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Detection of Genetic Variation and Gene Introgression in Potato Dihaploids Using Randomly Amplified Polymorphic DNA (RAPD) Markers

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Randomly amplified polymorphic DNAs were employed to study the genetic variation and gene introgression in potato dihaploids (2n=24) which were generated after interspecific pollination of tetraploid cultivars (2n=4X=48, *Solanum tuberosum* cv Irish Cobbler, Superior and Dejima) by haploid inducer clones (2n=2X=24, *Solanum phureja* 1.22, Hes-5 and Hes-6). Genetic variation and DNA marker segregation among dihaploids were observed. Most dihaploids contain *S. tuberosum* specific RAPD markers but haploid inducer-specific RAPD markers were also found in some dihaploids. Of six different arbitrary 10-mer oligonucletide primers which showed polymorphism between tetraploid cultivars and haploid inducers used, three generated amplification products which seemed to be derived from the *S. phureja* parent. Our results indicate that chromosomes of dihaploids may not be pure *S. tuberosum* and the dihaploids may not be produced by parthenogenesis.

Keywords: RAPDs, polymorphism, dihaploids, haploid inducer, gene introgression

Crosses that may be either interspecific or interploidal have been used frequently to produce haploid plants (Rowe, 1974), and parthenogenesis (gynogenesis), androgenesis (Lacadena, 1974) or chromosome elimination (Kasha and Kao, 1970) after hybridization has been suggested to induce haploid plants.

The haploids or dihaploids are valuable to geneticists and breeders since reduced ploids simplify the complex genetics of the the plants. In potato, dihaploids can be generated after interspecific pollination of tetraploid potatoes (2n=4X=48) by clones (2n=2X =24, *Solanum phureja*) known as dihaploid inducers. However, these crosses usually produce a range of dihaploid, triploid and tetraploid progenies (Clulow *et al.*, 1991; Cho *et al.*, 1993, 1995). The utility of haploids is based on the assumption that haploids are genetically pure and so can be regarded as gametic sample of their tetraploid parents (Wilkinson *et al.*, 1995).

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Although the detailed process of dihaploid induction is not known, it has been known that pollination using pollen from haploid inducer *S. phureja* clones stimulates unfertilized ovules in the tetraploid parent to develop parthenogenetically and that the haploid inducer does not contribute any genetical information to the dihaploid progenies (Rowe, 1974; Van Breukelen *et al.*, 1977). Bender (1960) and Montelongo-Escobedo and Rowe (1963) observed that in some *S. phureja* microspores the generative nucleus did not divide to form two sperm nuclei, instead a single restitution nucleus of 24 chromosomes was provided. Rowe (1974) suggested that the restitution nucleus combined with the central nucleus gave a hexaploid endosperm and helped parthenogenetic development of an unfertilized dihaploid embryo. The tetraploid progeny probably arise from the fertilization by diplandoids (2n pollen) of diploid pollinator (Quinn *et al.*, 1974) or doubling of dihaploid embryos (Wilkinson *et al.*, 1995).

However, pollinating the tetraploid cultivars with pollen from haploid-inducer produces aneusomatic dihaploids with a proportion of hyperploid cells and

some dihaploids contain variable numbers of triploid cells (2n=36) (Clulow *et al.*, 1991). It has been observed that the dihaploids with extra chromosomes usually contain DNA segments of the pollinator (Clulow *et al.*, 1993). RFLP analyses identified unique hybridizing fragments present in *S. phureja* but not in tetraploid cultivars in some of the dihaploid offspring (Clulow *et al.*, 1991; Waugh *et al.*, 1992). Thus, it was suggested that the ovules of tetraploid cultivars were fertilized by pollen from *S. phureja* and that the aneusomatic clones were derived from triploid zygotes from which some of the *S. phureja* chromosomes were eliminated (Waugh *et al.*, 1992). Recently, Wilkinson *et al.* (1995) reported that euploid (2n=24) generated from crossing *S. tuberodum* cv. Pentland Crown with a haploid inducer *S. phureja* clone IVP48 also contained and expressed DNA from dihaploid inducer (*S. phureja*) clone IVP48. Since genomic *in situ* hybridization clearly identified the inducer DNAs incorporated into dihaploid at the several sites on chromosomes, it was suggested that somatic translocation might occur during dihaploid induction (Wilkinson *et al.*, 1995). Both euploids and aneuploids containing alien DNA segments from haploid-inducer indicate that besides parthenogenesis other mechanisms are involved in the dihaploid induction in potato. Uses of such heterogenous euploid dihaploids as gametic samples for genetical study is limited.

In this study, we report that genetic variation among dihaploids can be identified easily using RAPDs and that introgression of haploid-inducer segments into dihaploids of several *S. tuberosume* cultivars which are economically important in Korea is very common. Furthermore, the information obtained in this study will help to verify the exact mechanism of haploid induction after interspecific crossing.

