Theor Appl Genet (1984) 68:515–519

Genetic instability in protoclones of potato (*Solanum tuberosum* L. cv. 'Bintje'): new types of variation after vegetative propagation

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Received January 31, 1984; Accepted June 16, 1984
Communicated by G. Wenzel

Summary. The transmission of variation from protoplast-derived plants of tetraploid potato cultivar 'Bintje' to tuber progeny was examined. The morphological alterations of a majority of the variant protoclones were transmitted to corresponding tuber progeny. Some of the normal and variant protoclones gave new phenotypes, or segregated into parental and new phenotypes after vegetative propagation. The ploidy levels of almost all these clones remained unchanged after propagation. It was concluded that the occurrence of variation after vegetative propagation was due to somatic segregation of chimeras resulting from gene mutations or chromosome structural rearrangements in only part of the regenerated plant. The origin of variation is discussed in the light of these results.

Key words: Potato protoclones – Transmission of variation – Chimeras – Somatic mutations

Introduction

Non-meristematic tissue explants, cells or protoplasts removed from the stabilizing environment of the intact plant and cultured in vitro, display genetic instabilities of various kinds. These include gross chromosome variation (polyploidy, aneuploidy), structural changes in chromosomes and gene mutations. It is evident from several studies that these changes are transmitted to regenerated plants and to their progeny (D'Amato 1975, 1978; Bayliss 1980; Larkin and Scowcroft 1981; Sree Ramulu 1982; Browers and Orton 1982; Barbier and Dulieu 1983; Lörz and Scowcrort 1983).

The variation occuring during the plant regeneration process stimulated interest in recent years, both from the point of using it in plant breeding, and because it may be undesirable when stable reproduction of a specific genotype is essential, as in the case of micropropagation and in genetic transformation studies. In potato, a wide range of variation in morphological characters, disease resistance and ploidy levels has been reported among plants regenerated from protoplasts or tissue cultures of several genotypes, including the Dutch cv. 'Bintje' (Wenzel et al. 1979; Behnke 1979, 1980; Shepard et al. 1980; Wenzel and Uhrig 1981; Van Harten et al. 1981; Jacobsen 1981; Thomas et al. 1982; Karp et al. 1982; Austin and Cassells 1983; Sree Ramulu et al. 1983). 'Bintje' is the most important cultivar in the Netherlands because of its excellent quality for domestic and industrial uses. However, it is susceptible to most potato diseases. The conventional breeding methods have not been successful until now due to male sterility and heterozygosity of the cultivar. Molecular and in vitro somatic approaches might be useful in this regard. However, this requires the development of cultures which could give efficient and stable regeneration of plants.

Recently, using the method of protoplast isolation and culture described for dihaploid clones of potato (Binding et al. 1978), plants have been regenerated from shoot culture-derived protoplasts of cv. 'Bintje' and analysed for variation (Bokelmann and Roest 1983; Sree Ramulu et al. 1983). This work was undertaken in order to evaluate stability among regenerated plants.

The present article reports new results on the occurrence of different types of variation after vegetative propagation of potato (cv. 'Bintje') protoclones.

Materials and methods

Plant material

The cultivar 'Bintje' of tetraploid *Solanum tuberosum* (2n=4x=48) was used. The method of protoplast isolation and culture described for dihaploid potato clones (Binding et al. 1978) was used with some modifications. For details on the procedures of propagation of shoot cultures, protoplast culture and plant regeneration, reference is made to Bokelmann and Roest (1983).

Morphological characterization

The morphological characterization described previously (Sree Ramulu et al. 1983) for protoplast-derived plants (protoclones) of cv. 'Bintje' was followed in this study to analyse the tuber progeny. The term "protoclone" is used to denote each individual shoot or plant obtained from protoplast regeneration process. Thus, a given protoplast-callus can produce one or more protoclones. The term is not intended to suggest identity with the mother plant cv. 'Bintje', as variation was found among protoclones (obtained from the same or different protoplast-callus) as well as within a protoclone. The growth and vigour of plants, leaf characters such as colour, shape, texture and size and axillary branching were studied. The characters of each of the plants were scored several times during the various periods of growth and compared with those of the control plants. They were classified into three types according to their morphological characters, 1) normal-looking plants (resembling the mother plant cv. 'Bintje'), 2) plants showing variation in a few (1–3) characters and 3) plants showing variation in several (> 3) characters (gross aberrants). All the plants were grown under greenhouse conditions.

The number of tubers grown from each regernated plant (protoclone) varied from 1–20, it being 3 in most cases. Thus, more than 2,000 tuber-propagated plants were scored from 613 protoclones.

