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学术园地

Preservation of Disease - freeShoot Tips of Potato Germplasm through in vitro System

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Abstract: The present investigation was undertaken to find out the best medium composition for medium-term preservation of disease free potato shoot tips in in vitro system. Thirteen potato genotypes and fourteen treatments were taken under consideration for the present experiment. Among fourteen treatments, mannitol and sorbitol containing media proved to be the best for medium-term preservation of potato shoot tips. High concentration of mannitol delayed root formation.

Key Words: medium; genotype; in vitro; Solanum tuberosum L

In vegetatively propagated crops, once systematically infected with a viral disease, the pathogen can passed from one generation to the next[1]. Especially in potato, contamination by a pathogen can severely reduce the total yield of the crop[2]. Traditionally, potato varieties have been and still maintained in a field gene bank. Maintenance of potato germplasm in the field is a major consumer of time, manpower and space aside from diseases and environmental stresses. The major disadvantage of a field gene bank is the risk to lose part of the collection through disease, pest, weather damage or other accidents. For long term storage (preservation) of potato varieties, tissue culture system is possible, in which potatoes are stored either as plantlets or as microtubers. This type of storage lowers the risk to lose of the material due to environmental stresses. Once viruses or other pathogens have been eliminated, cultures can be kept pathogen free. Preserving the disease-free potato is important in order that the breeders can select the best source materials available and it also serves to protect the gene pool of the potato. Since 1975, the International Potato Center (CIP) has contributed in developing tissue culture techniques for consecrating germplasm of potato[3]. In vitro conservation is now being the most useful and efficient way to describe clonal materials. It facilitates the availability of planting materials at any time avoiding the transfer of major pests and pathogens and makes virus eradication possible through meristem culture[4, 5]. In addition, in vitro conservation is less expensive than cryopreservation of field grown clonal materials[6]. In vitro preservations have been reported on potatoes, e.g. meristems freeze-preserved for 24 months[7], leaf callus of potato suspension cultures at -14℃[8], disease free minitubers [1, 9, 10, 11], and "storage" of potato material for three months[12]. Using the nodal explants, propagation of Syringa chinensis cv. Sangeana in vitro preservation is also possible[13]. Chuai Zheng reported the storage of the aseptic seedlings of economic plants in low temperature in vitro[14]. There are also reports on shoot tip of potato preserved in vitro system using MS medium supplemented with inositol, auxin, cytokinins and mainatol. In this investigation, no phytohormon was used

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because it is expected that the use of phytohormon may result in unexpected or abnormal morphological variation in the in vitro derived plants. In higher plants, initiation and growth of stems and roots are regulated by internal and external factors including light, temperature, hormones and sugars. A reduction in light intensity affects growth rate by lowering photosynthetic requirements and therefore metabolism [15]. Reducing growth temperature close to 0℃ for temperate plant species or several degrees below normal for tropical crops can also minimize the growth rate in many crops[16, 17]. The addition of osmotic or growth retardants to the medium has proved efficient for reducing growth rates of different plant species. Osmoticums such as mannnitol or sorbitol reduce mineral uptake by cells through differences in osmotic pressure, thereby retarding plant growth[16, 18]. Sugars are one of the most important factors[19] since they are used not only as an energy source but also for constructing structural components of cells and cell walls. In vitro plants growing in closed culture vessels having low concentration of CO_2, C absorption is maintained by supplementing the medium with sugar. The present investigation was conducted with a purpose to establish an efficient protocol for medium term in vitro preservation of disease free shoot tips of potato by using different growth inhibitors.

