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Somatic hybridization between anther-derived dihaploid clones of potato (*Solanum tuberosum* L.) and the identification of hybrid plants by isozyme analysis

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Summary. Green mesophyll protoplasts of the dihaploid potato line 198:2 (*Solanum tuberosum* L.) were fused with herbicide-bleached mesophyll protoplasts of the dihaploid potato line 67:9 using a polyethylene glycol protocol. Heterokaryons were identified under a fluorescence microscope using the dual fluorescence of carboxyfluorescein-stained, herbicide-bleached protoplasts and the autofluorescence of green mesophyll protoplasts. About 20% of the protoplasts survived the fusion treatment, and the fusion frequency was 3%–4%. Unfused and fused protoplasts were mass cultured for 6 weeks after which vigorously growing calli were selected and transferred to shoot regeneration medium. Somatic hybrids were identified by a combination of five isozyme markers, and the ploidy level was determined by flow cytometry. Out of 15 calli that regenerated shoots, 6 plants derived from 2 different calli were identified as hexaploid somatic hybrids, while one morphologically deviant plant from a third callus was identified as a mixoploid that had lost some enzyme markers after 4 months of culturing.

Key words: *Solanum tuberosum* – Protoplast fusion – Somatic hybrids – Isozymes – Ploidy level

Introduction

The development of protoplast culture and somatic hybridization techniques has made it possible to produce somatic hybrid plants between potato and several partly and completely sexually incompatible wild *Solanum* species such as *Solanum brevidens* (Barsby et al. 1984; Austin et al. 1985a; Fish et al. 1987), *Solanum chacoense* (Butenko and Kuchko 1980) and *Solanum phureja* (Puite et al. 1986). The wild *Solanum* species often carry resistance properties to diseases, and the transmission of resistance to potato leaf roll virus and late blight into interspecific somatic hybrids has been reported (Helgesson et al. 1986). By utilizing the technique of protoplast fusion, the gene pool of potato can be increased, thus providing a desirable diversity for the production of new cultivars.

A more direct approach to the incorporation of tissue culture techniques into practical potato breeding has been proposed by Wenzel et al. (1979). By the use of anther-derived or parthenogenically developed monohaploid and dihaploid lines, breeding could be achieved at a lower ploidy level. This could be followed by fusion of dihaploid clones thereby reconstituting the tetraploidy of potato without the meiotic segregation which would result if heterozygous dihaploids were sexually crossed (Wenzel et al. 1979).

Several methods have already been developed for efficiently using tissue culture in practical breeding. For example methods for the production of anther – derived or parthenogenically produced dihaploid and monohaploid clones have been reported (Foroughi-Wher et al. 1977; Johansson 1986; Uijtewaal et al. 1987a), as have protoplast isolation and culture protocols (for review, see Carlberg et al. 1987)

The objective of this study was to find an efficient and reliable method for identifying intraspecific somatic hybrids of potato produced by the fusion of anther-derived dihaploid clones. The usefulness of isozyme analysis as a method of potato was examined. This technique has already been utilized in the identification of potato cultivars (Oliver and Martínez-Zapater 1985) and somatic hybrids between other species (Sundberg and Glimelius

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1986). It is fast, easy and demands little material. The classification of potato cultivars has been based upon isozymes expressed in tubers, and in order to ensure a more rapid identification of hybrid plants after fusion, we decided to analyse the isozyme systems expressed in the leaves of in vitro-grown plants. Several dihaploid clones were examined for isozyme systems potentially useful for indentifying hybrid and parental plants, irrespective of which of our dihaploid clones we fused. Isozymes have previously been used as biochemical markers for identifying intraspecific somatic potato hybrids. For example, Austin et al. (1985b) analysed peroxidase; Hein and Schieder (1986), peroxidase and esterase; Uijtewaal et al. (1987b), malate dehydrogenase. This report presents a more detailed study of isozymes present in in vitro-grown potato plants which can be used in the identification of intraspecific somatic hybrids.

Materials and Methods

Plant material

Anther-derived dihaploid clones of potato (2n = 2x = 24) (Johansson 1986) were subcultured in vitro as described previously (Carlberg et al. 1983). The following dihaploid clones were used for isozyme analysis: 161:12, 161:14 and 161:15 derived from cv 'Stina'; 198:1, 198:2, 198:4 and 198:21 derived from cv 'Maria'; 309:2 derived from Swedish number var 'SVU 71111'; 536:2 derived from cv 'Magdalena'; and 67:9 derived from Norweigan number var 'Y-67-20-40'.

