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Genetic Variation in Potato Cv. Record: Evidence from *In Vitro* 'Regeneration Ability'

S. A. JUNED, M. T. JACKSON* and B. V. FORD-LLOYD

School of Biological Sciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

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Regeneration ability *in vitro* was studied in 170 individual tubers putatively derived from several or many parent plants of the potato cv. Record. Of these, 120 were sprouted and the sprouts used to establish *in vitro* shoot cultures for leaf disc production. The other 50 were grown in a glasshouse for the production of leaf discs. The reliable regeneration of somaclones from leaf disc calluses was successful from only 11 parental tubers. In ten of these, somaclones were derived from *in vitro* shoot cultures, and from a glasshouse-grown plant in the other. Four parental tubers gave the majority of somaclones, and one, R149, produced 85% of all somaclones at 15 months from initiation of leaf disc cultures. This differential regeneration ability may be due to genetic differences between tubers in this potato cultivar as it was found to be maintained in subsequent tuber generations. The results are discussed in terms of seed potato production and *in vitro* genetic conservation of vegetatively propagated species.

Key words: Potato, *Solanum tuberosum* cv. Record, regeneration ability, leaf disc culture, somaclonal variation.

INTRODUCTION

The generation of somaclonal variation (Larkin and Scowcroft, 1981) has been applied in plant breeding with the intention of inducing and exploiting useful and economically valuable characters that may not be readily available within other sources of germplasm. In a recent publication (Karp *et al.*, 1989) it has been estimated that up to 3% of potato somaclones may be valuable. The practical application of somaclonal variation, however, depends initially upon the ability to regenerate adventitiously plants *in vitro* at a high frequency over a long period of time. This requirement is essential for the production of large numbers of plants to screen for desirable characteristics which may be needed in a breeding programme.

The potato cv. Record (*Solanum tuberosum*) has been reported to have a low frequency of regeneration from callus (Wheeler *et al.* 1985), and to respond to regeneration techniques far more slowly than many other cultivars (Cassells, Austin and Goetz, 1987). However, it has been shown by Uhrig (1985) and Wenzel *et al.* (1987) that selection can be made *in vitro* for the character 'regeneration ability', clearly indicating a genetic difference between genotypes for this character. This was demonstrated in experiments aimed at the regeneration of isolated microspores from tetraploids and a range of dihaploid clones from which protoplast regeneration was intended. Following crosses between clones with good regeneration ability, it was possible to demonstrate that in the F_1 progenies the capacity for producing macroscopic structures from microspores was under genetic control.

In this paper we report that it is possible to carry out such selection for regeneration ability from within vegetatively reproduced material of one potato cultivar, cv. Record. The significance of this is discussed in terms of seed potato production and stability of *in vitro* cultures for genetic conservation, as well as for *in vitro* regeneration for the production of somaclones.

* For correspondence.

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MATERIALS AND METHODS

Plant material and in vitro *culture*

Seed tubers (Super Elite 1 grade) of the potato cv. Record were numbered R1 to R170; these were the individual parental tubers from which leaf disc explants were ultimately derived. Fifty (R1–R50) were grown in the glasshouse and the shoots from these tubers were never grown in culture.

The other 120 tubers (R51–R170) were taken immediately into culture from sprouts. A single sprout from each tuber was removed, surface sterilized and placed on Murashige and Skoog (1962) medium in 0·8% agar with the addition of NAA (0·2 mg l^{-1}) and BAP (0·05 mg l^{-1}), adjusted to pH 5·6, in a boiling tube (60 ml). Shoots were sub-cultured into jars every 4 weeks after the removal of leaves for leaf discs, using an alternating cycle of MS medium with and without NAA and BAP. Cultures were maintained at a constant 25 °C, with a 16-h photoperiod. No evidence of adventitious shoot production was noted during these cycles. The original tuber number was maintained throughout so that each shoot culture could be specifically identified in terms of tuber parentage as a shoot culture line.

