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In Vitro Meristem Culture and Regeneration of Three Potato Varieties of Bangladesh

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This paper describes a regeneration protocol by *in vitro* meristem culture to get disease free potato plantlets. Three recently released varieties of potato (*Solanum tuberosum* L.) namely Esprit, Lady Rosseta and Meridian were used for this work. In all the three varieties, the percentage of survived explants was the maximum in surface sterilization with 0.1% $HgCl_2$ for a period of 4 minutes when cultured in MS basal medium. After having DAS-Elisa test, the *in vitro* grown plantlets were being used for massive micropropagation. In order to get multiple shoot buds in shoot apex and nodal segment and their further elongation, MS medium supplemented with 1.0 mg / l BAP+1.0 mg / l GA_3 was proved best in case of Esprit and Meridian whereas in Lady Rosseta MS medium fortified with 1.0 mg/l BAP+0.5 mg/l GA_3 was the best. Various rooting media were used for induction of well-developed root system in elongated shoot buds. MS medium supplemented with 0.5 mg/l IAA was found appropriate for induction of well root system in all the three varieties.

Keywords: Meristem culture, regeneration, disease free plantlet, Plant Growth Regulators.

Potato is an important tuberous food crop. It is also the most productive economically important and widely grown vegetable crop cultivated and consumed in Bangladesh. In 2008, several international organizations highlighted the role of potato in world food production, in the face of developing economic problems. Due to perishability, only about 5% of the world's potato crop is traded internationally, its minimal present in world financial markets contributed to its stable pricing during the 2007-2008 world food price crises. Thus the United Nations officially declared the year 2008 as the International Year of the potato, to raise its profile in developing nations, calling the crop a "hidden treasure". In Bangladesh, area under potato cultivation has been increasing over the past several years with an enhanced production (BBS, 1999). However, the average yield of potato in Bangladesh is several times lower than that of many European countries.

Although potato is being considered as one of the main food crop in Bangladesh, its productivity is hampered due to infection of virus, fungus and bacterial diseases. The total loss caused by these diseases is 30-100% during cultivation and storage. Potato varieties with higher yield, better agronomic quantities, and resistance to disease are immense importance for increased production.

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Common potato diseases in Bangladesh are caused by potato viruses X, Y, A, M and leaf roll virus. To overcome these impediments, both conventional and biotechnological breeding programmes need to be applied. Disease free and genetically uniform plantlets may be produced by meristem culture through tissue culture techniques (Hoque *et al*., 2007). Plant tissue culture techniques have been employed in a large number of important potato varieties in agriculture (Hashem *et al*., 1990).

The application of tissue culture and rapid propagation method for potato production continues to become more widely used in both developed and developing countries. Tissue culture techniques can be applied not only to increase propagation rates, but also to modify the germplasm itself while conserving the present resources. For many years tissue culture has been applied to improve potato production by means of micropropagation, pathogen free propagule development, and germplasm conservation (Roca *et al*., 1978; 1979).

The present research study was conducted with aspects of *in vitro* meristem culture. The first major breakthrough in the field of tissue culture was made when Robbins (1922) and Kotte (1922) independently observed growth of root tips on mineral solutions supplemented with sugar, aspirin, and peptone. Later White (1943) grew cultures of tomato root tips for a longer period in a nutrient medium with sucrose, inorganic salts, and yeast extract.

Many improvements in the media have been made after that. Morel and Martin (1952) were the first to use meristem culture to eradicate viruses from potatoes. A useful nutrient medium is the one devised by Murashige and Skoog (1962). Increased concentrations were found to be critical for the culture of meristem tips of potato, which developed into plantlets, used lower concentrations of microelements (Morel and Muller, 1964).

Smith and Murashige (1970) accomplished the first true meristem culture of an isolated shoot angiosperm meristem into a complete plant.

