Acta Bot. Neerl. 39(2), June 1990, p. 163–169

Somaclonal variation in potato: a karyotypic evaluation

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SUMMARY

The data on somaclonal variation in potato are reviewed and discussed from a karyological point of view. Potato species are polysomatic. This pre-existing genetic variation is introduced into *in-vitro* cultures. Cultures of protoplasts, cell suspensions and calli show a high degree of nuclear instability leading to polyploidy, aneuploidy and structural chromosome alterations. Genetic and tissue culture factors influence the degree of instability. *In-vitro* cultures showing gross karyotypic changes have a decreased capacity to regenerate into whole plants. Cell selection takes place during the regeneration process, but most of the regenerated plants still show karyotypic alterations, which have most probably arisen before and during the *in-vitro* culture period. These alterations coincide with phenotypic variation and chimeras among the regenerated plants.

Key-words: chromosome variation, *in-vitro* culture, potato, regenerated plants, somaclonal variation.

INTRODUCTION

Somaclonal variation occurs at a high frequency among potato plants regenerated from *in-vitro* grown callus of non-meristematic tissues (reviews in Sree Ramulu 1986; 1987; Jacobsen 1987). Changes in nuclear and cytoplasmic genetic factors may underlie this variation (De Klerk 1990). Useful alterations in agronomically important traits can thus become available for crop improvement. However, somaclonal variation is a feature which is not yet controllable and is often accompanied by unfavourable genetic changes. Therefore, knowledge on the causes and mechanisms generating somaclonal variation is important. In this article, data on somaclonal variation in potato will be reviewed and discussed from a karyological point of view. For convenience, the term 'potato' is used to cover the commercial tetraploid cultivars and breeding lines of *Solanum tuberosum* L. ($2n = 4 \times = 48$) and the derived dihaploid and monohaploid lines; other potato species will be mentioned specifically.

GENETIC CONDITION OF THE DONOR MATERIAL

DNA measurements of nuclei from various organs (shoot, leaf, stem, root) have shown that potato and *S. phureja* Juz. et Buk. are polysomatic species, a class in which the tissues

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(excluding meristems) contain a mixture of cells with the expected ploidy level and cells with endoreduplicated nuclei (polysomaty). The frequency and degree of polyploidization is generally related to differentiation processes: organs differ in the percentages of endopolyploid cells and in the degree of polyploidy (number of endoreduplication cycles), depending on the genotype of the plant, culture conditions (*in-vivo* versus *in-vitro* cultured plants) and tissue age (Sree Ramulu & Dijkhuis 1986; Uijtewaal 1987; Pijnacker *et al.* 1989b).

Protoplasts or explants can thus introduce a genetically heterogeneous population of cells into the cell culture which will eventually contribute to somaclonal variation during *in-vitro* culture and in regenerated plants. Investigations should, therefore, be aimed at the identification of organs containing cells having the same ploidy level and at the utility of meristems. The factors triggering endoreduplication should be found. Furthermore, it would be important to know whether during differentiation cells have also changed genetically in other ways, for instance by point mutations, gene amplification or heterochromatinization.

GENETIC CHANGES DURING *IN-VITRO* CELL CULTURE

Cell cultures of potato show genetic changes from the onset of their development onwards, as revealed by DNA measurements and karyotypic analysis (Khvilkovskaya 1982; Jacobsen *et al.* 1983; Sree Ramulu *et al.* 1984; 1985; 1989; Carlberg *et al.* 1984; Tempelaar *et al.* 1985; Pijnacker *et al.* 1986a,b; Pijnacker and Ferwerda 1987).

The earliest mitoses of isolated cells (i.e. protoplasts with a newly formed cell wall) and of explant cells of potato and *S. phureja* may show diplochromosomes. These are the result of an endoreduplication cycle before (see above) or after the start of the culture. As a consequence, the next cell generation(s) will have a doubled number of chromosomes. It is not known why at an early stage during *in-vitro* culture some cells endoreduplicate and others do not. Controlling this type of polyploidization is crucial, because it occurs at high frequencies and may be repeated in the same cell. It should be mentioned here that in monohaploid explant cultures, polyploid cells are selectively triggered to mitosis (Pijnacker *et al.* 1986b, 1989b; W. van Everdink, personal communication). Anaphases in the single cells (protoplasts) may show one or more bridges indicating the occurrence of chromosome aberrations. These bridges are not found in the cells of explants. If such chromosome aberrations are due to a differentiation process in the plant, for instance, concomittant with partial chromosome replication, and not due to the culture conditions, the question remains why these cells do not divide in explants. Moreover, chromosome elimination, abnormal and multipolar spindles and acytokinesis leading to aneuploidy and multinuclear cells occur in single cells during the first divisions (Carlberg *et al.* 1984; W. van Everdink, personal communication). These types of aberrations are probably related to abnormal functioning of the cytoplasm, possibly because of destruction of the tissue integrity and disorganization of the cytoskeleton during protoplast isolation. Aneuploidization, chromosome aberrations and acytokinesis rarely occur during the first week of callus development in explant cultures.