Fusion and culture of protoplasts

Dihaploid clone 67:9 was chlorophyll bleached with the herbicide SAN 9789 (Sandoz AG, Basel, Switzerland) by placing nodal explants in shoot propagation medium (Carlberg et al. 1983) containing 3% sucrose and 2.8 mg/ml SAN 9789 (Uhrig 1981). Green shoot cultures of dihaploid clone 198:2 were subcultured as described previously (Carlberg et al. 1983). Protoplasts were isolated from 3-week-old, herbicide-treated plants and 4- to 6-week-old green plants according to Carlberg et al. (1983). Fluorescent staining of herbicide-bleached protoplasts was accomplished by the addition of carboxy-fluorescein (Eastman Kodak, Rochester/NY) at a concentration of 4 µg/ml enzyme solution 20 min before protoplast washing.

The fusion procedure was modified from Glimelius et al. (1978). Protoplasts were resuspended in W5 (Menczel et al. 1981) at a final concentration of 5×10^5 protoplasts/ml of each protoplast type. Small droplets of protoplast suspension were added to concanavalin A-treated petri dishes (Glimelius et al. 1978) and allowed to settle for at least 5 min. One or two drops of Hein's fusion solution (Hein et al. 1983) containing 40% w/v polyethylene glycol (PEG) (MW 6,000; Kebo AB, Stockholm, Sweden) at pH 9.0 was added to each protoplast drop and incubated for 7 min. The PEG solution was gradually diluted by the successive addition of a total of 2.0 ml 0.1 *M* $CaCl_2$ in 0.1 *M* sorbitol at pH 7.0 (Glimelius et al. 1978) during a period of 10 min. The PEG-calcium solution was rinsed out with calcium solution followed by a final rinse with culture medium before culture.

The PEG-treated protoplasts were cultured in 1.5 ml modified 8p medium (Glimelius et al. 1986) containing 0.4 *M* glucose, 1.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg/l N^6-benzylaminopurine (BA) (Carlberg et al. 1987) in 5-cm diameter plastic petri dishes. Cultures were kept in the refrigerator for a few hours before transfer to a growth chamber with continuous dim red light (Osram-L-Fluora, 0.5 $uEm^{-2}s^{-1}$) at 25 °C and cultured as described by Carlberg et al. (1987).

After 1 month of culture, fast growing calli were selected under the assumption that they were showing hybrid vigour, and they were transferred to shoot induction medium D (Shepard 1980). From 15 of these calli, plants were regenerated as described by Carlberg et al. (1983). Regenerating shoots were labelled with numbers designating the callus from which they originated and letters identifying separate shoots regenerating from the same callus.

Isozyme analysis

Fresh leaves (100 mg) from in vitro-grown shoots were homogenized in small plastic centrifuge tubes. Different types of extraction buffers and various methods of freeze storage of samples were also tested. However, extraction without extraction buffer and with fresh samples gave the best results. The homogenate was spun at 10,000 *g* for 10 min at +4 °C, and the supernatant was used for analysis. Starch gels (11%) were prepared and run as described by Shields et al. (1983). Three different buffer systems, termed G, H and I by Shields et al. (1983), were tested for all enzymes analysed by starch gel electrophoresis. The following condition for separation on starch gels were utilized for enzymes that showed activity in the leaves. Gels containing buffer G (IDH, MDH) were run for 5–6 h at 20 V/cm; H, for 3 (6PGD) or 6 (PGI) h at 15 V/cm; I, (PGM) for 4–5 h at 17.5 V/cm. For the separation of isozymes by isoelectric focussing (IEF), polyacrylamide gels containing pharmalyte establishing pH gradients of 3.0–10.0 (EST) or 4.0–6.5 (AP) were prepared and run according to Pharmacia's instructions (1982, Laboratory Manual, Pharmacia, Uppsala, Sweden). Prefabricated gels (LKB Produkter AB, Bromma, Sweden) were used for hybrid identification. Gels were prerun at 10 W for 300–500 Vh before the application of filter paper wicks each containing a 20 µl sample. Wicks were removed after about 100 Vh, and the separation was completed after 3,800 Vh.

