In vitro Performance of Sweet Potato (*Ipomoea batatus* L.) in Bangladesh

Shamim Ahmed[1], Labony Yeasmin[1] and Mohammad Nazrul Islam[2*]

[1]Department of Biotechnology, Bangladesh Agricultural University, Mymensingh, Bangladesh
[2]Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

Article History

Manuscript No. 127
Received in 27th February, 2011
Received in revised form 29th May, 2011
Accepted in final form 31st May, 2011

Correspondence to

E-mail: nazrul_bd5@yahoo.co.uk

Keywords

Sweet potato, *in vitro* regeneration, Bangladesh

Abstract

A study was conducted to observe an *in vitro* performance of 10 sweet potato germplasms at the Tissue Culture Laboratory, Tuber Crops Research Centre, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh during July to November, 2005. The explants of 10 sweet potato germplasms were cultured on MS media. Maximum 25% cultures developed shoot directly, though 90% cultures formed calli of which 70-85% developed complete microplants. Both development of direct shot and differentiation of callus occurred within 4-7 and 4-5 days, respectively, whereas it was 8-10 days for regeneration of microplant via callus. Within 35 days of culture period the microplants attained from 3.275 to 6.475 cm height and maximum number of 14.425 leaves and 3.825 roots microplants^{-1} were formed. Rooting of cultures occurred within 5-9 days in maximum 98% cultures and with 35 days culture period the maximum root length was 9.242 cm. The germplasms varied significantly for area of leaf in which maximum leaf area was 0.653 cm^2. The germplasms had an internode length of 3.825-5.302 mm and; the maximum rate of node production and microplant growth rate were 2.885 nodes week^{-1} and 1.850 mm day^{-1}, respectively.

1. Introduction

Sweet potato (*Ipomoea batatas* L.) belonging to the family Convolvulaceae is one of the most important food items in the world including Bangladesh. This crop is mainly cultivated by the marginal or subsistence farmers in a sporadic way in different river belts, char lands, deltas and seasonally inundated flood plains in the countries. It covers only 0.7% of the total cropped land of Bangladesh (BBS, 2003). Nutritionally, sweet potato is a good source of vitamins and minerals in addition to carbohydrate. The yellow flesh type cultivar contains high content of carotene, which could help reducing night blindness in the children. The 100g roots and leaves may content 15000 and 3000 IU carotene respectively (Rashid, 1999).

The sweet potato is conventionally propagated by vine cuttings in the tropics and sub-tropics. This method is ineffective for maintaining stock plants free from diseases and insect pests. In addition, considerable variation can occur within sweet potato over a period of time as a result of mutation. Most commonly used materials include excised embryos, shoot tips, pieces of stems, roots, leafs, protoplasts, etc. (Ushewokunze, 2000). *In vitro* regeneration of sweet potato has been reported utilizing storage root tissue (Yamaguchi and Nakajima, 1974), leaf tissue (Seghal, 1975) and meristem tip culture (Hossain et al., 1999). The techniques have not only the potential of producing a large number of propagules within a single year but also have added advantage of built-in disease protection. Rapid multiplication by tissue culture would enable newly selected varieties to be bulked up in disease-free condition for commercial use. According to Smith (2004), use of tissue-cultured sweet potatoes by smallholder farmers in Zimbabwe has been shown to improve household food security. Experiences in Zimbabwe on the impact of tissue-cultured sweet potatoes on yields and incomes have also been confirmed by other studies in developing countries (Moyo, 2004; Masumba, 2004). The economic implication of using tissue-cultured planting materials of sweet potato, in a context characterized by an artificial environment, has received little attention in Bangladesh. Hence, the present investigation was conducted to study the *in vitro* morphological growth characters and performance of 10 sweet potato germplasms under aseptic condition.

