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Elimination of *Solanum phureja* nucleolar chromosomes in *S. tuberosum* + *S. phureja* somatic hybrids

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Summary. The karyotype of the dihaploid SVP1 line of *S. tuberosum* ($2n = 2x = 24$) showed two nucleolar chromosomes with differently sized satellites. The diploid SVP5 line ($2n = 2x = 24$) and tetraploid regenerants of *S. phureja* had larger but similar satellites. Somatic hybrids between the diploid lines of these potato species with genome combinations 4 tub + 2 ph (plants 1-3), 2 tub + 4 ph (plants 4-7) and 4 tub + 4 ph (plant 8) had lost 2 *phureja* nucleolar chromosomes if 4 *phureja* genomes were present. One *phureja* nucleolar chromosome of plants 1-3 and both of plants 5 and 7 had rearranged satellites. Elimination of the two nucleolar chromosomes occurred preferentially, was under genetic control, and probably took place during early callus development. NOR activity resulting in rearrangements between NORs may have caused the elimination.

Key words: Potatoes – Somatic hybrid plants – Nucleolar chromosomes – Chromosome elimination

Introduction

Normally, chromosome elimination does not take place during mitosis of plant cells.

However, it has been reported as a regular phenomenon in some sexual hybrids (Lange 1971; Ramanna and Hermsen 1971; Subrahmanyam and Kasha 1973; Davies 1974), polyploids (Tan and Dunn 1977; Nirmala and Rao 1984) and plants with B-chromosomes (Jones 1975). In *Hordeum* hybrids, uniparental loss of the chromosomes may take place and in *H. maximum* × *H. vulgare* endosperm the *vulgare* nucleolar chromosomes are eliminated first for unknown reasons (Finch 1983).

Chromosome elimination is, however, a regular feature in in vitro plant cell cultures, whether cell suspensions or calli, and may even proceed in regenerants. The chromosomes become eliminated apparently at random (Bayliss 1980; D'Amato 1985). In somatic hybrid cells the chromosomes of one of the parents may be eliminated preferentially (Gleba and Sytnik 1984; D'Amato 1985; Pental 1986). Elimination in a certain order within a genome has not yet been described.

Chromosomal instability has been shown for cell cultures and regenerants of potato species (Karp and Bright 1985; Sree Ramulu 1986; Pijnacker et al. 1986; Gill et al. 1986) and their somatic hybrids (Austin et al. 1986; Puite et al. 1986). Elimination of particular chromosomes can be shown only if the chromosomes can be karyotyped individually. The nucleolar chromosomes of the species *Solanum tuberosum* and *S. phureja* differ in their C-band patterns (Pijnacker and Ferwerda 1984). Somatic hybrids of these species contained odd chromosome numbers, mostly aneusomatic, due to chromosome loss (Puite et al. 1986).

In this paper we present evidence that the nucleolar chromosomes of *S. phureja* are eliminated preferentially in *S. tuberosum* + *S. phureja* somatic hybrids with defined genome combinations.

Materials and methods

Eight somatic hybrid plants were used which originated from electrofused protoplasts of the dihaploid ($2n = 2x = 24$) SVP1 line (SH 77-78-1994) of *Solanum tuberosum* (T) and the diploid ($2n = 2x = 24$) SVP5 line (PH 77-1445-2242) of *S. phureja* (PH). Their origin, culture, cytology (including chromosome number) and morphology have been described by Puite et al. (1986). In addition, tetraploid ($2n = 4x = 48$) plants regenerated from shoot culture-derived protoplasts of the diploid SVP5 line of *S. phureja* were investigated (kindly supplied by Dr. K. Sree Ramulu, Research Institute ITAL, Wageningen).

Air-dried chromosome preparations were made from shoot and/or root tip cells and stained with Giemsa or by Giemsa C-banding as described earlier (Pijnacker and Ferwerda 1984) with a slight modification (Puite et al. 1986).

