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# Cytological and molecular observations on *Solanum phureja*-induced dihaploid potatoes

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**Summary**. Seventeen potato dihaploids, produced by pollinating the tetraploid (2n=48) cv 'Pentland Crown' with pollen from *Solanum phureja* (2n=24) dihaploid inducer clones, were studied. Since dihaploids are thought to develop parthenogenetically from unfertilized ovules they were expected to be euploid (2n=24), but somatic chromosome counts showed that 15 of the 17 dihaploids were aneusomatic. Ten of the clones were predominantly diploid (2n=24) with a proportion of hyperploid cells that contained 25 or 26 chromosomes. Five of the dihaploids contained variable numbers of triploid cells (2n=36). RFLP analysis was used to determine whether the additional chromosomes were from *S. phureja* or *S. tuberosum*. Unique hybridizing fragments present in *S. phureja* but not in 'Pentland Crown' were identified. These *S. phureja*-specific restriction fragments were present in some of the dihaploid offspring of 'Pentland Crown'. Of the 5 clones that contained triploid cells 4 had *S. phureja* type banding. Four of the 10 aneusomatic clones that contained hyperploid cells had the unique *S. phureja* hybridizing fragments. We propose that ovules of 'Pentland Crown' 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. We consider that this is an additional mechanism of dihaploid formation in potato.

*Key words*: Potato – Dihaploid – Parthenogenesis – Fertilization – Chromosome elimination

## Introduction

Cross pollinations between heterozygous tetraploid (2n=48) potato clones produce offspring that display complex segregations of agronomic and quality characters and pest and disease resistances. Consequently, it is difficult to study the genetics of interesting and economically important characters in potatoes. By reducing the ploidy level such genetic studies can be greatly simplified. This can be achieved by the production of dihaploid (2n=24) potatoes. A number of authors have proposed that breeding potato cultivars by combining complementary dihaploid genotypes and selecting at the diploid level could be simpler and more efficient than traditional tetraploid crossing approaches (Chase 1963; Peloquin et al. 1966).

Dihaploids can be produced by pollinating tetraploid potatoes with a *Solanum phureja* (2n=24) dihaploid inducer clone (Hougas and Peloquin 1957). These crosses produce a range of dihaploid, triploid and tetraploid progeny (Hougas et al. 1964; Caligari et al. 1988). To facilitate identification of the triploids and tetraploids *S. phureja* clones homozygous for the dominant embryo spot marker gene are currently used as dihaploid inducers (Hermsen and Verdenius 1973). Tetraploid and triploid hybrid offspring inherit a purple colouration at the base of the cotyledons (seed spot) and at the base of all plant organs that are analogous to leaves (nodal marker).

It is widely believed that pollination using pollen from *S. phureja* clones stimulates unfertilized ovules in the tetraploid parent to develop parthenogenetically and that the dihaploid inducer does not contribute any genetic information to the dihaploid progeny (Hermsen and Verdenius 1973; Rowe 1974; Van Breukelen et al. 1977). Cytological investigations have shown that developing dihaploid embryos are associated with hexaploid endosperm (Wagenheim et al. 1960; Bender 1963). Bender (1963) and Montelongo-Escobedo and Rowe (1969) reported that in some *S. phureja* microspores the generative nucleus did not divide to form two sperm nuclei, instead

**Table 1.** The total number of diploid, hyperploid and polyploid cells identified in root tips of 'Pentland Crown', the dihaploid inducer IVP 48, 17 'Pentland Crown' dihaploids and the putative hybrid clone PDH 591; and the presence (+) of DNA hybridizing fragments characteristic of *S. phureja* in these clones