2. Materials and Methods

The experiment was conducted in order to study the *in vitro* performance and *ex vitro* establishment of 10 sweet potato germplasms at the Tissue Culture Laboratory of Tuber Crops

Research Centre (TCRC), Bangladesh Agricultural Research Institute (BARI) during during July to November, 2005. Ready stock *in vitro* microplants of all germplasms having luxuriant vegetative growth were cut into nodal sections for setting the experiment. The germplasms used were 199004.2, 199014.6, 199035.7, 199057.4, 199062.1, 199069.1, 440262, 440443, 187012.12 and 187015.1. Under aseptic condition, one month old microplants (4-5 cm in height) were taken out from the test tube and placed on a sterilized tile. Leaf and shoot tip from each microplant were discarded. Only nodal sections of about 1.0 cm long were cut aseptically. The individual explant was directly placed on medium in each test tube containing 10 ml of prepared culture media. Murashige and Skoog (1962) medium supplemented with 30 gL^{-1} sucrose, 0.2 mgL^{-1} thiamine HCl 0.25 mg pyridoxine HCl, 100 mgL^{-1} myo-inositol, 5 mgL^{-1} BAP and 6 gL^{-1} agar for regeneration was followed. All the cultures were kept at 24±2°C and the room was illuminated 16 h daily with a light intensity of 3000 lux.

The number of plants that survived after hardening *in vitro* plants, plant height, numbers of leaves, number of branches and the leaf area (LA) of the most fully expanded leaf. The experiment was arranged in Completely Randomized Design (CRD) with four replications with five tubes in each replication. One microplant was grown in each tube. Analysis of variance for different parameters stated above was performed and the mean separation among the germplasms was done using LSD (p=0.05 and p=0.01) values (Zaman et al., 1982). All these analyses were done using the MSTAT-5 package computer program. The percentage data were subjected to square root transformation before analysis according to Zaman et al. (1982).

3. Results and discussion

3.1. Cultures developed shoot directly

The percentage of cultures that developed shoot directly did not vary significantly. However, the maximum 25% cultures of the germplasms 199069.1, 187012.12 and 187015.1 developed shoots directly and the minimum was 10% of the germplasm 199014.6, while others ranged from 15-20% (table 1 and plate 1). Cheng and Yeh (2003 and 2004) cultured different parts of sweet potato on MS culture media with different supplements and obtained maximum 96.3% direct shoot regeneration which was contradictory to the finding of the present investigation. Whereas, Kim et al. (1992) also obtained direct shoot regeneration without callus.

3.2. Percentage of callusing

The cultures of most of the germplasms developed calli *in vitro*. The maximum callusing occurred was 90% in germplasms 199014.6 and 440443 (table 1 and plate 2). The lowest percentage of callusing was 75 in four germplasms (199035.7, 199069.1, 187012.12 and 187015.1). Callusing was found to be enhanced in most of the germplasms (table 1). Hossain et al. (1999) studied the in vitro performance of five standard sweet potato cultivars such as, Daulatpuri, Tripti, Kamalasundari, BARI SP-4 and BARI SP-5 and obtained maximum 70% callus formation. Result of the present study was better than that of Hossain et al. (1999).

3.3. Development of microplants via callus

A good number of microplants developed via callus in culture. The percentage of callus forming cultures followed by development of microplants ranged from 70-85 (table 1). The germplasms 199014.6 and 440443 developed maximum 85% microplants via callus, whereas only 70% of germplasm 187012.12 developed the same (table 1). Hossain et al. (1999) and Xin and Zhang (1987) obtained maximum 31% and 17.6% shoot regeneration via callus but it was upward 85% is the present study.

3.4. Days to direct shoot development

The number of days required for direct shoot development in cultures ranged from 4.001 to 7.375 (table 2). The germplasm 199014.6 developed direct shoot in culture earliest of 4.001

Plate 1: Development of shoots from cultures without forming any callus and development of shoots

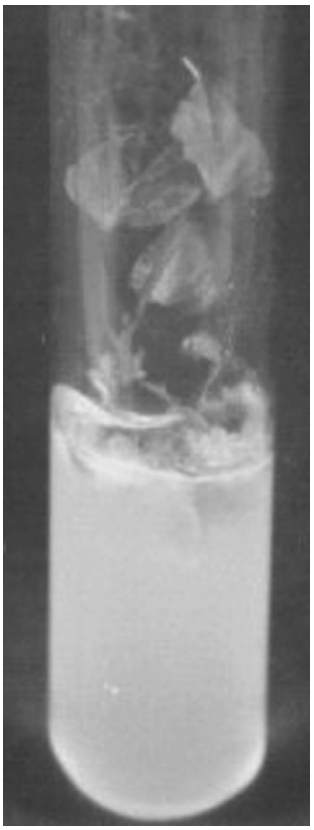

Plate 2: Development of shoots from cultures via callus

Table 1: The percentages of cultures of 10 sweet potato germplasms developed shoots directly or callus and microplants via callus

