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IN VITRO CONSERVATION OF POTATO

M. T. ISLAM[1], R. AFROZ[2], S. RAHMAN[3], M. A. HOSSAIN[4]

Abstract

A study was conducted to assess the effect of sorbitol on *in vitro* conservation of potato. MS medium supplemented with different concentrations of sorbitol @ 0, 20 and 40 g/l and nodal explants of potato varieties were used in this study and continued upto five months. Days to shoot and root initiation; number of leaf, number of shoot, number of root and length of shoot were assessed. BARI Alu-7 produced the maximum leaflets (29.8) while the BARI Alu-41 produced the minimum leaflets (16.2) in MS supplemented with 20 g/l sorbitol after five months of conservation. The varieties produced 6 to 13.8 shoots and 5 to 11.2 roots in the same medium. The maximum shoot length (12.2 cm) was exhibited in BARI Alu-25 and BARI Alu-28, where minimum (9.9 cm) was found in BARI Alu-37. Among the varieties, BARI Alu-7 and BARI Alu-37 performed better for shoot growth and development. MS medium supplemented with 20g/l sorbitol showed the best performance where the *in vitro* plantlet could be conserved for five months. Three studies were also conducted with or without sorbitol for six weeks, eight weeks and three months. The *in vitro* conservation protocol developed from nodal explants has applicability in improvement of potato.

Keywords: Potato, *Solanum tuberosum*, nodal explant, growth regulators, *in vitro* conservation.

Introduction

Potato (*Solanum tuberosum* L.) is the 3rd important food crop in Bangladesh after rice and wheat. It is a vegetable as well as an industrial crop. Bangladesh is producing about 96.06 lac tons of potatoes annually from 4.62 lac hectares of land with an average of 20.82 tons//ha (BBS, 2022). Potato cultivation contributes to global food security (FAOSTAT. 2022; Zsögön *et al.* 2022). Potato varieties are generally maintained through vegetative propagation in order to maintain their genetic integrity as the crop is highly heterozygous and segregates on sexual production. Factors related to climate change have led to a large *in situ* loss of biodiversity of the plant genetic resource (Kulak *et al.* 2022). Potato tubers are bulky and highly perishable; the crop is generally conserved as clones either in field genebanks (with annual replanting), *in-vitro* conservation in slow growth media for short-to-medium term and cryopreservation for long term. Field genebanks are expensive to maintain and the crop is exposed to many dangers; hence, cryopreservation is the only feasible method for long term conservation.

[1]Director, Training & Communication Wing, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur-1701, Bangladesh, [2&3]Senior Scientific Officer, Plant Genetic Resources Centre, BARI, Joydebpur, Gazipur-1701, Bangladesh, [4]Ex. Director, Support and Service Wing, BARI, Joydebpur, Gazipur-1701, Bangladesh.

However, given the high cost of cryopreservation, longterm conservation of potato genetic resources is poorly developed in most resource-poor countries leading to high rates of genetic erosion. *In vitro* techniques have thus been advocated as a useful alternative to conserve potato plantlets under diseases-free conditions (Golmirzaie *et al.* 1999; Gopal *et al.* 2002). Under optimum propagation conditions (MS medium with 30g sucrose, 16h photoperiod, 22 to 24^0C), the plantlets require sub-culturing every four to eight weeks (Gopal and Chauhan, 2010). *In situ* and *ex situ* conservation of potato collections are essential in breeding programs. Plant biotechnology offers different alternatives for germplasm conservation through plant tissue culture, which allows *in vitro* conservation and the regeneration of germplasm. The *in vitro* conservation is classified into three categories: short-, medium- and long-term. Medium-term *in vitro* conservation has more advantages than short-term periods, such as control over storage conditions, and compared to long-term periods that require a constant supply of liquid nitrogen for cryopreservation, which is not cost effective for use in commercial laboratories (Panis *et al.* 2020, Engels and Andreas, 2021). In addition, medium-term *in vitro* conservation allows for slow-growth storage, reduces labor costs and allows for the availability of the germplasm bank (Spinoso-Castillo *et al.* 2022). Slow-growth storage consists of modifying the physical and chemical conditions of the *in vitro* culture to reduce the metabolism and development of cells, tissues, organs or whole plants (Salgotra *et al.* 2023). Physical factors include incubation at low temperatures, the use of nutrient-poor culture media, increased osmotic potential of the culture medium and the use of growth inhibitors such as ABA and gibberellin synthesis inhibitors (GAs) (Ruta *et al.* 2020). BARI released 104 varieties of potato. They need *in vitro* conservation to reduce the sub-culturing strategies. Therefore, the present study was undertaken to develop an *in vitro* medium term conservation protocol of potato for potato propagation.