1　Materials and Methods

The shoot tips of the thirteen cultivars collected from 25-35 days old field grown seedlings were used as explants for meristem culture. The experiment was carried out in the Plant Breeding and Biotechnology Laboratory of Department of Botany, Rajshahi University, Bangladesh during the year 2002-2004. At first, the excised clean shoot tips were sterilized in savlon and tween-20 [polyoxuethelen (20) sorbitan, oleate] followed by 2-3 times washing with sterilized distilled water. Then, the explants were sterilized with 0.1% $HgCl_2$ solution with gently shaking for 2-8 minutes (3 min is best) followed by 3-5 times washing with sterilized distil water. From sterilized shoot tips, immature leaves and leaf primordial were snapped off. Then the isolated meristems(0.3 mm) were quickly transferred on the filter paper bridge in test tubes containing liquid MS [20] medium supplemented with BA, GA_3 and KIN either singly or in combination and MS_0(hormone free medium) for primary establishment. After 3 to 4 weeks of inoculation of meristem, the developed meristems were sub cultured on semi-solid MS_0 for 3 to 5 weeks for shoot elongation.

In vitro propagated nodes of thirteen cultivars of potato which consisted of stem segments 0.7 to 1.0 cm long with 2 to 3 nodes and 3 to 4 leaves were used for preservation study. The explants were subcultured on to fourteen different concentration and combination of MS, carbon source, mannitol and sorbitol. The medium compositions are listed in Table 1.

Table 1　Media used in conservation of disease free shoot tip explants

Medium code	Strength of MS media	Sucrose(%)	Sorbitol(%)	Manitol(%)
M_1	Full	3	-	-
M_2	1/3	3	-	-
M_3	1/4	3	-	-
M_4	1/5	3	-	-
M_5	Full	2	-	-
M_6	Full	1	-	-
M_7	Full	0.5	-	-
M_8	Full	3	3	-
M_9	Full	3	6	-
M_{10}	Full	3	9	-
M_{11}	Full	3	-	3
M_{12}	Full	3	-	6
M_{13}	Full	3	-	9
M_{14}	Full	3	6	5

The pH of the media was adjusted at 5.7. The culture bottles were autoclaved at 120℃ and 1.5 $kg \cdot cm^{-1}$ pressure for 20 minutes. All the equipments used were previously sterilized. All the bottles containing growth media were cultured first, and then brought into the laminar flow cabinet for sterilization under the ultraviolet lamp for 15 minutes. For the days to survival obser-

vation on different medium combination, the cultures were kept under 1.0 to 1.5 k lx fluorescent lamps for 16 h light and 8 h at darkness at 12-15 ℃. Data for growth rate of shoot tip and root elongation from the initial cultures were taken after four weeks of cultivation. Each treatment was repeated at least 13 times. The results were subject to analysis of variance (ANOVA).

2 Results and discussion

The effects of different media on growth of disease free shoot tips of potato are presented in Table 2. Among the fourteen treatments, highest shoot length was observed in treatments M_1 followed by M_2 and M_3; and the lowest shoot length was observed in treatments M_{13}. Highly significant difference in shoot length was exhibited on high concentration manitol containing MS medium. Shoot length was almost similar in the treatments M_2, M_3 and M_4. Strength of MS was not significantly affected on growth of potato shoot tips. Shoot length was significantly different among the treatments M_5, M_6 and M_7. Low concentration sucrose containing medium reduced the shoot and root growth.

Table 2 The effect of different media on controlling the stem, root and number of node growth of potato shoot-tips in vitro

