

SHORT-TERM CONSERVATION OF SWEET POTATO (*Ipomoea batatas* L.) *IN VITRO*

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Abstract: An Investigation was carried out to develop the techniques for short-term *in vitro* conservation using shoot tip and nodal explant of *Ipomoea batatas*. The culture conditions were 25-27°C room temperature under dark or light and refrigerator temperature 10-12°C under dark. The explants which were preserved on agar-sucrose-1/4MS medium at refrigerator (10-12°C) under dark condition showed the highest survival rates (60 & 70%) for shoot tip and nodal explants for variety 'white skinned' while 63 & 77% for 'red skinned' variety, respectively than other media (agar-sucrose medium and agar-sucrose-MS medium). For the test of resumption of growth of the *in vitro* preserved explants which were survived on preservation media for 3 months and 6 months conservation period, were cultured on MS medium supplemented with 1.0 mg/l BA (proliferation medium) at 25-27°C under light. It was evident that, the explant those were preserved at 25-27°C under light were more responsive than those were preserved at refrigerator (10-12°C) under dark for resumption of growth.

Key words: Sweet potato, Auxin, Cytokinin, Medium and Preservation.

Introduction

Sweet potato (*Ipomoea batatas*), belongs to the family Convolvulaceae is a large genus of about 400 species mainly annual and perennial herbaceous twiners in the tropics. It is an important economic plant under this genus and occupies an important place in the daily diet in many countries. It is also known as poor man's food in Bangladesh for low price and high nutritive value in comparison with potato (Sharfuddin and Voican, 1985). It is also used for canning, dehydrating flour and as a source of starch, glucose, syrup and alcohol (Samsuddin, 1983).

Ipomoea batatas is a hexaploid ($2n=6x=90$) and has been suggested that it or its progenitor was derived by amphiploidy from a tetraploid ($2n=4x=60$) and a diploid ($2n=2x=30$) to produce triploid ($2n=3x=45$), followed by subsequent chromosome doubling to produce the hexaploid. The existence of an ample genetic diversity among cultivated plant species and their wild relatives, as well as the availability of seeds and other organs of reproduction or propagation (germplasm) of plants in collection are the essential requirements for the safeguard and improvement of crop plants. The loss of genetic variability of cultured crops or "genetic erosion" results in the marked decrease of plant genetic resources submitted to natural selection and in the increase of crop susceptibility to unforeseen environmental change or to the appearance of new diseases and pests. Plant genetic resources are threatened by the destruction of the natural environment in many ways, and are also indirectly threatened by the spread of monocultures, either food crops or commercial plantation. Considering these alarming situation of plant genetic resources, many suitable conservation techniques have been developed in controlling genetic erosion.

Sweet potato has a very high genetic variability and thousands of varieties of sweet potato exist in germplasm collection. Since sweet potato is vegetatively propagated, the maintenance of its germplasm at gene banks can be very laborious task. A tissue culture technique assists the conservation of disease free collection and facilitates easier maintenance and distribution of germplasm. *In vitro* conservation techniques have been developed for many plant species. There are many reports on *in vitro* conservation of sweet potato from different countries (Ng, 1991). But little attempts have been made for *in vitro* conservation of sweet potato on Bangladeshi varieties. Therefore, the present investigation was undertaken to establish protocols for short-term preservation of sweet potato germplasm *in vitro*.

Materials and Methods

Plant materials: Two promising sweet potato cultivars of Bangladesh such as "red skinned" and "white skinned" were used as plant materials. The shoot tip and node were excised from two months old stem

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cutting from the Botanical garden of Rajshahi University. The explants were cultured aseptically for *in vitro* conservation on modified MS medium with different concentration of growth regulators.

Preservation condition: Shoot tip and nodal segments were used for short-term conservation under different media and culture conditions. Explants were placed either on a nutrient free medium, 0.6% (w/v) agar + 1.0% (w/v) sucrose (designated as agar-sucrose medium), or on minimal nutrient medium, 0.6% (w/v) agar + 1.0% (w/v) sucrose + MS organic nutrient only (designated as agar-sucrose MS organic medium), or 0.6% (w/v) agar + 1.0% (w/v) sucrose + ¼ MS inorganic and organic nutrients (designated as agar-sucrose ¼ MS medium). These cultures were kept in culture room at 25-27°C under light or dark or at 10-12°C under dark.