Materials and Methods

Eleven (11) varieties of potato viz. BARI Alu-7, BARI Alu-8, BARI Alu-13, BARI Alu-21, BARI Alu-24, BARI Alu-25, BARI Alu-27, BARI Alu-28, BARI Alu-30, BARI Alu-31 and BARI Alu-32 were used in this study at the *In vitro* Conservation Laboratory of Plant Genetic Resources Centre, BARI, Joydebpur Gazipur during 2012-13 to 2015-16. These are the introduced varieties from Netherlands and Germany and commercially cultivated in Bangladesh. In the first study, eight weeks old *in vitro* plantlets of 11 potato varieties were collected from Tuber Crop Research Centre of BARI TCRC on 22 August 2012. The MS medium was prepared. The pH of the medium was adjusted to 5.8 before autoclaving and the media were solidified with agar @ 8 g/l. The medium was autoclaved at 1.06 kg/cm^2 pressure and 121°C for 15 min. Nodal explants were prepared under Laminar Air Flow aseptically and inoculated into test tube (25 x 150 mm) containing 15 ml of the basal MS medium. Explant having one node and two leaves

was cultured in each test tube. Aluminum foil was used as closure. The explants were incubated for six weeks in a culture room at 23 ± 1°C under about 500 lux light provided by cool-white fluorescent tubes maintaining a 16/8hr alternate light/dark cycle and 60-70% relative humidity. Completely randomized design with four replications were applied in the first study where each replication contained of 5 test tubes. Data from the experiment were subjected to ANOVA and mean separations were carried out by the LSD using the MSTAT program.

During 2013-14 (2nd study), six varieties (BARI-Alu-7, BARI Alu-8, BARI Alu-12, BARI Alu-21, BARI Alu-25 and BARI Alu-28) of potato were tested (Table 2). Nodal explants of 4 weeks old plantlets were prepared and inoculated one explant of into test tube containing 15 ml MS medium supplemented with 0, 20 g and 40g/l sorbitol, plus 2% sucrose. The media were solidified with agar @ 8 g/l. The explants were incubated for eight weeks in a culture room at 22 ± 1°C temperature. The experimental set up was in a 3 X 6 factorial in a completely randomized design with five replications and each replication contained five explants.

During 2014-15, the 3rd study was conducted with 10 varieties (BARI Alu-7, BARI Alu-8, BARI Alu-12, BARI Alu-21, BARI Alu-25, BARI Alu-28, BARI Alu-32, BARI Alu-33, BARI Alu-37 and BARI Alu-41). The MS media containing 20 g/l sorbitol were prepared. Nodal explants of 4 weeks old were prepared and inoculated one explant into the test tube containing 15 ml MS medium supplemented with 20g/l sorbitol. The explants were incubated for three months at 22 ± 1°C temperature. The experimental set up was in a completely randomized design with five replications and each replication consisted of 5 explants. Data from the experiment were subjected to ANOVA and mean separation was carried out using the MSTAT program.

The 4th study was conducted with 9 varieties (BARI Alu-7, BARI Alu-8, BARI Alu-12, BARI Alu-25, BARI Alu-28, BARI Alu-32, BARI Alu-33, BARI Alu-37, BARI Alu-41) of potato from Study-3 during 2015-16. Nodal explants of 4 weeks old were used in this study. The MS medium supplemented with 20 g/l sorbitol was used. The explants were incubated for five months in a culture room at 22 ± 1°C temperature. The experimental set up was in a completely randomized design with five replications and each replication consisted of 5 explants. Data from the experiment were subjected to ANOVA and LSD values were calculated using the MSTAT program.