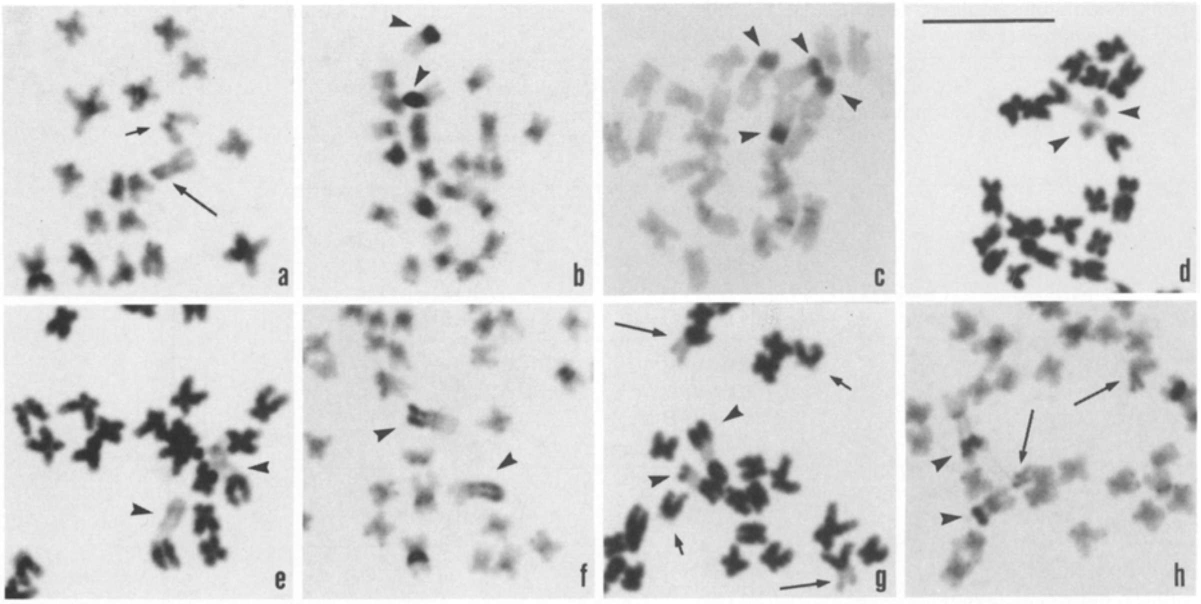

Fig. 1. Nucleolar chromosomes of (parts of) somatic metaphases of a dihaploid *Solanum tuberosum* line SVP1 (**a**), a diploid *S. phureja* line SVP5 (**b**), hexaploid somatic hybrids of these lines (**d–h**) and a tetraploid *S. phureja* (**c**). Arrows and arrowheads: *S. tuberosum* and *S. phureja* nucleolar chromosomes, respectively. **a, g** Heteromorphism of satellited arms of *S. tuberosum;* **d** Association of two satellited arms; **e, f** Two satellites longer through rearrangement with other satellites; **g, h** One satellite of *S. phureja* longer through distal rearrangement with euchromatic chromosome arm. **d, e, g** Giemsa stained; **a–c, f, h** Giemsa C-banded with positive staining of satellites. Bar represents 10 μm

Results and discussion

In the diploid karyotypes of the parent lines *S. tuberosum* and *S. phureja,* the nucleolar organizer region (NOR) is situated on the short arm of chromosome 2 (Fig. 1; cf. Puite et al. 1986). This region is usually visible as a constriction of variable length (connecting thread) near the centromeric chromatin and delimits distally a satellite. Centromeric chromatin and satellite stain by the C-banding procedure and thus consist of constitutive heterochromatin; the NOR does not stain. The satellites of the two T nucleolar chromosomes differ in size (Fig. 1a, g, h). The smallest satellite is visible only after C-banding and is hardly more than a telomere. The origin of this heteromorphism is not known. The two PH satellites are much larger (Fig. 1b–d). Between these two satellites a small difference in volume was observed in some metaphases but it could not be established whether this difference was constitutive. The sister satellites of a metaphase chromosome were often fused.

