Bangladesh J. Bot. **47**(3): 369-380, 2018 (September)

IN VITRO SLOW-GROWTH CONSERVATION FOR TWO GENOTYPES OF *SOLANUM TUBEROSUM* L.

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Keywords: Potato, Microplant, Slow-growth, Osmoticum, *In vitro* conservation, *Solanum tuberosum*

Abstract

To find out the most suitable culture medium formulation to induce slow-growth and reduce the frequency of sub-culturing of the *in vitro* conserved microplants of the two potato genotypes at 24 ± 1ºC was conducted. Growth was controlled by using different concentrations of sucrose, mannitol, sorbitol (30 g/l) alone or in combination with either mannitol (15, 20 and 25 g/l) or sorbitol (15, 20 and 25 g/l) in murashige and Skoog medium. The results showed that single treatment (Sucrose or mannitol or sorbitol alone) was not feasible for long-term conservation. Combined treatment was responding better and maximum microplant survived (80.82 - 83.15%) after 12 months of storage on (T-8) medium supplemented with 10 g/l sucrose and 20 g/l sorbitol. In this formulation microplants were in very good condition, without phenotypic abnormalities and had enough nodes for sub-culturing up to 12 months. Microplant survival and condition were closely associated with each other but not with root growth.

Introduction

Solanaceae is comprised of 3,000 - 4,000 species belonging to about 90 genera and potato (*Solanum tuberosum* L.), tomato (*S. lycopersicum* L.), aubergine or eggplant (*S. melongena* L.), chili pepper (*Capsicum* sp.), and husk tomato (*Physalis* sp.) which are well-known and most widely cultivated crops. Besides, a number of species are locally cultivated for their edible fruits, tubers, or leaves, and for horticultural purposes. Cultivated potato (Order: *Solanales*; *Solanaceae*) and its wild relatives belong to the genus *Solanum*, the largest genus with 1,500 - 2,000 species. Within the genus *Solanum*, over a thousand of species have been recognized (Burton 1989). Generally, tuber-bearing *Solanum* species are grouped in the *Petota* section. This section is subdivided into two subsections, *Potatoe* and *Estolonifera* (Hawkes 1990). The subsection *Potatoe* contains all tuber-bearing potatoes, including common potato (*S. tuberosum*, belonging to series *Tuberosa*).

The cultivated potato is a tetraploid (2n = 4x = 48) and exhibits complex tetrasomic inheritance. It is highly heterozygous and segregates on sexual reproduction. Elite parental lines and cultivars of potato are thus maintained through vegetative propagation in order to maintain their genetic integrity. Maintenance of potato germplasm through field clonal propagation is time-consuming and it requires large amounts of space and is labour-intensive. This also exposes the plants to disease, pests, and risks of loss due to abiotic stresses and natural calamities (Withers *et al*. 1990). Therefore, throughout the world, potato gene banks prefer to conserve elite parental lines and clones as *in vitro* propagated microplants under disease-free tissue culture conditions (Westcott *et al*. 1977, Golmirzaie *et al*. 1999, Gopal *et al*. 2002, Engelmann 2011). When grown under optimum propagation conditions (MS medium with 30 g/l sucrose 16 hrs photoperiod 22 – 25 ºC), the microplants require sub-culturing every after 4 - 8 weeks. In order to reduce the frequency of sub-culturing, growth of the microplants is restricted by employing growth retardants or osmotic stress in combination with a reduced energy source, low temperatures, low light

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intensity and varied photoperiod (Golmirzaie and Toledo 1997, Negri *et al.* 2000, Gopal *et al.* 2002, Gopal and Chauhan 2010). The use of low temperatures (6 - 8ºC) and 16 hrs photoperiod (15 - 30 µmol/m^2/s light intensity from cool white fluorescent lamps) is almost universal in potato gene banks for conservation (Gopal *et al.* 2005). However, in the tropics and sub-tropics, ambient temperatures in summer can be as high as 45 - 50ºC. Therefore, maintenance of walk-in-chambers at 6 - 8ºC in tropical and sub-tropical conditions is very energy demanding and costly.