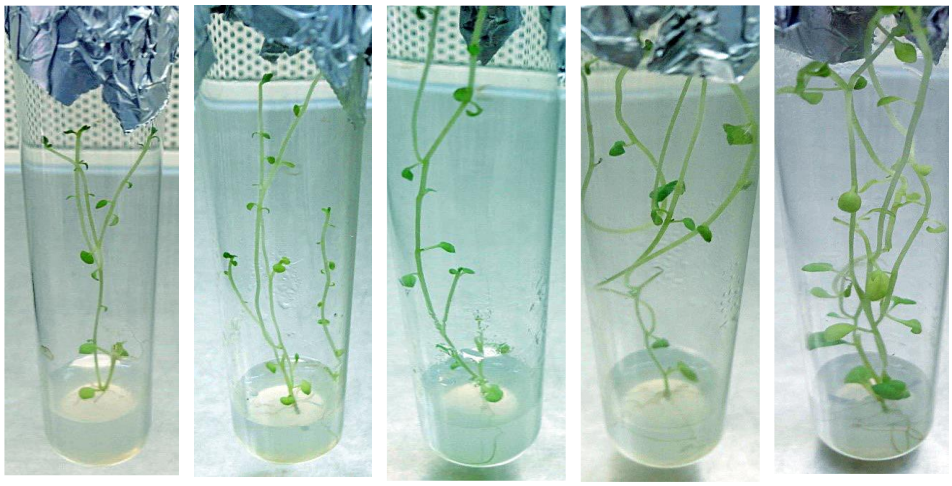
Result and Discussion

Growth and development of potato varieties, *in vitro* cultivated for six weeks in MS medium are shown in Table 1 and Fig.1. All the potato varieties initiated root and shoot within a week of culture. The number of roots produced from the varieties ranged from 2.5 to 6.25. However, no significant variations were observed in the ability of explants to develop roots. BARI Alu-30 (Meridian) took

the maximum number of days (6 days) to root initiation and the remaining varieties initiated roots within 4 to 5 days (Table 1). The maximum number of leaves was found in BARI Alu-32 (23.75), followed by BARI Alu-28 (23) and BARI Alu-8 (19.75) and the minimum leaves were found in BARI Alu-27 (7.75). The maximum shoot length (11.88 cm) was found in BARI Alu-8 (Cardinal) followed by BARI Alu-31 (Sagitla, 11.63cm) and BARI Alu-21 (Provento, 10.75cm). The minimum shoot length (6.5 cm) was found in BARI Alu-30 (Meridian). The highest number of shoot (6.5) was exhibited in BARI Alu-28 (Lady Rosseta) and the lowest (2.75) in BARI Alu-7 (Diamant) which was statistically significant.

Table 1. *In vitro* conservation of potato varieties for six weeks in MS medium-2012-13

Name of variety	Days to root initiation	No. of Leaves/plantlet	Length of shoot (cm)	No. of shoots	No. of roots
BARI Alu-7 (Diamant)	4	13.50	9.25	2.75	4.00
BARI Alu-8 (Cardinal)	4	19.75	11.88	5.25	4.50
BARI Alu-13 (Granola)	4	15.75	10.38	5.25	4.50
BARI Alu-21 (Provento)	5	14.25	10.75	3.50	4.75
BARI Alu-24 (Dura)	5	11.25	7.25	4.50	3.25
BARI Alu-25 (Asterix)	4	12.25	8.63	5.75	5.00
BARI Alu-27 (Esprit)	5	7.75	6.88	3.50	2.50
BARI Alu-28 (Lady Rosseta)	5	23.00	10.13	6.50	4.50
BARI Alu-30 (Meridian)	6	15.00	6.50	5.25	3.50
BARI Alu-31 (Sagitla)	4	11.50	11.63	4.00	5.25
BARI Alu-32 (Quincy)	4	23.75	10.00	4.75	6.25
LSD (0.01)	1.63	8.30	3.39	3.41	3.62



BARI Alu-8 BARI Alu-13 BARI Alu-28 BARI Alu-31 BARI Alu-32

Fig. 1 Response of potato varieties on MS medium at six weeks of culture.