Fourteen enzyme systems were analysed for activity in in vitro-grown leaves: acid phosphatases (AP) E.C. 3.1.3.2. (Soltis et al. 1983); alcohol dehydrogenase (ADH) E.C. 1.1.1.1. (Vallejos 1983); alkaline phosphatases (AKP) E.C. 3.1.3.1. (Shaw and Prasad 1970); esterases (EST) E.C. 3.1.1.1. using α-naphthyl acetate α-naphthyl propionate and β-naphthyl acetate as substrates in 0.2 *M* Tris-HCl buffer (pH 7.2) and staining with Fast Blue RR; glutamate dehydrogenase (GDH) E.C. 1.4.1.3. (Vallejos 1983); glutamate oxaloacetate transminase (GOT) E.C. 2.6.1.1. (Vallejos 1983); isocitrate dehydrogenase (IDH) E.C.1.1.1.42 (Solitis et al. 1983); leucine aminopeptidase (LAP) E.C. 3.4.11.1 (Vallejos 1983); malate dehydrogenase (MDH) E.C. 1.1.1.37 (Vallejos 1983); Phosphoglucoisomerase (PGI) E.C. 5.3.1.9 (Vallejos 1983); phosphoglucomutase (PGM) E.C. 2.7.5.1 (Vallejos 1983); 6-phosphogluconate dehydrogenase (6PGD) E.C. 1.1.1.43 (Vallejos 1983); shikimate dehydrogenase (SHDH) E.C. 1.1.1.25 (Soltis et al. 1983); superoxide dismutase (SOD) E.C. 1.15.1.1. (Vallejos 1983). Enhanced staining of some isozyme systems was accomplished by applying buffer containing the stain in an agarose overlay (2%). Starch gels were fixed in 5 parts distilled water; 5 parts methanol : 1 part glacial acetic acid, and the polyacrylamide gels were fixed in 2% glycerol.

For PGI, PGM and 6-PGD, the designation of enzyme systems follow the terminology of Oliver and Martínez-Zapater

(1985). All other enzyme systems have been labelled with a hyphenated number added to the abbreviation of each enzyme; the locus coding for the most anodal migration was designated 1, the next 2 and so forth.

Analysis of ploidy level

The DNA content of regenerated hybrid shoots and dihaploid clone 67:9 and 198:2 was measured quantitatively by subjecting propidium iodine-stained nuclei of mesophyll protoplasts to flow cytometry. The protoplasts were isolated from shoots grown in vitro as described above, and the nuclei were prepared, stained and analysed according to Fahleson et al. (1988).

Results

Fusion and culture of fusion products

Protoplasts were isolated from normal green and herbicide-bleached plants (Fig 1). The fusion protocol resulted in fusion frequencies of up to 4% and 20% survival of the protoplasts.

Fusion products could be identified up to 2 days after fusion using the dual fluorescence of carboxy-fluorescein stained, herbicide-treated protoplasts and the autofluorescence of chloroplasts from green mesophyll cells. Fluorescent staining of the herbicide-bleached protoplasts was necessary in order to separate fusion products from normal mesophyll protoplasts. The staining technique made it possible to modify the fusion procedure to obtain living fusion products after several days of culture. Attempts were made to isolate heterokaryons, but the agglutination of dead, unfused and fused protoplasts after 2 days of culture made it extremely difficult. However, agglutinating protoplasts could be separated after 4 days of culture when the protoplasts had regenerated a cell wall and were diluted. Unfortunately, at this time, the fluorescence was too weak to assure a reliable identification of the fusion products. Therefore, nonfused and fused cells were cultured together, and fast growing calli were selected under the assumption that they were showing hybrid vigour. Of these, 25 calli were transferred to shoot induction media, and plants were regenerated from 15 of them.

Enzyme activity in in vitro-grown leaves

Leaf samples were subjected to electrophoresis using starch gel electrophoresis and isoelectric focussing (IEF). In total, 14 isozyme systems were analysed. Of these, 12 were separated using starch gel electrophoresis. Activity was found in leaf tissue for GDH, GOT, IDH, MDH, PGM, 6PGD, PGI and SOD. Using IEF, the activities of six enzyme systems were analysed (Table 1). Of these, the enzyme AP, EST, GOT and SOD showed detectable activity.

An AKP system was also observed, but the staining ability varied from time to time, independent of the separation technique. The SOD pattern was both hard to interpret and inconsistent, and only weak activity of GDH was observed. Therefore, AKP, GDH and SOD were not used in hybrid identification.

