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Variation Amongst Protoplast-derived Potato Plants (*Solanum tuberosum* cv. 'Maris Bard')

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Summary. Plants were obtained from protoplasts of shoot cultures of potato (*Solanum tuberosum* L. cv. 'Maris Bard') and from in situ calluses upon plants of cv. 'Majestic'. None of the protoplast-derived plants resembled each other in all of ten morphological characteristics scored and only one resembled the parental 'Maris Bard' type. As there were a number of plants regenerated from each of ten protoplast-derived calluses it is concluded that variation arose after protoplast isolation during the cell culture phase. Plants regenerated from in situ calluses of cv. 'Majestic' were quite uniform. Reported cases of variation and uniformity from cultured potato tissues are discussed. It is concluded that the variation is not a consequence of using protoplasts and that the expression or induction of variation is controllable.

Key words: Potato protoplasts – Tissue culture – Variation

Introduction

Potatoes are a major world crop producing some of the highest obtainable yields of edible dry matter per hectare. Despite this there has been limited success, especially in comparison to that obtained with cereals, in breeding new cultivars and much of the acreage is planted with those released many years ago. In part this is because many important commercial cultivars are partly or completely infertile and thus cannot enter a breeding programme. Since around 20% of the world potato crop is lost annually through disease a major breeding aim is disease resistance. There has therefore been considerable interest in the report of Shepard et al. (1980) that amongst plants regenerated from single leaf protoplasts of the commercial tetraploid cultivar 'Russet Burbank', there is marked variation for several agronomically important characteristics including disease resistance because it offers the potential for improving existing cultivars without the need for breeding. In contrast Wenzel et al. (1979) found a lack of variation amongst plants regenerated from protoplasts of dihaploid potatoes. The reasons for the different results are the subject of some controversy. It has been suggested that the variation observed is present in the leaf cells, particularly of old cultivars, or may arise as a consequence of the culture techniques employed.

Materials and Methods

Protoplasts from in vitro propagated shoot cultures of *Solanum tuberosum* cv. 'Maris Bard' were induced to form callus and shoots by methods already described (Thomas 1981). Altogether 45 shoots were excised from 10 different calluses. These shoots were used to establish shoot cultures by placing a piece of stem with a single leaf attached on Murashige and Skoog (1962) medium (Flow Laboratories) containing 0.1 mg/l benzyl adenine and 6 g/l agar. The shoot cultures were propagated at 2–4 week intervals after growth at 25 °C in diffuse light (Thomas 1981). Plants were obtained from shoot cultures by transferring individual shoots to Murashige and Skoog medium containing 0.05 mg/l naphthalene acetic acid, 20 g/l sucrose, 6 g/l agar. Once rooted the plants were transferred to vermiculite and grown in 10 cm pots in a humid container within a growth room. Once established the plants were grown in 15 cm pots containing a mixture of 1 part Eff loamless compost to 1 part grit in the same growth chamber. Plants were grown in a 12 h day (20 °C/16 °C; RH 70%/80%) under a mixture of warm white fluorescent lights 450 $\mu em^{-2} s^{-1}$) and tungsten lights (11% of wattage). For a separate experiment the stems of 8 week old greenhouse-grown plants of *S. tuberosum* cv. 'Majestic' were excised 15 cm above soil level and the cut surface treated with 0.1 g/l chlorophenoxyacetic acid. Callus formed at the cut surface gave rise to shoots which were excised and rooted directly in moist sand before transfer to compost.

Results and Discussion

a) Protoplast-derived Plants

The experiment performed is outlined in Figure 1. Since the protoplasts were grown in agar with low plating efficiency (0.1% of the initial protoplast population eventually formed calluses) the possibility of calluses arising from more than one protoplast was excluded as far as was practicable. Several shoots were formed at different points on some calluses. The shoots and their resulting shoot cultures numbered 45 in all. Potted plants were only obtained from 23 of the shoot cultures. The remaining 22 showed slow growth rates, callus formation, variable chlorophyll pigmentation with any rooted plantlets dying on transfer to the growth chamber.