MATERIALS AND METHODS

Plant materials

Dihaploids were made by crossing *S. tuberosum* cultivars (2n=48) by diploid *S. phureja* haploid-inducers (2n=24) (Cho *et al.*, 1995). Dihaploids were identified and selected by counting chromosome numbers (data not shown). The cultivars, haploid-inducer clones and dihaploids used in this study are given in Table 1.

DNA extraction and polymerase chain reaction

Table 1. List of cultivars, dihaploid-inducer clones and dihaploids

	Source
Tetraploid cultivars (*Solanum tuberosum*)	
Irish Cobbler	R.D.A.
Dejima	R.D.A.
Superior	R.D.A.
Dihaploid-inducer clones (*Solanum phureja*)	
1.22	R.D.A.*
Hes-5	R.D.A.
Hes-6	R.D.A.
Dihaploids	
DH28-2	Dejima X Hes-5
DH28-3	Dejima X Hes-5
DH29-20	Dejima X Hes-6
DH29-21	Dejima X Hes-6
DH29-25	Dejima X Hes-6
DH29-42	Dejima X Hes-6
DH4-6	Superior X 1.22
DH2-10	Superior X Hes-5
DH3-24	Superior X Hes-5
HIC-139	Irish Cobbler X Hes-5
HIC-120	Irish Cobbler X Hes-5
HIC-48	Irish Cobbler X Hes-6
HIC-92	Irish Cobbler X 1.22
DH32-3	Irish Cobbler X 1.22

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Genomic DNA was extracted from 1 g of fresh leaf using extraction buffer containing cetyltrimethyl ammoniumbromide (CTAB) (Isabele *et al.*, 1993). PCR analyses were performed on DNA from *S. tuberosum* cultivars, *S. phureja* clones and dihaploids.

The arbitrary oligonucleotide (10-mers) primers used for the generation of RAPDs in this study were purchased from University of British Columbia, Canada. Only the primers showing polymorphism between cultivars and inducer clones were selected and used in this study. These were #420 (5'-GCAGGGTTCG-3'), 457 (5'-CGACGCCCTG-3'), 467 (5'-AGCACGGGCA-3'), 471 (5'-CCGACCGGAA-3'), 472 (5'-AGGCGTGCAA-3') and 474 (5'-AGGCGGGAAC-3'). Amplification reactions (25 μL final volume) contained 200 μM dNTPs (Pharmacia), 0.2 μM primer, 1 unit of Taq DNA polymerase in the incubation buffer provided by the manufacturer of the enzyme (Promega, USA), 1.25 mM $MgCl_2$ and 30 ng DNA. Amplification was achieved in a thermal cycler (Ericomp, USA) programmed as follows: Cycle 1, 30 s at 94°C; cycles 2-

46, 30 s at 94°C, 30 s at 35°C and 2 min at 72°C; cycle 47, 5 min at 72°C. Amplification products were subjected to electrophoresis in 1.3 percent agarose (SeaKem) and detected by ethidium bromide staining, viewing under ultraviolet light and photographed using Polaroid 667 film. Molecular weight were estimated using pBR328 DNA·Bgl I+ pBR328 DNA·Hinf I marker (Boehringer Mannheim, Germany).

RESULTS AND DISCUSSION

The profiles of the amplified DNA products from cultivars and haploid inducer clones were compared to each other for identification of specific markers, and six primers showing polymorphism were selected and used for further studies. When the transmission of amplified polymorphic DNA was monitored in dihaploids, two categories of dihaploids can be identified, those with polymorphic amplified products derived only from tetraploid parents and those with polymorphic products from both of the tetraploid parent and *S. phureja* haploid inducer (Fig. 1a, b, c, d and e).

Some amplified products were found to segeregate in the dihaploid progeny indicating that these loci are in the heterozygous condition in the tetraploid parent (Fig. 1a). Thus, the identification of these segregating markers could be used in genetical analysis such as marker-assisted selection, linkage, tagging and changes of allele frequencies.

Most dihaploids contain *tuberosum* specific RAPD markers but haploid inducer-specific RAPD markers were also found in some dihaploids (Fig. 1b, c and e). Of 6 RAPD primers found to discriminate between parents, three (UBC-467, 472, 474) revealed an haploid inducer-specific marker in dihaploids. As Waugh *et al.* (1992) and Wilkinson *et al.* (1995) revealed an inducer-specific marker in dihaploids and Southern blot analysis confirmed that bands in dihaploids and inducer were homologous, haploid inducer-specific markers detected in dihaploids in this study appeared to be from the *S. phureja* haploid inducer origin although Southern blot analyses were not conducted in this study. Therefore, the chromosomes of dihaploids are not pure *S. tuberosum*. This conclusion is also consistent with a recent cytological and molecular studies (Clulow *et al.*, 1991; Waugh *et al.*, 1992; Wilkinson *et al.*, 1995) which indicated that the aneusomatic- or euploid-derived dihaploids were heterogenous.