PGM-A and PGM-B patterns (GOT, 6-PGD, PGI and PGM enzymes were labelled according to Oliver and Martínez-Zapater 1985) were identical for all the dihaploid clones tested except clone 161:12 which showed an extra PGM-A band. The GOT-B pattern was found to be identical in the two dihaploid clones tested (198:2 and 67:9): both showed a three-banded phenotype. The separation and staining of GOT-B was comparable for both starch gel electrophoresis and IEF. The isozyme

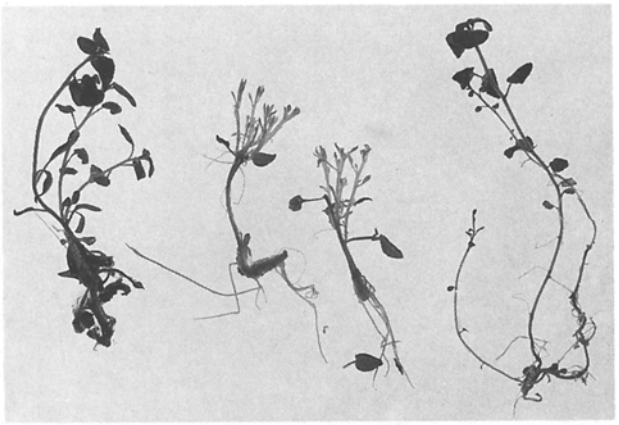

Fig. 1. In vitro-grown shoots used for protoplast isolation: *left*, a green shoot of dihaploid clone 67:9; *middle*, herbicide-bleached shoots of 67:9; and *right*, a green shoot of dihaploid clone 198:2

Table 1. Enzyme activity in in vitro-grown shoots. Separation of enzymes was achieved using starch gel electrophoresis (GE) or isoelectric focussing (IEF)

Enzyme	Abbreviation	GE	IEF
Acid phosphatase	AP	N.T.	+
Alcohol dehydrogenase	ADH	−	−
Alkaline phosphatase	AKP	*	*
Esterase	EST	N.T.	+
Glutamate dehydrogenase	GDH	−	N.T.
Glutamate-oxaloacetate transaminase	GOT	+	+
Isocitrate dehydrogenase	IDH	+	N.T.
Leucine aminopeptidase	LAP	−	−
Malate dehydrogenase	MDH	+	N.T.
6-Phosphogluconate dehydrogenase	6-PGDH	+	N.T.
Phosphoglucomutase	PGM	+	N.T.
Phosphoglucose isomerase	PGI	+	N.T.
Shikimate dehydrogenase	SHDH	−	N.T.
Superoxide dismutase	SOD	+	+

− = no or weak activity; + = strong activity; * = activity varies; N.T. = not tested

