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IN VITRO AND *IN VIVO* MULTIPLICATION OF VIRUS-FREE 'SPUNTA' POTATO

RIDA A. SHIBLI, ABDULLAH M. ABU-EIN AND MOHAMMED M. AJLOUNI

Plant Tissue Culture & Physiology Laboratory,
Faculty of Agriculture,
Jordan University of Science and Technology,
P.O. Box 3030, Irbid-Jordan.

Abstract

In vitro shoots of *Solanum tuberosum* cv 'Spunta' were subcultured on liquid MS media containing 0.0, 0.5, 1.0, 1.5 and 2.0 mg/l of benzyl adenine (BA) or kinetin. Significant reduction in stem and internode length was observed by increasing BA and kinetin concentrations. BA up to 1.0 and 1.5 mg/l showed an increase in number of proliferated shoots and number of nodes per flask. Single node cuttings were rooted on solid media containing NAA, IBA, or IAA @ 0.0, 0.5, 1.0, 1.5, 2.0 mg/l, individually with or without 1.0 mg/l GA_3. Sucrose concentration from 30 to 90 g/l gave significant enhancement of microtuberization. *In vitro* rooted shoots were successfully acclimatized. Cuttings, 3.0 cm from glasshouse grown plants were successfully rooted by treating 0.5 cm base with 1 g/l IBA + 0.5 g/l IAA for five seconds.

Introduction

Conventional vegetative propagation of potato is usually subjected to cumulative bacterial, fungal and virus infections, a process called "degeneration". Degeneration is a common problem in potato seed production, decreasing yield, quality and international distribution of potato germplasm. Tissue culture has been used to decrease seed tuber degeneration through virus elimination and germplasm conservation (Amirouche *et al.*, 1985; Dodds, 1988; Kwaitkowski *et al.*, 1988; Lizarraga *et al.*, 1980; Montiel & Saldana, 1987; Silva, 1985; Slimmon *et al*, 1989; Tovar *et al.*, 1985). The present report describes the micropropagation of virus-free 'Spunta' the most planted potato cultivar in Jordan. It also explored the *in vivo* acclimatization of tissue culture produced plants and rooting of stem cuttings.

Materials and Methods

In vitro cultures of potato cultivar 'Spunta' were received from the International Potato Center (CIP), Lima, Peru. The plants were multiplied by culturing of single node cuttings on solid Murashige & Skoog (MS) (1962) medium supplemented with either 1.0 mg/l BA and 0.1 mg/l NAA (Naphthalene acetic acid) in 25 X 150 mm test tubes. Cultures were kept under the growth room conditions at 22±2 °C and 16 hrs light (photosynthetic photon flux PPF = 40-50 μmol m^{-2} s^{-1}).

***In Vitro* shoot multiplication and rooting:** Shoots, 3.0 cm long, were subcultured on 40 ml liquid MS medium in 250 ml flasks containing different BA or kinetin concentrations (Table 1). Treatments were randomized and replicated 10 times. Data was recorded after four weeks on shoot length, number of proliferated shoots, number of nodes, internode length and rooting (+/-). Experiment was repeated twice.

Table 1. Effect of BA or kinetin on shoot proliferation and growth of '*Spunta*' potato grown *in vitro* on liquid MS media.

Response	Concentration of growth regulator (mg/l)					
	0.0	0.5	1.0	1.5	2.0	LSD (0.05)
				BA		
Shoot length (cm)	8.6	5.6	5.4	5.3	4.6	1.59
Number of shoots	7.5	9.1	10.3	12.1	8.0	3.73
Number of nodes	40.9	48.9	55.6	82.7	46.2	21.18
Internode length (cm)	1.3	1.1	1.1	0.8	0.8	0.19
Rooting (+/-)	+	-	-	-	-	-
				(Kinetin)		
Shoot length (cm)	8.6	7.6	6.4	5.7	5.6	2.11
Number of shoots	10.3	11.0	11.6	7.8	9.0	4.50
Number of nodes	40.9	59.6	64.1	45.1	48.3	27.31
Internode length (cm)	1.3	1.4	1.3	1.4	1.3	0.19
Rooting (+/-)	+	-	-	-	-	-

