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The use of hormonal and osmotic growth retardants in media used for the storage of potato germplasm in-vitro

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Summary

Cultures of 23 potato cultivars were examined for their responses to hormonal and osmotic growth inhibitors incorporated in media used for storage in-vitro. Cultivars differed in their tolerance of inhibitors and also in their growth on a Murashige and Skoog medium with 3 % sucrose. Both osmotic and hormonal growth retardants restricted the development of shoot tips but there were significant cultivar × treatment interactions, indicating that the ranking of cultivars will vary with the media used. Such interactions may complicate the establishment of facilities for storage in-vitro. However, addition to the basal medium of 5 mg l^{-1} abscisic acid reduced both the overall mean scores and, more importantly, the between cultivar variance; its use in conjunction with low temperature shows promise as a method for conserving potato germplasm in-vitro.

Introduction

The potato is an important tuber crop worldwide that includes a range of species related to *Solanum tuberosum,* with several ploidy levels (Hawkes, 1978). Much of the germplasm is maintained as clones because of its heterozygous nature and problems of low fertility. Maintenance by tuber propagation is expensive and a cause of losses, for example from diseases, especially those caused by viruses. Other methods of storage based on in-vitro tissue culture methods have been proposed (Henshaw et al., 1980) and these have the advantage that the same procedures may be employed to eliminate disease as to maintain disease-free stocks, for example viruses may be eliminated by heat treatment of shoot tip cultures. Other advantages include high multiplication rates and the ease of international distribution of potato germplasm for breeding programmes (Roca et al., 1979). Clearly, storage in-vitro offers many advantages over conventional systems for the genetic conservation of potato germplasm. Indeed, the application of cellular and molecular techniques to potato improvement should lead to greater use of this method.

Culture storage techniques, based on the principle of retarding growth, have included the use of low temperature, usually of about 10 °C. A potentially more widely applicable approach, that could be used in less developed or remote sites, is the use in

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media of growth retardant chemicals including osmotically active compounds and natural or synthetic hormones. The experiment reported here was designed to examine the response of a range of potato cultivars to storage conditions in-vitro that involved the use of one osmotic and two hormonal growth inhibitors.

Materials and methods

Shoot cultures were established in-vitro from surface-sterilised axillary buds of the cultivars: Baillie, Cara, Croft, Désirée, Estima, Fiona, Foxton, Golden Wonder, Home Guard, Kerrs Pink, Kingston, Maris Bard, Maris Page, Maris Piper, Moira, Morag, Pentland Crown, Pentland Dell, Pentland Javelin, Record, Stormont Enterprise, Teena and Wilja. They were maintained on a basal medium of Murashige and Skoog (1962) with 2 % sucrose in jars fitted with 9 cm diameter Petri dish lids and covered with nescofilm (Bando Chemical Ind. Ltd., Japan), slitted to allow ventilation (Foulger & Jones, 1986).

Nodal segments, i.e. stem cuttings containing one axillary bud and its subtending leaf, were excised aseptically from the shoot cultures and used for the storage experiments. Four central, nodal segments were obtained from individual potato cultures and distributed at random between the treatment regimes. The explants were cultured on 'M' shaped filter paper bridges in Kimble tubes (150×15 mm) and covered with polypropylene caps. The following liquid media were used:

A. Murashige and Skoog (MS) medium and 3 % sucrose (Basal medium);
B. MS medium with 3 % sucrose and 6 % mannitol (Sigma Chemical Co. Ltd., Dorset, UK);
C. MS medium with 3 % sucrose and 5 mg l^{-1} of abscisic acid (Sigma Chemical Co. Ltd., Dorset, UK);
D. MS medium with 3 % sucrose and 50 mg l^{-1} of B-nine (N-dimethyl-succinamic acid), (Aldrich Chem. Co. Ltd., Dorset, UK).

Each cultivar in each treatment was represented by cultures in five Kimble tubes stored at 10 °C and a 16 h. photoperiod using florescent lights (30 μE m^{-2} s^{-1}).