The somatic metaphases of all the tetraploid (protoplast-derived) *S. phureja* plants showed 4 nucleolar chromosomes. The morphology of the satellites was similar to that of the diploid parent plants (Fig. 1c).

The 8 somatic hybrids had various chromosome numbers and chromosome sets according to Puite et al. (1986; see Table 1). Based on karyotype analysis they established that plants 1-3 contained 4T+2PH genomes (hexaploidy), plants 4-7 2T+4PH genomes (hexaploidy) and plant 8 4T+4PH genomes (octoploidy). Plant 1 was euploid, plant 6 aneuploid and the others aneusomatic; plant 2 was also mixoploid for some hypotetraploid cells. Thus, except for plant 1, the hybrid cells had lost one or more chromosomes. With the aid of the structural characteristics of the nucleolar chromosomes, particularly of the satellites, it was investigated whether, and if so which, nucleolar chromosomes of the somatic hybrids were involved in the chromosome loss. As can be seen from Table 1, the karyotypes of the hexaploid hybrids with genome combinations 4T+2PH (plants 1-3) had retained their satellites. However, 2 PH satellites were always absent if 2T+4PH were present (plants 4-7). Some hypotetraploid metaphases of plant 2 had only 1 PH satellite. In the octoploid hybrid (plant 8) maximally 6 satellites were present, i.e. 2 large T+1 small T+3 PH satellites. A metaphase with 2 small T satellites was not found in this hybrid and most of the metaphases showed 3 T+2 PH satellites.

Relying upon the following considerations, it was investigated whether only the satellites or the complete PH nucleolar chromosomes (two chromosomes will be considered) had got lost. Two satellites can be eliminated as acentric fragments after deletion (or diminution) (cf. Armstrong et al. 1983; Lapitan et al. 1984; Brettell et al. 1986) and after a break in two NORs or an asymmetrical reciprocal translocation between two NORs (cf. Marchant and Brighton 1971; Sato et al.

Table 1. Composition of satellites and nucleolar chromosomes in 8 somatic hybrids of *Solanum tuberosum* (T) + *S. phureja* (PH)

Callus no.	Plant no.	No. of chromosomes[b]	No. of T and PH satellites = nucleolar chromosomes	
			Expected	Observed
1	1	72	4T+2PH	4T+1PH+1PH[a]
	2	45–47	2T+2PH	2T+1PH+1PH[a] 2T+1PH or 1PH[a]
		72, some 71	4T+2PH	4T+1PH+1PH[a]
	3	67–69	4T+2PH	4T+1PH+1PH[a]
2	4	65–66	2T+4PH	2T+2PH
	5	69, some 68	2T+4PH	2T+2PH[a]
	6	71	2T+4PH	2T+2PH
	7	68–69	2T+4PH	2T+2PH[a]
3	8	90–96	4T+4PH	3T+3PH 3T+2PH

[a] Structurally rearranged satellite

[b] From Table 2, Puite et al. (1986)

1983). In case of deletion two deleted chromosomes must appear in the karyotype shaped as acrocentric chromosomes with a short arm consisting of centromeric chromatin, a NOR and a telomere. These chromosomes were not found. Similar chromosomes but without a telomere are formed after breakage. They also were not observed but, if they had been formed, could have been eliminated through breakage-fusion-bridge cycles because of the absence of a telomere. The asymmetrical reciprocal translocation leads to the formation of a dicentric chromosome with the two long arms of nucleolar chromosomes. This dicentric chromosome was not found but, if formed, could also have been eliminated through a breakage-fusion-bridge cycle.