Hence, there is a need to develop protocols for conserving potato microplants at temperatures of 24 ± 1ºC that are normally available in tissue culture rooms used for micropropagation. Non-metabolisable sugar-alcohols (osmoticums) reduce the water availability to the growing cultures by imposing a water-deficit stress (Gopal *et al.* 2002). This stress may perhaps be responsible for slow-growth of potato microplants. In fact, *in vitro* technique may be used to achieve medium-term conservation to allow storage of biological material from several months to 2 - 3 years without subculture, depending on the technique used and the plant material (Cruz-Cruz *et al.* 2013).

Thus, the main objective for the present study was to conserve potato germplasm *in vitro* at 24 ± 1°C by employing different concentrations and combinations of metabolically inactive sugar-alcohols (osmoticums) to produce osmotic stress for reducing sub-culture time of the microplants.

Materials and Methods

Single nodal segments from *in vitro* grown shoots of two potato genotypes *viz.*, Asterix and Diamant were used to conduct different experiments. These genotypes were selected for their contrasting response to *in vitro* minimal growth. Further, they have wider genetic base.

Table 1. Media composition of different treatments

Treatment code	MS media with		
	MS + sucrose (g/l)	MS + mannitol (g/l)	MS + sorbitol (g/l)
T1	30	0	0
T2	0	30	0
T3	0	0	30
T4	15	15	0
T5	10	20	0
T6	5	25	0
T7	15	0	15
T8	10	0	20
T9	5	0	25

After sprouting from potato disease free microplants were cultured and maintained on MS (Murashige and Skoog 1962) medium supplemented with 30 g/1 sucrose under standard culture conditions (16 hrs photoperiod, 40 µmol/m^2/s light intensity and 24 ±1ºC). Single nodal cuttings (SNCs) from primary culture were sub-culture on MS medium supplemented with different concentrations of sucrose, mannitol, sorbitol (30 g/l) alone or in combination with either mannitol (15, 20 and 25 g/l) or sorbitol (15, 20 and 25 g/l) (Table 1). Every sub-culture vessels containing

20 ml of medium solidified with 7 g/l Nobel agar (Merck, India). Culture tubes were closed with polypropylene caps and sealed with parafilm M (Laboratory Film) (Chicago, II. 60631, USA), and incubated at 16 hrs photoperiod (From cool white fluorescent lamps, approx. 20 µmol/m^2/s light intensity) at 24 ± 1ºC in tissue culture room. The experiment was conducted in factorial (9 medium × 2 cultivars) randomized complete block design with 6 replicate culture tubes which were used in this investigation.

Microplant survival, microplant condition (on a visual 0 - 5 preference scale: 0 = dead, 1 = very poor, 2 = poor, 3 = moderate, 4 = good to 5 = very good) for suitability of sub-culturing, root growth (on a visual 0-5 preference scale: 0 = nil, 1 = very poor, 2 = poor, 3 = moderate, 4 = good to 5 = very good), shoot length (cm) and number of nodes per microplants data were recorded after 3, 6 and 12 months of incubation. Observations were also made on the presence or absence of aerial roots, microtubers or phenotypic abnormality. The data on percent microplant survival were transformed into arec sine and those of microplant condition and root development into square roots ($\sqrt{x} + 0.5$). Both non-transformed and transformed data used to similar results, so only non transformed data were analyzed. Analysis of variance and correlation coefficient were carried out according to the standard procedures (Gomez and Gomez 1984). Experimental values are given as mean, the mean were compared using Duncan's multiple-range test (DMRT) as outlined by IBM SPSS software version 20 (SPSS Inc. USA).

Results and Discussion

The single nodal explants of two potato genotypes *viz.*, Asterix and Diamant from *in vitro* generated shoot cultures were excised and sub-cultured on MS medium solidified with agar and supplemented with different concentrations of sucrose, sorbitol and mannitol alone and sucrose with mannitol, sucrose with sorbitol combination in order to find out the most suitable culture media formulation to induce slow-growth of the conserve potato microplants and to reduce the interval time between the sub-cultures. Five parameters such as percentage microplant survival, microplant condition for suitability of sub-culturing, root growth, shoot length (cm) and number of nodes per microplant were considered to evaluate the performance of the conserved microplants.