Germplasms	% culture developed		
	Shoot directly	Callus	Microplants via callus
199004.2	20.00 (3.817)	80.00 (8.909)	80.00 (8.909)
199014.6	10.00 (2.236)	90.00 (9.472)	85.00 (9.208)
199035.7	25.00 (4.280)	75.00 (8.609)	75.00 (8.609)
199057.4	20.00 (3.817)	80.00 (8.909)	75.00 (8.645)
199062.1	15.00 (3.354)	85.00 (9.208)	80.00 (8.909)
199069.1	25.00 (4.935)	75.00 (8.633)	75.00 (8.645)
440262.0	15.00 (3.354)	85.00 (9.173)	80.00 (8.909)
440443.0	15.00 (3.354)	90.00 (9.472)	85.00 (9.208)
187012.12	25.00 (4.935)	75.00 (8.645)	70.00 (8.345)
1897015.1	25.00 (4.935)	75.00 (8.645)	75.00 (8.645)
LSD (p=0.05)	NS	NS	NS

Figures in parenthesis are square root transformed data; NS=Not significant

Table 2: The number of days required for direct shoot development or callus differentiation and shoot differentiation via callus in 10 sweet potato germplasms

Germplasms	Days to direct shoot development	Days to callus initiation	Days to shoot differentiation via callus
199004.2	4.750	4.801	9.350
199014.6	4.001	5.201	9.702
199035.7	5.502	4.502	8.901
199057.4	5.875	5.003	10.400
199062.1	4.250	5.701	10.951
199069.1	7.375	4.502	8.351
440262	2.751	5.210	9.852
440443	5.012	5.812	10.902
187012.12	6.751	5.051	8.851
1897015.1	7.252	5.212	9.352
LSD (p=0.05)	NS	NS	NS

days compared to maximum days required by 199069.1 (7.252 days). Among 10 germplasms, three required between 4-5 days for shoot development, four between 5-6 days, one between 6-7 day and two above 7 days (table 2). In an experiment, Hossain and Sultana (1997) cultured three promising sweet potato germplasm namely, SP-412, SP-434 and SP-454 on MS and MMS media and recorded maximum 30.0% shoot development directly on MS culture media. Whereas, SP-434 showed 32% direct shoot development. These results support the findings of the present investigation.

3.5. Days to callus initiation

All the germplasms required statistically the same number of days for callus initiation (4-5 days). However, among 10 germplasms, three germplasms showed callus initiation within 4-5 days. The rest seven germplasms took more than 5 days for callus initiation. Callus initiation was earlier in germplasms 199035.7 and 199069.1, each within 4.502 days, whereas, the process was delayed by 5.812 days in case of germplasm 440443 (table 2). Gradual callus formation in culture was observed by Hossain et al. (1999). They recorded 35% callusing in 10 days period which arose to 70% in 30 days. These wide variations may be due to using different cultivars. The results were contradictory to the finding of the present investigation.

3.6. Days to shoot regeneration from callus

Shoot formation in culture via callus took longer time, ranging from 8.351-10.951 days. The germplasm 199069.1 developed shoots via callus earliest (8.351 days) compared to maximum days required by 199062.1 (10.951 days). Among to germplasms, three developed shoots via callus within 8-9 days, four within 9-10 days and three took more than 10 days (table 2). Hossain et al. (1999) studied *in vitro* growth of five sweet potato cultivars and obtained regeneration of shoot via callus up to 70%. They recorded first shooting after 30 days of cultures. But, in the present investigation, shoot development via callus completed within 10.951 days.