Treatment No	Shoot length (cm)	Root Length (cm)	No of Node per plant
	Mean±SE	Mean±SE	Mean±SE
M_1	6.27±0.45 a	6.97±0.42 a	5.56±0.27 a
M_2	4.78±0.28 b	5.88±0.30 ab	4.80±0.29 ab
M_3	4.48±0.29 b	4.94±0.43 bc	4.20±0.18 bcd
M_4	4.16±0.29 bc	3.86±0.34 c	3.79±0.28 cd
M_5	4.15±0.26 bc	4.68±0.32 bc	4.59±0.29 bc
M_6	2.78±0.31 d	1.50±0.39 def	4.20±0.29 bcd
M_7	1.64±0.14 fg	0.59±0.21 fg	2.59±0.27 fg
M_8	3.56±0.05 c	2.56±0.07 d	4.44±0.07 bc
M_9	2.63±0.06 d	1.86±0.05 def	3.51±0.12 de
M_{10}	1.74±0.05 ef	1.41±0.06 def	2.82±0.13 efg
M_{11}	1.86±0.02 ef	1.22±0.07 efg	4.11±0.12 bcd
M_{12}	1.23±0.03 fg	0.26±0.07 fg	3.42±0.06 def
M_{13}	0.81±0.03 g	0.00±0.00 h	2.52±0.06 g
M_{14}	2.43±0.04 de	1.46±0.05 def	3.87±0.13 cd

The maximum root length was noted in the treatment M_1 followed by M_2 and M_3. The lowest root length was found in the treatment M_{12} and no root growth on the treatments M_{13}. Among the fourteen treatments, lower root growth was observed on manitol containing medium than that of sorbitol and low concentration of sucrose containing medium. Maximal number of node was found in the treatment M_1, while minimal number of node per plant was found in the treatments M_{13}.

The present investigation was undertaken to find out the suitable media for medium-term in vitro preservation of potato shoot tips. For this reason, different strength of MS, low concentration of sucrose, and high concentration of manitol and sorbitol were used as growth inhibitor. Among them, high concentration of manitol showed the most effective as growth inhibitor for potato shoot tips. The efficiency of in vitro preservation of potato shoot tip using MS medium added with manitol was depended on the concentra-

tion of manitol. Significant difference of growth on manitol containing medium at various concentrations was also observed. MS with 9% manitol showed slower growth of potato.

Analysis of variance revealed that the treatment sources were highly significant for all the characters; on the other hand, variety sources were highly significant for shoot length and number of nodes per plant. Variety source was significant at 5% level of probability for the character root length(Table 3) . This result indicated that both varieties and treatments were significantly different among them.

Table 3　Analysis of variance for shoot length, root length and number of nodes per plant after 4 weeks of culture

Source of variation	df	Shoot length MS	Root length MS	No of nodes per plant MS
Variety	12	1.98***	1.64*	1.53***
Treatment	13	32.22***	63.74***	9.78***
Error	156	0.48	0.76	0.47
Total	181			

*, **, *** = significant at probability of 5%, 1% and 0.1%, respectively.

Data in Figure 1 indicates that mannitol concentration had an action of delaying root development. It took only 4-5 days after inoculation of the explants in the treatment M_1-M_9 to begin having roots while those in the treatments M_{10}, M_{11} and M_{12} needed 6, 7 and 10 days respectively after inoculation. Within 4 weeks, the explants in the treatment M_{13} were not found to form roots at all.

To find out the best medium composition for medium-term conservation of potato shoot tips in in vitro system, potato shoot tips were tested on fourteen different treatments and observed for up to 12 months. Percentage of survival rate was recorded and presented in a graphical form(Figure 2) . The Figure 2 indicated that maximum survival rate(58%) after 12 month was achieved in treatment M_{13}. A culture media containing sorbitol(3%-9%) or mannitol(3%-9%) successfully maintained potato shoot tips for up to 12 month without subculture.

CIP conserved some potato varieties for up to 2-4 years without subculture in in vitro system using 4% sorbitol containing medium at a temperature of 6-8℃ and light intensity of 1 000 lx. We were able to preserve potato shoot tips(41%-47%) for up to 12 months without subculture using 3%-9% sorbitol containing medium at a temperature of 12-15℃ and light intensity of 3000 lx.

This conservation method is one of the most efficient ways for managing a large number of potato accessions. This method is also efficient for developing countries where freezing equipments for cryopreservation are not available.

