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Mixoploidy and Chimeric Structures in Somaclones of Potato (*Solanum tuberosum* L.) cv. Bintje

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Summary

Plant tissue culture is recognised as a disposable tool to generate useful genetic variability for crop improvement. Nine somaclones of tetraploid potato cv. Bintje regenerated from stem, tuber or protoplast-derived callus were analysed *in vitro* and in the field. All of them were morphological and cytological chimeras. They had alterations in general appearance, leaf morphology and tuber characteristics. The phenotypic variations of the first vegetative progeny (SC2 plants) were in most cases different in comparison with the primary regenerants (SC1 plants). The observed phenotypic instability was in correlation with chromosome mosaicism (chromosomal instability) as aneuoploid and polyploid cells were detected at high frequency (57-89 %) in root tips of all somaclons investigated.

Key words: *Solanum tuberosum* L. potato, callus culture, regenerants, somaclonal variation, chimeras, mixoploidy

Introduction

Plants regenerated from protoplasts, cells or tissues from adventitious sources often go through a callus phase and exhibit phenotypic and genetic variation termed somaclonal variation *(1-3).* Several mechanisms governing somaclonal variation induced during cell culture include gene amplification, single nucleotide base change, transposon migration, altered methylation states, chromosome instability, chromosome inversions, single gene mutations, translocations, cytoplasmic genetic changes, ploidy changes, rearrangements and partial chromosome deletion *(4).* The generation of somaclonal variation has been applied in crop improvement with the intention of inducing and exploiting useful and economically valuable characters that may not be readily available within other sources of germplasm *(5).* Tissue culture variants for a specific trait are generated at a frequency of up to 30 %, while spontaneous mutation rates from mutation breeding methods occur at approximately one in 10^6 *(4).* In vegetatively propagated plants such genetic modifications can be directly incorporated into new varieties.

In several commercial varieties of potato, tissue culture induced variations were observed in a wide range of characters, such as plant morphology, tuber characteristics, disease resistance, isoenzymatic pattern, tuber proteins and chromosome number and structure *(6-14).* Factors affecting somaclonal variation in cultured cells include time in culture, explant source, pathway of regeneration, genotype of the donor plant, environmental conditions during culture, concentration and type of plant growth regulators in the culture media and presence or absence of *in vitro* selective agents *(4,15).*

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The purpose of the present work was to screen plants regenerated from calli of different origins (stem, tuber and protoplast) of potato cv. Bintje, for any useful agronomic or commercial trait under the field condition. Many unstable phenotypic variations were observed among regenerated plants, often accompanied by changes in chromosome number *(3,15)*, so we have undertaken a cytogenetic analysis of regenerated plants to assess this factors.

Material and Methods

Plant material and tissue culture

Potato plants (*Solanum tuberosum* cv. Bintje), micropropagated by nodal segments in long term culture, and potato tubers grown in the field were used as a source of explants for callus induction. Callus proliferation was obtained from:

a) Stem internodal tissue cultured on medium MS (*16*) supplemented with: 0.05 mg/L D-biotin, 0.5 mg/L folic acid, 5 % sucrose, 0.9 % Difco Bacto agar, and growth regulators (μM): 4.4 BA (N^6-benzyladenine), 5.7 IAA (indole-3-acetic acid) and 28.9 GA_3 (gibberellic acid a3).

b) Subepidermal tuber tissue inoculated on medium MS with 825 mg/L NH_4NO_3, and supplemented with: 0.05 mg/L D-biotin, 0.5 mg/L folic acid, 2.5 % sucrose, 0.9 % Difco Bacto agar, and growth regulators (μM): 13.3 BA, 0.05 NAA (α-naphtalenacetic acid), 5.7 IAA, 1.4 2,4-D (2,4-dichlorophenoxyacetic acid) and 5.7 GA_3. Adventitious buds induced in callus cultures were developed in plantlets on regenerative medium MS with 8.9 μM BA, and were routinely micropropagated on medium MS with 0.29 μM GA_3, 2 % sucrose and 0.8 % agar.

c) Mesophyll protoplasts of *in vitro* grown plants were isolated and cultured using the method of Shepard and Totten *(17).*

Nine somaclones were analysed in details. Two of these (15C2 and 17C) were regenerated from tuber callus, six (10/15d, 24d, 10/12d, 10/14d1 and 10/10d) from stem segment callus and one (St4A3/9) from protoplast callus (protocallus). Somaclones were propagated by single node culture on agarified MS medium supplemented with 2 % sucrose and 1.4 μM GA_3.

Morphological analysis

After 90 days growing *ex vitro*, evaluations were made of stem height, number of nodes and changes in leaf morphology of primary regenerants (SC1 plants) trained in glasshouse conditions, and of their vegetative progenies (SC2 plants, the first tuber generation) in the field. Number of tubers, skin colour, size and shape of tubers of SC1 and SC2 plants were also analysed.