3.7. Height of microplants

The germplasms varied significantly for their height *in vitro*. The germplasm 199035.7 attained the maximum height of 6.475 cm compared to minimum of 3.275 cm by the germplasm 199069.1 (table 3). The height of microplants ranged from 3.275-6.475 cm. The germplasms 199057.4, 199069.1 and 440443 attained the minimum height of 3.412, 3.275 and 3.551 cm, respectively; whereas, the germplasms 440262, 187012.12 and 187015.1 attained height of more than 6.0 cm (table 3). Sweet potato appeared to have very slow growth *in vitro*, through *in vitro* growth rate of microplants of the present investigation was quite good, ranging from 3.275-6.475 cm after 35 days. While Hossain et al. (1999) recorded 4.0 cm height of microplants of sweet potato after 50 days.

3.8. Number of leaves microplant^{-1}

The number of leaves microplant^{-1} varied significantly among the germplasms. The maximum number of leaves was produced

Table 3: *In vitro* growth of microplants of ten sweet potato germplasms

Germplasms	Microplants growth parameters at 35 days		
	Height (cm)	No. of leaves	No. of roots
199004.2	4.425^{ab}	7.751^{d}	2.075
199014.6	6.125^{a}	13.701^{ab}	3.825
199035.7	6.475^{a}	8.175^{cd}	1.151
199057.4	3.412^{b}	11.802^{abc}	1.825
199062.1	4.702^{ab}	11.425^{abcd}	2.802
199069.1	3.275^{b}	9.001^{cd}	1.525
440262	6.325^{a}	9.125^{cd}	2.751
440443	3.551^{b}	14.425^{a}	1.251
187012.12	6.125^{a}	13.102^{ab}	2.025
1897015.1	6.101^{a}	9.925^{bcd}	1.575
LSD (p=0.05)	1.68	2.85	NS
LSD (p=0.05)	2.26	3.85	NS

Same letter (s) in a column do not vary significantly

by the germplasm 440443 (14.425) which was statically similar 199014.6 (13.701), 199057.4 (11.802), 199062.1 (11.425) and 187012.12 (13.102). The germplasm 199004.2 formed the least number of leaves microplant^{-1} (7.751), which was statistically similar to 199062.1 (11.425) (table 3). In the present investigation, the rate of leaf production was vary high because Hossain et al. (1999) obtained 3-4 leaves microplant^{-1} in 5-7 weeks.

3.9. Number of roots culture^{-1}

The germplasms varied insignificantly for the number of roots microplant^{-1} (table 3). However, the maximum number of roots microplant^{-1} was 3.825 in germplasm 199014.6. The germplasm 199004.2, 199062.1, 400262 and 187012.12 formed respectively 2.075, 2.802, 2.751 and 2.025 roots microplant^{-1}, the rest formed roots between 1 and 2 (table 3). The minimum number of roots microplant^{-1} was found in 199035.7 (1.151). Carswell and Locy (1984) found profuse root formation in sweet potato explants in culture. But, in the present study, the number of root was maximum 3.825, which was very low.

3.10. Area leaf^{-1}

The average leaf area of the germplasms varied significantly. The maximum average leaf area was 0.653 cm^2 in germplasm 199004.2 which was statistically similar to 199069.1 (0.461 cm^2). The rest of the germplasms were statistically in the same bracket, ranging from 0.291 cm^2 to 0.433 cm^2 leaf^{-1} (figure 2).

3.11. Average length (mm) of internodes

The germplasms did not vary significantly for the average length of internode. The average length of internode varied in the range of 3.825-5.302 mm. The germplasm 199014.6 scored the highest average length of internode (5.302 mm) and the lowest was with the germplasm 199057.4 (3.825 mm). Only two germplasms, 199014.6 and 187012.12 had an average length of internode above 5.0 mm and six had above 4.0 mm and two above 3.0 mm (table 4). Length of internode was found to be closely related to the growth of microplants, which is governed by a number of chemical and physical factors. Moreover, low light intensity makes plant taller and lanky, resulting in increasing larger internode. In the present investigation, the length of internode was too small, which might be due to high light intensity or might be of germplasms-specific.

