Plant Cell Reports (1992) 11:466–469

Plant Cell Reports

The use of RAPD markers for the detection of gene introgression in potato

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Received January 2, 1992/Revised version received May 7, 1992 – Communicated by M. R. Davey

Abstract. Randomly Amplified Polymorphic DNAs were employed to demonstrate that potato dihaploids generated after interspecific pollination of a tetraploid *Solanum tuberosum* cultivar (Pentland Crown) by *Solanum phureja* dihaploid inducer clones could not be of parthenogenetic origin. Of six different 10-mer oligonucleotides, four generated products from total potato dihaploid genomic DNAs which were not derived from the *S. tuberosum* parent. Gel electrophoresis and Southern analysis indicated that these amplified bands originated from *S. phureja*. The results are discussed in the context of recent cytological and molecular evidence which demonstrates that potato dihaploids are aneusomatic (Clulow et al. 1991) and emphasises this approach as a general methodology for the detection of alien gene introgression in both natural and cultivated plant populations.

Introduction

The plasticity or fluidity of the plant genome results in its ability to tolerate and generate diversity which is a key component of conventional crop improvement programmes. In addition to being a feature of crop improvement and a major factor in angiosperm evolution, interspecific and intergeneric hybridisation have also been used for the *in vitro* induction of haploids (Hermsen and Ramana 1981). Interspecific hybridisation followed by normal double fertilisation and preferential elimination of chromosomes during the early stages of embryo development is widespread. The main examples include: barley following interspecific hybridisation with *H. bulbosum* (Kasha and Kao 1970), wheat (*Triticum aestivum*) following intergeneric hybridisation with *H. bulbosum* (Barclay 1975) and maize (Laurie and Bennett 1986). Pseudogamy is another route to ploidy reduction and has been described for: *Zea mays* (Chase 1969), *Solanum tuberosum* (Hougas and Peloquin 1958), *Medicago sativa* (Bingham 1969), and *Populus* species (Stettler et al. 1969).

The extraction of dihaploids (2n = 2x = 24) from *Solanum tuberosum* following pollination with *S. phureja* is particularly relevant to potato breeders. The tetraploid, highly heterozygous nature of potato limits the predictability of identifying genotypes with desirable attributes in sexual progeny. In order to overcome this problem, dihaploids have been used extensively in potato breeding and genetics (Ross 1986). In addition, wild diploid *Solanum* species are an important source of abiotic and biotic resistance genes. Sexual hybrids between dihaploids and wild species provide a vehicle for gene transfer into adapted potato germplasm. Dihaploids may therefore be used to expedite and refine the breeding of improved potato cultivars. Recently, an alternative mechanism for dihaploid production in potato has been proposed (Clulow et al. 1991). In this manuscript we report the use of randomly amplified polymorphic DNA markers (RAPDs) (Williams et al. 1990, Welsh and McClelland 1990, Waugh and Powell 1992) for the characterisation of dihaploid

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Table 1. A summary of the genetic constitution of Pentland Crown dihaploids for the presence (+) and absence (–) of polymorphic amplification products specific to *S. phureja* for four primers.

	Dihaploid inducer	SC10–15 GCTCGTCAAC *S. phureja*	SC10–19 CGTCCGTCAG *S. phureja*	SC10–20 ACTCGTAGCC *S. phureja*	SC10–4 TACCGACACC *S. phureja*
Pentland Crown		–	–	–	–
S. phureja	IVP 48	+	+	+	+
PDH 7	EC 90	–	+	+	–
PDH 35	EC 90	–	+	+	–
PDH 40	PI 1	–	–	+	–
PDH 41	PI 22	+	–	+	–
PDH 51	IVP 48	–	+	–	–
PDH 52	IVP 48	–	+	+	–
PDH 55	IVP 48	+	–	–	–
PDH 87	IVP 48	–	+	+	–
PDH 425	IVP 48	–	Bands from neither parent	–	+
PDH 440	IVP 48	+	*	–	–
PDH 452	IVP 48	+	–	–	+
PDH 590	80CP12	+	–	+	+
PDH 591	80CP12	+	+	–	+
PDH 593	80CP12	–	–	–	–
PDH 595	80CP12	–	*	*	–

* Not done.

potato genotypes. The detection of *S. phureja* specific amplification products in the dihaploids derived from *S. tuberosum* provides further molecular proof for the origin of these dihaploids via double fertilisation and subsequent chromosome elimination.

Materials and Methods

Plant material

The dihaploids generated from the tetraploid cultivar Pentland Crown by interspecific pollination with *S. phureja* are given in Table 1. Five *S. phureja* dihaploid inducing clones were used to construct the dihaploids (IVP48, EC90, PI1, PI22 and 80CP12). Only one, IVP48, was available for inclusion in this study.

DNA extraction and polymerase chain reaction

DNA was isolated from fresh leaf material by the procedure of Saghai–Maroof et al. (1984) but with additional phenol and chloroform extractions, and RNase A treatment at 68°C.