| | Dihaploid inducer | Year of induction cross | Number of diploid, aneuploid and polyploid cells identified[c] Number of chromosomes per cell | | | | | Total number of cells karyotyped | Presence of *S. phureja*-hybridizing fragments | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 24 | 25 | 26 | 36 | 48 | | pSTC34 | pSOD[1] |
| P. Crown | | | | | | | 22 | 22 | | |
| IVP 48 | | | 153 | | | | | 153 | + | +[d] |
| PDH 591[a] | 80CP12 | 1982 | 2 | 1 | | 37 | | 40 | | + |
| PDH 4 | –[b] | 1970 | 15 | | | | | 15 | | |
| PDH 5 | – | 1970 | 46 | 3 | | | | 49 | | |
| PDH 7 | EC 90 | 1970 | 45 | 5 | | | | 50 | *[e] | |
| PDH 35 | – | 1969 | 43 | 2 | | | | 45 | + | |
| PDH 36 | – | 1969 | 40 | 2 | 1 | | | 43 | * | + |
| PDH 40 | PI 1 | 1970 | 32 | 2 | 1 | 36 | | 71 | + | * |
| PDH 41 | PI 22 | 1970 | 44 | 7 | | 2 | | 53 | + | |
| PDH 51 | IVP 48 | 1972 | 24 | 15 | 1 | | | 40 | + | |
| PDH 52 | IVP 48 | 1972 | 46 | 3 | | | | 49 | | |
| PDH 55 | IVP 48 | 1972 | 38 | | | 1 | | 39 | | |
| PDH 87 | IVP 48 | 1972 | 36 | 4 | | | 3 | 43 | | |
| PDH 425 | IVP 48 | 1973 | 43 | 4 | | | | 47 | | |
| PDH 440 | IVP 48 | 1973 | 40 | 4 | | | | 44 | + | |
| PDH 452 | IVP 48 | 1973 | 42 | | | | | 42 | | |
| PDH 590 | 80CP12 | 1982 | 3 | | | 41 | 18 | 62 | + | + |
| PDH 593 | 80CP12 | 1982 | 13 | | | 26 | | 39 | + | + |
| PDH 598 | 71P10 | 1982 | 40 | 2 | | | | 42 | * | |

[a] Putative hybrid clone
[b] Unknown *S. phureja* dihaploid inducer clone
[c] Three or four roots examined per clone
[d] Presence of hybridizing fragments characteristic of *S. phureja*
[e] Unique hybridizing fragments not present in IVP 48

a single restitution nucleus of 24 chromosomes was produced. Wagenheim et al. (1960) and Rowe (1974) proposed that the restitution nucleus combined with the central nucleus giving an unfertilized dihaploid embryo with viable hexaploid endosperm thereby allowing parthenogenesis to occur.

Triploid progeny are formed by direct fertilization of the tetraploid parent's ovules by gametes from *S. phureja*, whilst tetraploid hybrid progeny most probably arise from ovules fertilized by unreduced *S. phureja* pollen (Wagenheim et al. 1960; Rowe 1974).

In this paper we propose an alternative mechanism for dihaploid formation. We present cytological and molecular evidence demonstrating that potato dihaploids can contain additional chromosomes from the *S. phureja* dihaploid inducer. Such plants are predominantly dihaploid but have a small proportion of cells containing some or all of the dihaploid inducer's chromosomes. We propose that these dihaploids are derived from fertilized ovules from which *S. phureja* chromosomes were eliminated during somatic cell division. Similar mechanisms of haploid production have been reported in wheat (Barclay 1975; Laurie and Bennet 1986) and barley (Kasha and Kao 1970).

## Materials and methods

### *Plant material*

Seventeen dihaploids derived from the cv 'Pentland Crown' were used in this study (Table 1). They were produced by pollinating 'Pentland Crown' with various *S. phureja* dihaploid inducer clones. Seeds from these pollinations were germinated on filter paper and allowed to continue growing in glasshouses. The ploidy level of the offspring was confirmed by chloroplast counts and cytological estimations of ploidy level. Initially accurate chromosome counts were not made. Dihaploid clones were maintained by producing tubers under glasshouse conditions. All of the dihaploids lacked nodal banding. PDH 591, which exhibited weak nodal banding, was also studied.