Cytological analysis

To determine ploidy levels of plants, root-tips collected from young potted plants were pretreated with 8-hydroxy-quiniline (0.002 M) for 4 h at 18 °C and then fixed in absolute alcohol: glacial acetic acid (3 : 1 v/v) for 24 h. The roots were hydrolysed in 1 N HCl for 8 min at 60 °C and stained in Feulgen. Chromosome counts greater than 50 were ± 2 chromosomes.

Results

The data on the types of protoclones and their tuber progeny are given in Table 1. There were altogether 391 normal-looking protoclones. After tuber propagation, 373 resembled the normal type, while the remainder (18) showed variation: 14 protoclones showed new phenotypes and 4 segregated each into new and normal phenotypes. In all, 11 different types occurred, each varying in one or a few morphological characters.

Out of 125 protoclones showing variation in a few characters, 96 resembled the parental types (i.e. parent-protoclones) in the next vegetative generation, 13 reverted to normal type and 16 segregated into parental types and new or normal types (Table 1). The new phenotypes (12 different types) varied in one or a few traits.

A feature of interest was the tuber progeny of a chlorophyll chimera with a small yellow sector on one of the leaves. The progeny segregated into 1 normal green and 2 variegated (chimeric) plants, all being tetraploid and normal in other morphological characters. One of the chimeric plants (Fig. 1 A) consisted of 5 shoots: 2 chimeric, 1 completely yellow shoot and 2 normal green. The other chimeric plant (Fig. 1 B) had four shoots, all being chimeric. Tubers raised from the former plant gave 1 completely yellow plantlet (Fig. 1 C), 1 chimera with completely yellow and green shoots (Fig. 1 D) and 2 completely green plants (not shown in Fig. 1). As the yellow plant is weak and slow growing, it is being propagated in vitro in order to use as a marker in protoplast fusion and transformation studies.

The other 103 protoclones were grossly aberrant in phenotype, 61 showing chimeric structures within the plant, i.e. chlorotic spots or sectors of different size and colour on leaves and morphologically abnormal and normal shoots. A high frequency (ca. 80%) of the protoclones had altered ploidies (aneuploid, octoploid, mixoploid). After vegetative propagation, 99 protoclones resembled the respective parental type, while 4 showed new phenotypes differing in one or more traits (Table 1).

Thus, in the total material 18 normal-looking protoclones and 20 variant protoclones segregated or showed new phenotypes after vegetative propagation. They were derived from 35 different protoplast-calli. Each of these calli also gave rise to one or more other protoclones (normal or variant) which did not segregate after tuber propagation.

Table 1. Types of protoclones (regenerated plants) and their tuber progeny in tetraploid potato cv. 'Bintje' (2n = 4x = 48)

Protoclones			Tuber progeny					
Type	No.	%	Types					
			Normal	Parental	New	New + normal	New + parental	Normal + parental
Normal-looking	391	63.2	373	–	14	4	–	–
Varying in a few characters	125	20.2	13	96	–	11	2	3
Varying in several characters (gross aberrants)	103	16.6	–	99	4	–	–	–
Total	619	100.0	386	195	18	15	2	3

Normal and parental types refer to plants resembling cv. 'Bintje' and variant protoclones, respectively