In another experiment, different rooting hormones NAA, indole butyric acid [IBA] and indole acetic acid [IAA] in combination with GA_3 at 0.0 or 1.0 mg/l were used to study their effects on rooting (Table 2). Solid media 10 ml was used in 25 X 150 mm test tubes. Data was recorded on shoot growth and root number. Experiment was repeated twice with 10 replicates for each treatment.

2. Microtuberization: Shoots, 3.0 cm long, were subcultured on 40 ml liquid MS media in 250 ml flasks supplemented with different BA or kinetin concentrations and kept in dark conditions (Table 3). Data was recorded after eight weeks on number of microtubers formed and number of proliferated shoots.

In another experiment, shoots were subcultured on 40 ml liquid MS media containing different sucrose concentrations with BA or kinetin at 1.0 mg/l (Table 4). Data was recorded for microtubers number and number of proliferated shoots. Experiment was repeated twice and treatments were replicated ten times in a completely randomized design (CRD).

Microtubers were dipped for 30 minutes in 50 ppm GA_3, removed and cleaned from surface water and stored in dark at room temperature (24±2°C) for dormancy breaking and compared with non treated microtubers.

3. Acclimatization and rooting of stem cuttings: *In vitro* rooted plantlets were removed from test tubes, thoroughly cleaned from agar under running tap water and planted in 1 peat : 1 perlite mixture in an 84 cell polystyrene trays. The plantlets were acclimatized under a special stage which has 16 hrs supplementary light of 450 µmol $m^{-2}s^{-1}$ and a frequent water misting for two weeks at 24 ± 2°C. Relative humidity decreased frequently from 95 at the beginning of acclimatization to 75% by the second week of acclimatization.

Stem cuttings, 3.0 cm long were taken from the acclimatized plants after two weeks and basal 0.5 cm treated for 5 seconds with a rooting hormone (1.0 g/l IBA + 0.5 g/l IAA) and planted under the conditions of the same stage for multiplication of plants *in vivo*. Other acclimatized plants were moved to the glasshouse (25 ± 2 [day]/18 ± 2 °C [night]) for further growth. Plants were grown in a bed in 1 peat : 1 perlite mixture and covered with muslin cloth to protect them from insect attack.

Table 2. Effect of different growth regulators on *in vitro* growth and rooting of *'Spunta'* potato grown on solid MS media.

Growth regulator (mg/l)				Response			
				No. of shoots	Shoot length	No. of nodes	No. of root
NAA	IBA	IAA	GA_3				
0.0	0.0	0.0	0.0	1.3	5.1	5.6	4.5
0.5	0.0	0.0	0.0	1.2	6.1	5.5	4.0
1.0	0.0	0.0	0.0	1.3	6.9	6.7	4.7
1.5	0.0	0.0	0.0	1.4	7.2	7.4	4.2
2.0	0.0	0.0	0.0	1.6	9.6	8.7	6.2
0.0	0.5	0.0	0.0	1.0	4.5	5.2	7.2
0.0	1.0	0.0	0.0	1.6	5.2	5.0	16.2
0.0	1.5	0.0	0.0	1.3	5.2	5.6	14.0
0.0	2.0	0.0	0.0	1.2	4.5	6.0	13.6
0.0	0.0	0.5	0.0	2.0	5.6	6.5	8.6
0.0	0.0	1.0	0.0	1.1	7.8	7.8	11.0
0.0	0.0	1.5	0.0	1.3	4.2	6.2	6.4
0.0	0.0	2.0	0.0	1.2	8.7	5.9	11.6
0.5	0.0	0.0	1.0	1.3	8.7	7.2	4.7
1.0	0.0	0.0	1.0	1.2	8.3	7.4	4.6
1.5	0.0	0.0	1.0	1.5	9.2	8.5	5.7
2.0	0.0	0.0	1.0	1.4	9.4	8.6	6.0
0.0	0.5	0.0	1.0	1.3	9.6	8.8	8.1
0.0	1.0	0.0	1.0	1.7	10.2	10.2	9.2
0.0	1.5	0.0	1.0	1.2	7.9	7.8	7.5
0.0	2.0	0.0	1.0	1.1	7.5	7.5	10.2
0.0	0.0	0.5	1.0	1.1	4.1	5.3	1.8
0.0	0.0	1.0	1.0	1.1	5.5	6.5	2.4
0.0	0.0	1.5	1.0	1.0	5.2	6.7	1.9
0.0	0.0	2.0	1.0	1.0	1.3	3.5	0.3
LSD	(0.5)			0.392	1.706	1.315	2.562