### *Cytology*

Somatic chromosome counts were prepared from root tip squashes. The roots were pretreated by incubation in a saturated aqueous solution of 8-hydroxyquinoline for 5 h, then fixed in Carnoy's fluid (3 ethanol:1 glacial acetic acid). Preparations were mounted, and stained and squashed in a 1% aqueous solution of crystal violet with a small drop of 45% acetic acid acting as mordant. Three or four roots from each plant were examined.

### *DNA isolation and RFLP procedures*

DNA was isolated from either freeze-dried or freshly frozen leaf material by a modification of the procedure of Saghai-Maroof

et al. (1984). Isolated DNA (5–10 μg) was digested with approximately 2 units of restriction enzyme per microgram DNA at 37 °C for 16 h. Restriction fragments were separated on 1% agarose gels run in 1 × TBE buffer (89 m*M* TRIS-HCl pH 8.3, 89 m*M* boric acid, 10 m*M* EDTA). The gels were subsequently stained with ethidium bromide, photographed, and the DNA transferred to Hybond $N^+$ (Amersham) nylon membrane by the alkaline method of Reed and Mann (1985). Membranes were washed in 6 × SSC, air dried and baked at 80 °C for approximately 2 h. The DNA was fixed to the membrane by exposure to UV (312 nM) light for 1 min.

Clone inserts were separated from the plasmid vector prior to labelling by the random priming method of Feinburg and Vogelstein (1983) using α[$^{32}$P]-dCTP (3000 Ci/mMol, ICN Biomedical). Unincorporated nucleotides were removed by spinning through sepharose CL-6B columns. Pre-hybridization and hybridization with 10% dextran sulphate were as described by Maniatis et al. (1982). After a final wash at 65 °C in 0.1 × SSC, 0.1% sodium dodecyl sulphate, hybridizing fragments were detected by autoradiography following exposure to Fuji RX film with two intensifying screens at −80 °C for 2–10 days.

## Results

Potato dihaploids are thought to result from the autonomous growth of unfertilized ovules in a tetraploid clone (Hermesen and Verdinius 1973), therefore they are expected to have 24 chromosomes per cell. Roots from the 'Pentland Crown' dihaploids were examined cytologically to test this assumption. The chromosome counts showed that 15 of the 17 dihaploids were aneusomatic with a high proportion of root cells containing more than 24 chromosomes (Table 1, Fig. 1 c–f). Twelve of the dihaploid clones had root cells containing 25 chromosomes, and in 3 of the dihaploids a single root cell with 26 chromosomes was observed (Table 1, Fig. 1 d, e). Triploid cells (2n = 36) were found in 5 of the dihaploid clones, and tetraploid cells (2n = 48) were identified in 2 clones (Table 1, Fig. 1 f). The putative hybrid clone that showed weak nodal banding (PDH 591) was predominantly, but not exclusively, triploid (Table 1).

In general, the frequency of aneuploid cells was similar in different roots taken from the same plant (for example PDH 7, Table 2). In some cases, however, variation in the frequency of aneuploid cells between roots was observed. This variation was generally small (for example PDH 440), except in PDH 40, where aneuploid and triploid cells were found in only one root (Table 2).

The genetic constitution of the aneusomatic dihaploid clones was investigated to determine whether the additional chromosomes were from *S. phureja* or from 'Pentland Crown'. Since few cytological markers can be used to distinguish between *S. tuberosum* and *S. phureja* chromosomes (Pijnacker and Ferwerda 1984), molecular markers were used. When filters were hybridized with a radioactively labelled potato cDNA clone (pSTc 34),