The analyses of variance showed that the variation between the two genotypes were statistically non significant (p = 0.05) for most of the characters except in microplant conditions. The treatment effects were highly significant (p = 0.05) for most of the characters indicating that significant differences existed among the treatments (Table 2). Mean squares due to interaction (G × T) effects were also highly significant for most of the characters except in microplant survival after 3 and 12 months of culture, microplant condition after 3 and 6 months of culture, root growth after 3 and 12 months, microplant shoot length after 6 months of culture, indicated that the two genotypes responded differently in different treatments. This indicates there is on need for developing genotype specific protocol to improve the efficiency of tissue culture systems. However, it may be difficult to develop genotype specific protocols for germplasm conservation.

The results of correlation coefficients showed that microplant survival percentage was highly and positively correlated with microplants condition score (r = 0.94) and number of shoots per microplant (r = 0.81). Microplant condition score also showed significant and positive correlation with number of shoot per microplants (r = 0.68) after 12 months of *in vitro* conservation (Table 3).

Microplant survival rates (average over genotypes) after 3 months of culture ranged from 83.91 - 85.50 per cent, and decreased to 58.88 - 59.62 and 51.66 - 54.21 per cent after 6 and 12 months of conservation, respectively (Table 4). The highest value of microplant survival rates (average over periods) was after 3 months of culture (84.71%), followed by after 6 months of culture (59.25%). The lowest was observed after 12 months of culture (52.93%). The highest value

of microplants survival rates (average over treatments) was recorded in T8 (83.01%) and statistically similar results was observed in T9 (78.76%), T5 (80.51%), T6 (80.34%), T4

Table 2. Mean squares in the analysis of variance of different characters of two potato genotypes grown *in vitro* under different osmotically stress conditions, data were recorded after 3, 6 and 12 months of culture.

Item	Duration	Degree of freedom	Percent microplant survival	Microplant condition	Root growth	Shoot length (cm)	Number of buds per shoot
Genotypes	3 months	1	0.04 ns	0.6891***	0.06 ns	0.19***	0.17 ***
	6 "	1	7.40 ns	0.90 ***	0.02 ns	0.00 ns	1.10 ns
	12 "	1	88.17***	0.85 ****	0.00 ns	0.02 ns	0.00 ns
Treatments	3 months	8	131.9 ***	2.46***	7.55***	11.73***	10.66 ***
	6 "	8	4965 ***	6.98***	0.49 ***	4.68 ***	26.6 ***
	12 "	8	5404 ***	3.05 ***	7.03 ***	5.46 ***	29.25 ***
G × T	3 months	8	3.74 ns	0.09 ns	0.03 ns	0.13***	0.15 ***
	6 "	8	13.37***	0.04 ns	2.70 ***	0.01 ns	0.19 ***
	12 "	8	13.08 ns	0.12 ***	0.04 ns	0.17 ***	0.10 ***

*** and ns indicate significant at 5% level and non-significant.

(79.45%) followed by T7 (69.83%), T2 (44.07%), T3 (43.62%). The lowest value of microplant survival rates was found in T1 (39.88%) after 12 months of culture. Between the two treatment groups highest microplant survival rate was found in combined group of MS medium with sucrose and mannitol (80.15%), followed by combined treatment with MS medium and sorbitol (77.06%), and the lowest was observed in single treatment group (42.72%) (Table 4).

Table 3. Correlation coefficients among the five characteristics after 12 months of conservation.

Item	Microplant survival %	Microplant condition	Root growth	Shoot length (cm)	Number of nods/shoot
Microplant survival %	0.00				
Microplant condition	0.94 ***	0.00			
Root growth	0.40	0.48	0.00		
Shoot length (cm)	0.23	0.30	0.90 ***	0.00	
Number of nods/shoot	0.81 **	0.68 *	0.17	0.10	0.00

***, **,* indicate significant at 5, 1, 0.5% level.

Interestingly, T8 gave maximum microplants survival and favorable condition, resulted in significantly less root growth than rest of the treatments in which microplants condition and survival rates was lower (Tables 4, 5, 6 and 7). These findings support the findings of Westcott's (1981b) and Gopal *et al.* (2002) who opined that reduced root growth increases longevity of cultures by delaying the time when medium components become limiting for growth. However, the present results also indicate that there is a limit and an optimal level of root growth is required to achieve maximum microplant survival.

