SUMMARY AND CONCLUSIONS

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Plant Biotechnology is increasingly contributing to the improvement in biological productivity, especially in the field of production and propagation of new cultivated disease-free varieties of plants. In the case of root and tuber crops, continuous vegetative propagation often results in deterioration in quality of planting material. Improper selection of planting materials leads to perpetuation of viral, bacterial or fungal pathogens through clonal regenerations, resulting in severe crop loss. Thus, production of healthy planting materials and their rapid multiplication is necessary for the management of genetic resource of tuber crops.

Potato (*Solanum tuberosum* L.) is one of the most important food crops in the world after wheat, rice and maize. As it is propagated vegetatively, there are several problems for its breeding, including a high level of heterozygosity, the common occurrence of pollen sterility, selection difficulties in the seedlings and first clonal years, slow rate of increase, difficulties in germplasm storage and transport. Moreover, potatoes are infected by more than 30 viruses. Once a potato clone is infected by a virus, the disease gets perpetuated and amplified during successive clonal generations, leading to a significant reduction in the yield potential of the crop.

Such requirements have led to the utilization of micropropagation of potatoes by using biotechnological approaches. An alternative method to the micropropagation of potato is the production of microtubers, which is produced by allowing *in vitro* plantlets to grow under tuber-inducing conditions. Microtubers are particularly convenient for handling, storage and transport of germplasm. Also, unlike *in vitro* propagated plants, they do not require any hardening treatment. But the major limitation of the microtubers is their small size. Small microtubers are more vulnerable to storage damage and are unsuitable for direct field planting. For commercial seed potato production, microtuber size is more important than microtuber number.

Keeping all these points in mind present studies were undertaken with a view to–

1. standardize a protocol for *in vitro* establishment of sprout tips
2. standardize a protocol for shoot multiplication
3. standardize conditions for optimum production of microtubers

4. understand the development of microtubers
5. understand the histochemical changes during microtuberization viz. changes in the levels of carbohydrates
6. standardize the storage condition, shelf life and germination of microtubers
7. produce synthetic seeds of potato using nodal segments and standardizing the conditions for their storage and germination
8. produce minitubers from microtubers

of two varieties of potato viz. var Dheera (late maturing) and var Heera (early maturing), which are the most preferred varieties in Bangladesh.

0.5-1cm long sprout tips were collected for the initial establishment of *in ivtro* shoots. Lowest percent of contamination was observed when explants have been given a pre-treatment with a combination of Chloramphenicol, bavistin and activated charcoal for a period of 4-6 hours (4 h. for var DR, 6 h. for var HR) prior to their sterilization with 0.08% $HgCl_2$ for 4-6 min. (4 min for var DR, 6 min for var HR). Pre-treated and surface sterilized sprout tips were established on MS basal medium. Single node cuttings from these *in vitro* shoots were cultured on MS medium supplemented with various concentration and combination of hormones. It was observed that, NAA alone at 0.1 mg/l significantly increased the length of shoot and number of nodes per shoot compared to the control. However, combinations of NAA and BAP did not show any significant effect on production of healthy shoots and leaves in both the varieties. A combination of GA_3, NAA and Ca-pantothenate showed significant increase in the length of the shoots as well as number of nodes per shoot.

Production of healthy shoots with large green leaves (Mean length 4.4 cm; Mean number of nodes, 3.8) was observed in var DR under the influence of 0.1 mg/l GA_3, 0.01 mg/l NAA and 2 mg/l Ca-pantothenate. However, significant improvement in both the length of the shoot (5.1 cm) and number of nodes per shoot (3.4) was observed in var HR in a medium supplemented with 0.25 mg/l GA_3 0.01mg / l NAA and 2mg/l Ca-pantotharate.

A combination of IBA (0.5 mg/l), ADS (0.5 mg/l) and BAP (1.5 mg/l) showed significant increase in the length of the shoots (8.0), as well as in the number of nodes

per shoot (6.0) in var DR, but produced thin and unhealthy shoots. Similar results were observed in var HR.

Healthy shoots with green leaves were observed in var DR when single nodes were cultured on MS liquid medium supplemented with 0.1 mg/l GA_3, 0.01 mg/l NAA, 2 mg/l Ca-pantothanate and 1 mg/l Thiamine HCl. Mean length of the shoots was 7.1 cm and number of nods per shoot 5.4. Similar results were observed in var HR when MS liquid medium was supplemented with 0.25 mg/l GA_3, 0.01 mg/l NAA, 2 mg/l Ca-pantothenate and 1.0 mg/l Thiamine-HCl. It was observed that shoot production was better and faster in liquid medium than in solid medium.

