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NOTE

EFFECTS OF ABSCISIC ACID, MANNITOL AND TEMPERATURE ON *IN VITRO* STORAGE OF POTATO GERMPLASM

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ABSTRACT

In vitro storage of eight potato genotypes (four cultivars and four wild species) using either abscisic acid (ABA) (5 mg l^{-1}) or mannitol (3%) as growth retardant added to Murashige and Skoog (MS) medium was examined at two storage temperatures (8 °C and 25 °C). After 10 months, the cultures were evaluated. Compared with control, medium containing ABA or mannitol significantly retarded growth. Genotypes were significantly different for most characteristics measured with cultivars 'Cosima' and 'Draga' superiority to others. The survival rate was significantly affected by culture medium and was the highest with mannitol treatment. Significant two-factor interactions for some characters and also different responses of cultivars and wild species for regeneration following transfer to normal condition may complicate the establishment of facilities for *in vitro* storage. However, MS medium supplemented with 3% mannitol showed to be superior for storing potato germplasm *in vitro* at room temperature.

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Key words: ABA, *In vitro* storage, Mannitol, Potato genotypes.

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اثر ابسیسیک اسید، مانیتول و دما بر نگهداری ژرم پلاسم سیب زمینی

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چکیده

در این پژوهش اثر محیط کشت های دارای ابسیسیک اسید (۵ میلی گرم در لیتر) و مانیتول (۳ درصد) و دما (۸ و ۲۵ درجه سانتیگراد) بر رشد گیاهچه های هشت نژادگان سیب زمینی (چهار رقم و چهار گونه وحشی) در کشت درون شیشه ای بررسی شد. پس از ده ماه، کشت ها ارزیابی شدند. در مقایسه با شاهد، محیط های کشت دارای مانیتول و ابسیسیک اسید به طور معنی داری باعث کاهش رشد گیاهچه ها شدند. نژادگان ها برای بیشتر صفات اندازه گیری شده، با یکدیگر تفاوت معنی داری نشان دادند و رقم های 'دراگا' و 'کوزیما' نسبت به سایر ارقام برتر بودند. میزان زنده ماندن گیاهچه ها پس از انتقال به محیط کشت ریز افزایی، به طور معنی داری در محیط کشت دارای مانیتول بالاتر از دیگر محیط های کشت بود. با توجه به واکنش متفاوت ارقام و گونه های وحشی به محیط های کشت گوناگون، پیشنهاد استفاده از یک محیط کشت و یک دما برای نگهداری همه نژادگان ها عملی نیست ولی با به

کارگیری محیط کشت MS دارای ۳ ٪ مانیتول و دمای اتاق، می توان ژرم پلاسم سیب زمینی را برای دراز مدت نگهداری کرد.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important tuber crop worldwide that has a range of related species with several ploidy levels (1). Much of the germplasm is maintained as clones because of its heterozygous nature and low fertility. Maintenance by tuber propagation is expensive and perpetuates viruses and certain other diseases agents. Other methods of storage based on *in vitro* tissue culture have the advantages of eliminating the disease agents and maintaining the disease free stocks (2). Other advantages includes high multiplication rates and the ease of international distribution of potato germplasm for breeding programs (6).

Culture storage techniques based on the principle of retarding growth have included the use of low temperatures (4, 8). A potentially more widely applicable approach with growth retarding chemicals including osmotically active compounds and natural synthetic growth regulators.

The purpose of this study was to examine the response of some potato cultivars and wild species to *in vitro* storage conditions using an osmoticum (mannitol) and a growth retardant (ABA) in culture media and two temperature regimes.

MATERIALS AND METHODS

Initiation of *In Vitro* Cultures

Cultivars. The following procedure was followed to initiate the cultures. Tubers were washed in a jet of water and rubbed gently to remove soil and then left in a growth room with low light intensity, at 15 °C until the tubers produced young shoots. The shoots between 2-15 mm length were suitable for culturing. The medium used for propagation was that of Murashige and Skoog

(MS) (5) without growth regulators. The cultivars used in experiments were 'Draga', 'Cosima', 'Aula' and 'Morene'.