**Table 2.** The number of diploid, hyperploid and polyploid cells identified in different root tips of three 'Pentland Crown' dihaploids

| | Number of diploid, hyperploid and polyploid cells identified Number of chromosomes per cell | | | | | Total number cells karyotyped |
|---|---|---|---|---|---|---|
| | 24 | 25 | 26 | 36 | 48 | |
| PDH 7 | | | | | | |
| Root 1 | 4 | 1 | | | | |
| Root 2 | 6 | 1 | | | | |
| Root 3 | 21 | 2 | | | | |
| Root 4 | 14 | 1 | | | | |
| Total | 45 | 5 | | | | 50 |
| PDH 40 | | | | | | |
| Root 1 | 8 | | | | | |
| Root 2 | 11 | 2 | 1 | 36 | | |
| Root 3 | 12 | | | | | |
| Root 4 | 1 | | | | | |
| Total | 32 | 2 | 1 | 36 | | 71 |
| PDH 440 | | | | | | |
| Root 1 | 9 | 2 | | | | |
| Root 2 | 14 | 2 | | | | |
| Root 3 | 17 | | | | | |
| Total | 40 | 4 | | | | 44 |

*S. phureja* had hybridizing fragments that were not present in 'Pentland Crown' (Fig. 2). These additional bands were also present in a number of the dihaploid clones (Fig. 2, Table 1). Similarly, when filters were probed with a clone of tobacco superoxide dismutase (pSOD1, Bowler 1989), *S. phureja* had characteristic hybridizing fragments which did not occur in 'Pentland Crown', but appeared in some of its dihaploid progeny (Fig. 3, Table 1). This indicated that the aneusomatic dihaploids contained genetic material from the *S. phureja* dihaploid inducers.

DNA restriction fragments characteristic of *S. phureja* were not detected in either of the euploid clones (PDH 4, PDH 425), but pSOD1 hybridizing fragments characteristics of *S. phureja* were detected in the predominantly triploid clone that exhibited weak nodal banding (PDH 591, Table 1, Fig. 3).

Of the 5 dihaploids that contained triploid cells, 4 had *S. phureja* type banding with pSTc 34 and 2 of these also showed *S. phureja* type banding with pSOD1 (Table 1). Of the 10 aneusomatic clones that did not contain triploid cells but contained cells with 25 or 26 chromosomes, 3 had the *S. phureja* hybridizing fragments when probed with pSTc 34, and 1 had pSOD1 bands characteristic of *S. phureja* (Table 1, Figs. 2 and 3).

When probed with pSTc 34 and pSOD1 4 clones had unique hybridizing fragments that were not present in 'Pentland Crown' or *S. phureja* IVP48 (Table 1). The