After nine months cultures were scored for:

1. Vigour (Vigour), based on a visual assessment recorded on a 1 to 9 scale, with 9 for the most vigorous growth.
2. Chlorophyll intensity (Green), pigmentation scored on a 1–9 scale with 9 indicating the normal green colour for a shoot culture in-vitro.
3. Length of shoot in mm (Shoot length).
4. Number of shoots (Shoot number).
5. Number of nodes (Node number).
6. Number of nodes usable for further propagation (Usable nodes).
7. Number of mini-tubers (Tuber number).
8. Length of root in mm (Root length).
9. Fresh weight of total plant material in mg (Fresh weight).
10. Mean inter-nodal distance in mm (calculated from 3 and 5 above) (Internode length).

A preliminary analysis of the data showed that the distribution for the character shoot length was heavily skewed. A square root transformation restored normality, the means are shown and analyses were carried out for shoot length on data transformed in this way (Sq.SL).

Results

The mean performance of the 23 cultivars on the four media show that the growth retardants reduced growth (Table 1). The highest mean scores, including those for Vigour and Green, were consistently obtained on the basal medium (medium A) which did not contain growth retardants. These visual assessments also showed that this medium was better in this respect than those containing mannitol, abscisic acid and B-nine. Moreover medium A, in conjunction with low temperature, maintained the viability of plantlets for nine months. However these overall assessments do not include any measure of clonal variation.

Observations on the 10 characters of the 23 clones in the basal medium (medium A), showed considerable variation between clones for Shoot length, the maximum and minimum responses being recorded for Cara and Baillie respectively. On this medium some cultivars, Home Guard, Maris Page, Pentland Javelin, Record, Stormont Enterprise and Teena, formed between two to three micro-tubers per vessel. Dodds et al. (1986) suggested that micro-tuber production may help to promote the international distribution of germplasm and assist in the storage of elite genotypes. Our data would suggest that there is genetic variation for micro-tuber production and that some cultivars readily form them in-vitro in the absence of cytokinin.

The analyses of variance testing for differences between the means of the cultivars grown from nodal segments (Table 2) shows that, with the exception of Internode length, there were significant differences between the means of all the characters. The one degree of freedom comparison between the mean of the cultivars grown on medium A and the mean of the cultivars grown on media containing growth retardants was also significant for all the characters measured. There were significant treatment × cultivar interactions; Vigour, Green, Node number, Usable nodes, Tuber number, Root length, Fresh weight and Sq.SL. These show that cultivars responded differentially to the four media and this is illustrated in Fig. 1 for Fresh weight. The maximum Fresh

Table 1. Overall mean scores for the cultivars evaluated in the four different media (see text).

Characters [a]	Media			
	A	B	C	D
Vigour	3.03	2.12	1.28	1.97
Green	2.33	1.86	1.16	1.64
Shoots number	1.14	1.03	0.68	0.98
Node number	3.60	2.18	0.97	2.29
Usable nodes	1.44	0.84	0.26	0.72
Tuber number	1.07	0.89	0.36	0.93
Root length	1.90	1.44	0.78	1.37
Fresh Weight	303.59	164.14	43.71	162.14
Internode L^{th}	3.55	2.98	2.66	2.92
Sq.SL [b]	3.31	2.44	1.48	2.48

[a] For full details see 'Materials and methods'.
[b] Square root transformation of Shoot length (mm).

Table 2. Overall analyses of variance for the 23 cultivars.

Item	df	M.S. Vigour	M.S. Green	M.S. Shoot number	M.S. Nodes number	M.S. Usable nodes	M.S. Tuber numbers	M.S. Root length	M.S. Fresh weight	M.S. Internode length	M.S. Sq.SL
Media (M)	3	61.26***	28.11***	4.92***	137.94***	28.24***	11.56***	24.76***	133.31***	16.09**	63.78***
(i) A v B+C+D	1	130.50***	50.50***	4.16***	273.78***	59.92***	8.54**	40.31***	281.54***	41.57*	119.01***
(ii) Rest	2	26.64***	16.92**	5.30***	70.03***	12.39**	13.07***	16.98***	59.20***	3.35 n.s.	36.16**
Cultivars (C)	22	13.56***	8.77***	0.36 n.s.	19.72***	9.82**	4.94***	2.83*	18.47***	9.61 n.s.	9.13 n.s.
Replicates	4	4.06 n.s.	3.78 n.s.	0.83 n.s.	20.49*	0.63	2.10 n.s.	6.26**	1.58 n.s.	37.10**	1.14 n.s.
M×C	66	3.03***	3.04***	0.33 n.s.	8.02***	4.94***	1.15***	1.58***	6.50***	8.64 n.s.	4.08***
(i) ×C	22	5.71***	5.60***	0.27 n.s.	18.98***	12.96***	2.14***	2.34***	9.86***	8.17 n.s.	8.77***
(ii) ×C	44	1.69*	1.77**	0.36 n.s.	2.53 n.s.	0.93 n.s.	0.66 n.s.	1.20*	4.81***	8.87 n.s.	1.73 n.s.
Rep. Ints.	364	1.138	1.028	0.301	3.192	1.239	0.810	0.825	1.535	8.235	1.467