Wild species. The *in vitro* culture establishment of wild species was achieved by seed culture. Seeds supplied by the Commonwealth Potato Collection, held by the Scottish Crop Research Institute, were used to initiate cultures. Seeds were surface sterilized by submerging in ethanol for 10 s followed by an 8 min treatment with bleach (2% Domestos). This was followed by several rinsings with sterile distilled water. Each seed was introduced into a glass tube (150×20 mm) which contained 8 ml of MS + sucrose (3%) + agar (0.8%). After 5-8 wk, each seed produced an initial shoot, which was not suitable for experiments. After sub-culturing of these delicate shoots, acceptable culture establishment was achieved for further experiments. The wild species used for experiments were: *S. hjertingee* Hawkes (2n=48), *S. stoloniferum* Scheclitd and Bche (2n=48), *S. papita* Rydb (2n=48) and *S. demissum* Lindi (2n=72).

Nodal segments, i.e., stem cutting containing one axillary bud and its subtending leaf, were excised aseptically from the shoot cultures and used for the storage experiments. Central noded segments from individual potato cultures were randomly distributed between the treatments. The explants were cultured in glass jars (4×11 cm) filled with 25 ml of culture medium. The following solid media were used:

1) MS medium and 3% sucrose (basal medium).
2) MS medium with 3% sucrose and 3% mannitol.
3) MS medium with 3% sucrose and 5 mg l^{-1} ABA.

All treatments were stored at 8 °C or 25 °C and a 16 hr photoperiod using fluorescent lights (30 E m^{-1} s^{-1}). Each cultivar in each treatment was represented by cultures in 6 glass jars containing 2 explants each.

After 10 months, cultures were scored for:

1. Vigor based on a survival assessment recorded on a 1 to 9 scale, with 9 for the most vigorous growth.
2. Chlorophyll intensity (Green) pigmentation scored on a 1 to 9 scale with 9 indicating the normal green color for a shoot culture *in vitro*.

3. Length of shoot in mm.
4. Root score based on a 1 to 9 scale, with 9 for the most vigorous growth.
5. Length of root in mm.
6. Number of mini tubers.

After 10 mo, plantlets belonging to all treatments were micropropagated on a medium composed of MS medium + calcium panthotenate (2 mg l^{-1}) + GA_3 (0.1 mg l^{-1}) + NAA (0.1 mg l^{-1}) + sucrose (3%) + agar (0.8%). The number of surviving explants were counted 4 wk later and converted to percentage.

A preliminary analysis of data showed that the distribution for the characters was not normal. An arcsine transformation for survival percentage and a square root transformation for other characters restored normality. Therefore, analyses were carried out on transformed data.

RESULTS

The mean performance of cultivars and wild species on the three media showed that the growth retardants significantly reduced growth (Table 1). The highest mean scores, including those for vigor, shoot length, root score and root length were consistently obtained on the basal medium which did not contain growth retardant (control). Survival rate was highest with the medium containing mannitol followed by basal medium and with the ABA containing medium. The effect of genotype was significant for most characters. The performance of cultivars, 'Draga' and 'Cosima' was generally superior for characters such as vigor, green, root score and root length. Generally, the performance of wild species was inferior for most characters when compared with cultivars (Table 2). Interaction of temperature and growth retardant was significant for all characters except for shoot length (Table 3).

Table 1. Effect of different media on mean scores for the genotypes.

	Medium			
Characters	ABA	Mannitol	Control	LSD 1%
Vigor	1.21	1.33	1.85	0.20
Green	1.50	1.60	1.83	0.30
Shoot length (mm)	1.17	1.17	1.76	0.50
Root Score	0.71	0.90	0.91	0.35
Root length (mm)	1.21	1.33	1.84	0.3
Tuber number	1.50	1.60	0.93	NS
Survival (%)	1.17	1.17	36.5	9.00

† NS= not significant.