3.12. Rate of node production

The rate of node production varied significantly among the germplasms. The germplasm 440443 had the maximum rate of node production week^{-1} (2.885), which was statistically similar to 199014.6 (2.741), 199057.4 (2.361), 199062.1 (2.285) and 187012.12 (2.620) (table 4). The rest of the germplasm had rate of node production ranging from 1.551-1.985 nodes week^{-1}. Calculation of rate of node growth is an important parameter related to *in vitro* growth of microplant. Production of more

Table 4: *In vitro* measurement of internode and microplants growth of ten sweet potato accessions

Germplasms	Length of internode (mm)	Rate of node production (number week^{-1})	Microplant growth rate (mm day^{-1})
199004.2	4.225	1.551^{d}	1.264^{ab}
199014.6	5.302	2.741^{ab}	1.751^{a}
199035.7	4.351	1.635^{cd}	1.850^{a}
199057.4	3.825	2.361^{abc}	0.971^{b}
199062.1	4.402	2.285^{abcd}	1.343^{ab}
199069.1	4.975	1.802^{cd}	0.936^{b}
440262	4.701	1.825^{cd}	1.807^{a}
440443	3.925	2.885^{a}	1.014^{b}
187012.12	5.125	2.620^{ab}	1.751^{a}
1897015.1	4.625	1.985^{bcd}	1.743^{a}
LSD (p=0.05)	NS	0.57	0.479
LSD (p=0.05)	NS	0.77	0.646

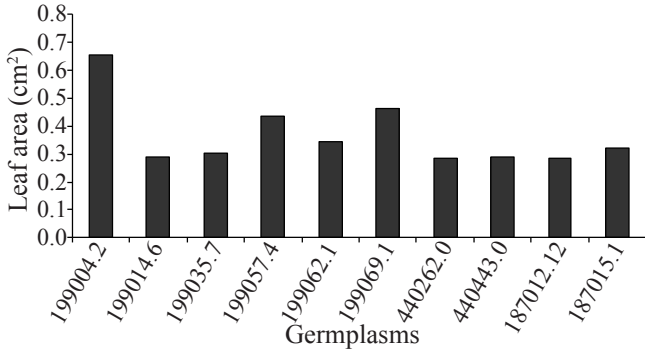

Figure 1: The average leaf area (cm^2) of ten sweet potato germplasms at the age of 35 days after culture on MS culture media and under 16-h photoperiod from 3000 lux light intensity. Bar indicates LSD values at 1% level.

than 2 nodes $week^{-1}$ was quite good, though many reported much less (Hossain et al., 1999; Carswell and Locy, 1984). Liu et al. (1999 a,b) micropropagated sweet potato nodes on MS culture media and incubated under 16-18 h photoperiod and obtained a very good rate of node production (ca. 2.0 $week^{-1}$) which is in agreement with the findings of the present investigation.

3.13. Microplant growth rate

In vitro microplant growth rate varied significantly among the germplasm of sweet potato. The maximum growth rate of microplant was recorded 1.850 mm day^{-1} in germplasm 199035.7, which as statistically similar to germplasms 199014.6 (1.751 mm day^{-1}), 199062.1 (1.343 mm day^{-1}), 440262 (1.807 mm day^{-1}), 187012.12 (1.751 mm day^{-1}) and 187015.1 (1.743 mm day^{-1}) (table 4). The lowest growth rate of microplant was 0.936 mm day^{-1} in germplasm 199069.1, which was also statistically inferior (table 4). According to de Fossard (1978), growth of microplant *in vitro* is dependent to a number of factors such as, nutrient culture media composition, hormone used and environmental factor. In the present, the growth rate of microplant was quite good (max. 1.807 mm day^{-1}) through much less reported elsewhere (Hossain et al., 1999).

4. Conclusion

Growth performance of 10 germplasms would help further *in vitro* propagation and *in vitro* maintenance breeding of valuable sweet potato germplasm. In addition, *in vitro* growth performance and *ex vitro* establishment would help large-scale multiplication the same under *ex vitro* condition. In Bangladesh context, attempts for growth performance of the tissue-cultured sweet potato germplasms in farmer levels are suggested as challenges like food crisis and climate changes are more intensifying now-a-days.

