Theor Appl Genet (1985) 71:172–175

Intra-specific fusions in *Solanum tuberosum*

S. Austin, M. Baer, M. Ehlenfeldt, P. J. Kazmierczak and J. P. Helgeson *

Agricultural Research Service, U.S. Department of Agriculture and Department of Plant Pathology, University of Wisconsin, Madison, WI 53706, USA

Received May 1, 1985; Accepted May 14, 1985
Communicated by H. F. Linskens

Summary. Plants were regenerated from callus arising from protoplast fusion of two *S. tuberosum* diploids. Tetraploid progeny from the fusion of the two diploid partners had increased vigor. Isozyme analysis confirmed the presence of proteins from both partners in the fusion progeny. Pigmentation of tubers and anthers was heightened substantially in the fusion products. This fusion, the first intra-specific fusion within *S. tuberosum*, indicates that somatic fusion may be useful for transferring traits within this group.

Key words: Protoplast – Somatic fusion – Overcoming incompatibility – *S. tuberosum*

Introduction

Interspecific fusions of leaf mesophyll protoplasts can provide a means by which the genomes of sexually incompatible species may be combined (Evans 1983; Shepard et al. 1983). To this end, we have been examining interspecific fusions within the genus *Solanum*, the common potato and its relatives. Incompatibility can also occur within the same species. In addition, male or female sterility of one or both of the partners may hamper breeding efforts. In these cases, somatic fusions might provide combinations that are highly desirable. If the crop is clonally propagated, as is potato, these novel gene combinations could be useful even if passage of the combination through meiosis proved difficult. Therefore, we undertook the fusion of two *S. tuberosum* diploids to evaluate the potential for intraspecific hybridization.

The two lines chosen for fusion possessed distinctive morphological characteristics. This was essential for easy recognition of parental lines should self-fusions or regeneration of unfused materials occur in addition to somatic fusion. Also, these lines had different behavior in culture. One clone (US-W9545.99) gave protoplasts which were capable of sustained division, but in only one of four experiments was the clone capable of limited shoot regeneration under our standard cultural conditions (Haberlach et al. 1985). In contrast, approximately 10% of calli from the other fusion partner (US-W9310.3) yielded shoots. Thus, any shoot-producing calli obtained from a fusion experiment of the above lines would be expected to be either US-W9310.3 or the result of a fusion event. It was also anticipated from all our earlier results (Baer et al. 1984; Austin et al. 1985) that the additional vigor of tetraploids resulting from the fusion of two different diploid lines would favor survival of fusion progeny.

* To whom offprint requests should be addressed

Methods

Plant material and protoplast fusion

Diploid hybrids US-W9545.99 and US-W9310.3 from Gp. Tuberosum – Gp. Phureja crosses were obtained from the Inter-Regional Potato Introduction Project, Sturgeon Bay, WI. Leaf mesophyll protoplasts were isolated from shoot tip cultures and cultured following the procedure of Shepard (1980) as modified by Haberlach et al. (1985). Protoplast fusion was achieved by a refinement (Austin et al. 1985) of the procedure used by Melchers et al. (1978). Our method generally resulted in the production of approximately 2–3% heterokaryons as determined by dual fluorescent labelling (Galbraith and Galbraith 1979). After fusion and subsequent culture, representative plantlets were excised from any calli that produced shoots.

Plant establishment and morphological assessment

Shoots taken from different calli were individually transferred to propagation medium for rooting (Haberlach et al. 1985). After 3–4 weeks, rooted plantlets were established in small peat

cylinders ("Jiffy-7") under greenhouse conditions for 3 weeks before transplantation to the field at the Hancock Experiment Station. Clonal representatives of each parent and protoplast-derived plants of US-W9310.3 that had not been subjected to fusion conditions were also included in field evaluations. The morphology of plants was assessed throughout the growing season and tubers were harvested from all of the plants.

Chromosome counting and isozyme analyses

Chromosome counts were done on root tips treated with an 8-hydroxyquinioline solution (0.29 g/l) and fixed in a 3:1 (v:v) solution of methanol and acetic acid. Root tips were stained with a solution of 1% (w:v) acetocarmine.

Polyacrylamide gels were prepared and run according to Laemmli (1970) and Hagar and Burgess (1980). Peroxidase staining was performed according to Quiros (1981).