Leaf explants and culture media

Leaf explants were taken from the 4- to 20-week-old glasshouse-grown plants, using the youngest fully expanded leaves, and surface sterilized following standard procedures. Leaf explants from *in vitro* grown plants (maintained as described above) were used after growth in culture for 4

TABLE *Media used in the* in vitro *production of somaclones from leaf discs from potato cv. Record*

Medium no.	Basal medium	Agar (%)	Sucrose (%)	Growth regulators (mg l^{-1})
	MS	0·8	2·5	NAA 0·5 Zeatin 5·0
2	MS	0·8	2·0	GA_3 5·0
3	MS	0·8	2·5	BAP 0·2 GA_3 5·0
4	MS	1·0	3·0	GA_3 5·0

weeks. Leaf explants were taken after pre-treatment with a dark period of 24 h (George and Sherrington, 1984). Only the uppermost two to three leaves were used from each shoot culture or plant.

Each leaf was cut to provide a single 1-cm^2 square with a central vein in each piece. Five leaf squares were placed in each Petri dish on Medium 1 (Table 1). The dishes were kept in the dark for a further 24 h before exposure to a 16-h day at 25 °C. Leaf explants from *in vitro* shoot cultures were more successfully established than explants from glasshouse-grown material. Bacterial contamination and phenolic browning were problems in explants from glasshouse material.

In order to initiate and maintain calluses capable of regenerating over a long period of time, four culture media were used (Table 1). The basal medium consisted of the salts, vitamins and organic compounds of MS medium; the concentrations of sucrose, agar and growth regulators varied for each medium and are specified separately. A two-step regeneration method (Webb, Osifo and Henshaw, 1983) was generally used, with a third step added where regeneration response was slow.

Explants were kept on Medium 1 for 14–21 d, after which they were transferred to Medium 2. Thereafter, they were transferred to fresh Medium 2 every 4 weeks. Two to three such transfers were usually required before multiple shoot production was initiated. Medium 3 was used where the regeneration response was slow, i.e. 28 d on Medium 2 with no response. After the initiation of multiple shoots, selected calluses were maintained on Medium 4 for further shoot production.

After the removal of shoots, calluses were subcultured on Medium 2 every 4 weeks until multiple shoot production was established. Transfer to Medium 4 (with a raised agar concentration) maintained this production. This stage was reached approx. 16 weeks from callus initiation. In these subsequent transfers the callus could be divided in the following way.

(1) Shoots approx. 15 mm long were removed together with a small piece of callus. The shoots were then cut free from the callus individually, subcultured and rooted on half-strength MS medium with the addition of NAA (0·1 mg l^{-1}) and BAP (0·025 mg l^{-1}). A sucrose concentration of 2% and agar at 0·65% were used.

(2) Areas of the callus producing shoots were divided from non-regenerative callus, and were subdivided into pieces approx. 10 mm^2 on fresh Medium 4.

TABLE 2. *Selection procedure during the production of somaclones from potato cv. Record from leaf discs. Each selection cycle was repeated twice before rejection*

Cycle	No. parental tubers	Rejected	Reason for rejection	Remainder
1	170	20	1	150
2	150	50	2	100
3	100	45	3	55
4	55	28	4	27
5	27	16	5	

Reasons for rejection:
1. Plants were weak or shoot cultures failed to grow.
2. Failure to produce callus from leaf discs or repeatedly infected.
3. Little or unreliable callus production from leaf discs.
4. No shoot production from callus after 16 weeks.
5. Failure to produce shoots in duplicate on callus.

Each shoot successfully removed from the callus and rooted was given a unique number.

Selection of somaclones from culture

A carefully controlled selection process was carried out at each stage of sub-culture or growth. Weak or abnormal plants were removed and rejected. Explants producing poor callus growth after 21 d on Medium 1 were rejected, as were callus cultures not producing shoots after two transfers to Medium 2. Calluses producing multiple shoots were maintained on Medium 4 as described above. Table 2 summarizes the selection process. Steps one to three were repeated twice for each shoot culture line or plant before its final rejection as well as rejection of the parental tuber.