Results

Survival of *in vitro* preserved explants on different culture media and conditions: Shoot tips and nodal explants were cultured on different preservation media viz. agar-sucrose medium, agar-sucrose MS organic medium and agar-sucrose ¼ MS medium at different culture conditions viz. in culture room temperature (25-27°C) under dark. The results of *in vitro* preservation of sweet potato culture have been shown in Table 1. Among these culture media and culture conditions, the best survival of the explant was observed on the medium containing agar-sucrose ¼ MS medium when the cultured explants were preserved in refrigerator at 10-12°C under dark. On this medium and under this preservation condition, the survival rates were 86 and 93% for shoot tip and nodal explants of 'white skinned' variety while 93 and 100% for shoot tip and nodal explants of 'red skinned' variety when evaluated after 3 months of culture. After 6 months of conservation on the same nutrient medium and condition, the survival rates were found to be decreased. The survival rates were 60 and 66% for shoot tip and nodal explants of 'white skinned' variety and 65 and 77% for shoot tip and nodal explants of 'red skinned' variety. The lowest survival percentage was found both in 3 months and 6 months of conservation periods on the nutrient free *agar-sucrose medium* in refrigerator at 10-12°C under dark.

Table 1. Survival percentage of *in vitro* preserved explants on different culture media and culture conditions after 3 and 6 months of culture.

Culture conditions	Types of explant	% explant survived on different media and conditions					
		"white skinned"			"red skinned"		
		Agar-sucrose medium	Agar-sucrose-MS organic medium	Agar-sucrose-1/4 MS inorganic & organic medium	Agar-sucrose medium	Agar-sucrose-MS organic medium	Agar-sucrose-1/4 MS inorganic & organic medium
% Survival after 3 months							
Culture room (25-27°C)							
(i) Light	ST	27	47	58	33	52	67
	N	33	53	67	40	60	74
(ii) Dark	ST	14	33	46	20	33	46
	N	20	47	54	27	40	54
Refrigerator (10-12°C)							
(i) Dark	ST	47	74	86	67	87	93
	N	53	80	93	73	93	100
% Survival after 6 months							
Culture room (25-27°C)							
(i) Light	ST	14	26	33	20	33	40
	N	20	34	40	26	40	53
(ii) Dark	ST	07	13	20	13	20	27
	N	13	20	34	20	27	33
Refrigerator (10-12°C)							
(i) Dark	ST	26	47	60	33	54	65
	N	33	53	66	47	63	77

ST=Shoot tip
N=Node

The culture room condition: Light was preferable than dark for the survival of nodal explant. The survival rates at 25-27°C under light were 58 and 67% for shoot tip and nodal explants of white variety and 67 and 74% for shoot tip and nodal explants of red variety after 3 months of conservation. Whereas, under dark condition it was 46 and 54% for shoot tip and nodal explants of white and red variety respectively on the agar-sucrose ¼ MS medium. After 6 months of culture on the same medium and condition, the results were 33 and 40% for white variety and 40 and 53% for red variety under light. Under dark condition it was 20 and 34% for white variety and 27% and 33% for red variety.

From this *in vitro* conservation experiment, it was evident that conservation in refrigerator (10-12°C) under dark was superior than conservation at culture room temperature (25-27°C) under both light and dark for longer preservation of explants. Among different culture media, agar-sucrose ¼ MS medium was found to be the most suitable compared to other media for *in vitro* conservation purpose.

Resumption of growth of *in vitro* preserved explants on normal culture medium: The explants that survived 3 and 6 months conservation period on the preservation medium at culture room temperature (25-27°C) under light and refrigerator (10-12°C) at dark conditions, were separately transferred on the normal culture medium (MS+ BA1.0 mg L⁻¹) at culture room temperature (25-27°C) condition under light for the test of resumption of growth. The results of resumption studies have been shown in Table 2. In these studies *in vitro* preserved nodal explant showed better response on normal culture medium than shoot tip explant in all treatments except agar-sucrose medium and white variety of potato. The explants which were preserved at culture room temperature (25-27°C) condition under light on agar-sucrose-¼ MS medium showed the highest degree of resumption of growth. After 4 weeks of culture on normal culture medium, growth resumption were 88 and 100% for shoot tip and nodal explants of white variety and 75 and 88% for red variety in case of explant preserved for 3 months. Whereas, in case of explant preserved for 6 months, they were 63 and 75% white variety and 50 and 63% in red variety for shoot tip and nodal explant respectively. Within culture room condition the lowest percentage of resumption of growth was shown by the explants those preserved on agar-sucrose medium at 25-27°C under light condition were cultured on normal proliferation medium.