Basal medium, in conjunction with lower temperature (8 °C) had the highest mean scores for most characters. Survival rate was high in mannitol containing medium and low in ABA containing medium at both temperature regimes and was also high in basal medium at 8 °C.

Table 4 shows the interaction between temperature and growth retardant for cultivars and wild species. Cultivars could not survive at 25 °C on basal and ABA containing media whereas wild species could not survive at 8 °C on ABA containing medium and had poor performance at 25 °C on basal medium and to a lesser extent on ABA containing medium. The performance of genotypes to other treatments was generally comparable.

DISCUSSION

The results showed that cultivars and wild species differed significantly in their ability to withstand conditions that retarded growth. Both ABA and mannitol had restricted the development of potato shoot tips *in vitro*. Addition of ABA to the basal medium resulted in a marked reduction for most characters, a finding similar to that of Westcott (8). An effective technique for storing potato

Table 2. Response of genotyes to characters measured.

Character	Genotypes								
	'Aula'	'Cosima'	'Morene'	'Draga'	*S. hjertingee*	*S. stoloniferum*	*S. Papita*	*S. demissum*	LSD 1%
Vigor	1.4	1.7	1.4	1.8	1.3	1.2	1.4	1.3	0.34
Green	1.6	1.7	1.8	1.9	1.4	1.6	1.3	1.0	NS
Shoot length (mm)	1.3	1.5	1.4	1.3	1.1	1.3	1.2	1.0	0.25
Root.score	1.3	1.3	1.0	1.3	0.9	0.9	1.2	1.0	0.35
Root length (mm)	1.2	1.4	1.0	1.3	0.9	1.0	1.1	1.0	0.29
Tuber number	0.9	0.8	0.7	0.7	0.7	0.7	1.2	0.7	0.35
Survival (%)	7.0	22.0	13.0	7.0	11.0	9.0	17.0	21.0	12.0

Table 3. Effect of different media and temperature regimes on mean score for the genotypes.

	Medium						
	ABA		Mannitol		Control		
Characters Temp.	8	25	8	25	8	25	LSD 1%
Vigor	1.03	1.33	1.26	1.42	2.03	1.52	0.22
Green	1.78	1.08	1.71	1.48	2.08	1.37	0.58
Shoot length (mm)	1.98	1.13	1.14	1.21	1.78	1.73	NS†
Root score	0.72	0.71	0.86	0.94	2.00	1.74	0.50
Root length (mm)	0.71	0.73	0.86	0.94	2.00	1.73	0.46
Tuber number	0.80	0.71	0.80	0.82	1.02	0.77	0.32
Survival (%)	29.00	16.70	48.00	56.20	64.60	8.30	12.00

† NS= not significant.

Table 4. Effect of temperature and media on survival rate of genotypes.

	Temperature					
	8			25		
Genotype/ Medium	Control	Mannitol	ABA	Control	Mannitol	ABA
Cultivar	58.3	41.7	58.3	0	46.0	0
Wild species	71.0	54.2	0	16.7	66.7	33.3

LSD 1% = 15.

germplasm *in vitro* will need to combine a high degree of growth retardation whilst maintaining maximum viability for a wide range of potato genotypes. The significant interaction between genotype and growth retardant detected in this experiment for characters may to some extent complicate the establishment of facilities for *in vitro* storage. However, addition of ABA or mannitol to the media consistently reduced overall growth indicating their potential use for

long-term storage. The protocols recommended for routine use, is the application of growth retardant in conjunction with low temperature in the range of 2 to 10 °C (3), which is not easily feasible in many developing countries. Therefore, our results indicated that it was possible to use higher storage temperature (25 °C) which is routinely used in culture rooms with comparable results for the wide range of genotypes tested. However, any strategy for *in vitro* storage 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 changes may occur. Therefore, furture research should include evaluation of progenies and be carried out at the molecular as well as whole plant level.

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