Microplant condition score (average over genotypes) after 3 months of culture ranged from 3.27 - 3.49, and it was decreased to 2.55 - 2.68 and 1.90 - 2.04 after 6 and 12 months of conservation, respectively (Table 5). The highest microplant condition score (average over periods) was recorded after 3 months of conservation (3.38), followed by after 6 months of conservation (2.62), and the lowest was observed after 12 months of conservation (1.97). The highest microplant condition score (average over treatments) was recorded in T8 (4.01), T9 (3.19), T5 (3.19), T6 (2.99), T7 (2.71), T4 (2.66), T1 (2.20), T3 (1.83). The lowest values of microplant condition score were obtained in T2 (1.77). Among the different treatments highest microplants condition was obtained in combined group of MS medium with sucrose and sorbitol (3.24), followed by combined group of MS with sucrose and mannitol (2.96). The lowest microplant condition

Table 4. Percentage of microplant survival of two potato genotypes grown under *in vitro* in different treatment and different durations.

Treatment group	Treatment	Per cent survival						Mean treatment	Mean treatment group
		3 months		6 moths		12 months			
		Asterix	Diamond	Asterix	Diamond	Asterix	Diamond		
Single treatment	T1	93.53	93.86	14.92	15.58	10.21	11.21	39.88 d	42.72 c
	T2	81.66	82.66	31.48	28.15	20.59	19.92	44.07 c	
	T3	79.85	79.19	20.89	18.89	12.41	11.41	43.62 c	
	Mean	85.01	85.23	22.43	20.87	14.40	14.18		
Combined (Sucrose + mannitol)	T4	82.72	80.72	78.37	80.70	77.63	76.63	79.46 ab	80.15 a
	T5	91.50	88.50	79.99	85.99	69.03	68.03	80.51 a	
	T6	88.00	85.34	77.00	78.00	75.07	67.73	80.34 a	
	Mean	87.41	84.85	78.45	81.57	73.91	70.80		
Combined (Sucrose + sorbitol)	T7	86.22	83.22	66.63	70.63	57.80	54.47	69.83 b	77.06 b
	T8	85.30	81.96	83.76	83.10	83.15	80.82	83.01 a	
	T9	80.75	79.75	76.87	75.54	82.04	74.70	78.76 ab	
	Mean	84.09	81.65	75.76	76.42	74.33	70.00		
	Mean genotypes	85.50 a	83.91 a	58.88 a	59.62 a	54.21a	51.66 b		
	Mean periods	84.71 a		59.25 b		52.93 c			

Means followed by the same letters, within genotypes, within in a period, within treatments and within treatment groups do not differ significantly by DMRT test at 5% probability.

was found in single treatment group (1.91) (Table 5). Among the three treatment groups microplant conserved in MS supplemented with sucrose and sorbitol performed better. The microplant survivability with better microplants condition (score: 3.24), poor root growth (score: 1.91), satisfactory shoot length and number of nodes per microplant were observed in this group after 6 and 12 months of conservation. Thus, it might be practicable treatment group. The combined group of MS medium with sucrose and mannitol was also effective treatment for long term conservation but it was comparatively less effective than MS medium with sucrose and sorbitol.

Root growth (average over genotypes) after 3 months of culture ranged from 1.19 - 1.23, and it was increased to 1.53 - 1.60 and 1.90 - 1.92 after 6 and 12 months of conservation respectively (Table 6). The highest root growth score (average over periods) was achieved 12 months after conservation (1.91), followed by 6 months after conservation (1.57). The lowest was observed after 3 months of conservation. The highest root growth score (average over treatments) were achieved in T1 (2.83), T8 (2.42), T9 (1.77), T6 (1.71), T7 (1.47), T4 (1.44), T5 (1.42), T3 (0.68). The lowest microplant root growth score was obtained in T-2 (0.33). Among the different treatments highest microplant root growth score was found in combined group of MS medium with sucrose and sorbitol (1.91), followed by combined group of MS medium with sucrose and mannitol (1.56). The lowest microplant root growth score was recorded in single treatment groups (1.17) (Table 6).

Table 5. Microplant condition (on 0 - 5 scale, 0 = dead, 1 = very poor, 2 = poor, 3 = moderate, 4 = good, 5 = very good) of two potato genotypes under *in vitro* conservation in different treatments and different durations.