An *in vivo* rooting experiment was done when stem cuttings were taken from glasshouse grown plants. Data was recorded after two weeks on rooting percentage. Rooted cuttings were cleaned from soil-less mixture by dipping roots in water with light hand shaking. Root number was counted in each transplant.

4. Virus Testing: The *in vivo* plants grown under acclimatization and glasshouse conditions were subjected to direct double antibody sandwich enzyme linked immuno sorbent assay [ELISA] (Hill, 1984) for testing of potato A,S,X,Y and potato leaf roll (PLR) virus.

Results and Discussion

1. In vitro shoot multiplication and rooting: Increasing BA concentration to 0.5 mg/l or more significantly decreased shoot length (Table 1). Shoot proliferation responded positively to increased BA up to 1.5 mg/l and decreased at 2.0 mg/l. Total number of nodes per flask increased significantly with BA @ 1.5 mg/l (82.7 nodes) and decreased at higher concentrations. Dodds (1988) reported that using a shoot consisting 3-4 nodes can rapidly proliferate on liquid media and give 60-70 nodes per flask. Tovar *et al.*, (1985) reported rapid proliferation of potato stems on liquid media and flasks were filled after two to three weeks of growth.

Increasing kinetin concentration decreased shoot length but did not have any significant effect on number of proliferated shoots, number of nodes and internode length (Table 1). Both BA and kinetin inhibited root formation. *In vitro* multiplication rates were reported to vary with potato cultivar (Hussey & Stacey, 1981; Miller *et al.*, 1985), media components (Miller *et al.*, 1985; Mumtaz & Quraishi, 1989) and method of clonal micropropagation (Hussey & Stacey, 1981; Wang & Hu, 1982).

In vitro rooting on solid media showed that most plants gave low number of shoots which ranged from 2.0 to 1.0 per test tube (Table 2). Longest shoot was obtained at 1.0 mg/l IBA (10.2 cm) and lowest (1.3 cm) at 2.0 mg/l IAA + 1.0 mg/l GA_3. Total number of nodes varied from 10.2 (at 2.0 mg/l IBA + 1.0 mg/l GA_3) to 3.5 nodes/test tube (at 2.0 mg/l IAA + 1.0 mg/l GA_3). Root number varied with treatments and ranged from 16.2 (at 1.0 mg/l IBA) and 14.0 (at 1.5 mg/l IBA) to 0.3 (at 2.0 mg/l IAA +1.0 mg/l GA_3). Dodds (1988) reported that a single potato node if subcultured on solid media, the axillary bud will grow and give a plantlet with six or seven nodes in 3-4 weeks and rooting will occur.

2. Microtuberization: Increasing BA or kinetin from 1.0 to 3.0 mg/l did not show any significant effect on microtuber formation and shoot growth under dark conditions (Table 3). BA @ 10 mg/l has been reported to induce the highest number of microtubers (Wang & Hu, 1982). It is expected that the BA concentration in the present study was low that could not induce microtuberization. Wang & Hu (1982) reported that a lower dosage of 2.5 mg/l BA is needed for inducing microtuber in apical potato stolons. Kinetin is reported to have very little effect on *in vitro* tuberization (Forsline & Langille, 1976).

