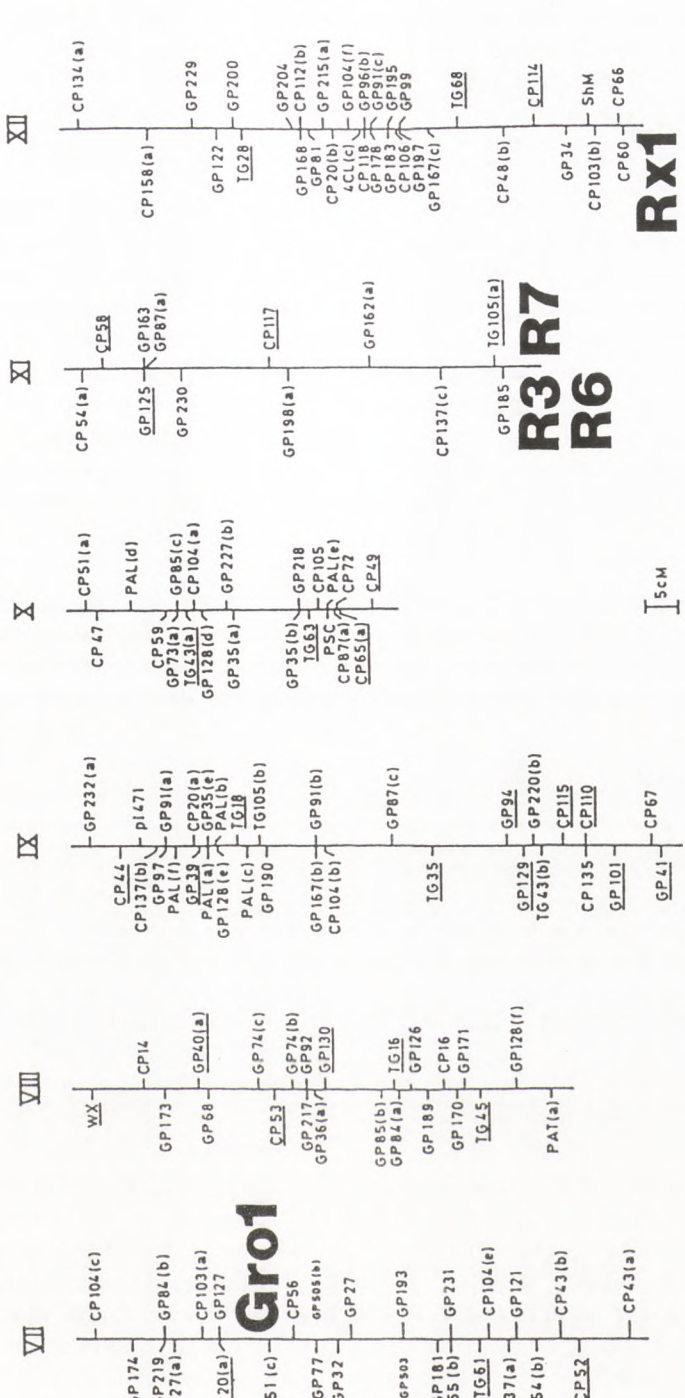

Fig. 3. RFLP linkage map of potato, modified according to Gebhardt et al. (1991). The map includes the approximate genomic positions of six resistance loci, Gro1, H1, Rx1, Rx2, R1 and R3, that were identified in independent mapping experiments. The twelve potato chromosomes numbering is the same as for the homoeologous tomato chromosomes GP and CP markers originate from genomic DNA and cDNA of potato, respectively. TG markers are from tomato and were provided by S.D. Tanksley (Cornell University, Ithaca, N.Y. USA). Markers based on functional genes are indicated by their proper names (for identification see Gebhardt et al., 1991). When more than one RFLP locus is detected whit the same marker probe, the loci are discriminated by small letters in parenthesis. RFLP loci that have been mapped to homoeologus positions in potato and tomato are underlined.

for designing PCR primers by which only a specific marker allele closely linked with the resistance allele was amplified. Marker assisted selection using a PCR assay will be possible in large numbers of plants, if the marker allele is linked in coupling phase with the resistance allele. In unrelated germplasm, however, PCR assays have to be adapted to the particular source of resistance gene present.