The characters scored are as given in Table 1.
(*** $P<0.001$; ** $P<0.01$; * $P<0.05$; n.s. $P>0.05$ i.e. not significant).

Table 3. The analyses of variance for the 23 cultivars for the four different media (see text).

Medium		df	M.S. Vigour	M.S. Green	M.S. Shoot number	M.S. Node number	M.S. Usable nodes	M.S. Tuber numbers	M.S. Root length	M.S. Fresh weight	M.S. Internode length	M.S. Sq.SL
A	Cults	22	14.05***	11.39***	0.42 n.s.	33.67***	20.99***	4.79***	3.76***	19.82***	9.30***	15.55***
	Reps	88	1.71	1.36	0.44	7.65	3.08	1.33	1.35	3.12	2.79	2.28
B	Cults	22	4.59***	3.98**	0.53 n.s.	4.12**	1.44**	1.02*	2.20***	11.57***	2.07 n.s.	1.95*
	Reps	88	1.22	1.48	0.39	1.68	0.70	0.65	0.77	1.12	2.14	0.97
C	Cults	22	0.36**	0.30 n.s.	0.27 n.s.	1.02 n.s.	0.27 n.s.	0.69**	0.42 n.s.	0.24**	21.78 n.s.	1.69 n.s.
	Reps	88	0.22	0.24	0.27	0.93	0.23	0.28	0.30	0.05	25.00	1.39
D	Cults	22	3.65***	2.24**	0.14 n.s.	4.96*	1.94*	1.90*	1.20*	6.34***	2.36 n.s.	2.19*
	Reps	88	1.37	1.03	0.12	2.43	1.01	0.98	0.84	1.87	2.90	1.27

The characters scored are as given in Table 1.
(*** $P<0.001$; ** $P<0.01$; * $P<0.05$; n.s. $P>0.05$ i.e. not significant).

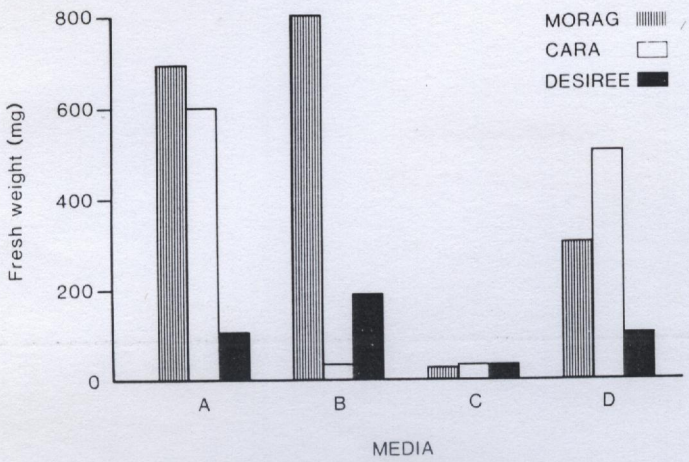

Fig. 1. The response of the cultivars Cara, Désirée and Morag on the four different media (see text) for the character fresh weight (mgs).

weight was that of Cara in medium A, but medium B produced the highest fresh weight of cultivar Morag and Désirée. The lowest fresh weight for all three cultivars was produced in medium C which contained 5 mg l^{-1} of abscisic acid.