Only a fraction (in general less than 2%) of the differentiated cells of the donor material de-differentiates and undergoes mitosis in protoplasts or explant culture. It is important to know why the majority of the cells do not divide. It is not known whether this is due to their degree of differentiation or to culture conditions.

Established cell suspensions and callus cultures maintain high levels and rates of polyploidization, aneuploidization and chromosome mutations, even after 2 years of culture. In these cultures, polyploidization is caused by restitution rather than by endoreduplication, and aneuploidization by chromosome elimination rather than by multipolar spindles. Mitosis is not blocked by a high number of chromosomes: cells with 500 or more chromosomes may divide normally. Chromosome numbers and DNA values of interphase nuclei indicate that polyploidization can reach beyond the 32-ploidy level. Chromosome aberrations become visible as dicentric chromosomes, acentric fragments and deletions, and are thus the result of breaks. Moreover, in cell suspensions metaphases show chains of chromosomes by telomere–telomere connections. Acytokinesis occurs in both types of culture but at lower frequencies than at the start of the protoplast culture (Khvilkovskaya 1982; Sree Ramulu *et al.* 1984, 1985; Pijnacker *et al.* 1986a,b). Cell suspensions or callus cultures with unchanged karyotypes have not yet been established, and subcultures may become karyotypically different in time. The cell culture period thus has a large impact on the frequency of occurrence of somaclonal variation because various mutations are generated with increasing culture age.

In *in-vitro* culture of wild and cultivated potato species the cells tolerate a high degree of genome and chromosome mutations which may be related to polysomaty, polyploidy or heterozygosity. Point mutations, position effects and activation of transposable elements can therefore occur at a high frequency.

FACTORS INFLUENCING GENETIC (IN-)STABILITY DURING *IN-VITRO* CULTURE

During the initial stages of callus induction in explants the number of endoreduplication cycles is dependent on the species (potato, *S. phureja*), and the degree of polyploidization on the ploidy level of the donor material (Sree Ramulu & Dijkhuis 1986; Pijnacker *et al.* 1989b).

The type of *in-vitro* culture of potato determines the onset and presence of certain nuclear instabilities. As mentioned above, chromosome mutations occur at an earlier time during culture of single cells (protoplasts) than during culture of explants. In addition, multinucleate cells are found at early stages of callus formation from protoplasts, but not from explants. It should be noted that during protoplast isolation a selective loss of cells with a certain ploidy level may take place (Uijtewaal 1987).

Medium components and physical environment influence the growth of cell cultures of potato and various other species (Opatrný *et al.* 1980; Creissen & Karp 1985; Haberlach *et al.* 1985; Nelson *et al.* 1986; de Vries & Bokelmann 1986; Foulger & Jones 1986; Masson *et al.* 1987). Cell proliferation (mitotic activity) and cell size may be altered but how the various factors exert their influence has not yet been established. Polyploidization by endoreduplication in monohaploid potato explants is dependent on the sucrose concentration and not on the osmolality. Diploid and tetraploid explants do not react as such to sucrose (Pijnacker & Ferwerda 1990). There are indications that hormones added to the medium cause polyploidization but the results are contradictory (Wenzel *et al.* 1979; Shepard *et al.* 1980; Jacobsen 1981; Sree Ramulu *et al.* 1983; Carlberg *et al.* 1984; Fish & Karp 1986; Hänisch ten Cate & Sree Ramulu 1987). However, in general, it is not known which factors cause instability and which mechanisms are involved.

REGENERATION PROCESS

Depending on the hormonal status of the medium, calli regenerate shoots or roots. Regeneration capacity of potato is under genotypic control (Bragdø-Aas 1977; Surikov 1985; Debnath *et al.* 1986; Fish & Jones 1988). Ageing of the cell cultures may be accompanied by a decreasing capacity to regenerate. The latter is probably caused by the genetic changes occurring during the *in-vitro* culture period (Pijnacker *et al.* 1986a). Whether or not regeneration capacity can be increased by varying the medium composition, subculturing or initiation of regeneration at various environmental/seasonal conditions need to be investigated.