The presence of specific *S. phureja* amplification products in the dihaploid progenies indicates that ovules of tetraploid parent were fertilized by pollen

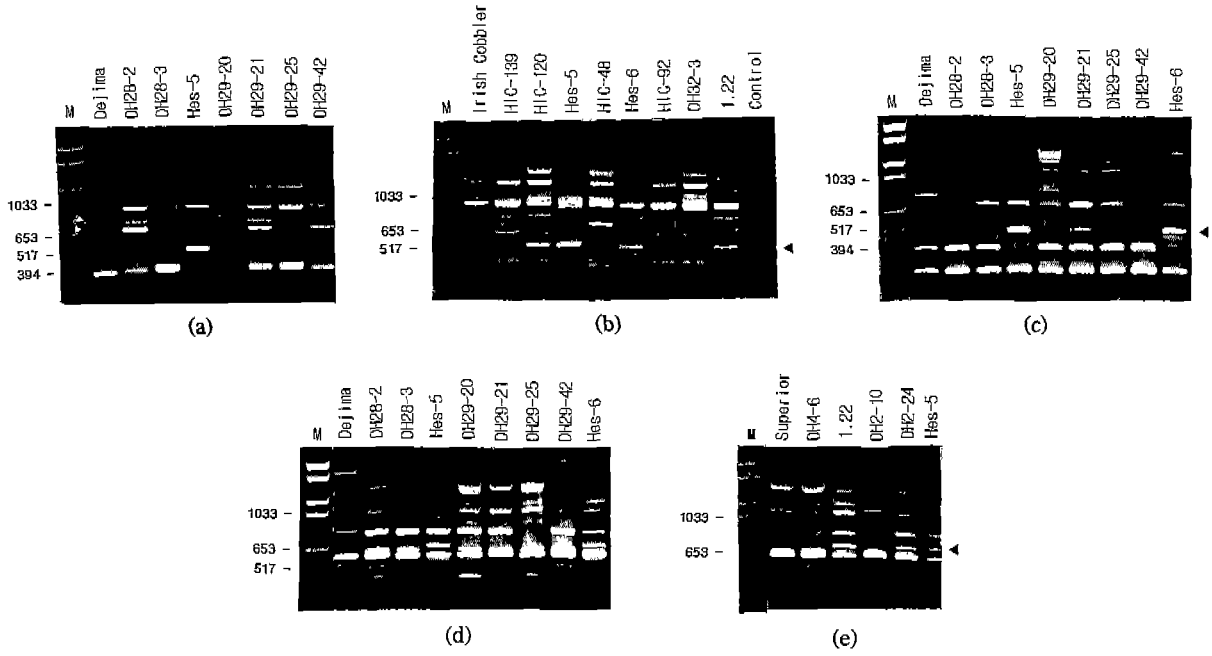

Fig. 1. Amplification products using DNA extracted from several tetraploid potato cultivars (*S. tuberosum*) and dihaploid inducer clones (*S. phureja*). a) and b) primer UBC-467, c) primer UBC-472, and d and e) primer UBC-474 ▷ Segregation of RAPD markers specific to *S. tuberosum*. ▶ Presence and expression of DNA from inducers in potato dihaploids.

from *S. phureja* haploid inducer and that the dihaploid clones might be originated from triploid zygotes following elimination of some of the *S. phureja* chromosomes as in haploid induction after crossing between *Hordeum vulgare* and *Hordeum bulbosum* (Kasha and Kao, 1970). *In situ* hybridization of chromosome by haploid inducer(IVP48)-specific DNA band as a probe showed that signals were present as large segments on several chromosomes of dihaploid (PDH55) and inducer (IVP48) DNA has not incorporated into dihaploid (PDH55) as entire chromosomes (Wilkinson *et al.*, 1995). Thus, translocation of DNA segments may occur during dihaploid induction. However, it is not known whether the introgression of haploid-inducer specific DNA segments into *S. tuberosum* chromosomes occurs after parthenogenesis.

Our results also indicate that the potential of RAPD markers for monitoring and identifying the presence of alien genes in potato cultivars. RAPD techniques used in this study give several advantages over the polymorphism assays including simplicity and rapidity of operation and a non-isotopic procedure for the detection of polymorphism. The small amount of genomic DNA (30 ng) used in the PCR is also a significant advantage as a method of identification of potato clones.

We are now continuing further researches on the useful traits of dihaploid inducer, a pattern of gene introgression and detailed mechanism of dihaploid induction using RAPD and *in situ* hybridization.

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