Treatment group	Treatment	Microplant condition						Mean treatment	Mean treatment group
		3 months		6 months		12 months			
		Asterix	Diamond	Asterix	Diamond	Asterix	Diamond		
Single treatment	T1	4.50	4.00	1.77	1.58	0.63	0.70	2.20 d	1.91 c
	T2	2.20	2.53	1.93	1.98	1.02	0.94	1.77 e	
	T3	2.28	2.61	1.33	1.70	0.38	0.52	1.83 e	
	Mean	2.99	3.05	1.68	1.76	0.68	0.72		
Combined (Sucrose + mannitol)	T4	2.76	3.09	2.51	2.74	2.59	2.26	2.66 c	2.96 b
	T5	3.45	3.65	3.14	3.27	2.76	2.85	3.19 b	
	T6	3.26	3.59	2.74	2.96	2.33	2.69	2.99 b	
	Mean	3.16	3.44	2.80	2.99	2.56	2.60		
Combined (Sucrose + sorbitol)	T7	3.48	3.81	2.69	2.91	1.50	1.89	2.71 c	3.24 a
	T8	4.25	4.58	4.09	3.89	3.54	3.68	4.01 a	
	T9	3.23	3.57	2.80	3.06	2.33	2.80	3.19 b	
	Mean	3.65	3.99	3.19	3.29	2.46	2.79		
	Mean genotypes	3.27 b	3.49 a	2.55 b	2.68 a	1.90 b	2.04 a		
	Mean periods	3.38 a		2.62 b		1.97 c			

Means followed by the same letters, within genotypes, within in a period, within treatments and within treatment groups do not differ significantly by DMRT test at 5% probability.

The shoot length (cm) (average over genotypes) of conserved microplant after 3 months of conservation ranged from 1.84 - 1.92 cm, and increased to 2.29 - 2.30 and 2.70 - 2.72 cm after 6 and 12 months of conservation respectively (Table 7). The highest microplant shoot length (cm) (average over periods) was recorded after 12 months after conservation (2.71), followed by 6 months after conservation (2.30). The lowest was observed after 3 months of conservation (1.88). The highest microplant shoot length (cm) (average over treatments) was recorded in T1 (4.89 cm), followed by T8 (3.31 cm), T9 (2.84 cm), T7 (2.81 cm), T4 (1.87 cm), T5 (1.83 cm), T6 (1.73 cm), T3 (1.14 cm). The lowest shoot length (cm) was obtained in T2 (0.59 cm). Among the different

treatments highest microplant shoot length (cm) was found in combined groups of MS medium with sucrose and sorbitol (3.01 cm), followed by single groups (2.01 cm). The lowest microplant shoot length (cm) was recorded in combined groups of MS medium with sucrose and mannitol (1.80 cm) (Table 7).

Table 6. Root growth (on 0 - 5 scale, 0 = nil, 1 = very poor, 2 = poor, 3 = moderate, 4 = good, 5 = very good) of two potato genotypes under *in vitro* conservation in different treatments and different durations.

Treatment group	Treatment	Root growth						Mean treatments	Mean treatment group
		3 months		6 moths		12 months			
		Asterix	Diamond	Asterix	Diamond	Asterix	Diamond		
Single treatment	T1	2.55	2.42	2.77	2.47	3.48	3.31	2.83 a	1.17 b
	T2	0.22	0.40	0.19	0.42	0.39	0.35	0.33 f	
	T3	0.53	0.20	0.49	0.64	0.18	0.36	0.68 e	
	Mean	1.10	1.00	1.15	1.17	1.35	1.34		
Combined (Sucrose + mannitol)	T4	1.21	1.41	1.28	1.35	1.65	1.75	1.44 d	1.56 a
	T5	1.04	1.17	1.52	1.66	1.50	1.63	1.42 d	
	T6	1.21	1.31	1.77	1.84	2.61	2.78	1.71 c	
	Mean	1.15	1.30	1.53	1.62	1.92	2.05		
Combined (Sucrose + sorbitol)	T7	1.13	1.31	1.48	1.59	1.77	1.53	1.47 d	1.11 c
	T8	1.73	1.26	2.47	2.57	1.30	1.20	1.42 d	
	T9	1.47	1.27	1.81	1.88	2.42	2.19	1.77 c	
	Mean	1.44	1.28	1.92	2.01	2.50	2.31		
	Mean genotypes	1.23 a	1.19 a	1.53 a	1.60 a	1.92 a	1.90 a		
	Mean periods	1.21 c		1.57 b		1.91 a			

Means followed by the same letters, within genotypes, within a period, within treatments and within treatment groups do not differ significantly by DMRT test at 5% probability.