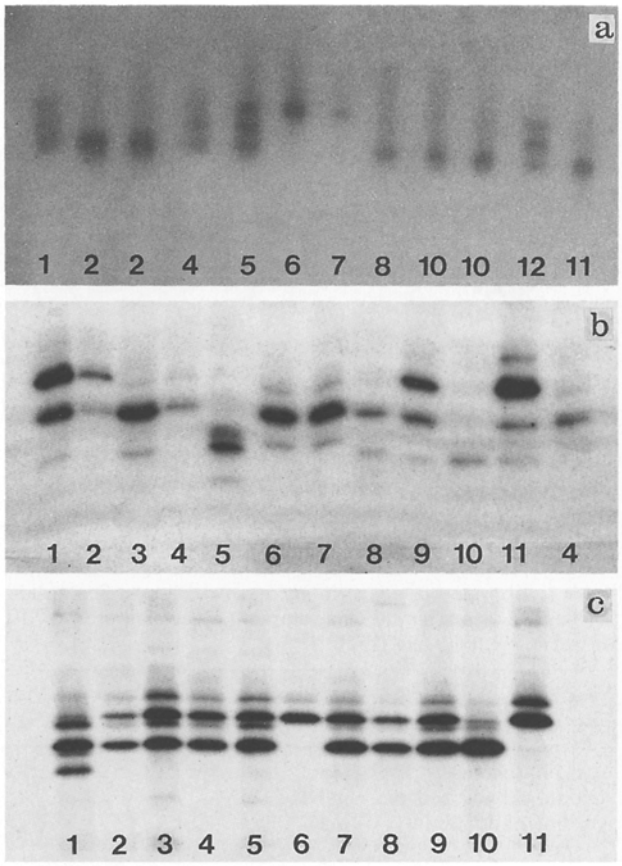

Fig. 2a–c. Isozyme expression in in vitro-grown leaves shown by different dihaploid potato clones. **a** Isocitrate dehydrogenase, starch gelelectrophoresis; **b** acid phosphatases, IEF; and **c** esterases, IEF. Explanation of symbols: *1* = 161:12, *2* = 161:14, *3* = 161:15, *4* = 198:1, *5* = 198:2, *6* = 198:4, *7* = 198:21, *8* = 309:2, *9* = 309:6, *10* = 536:2, *11* = 67:9, *12* = P536 cv 'Magdalena'. *Lane 5* (clone 198:2) and *lane 11* (clone 67:9) represent dihaploid fusion parents

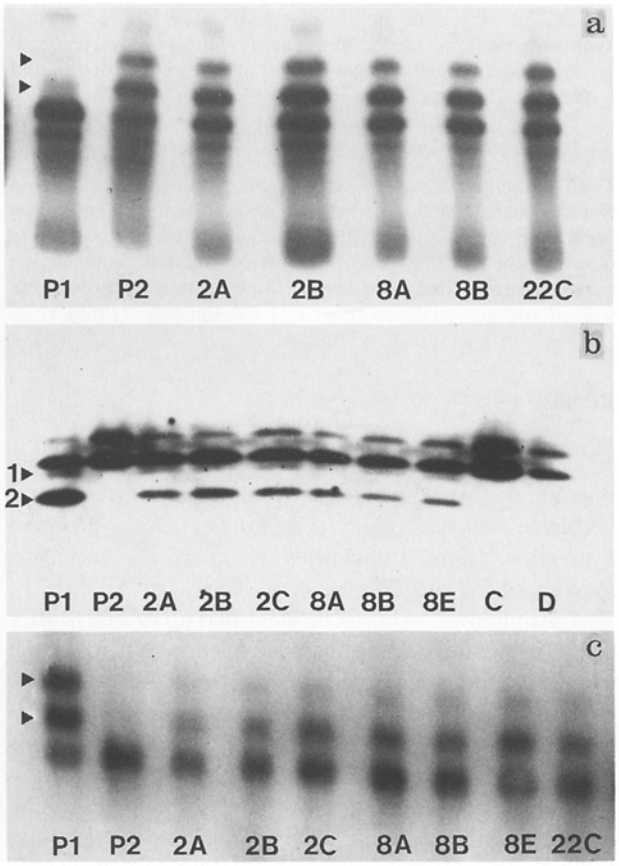

Fig. 3a–c. Isozyme expression in in vitro-grown leaves from dihaploid potato clones used as fusion parents, somatic hybrids and protoclones of 67:9. **a** Phosphoglucose isomerase, starch gel electrophoresis; **b** esterases, IEF; and **c** isocitrate dehydrogenase, starch gel electrophoresis. Explanation of symbols: parental clones: *P1* = 198:2, *P2* = 67:9; hybrid plants (as described in text): *2A, 2B, 2C, 8A, 8B, 8C, 22C;* protoclones of 67:9: *C* and *D*

pattern of IDH varied between dihaploid clones (Fig. 2a) consisting of one out of two bands with different electrophoretic mobility or of both bands and a band with intermediate electrophoretic mobility. Isozyme patterns also varied for the dimeric enzyme systems MDH-2, PGI-B and 6PGD-C (data not shown). The AP pattern separated by IEF consisted of a total of six bands (Fig. 2b), the number varying between dihaploid clones. The three more anodal bands were consistent in intensity and did not vary between different runs; the three more cathodal bands were more variable. The pI of this AP system was estimated to pH 5.5–6.2. A more cathodal AP system was also present, but not recorded. Two EST systems were recorded, EST-1 and EST-2, but EST-2 showed higher activity, and clonal differences were recorded among the six bands (Fig. 2c). By comparing the clonal isozyme patterns for EST, AP, PGI, MDH, IDH, PGM and 6PGD, we found that hybrids are possible to identify irrespective of which of the ten dihaploid clones are fused.