Cytological analysis

For cytogenetic analysis seven-day-old roots of microcloned somaclones were used. Roots obtained from potato tubers of the seed potato cv. Bintje were used as a control. Root-tips were pretreated with 8-hydroxy-quinoline (0.002 M) for four hours at 23 °C and then fixed in absolute alcohol:glacial acetic acid (3:1). Before staining in Feulgen the samples were hydrolysed in 1M HCl for 8 min at 60 °C. Permanent slides were prepared by means of liquid carbon dioxide and mounted in DePex.

Results

Morphological characteristics of the regenerated plants

Early changes in the growth rate and general appearance of plants were established within two weeks of development. After 90 days plants reached the height in range from 23 to 77 cm. The number of nodes per plant was different, and did not correlate with the height of stem. Variations in maturity, tuber shape and tuber yield were also recognised (Table 1). Three primary regenerants (SC1 plants), St4A3/9 obtained from protoplasts, 24d-2 from stem and 15C2-2 from tuber tissue failed to form tubers. Violet (anthocyanin pigmentation) and/or green colour of skin were found only in the first generation of tubers. Tubers planted in the field showed a high level of generative ability. The first tuber progeny (SC2 plants) of all somaclones yielded a small number of large, oval shaped and white skin tubers under field conditions. Among them two plants (10/12d-1 and 17C-1) failed to produce tubers.

Leaf morphology was also studied (Table 1). Greatly altered leaf morphology was found in all the plantles regenerated in tuber tissue callus culture. The plants analysed cytologically in this work (15C2-2 and 17C) had small, simple asymmetric leaves. In one SC1 plant (15C2-2) very small simple leaves with dark-violet sectors were found. These traits were found only in primary regenerants. Vegetative progeny of these somaclones had simple leaves or compound leaves with coalesced leaflets. Among the plants derived from stem callus only the somaclone 10/15d showed greatly altered leaf morphology (coalesced leaflets). The other investigated plants had normal leaves with distinct asymmetry. In their vegetative progenies (SC2 plants) grown in field conditions, new leaf forms were observed – compound leaves with coalesced leaflets or simple asymmetric leaves. The protoplast-derived primary regenerant St4A3/9, cultivated in growth chamber conditions, had small, simple epinastic leaves.

Cytological analyses

Ploidy level was analysed in 17-24 well-spread metaphases in each somaclone. All somaclones were mixoploids containing cells with chromosome numbers that deviated from the normal tetraploid (2n=4x=48). Each somaclone contained aneuploid and polyploid cells together with normal tetraploid cells. The aneuploidy level varied from hypotetraploidy (2n=4x=42-47) to hypertetraploidy (2n=4x=49-50) (Table 2). Hypotetraploid cells appeared more frequently than hypertetraploid ones in all somaclones except in the St4A3/9 regenerated from protocallus. Increased polyploidy was noticed in all somaclones (except St4A3/9) but in a lower frequency than aneuploidy. All polyploid cells were at the octoploid level (2n=8x=96).

Table 1. Characteristics of plants regenerated from callus tissues of potato cv. Bintje (SC1 plants), and their first vegetative progeny (SC2 plants)

SC1									SC2					
Somaclone origin, code	Plant No.	[1]Height (cm)/ node number	Leaf morphology	Tubers				Tubers in the field	Sprouted SC2 plants	Leaf morphology	Tubers			
				No.	Size (mm)	[2]I.E.	Colour				No.	Size (mm)	I.E.	Colour
Stem tissue														
10/15d	1	41/17	coalesced leaflets	11	6–17	1.0	white	yes	yes	coalesced leaflets	5	13–43	1.4	white
	2	43/23	coalesced leaflets	14	3–10	1.4	white	yes	yes	normal	1	50	1.5	white
24d	1	[3]nd	nd	8	3–8	1.0	violet	yes	no	–	–	–	–	–
	2	48/22	simple-compound, asymmetry	0	–	–	–	–	–	–	–	–	–	–
	3	nd	nd	2	4–6	1.0	white	yes	yes	simple, asymmetry	4	12–23	1.3	white
10/12d	1	34/18	simple-compound, asymmetry	6	4–7	1.1	white	yes	yes	nd	0	–	–	–
	2	40/22	simple-compound, asymmetry	2	8–12	1.2	white	yes	yes	simple, asymmetry	1	29	1.7	white
10/4d	1	77/33	normal	7	4–19	1.0	white	yes	yes	coalesced leaflets	4	24–36	1.6	white
	2	70/26	normal, asymetry	15	3–15	1.0	white	yes	no	–	–	–	–	–
	3	nd	nd	11	4–22	1.1	white	yes	yes	simple, asymmetry	5	10-60	1.9	white
10/14d	1	50/31	normal, asymetry	4	8–9	1.1	violet	yes	yes	coalesced leaflets, asymmetry	5	10-24	1.4	white
10/10d	1	59/28	normal, asymetry	4	4–7	1.0	white	no	–	–	–	–	–	–
Tuber tissue														
15C2	1	50/22	simple, asymmetry	7	3–8	1.0	white	yes	yes	coalesced leaflets	3	10-34	1.4	white
	2	26/27	very small, simple, dark violet sectors	0	–	–	–	—	–	–	–	–	–	–
17C	1	23/19	normal, asymetry	1	9	1.0	white	yes	yes	nd	0	–	–	–
	2	nd	nd	6	4–15	1.3	green	yes	yes	simple	1	45	1.6	white
	3	nd	nd	4	4–9	1.1	white	yes	yes	coalesced leaflets	4	10-25	1.3	white
Protoplast														
St4A3/9	1	35/16	small, simple, epinastic	0	–	–	–	–	–	–	–	–	–	–
Control	1	70/31	normal	5	9-19	1.7	white	yes	yes	normal	10	28-45	1.7	white