Table 2. Growth resumption percentage of *in vitro* preserved explants on proliferation medium (MS + 1.0 mg/l BA) after 4 weeks of culture under light at 25-27°C.

Preservation media	Previous culture condition	Type of explant	% resumption of growth			
			Explant preserved for 3 months		Explant preserved for 6 months	
			White	Red	White	Red
Agar-sucrose Medium Agar-sucrose-MS organic medium Agar-sucrose-1/4 MS inorganic & organic medium	25-27°C (light)	ST	25	12	12	-
		N	12	25	25	12
		ST	63	50	38	25
		N	75	63	50	38
		ST	88	75	63	50
		N	100	88	75	63
Agar-sucrose Medium Agar-sucrose-MS organic medium Agar-sucrose-1/4 MS inorganic & organic medium	10-12°C (dark)	ST	-	-	-	-
		N	-	-	-	-
		ST	12	-	-	-
		N	25	12	12	12
		ST	25	12	12	-
		N	37	25	25	12

ST= Shoot tip, N= Node

All explants those were preserved in refrigerator (10-12°C) under dark condition for longer period on agar-sucrose medium failed to show resumption of growth on normal culture medium. When agar-sucrose-MS organic medium was used for preservation of explants in refrigerator (10-12°C), only nodal explant showed poor growth on normal culture medium for both the varieties of sweet potatoes. The explants, which were preserved under the same condition on agar-sucrose ¼-MS medium, showed good resumption of growth on the normal

culture medium. Here resumption of growth were 25 and 37% for shoot tip and nodal explants of white variety and 12 and 25% for shoot tip and nodal explants of red variety after 3 months of preservation. Whereas, in case of the explants preserved for 6 months, they were 12 and 25% for shoot tip and nodal explants of white variety while, 12% for nodal explant of red variety, but shoot tip explant of red variety failed to show the resumption of growth.

From this experiment, it was evident that explants those were preserved at culture room (25-27°C) under light condition is preferable than those preserved in refrigerator (10-12°C) under dark condition for resumption of growth under optimal culture condition. Among different preservation media, agar-sucrose-1/4 MS medium was found to be the most suitable than other conservation media. The *in vitro* conserved plant material of "white skinned" variety showed better results than those of "red skinned" variety for the resumption of growth under optimal culture condition.

Discussion

For short-term conservation methods, explants were preserved *in vitro* with different culture media, culture conditions and different duration of times viz, 3 months and 6 months. Among different media and culture conditions, the explant preserved on agar-sucrose-1/4 MS medium in refrigerator (10-12°C) under dark condition was found to be superior over rest of the media and culture conditions in respect to survival rate. In this experiment nodal explants showed better survival than shoot tip explants. The survival frequency was 93 and 100% for nodal explants of "white skinned" and "red skinned" varieties respectively when evaluated after 3 months on preserved media. Six months conservation on the same media and culture condition, survival rate was decreased and it was 63% and 77% for "white skinned" and "red skinned" varieties, respectively. Results of this conservation are in agreement to those of Kriuk *et al.*, (1990) and Ng (1991) who used short-term conservation of sweet potato and other economic plants. Lal and Ahuja (1995) also used short-term preservation of *Picrorhiza korroa*, *Rheum emodi* explants. They used MS + agar (0.7% w/v), only agar (1.0% w/v) and agar (1.0% w/v) + mineral oil overlay and also obtained satisfactory preservation results on medium containing MS salt and under dark condition at low temperatures.

In the present study, the lowest survival rate was observed when explants were preserved on agar-sucrose medium in culture room temperature (25-27°C) under light condition. But Lal and Ahuja (1995) reported the lowest survival rate of two medicinal plants on media without any MS salt at culture room temperature under dark. The explants which survived on preservation media under *in vitro* conditions for commanded times were sub cultured on proliferation medium (MS + 1.0 mg/l BA) for testing resumption of their growth. The explants which were preserved on agar-sucrose-1/4 MS medium at culture room temperature (25-27°C) under light, showed the best results regarding resumption of growth on proliferation media than those preserved under dark. On the other hand, explants, those survived on preservation media in refrigerator (10-12°C) under dark, showed the lowest growth resumption on proliferation medium. Moreover, all shoot tip explants preserved under such condition (in refrigerator under dark) failed to show resumption of growth.