The number of nodes per microplant (average over genotypes) of conserved microplants after 3 months of incubation ranged from 2.93 - 3.02, which increased to 3.72 - 3.72 and 4.06 - 4.08 after 6 and 12 months of conservation, respectively (Table 8). The maximum number of nodes per microplant (average over periods) was achieved after 12 months after conservation (4.07), followed by 6 months after conservation (3.72). The lowest was observed after 3 months of conservation (2.98). The maximum number of nodes per microplant (average over treatments) was achieved in T7 (6.93), followed by T4 (5.43), T6 (4.85), T8 (4.03), T5 (4.02), T9 (3.33), T1 (1.69), T3 (1.45). The minimum number of nodes per microplant was obtained in T2 (0.58). Among the different treatments maximum number of nodes per microplant was obtained in combined groups of MS medium with sucrose and sorbitol and it was statistically similar to combined group of MS medium with sucrose and mannitol. The minimum number of nodes per microplant was recorded in single treatment group (Table 8).

The potato microplants in medium without sucrose were less responsive in microplant survivability, condition, and root growth, shoot length, number of nodes per microplant in both the cultivars. The results also showed that in medium with sucrose alone the microplants failed to

survive after 6 and 12 months of conservation. So, it can be concluded that single treatment is not feasible for long term conservation. In contrast the medium with sucrose and mannitol or sucrose and sorbitol the microplants performed better in case of microplants survivability, condition, root growth, and shoot length and number of nodes per microplant. These microplants were healthy and were suitable for further cultures.

The objective of the present study was to induce slow-growth or reduce the growth employing osmotic stress for conservation of potato microplants at normal (24 ± 1ºC) propagation temperature. Optimum results were achieved with MS medium with 10 g/l sucrose plus 20 g/l sorbitol. After 12 months without sub-culturing, maximum survival (80.82% in Diamant and 83.15% in Asterix) was obtained with moderate condition score (score 3.54 to 3.68) in both the cultivars and microplants had enough nodes for sub culturing. During *in vitro* conservation, some microplants presented their shape change which showed a certain degree of instability, such as generating base bulbs, turning into purple color, which were the physiological changes produced

Table 7. Shoot length (cm) of two potato genotypes under *in vitro* conservation in different treatments and different durations.

Treatment group	Treatment	Shoot length (cm)						Mean treatments	Mean treatment group
		3 months		6 months		12 months			
		Asterix	Diamond	Asterix	Diamond	Asterix	Diamond		
Single treatment	T1	4.61	4.19	5.00	5.07	5.29	5.16	4.89 a	2.01 b
	T2	0.48	0.42	0.61	0.66	0.66	0.68	0.59 g	
	T3	0.58	0.53	0.61	0.66	0.65	0.60	1.14 f	
	Mean	1.89	1.71	2.07	2.13	2.20	2.15		
Combined (Sucrose + Mannitol)	T4	1.51	1.50	1.81	1.77	2.26	2.36	1.87 d	1.80 c
	T5	1.39	1.34	1.67	1.73	2.41	2.48	1.83 d	
	T6	1.39	1.49	1.79	1.75	2.23	2.33	1.73 e	
	Mean	1.43	1.44	1.76	1.75	2.30	2.39		
Combined (Sucrose + Sorbitol)	T7	2.35	2.43	2.82	2.74	3.25	3.27	2.81 c	3.01 a
	T8	2.55	2.35	3.29	3.14	4.23	4.33	3.31 b	
	T9	2.42	2.28	3.16	3.06	3.36	3.29	2.84 c	
	Mean	2.44	2.35	3.09	2.98	3.61	3.63		
	Mean genotypes	1.92 b	1.84 a	2.30 a	2.29 a	2.70 a	2.72 a		
	Mean periods	1.88 c		2.30 b		2.71 a			

Means followed by the same letters, within genotypes, within in a period, within treatments and within treatment, groups do not differ significantly by DMRT test at 5% probability.