A QTL mapping experiment has been also performed which resulted in the identification of several chromosomal segments of the potato genome having a quantitative effect on *P. infestans* resistance (Leonards-Schippers et al., 1994). Ca. 200 F1 hybrids derived from crossing a highly resistant with a highly susceptible dihaploid line were analysed phenotypically for the resistance level to *P. infestans* races o and 1. Resistance tests were performed on leaf disks under environmentally controlled conditions and the percentage of infected leaf disk area was scored. The same F1 population was analysed with 104 RFLP marker probes and chromosome maps were constructed from the segregation data of RFLP alleles. The analysis revealed eleven chromosome segments on nine potato chromosomes which showed significant contrasts between marker genotypic classes. QTL mapping identified at least one QT locus on chromosome IV which was specific for race 1. Two QT regions correlated with the two small segments on chromosomes V and XII to which the dominant allele *R1* as well as alleles *Rx1* and *Rx2*, both inducing extreme resistance to potato virus X, have been localized in independent mapping experiments. The molecular cloning and characterization of resistance genes of potato and other plant species will open up the possibility of directly engineering disease resistance in the potato. Our target loci are *R1* possibly encoding multiple resistances and the nematode resistance gene *Gro1*.

The dominant allele *Gro1* confers to potato resistance to the root cyst nematode *Globodera rostochiensis*. The *Gro1* locus has been mapped to chromosome VII on the genetic map of potato using RFLP markers (Barone et al., 1990). This makes possible the cloning of *Gro1* based on its map position. As part of this strategy we have constructed a high resolution genetic map of the chromosome segment surrounding *Gro1* based on RFLP; RAPD and AFLP markers (Ballvora et al., 1985). RAPD and AFLP markers closely linked to *Gro1* were selected by bulked segregant analysis and mapped relative to the *Gro1* locus in a segregating population of 1105 plants. Three RFLP and one RAPD marker were found linked to *Gro1* without recombination. Two AFLP markers were identified that flanked *Gro1* at a genetic distance of 0.6 cM and 0.8 cM, respectively. A genetic distance of 1 cM in the *Gro1* region corresponded to a physical distance of ca. 100 kb, as judged from long range restriction analysis. Marker assisted selection for nematode resistance was practised in the course of constructing the high resolution map. Plants carrying the resistance allele *Gro1* were distinguished from susceptible plants by marker assays based on the polymerase chain reaction (PCR).

The *R1* allele confers on potato a race-specific resistance to *Phytophthora infestans*. The corresponding genetic locus maps on chromosome V in a re-

gion in which several other resistance genes are also located (Leonards-Schippers et al., 1990). As part of a strategy for cloning *R1*, a high-resolution genetic map was constructed for the segment of chromosome V that is bordered by the RFLP loci *GP21* and *GP179* and includes the *R1* locus (Meksem et al., 1995). Bulked segregation analysis and markers based on amplified fragment length polymorphisms (AFLP markers) were used to select molecular markers closely linked to *R1*. Twenty-nine of approximately 3200 informative AFLP loci displayed linkage to the *R1* locus. Based on the genotypic analysis of 461 gametes, eight loci mapped within the *GP21-GP179* interval. Two of those could not be separated from *R1* by recombination. For genotyping large numbers of plants with respect to the flanking markers *GP21* and *GP179* PCR based assays were also developed which allowed marker-assisted selection of plants with genotypes *Rr* and *rr* and of recombinant plants.

References

Afonso C., Harkins K., Thomas-Compton M., Krejci A., Galbraith D., (1985), *Selection of somatic hybrid plants in Nicotiana through fluorescence activated sorting of protoplasts*, Bio/Technology, 3, 811-816.

Austin S., Baer M. A., Helgeson J. O., (1985a), *Transfer of resistance to potato leaf roll virus from Solanum brevidens into Solanum tuberosum by somatic fusion*, Plant Sci., 39, 75-82.

Austin S., Pohlmann J. D., Brown C. R., Mojtahedi H., Santo G. S., Douches D. S., Helgeson J. P., (1993), *Interspecific somatic hybridization between Solanum tuberosum L. and Solanum bulbocastanum Dun. as a means of transferring nematode resistance*, American Potato Journal, 70, 485-495.