RESULTS

Callus induction and plant regeneration

On Medium 1, callus formation was initiated at the cut surfaces of leaf discs, particularly around the leaf veins. On Medium 2, the developing callus appeared nodular. Shoots emerged by organogenesis after approx. 12 weeks from callus initiation, and these showed subsequent elongation.

After the selection process for sustained regeneration, only material derived originally from 11 parental tubers remained at 15 months from initiation of leaf disc cultures. From these, shoots were produced between 3 and 6 months from induction (Table 3). Further work was concentrated on material from four parental tubers showing multiple shoot production, namely R68, R110, R149 and R150. Shoots were removed, and calluses split and replated regularly. After 15 months, a total of 3251 regenerants had been produced, the majority (85%) coming from R149 (Table 3). The calluses derived from this parent and the other three were vigorous over a long period. Callus derived from only one of the glasshouse-grown plants, R45, showed any consistent signs of regeneration after 6 months.

TABLE 3. *The production of somaclones from original parental tubers of potato cv. Record which showed regeneration at 15 months from initiation of leaf disc cultures in July 1987*

Original parent tuber R no.	Somaclone production (months from initiation of cultures) 3	6	9	12	15	Source of leaf discs
45	1	1	4	4	4	
68	4	31	57	58	59	
94	2	3	3	3	3	
95		2	2	2	2	
96		3	4	33	34	
110	2	14	46	64	112	Glasshouse-grown plants
132		2	4	4	4	*in vitro* shoot cultures
137		2	2	2	2	
148		1	2	2	2	
149	11	168	781	1637	2777	
150	1	51	165	197	242	
Other 159 tubers					10	Glasshouse-grown plants or *in vitro* shoot cultures
Total	21	278	1070	2006	3251	

'Inherited' differences between original tubers

Since there was a clear differential response between material derived from different original parental tubers, it was taken as strong evidence that these tubers were genetically heterogeneous with regard to 'regeneration ability'. In order to test this hypothesis, the superior parental tubers, as well as several of those that had not responded in terms of *in vitro* regeneration in culture, were put through a second cycle of vegetative multiplication in the glasshouse, using tubers produced from the original glasshouse-grown plants as well as from plants which had been produced *in vitro*. Following the same successful *in vitro* protocols, leading to the regeneration of somaclones, it was possible to demonstrate that 'regeneration ability' was evident in those plants derived from the original parental tubers in contrast to those which were consistently non-regenerant (Table 4).

TABLE 4. *Production of somaclones from second-cycle tubers derived from original parental tubers, at 3 months from initiation of leaf disc cultures in July 1988. All material derived from* in vitro *shoot cultures*

Tuber line	No. somaclones
Responsive lines	
R45	2
R68	2
R110	3
R149	41
R150	2
Non-responsive lines	
15 other individual tubers	0

DISCUSSION

The research reported in this paper has two important aspects. First, we identified differences in regeneration ability in material derived from a large sample of potato tubers of a single cultivar. Secondly, the decision to treat each parental tuber as potentially different from the initiation of this project has facilitated the identification and maintenance of superior individual lines from these tubers. Consequently, we can postulate that variation, putatively of a genetic nature, exists within the cv. Record for the character 'regeneration ability'. Given the vegetative nature of the potato, this hypothesis cannot be tested by making sexual crosses, since this will lead to disruption of the so-called superior genotypes with respect to this character. Nevertheless, the maintenance of the characteristic through two vegetative generations of these tuber lines gives a clear indication of genetic differences between them. It has also been shown that these original parental tubers responded better, in general, in terms of protoplast isolation and plating efficiency (Coleman *et al.*, 1990). It is interesting to note, however, that one of the parental tubers (R16), which was grown at Birmingham only in the glasshouse, and was not identified as a superior 'clone', nevertheless responded to protoplast isolation once plants were grown *in vitro*.