To evaluate performance in-vitro more completely, second degree statistics were examined, i.e. the between cultivar variation observed in each of the four media. The between cultivar mean squares (MS) together with the cultivar × replicate interaction terms (Table 3) show the addition of growth retardants to the basal medium significantly reduced the magnitude of the between cultivar MS when compared to the response to medium A except for Shoot number. This aspect of response in-vitro is presented as histograms (Fig. 2) which demonstrate: first, the reduction in the mean of the cultivar samples when growth retardants were included, and, second, the effect of the retardants on the between cultivar variance. Thus the response of the 23 cultivars on medium B, containing 6 % mannitol, identifies the extreme response for one of the cultivars, Morag, in the presence of an osmotic inhibitor. These variations emphasise the importance of examining the range as well as the mean responses of shoot tips in culture.

The relationship of pairs of characters is of interest because differences in them between treatments indicates the scope for controlling some aspects of development by manipulation of the media. For this purpose, phenotypic correlations (i.e. the correlations between the observed means) have been calculated and are tabulated for the four media in Table 4. These correlations, containing both environmental and genetic components, are mostly positive and significant indicating pleiotropic and/or linkage between the genes controlling the characters of interest (Caligari et al., 1986). The relative magnitude of the coefficients in the four media can also be compared in Table 4. Those between characters from micro-propagated plants in medium containing abscisic acid appeared much reduced when compared to the coefficients in the other three media. This may be exemplified by reference to the correlations obtained between the number

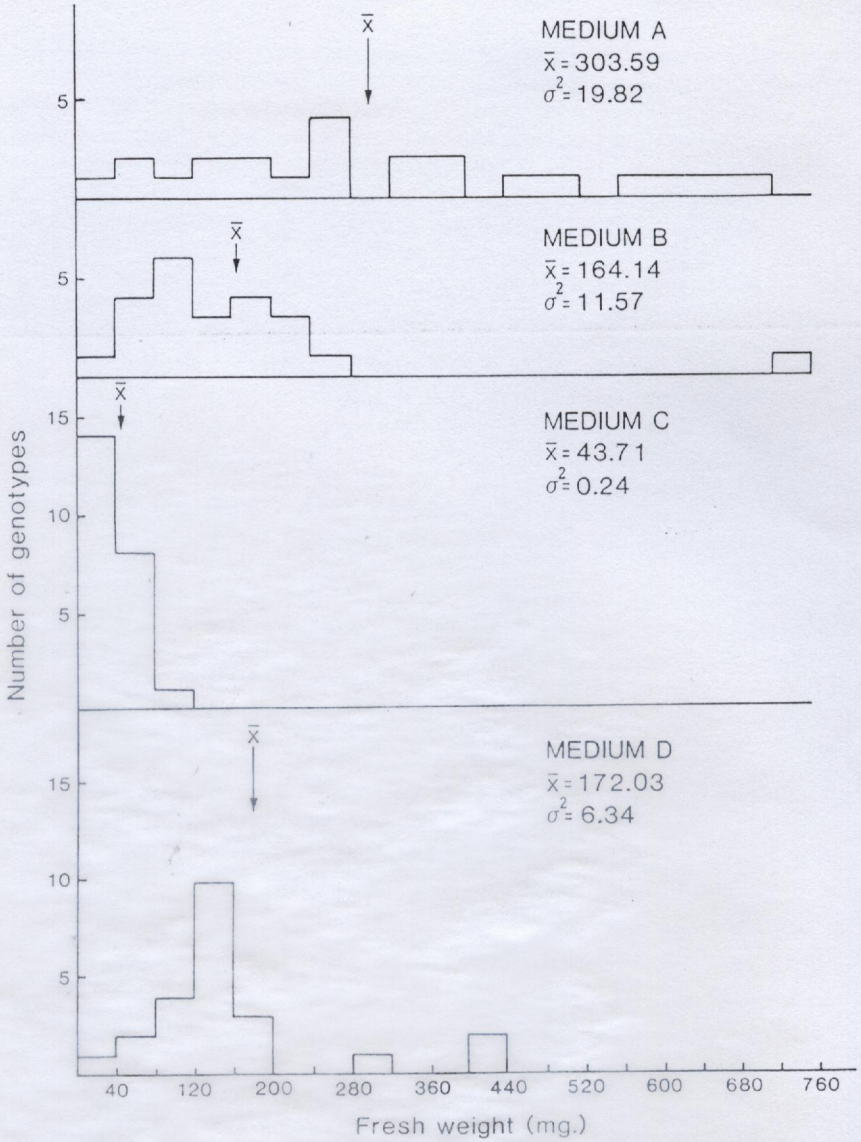

Fig. 2. The numbers of cultivars falling into the different fresh weight categories in the four media (see text).

of usable nodes and the plant height. Significant positive correlations were obtained between this pair of characters in media A, B and D, but not medium C. It thus appears that different growth retardants may also affect the relationships between the developmental characters in culture.