Identification of somatic hybrids

Leaves from 28 regenerated shoots of putative hybrids (derived from 15 calli) and from the fusion parents, dihaploid clones 67:9 and 198:2, were subjected to electrophoresis using the methods described in the previous section. The PGI-B patterns of the putative hybrids and the dihaploid parents were compared. In this isozyme system, dihaploid parents were compared. In this isozyme system , dihaploid clone 67:9 contains 2 alleles coding for proteins of different electrophoretic mobility. Since PGI-B is a dimeric enzyme, a heterodimeric band is also formed, resulting in a three-banded phenotype.

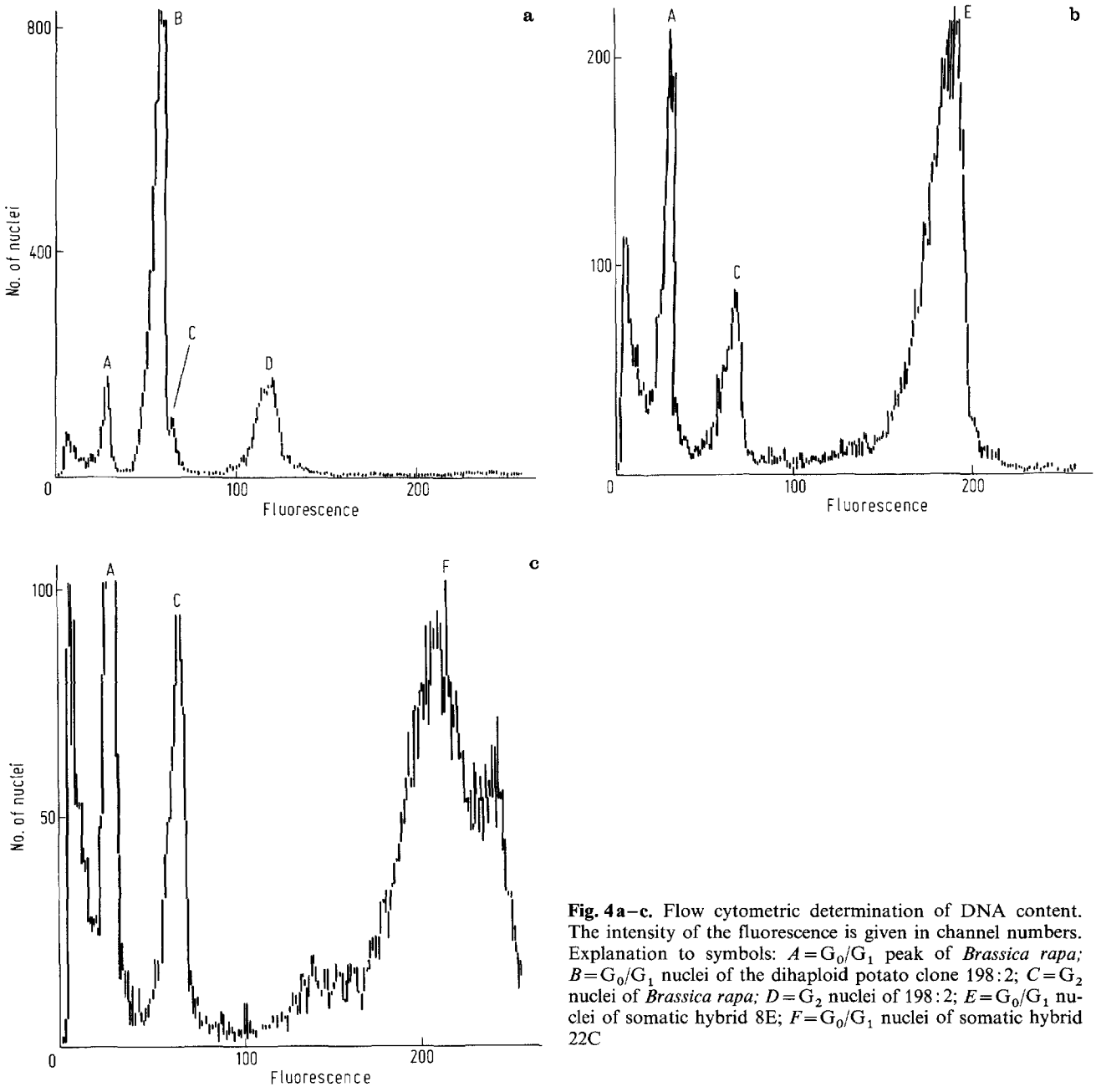

Fig. 4a–c. Flow cytometric determination of DNA content. The intensity of the fluorescence is given in channel numbers. Explanation to symbols: $A = G_0/G_1$ peak of *Brassica rapa*; $B = G_0/G_1$ nuclei of the dihaploid potato clone 198:2; $C = G_2$ nuclei of *Brassica rapa*; $D = G_2$ nuclei of 198:2; $E = G_0/G_1$ nuclei of somatic hybrid 8E; $F = G_0/G_1$ nuclei of somatic hybrid 22C