Apparent genetic variation within four North American potato cultivars, Kennebec, Norchip, Red Pontiac and Superior, has been described by May, Staub and Kuhns (1982) on the basis of phenotypic variability for the enzymes alkaline phosphatase and glucosephosphate isomerase. Comparison was also made between cv. Red Pontiac and 16 other red-skinned cultivars. One of the principal conclusions of this work was that cultivars of a given name could no longer be considered clones with identical genotypes. However, whilst considering several explanations to account for the observed variation, such as mutation and subsequent

selection, May *et al.* (1982) suggested that cultivar mislabelling was the most probable source of the variation, particularly in the most variable of the four cultivars, namely cv. Red Pontiac. Our data clearly indicate otherwise in cv. Record.

The individual characteristics of any potato cultivar can only be maintained through asexual reproduction. Variation within potato cultivars has long been recognized, however, at the field level. Under such conditions the presence of variants or sports (usually undesirable) is a well-known phenomenon. Salaman (1926) described variants known as the 'Bolter' and the 'Wilding'. Burton (1966) recorded several other minor variants such as flower and tuber colour changes known to occur with a measurable frequency. Such 'sports' may occur frequently and escape detection if they show only minor variation, or may not be expressed in the phenotype under normal growth conditions. Since potato cultivars are presumably initially derived from a single tuber, the assumption is generally made that a cultivar is genetically homogeneous. This does not take account, however, of many of the seed potato production schemes operated in many countries. In order to maintain high levels of phytosanitary quality, potato cultivars are propagated from a range of virus-free 'clones' which are maintained *in vitro*. In the subsequent production stages, from seeds derived from virus tested stem cuttings (VTSC), to the eventual production of seed tubers for ware crop production, these 'clones' become mixed. Since seed potato production in the United Kingdom follows this scheme, we can be confident, therefore, that the parental tubers from which the somaclones were produced came from several or many of these cv. Record 'clones'.

Historically, there have been reports of spontaneous mutations occurring *in vivo*. Many examples of these have been reported by van Harten (1978). However, the total observed frequency of spontaneous mutations appears to be very low. East (1917) described only 12 clear cases out of 100000 plants examined. Only five leaf mutants out of 350000 plants were recorded by Folsom (1923). More recently, Heiken (1960) found spontaneous mutants at the rate of between $1{\cdot}5 \times 10^{-3}$ and $1{\cdot}2 \times 10^{-5}$.

There have also been reports that the morphotypes of particular potato cultivars have indeed been shifted, when these have been subjected to different selection criteria during seed production. These field reports, as well as the results in this paper, hold important implications for seed production, and perhaps also for the genetic conservation of vegetatively propagated crops such as potato using *in vitro* techniques. The field multiplication of a particular cultivar in seed production programmes is significant in as much as the production and marketing of commercial varieties is subject to criteria which ensure quality. Trueness-to-type is one important criterion. Nevertheless, it is clear that unless care is taken during the *in vitro* stage of seed multiplication there is a strong possibility that a shift in the pattern of variation could occur in some cultivars.

In terms of genetic conservation, the results presented in this paper have serious implications. Genetic conservation of vegetatively propagated crops is achieved by field gene banks and *in vitro* culture. The former is akin to seed multiplication, but on a much smaller scale. *In vitro* techniques are advantageous for many reasons, since field maintenance of germplasm has many risks attached to it. However, one of the criteria for efficient and acceptable *in vitro* storage of germplasm is the maintenance of the original genotype over a long period. The choice of material from which to establish an *in vitro* germplasm collection may therefore have long-term consequences since we may not be dealing with a random sample of plants within a genotype at the culture stage. What is actually conserved may be a genetically biased sample favouring an inherent ability to regenerate and multiply under *in vitro* conditions.

The results further lead us to question the nature of potato cultivars. These are propagated vegetatively, yet there is strong evidence that cv. Record, at least, is genetically heterogeneous. As a consequence it might be wise in the future to determine the degree to which variation exists for characters of economic importance, such as yield, within potato cultivars.

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