by environmental stimulation under stress conditions, rather than genetic variation. Chen *et al.* (2006) observed similar results. Conservation under *in vitro* slow-growth represents a possible solution for medium to long-term storage of plant material in limited space and at reduced costs. *In vitro* slow-growth procedures allow clonally propagated plant conservation for 1 - 15 years (depending on the species) under tissue culture conditions, requiring only infrequent sub-culturing (Rao 2004). The observation could not be recorded beyond 12 months of conservation as medium had exhausted and desicated in all culture tubes by this time. Perhaps cultures can be stored for a longer period if more medium and bigger culture tubes or other suitable vessels are used (Westcott 1981a, b). The conservation period may also be extended by using liquid medium or by adding

fresh medium before the initial medium is completely exhausted. In the latter case, liquid medium would be the obvious choice. It will be interesting to explore these possibilities.

In most of the previous studies, *in vitro* conservation of potato microplants has been tried at low temperatures. When MS medium was supplemented with growth inhibitors such as abscisic acid, maleic hydrazide, N-dimethylaminosuccinamic acid, phosphon D (Radatz and Standke 1978, Westcott 1981b, Gopal *et al.* 2002) and acetylsalicylic acid (Lopez-Delgado *et al.* 1998), the interval between sub-cultures ranged from 6 to 16 months depending on genotype and the type and concentration of the retardant used (Njoroge 2000). Westcott (1981 a) and Naik and Karihaloo 2007 reported that interval between sub-culture could be increased from 4 to 12 months by storing

Table 8. Number of nodes per microplant of two potato genotypes under *in vitro* conservation in different treatment and different durations.

Treatment group	Treatment	Number of nodes per microplant						Mean treatments	Mean treatment group
		2 months		6 moths		12 months			
		Asterix	Diamond	Asterix	Diamond	Asterix	Diamond		
Single treatment	T1	4.93	5.22	0	0	0	0	1.69 g	1.23 b
	T2	0.46	0.36	0.4	0.63	0.8	0.81	0.58 i	
	T3	1.33	1.46	1.29	1.56	1.4	1.65	1.45 h	
	Mean	2.24	2.35	0.56	0.73	0.73	0.82		
Combined (Sucrose + Mannitol)	T4	3.7	3.56	6.53	5.84	6.86	6.06	5.43 b	4.76 a
	T5	2.56	2.7	4.4	4.7	4.8	4.93	4.02 e	
	T6	3.33	3.3	5.39	5.52	5.68	5.89	4.85 c	
	Mean	3.20	3.19	5.44	5.35	5.78	5.63		
Combined (Sucrose + Sorbitol)	T7	5.53	5.5	7.46	7.38	7.8	7.93	6.93 a	4.76 a
	T8	2.6	2.56	4.48	4.26	5.15	5.12	4.03 d	
	T9	1.93	2.53	3.51	3.6	4.08	4.32	3.33 f	
	Mean	3.35	3.53	5.15	5.08	5.68	5.79		
	Mean genotypes	2.93 b	3.02 a	3.72 a	3.72 a	4.06 a	4.08 a		
	Mean periods	2.98 c		3.72 b		4.07 a			

Means followed by the same letters, within genotypes, within in a period, within treatments and within treatment groups do not differ significantly by DMRT test at 5% probability.

the microplants at 6ºC instead of 22ºC when normal MS medium with 30 g/l sucrose was used. The present study shows that a similar sub-culture period can be achieved even at 24 ± 1ºC by using MS medium with 10 g/l sucrose and 20 g/l sorbitol. The microplants conserved in this way had normal phenotype with thick stems and broad leaves (Fig. 3). In contrast, microplants conserved at low temperatures generally look abnormal with stunted growth, thin stems and leaves that are reduced or absent. Such microplants have also been reported to have a high incidence of abnormalities such as cholorosis, vitrification and flaccidity, particularly when low temperature is combined with media with osmotic stress or growth retardants (Lopez-Delgado *et al.* 1998, Naik 2000, Santana-Buzzy 2006). Such morphological abnormalities make the recovery of normal plants difficult on sub-culture. This raises concern about the genetic stability of plants conserved in this way for prolonged periods (Harding 1994, 1999, Kaviani 2011, Rajasekharan and Sahijram 2015).