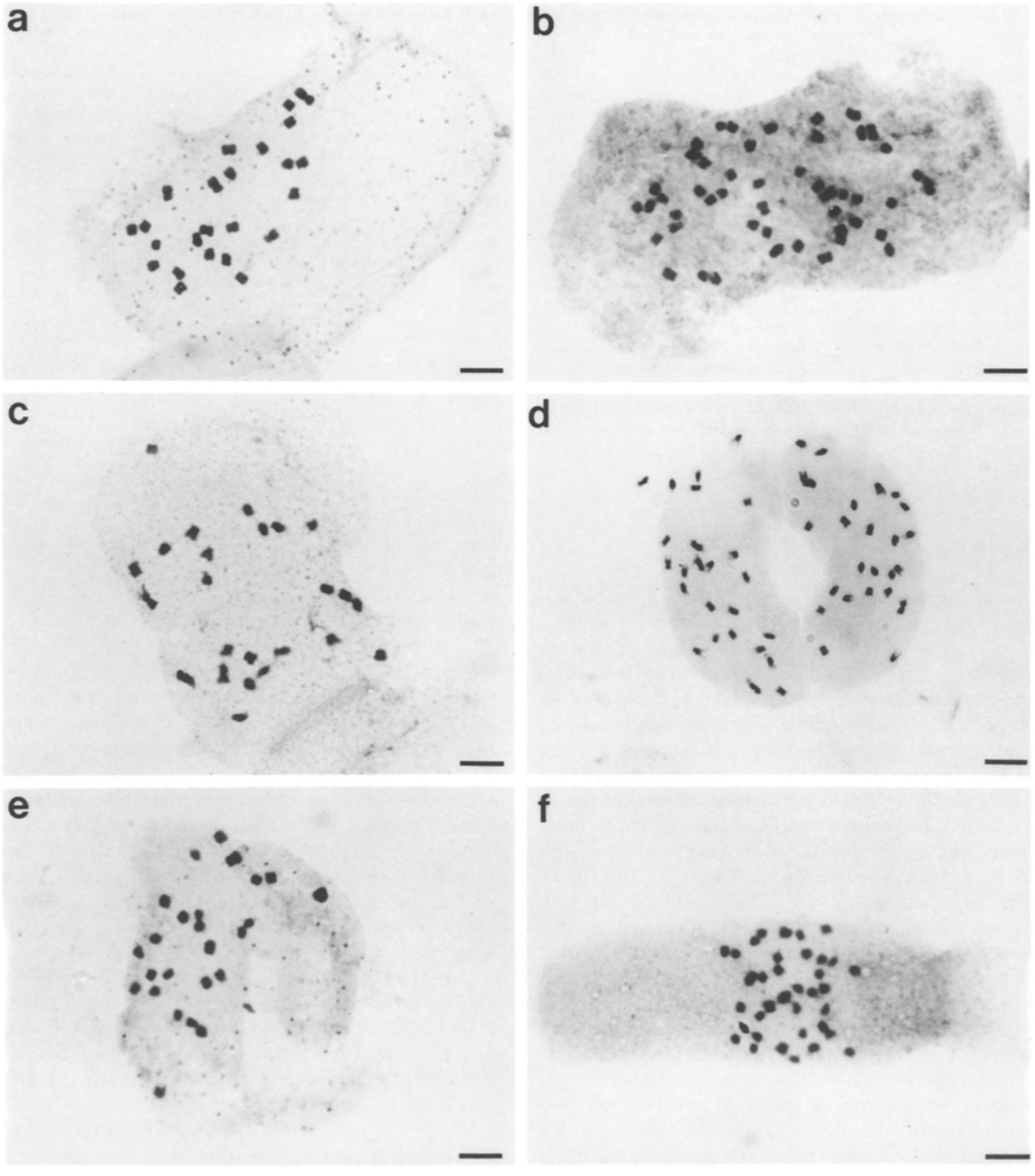


**Fig. 1.** Squash preparations of **a** *S. phureja* IVP 48, **b** cv 'Pentland Crown' and **c–f** potato dihaploids: **c** PDH 7, 24 chromosomes; **d** PDH 7, 25 chromosomes; **e** PDH 51, 26 chromosomes; **f** PDH 41, 36 chromosomes. *Bars* = 5 μm

aneusomatic clones PDH 7, PDH 36 and PDH 598 had pSTc 34 hybridizing fragments that were not present in either of the parental clones (Fig. 2), and PDH 40, which contained a high proportion of triploid cells, had a unique pSOD1 hybridizing fragment (not shown).

## Discussion

The results demonstrate that some of the 'Pentland Crown' dihaploids used in this study were aneusomatic and contained genetic material from *S. phureja* (Table 1).

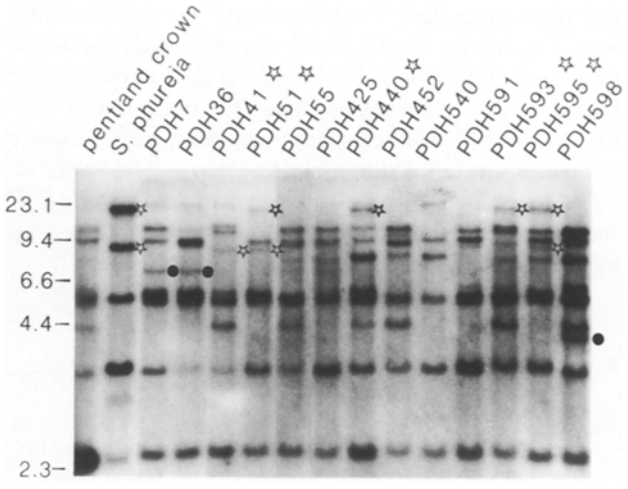


**Fig. 2.** Autoradiograph of DNA from 'Pentland Crown', *S. phureja* IVP 48 and 13 dihaploids, digested with EcoRI and probed with the anonymous potato cDNA clone pSTc 34. * indicates the bands present in *S. phureja* IVP 48 that can be detected in the dihaploids; • indicates bands not present in either 'Pentland Crown' or *S. phureja* IVP 48