Effect of sorbitol on *in vitro* growth and development from nodal explant of six potato varieties are shown in Table 2 and Fig.2. The highest, 97% explants produced roots initiation was seen in MS medium supplemented with 20g sorbitol/l while the lowest 87% explant produced roots in 40g sorbitol/l (Table 2). The 6 potato varieties had 87 to 93% root initiation (Table 3).The combination of 20 and 40g/l sorbitol with the varieties produced 80 to 100% root initiation (Table 4). Cent percent explants (100%) produced shoots in both 0 and 20 g/l sorbitol and the lowest shoot (93%) was found in 40 g/l sorbitol containing MS medium (Table 4). The varieties produced 93 to 100% shoot proliferation (Table 3). Potato varieties produced 100% shoots in MS medium supplemented with 20 g/l sorbitol. Control treatment also produced 100% shoots (Table 3). However, the lowest (80%) shoot initiation was exhibited in 40g/l sorbitol supplemented MS medium with BARI Alu-25 and BARI Alu-28 (Table 4). Earlier root initiation within (4-5 days) was observed in 0 and 20g/l sorbitol supplemented MS medium. Without sorbitol the plantlets were produced the maximum number (16.3) of leaves per shoot while the minimum number of leaves (10) was obtained from 40g sorbitol/l (Table 4). BARI Alu-28 produced the maximum (19.3) of leaves. The minimum number of leaves (10.9) was obtained from BARI Alu-21. Sorbitol-free MS medium produced the higher number (2.0) of shoots. The remaining treatments produced 1.0 to 1.2 shoots. Potato varieties produced 1.0 to 2.5 shoots per plantlet. The highest (8.0 cm) and the lowest (7.9 cm) shoot lengths were obtained from sorbitol free MS media and 40 g/l sorbitol, respectively. Maximum shoot length (11.3 cm) was obtained from BARI Alu-28 with MS while the minimum (2.5 cm) from BARI Alu-8 and BARI Alu-28 with 40 g/l sorbitol. Sorbitol (40.0 mg/l) produced the highest number of 11.3 roots per shoot while the lowest number of roots (7.9) was obtained from without sorbitol. Sorbitol metabolism plays multiple roles in plants, including energy and carbon enrichment, effective defence against various stresses and other emerging specific roles. It has been clear that enriching carbohydrate metabolism with a sorbitol branch improves plant fitness under stress. Nevertheless, this is probably valid only when appropriate growth and defence trade-offs are ensured. Information on the ectopic expression of sorbitol metabolism genes has contributed substantially of the sorbitol roles and raises new questions regarding sorbitol signalling potential (Pleyerová *et al*. 2022).The maximum number (12.1) of roots was obtained from BARI Alu-12 while the minimum number (8.5) of roots was obtained from BARI Alu-8 (Table 4). After 8 weeks, the terminal leaves of some varieties were become yellow. BARI Alu-28 was the superior for shoot proliferation through node culture.

Table 2. Effect of sorbitol on *in vitro* conservation of potato from nodal explant at eight weeks -2013-14

Sorbitol (g/l)	% of root initiation	% of shoot initiation	Days of root initiation	Days to shoot initiation	Number of leaf/shoot	Number of shoot/ explant	Length of shoot (cm)	Number of root/ plantlet
0	93	100	5	8	16.3	2.0	8.0	7.9
20	97	100	5	8	12.1	1.0	6.6	10.2
40	87	93	8	11	10.2	1.2	3.4	11.3

Table 3. Effect of potato varieties on *in vitro* conservation from nodal explant at eight weeks