Ballvora A., Hesselbach J., Niewöhner J., Leiser D., Salamini F., Gebhardt C., (1995), *Marker enrichment and high resolution map of the segment of potato chromosome VII harbouring the nematode resistance gene Gro1*, Mol. Gen. Genet., (in press).

Barone A., Ritter E., Schachtschabel U., Debener T., Salamini F., Gebhardt C., (1990), *Localization by restriction fragment length polymorphism mapping in potato of a major dominant gene conferring resistance to the potato cyst numatode Globodera rostochiensis*, Mol. Gen. Genet., 224, 177-182.

Binding H., Nehls R., (1977), *Regeneration of isolated protoplasts to plants in Solanum dulcamara L.*, Z. Pflanzenphysiologie, 85, 279-280.

Binding H., Jain S., Finger H., Mordhorst G., Nehls R., Gressel J., (1982), *Somatic hybridization of an atrazine resistant biotype of Solanum nigrum with Solanum tuberosum*. Theor. Appl. Genet., 63, 273-277.

Butenko R.G., Kuchko A., (1980), *Somatic hybridization of Solanum tuberosum and Solanum chacoense Bitt. by protoplast fusion, in: Advances in protoplast research*, Eds. L. Ferency, G. L. Farkas, Pergamon Press, Oxford, 293-330.

Cockerham G., (1970), *Genetical studies on resistance to potato virus X and Y*, Heredity, 25, 309-348.

Cocking E. C., George D., Price-Jones M. J., Power J. B., (1977), *Selection procedures for the production of interspecies somatic hybrids of Petunia hybrida and Petunia parodii II. Albino complementation selection*, Plant Science Letters, 10, 7-12.

Dörr I., Miltenyi S., Salamini F., Uhrig H., (1994), *Selecting somatic hybrid plants using magnetic protoplast sorting*, Bio/Technology, 12, 511-515.

Dunwell J. M., Sunderland H., (1973), *Anther culture of solanum tuberosum L.*, Euphytica, 22, 317-323.

El-Kharbotly A., Leonards-Schippers C., Huigen D. J., Jacobsen E., Pereira A., Stie-

kema W. J., Salamini F., Gebhardt C., (1994), *Segregation analysis and RFLP mapping of the R1 and R3 alleles conferring race-specific resistance to Phytophthora infestans in progeny of dihaploid potato parents*, Mol. Gen. Genet., 242, 749-754.

El-Kharbotly A., Palomino-Sánchez C., Salamini F., Jocabsen E., Gebhardt C., *R6* and *R7* alleles of potato conferring race-specific resistance to *Phytophthora infestans* (Mont.) de Bary cluster with the *R3* locus on chromosome XI, (in preparation).

Ellenby C., (1954), *Tuber-forming species and varieties of the genus Solanum tested for resistance to the potato root eelworm Heterodora rostochiensis Wollenweber*, Euphytica, 3, 195-202.

Fish N., Karp A., Jones M.G.K., (1988), *Production of somatic hybrids by electrofusion in Solanum*, Theor. Appl. Genet., 76, 260-266.

Gebhardt C., Mugniery D., Ritter E., Salamini F., Bonnel E., (1993), *Identification of RFLP markers closely linked to the H1 gene conferring resistance to Globodera rostochiensis in potato*, Theor. Appl. Genet., 85, 541-544.

Gebhardt C., Ritter E., Salamini F., (1994), *RFLP map of the potato*, in: *DNA-based markers in plants*, Eds. R. L. Phillips, I. K. Vasil, Kluwer Acad. Publ., 271-285.

Gebhardt C., Ritter E., Barone A., Debener T., Walkemeier B., Schachtschabel U., Kaufmann H., Thompson R. D., Bonierbale M. W., Ganal M. W., Tanksley S. D., Salamini F., (1991), *RFLP maps of potato and their alignment with the homoeologous tomato genome*, Theor. Appl. Genet., 83, 49-57.

Gebhardt C., Ritter E., Debener T., Schachtschabel U., Walkemeier B., Uhrig H., Salamini F., (1989), *RFLP analysis and linkage mapping in Solanum tuberosum*, Theor. Appl. Genet., 78, 65-75.