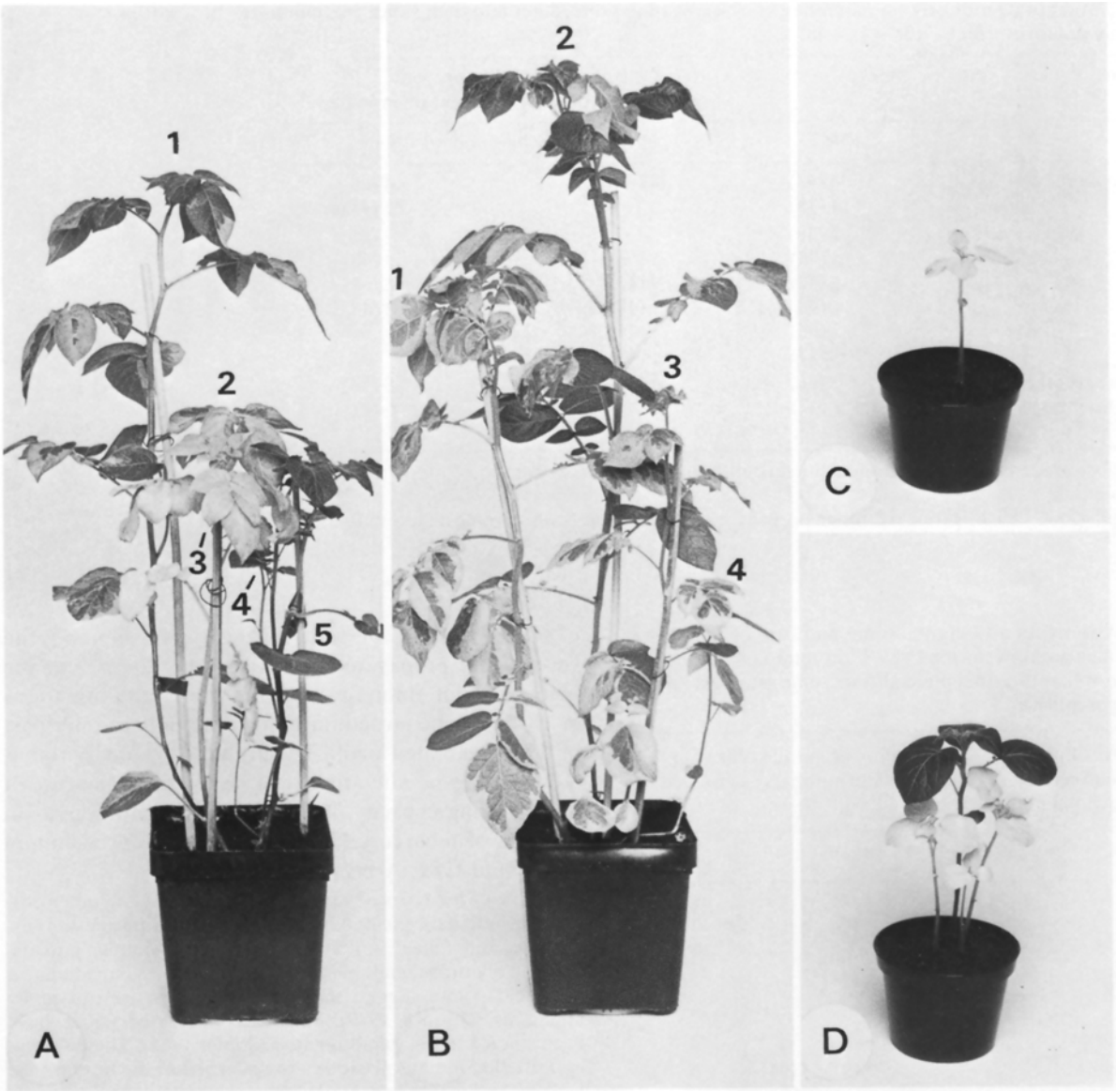

Fig. 1 A–D. Tuber progeny from a protoplast-derived chlorophyll chimera in tetraploid potato cv. 'Bintje'. **A** variegated plant with five shoots: *1* and *2* chimeric shoots, *3* a completely yellow shoot, *4* and *5* green shoots; **B** variegated plant with four chimeric shoots; **C** a completely yellow plantlet; **D** a chimeric plantlet with completely yellow and green shoots, both derived from the tubers of plant A

Table 2 gives data on chromosome numbers of all the 38 segregating protoclones and their tuber progeny. All 18 normal-looking protoclones and their progeny (new and normal types) were tetraploid (2n = 48). Among the protoclones showing variation in a few or several characters, there were not only tetraploids, but also aneuploids, mixoploids and an octoploid. After tuber propagation, the chromosome numbers of all the protoclones remained the same, except in 2 mixoploids which segregated into different ploidy-types. One of the mixoploid clones ("89-J") gave 12 progeny plants of 6 different phenotypes, each varying in one or a few morphological characters (Table 3). Four of these were mixoploids, one was tetraploid and one octoploid.

Discussion

The results obtained show that the morphological alterations of a majority of the variant protoclones were transmitted to the tuber progeny. Furthermore, the analysis of the tuber progeny revealed the following features.

1) Some of the normal and variant protoclones segregated or showed new phenotypes after vegetative

Table 2. Chromosome numbers (in parentheses) of segregating protoclones and their tuber progenies in tetraploid potato cv. 'Bintje' (2n = 4x = 48)

Protoclones		Distribution of tuber progeny plants[a] showing different phenotypes		
Type	No.	Normal	Parental	New
Normal-looking	4 (48)	8 (48)		4 (48)
	14 (48)			35 (48)
Varying in a few characters	2 (48)		3 (48)	2 (48)
	5 (48)	5 (48)		7 (48)
	5 (46)	9 (46)		6 (46)
	1 (48, 96)[b]	1 (48)		2 (48, 96)[b]
	2 (48)	3 (48)	2 (48)	
	1 (45)	2 (45)	1 (45)	
Varying in several characters (gross aberrants)	1 (96)			8 (96)
	2 (47)			6 (47)
	1 (48, 94, 96)[c]			12[c]