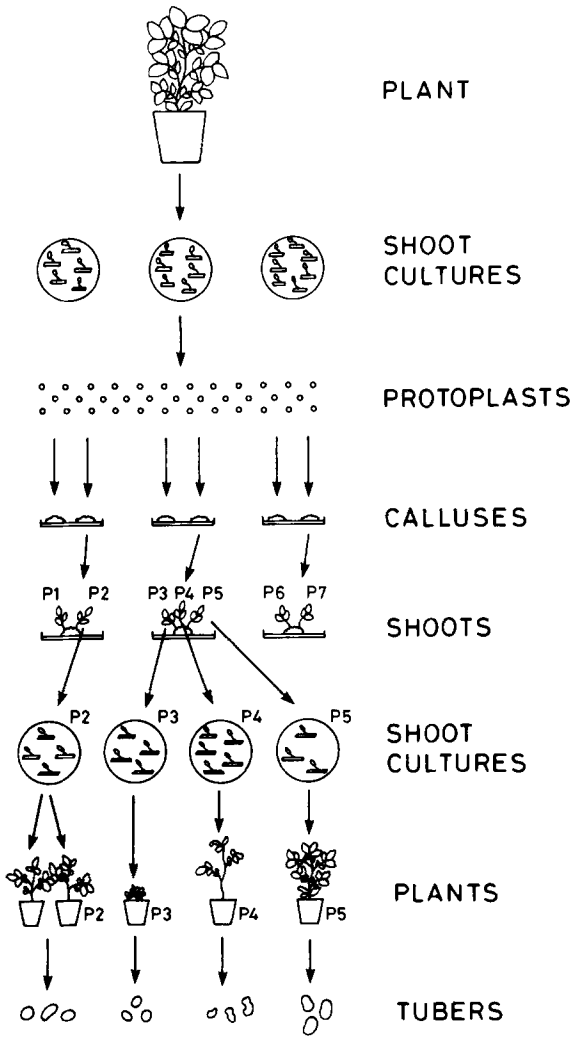

Fig. 1. Diagrammatic representation of experimental approach for testing the origin of variation of protoplast-derived plants of *S. tuberosum* cv. 'Maris Bard'

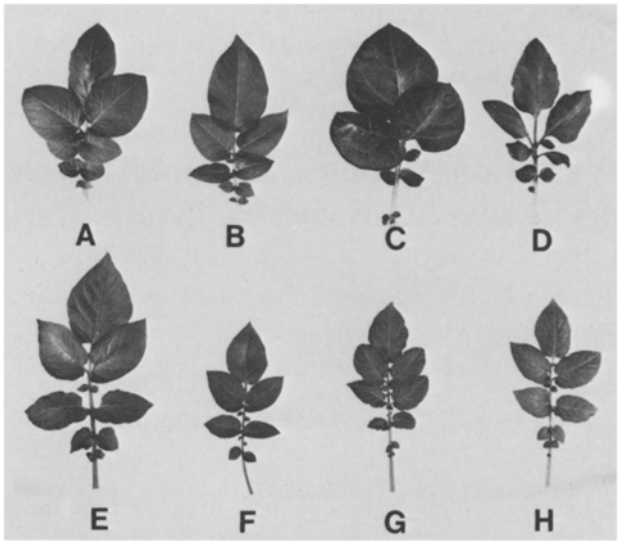

Fig. 2A–H. Leaves from plants of 'Maris Bard' (**A**) three protoplast-derived shoots (**B:** P40, **C:** P33, **D:** P5), 'Majestic' (**E**) and three plants (**F, G, H**) from an in situ callus

The plants obtained were examined for ten morphological characters used in identification of commercial cultivars (NIAB 1975). They varied in most aspects of vegetative anatomy (Table 1), and just one shoot (P40) gave plants resembling 'Maris Bard' in its morphology. Some indications of leaf shape (categories DEFG of Table 1) are shown in Figure 2: note the similarity of 'Maris Bard' (A) and P40 (B), the rounder leaflet shape of P33 (C) and the narrower, fully separated leaflets of P5 (D). The variation was such that none of the original selected shoots gave rise to plants that were the same as those from any other shoot, showing that, because some shoots came from the same callus, variation existed between plants derived from a single callus and hence a single protoplast.

In general, plants showed uniformity after shoot culture propagation e.g. the nine propagations from shoot P4 were similar (Table 1) as were those derived from P40 and P35 (Fig. 3) and 10 other shoots. However, 5 shoots gave varied plant types on propaga-

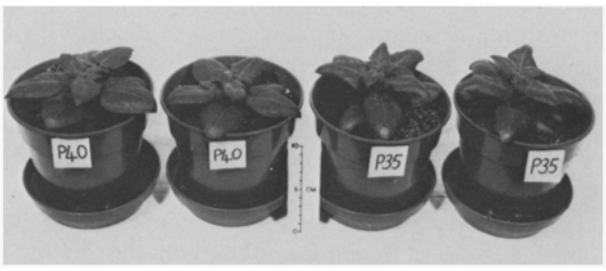

Fig. 3. Young plants derived from shoot cultures of P40 and P35

Table 1. Assessment of 10 morphological characters in 'Maris Bard' and plants derived from it by protoplast culture