Meristem tip culture of cassava became popular and research was oriented towards elimination of virus disease for recovery of healthy clones in 1980s (Kartha and Gamborg, 1975). Meristem culture technique has been applied to many crops, especially vegetative propagated crops such as potato, over the last 40 years to eliminate viruses from important cultivars (Bhojwani and Razdan, 1983; Hartmann *et al*., 1990). Tissue culture has been used for both basic and applied purposes in potato programmes (Dodds, 1988). Over 20 viruses may infect potatoes and hence meristem culture and tissue culture techniques have been adopted for virus elimination and maintenance and propagation of nuclear seed stocks, respectively (Bryan, 1988; Jones, 1988).

Huda and Sikdar (2006) developed a protocol for *in vitro* plant production through apical meristem culture of bitter gourd (*Momordica charantia* L.). The growth of meristem was observed on semisolid MS medium supplemented with 0.05-mg/l Kn + 0.1mg/l GA_3. After three weeks meristems were transferred to MS supplemented with BA, Kn, IBA, NAA and IAA singly or in combination for shoot elongation and root induction. Among different treatments for shoot initiation with elongation were obtained in MS medium supplemented with 1.0 mg/l BA+0.1 mg/l IBA+0.3 mg/lGA_3.

Aasim *et al*. (2008) established a protocol for *in vitro* micropropagation from shoot meristems of Turkish cowpea (*Vigna unguiculata* L.). Multiple shoots from shoot meristem of three-five-day-old *in vitro* grown seedlings of Turkish cowpea (*Vigna unguiculata* L.) cv. Akkiz was obtained in MS supplemented with 0.50 mg/l BAP-0, 0.10, 0.30 and 0.50 mg/1 NAA.

Materials and methods

Plant materials

Three potato varieties Esprit, Lady Rosseta, and Meridian were used in this experiment. Tubers were collected from Tuber Crop Research Centre of Bangladesh Agricultural Research Institute (BARI) and maintained in the Plant Tissue Culture and Biotechnology Laboratory of the Department of Botany, University of Chittagong.

Sprout initiation

Collected potato tubers were kept in the incubator at 18°C. The distinct sign of sprouting was visible within 15 to 20 days. The sprouts were collected at optimum size and the tubers were kept in the incubator as usual to get further sprouts, as shown in Fig.-1. A single tuber could be used several times to collect sprouts.

Surface sterilization

For surface sterilization, the potato sprouting measuring 1.0 to 2.0 cm in length were cut from the tubers and washed with distilled water followed by dipping in 70% alcohol for 30 seconds and immediately thereafter washed with distilled water and subsequently sterilized in the laminar air flow cabinet with 0.1% aqueous solution of $HgCl_2$ for 4 to 7 minutes. Surface sterilized sprouts were washed 4 to 5 times with sterilized distilled water.

Inoculation of sprouts

The surface sterilized sprout cuttings were transferred into a sterile Petri dish. About 2.0 to 3.0 mm long sprouted apexes were excised with the help of sterilized scalpel and forceps. The cuttings were inoculated into agar solidified MS medium (Basal). In each test tubes and flask a single cutting and two or three cuttings were placed in the medium respectively keeping the position of the nodes upside as shown in Fig.-2(a).

Incubation of inoculated culture flasks

The inoculated test tubes and conical flasks were incubated under florescent light at 25±2°C temperature. The sprout cuttings give raise shoot bud within 2 to 3 weeks as shown in Fig.-2(b). The shoot bud was sub-cultured regularly in order to get large scale of shoot apex and nodal segments.

Fig.-1: Lady Rosseta with sprout

Fig. 2: (a) Sprouted apex culture (var. Meridian), (b) Regeneration from cultured sprouted apex (Lady Rosseta).

DAS-ELISA test

Before shoot multiplication, a serological identification was done in the cultured plants and to detected virus. In this detection, the double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) methods were followed. Virus free plantlets were used for mass propagation in

MS medium supplemented with different kinds of PGRs.

Subculture of shoot apex and nodal segments

Shoot apex from shoot bud and nodal segments from shoot bud were transferred to fresh MS media regularly at an interval of 3-4 weeks for maintenance and was regularly examined for different morphogenesis development.

Multiple shoots regeneration media

For the purpose of shoot initiation, MS basal medium (Murashige and Skoog, 1962) and MS medium supplemented with various plant growth regulators (PGRs) combination and concentrations of BAP, Kn, GA_3 were used for three weeks of culture.