[a] No. of tuber progeny plants per protoclone ranged from 2–12, it being mostly 3
[b] Mixoploid
[c] Mixoploid clone "89-J" giving 12 tuber progeny plants (for details on chromosome numbers, see Table 3)

Table 3. Phenotypes and chromosome numbers of tuber progeny obtained from protoclone "89-J" in tetraploid potato cv. 'Bintje' (2n = 4x = 48). Aneuploid chromosome numbers are reported in parentheses

Phenotypes	No. of plants[a] analysed in each phenotype	Distribution of mitotic cells with chromosome numbers as grouped below		
		48 (4x)	48–95	96 (8x)
1	3	5	1 (50), 3 (52), 2 (83), 2 (92)	6
2	2	4	2 (60), 3 (72), 2 (85)	5
3	1	8	–	–
4	2	7	–	4
5	3	–	5 (91), 4 (94)	8
6	1	–	–	7

[a] All plants of phenotypes 1, 2, 4 and 5 were mixoploid

propagation (within-clone variation). The new phenotypes differed in one or a few traits.

2) Most of the segregating protoclones were derived from different calli which also produced one or more non-segregating protoclones (normal or variant).

3) The gross-aberrant protoclones were associated, in general with ploidy alteration. However, this does not appear to be the source of variation found after vegetative propagation, because the ploidies of almost all the protoclones remained unchanged after propagation and the progenies differing in phenotype had the same ploidy level.

These results suggest that variation among the vegetatively propagated plants may be due to somatic segregation of chimeras resulting from gene mutations or chromosome structural rearrangements in only part of the regenerated plant. In tetraploid potato, chromosomal and gene alterations can be tolerated because of the buffering capacity of the polyploid condition, as in the case of tobacco and wheat (Ogura 1976; Ashmore and Gould 1981; Armstrong et al. 1983).

Some of the types of variation found among the tuber progeny include dark green, virescent, tiny dwarf, purple sprouts, wildings and foliage types such as narrow, simple, pinnate, spinach, glabrous and blistered. Genetical investigations in dihaploid potato suggest that such alterations are caused by mutations (Howard 1970). Similarly, variegated plants have been studied in dihaploid potato (Klopfer 1965). These studies indicate that mutant plastomes were carried in the histogenetic L_2 layer and that they exhibited maternal inheritance.

Due to difficulties in cv. 'Bintje', of performing sexual crosses (male sterility), and because of heterozygous nature of the tetraploid potato genome, it is not possible to perform genetic analysis at present. Advances in molecular methods (DNA probes, isozyme characteristics, banding techniques) might help in obtaining additional information in this regard.

As to the source of chimerism in the regenerants (protoclones) which segregated after tuber propagation, the most likely explanation is that they are initially derived from more than one cell. A high frequency of protoplast-derived calli comprised heterogeneous populations of cells as a consequence of instability of the mitotic process (abnormal DNA synthesis and mitosis) (Sree Ramulu et al. 1984). Therefore, it is likely that in the case of chimeras, one or more genetically different (mutated) cells participate in the organization of an adventitious bud. Previously, evidence for the multicellular origin of adventitious buds and plantlets in vitro has been obtained in the case of morphological and cytological chimeras, including mixoploids (chromosome mosaics) (Ogura 1976; Bennici 1976; Sree Ramulu et al. 1976; Mix et al. 1978; Bennici and D'Amato 1978; Browers and Orton 1982).

Several factors, such as genetic architecture of species or genotype, explant origin, composition of the medium and culture age are associated with genetic instability during the plant regeneration process (Torrey 1967; D'Amato 1975; Wenzel et al. 1979; Larkin and Scowcroft 1981; Evans and Gamborg 1982). Therefore, appropriate conditions have to be established in order to reduce or avoid undesirable gross aberrants and chimeras. This is of practical importance for the proper utilization of genetic manipulation techniques and to exploit the most promising source of somaclonal variation for crop improvement, i.e. point mutations.

The appearance of progeny (tuber) plants varying in a few characters which were derived from completely normal-looking protoclones, stresses the need of a greater insight into the chimeric fine structure of protoplast-calli. A note on the geneology in regards to the segregation in vegetatively propagated plants and chimeric composition of the protoplast-calli is in progress.

Acknowledgements. Several suggestions for improvement of the manuscript were given by Prof. B. de Groot and Prof. J. Sybenga.

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