Hammatt N., Lister A., Blackhall N. W., Gartland J., Ghose T. K., Gilmour D. M., Power J. B., Davey M. R., Cocking E. C., (1990), *Selection of plant heterokaryons by flow cytometry*, Protoplasma, 154, 34-44.

Helgeson J. P., Hunt G. J., Haberlach G. T., Austin S., (1986), *Somatic hybrids between Solanum brevidens and Solanum tuberosum: Expression of a light blight resistance gene and potato leaf roll resistance*, Plant Cell Reports, 3, 212-214.

Hermsen J. G. Th., Verdenius J., (1973), *Selection from Solanum tuberosum group phureja of genotypes combining high-frequency haploid induction with homozygosity for embryo spot*, Euphytica, 22, 244-259.

Hougas R. W., Peloquin S. J., Gabert A. C., (1964), *Effect of seed parent and pollinator on the frequency of haploids in Solanum tuberosum*, Crop. Sci., 4, 593-595.

Leonards-Schippers C., Gieffers W., Salamini F., Gebhardt C., (1992), *The R1 gene conferring race-specific resistance to Phytophthora infestans in potato is located on potato chromosome V*, Mol. Gen. Genet., 233, 278-283.

Leonards-Schippers C., Gieffers W., Schäfer-Pregl R., Ritter E., Knapp S. J., Salamini F., Gebhardt C., (1994), *Quantitative resistance to Phytophtora infestans in potato: a case study for QTL mapping in an allogamous plant species*, Genetics, 137, 67-77.

Lo Schiavo F., Giovinazzo G., Terzi M., (1983), *8-Azaguanine resistant carrot cell mutants and their use as universal hybridizers*, Mol. Gen. Genet., 192, 326-329.

Masson J., Lancelin D., Bellini C., Lecerf M., Guerche P., Pelletier G., (1989), *Selection of somatic hybrids between diploid clones of potato (S. tuberosum L.) transformed by direct gene transfer*, Theor. Appl. Genet., 78, 153-159.

Mastenbroek C., (1953), *Experiments on the inheritance of blight immunity in potatoes derived from Solanum demissum Lindl*, Euphytica, 2, 197-206.

Matteij W. M., Puite K-J., (1992), *Tetraploid potato hybrids through protoplast fusion and analysis of their performance in field*, Theor. Appl. Genet., 83, 807-812.

Matteij W. M., Eijlander R., de Koning J. R. A., Louwes K. M., (1992), *Interspecific hybridization between the cultivated potato Solanum tuberosum subspecies tuberosum L. and the wild species S. circaeifolium subsp. circaeifolium Bitter exhibiting resistance to Phytophthora infestans (Mont.) de Bary and Globodera pallida (Stone) Behrens*, Theor. Appl. Genet, 83, 459-466.

Meksem K., Leister D., Peleman J., Zabeau M., Salamini F., Gebhardt C., (1995), *A high-resolution map of the vicinity of the R1 locus on chromosome V of potato based on RFLP and AFLP markers*, Mol. Gen. Genet., 248, (in press).

Miltenyi S., Müller W., Wichel W., Radbruch A., (1990), *High gradient magnetic cell separation with MACS*, Cytometry, 11, 231-238.

Niewöhner J., Salamini F., Gebhardt C., (1995), *Development of PCR assays diagnostic for RFLP marker alleles closely linked to alleles Gro1 and H1, conferring resistance to the root cyst nematode Globodera rostochiensis in potato*, Molecular Breeding, 1, 65-78.

Novy R. G., Hannemann R. E., (1991), *Hybridization between gp. Tuberosum haploids and 1 EBN wild potato species*, Am. Pot. J., 68, 151-169.

Pehu E., Karp A., Moore K., Steele S., Dunckley R., Jones M. G. K., (1989), *Molecular, cytogenetic and morphological characterization of somatic hybrids of dihaplid S. tuberosum and diploid S. brevidens Phil*, Theor. Appl. Genet., 78, 696-704.

Pijnacker L. P., Ferwerda M. A., Puite K. J., Roest S., (1987), *Elimination of S. phureja chromosomes in S. tuberosum + S. phureja somatic hybrids*, Theor. Appl. Genet., 73, 878-882.