Name of variety	% of root initiation	% of shoot initiation	Days of root initiation	Days to shoot initiation	Number of leaf/shoot	Number of shoot/ explant	Length of shoot (cm)	Number of root/plantlet
BARI Alu-7	87	100	5.5	9.7	13.1	1.8	5.9	9.6
BARI Alu-8	93	100	6.9	10.1	11.3	1.1	5.6	8.5
BARI Alu-12	93	100	6.3	10.8	11.1	1.0	4.8	9.5
BARI Alu-21	93	100	5.2	9.1	10.9	1.1	6.1	12.1
BARI Alu-25	93	93	5	5.9	11.5	1.0	6.8	10.3
BARI Alu-28	93	93	8.3	8.8	19.3	2.5	7.0	8.9

Table 4. Combined effect of sorbitol and potato varieties on *In vitro* conservation from nodal explant at eight weeks

Sorbitol (g/l)	Name of variety	% of root initiation	% of shoot initiation	Days of root initiation	Days to shoot initiation	Number of leaf/shoot	Number of shoot	Length of shoot (cm)	Number of root/ plantlet
0	BARI Alu-7	80	100	5	7	16.4	2.8	8.4	5.0
	BARI Alu-8	100	100	4	7	15.2	1.2	8.2	10.0
	BARI Alu-12	80	100	6	12	10.2	1.0	5.4	6.2
	BARI Alu-21	100	100	5	7	10.8	1.0	5.8	8.0
	BARI Alu-25	100	100	4	6	11.6	1.0	9.1	10.4
	BARI Alu-28	100	100	7	7	33.8	5.0	11.3	7.6
20	BARI Alu-7	80	100	5	11	12.2	1.2	5.5	9.6
	BARI Alu8	100	100	6	10	11.4	1.0	6.1	10.6
	BARI Alu-12	100	100	4	9	12.6	1.0	4.7	9.8
	BARI Alu-21	100	100	5	7	10.8	1.0	8.6	10.0
	BARI Alu-25	100	100	5	5	11.0	1.0	7.7	11.6
	BARI Alu-28	100	100	7	7	14.6	1.0	7.1	9.8
40	BARI Alu-7	100	100	7	11	10.8	1.4	3.8	14.2
	BARI Alu8	80	100	10	13	7.4	1.2	2.5	4.8
	BARI Alu-12	100	100	9	12	10.6	1.0	4.2	12.6
	BARI Alu-21	80	100	5	13	11.0	1.2	3.9	18.2
	BARI Alu-25	80	80	6	6	11.8	1.0	3.6	8.8
	BARI Alu-28	80	80	11	12	9.4	1.4	2.5	9.2

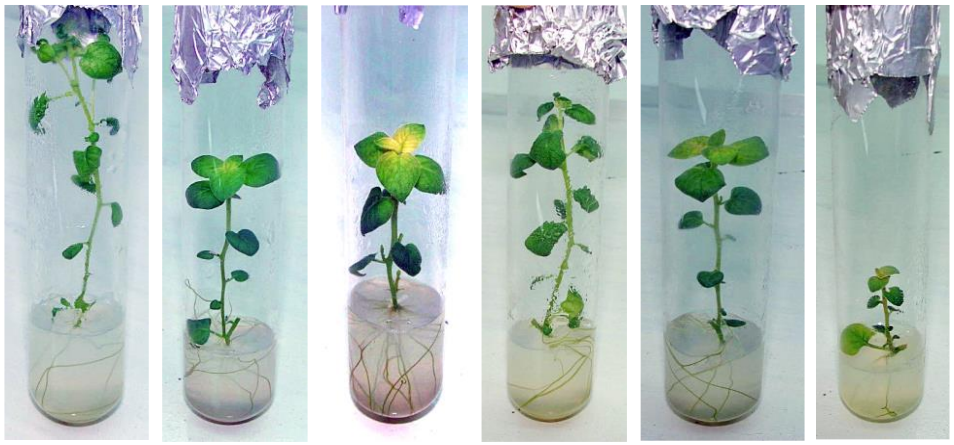
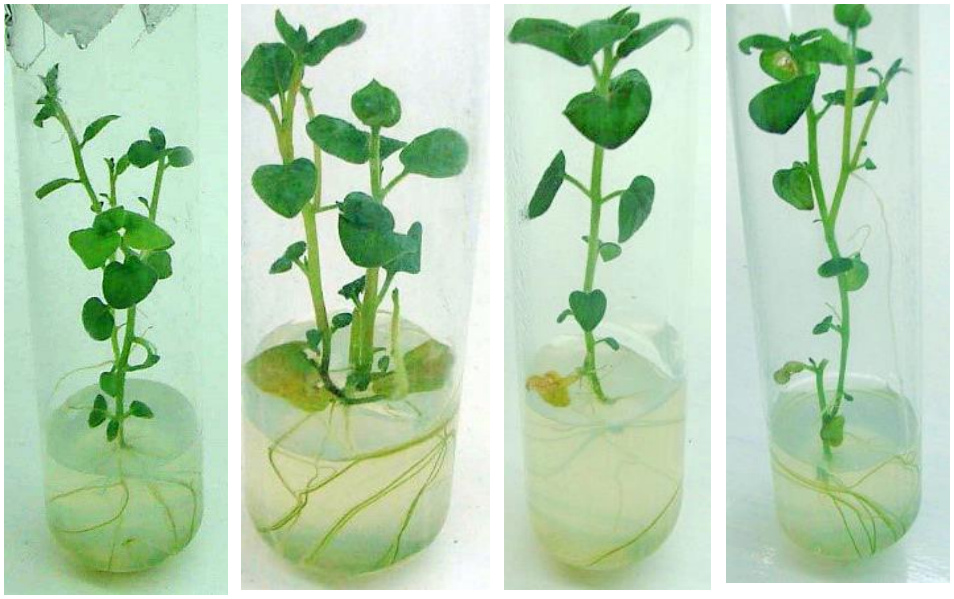