Rooting

For the induction of roots of the *in vitro* grown shoots, full strength MS medium, half strength MS medium and ¼ strength MS medium were used. Different combinations of different auxins (IAA, IBA) were used with this strength of MS medium.

Results and Discussion

The present investigation was carried out with a view to develop efficient protocols for *in vitro* regeneration. At present, food crisis is arising because of high rate of population growth particularly in developing countries. The techniques of plant cell and tissue culture have become popular and useful methods in solving many of the related problems in agriculture and forestry (Rao and Lee, 1986).

Sprouted apex was used as a source of meristem. Before incubation for regeneration and controlling contamination, the explants were decontaminated by 0.1% mercuric chloride ($HgCl_2$) and the effects of the duration of surface sterilization periods with $HgCl_2$ were observed during the regeneration from sprouted apex. The Sterilization period for these explants has been found to be important in obtaining desired regeneration. Before treating with 0.1% $HgCl_2$, collected explants from tuber were washed thoroughly under running tap water and then washed with continuous shaking in savlon water for 5-10 minutes. The sprouts were excised and decontaminated by 0.1% $HgCl_2$ and grown aseptically in MS medium. In the regeneration experiments the effects of the duration of surface sterilization periods with 0.1% mercuric chloride ($HgCl_2$) was investigated for 20 explants of each variety. Percentage of contaminated explants decreased with increased sterilization period in all the varieties. However, an increase in sterilization period also caused a decrease in the survivability of the non-contaminated explants as presented in Fig.-3. Islam (1990) and Mila (1991) used 20 minutes for surface sterilization periods with 0.1% $HgCl_2$. Whereas Khan and Rabbani (1999) used a 15-minute sterilization period and Sarker and Mustafa (2002) used a 15-minute sterilization period in case of different potato varieties for sterilization during regeneration. In the present study an optimum response, in terms of reduced percentage of contamination was observed with 7 minute of surface sterilization and increased percentage of responsive explants (survivability) was noted with 4 minutes of surface sterilization in case of Esprit, Lady Rosseta and Meridian. The effects of different treatment duration on surface sterilization of sprout apex are shown variety wise in Fig.-3.

In vitro regeneration was done with the use of apical meristem of *in vitro* grown potato sprouts. Potato is usually vegetative propagated and very much susceptible to the number of viral diseases, which cause remarkable decrease in yield. The technique of meristem culture is one of the important methods of production of virus free plants (Morel and Martin, 1952). This technique has been widely used in development of virus free plants in different species including

potato, pineapple, sweet potato, soyabean, tobacco, zinger and cabbage.

Different concentrations of BAP (0.5-2.5 mg/l) and Kn (0.5-2.5 mg/l) were used separately to see their effect on multiple shoot regeneration in 50 explants. Between the two cytokinins used here BAP showed better response in terms of number of shoots per explants as well as shoot length as shown in Fig.-4. A Similar effect of BAP was also observed by Islam (1990), Mila (1991), Sarkar and Mustafa (2002). In case of Esprit and Meridian maximum number of shoots per shoot apex and nodal segment was formed at 1.0 mg/l BAP with MS media. In case of Lady Rosseta it was MS medium containing 0.5 mg/l BAP. Bhuiyan *et al*. (1992) observed the positive effects when they used BAP in MS medium for shoot proliferation in peanut. In the present investigation results also reveal that the number of shoots and shoot length decreased with higher concentrations of Kn and BAP. It was also observed that either very low (0.5 mg/l) or very high (2.5 mg/l) concentrations of BAP or Kn decreased shoot length and shoot per explants. Similar findings were obtained by (Hoque *et al*., 1996b) in some other potato varieties.

Different concentrations of GA_3 were used in combination with BAP or Kn to see their combined effect on induction and elongation of regenerated shoots in 20 explants. In Esprit and Meridian maximum numbers of healthy shoots with well-expanded leaves per explants were produced on MS medium with 1.0 mg/l BAP+1.0 mg/l GA_3. For Lady Rosseta, it was 1.0 mg/l BAP+0.5 mg/l GA_3 as shown in Fig. 5. GA_3 was found to be the most effective in increasing shoot length as illustrated in Fig.6. These observations are in agreement with those of Goodwin *et al*. (1980), Hoque *et al*. (1996a), Mila (1991) and Novak and Zadina (1987) in different potato varieties.