Powell W., Uhrig H., (1987), *Anther culture of Solanum genotypes*, Plant Cell Tissue and Organ Culture, 11, 13-24.

Preiszner J., Feher A., Veisz O., Sutka J., Dudits D., (1991), *Characterization of morphological variation and cold resistance in interspecific somatic hybrids between potato (Solanum tuberosum) and S. brevidens Phil*, Euphytica, 57, 37-49.

Puite K. J., Roest S., Pijnacker L., (1986), *Somatic hybrid potato plants after electrofusion of diploid Solanum tuberosum and Solanum phureja*, Plant Cell Reports, 5, 262-265.

Ramana N. S., Hermsen J. G., (1979), *Genome relationship in tuber bearing Solanum, in: The biology and taxonomy of the Solanaceae*, Eds. J. G. Hawkes, R. N. Lester, A. D. Skelding, Academic Press, London, 647-655.

Ritter E., Debener T., Barone A., Salamini F., Gebhardt C., (1991), *RFLP mapping on potato chromosomes of two genes controlling extreme resistance to potato virus X (PVX)*, Mol. Gen. Genet., 227, 81-85.

Ritter E., Gebhardt C., Salamini F., (1990), *Estimation of recombination frequencies and construction of RFLP linkage maps in plants from crosses between heterozygous parents*, Genetics, 125, 645-654.

Rohde W., Gramstat A., Schmitz J., Tacke E., Prüfer D., (1994), *Plant viruses as model systems for the study of noncanonical translation mechanisms in higher plants*, J. Gen. Virol., 75, 2141-2149.

Ross H., (1962), *Über die Vererbung der Resistenz gegen den Kartoffelnematoden (Heterodera rostochiensis Woll.) in Kreuzungen von Solanum famatinae Bitt. et Wittm. mit Solanum tuberosum L. und mit S. chacoense Bitt.*, Der Züchter, 32, 74-80.

Ross H., (1986), *Potato breeding — problems and perspectives*, M Plant Breed., Supplement 13.

Seraaf I., Sikachkr D., Ducreux G., Brown S., Allot M., Barghi N., Rossigol L., (1991), *Interspecific somatic hybridization in potato by protoplast electrofusion*, Plant Science, 76, 115-126.

Tacke E., Prüfer D., Salamini F., Rohde W., (1990), *Characterization of a potato leafroll luteovirus subgenomic RNA: Differential expression of viral proteins by internal translation initiation and UAG suppression*, J. Gen. Virol., 71, 2265-2272.

Tacke E., Prüfer D., Schmitz J., Rohde W., (1991), *The potato leafroll luteovirus 17 kDa protein is a single-stranded nucleic acid-binding protein*, J. Gen. Virol., 72, 2035-2038.

Tacke E., Prüfer D., Schmitz J., Rohde W., (1993), *Mutational analysis of the nucleic acid-binding 17K protein of potato leafroll leteovirus identifies an amphipathic helix as the domain for protein/protein interactions*, Virology, 197, 274-282.

Toxopeus H. J., Huijsman C. A., (1953), *Breeding for resistance to potato root eelworm.*

I. Preliminary data concerning the inheritance and the nature of resistance, Euphytica, 2, 180-186.

Waara S., Tegelström H., Wallin A., Erikson T., (1989), *Somatic hybridization between anther derived dihaploid clones of potato (S. tuberosum L.) and the identification of hybrid plants by universal hybridizers*, Mol. Gen. Genet., 192, 326-329.

Modern approaches to the breeding of potato (*Solanum tuberosum* L.)

Summary

Solanum tuberosum, the potato, a crop of the family *Solanaceae*, is cultivated in temperate and subtropical zones of the world. After wheat, rice and corn it occupies the fourth position in terms of world production per year. We summarize the work carried out in our department concerning the use of cellular and molecular techniques in potato breeding.

Key words:
breeding, potato, somatic hybrids, transgenic.

Address for correspondence:
F. Salamini, Max-Planck-Institut für Züchtungsforschung, Abteilung Pflanzenzüchtung und Ertragsphysiologie, Carl-von-Linné-Weg 10, D-50829 Köln, Germany.