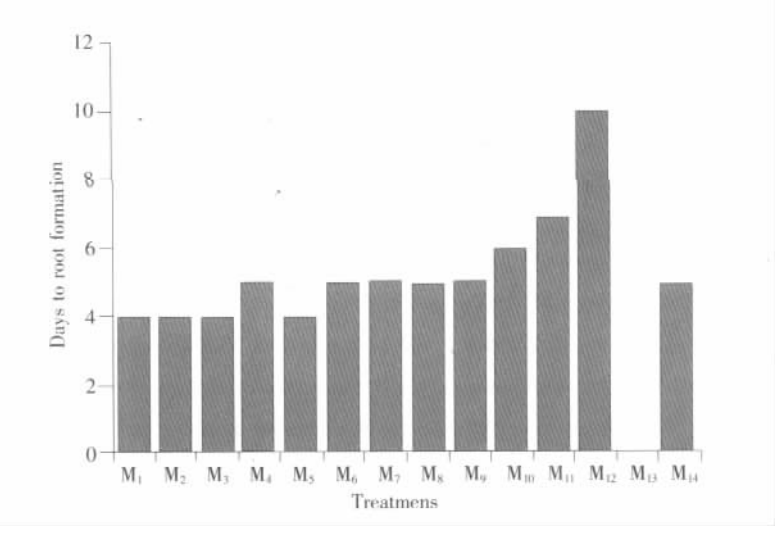

Fig. 1　Days after induction for root development of the potato shoot tips

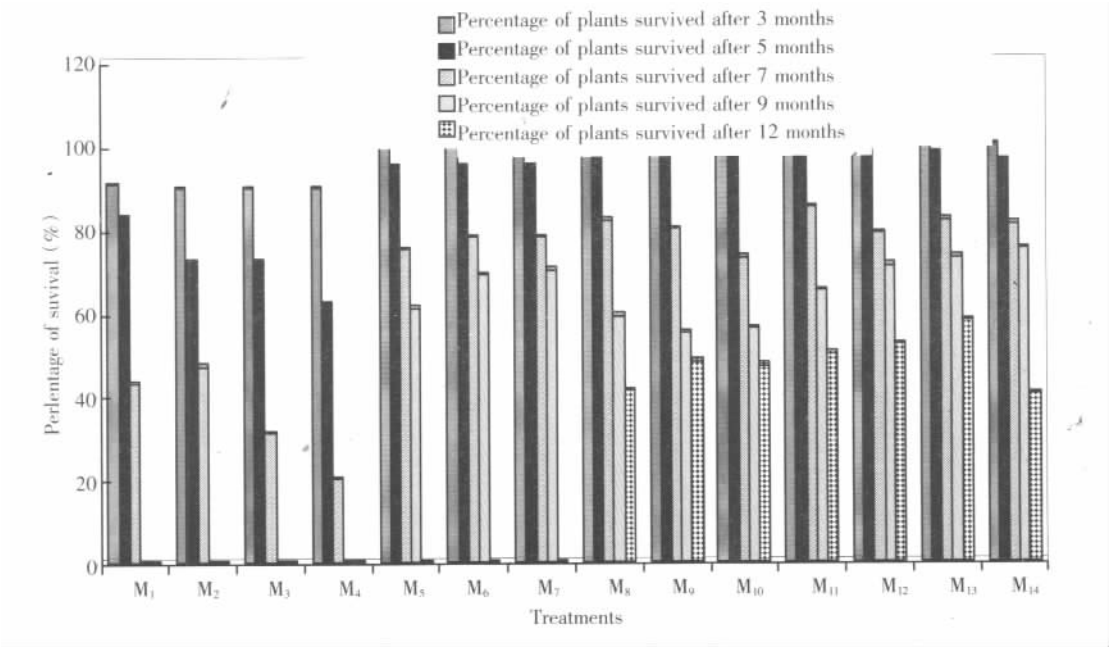

Fig. 2 Effect of different treatments on percentage of survival of plants up to 12 months of storage for medium-term preservation of potato shoot-tips

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