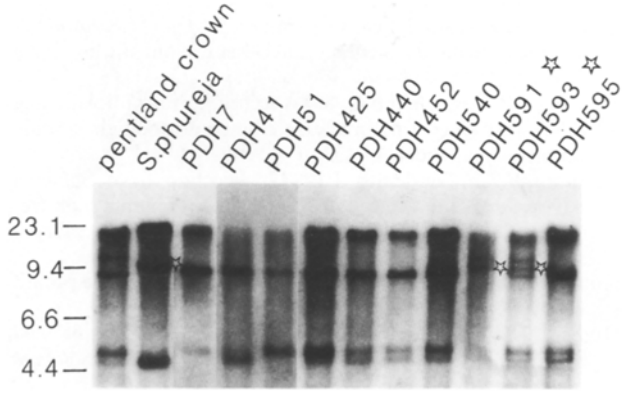


**Fig. 3.** Autoradiograph of DNA from 'Pentland Crown'-derived dihaploids digested with EcoRI and probed with pSOD1: * indicates *S. phureja* IVP 48 bands present in the dihaploids

It is extremely unlikely that such large numbers of hyperploid and triploid cells could have arisen by abnormal somatic cell division in an exclusively diploid individual. Moreover, the appearance of genetic material from *S. phureja* in these clones excludes this possibility. The occurrence of triploid cells implies that the plants were derived from triploid embryos. Some 'Pentland Crown' ovules therefore must have been fertilized by pollen from *S. phureja* to produce triploid zygotes. Hybrid triploid offspring are often produced from interploidy crosses between *S. tuberosum* and *S. phureja*, and the frequency of triploid offspring can be as high as 34% (Caligari et al. 1988). The clones used in this study are unusual as they are aneusomatic rather than exclusively triploid, thus chromosome elimination must have occurred during somatic cell division. Since these plants are phenotypically recessive for the nodal banding gene, some *S. phureja* chromosomes must have been eliminated. Many of the aneusomatic clones have RFLP bands characteristic of *S. phureja*, however, which indicates that DNA from *S. phureja* was present in these clones. The most likely explanation of the data is that preferential elimination of *S. phureja* chromosomes from triploid zygotes gave rise to predominantly diploid, aneusomatic individuals containing a low frequency of *S. phureja* chromosomes. Preferential elimination of *S. phureja* chromosomes has been shown to occur in somatic hybrids between *S. tuberosum* and *S. phureja* (Pijnacker et al. 1989). Similarly, when hexaploid $F_1$ clones from the cross *S. acaule* × *S. bulbocastanum* were pollinated with pollen from *S. phureja*, chromosome elimination occurred in some individuals resulting in chimeric offspring with euploid (2n = 48) and hypoploid cells (Ramanna and Hermsen 1971). Therefore, it is possible that chromosome elimination could have occured in hybrid triploid embryos from the cross 'Pentland Crown' × *S. phureja*. At present we cannot exclude the possibility that *S. tuberosum* chromosomes are also eliminated during the development of the aneusomatic dihaploids (Lange 1969).

Chromosome elimination during embryo development has been shown to occur in other systems (see Kasha 1974). When wheat and barley plants are pollinated with *Hordeum bulbosum* pollen, fertilization occurs but the *H. bulbosum* chromosomes are rapidly eliminated during the early stages of development and the resulting offspring are haploid (Kash and Kao 1970; Subrahmanyam and Kasha 1973; Barclay 1975). Similarly, the cross between wheat and maize results in a hybrid zygote from which the maize chromosomes are subsequently eliminated (Laurie and Bennett 1986).