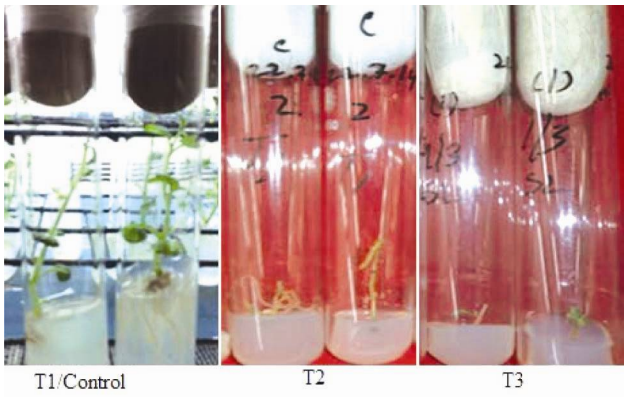

Fig. 1. Comparison of microplants of single treatment: T1/control (MS medium with 30 g/l sugar; after 3 months); T2 (MS medium with 30 g/l mannitol; after one year of conservation) and T3 (MS medium with 30 g/l sorbitol; after one year of conservation).

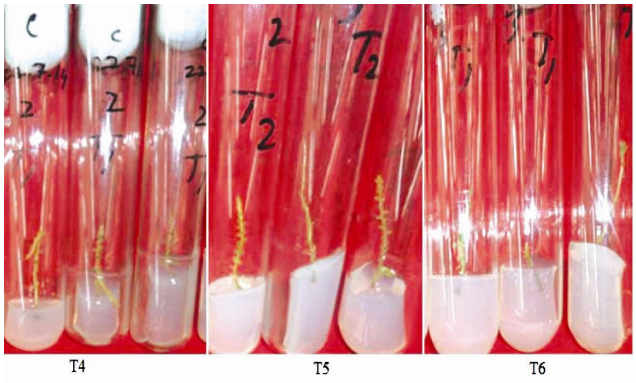

Fig. 2. Comparison of microplants of combined treatment (MS + sugar + mannitol): T4 (MS medium with 15 g/l sugar and 15 g/l mannitol); T5 (MS medium with 10 g/l sugar and 20 g/l mannitol) and T6 (MS medium with 5 g/l sugar and 25 g/l mannitol). Photographs were taken after one year of conservation.

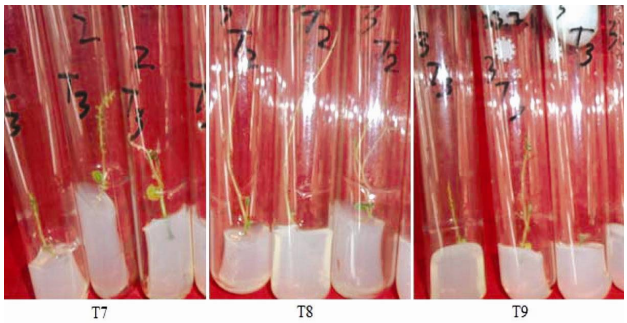

Fig. 3. Comparison of microplants of combined treatment (MS + sugar + sorbitol): T7 (MS medium with 15 g/l sugar and 15 g/l sorbitol); T8 (MS medium with 10 g/l sugar and 20 g/l sorbitol) and T9 (MS medium with 5 g/l sugar and 25 g/l sorbitol). Photographs were taken after one year of conservation.

Till date the method of slow-growth conservation *in vitro* at normal propagation temperatures has been used to conserve germplasm of potato (*Solanum tuberosum* L.) (Yun-peng *et al.* 2012). Therefore, the method followed in this investigation might be a very promising method and desirable not only to save on the cost of energy and maintenance etc., but also for better genetic stability of the germplasm. *In vitro* slow-growth storage techniques are being routinely used for medium-term conservation of numerous species, both from temperate and tropical origin, including crop plants, e.g. potato, musa, yam, cassava (Ashmore 1997, Razdan and Cocking 1997, Engelmann 1999) and rare and endangered species (Fay 1992, Sarasan *et al.* 2006). However, this method also has some problems yet to be resolved. Further, it has been grown awareness in the last decade and offer many advantages as a complement to field maintenance.

Acknowledgements

The authors express their deepest sense of appreciation and heartfelt thanks to the Chairman, Department of Botany, University of Rajshahi and to the University Grants Commission (UGC) for granting them a financial support to conduct the research.

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(Manuscript received on 21 August, 2017; revised on 8 March, 2018)