Results

Eleven shoot-producing calli were recovered. Representative plants from each callus (a total of 89 plants) were assessed under field conditions (Table 1). Ten of the 11 calli yielded plants that clearly had the morphology of the parent US-W9310.3. However, the two representative plants of the remaining callus had a distinctive morphological appearance that was different from either parent. The most spectacular feature of the hybrid plants was the production of dark-purple skinned tubers with flesh that had a heavy purple mottle or was solid purple (Fig. 1). The hybrid plants appeared to possess the purple skin pigment from one parent (US-

Table 1. Vegetative characteristics of parent lines and fusion progeny

US-W9545.99	Fusion progeny	US-W9310.3
Green stems	Purple stems	Green stems with purple axils
Thick, mid-green leaves with crinkled edges	Thick dark-green leaves with smooth edges	Thin mid-green leaves with smooth edges
Pentagonal white flowers with pale pink acumen on lower surfaces	Intermediate pentagonal-stellate lavender-purple flowers with green-yellow star	Stellate purple flowers with green-yellow star
Red-orange anthers	Dark orange-brown anthers	Yellow anthers
Red-purple fruit	Black-purple fruit	Red-purple fruit
Tubers with red skin	Tubers with black-purple skin	Tubers with yellow skin and purple splashes
Tuber flesh mottled red	Tuber flesh heavy solid purple or mottled purple	Tuber flesh solid gold-yellow

Fig. 1. Details of external (*lower row*) and internal (*upper row*) tuber appearance in *1* Diploid clone US-W9310.3 (*left*), *2* Tetraploid somatic hybrid (*center*), *3* Diploid clone US-W9545.99 (*right*)

Table 2. Ploidy levels of plants obtained from several protoplast-derived calli either after fusion attempt or from clone US-W 9310.3 alone

Protoplast derived calli from fusion experiment		Ploidy of plants examined	Protoplast-derived calli from unfused material of clone US-W 9310.3	Ploidy of plants examined
Callus 1	Fusion type	4×, 4×	Callus 1	4×
2	US-W 9310.3 type	4×, 4×	2	4×
3	US-W 9310.3 type	4×, 2×	3	4×
4	US-W 9310.3 type	2×, 2×	4	4×
5	US-W 9310.3 type	2×, 4×	5	4×
6	US-W 9310.3 type	2×, 2×	6	4×
7	US-W 9310.3 type	4×, 4×		
8	US-W 9310.3 type	4×, 4×		
9	US-W 9310.3 type	4×, 4×		

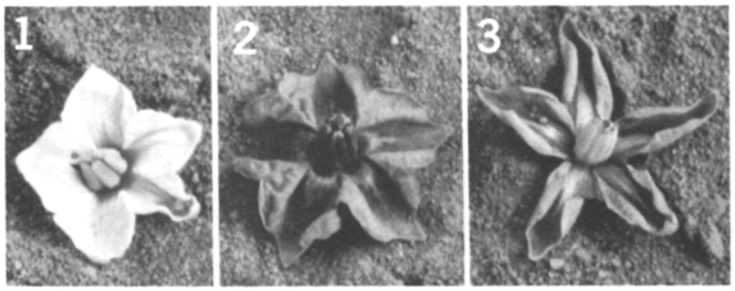

Fig. 2. Flower morphology of *1* Diploid clone US-W9545.99, *2* Tetraploid somatic hybrid, *3* Diploid clone US-W9310.3

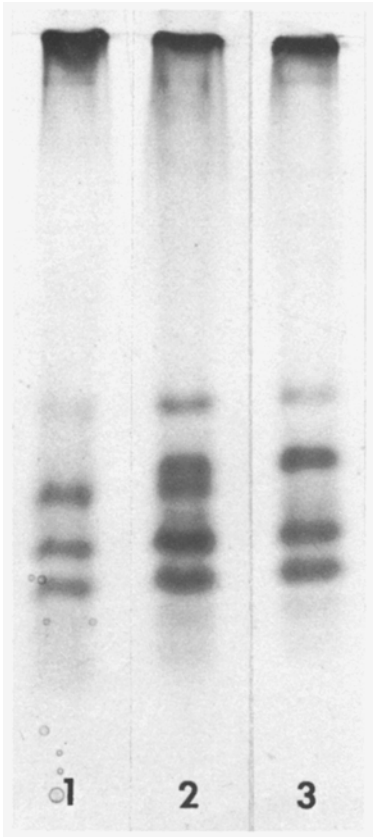

Fig. 3. Peroxidase isozymes separated by polyacrylamide gel electrophoresis using extracts from *1* Diploid clone US-W9545.99, *2* Tetraploid somatic hybrid, *3* Diploid clone US-W9310.3

W9310.3) and pigment distribution from the other (US-W9545.99). Figure 2 shows details of flower morphology. Hybrid flowers had a clearly intermediate shape as compared to parents; their most striking feature was heavily pigmented anthers.

The result of the polyacrylamide gel electrophoresis (peroxidase stain) is shown in Fig. 3. Plants displaying the hybrid morphology possessed peroxidase bands for both parents.