Table 3. Effect of BA or kinetin on *in vitro* microtuberization and shoot proliferation of '*Spunta*' potato grown on MS liquid media under dark conditions.

Response	Growth regulator (mg/l)			
	1.0	**2.0**	**3.0**	**LSD (0.05)**
		BA		
Number of microtubers	1.5	0.8	0.6	1.650
Number of shoots	7.4	6.0	6.6	1.801
		Kinetin		
Number of microtubers	1.3	2.3	1.4	1.264
Number of shoots	9.7	11.5	11.2	4.998

Increasing media sucrose concentration to 60 and 90 g/l showed significant increase in number of produced microtubers and shoot growth (Table 4). An overall microtuber number and shoot growth were better on media containing BA than kinetin. Leaves and shoots showed yellowing because plants were kept under dark. Potato cultivars were reported to have positive microtuberization response to increased sucrose in the media (Garner & Blake, 1989; Lillo, 1989; Tovar *et al.*, 1985; Wang & Hu, 1982). Influence of darkness on *in vitro* tuberization has also been reported (Hussey & Stacey, 1981; Slimmon *et al.*, 1989).

Table 4. Effect of sucrose concentration on *in vitro* microtuberization of 'Spunta' potato grown on liquid MS media under dark conditions.

Response	Sucrose (g/l)			LSD (0.05)
	30	60	90	
BA (1.0 mg/l)				
Number of microtubers	1.5	0.8	0.6	1.650
Number of shoots	7.4	6.0	6.6	1.801
Kinetin (1.0 mg/l)				
Number of microtubers	1.3	2.3	1.4	1.264
Number of shoots	9.7	11.5	11.2	4.998

All microtubers had an oblong shape, 4-8 mm long, representing the whole tuber shape of 'Spunta' cultivar. Dormancy was broken when buds developed and sprouts began after 5-6 weeks of storage under dark in GA_3 treated microtubers. Untreated microtubers took about 10-12 weeks to start bud growth and about 40% of untreated microtubers were lost because of weight loss and deterioration.

Table 5. Effect of soil-less mixture on root number of *in vivo* rooted '*Spunta*'' potato stem cuttings.

Mixture		
Peat	Perlite	Root Number
0	1	15.70
1	0	1.60
1	1	9.90
1	2	6.90
1	3	2.80
1	4	6.30
2	1	2.70
2	3	2.60
3	1	2.10
3	2	2.60
3	4	3.20
4	1	1.60
4	3	3.80
LSD	(0.05)	1.68

3. Acclimatization and rooting of stem cuttings: About 95 % survival was achieved in all transferred plants to acclimatization conditions. Cuttings which were taken from acclimatized plants showed 100% rooting in two weeks. Acclimatization was reported successfully for other potato cultivars when moved from tissue culture to organic mixtures (Dodds, 1988; Levy, 1988). Cuttings which were taken from the glasshouse and planted in different soil-less mixtures showed 100% rooting (Table 5). Root number was highest in perlite alone and was lowest in peat. This can be attributed to good aeration in perlite. Successful rooting of potato stem cuttings was reported previously (Levy, 1988) when *in vitro* stems were moved to 1 peat: 1 perlite mixture. The procedure of using stem cuttings helps potato seed production programs to produce large amounts of pathogen-free seed tubers (Bryan, 1988; Cole & Wright, 1967).

4. Virus testing: *In vivo* grown plants were tested as free of the five indicated viruses (A,S,X,Y, and PLR)

The results of the present study showed that total number of about 40 cuttings can be taken from a plant grown under glasshouse condition as well as an average of about five to six minitubers can be produced from the same plant. This method can increase the production from a single plant to 40 folds.

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