Pakistan J. Agric. Res. Vol. 17 No. 1, 2002

IN VITRO AND IN VIVO MULTIPLICATION OF VIRUS-FREE SPUNTA POTATO CLONE

Rida A. Shibli, Abdullah M.Abu-Ein and Mohammad M.Ajlouni*

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) 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) resulted in increased number of proliferated shoots and number of nodes per flask. Single node cuttings were rooted on solid media containing Naphthalene Acetic Acid (NAA), Indole Butyric Acid (IBA) or Indole Acetic Acid (IAA) (at 0.0,0.5,1.0,1.5 and 2.0 mg/l, individually) with or without 1.0 mg/l, Gibberellic acid (GA_3). Sucrose concentration from 30 to 90 g/l gave significant enhancement of microtuberization. In vitro rooted shoots were successfully acclimatized to in vivo conditions when moved to 1 peat: 1 perlite mixture. Cuttings (3.0 cm) from glasshouse grown plants were successfully rooted by treating them (basal 0.5 cm) with 1g/l IBA+0.5 g/l IAA for five seconds.

Key Words: Solanum tuberosum; Virus-free Plants; Mutiplications; Culture Media; Tissue Culture; Acclimitization; Jordan.

INTRODUCTION

Conventional vegetative propagation of potato is usually subjected to accumulative bacterial, fungal, virus and viroids infections, a process called degeneration. Degeneration is a common problem in potato production and distribution. Tissue culture has been used to decrease seed tuber degeneration through virus elimination and germplasm conservation (Slimmon et al., 1989; Dodds, 1988; Amirouche et al., 1985; Tovar et al., 1985). This study was aimed to examine the micropropagation of virus-free spunta, (most planted cultivar in Jordan) potato and also to explore the in vivo acclimatization of tissue culture produced plants as well as rooting of stem cuttings.

MATERIALS AND METHODS

In vitro cultures of potato cultivar 'Spunta, were received from the International Potato Centre (CIP), Lima, at Peru. The plants were multiplied by culturing of single node cuttings on solid media Murashige and Skoog (1962) supplemented either by 1.0 mg/l benzyl adenine (BA) and 0.1 mg/l Naphthalene Acetic Acid (NAA). Cultures were kept in room conditions at 22 ± 2 °C and 16 hours light (photosynthetic photon flux PPF = 40-50 μmol/m²/S.

In Vitro Shoot Multiplication and Rooting

Shoots (3.0 cm long) were subcultured in 40 ml liquid MS media (in 250 ml flasks) containing different BA or kinetin concentrations (Table 1). Treatments were completely randomized and replicated ten times. Observations were recorded after four weeks on shoot length, number of proliferated shoots, number of nodes, internode length and rooting (+/-). Experiment was repeated twice. In another experiment different rooting hormones NAA, IBA, and IAA in combination with GA_3 at 0.0 or 1.0 mg/1 were used to study their effects on rooting (Table 2). Observations were recorded on shoot growth and root numbers. Experiment was repeated twice with ten replicates for each treatment.

Microtuberization

Shoots (3.0 cm long) were subcultured in 40 ml liquid MS media (in 250 ml flasks) supplemented with different BA or kinetin concentrations and kept in dark conditions (Table 3). Data were 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

Plant Tissue Culture and Physiology Laboratory, Faculty of Agriculture, Jordan University of Science & Technology, Irbid, Jordan.

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

	Growth regulator (mg/l)					
Response	0.0	0.5	1.0	1.5	2.0	LSD (0.0t)
BA						
Shoot length (cm)	86	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 (+/-)	+	-	-	-	-	-

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)				Number of shoots	Shoot length	Number of nodes	Number of roots
NAA	IBA	IAA	GA3				
0.0	0.0	0.0	0.0	1.3	5.1	5.6	4.5
0.5	00	00	00	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	96	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

(Table 4) (media had BA or kinetin at 1.0 mg/l). Data were recorded for microtubers and 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 no treated microtubers.

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

	Growth regulator (mg/l)			
Response	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

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

	Sucrose (g/l)			
Response	30	60	90	LSD(0.0)
BA (1.0 mg/l)				
Number of microtubers	1.5	4.8	7.9	1.982
Number of shoots	7.4	13.4	12.6	2.727
Kinetin (1.0 mg/l)				
Number of microtubers	1.3	3.3	3.5	1.214
Number of shoots	9.7	12.5	11.8	2.834

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 h supplementary light of 450 μmol/m^2/S and a frequent water misting for two weeks at 24±2°C. Relative humidity was decreased frequently from 95% (at the beginning of acclimatization) to 75% (by the end of second week of acclimatization).

Stem cuttings (3.0 cm long) were taken from the acclimatized plants after two weeks and treated (basal 0.5 cm) for five 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 glasshouse (25±2°C [day)]/18±2°C [night] for further growth. Plants were grown in a bed (1 peat : 1 perlite) covered with muslin cloth to protect them from insect attack.

An in vivo rooting experiment was conducted and stem cuttings were taken from glasshouse grown plants. Data was recorded after two weeks on rooting percentage. Rooted cuttings were cleaned from soilless mixture by dipping roots in water with light hand shaking. Root number was counted per each transplant.

Virus Testing

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

RESULTS AND DISCUSSION

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/1. Total number of nodes per flask was increased significantly with BA upto 1.5 mg/l (82.7 nodes) and decreased at higher concentrations. Dodds (1988) reported that a shoot consisting of 3-4 nodes rapidly proliferate on liquid media and gave 60-70 nodes per flask. Tovar et al. (1985) reported rapid proliferation of potato stems in liquid media after two to three weeks of culture.