Ploidy levels of plants obtained either from calli after the fusion experiment or from the regeneration of protoplasts from clone US-W9310.3 that were not subjected to fusion conditions are given in Table 2. Plants from 9 calli originating from the fusion experiment were assessed. Of these, 5 calli produced only tetraploids; 2 produced only diploids and 2 produced both tetraploids and diploids. As expected, plants showing morphology different from either parent were tetraploids. Surprisingly, however, plants from protoplasts that had not been subjected to fusion conditions were also tetraploid even though the clonal plants from which the protoplasts were isolated were diploids.

Discussion

Morphological markers have been used by several workers as an indication of protoplast fusion (e.g. Schieder 1978). In our study, which is the first report of

fusion between two tuber-bearing diploid hybrids of *S. tuberosum*, the very different morphological markers of parents and fusion progeny made hybrid identification simple. For this reason, these two lines could be very useful in future studies to evaluate alternative fusion methods.

There are several reports of spontaneous chromosome doubling during the culture of potato protoplasts (e.g. Karp et al. 1982). Even though we examined only a relatively small sample (18 out of 89) of plants, the data show that an individual callus can give rise to plants of different ploidy. This is in agreement with the results shown by Ramulu et al. (1983) and Karp et al. (1982).

In this fusion experiment, in contrast with other experiments, (Austin et al. 1985; Helgeson et al., in preparation) progeny from parental plants as well as somatic hybrids were recovered. Since we obtained only one fusion callus from this experiment, we cannot determine whether or not fused material in this case had a strong cultural advantage over unfused parental material which we would expect from our other work.

Wenzel (1980) suggested a novel potato breeding strategy in which the final step would be protoplast fusion of two highly selected diploids with desirable disease resistances and agronomic traits to give rise to a tetraploid plant to be considered as a cultivar. He anticipated that heterosis exhibited by fusion products at the callus stages would aid in their selection. Our results to date would support the feasibility of this system. It also appears that a specific marker such as antibiotic resistance may not be required for selection of fusion products of *S. tuberosum* lines.

Acknowledgements. We thank Beth Howman-Combs for technical assistance, R. E. Hanneman, Jr. for tubers and helpful discussions and Steve Vicen for photographs.

References

Austin S, Baer MA, Helgeson JP (1985) Transfer of resistance to potato leaf roll virus from *Solanum brevidens* into *Solanum tuberosum* by somatic fusion. Plant Sci Lett 39: 75–82

Baer MA, Austin S, Helgeson JP (1984) Somatic fusions of protoplasts of *Solanum* species. Plant Physiol (Suppl) 75: 133

Evans DA (1983) Agricultural application of plant protoplast fusion. Biotechnology 1:253–261

Galbraith DW, Galbraith JEC (1979) A method for identification of fusion of plants protoplasts derived from tissue cultures. Z Pflanzenphysiol 93: 149–158

Haberlach GT, Cohen BA, Reichert NA, Baer MA, Towill LE, Helgeson JP (1985) Isolation, culture and regeneration of protoplasts from potato and several related *Solanum* species. Plant Sci Lett 39:67–74

Hager DA, Burgess RA (1980) Elution of proteins from sodium dodecyl/sulphate-polyacrylamide gels, removal of sodium dodecyl sulphate, and renaturation of enzymatic activity: results with sigma subunit of *Escherichia coli* RNA polymerase, wheat germ DNA topoisomerase, and other enzymes. Anal Biochem 109:76–86

Karp A, Nelson RS, Thomas E, Bright SWJ (1982) Chromosome variation in protoplast-derived potato plants. Theor Appl Genet 63: 1–7

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227:680–685

Melchers G, Sacristan MD, Holder AA (1978) Somatic hybrid plants of potato and tomato regenerated from fused protoplasts. Carlsberg Res Commun 43:203–218

Quirios CF (1981) Starch gel electrophoresis technique used with alfalfa and other *Medicago* species. Can J Plant Sci 61:754–749

Ramulu KS, Dijkhuis P, Roest S (1983) Phenotypic variation and ploidy level of plants regenerated from protoplasts of tetraploid potato (*Solanum tuberosum* L. cv. 'Bintje'). Theor Appl Genet 65:329–338

Schieder O (1978) Somatic hybrids of *Datura inoxia* Mill + *Datura discolor* Bernh. and of *Datura inoxia* L. + *Datura stramonium* L. var 'tatula'. 1. Selection and characterization. Mol Gen Genet 162:113–119

Shepard JF (1980) Mutant selection and plant regeneration from potato mesophyll protoplasts. In: Rubenstein I, Gengenbach B, Phillips RL, Green CE (eds) Genetic improvement of crops: emergent techniques. University of Minnesota press, Minneapolis, pp 185–219

Shepard JF, Bidney D, Barsby T, Kemble R (1983) Genetic transfer in plants through interspecific protoplast fusion. Science 219:683–688

Wenzel G (1980) Protoplast techniques incorporated into applied breeding programs. In: Ferenczy L, Farkas GL (eds) Advances in protoplast research. Pergamon Press, Oxford, pp 327–340