Dihaploid clone 198:2 contains only one allele (one-banded phenotype) which codes for the slower moving protein (Fig. 3a). The isozyme analysis of PGI-B demonstrated that every regenerated plants showed the three-banded phenotype (Fig. 3a). The presence of 67:9 specific isozymes in all regenerated putative hybrid plants was further confirmed by isozyme analysis of AP (data not shown).

To check if 198:2 specific isozymes were also present in all regenerated plants, EST-2, IDH and MDH-2 patterns were studied. In EST-2, dihaploid clone 198:2 could be separated from dihaploid clone 67:9: the former showed an extra band (b1) not present in 67:9 and a band (b2) of strong activity that showed very low activity in 67:9. However, of the regenerated plants only seven plants arising from three different calli showed both 67:9 specific isozymes and 198:2 specific isozymes. These plants (2A, 2B, 2C, 8A, 8B, 8E and 22C) contained the b1 esterase band and the b2 esterase bands of intermediate activity (Fig. b). The IDH (Fig. 3c). as well as the MDH-2 patterns confirmed the presence of 198:2 specific isozymes in the same plants as those showing

198:2 specific EST-2 isozymes. No other plant showed the presence of 198:2 specific isozymes for the enzymes analysed. From callus 22, three plants were regenerated, but only one (plant 22C) showed both 198:2 and 67:9 specific isozymes. There was no change in the isozyme patterns after 4 months of further in vitro culture except in plant 22C which had then lost its 198:2 specific MDH-2 isozymes.

DNA determination

The 2x ploidy level of dihaploid clones 67:9 and 198:2 was confirmed by flow cytometric measurement of total DNA. Plants 2A, 2B, 2C, 8A, 8B, 8E and 22C, which showed both 67:9 and 198:2 specific isozymes, were considered to be hybrid plants, and their DNA content was measured. Plants arising from calli 2 or 8 were all hexaploids, while plant 22C was highly mixoploid containing nuclei with ploidy levels ranging from tetraploidy to octoploidy. The ploidy level was stable after 4 months of further in vitro shoot culture. DNA histograms of dihaploid clone 198:2 hexaploid hybrid 8E and mixoploid hybrid 22C is shown in Fig. 4.

Morphology of hybrid plants

No detailed morphological study of the hybrid plants was carried out since it was not possible to separate the dihaploid clones on superficial morphological characters alone. However, hybrid plants from calli 2 and 8 looked quite similar and grew more vigourously than their parental plants, while plant 22C showed stunted growth and had dark green leaves.

Discussion

Fusion products could be identified up to 2 days after fusion based upon the dual fluorescence of the hybrid cells. By this time, a new cell wall had been regenerated, indicating survival of the hybrid cells. Several PEG fusion protocols were tested, but all resulted in an agglutination of the protoplasts, which made the mechanical isolation of the heterokaryons extremely difficult. Because the development of hybrid cells can only be monitored if heterokaryons are isolated and grown separately, we would have liked to have isolated the heterokaryons by either micromanipulation or flow sorting instead of by selecting putative hybrids based on the criterion of vigourous growth. To reduce the damage caused by PEG to potato mesophyll protoplasts, other types of protoplasts, such as callus protoplasts, have been used as one fusion partner, as described by Hein and Schieder (1986). They isolated heterokaryons using a micromanipulator and cultured them separately. Several hybrid calli were identified, but no shoots were obtained. We were reluctant to use cell culture protoplasts since chromosomal modifications are known to occur during the cell culture of potato (e.g. Jacobsen et al. 1983; Sree Ramulu 1986). Electrofusion rather than chemical fusion could also be used as an alternative. Somatic hybrids have been produced using electrofusion followed by the mechanical isolation of heterokaryons between mesophyll protoplasts of *Solanum phureja* and *Solanum tuberosum* (Puite et al. 1986). Using electrofusion, we have been able to mechanically isolate and culture potato heterokaryons (unpublished observations). More recently, we observed that if the PEG concentration is lowered from 40% to 35% w/v, there is an improved survival of protoplasts, and consequently, it is now also possible to isolate heterokaryons from PEG-fused material. The improved conditions for fusion will be presented elsewhere.