Increasing kinetin concentration decreased shoot length (Table 1) but did not have any significant effect on number of proliferated shoots, number of nodes and internode length. Both BA and kinetin inhibited root formation. In vitro multiplica-

tion rates were reported to vary with potato cultivar (Hussey and Tacey, 1981, Miller et al., 1985), media components (Miller et al., 1985; Mumtaz and Quraishi, 1989) and method of clonal micropropagation (Hussey and Stacey, 1981; Wang and Hu, 1982).

In vitro rooting on solid media (Table 2) showed that most plants gave low number of shoots which ranged from 2.0 to 1.0 per test tube. Longest shoot was that obtained at 1.0 mg/1 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/a IBA + 1.0 mg/l GA_3) to 3.5 nodes/test tube (at 2.0mg/l IAA + 1.0 mg/ 1 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/1 IBA)to 0.3 (at 2.0 mg/1 IAA+1.0 mg/l GA_3). It was reported by Dodds (1988), that a single potato node if subcultured on solid media, will trigger the axillary bud to grow and give a plantlet with six or seven nodes with well developed roots in 3-4 weeks of culture.

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). At 10 mg/1 BA, the highest number of microtubers was induced (Wang and Hu, 1982). It was expected that the BA concentration in the present study was low and could not induce microtuberization. It was reported by Wang and Hu, (1982) that a lower dosage of 2.5 mg/l BA is needed for inducing microtuber in apical potato stolons. Kinetin was reported with very little effect on in vitro tuberization (Forsline and Langille, 1976)

Increasing sucrose concentration to 60 to 90 g/l showed significant increase in the number of microtubers produced 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 and Blake, 1989; Lillo, 1989; Tovar et al., 1985; Wang and Hu, 1982). Influence of darkness on in vitro tuberization was reported by others (Hussey and Stacey, 1981; Slimmon et al., 1989).

All microtubers had an oblong shape (4-8 mm long) representing the actual tuber shape of Spunta cultivar. Dormancy was broken i.e., buds were developed and sprouting began after 5-6 weeks of storage under dark for GA_3 treated microtubers. About 40% of untreated microtubers were lost because of weight loss and deterioration.

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

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

Mixture		Root Number
Peat	Perlite	
0	1	15.7
1	0	1.6
1	1	9.9
1	2	6.9
1	3	2.8
1	4	6.3
2	1	2.7
2	3	2.6
3	1	2.1
3	2	2.6
3	4	3.2
4	1	1.6
4	3	3.8
LSD (0.05)		1.68

good aeration in perlite. Successful rooting of potato stem cuttings was reported previously (Levy, 1988) when in vitro stems were moved to a soil mixture containing peat and perlite in a ratio of 1: 1. The procedure of using stem cuttings helps potato seed production programme to produce large amounts of pathogen-free seed tubers (Bryan, 1988; Cole and Wright, 1967).

A total number of about 40 cuttings can be taken from one plant grown under glasshouse condition as well as 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.

LITERATURE CITED

Amirouche, L. Stuchbury, T. and Matthews, S. 1985. Comparison of cultivar performance on different nutrient media in a routine method for potato micropropagation. Potato Res. 28:469-478.

Bryan, J.E. 1998. Implementation of rapid multiplication and tissue culture methods in third world countries. Am. Potato. J. 65: 199-207.

Cole, F. and Wright, N.S. 1967. Propagation of potato by stem cuttings. Am. Potato J. 44:301-304.

Dodds, J.H. 1988. Tissue Culture Technology; practical application of sophisticated methods. Am. Potato J. 65: 167-180.

Forsline, P.L. and Langille, A.R. 1976. An assessement of modifying effect of kinetin on in vitro tuberization of induced and non-induced tissues of *Solanum tuberosum*. Can. J. Bot. 54: 2513-2516.

Garner, N. and Blake, J. 1989. The induction and development of potato microtubers in vitro on media free of growth regulating substances.Ann. Bot. 63: 663-674.

Hill, S.A. 1984. Methods in Plant Pathology.Vol. I. Methods in Plant Virology. 1st edn.Centre for Agricultural Publishing and Documentation, Wageningen.

Hussey, G. and Stacey, N.J. 1981. In vitro propagation of potato (*solanum tuberosum* L.). Ann . Bot. 48: 787-796.

Levy, D. 1988. Propagation of potato by the transfer of transplants of in vitro proliferated shoot cuttings into the field. Scientia Horticulturae. 36: 165-171.

Lillo, C. 1989. A simple two-phase system for efficient in vitro tuberization in potato. Norwegian J. Agric.Sci. 53: 23-27.

Miller, P.R. Amirouche, L. Stuchbury, T. and Matthews, S. 1985. The use of plant growth regulators in micro-propagation of slow-growing potato cultivars. Potato Res. 28: 479-486.

Mumtaz, A. and Quraishi, A. 1989. In vitro performance of selected potato cultivars.Sarhad J.Agric. 363-367.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-479.

Slimmon, T. Machado, V.S. and Coffin, 1989. The effect of light on in vitro microtuberization of potato cultivars. Am. Potato J. 66: 843-848.

Tovar, P. Estrada, R. Schilde Rentschler, L. and Dodds, J.H. 1985. Induction and use of in vitro potato tubers.CIP Circular. 13: 1-5.

Wang, P. and Hu, C. 1982. In vitro mass tuberization and virus-free seed potato production in Taiwan. Am. Potato J. 59: 33-37.