It is probably due to erratic change of conditions prevailed during preservation and proliferation periods. Furthermore, nodal explants were found to be more tolerant to *in vitro* conservation than the shoot tip explants, probably due to their favorable tissue hardness and fast growing potential. Similar results reported earlier else where (Bajaj, 1986; Zakir *et al.*, 1990).

Conclusion

Among three culture media and culture conditions the best survival of the explant was observed on the medium containing agar-sucrose ¼ MS medium when the cultured explants were preserved in refrigerator (10-12°C) under dark. The explants those were preserved at culture room (25-27°C) condition under light on agar-sucrose-¼ MS medium showed the highest percentage of resumption of growth proliferation. All explants those were preserved in refrigerator (10-12°C) under dark condition for longer period on *agar-sucrose medium* failed to show resumption of growth on normal culture medium. In this experiment, *in vitro* preserved nodal explant showed better response on normal culture medium than shoot tip explant in almost all treatments.

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Keywords: Bifid, Factors, proteins, probiotic, Sweetpotato, storage

Introduction

The beneficial microorganisms that inhabit in human intestine are known as probiotics. These include lactic acid bacteria, actinobacteria and bifidobacteria (Zinner and Gibson, 1999). Bifidobacteria are beneficial lactic acid bacteria that constitutes 25% of the normal flora (Mitsuoka, 1984; Ruzicka, 1991 and Kowalek, 1996). The species *Bifidobacterium longum* has recently been developed (Jelic et al, 1997) and is now being used in some yogurt production and other commercial purposes (Zinner and Gibson, 1999). One of the major advantages of the probiotics is to destroy the pathogens in gut (Madsforn and Gibson, 1994). It has been known that several food materials relatively help to grow the probiotics in the intestine, which are known as prebiotics. Different types of liliac monitah and oligosaccharides are the best known prebiotics (Hansen et al., 1999). Using these prebiotics the growth increases with the production of organic acids. As prebiotic is common substrate for all bacteria, FOS that is already reported as prebiotic, inulin was found to be useful for this organism to grow (Holt, 2001).

Acetic acid and L (+) lactic acid are produced in their metabolic pathway, but no CO₂ is formed. Small amounts of formic acid, ethanol and succinic acid are also produced. There is no production of butyric and propionic acid. Glucose is degraded by the fructose-bisphosphate cleave in this process. Fructose-bisphosphate cleavage fructose-bisphosphate to fructose-6-phosphate and erythrose-4-phosphate. Through the sequential action of transketolase, transaminase, xylulose-5-phosphate epimerase, and enzymes of EMP leading on pyruvate-2-phosphate, final products are formed. Except *Bifidobacterium longum* and *Bifidobacterium infantis*, all other strains are oxidase negative. They are catalase positive when grown in presence of air with or without added lactic. Among the species, *Bifidobacterium infantis* is unique because of utilization of different kind of carbohydrate sources (Gardovys, 1998).

The present investigation was carried out to show the production of acetic and lactic acid by *Bifidobacterium longum* with respect to utilization of different substrates: glucose, FOS, inulin etc.

Materials and Methods

The experiment was conducted in the Bioprocess Technology Laboratory of Asian Institute of Technology (AIT), Thailand. Pure strain *Bifidobacterium longum* (DB-12), which was obtained from Christian Hansen, Denmark through Asia Company, Thailand, was used throughout the investigation.

Cell Growth: Culture of the cells were grown in MRS-DM agar plate to confirm the identity of the strain at 37 °C. Distinguished colonies were stored into MRS-DM agar with and without Tetracycline for further use. Cells from Agar wells were aseptically cultured in 5 ml test tube, 20 ml flask and then 100 ml fermenter. The media was cultured in anaerobic condition and temperature was maintained at 37 °C. From the fermenter samples were collected in centrifuge tubes in every few-hour intervals. Cell density was measured by UV-vis

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