A general method for identifying intraspecific somatic hybrids of potato using isozyme patterns is presented in this report. By comparing isozyme patterns for EST and AP using isoelectric focussing, and PGM, PGI, 6-PGD, IDH and MDH patterns using starch gel electrophoresis, it should be possible to identify not only the intraspecific hybrids produced, but also other intraspecific potato hybrid plants irrespective of which of the dihaploid clones that are fused.

Based upon the combination of isozyme patterns from both parents, seven plants arising from three different calli were considered to be somatic hybrids. The remaining plants from 12 calli all showed isozyme patterns identical to dihaploid clone 67:9. The reason for this lack of plants with only parent 198:2 isozyme could be due to our selection of fast growing calli with hybrid vigour. We know from other experiments on protoplast culture (Waara and Eriksson, in preparation) that 67:9 protoplasts show a higher plating efficiency than 198:2 in the the selected medium. Either only a few 198:2 protoplasts formed callus, and these were discarded during hybrid selection, or calli were obtained, but no shoots regenerated.

The data presented by Austin et al. (1985b), Fish et al. (1987) and Uijtewaal et al. (1987b) indicate that at least some hybrid vigour is expressed in hybrid potato calli. This is in contrast to the report by Wenzel et al. (1982): they regenerated 2,000 plants from fusion of dihaploid potato after selecting for fast growing calli and detected no hybrid plants. We obtained hybrid plants from 3 out 15 shoot-regenerating calli which were selected as showing hybrid vigour. However, it is difficult to ascertain if this was a result of hybrid cells showing more vigorous growth than parental cells or a result of the poor division frequency of 198:2 protoplasts using the selected culture protocol.

In this study, only plants from calli 2, 8 and 22 showed isozymes corresponding to those of parent 198:2 (in three separate isozyme systems) and parent 67:9 (in 2 separate isozyme systems). Neither the apparence of new bands nor the loss of bands was detected in hybrid plants, except in plant 22C which lost 2 MDH-2 bands after 4 months of culture. Although somaclonal variation can give rise to new isozyme variants (Copper et al. 1986; Allicchio et al. 1987) the use of several enzyme systems reduces the risk of identifying a somaclonal variant shoot as a hybrid.

Flow cytometric DNA analysis revealed that while hexaploid and mixoploid hybrid plants were obtained, no hybrid plants with the excepted ploidy level were found. Other researchers have also found considerable variations in chromosome number in somatic hybrids. Uijtewaal et al. (1987b) found that after fusion between monohaploid lines of potato, only tetraploid plants were regenerated. Puite et al. (1986) cytologically analysed somatic hybrids between dihaploid *S. tuberosum* and diploid *S. phureja* and showed that primarily hexaploid and octoploid plants were obtained. Furthermore, Giemsa-C-banding of chromosomes in the hybrid plants demonstrated that both parents can contribute more than one chromosome set to the somatic hybrid. Other reports indicate that at least some plants with the expected chromosome number can be obtained (Austin et al. 1985a,b; Fish et al. 1987). These deviations in chromosome number may be caused by the survival of multiple fusion products or they are induced during cell culture. According to Carlberg et al. (1984), protoplasts containing an abnormally high DNA content occur as early as the initial stages of potato protoplast culture. We have also noted that all plants regenerated from protoplasts from dihaploid clone 67:9 are aneuploid or tetraploid (Waara and Eriksson, unpublished observation). Since the changes induced during cell culture are difficult to regulate, the only way to obtain stable, tetraploid somatic hybrids seems to be to select them from large populations of hybrids. The large scale production of somatic hybrids of potato is possible using the methods described in this paper. The identification of hybrid plants using isozyme analysis is especially rapid and allows us to screen 25 plants a day for up to 6 isozyme systems. However, a more efficient approach would be to combine the methods for identifying heterokaryons, for identifying hybrid plants using isozyme analysis, and DNA content measurements, as presented in this paper, with a more efficient fusion technique (i.e. electrofusion or our PEG fusion protocol with the reduced concentration of PEG) and a selection of hybrid cells by flow sorting. An analogous system has already proven useful for the selection of normal somatic hybrids of *Brassica napus* (Sundberg and Glimelius 1986; Sundberg et al. 1987).

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