It is well known that the multiplication and the growth of shoots under *in vitro* condition varied with media composition (Hussey and Stacey, 1981; Miller *et al*., 1985; Mumtaz and Quraishi, 1989). It may be mentioned here that among the three potato varieties used in the present investigation Lady Rosseta showed best response. Variable responses of different potato varieties due to genetic makeup *in vitro* shoot multiplication and their development was also reported by Hussey and Stacey (1981), Miller *et al*. (1985), and Bajaj (1981).

Induction of healthy roots from the base of regenerated shoot buds is an essential part of successful development of plantlet. During the present study, it was noticed that several roots spontaneously from *in vitro* grown shoots. These were inadequate for transplantation of the *in vitro* grown plants. Among the various auxins IAA and IBA were used for root induction. MS medium supplemented with 0.5 mg/lAA showed the best response in producing roots in 20 shoot buds 18-21 days of culture as presented in Fig. 7 and Fig. 8. However, Sarker and Mustafa (2002) found best response in 1.00 mg/l IAA supplemented MS medium for root induction. On the other hand, MS medium supplemented with 2.0 mg/l BAP to be best for rooting in Cardinal variety by Islam (1990).

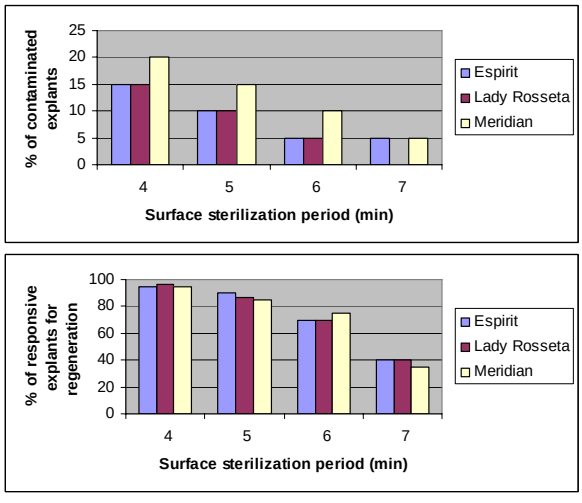

Fig. 3: Effects of different time duration of 0.1% $HgCl_2$ treatments.

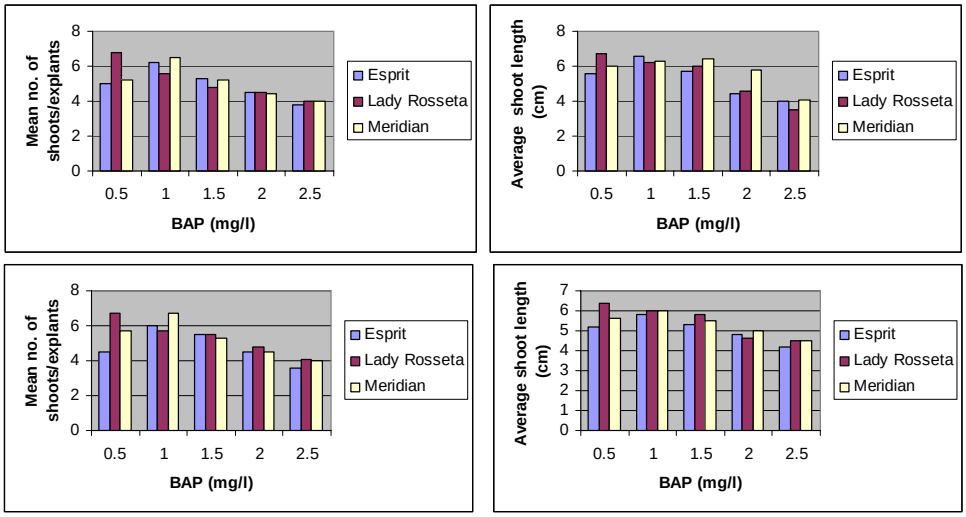

Fig. 4: Effects of different concentrations of BAP with MS medium (a) shoot apex (top), (b) nodal segments (bottom).