There seems to be a rigorous selection in favour of cells with a certain genetic constitution during the regeneration process: regenerating calli and regenerated plants of potato show a lower degree of karyotype alterations than the calli from which they are derived (see below; Pijnacker & Ferwerda 1987). It is not known how the cells with gross karyotypic changes are selected against. This may have a genotypic origin or be caused by differences in mitotic rates. In spite of the selection, shoots with different karyotypes can be regenerated from one callus (Karp *et al.* 1982; Sree Ramulu *et al.* 1986).

Shoots and roots normally do not regenerate simultaneously, often depending on the medium composition. Shoots of potato with different genetic constitution, but regenerated from the same type of cell culture, may require different media in order to regenerate roots (Fish & Karp 1986). A knowledge of the regeneration process is required, because controlling this process could lead to the production of (more) genetically uniform regenerants.

REGENERATED PLANTS

The karyotype of regenerated plants has been analysed in different materials (isolated cells, meristems, meiotic cells) by various methods (DNA measurements, karyotyping, chloroplast counts). The resolutions of these methods all differ. It should be noted that as chimeras occur among regenerated plants (see below), it is desirable to analyse the karyotype of the germ cells and of tuber-derived progeny. However, for convenience, root and shoot meristems are generally used for analysis.

The percentages of regenerated plants of potato, *S. phureja* and *S. brevidens* Phil. with deviating karyotypes vary considerably from one experiment to the other, and cell cultures which constantly give rise to clones with similar karyotypes have not been found. The plants show a polyploidized number of chromosomes, aneuploidy and chromosome rearrangements. The karyotypic alterations reflect those found in the cell cultures and it is tempting to suppose that they arose before regeneration started (reviews in Sree Ramulu 1986; Jacobsen 1987; and: Quraishi 1985; Sree Ramulu *et al.* 1986, 1989; Fish & Karp 1986; Nelson *et al.* 1986; Gill *et al.* 1986; Uijtewaal 1987; Zhila *et al.* 1987; Hovenkamp-Hermelink *et al.* 1988; Jones *et al.* 1989). As a rule, ploidy does not reach beyond the octoploid level. Aneuploidy often involves the addition or loss of one to three, and infrequently more, chromosomes. Monosomic and trisomic plants ($2x \pm 1$) have not been obtained. Chromosome structural changes in regenerated potato plants include translocations, deletions, duplications, inversions and amplified heterochromatin. The occurrence of these changes has also been demonstrated in meiotic cells of potato and may negatively influence the production of germ cells (Gill *et al.* 1987; Pijnacker & Ferwerda 1987). Nucleolar chromosomes may undergo structural changes in the nucleolar

organizer region (Ooms *et al.* 1985), and changes in the ribosomal DNA composition have also been detected (Landsmann & Uhrig 1985). It is not known whether certain chromosomes have an increased instability. At least in the case of somatic hybrids of potato + *S. phureja*, nucleolar chromosomes are more prone to breakage and elimination (Pijnacker *et al.* 1987, 1989a).

Chimeras have frequently been detected among regenerated plants of potato (review in Sree Ramulu 1986; Sree Ramulu *et al.* 1989). They are mosaic with two or more different karyotypes (aneusomaty, mixoploidy). It should be noted that the additional karyotypes occur at higher frequencies in the same or different tissues than those expected from the spontaneously occurring (rare) mutational events. The difference may involve one or more chromosomes as well as complete genomes. Chimeras may arise from two or more callus cells with different karyotypes which participate in the generation of the new apical meristem. It is also possible that apical meristems derive from single cells and that the descendants of these cells undergo genome or chromosome mutations during plant development.

Various phenotypic changes, e.g. habitus, leaf characters, tuber colour and form, disease resistance, etc. have been observed among regenerated plants of potato, *S. phureja* and *S. brevidens*. They often coincide with karyotypic changes (reviews in Sree Ramulu 1986; Jacobsen 1987; and: Quraishi 1985; Sree Ramulu *et al.* 1986, 1989; Fish & Karp 1986; Nelson *et al.* 1986; Gill *et al.* 1986; Uijtewaal 1987; Zhila *et al.* 1987; Hovenkamp-Hermelink *et al.* 1988; Jones *et al.* 1989).

CONCLUSIONS

Potato plants derived from callus cells show a high degree of unpredictable somaclonal variation. Polyploidization, aneuploidization and chromosomal rearrangements underlie this variation. Further studies on the nature and causes of somaclonal variation are urgently required so that beneficial somaclonal variation can be used in breeding programmes for crop improvement.

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