A decrease in the number of satellites also takes place if the nucleolar chromosomes are simply eliminated without interference of a structural mutation. In the present material, however, elimination of a chromosome was not observed during ana-telophase. A decrease also occurs through a reciprocal translocation between the distal region of the NOR and the telomeric region of a satellite (Marchant and Brighton 1971). This results in a non-satellited nucleolar chromosome and a nucleolar chromosome with two tandemly arranged satellites. This type of translocation apparently had taken place twice in plant 5, for the satellites of the two PH nucleolar chromosomes are about two times longer (Fig. 1 e–f). The satellites often demonstrate a constriction proximal to the middle and both parts stain for constitutive heterochromatin. The karyotype of this plant did not show the deleted nucleolar chromosomes, which must have got lost. These considerations and observations make it likely that together with the elimination or transposition of the satellites the remainder of the PH nucleolar chromosomes were eliminated.

The karyotype of plant 5 indicates that rearrangements, i.e. breakage, in the NORs or the satellites precede and may be involved in the elimination of PH nucleolar chromosomes. This is substantiated by the fact that 5 out of 8 plants had structurally changed PH satellites (Table 1). One of the two satellites of plants 1-3 is about two times longer, apparently through a translocation between the telomeric region of that satellite and a distal part of a euchromatic chromosome arm, because this part was not C-band positive (Fig. 1 g–h). The satellites of plant 7 differed in size through, apparently, an exchange of differently sized segments between these satellites. Rearrangements in satellited chromosomes have been reported earlier for cell cultures or regenerants of *Crepis capillaris* (Sacristán 1971), oat (McCoy et al. 1982) and potato (Ooms et al. 1985). Deletion of parts of the NOR, i.e. rRNA genes, has been described for regenerants of potato (Landsmann and Uhrig 1985) and triticale (Brettell et al. 1986).

Plants 1-3 had similarly rearranged PH nucleolar chromosomes and originated from the same callus (Table 1). This indicates that elimination of the nucleolar chromosomes took place during the formation of the hybrid cell or during the early callus phase. The differences between the nucleolar chromosomes of plants 4-7, also regenerated from one callus, do not contradict this assumption because plants may regenerate from karyotypically different cells which may be present within one callus (for potatoes: Austin et al. 1985; Creissen and Karp 1985; Sree Ramulu et al. 1986).

Table 1 shows that the PH nucleolar chromosomes were always involved in the loss of chromosomes of plants 4-8, even if 1-3 chromosomes were lacking. They were, therefore, eliminated preferentially. Plant 6 and several metaphases of plant 8 had retained higher chromosome numbers than expected after loss of the nucleolar chromosomes (71 instead of 70, 95–96 instead of 94). This cannot be explained yet, but nondisjunction might have happened.

The PH nucleolar chromosomes were eliminated only if 4 PH genomes were present in the hybrids. In combination with 4 T genomes the elimination was expressed less than with 2 T genomes. In the tetraploid *S. phureja* plants neither the nucleolar chromosomes nor the satellites were eliminated. This means that the elimination was under genetic control. Whether such a mechanism also operates in somatic hybrids with other genome combinations or in somatic hybrids between other (potato) species remains to be investigated.

Verma et al. (1983) found that, in silver stained human lymphocytes, only those nucleolar chromosomes that had active NORs were associated in the NOR-bearing chromosome arms, which could lead to rearrangement (or nondisjunction). In some (< 1%) metaphases of plants 3-7 the NORs of two retained PH chromosomes were associated (Fig. 1 d). It is then tempting to suppose that before elimination of the PH nucleolar chromosomes began the NORs of all the PH chromosomes were active and that, consequently, these chromosomes were eliminated through association and rearrangement in the NOR until two were left. Elimination of more PH nucleolar chromosomes was not tolerated and led to cell death. Nucleolar competition based on suppression of NOR activity, as occurs in sexual hybrids (Cermeño et al. 1984), apparently did not work in the hybrid cells. Information about elimination of nucleolar chromosomes, induced by their own NOR activity, could be obtained by silver staining of the NORs during early development of hybrid calli.

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