5. Acknowledgements

The authors are highly grateful to Dr. Lutful Hassan, Professor of Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh for his guidance to perform this research work. Authors also extend their thanks to Md. Jahangir Hossain, Senior Scientific Officer of Bangladesh Agricultural Research Institute, Gazipur, Bangladesh for supplying germplasm materials and providing laboratory facilities to conduct the experiment.

6. References

BBS, 2003. Statistical Year Book of Agriculture. Bangladesh Bureau of Statistics. Govt. of the Peoples' Republic of Bangladesh.

Carswell, G.K., Locy, R.D., 1984. Root and shoot initiation by leaf, stem and storage root explants of sweet potato. Plant Cell Tissue and Organ Culture 3, 229-236.

Cheng, H.H., Yeh, M.S., 2003. Studies on tissue Culture of Sweet Potato. The effects of different kinds and concentration of auxin on plant regeneration of explants from different parts. Journal of Agriculture and Forestry 52, 63-79.

Cheng, H.H., Yeh, M.S.. 2004. *In Vitro* Culture of Sweet Potato. III. Histological studies on root and shoot regeneration from leaf cultures of *Ipomoea batatas* (L.). Crop Environment and Bioinformatics 1, 102-111.

de Fossard, 1976. Tissue Culture for Plant Propagators. Department of Botany, University of New England. Armidale, N.S.W. 2351, Australia, 36-94.

Hossain, M.J., Sultana, N., 1997. *In vitro* propagation of sweet potato (*Ipomoea batatas* L.). Plant Tissue Culture 7, 89-95.

Hossain, M.J., Sultana, N., Ahmad, A.U., 1999. *In vitro* performancr of five standard sweet potato cultivars (*Ipomoea batatas* L). Plant Tissue Culture 9:151-157.

Kim, J.H., Jung, H., Park, S.W., Jeon, J.H., 1992. Plant regeneration of sweet potato (*Ipomoea batatas* (L.) Lam.). from root tissue cultured *in vitro*. Journal of Korean Society of Horticultural Sciences 33, 111-117.

Liu, X.T., Hang, B.Y., Liu, W.X., Hai, Y., He, X.C., Luo, P., 1999a. Determination of suitable media for the rapid multiplication of virus free sweet potato plants. Journal of Henan Agricultural Sciences 4, 4-5.

Liu, X.T., Huang, B.Y., Liu, W.X., Luo, P., Hai, Y., 1999b. Effect of nutrient elements in the medium on rapid multiplication of seedlings *in vitro* in virus-free sweet potato. Journal of Henan Agricultural Sciences 4, 6-7.

Masumba, E.A., 2004. Participatory evaluation of improved

sweet potato varieties in Tanzania. African Crop Science Journal 12(3), 259-266.

Moyo, C., 2004. Cassava and sweet potato yield assessment in Malawi. African Crop Science Journal 12(3), 295-303.

Murashige, T., Skoog, S., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologia Plantarum 15, 473- 497.

Rashid, M.M., 1999. Vegetables of sweet potato family (*Mitha alur paribarar sabji* in bengali). Shabji Biggan, Rashid Publishing House, DOHS, Dhaka, 410-435.

Seghal, C.B., 1975. Hormonal control of differentiation in leaf cultures of *Ipomoea batatas*. Biochemie und Physiologie der Pflanzen 51, 47-51.

Smith, M., 2004. Born-again crops give hope to Zimbabwean farmers: Ian Robertson and his colleagues have found a way to free staple crops from viruses, with dramatic results for their growers. *The Free Library*. Available on the World Wide Web: http://www.thefreelibrary.com/Born-again+crops+give+hope+to+Zimbabwean+ farmers: + and …a0126583061.

Ushewokunze, U., 2000. Potentials of biotechnology in animal science development in Zimbabwe. In: Workshop on State of Biotechnology Research and Priorities in Zimbabwe held by the Biotechnology Trust of Zimbabwe, Harare, Zimbabwe.

Xin, S.Y., Zhang, Z.Z., 1987. Explant tissue culture and plantlet regeneration of sweet potato. Acta Botanica Sinica 29, 114-116.

Zaman, S.M.H., Rahim, K., Howlader, M., 1982. Simple Lessons from Biometry. Publication no. 54. Bangladesh Rice Research Institute, Dhaka, Bangladesh, 82-92.