Fig. 5: (a) Nodal segment culture of Lady Rosseta in 1.0 mg/l BAP+0.5 mg/l GA_3 with MS medium, (b) Culture of nodal segment of Meridian in 1.0 mg/l BAP+1.0 mg/l GA_3 with MS medium.

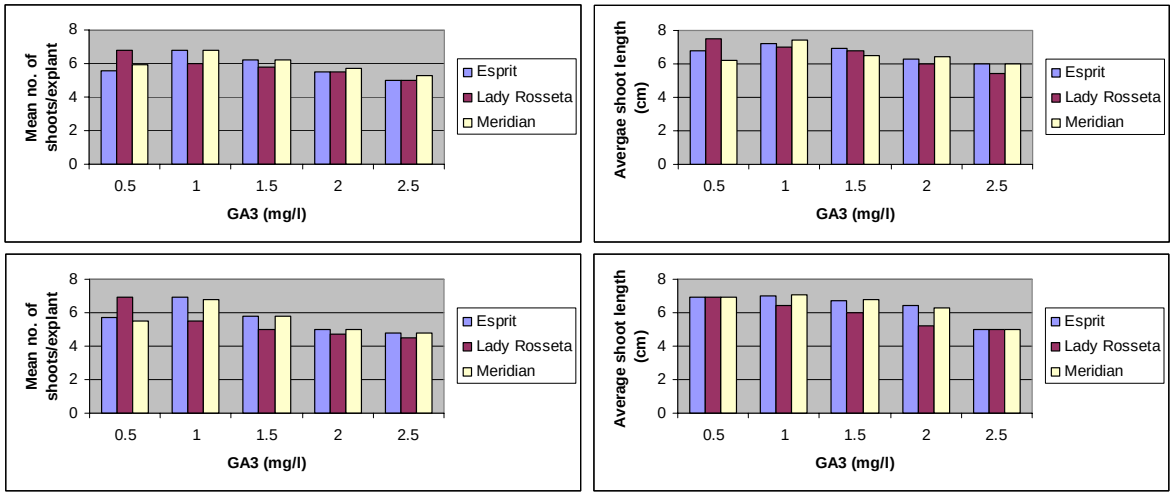

Fig. 6: Effects of various concentrations of GA_3 added with BAP (1 mg/l) in MS medium on induction and elongation of regenerated shoots from shoot apex (top) and nodal segments (bottom) of potato varieties.

Fig. 7: (a) Development of roots of regenerated shoots of Meridian on MS medium supplemented with 0.5 mg/l IAA, (b) Rooting of regenerated shoots of Esprit on MS medium supplemented with 0.5 mg/l IAA.

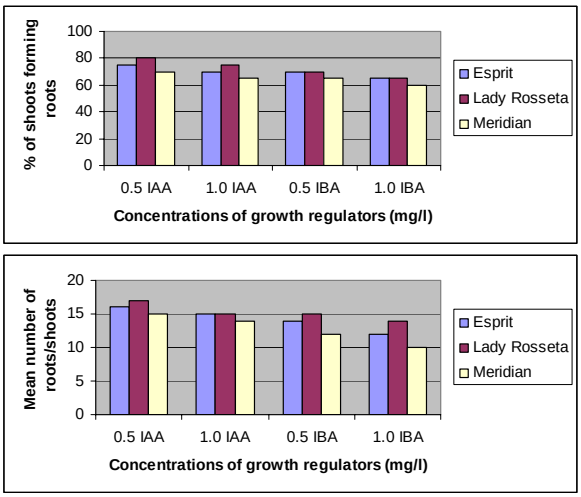

Fig. 8: Effects of different growth regulators supplemented in MS medium.

Conclusion

The present study was undertaken with a view to optimizing *in vitro* propagation technique considering for meristem culture in three potato varieties of Bangladesh and reveals that three varieties of potatoes are disease free and can be propagated in best performance showing media for large-scale production and also can be conserved as virus free germplasm.

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