[1] Plants after 90 days growth *in vivo*

[2] I.E. index of elongation=length/width

[3] nd – not determind

Table 2. Frequency of euploid, aneuploid and polyploid cells in potato cv. Bintje somaclones

Somaclone		% of metaphases				Number of analysed metaphases
origin	code	normal 2n=8x=48	polyploid 2n=8x=96	hypoaneuploid 2n=4x=42-47	hyperaneuploid 2n=4x=49-50	
Stem	10/15d	43.00	4.80	52.20	0.00	23
	24d	26.00	5.60	42.10	26.30	19
	10/12d	17.00	9.10	65.20	8.70	23
	10/4d	22.00	12.80	43.50	21.70	23
	10/14d	20.00	5.00	45.00	30.00	20
	10/10d	18.00	23.20	35.30	23.50	17
Tuber	15C2	33.00	17.00	29.20	20.80	24
	17C	12.00	17.40	70.60	0.00	17
Protoplast	St4A3/9	11.00	0.00	44.50	44.50	18
Control	C	100.00	0.00	0.00	0.00	30

Mitotic abnormalities such as lagging chromosomes and bridges at anaphase and telophase were observed in all regenerants, but not in the control (data not shown).

Discussion

Nine somaclones of tetraploid potato cultivar Bintje regenerated from stem, tuber or protoplast derived calli were analysed in this study. A wide range of phenotypic variations were observed among regenerants from different explant calli and also within plants regenerated from the same callus. All regenerants were morphological and cytological chimeras (chromosome mosaicism) with high frequency of cells (57-89 %) at abnormal ploidy level.

The observation that plants regenerated from the same callus were different in phenotype and ploidy implies that variation arose during culture phase. Sree Ramulu *et al. (18)* noticed that both poliploidy and aneuploidy occurred during the initial stages of cell division and callus induction of the cultivar Bintje. The phenomenom of cytochimera formation in regenerants of several cultivars of potato was observed sporadically also by some other authors *(6,7,19)*. Chromosomal changes occuring in undifferentiated callus of many species were discussed by Bayliss *(20)*. The primary processes *in vitro* which lead to polyploidy, aneuploidy, chromosome structural changes and mutations are still unclear, but a great number of influential factors have already been discussed *(11,20-24)*. In the case of chimeric regenerants the cells involved in the organization of an adventitious bud probably are cytologically different *(15,22,25)*. Our investigations show that mixoploidy is a very frequent occurence in tetraploid potato cv. Bintje regenerants generated from calli of diverse origin, but this does not prevent regeneration process.

The cytological variability of the regenerants from stem and tuber calli was not in correlation with the origin of callus. The regenerants of both origins were highly mixoploid, predominantly hypotetraploid. The fact that hypotetraploid cells occurred at higher frequency than that of hypertetraploid cells could be explained by Sree Ramulu's *(15)* suggestion that the buffering capacity of the polyploid condition might be responsible for the toleration of chromosomal and gene alterations. It is possible that loss of a few chromosomes can be tolerated more easily than a surplus because of the tetraploid level.

All of the plants obtained in this study were cytochimeras with unstable phenotypic characteristics during vegetatively propagated generations and therefore it was difficult to predict which characteristics would occur in the next vegetative progeny. In contrast to our results, useful variations must be stable, durable and should not alter other agronomic or economically important traits of the donor parent.

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Miksoploidija i himerne značajke somaklonova krumpira *Solanum tuberosum* L. cv. Bintje

Sažetak

Kultura biljnih stanica i tkiva jedna je od metoda kojom se može postići genetička varijabilnost u svrhu oplemenjivanja poljoprivrednih kultura. Devet somaklonova tetraploidnog krumpira sorte Bintje, regeneriranih iz kalusa potaknutih na eksplantatima stabljike i gomolja, te u kulturi protoplasta, analizirano je u uvjetima *in vitro* i u polju. Svi somaklonovi bili su morfološke i citološke himere. Fenotipske promjene očitovale su se na razini cijele biljke, uključujući listove i gomolje. U većini slučajeva utvrđene su morfološke razlike između SCl-biljaka (primarni regeneranti) i njihovih potomaka (SC2-biljke) dobivenih vegetativnim razmnožavanjem. Uočene fenotipske nestabilnosti mogu se povezati s mozaicizmom u broju kromosoma jer je u svih somaklonova utvrđena visoka učestalost (57–89 %) aneuploidnih i poliploidnih stanica (analizirani su meristemi vrška korijena).