The long established theory of dihaploid production in potato is that dihaploids develop from unfertilized ovules by parthenogenesis providing they are associated with viable endosperm (Wagenheim et al. 1960; Bender 1963; Hermsen and Verdenius 1973; Rowe 1974). Our data indicates dihaploids can also be formed by chromosome elimination from triploid zygotes. We propose that this should be regarded as an additional method of dihaploid formation and consider it likely that both systems occur. Since the frequency of triploids produced by interploidy crosses can be quite high (Caligari et al. 1988), dihaploid formation by chromosome elimination from triploid zygotes could produce a significant number of dihaploids. However, poor endosperm development may stop many triploid zygotes from developing into mature viable seed (Wagenheim et al. 1960; Kasha 1974).

As the aneusomatic dihaploids originated from 'Pentland Crown' ovules fertilized by *S. phureja*, the 6 clones in which triploid cells were identified (Table 1) should

still contain half of the dihaploid inducer's genome. Four of these clones showed the *S. phureja* hybridizing fragments when probed with pSTc 34, and two showed bands characteristic of *S. phureja* with pSOD1. Since the sequences in potato that hybridize to pSTc 34 and pSOD1 are highly heterozygous (unpublished observations), it is likely that the *S. phureja* clones were heterozygous for the characteristic pSTc 34 and pSOD1 fragments; therefore the unique *S. phureja* bands would only occur in some of the offspring.

Of the 10 aneusomatic clones that did not contain triploid cells, 1 showed *S. phureja* type banding with pSOD1 and 3 showed *S. phureja* banding with pSTc 34. Although these clones did not exhibit nodal banding they contain genetic information from *S. phureja*, suggesting that the additional chromosomes present in some of the cells were from *S. phureja*. The failure to detect hybridizing fragments characteristic of *S. phureja* in all of these clones probably reflects the heterozygosity of the probes, but it may also indicate that certain chromosomes were eliminated from some of the progeny while retained in others. Further work using chromosome-specific probes will enable a more complete analysis of the rate and nature of the elimination of *S. phureja* chromosomes.

The unique hybridizing fragments identified in PDH 7, PDH 36, PDH 40 and PDH 598 may have come from their respective dihaploid inducers, EC 90, PI 1 and 71P10 (Fig. 2, Table 1). These clones are no longer maintained in our collections and therefore we cannot verify this possibility.

There are considerable male and female parental effects on the frequency of dihaploid induction (Hougas et al. 1964). There may also be parental, chromosomal or gene balance effects on the stability of triploid progeny, the occurrence and rate of chromosome elimination and the length of time additional chromosomes are retained in aneusomatic offspring (Hougas et al. 1964; Kao and Kasha 1969). The factors that permit triploid embryo development and the parental effects on the rate and nature of chromosome elimination are presently unknown, but must be determined in order to fully characterize this system. It is surprising that the additional *S. phureja* chromosomes have been retained in some of the aneusomatic individuals for up to 20 years of clonal reproduction.

This mechanism of dihaploid formation has several important practical implications. Progeny from dihaploid induction crosses that do not exhibit embryo spot marker or nodal banding may nevertheless contain genetic information from *S. phureja*. Dihaploids derived by interspecific induction crosses may therefore contain some characteristics of the inducer species, and this should be considered when interpreting the results of genetic, biochemical or physiological research involving these dihaploids. Similarly, progeny from dihaploid induction crosses that have embryo spot or nodal banding would normally be discarded, however they could be predominantly diploid aneusomatics that have retained the particular chromosome carrying the embryo spot marker gene. If this occurred it would represent a waste of potentially useful 'dihaploid' genotypes. The availability of new molecular genetic markers in potato will allow the genetic constitution of dihaploids to be characterized more fully.

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