Discussion and conclusions

Our results, from observations on a range of characters, show that potato cultivars differ significantly in their ability to withstand conditions that retard growth, and that they also vary in their performance on a basal medium. Factorial experiments showed that both osmotic and hormonal growth retardants can be successfully used to restrict the development of potato shoot tips cultured in-vitro. Experiments by Powell & Dunwell (1987) have shown that a barley genotype's mean performance and sensitivity should both be taken into account when describing genotypic performance in-vitro. In our experiment adding 5 mg l^{-1} of abscisic acid to the basal medium resulted in a marked reduction in the between cultivar variance for most of the characters measured, a finding similar to that of Westcott (1981b) who noted that the addition of abscisic acid enhances the survival of nodal cultures. Perhaps more importantly he also noted that there were no evident differences in survival between cultivars.

Significant variation between cultivars was detected for the number of tubers formed and for fresh weight in medium C (containing 5 mg l^{-1} of abscisic acid). Westcott (1981a) speculated that the different survival rates of species may be related to their ability to form tubers in-vitro and it is relevant that the phenotypic correlation between tubers and vigour scores was significant ($r=0.55$) in medium C. There were also significant correlations between fresh weight and tuber number in media C and D (Table 4).

An effective technique for storing potato germplasm in-vitro will need to combine a high degree of growth redardation whilst maintaining maximum viability for a wide range of potato genotypes. The significant cultivar × treatment interaction detected in this experiment for several characters showed that the ranking of cultivars for performance varied with the medium used. However, the hormonal growth retardant abscisic acid consistently reduced both the mean and variance indicating its potential merit, in conjunction with low temperature (10 °C), for routine use. Any strategy for storage in-vitro must also take account of the effects of a given technique on the genetic integrity of the cultures since a selection pressure is being imposed and directional genetic change may occur. Future research must therefore include evaluation of progenies and should be carried out at the molecular as well as the whole plant level.

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The effect of netted scab (*Streptomyces* spp.) and *Verticillium dahliae* on growth and yield of potato

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Summary

The effect of *Streptomyces* spp. (netted scab) on the growth of potato was investigated in three pot experiments, in two of which the effect of *Verticillium dahliae* was also assessed.

The netted scab organisms attacked all underground plant parts of susceptible potato cultivars early in the growing season; the roots were especially seriously attacked, markedly reducing tuber yield and number but prolonging the duration of the growing season. The nematicide oxamyl had little effect on the incidence of netted scab. Repeated growing of the susceptible cv. Bintje greatly increased soil contamination with the netted scab pathogens.

V. dahliae reduced haulm growth before wilt symptoms were evident and it reduced tuber yield but not number. Oxamyl delayed infection by *V. dahliae* by controlling parasitic *Pratylenchus* nematodes (mainly *P. thornei*).

Introduction

Netted scab caused by *Streptomyces* spp., occurs mainly in areas where susceptible cultivars of potato (*Solanum tuberosum*) are grown on a large scale. It differs from common scab in many ways (Scholte & Labruyère, 1985); the most important is that the pathogens attack the roots as well as the tubers, resulting in lower yields.

Because cv. Bintje is one of the few susceptible cultivars (Scholte & Labruyère, 1985), the disease is found most commonly in the Netherlands (Scholte et al., 1985), Sweden (Bång, 1979), Denmark (Mygind, 1965) and Switzerland (Salzman, 1960). It occurs too in those parts of Austria where the susceptible cultivar Allerfrüheste Gelbe is grown (Wenzl, 1970). There are no reports from countries outside Europe where another susceptible cultivar, Désirée, is grown on a large scale.

Frequent cropping of susceptible cultivars of potato leads not only to an increasing contamination of the soil with the netted scab pathogens, but also to higher inoculum densities of other pathogens such as *Verticillium dahliae*. Therefore, attention was also paid to this fungus in two of the three pot experiments reported here that were designed to study the effects of netted scab on growth and tuber yield.