Fig.2. *In vitro* response of potato varieties on different concentrations of sorbitol after eight weeks of culture.

In vitro performances of ten potato varieties in 20g/l Sorbitol supplemented MS medium from nodal explant are shown in Table 5 and Fig.3. Cent percent shoots and roots were initiated from BARI Alu-28 and BARI Alu-32 produced after three months conservation. BARI Alu-8 produced the lowest roots (78%) and BARI Alu-7 produced the lowest (50%) shoots (Table 5).

Table 5. In vitro conservation of potato in sorbitol from nodal explant after three months of culture during 2014-15

Name of variety	% Root initiation	% shoot initiation	Days to root initiation	No. of leaflet	No. of shoot	Length of shoot (cm)	No. of root
BARI Alu-7	90	50	9.4	10.2	3	6.2	5.2
BARI Alu-8	78	78	9.4	18.6	3.4	11.7	11.6
BARI Alu-12	82	80	9.6	15.2	3	5.3	5.4
BARI Alu-21	80	80	9.2	15.8	2	5	3.4
BARI Alu-25	83	83	9.8	14.4	4	11.9	12.4
BARI Alu-28	100	100	10.6	20	3	9.1	7.2
BARI Alu-32	100	100	8.8	16.2	3.4	8.3	8
BARI Alu-33	83	84	8.4	20.2	2	11.6	5.4
BARI Alu-37	81	83	8.6	13.8	2	5.1	5.8
BARI Alu-41	83	82	8.6	19	2.6	6.5	6.8
Average	86	82	9.2	16.3	2.8	8.1	7.1
LSD (1%)	1.83	1.23	2.21	9.96	2.86	3.1	2.61



BARI Alu-8 BARI Alu-7 BARI Alu-32 BARI Alu-28

Fig. 3. *In vitro* conservation potato varieties for three months culturing in MS medium with 20g/l sorbitol

All the varieties initiated roots and shoots within 8.4 to 10.6 days. BARI Alu-28 and BARI Alu-33 produced the maximum leaflets (20 and 20.2) while the BARI Alu-7 produced the minimum leaflets (10.2). The varieties produced 2 to 4 shoots and 3.4 to 12.4 roots. The maximum shoot length (11.9 cm) was exhibited in BARI Alu-25, BARI Alu-8 (11.7) and BARI Alu-33 (11.6), and minimum (5 cm) obtained from BARI Alu-21. Considering all parameters, BARI Alu-28 and BARI Alu-32 performed better for shoot proliferation through node culture.