Shoot numbers from which plants are derived (number of plants scored in brackets)		A Leaf colour	B Leaf gloss	C Variegation	D Terminal leaflet shape	E Lateral leaflet shape	F Fusion of terminal & lateral leaflets	G Overlapping of terminal & lateral leaflets	H Anthocyanin pigment on leaf	I Vigour	J Height
'Maris Bard' tuber	(1)	2	2	0	2	2	0	3	0	4	3
P1	(1)	3	2	0	1	none	–	–	0	1	1
P2	(5)	V1–4	V1–3	1	V1–2	V2–3	0	V1–3	V0–1	V1–4	V1–3
P23X	(1)	4	3	0	2	2	1	3	0	2	3
P3	(7)	4	3	1	V1–2	V1–2	1	V1–3	0	3	3
P4	(9)	4	2	0	2	2	0	2	0	3	3
P45X	(11)	4	2	0	V2–3	V2–3	0	2	0	3	3
P5	(6)	4	2	1	2	2	0	2	0	3	3
P7	(6)	4	2	0	1	1	2	3	0	3	3
P8	(8)	4	2	0	2	2	0	3	0	3	3
P910	(2)	4	3	0	1	1	2	3	0	2	3
P11	(1)	4	3	0	2	2	0	3	0	3	3
P12	(2)	4	2	0	1	2	2	3	0	2	3
P13	(3)	V1–4	2	0	2	2	2	3	0	2	3
P16	(5)	V1–4	V1–3	1	2	2	0	2	1	V1–3	V2–3
P17	(1)	2	2	0	2	2	2	3	0	3	3
P22	(3)	4	3	0	1	1	3	–	0	3	3
P30	(6)	4	3	2	1	1	2	3	0	3	3
P33	(8)	4	3	0	1	1	0	3	1	3	3
P35	(5)	2	2	0	2	2	2	2	0	4	3
P38	(2)	4	3	0	n. d.	n. d.	0	n. d.	1	2	3
P39	(1)	2	2	0	2	2	2	1	0	3	3
P40	(7)	2	2	0	2	2	0	3	0	4	3
P41	(1)	4	2	0	1	1	0	3	0	2	3

A: 1 pale – 4 dark green; B: 1 matt – 3 heavy gloss; C: 0-none; 1-some plants with; 2-all plants with variegated patches; D & E: 1-round (length over width 1.0–1.2); 2-broad (ratio 1.3–1.5); 3-narrow (ratio 1.6–2); F: 0-no fusion; 1-some plants with some fused terminal and laterals; 2-all plants with some fusion; 3-both laterals fused; G: 1-terminal solitary – 4 terminal large overlap with laterals; H: 0-none; 1-some anthocyanin; I: 1 very slow – 4 vigorous growth; J: 1-prostrate form; 2-low; 3-normal height; V = score varies between different plants

tion. The most marked differences occurred among plants from shoot P2 (Fig. 4). This variability was unexpected because clonal propagation via shoot cultures is a common method for maintaining uniformity in many species including potato. The segregation in these cultures could be due to chromosomal instability of the original shoot or an indication of its chimeral nature (i.e. it had arisen from several genetically different cells of the callus).

Fig. 4. Four plants derived from shoot cultures of P2 vary in many characters and are all different from a plant derived from P5

b) In situ Callus-derived Plants

In a separate experiment the stems of greenhouse-grown plants of *S. tuberosum* cv. 'Majestic' were excised 15 cm above soil level and the cut surface induced to form callus and shoots. In all, 17 shoots were excised, rooted and grown further. They were then examined for the same range of morphological characters as before. The plants were relatively uniform: examples of leaf shapes are shown in Figure 2 (F–H). With one exception the plants fell in the same class (characters BCEIJ in Table 1) or in two adjacent classes (characters FIDFG in Table 1). Anthocyanin pigmentation was present in young leaves of 4 of the 17 plants and also in a number of control 'Majestic' plants. This degree of va-

riability is not outside that often found in glasshouse-grown material.

The experiments reported here and the previous work of Shepard et al. (1980) and Wenzel et al. (1979) allow us to draw the following conclusions.

(1) The differences between previous results is not due to differences in technique since we have followed Wenzel's method and obtained considerable variation.

(2) The variation observed by Shepard et al. is not due solely to the cultivar used nor is it a property of old cultivars (e.g. 'Russet Burbank' released ca. 1900) since 'Maris Bard' was only released in 1974.

(3) The observation that different plants from the same callus vary cannot be explained on the basis of genetic differences between cells in the leaf, but indicates that the variation arose during callus growth. Genetic and chromosomal instability in cultured cells has been well documented (Bayliss 1980). There is thus no need to posultate that the variation observed in these protoplast-derived plants results from genetic differences in the starting material. Furthermore, similar variation has been observed in plants regenerated from rachis, petiole or leaflet discs of *S. tuberosum* cv. 'Desiree' (van Harten et al. 1981). Variation is therefore not a consequence of the protoplast technique. This observation has obvious practical consequences since the production of plants from leaf or rachis pieces is relatively simple and may be a more rapid and reliable way of obtaining variation.

(4) In certain situations little variation occurs. Instances of this are the use of dihaploid protoplasts (Wenzel et al. 1979) or in situ calluses of normal tetraploid cultivars (even with the old cultivar 'Majestic' released in 1911). The induction or expression of variation is therefore controllable.

There is clearly a need to explain the apparent contradiction between the variability of plants derived from tetraploid protoplasts and the relative uniformity of those plants (97% of which were tetraploid) derived from dihaploids (Wenzel et al. 1979).

A number of hypotheses are available which lead to testable consequences and some of these are under experiment. Only with more work will the full basis of the variation be realised. For the present it is clear that variation arising from tissue cultures is of practical significance (Larkins and Scowcroft 1981) not only in potato but other crop species such as sugar cane (Heinz et al. 1977) and forage grasses (Ahloowalia 1978).

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