The oligonucleotide primers (10–mers) used for the generation of RAPDs in this study were synthesised on an Applied Biosystems PCR–mate oligonucleotide synthesiser. These were SC10–4 (5'–TACCGACACC–3'), SC10–15 (5'–GCTCGTCACC–3'), SC10–19 (5'–CGTCCGTCAG–3'), SC10–20 (5'–ACTCGTAGCC–3'), SC10–18 (5'–GCCCTACGCG–3') and SC10–2 (5'–GGTCCTCAGG–3'). Amplification reactions (100 µl final volume) contained 20 ng dNTPs, 1X Taq

polymerase buffer (10 mM Tris HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% Gelatin (w/v), 0.01% Tween 20, 0.01% NP40) and 1.3 units of Taq DNA polymerase (IBI). Each reaction mix was overlaid with 75 µl of mineral oil to prevent evaporation. For enzymic amplification, samples were subject to 45 repeats of the following thermal cycle: one minute at 92°C followed by one and a half minutes at 35°C then two minutes at 72°C. The extension time was increased to 5 minutes on the final cycle. Amplification products were separated according to length by running 20 µl of product on 1.5% agarose gels, stained with ethidium bromide and visualised by illumination with UV light.

Results and Discussion

Polymorphism was detected between *Solanum phureja* and *S. tuberosum* with four of the six primers evaluated. The transmission of amplified polymorphic DNA was monitored in dihaploids extracted from a common tetraploid clone (Pentland Crown). Amplified products were found to segregate in the dihaploid progeny indicating that these loci must be in the heterozygous condition in the tetraploid parent. The polymorphism detected between *S. phureja* (IVP 48) and Pentland Crown is illustrated in Fig. 1a with SC10–4. With this primer, Pentland Crown derived dihaploids PDH 452 and 590 also generate amplification products which are not present in the tetraploid parent but are characteristic of *S. phureja* IVP 48.

In order to provide further molecular proof for the presence of *S. phureja* IVP 48 products in the dihaploids, the amplification product (*) indicated in Fig. 1a was excised, labelled with [^{32}P] dCTP and used to probe Southern blots of the SC10–4 specific amplification products and restriction endonuclease digested Pentland Crown and *S. phureja* IVP 48 DNA. Fig. 1b demonstrates that the labelled product hybridises to *S. phureja* IVP 48 specific products present in PDH 452 and PDH 590. In genomic Southern blots, the excised fragment hybridised to a polymorphic highly repetitive sequence in both Pentland Crown and *S. phureja* and was therefore difficult to interpret (not shown). The results from similar studies with the other primers are summarised in Table 1. Two categories of dihaploids can be identified,

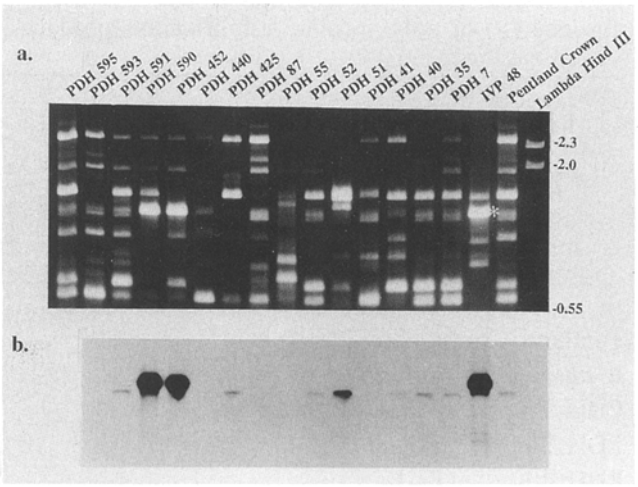

Fig. 1a. Detection of gene introgression with primer SC10–4 which gives an amplification product (*) unique to *S. phureja* IVP 48. This product also appears in PDH 452 and PDH 590.

Fig. 1b. The unique band (Fig. 1a*) from *S. phureja* IVP 48 was used to probe a Southern blot of the amplified fragments generated by SC10–4 (Fig. 1a). Hybridisation occurs only to the *S. phureja* IVP 48 specific products present in PDH 452 and PDH 590.

those with polymorphic amplified products derived only from Pentland Crown (e.g. PDH7) and those with polymorphic products from *S. phureja* and from P. Crown (e.g. PDH 452). Also included in Table 1 is the specific *S. phureja* genotype used in the dihaploid induction cross. Certain dihaploids (e.g. PDH 425) contain amplification products which are not found in either P. Crown or *S. phureja* IVP 48. Although these products may be specific to the particular *S. phureja* clone used as the male parent and reflect inherent polymorphism within *S. phureja* the possibility that they are simply spurious amplification products cannot be excluded. Nevertheless, the results suggest that the Pentland Crown derived dihaploids could not have arisen via a parthenogenetic mode of development. This conclusion is consistent with a recent cytological and molecular study (Clulow et al. 1991) which indicated that the Pentland Crown dihaploids are aneusomatic and could not be of parthenogenetic origin. The presence of specific *S. phureja* amplification products in the dihaploid

progeny indicates that ovules of Pentland Crown were fertilised by pollen from *S. phureja* and that the aneusomatic clones originated from triploid zygotes following elimination of some of the *S. phureja* chromosomes. These results demonstrate the potential of RAPD markers for monitoring and identifying the presence of alien genes in adapted potato cultivars. Furthermore RAPD markers may be used to study changes in allele frequencies under various selection pressures and investigate the stability of potato material originating from tissue culture. They offer several advantages over other polymorphism assays including simplicity of operation and a non-isotopic procedure for the detection of polymorphism. The small amount of genomic DNA (20 ng) used in the PCR is also a significant advantage and will allow tuber-extracted DNA to be used as a method of fingerprinting potato clones. In addition, the primers used in PCR are species-independent and may therefore be used as polymorphic markers in other organisms to monitor gene introgression and establish the origin and mode of ploidy reduction.

Acknowledgements

The Authors are supported by the Scottish Office Agriculture and Fisheries Department. We wish to thank Mr. M. De,Maine for access to the dihaploids used in this study.

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