In vitro performances of nine potato varieties in sorbitol (20 g/l) from nodal explant are shown in Table 6 and Fig.4. The varieties produced 86 to 100% roots within 7 days and 90 to 100% shoots within 11 days. After five months conserved, BARI Alu-7 produced the maximum leaflets (29.8) while the BARI Alu-41 produced the minimum leaflets (16.2).

The varieties produced 6 to 13.8 shoots and 5 to 11.2 roots. The maximum shoot length (12.2 cm) was exhibited in BARI Alu-25 and BARI Alu-28, and minimum (9.9 cm) obtained from BARI Alu-37. Among the varieties, BARI Alu-7 and BARI Alu-37 performed better for shoot proliferation (minimum growth) through node culture.

Table 6. *In vitro* conservation of potato in sorbitol from nodal explant for five months during 2015-16

Name of variety	% Root initiation	% Shoot initiation	Days to root initiation	Days to shoot initiation	No. of leaflet	No of shoot/ plantlet	No. of root/ plantlet	Shoot length (cm)
BARI Alu-7	100	100	6	11	29.8	7	6.2	10.7
BARI Alu-8	86	100	7	8	26.8	6	5	11.1
BARI Alu-12	100	100	5	6	27	6.6	6.4	11
BARI Alu-25	100	90	6	11	26	10	7.4	12.2
BARI Alu-28	100	100	7	7	24	9.4	8.8	12.2
BARI Alu-32	100	100	7	6	27	10.2	9.8	12
BARI Alu-33	100	100	5	5	28	13.2	13	11.6
BARI Alu-37	90	100	3	4	18.8	12.6	11.2	9.9
BARI Alu-41	100	100	5	6	16.2	13.8	11.2	11.2
Average	97.3	98.9	5.6	7.2	24.8	9.9	8.7	11.3
LSD (0.01)	-	-	2.33	2.77	9.29	5.11	1.74	2.94

BARI Alu-7 BARI Alu-8 BARI Alu-28 BARI Alu-37 BARI Alu-41

Fig. 4. *In vitro* conservation of potato varieties for five months culturing in MS medium with 20g/l sorbitol.

The efficacy of mannitol versus sorbitol for *in vitro* conservation of potato microplants at low (7±1 °C) temperature was previously studied. Two concentrations of sucrose (20 and 40 g/l) in combination with two concentrations (20 and 40 g/l) of either mannitol or sorbitol in Murashige and Skoog (MS) medium were tested. Microplant survival, microplant condition, and root growth in three potato genotypes belonging to different maturity groups were studied up to 18 months of *in vitro* storage without subculturing. Best results were achieved with MS medium having 20 g/l sucrose plus 40 g/l sorbitol. After 18 months without subculturing, maximum survival 58.0%) coupled with a microplant condition good enough to provide suitable nodes for subculturing was observed with the use of this medium (Gopal and Chauhan, 2010).

Many potato conservation units use low temperatures (6 to 8^0C) and 16 h photoperiod (15 to 30 µmol m^2 s^1 light intensity from cool white fluorescent lamps) for potato conservation despite the fact that maintenance of plantlets at 6 to 8^0C can be energy demanding due to high electricity charges. Previous report also showed that *in vitro* plantlets can be effectively conserved at 24 ± 1^0C using 20g/l sucrose and 40g Sorbitol for 12 months (Gopal *et al.* 2002). Thus minimizing cooling costs associated with low temperature conservation. Present study demonstrated that potato can be conserved efficiently at 22 ± 1^0C for 5 months.

Conclusion

The results of the present study suggested that sorbitol (20 g/l) with 3% sucrose showed good performance in *in vitro* conservation of potato varieties. The minimal

shoot proliferation was achieved in BARI Alu-7 and BARI Alu-37 through node culture. A protocol for *in vitro* conservation from nodal explants of potato varieties has been developed in this study. This protocol can be used for *in vitro* medium term conservation of potato varieties for germplasm and variety maintenance.

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