Highest number of shoots was obtained when *in vitro* shoot segments with three nodes were cultured in liquid MS medium with agitation. (10.6 for var DR, 8.6 for var HR). Incorporation of $AgNO_3$ in this liquid propagation medium though did not bring about any increase in the total number of shoots per flask in var DR but produced healthy green shoots with larger leaf lamina. Incorporation of $AgNO_3$ at 0.5 mg/l concentration significantly increased the number (10.6) as well as produced healthy shoots in var HR.

For tuber induction, optimum sucrose level observed was 8% for both the varieties. Continuous dark condition and low temperature increased the number as well as size of the tubers in both the varieties. Production of microtubers also depends on the types of medium used for tuberization. Good size of tubers with satisfactory numbers were produced when shoots produced in liquid agitated culture were supplied with liquid tuberization medium. Varietal difference was observed in the requirement of BAP and CCC in tuberization medium. Maximum number of tubers were produced in var DR in medium supplemented with 5.0 mg/l BAP and 500 mg/l CCC (11.66). However, var HR required 7.5 mg/l BAP and 750 mg/l CCC for maximum number of tubers (15.33). Addition of $AgNO_3$ in tuberization medium resulted in significant increase in the size of the tubers. It was observed that shoots produced in medium supplemented with 0.5 mg/l $AgNO_3$ along with GA_3, NAA, Ca-pantothenate and Thiamine-HCl when exposed to tuberization medium containing 15 mg/l $AgNO_3$, produced large number of tubers with maximum total fresh weight and diameter. It was also observed that concentration of GA_3 in propagation medium, more than 2 mg/l has

inhibitory effect on tuberization even though tuberization medium was supplemented with $AgNO_3$, BAP and CCC.

After harvesting, microtubers were stored at 4°C in small petridishes sealed with parafilm. Both the varieties showed maximum percentage of germination after the end of four months storage. Percentage of germination decreased from five months onward. One week before planting microtubers were removed from the refrigerator for sprouting. A grade (>0.800 gm) and B grade (>0.400 gm) microtubers showed 100% germination as well as 100% survival rate during the field trial. However, survival rate of the plants raised from C grade (<0.400 gm) was comparatively less than A and B grade tubers in both the varieties. Plants emerged from microtubers had only single shoot. Within 100 days minitubers reached to its maturity and was ready for harvesting. Highest yield in case of both the varieties has been obtained from plants raised from A grade microtubers.

Nodal segments of shoots cultured on MS medium were encapsulated using 3% sodium alginate. They exhibited 73 and 66% of sprouting in var DR and var HR respectively when cultured on MS basal medium on the first day of encapsulation. The performance of these encapsulated nodal segments were very poor when planted in a mixture of soil and peat (1:1).

During the formation of microtubers, four developmental stages were observed, (a) bud initiation, (b) bud growth, (c) cessation of bud or axillary shoot growth and (d) tuber initiation. Starch accumulation was noticed from the 1st day itself. The size and number of the starch grains increases with the increase of the size of microtubers. During the initial stages of microtuber development starch deposition was more in the cortical region than that of the pith region. Periderm formed gradually on the outside of the tuber by divisions in both epidermal and subepidermal cells. At the mature stage of the tuber, periderm reached to a 9-10 layered files of cells which form the skin of the microtubers.

It is concluded from the present studies that

1. The best medium for shoot growth of var DR is MS liquid medium supplemented with 0.25 mg/l GA_3, 0.01 mg/l NAA, 2 mg/l Ca-pantothanate, 1.0 mg/l Thiamine-HCl and 0.5 mg/l $AgNO_3$.

2. The best medium for shoot growth of var HR is MS liquid medium supplemented with, 0.25 mg/l GA_3, 0.05 mg/l NAA, 2 mg/l Ca-pantothanate, 1.0 mg/l Thiamine-HCl and 0.5 mg/l $AgNO_3$.
3. Maximum production of microtubers in var DR was observed when the cultured shoots were exposed to MS liquid medium containing 8% sucrose, 5mg/l BAP, 500 mg/l CCC and 15 mg/l $AgNO_3$.
4. However, best performance of var HR with the respect to microtuber production was observed under the influence of 8% sucrose, 7.5 mg/l BAP, 750 mg/l CCC and 15 mg/l $AgNO_3$.
5. Silver nitrate (15 mg/l) along with, BAP, CCC and 8% sucrose has been found to be very effective in inducing the formation of bigger microtubers.
6. Induced microtubers for their development and maturity took about 60 days.
7. Microtubers thus produced showed a storability of 4 months at 4°C.
8. Microtubers thus stored for four months gave cent percent germination under field condition.
9. Microtubers of A and B grade of both varieties under field conditions develop into plants and produced minitubers within a period of 100 days.
10. Nodal segments of *in vitro* shoots, encapsulated in 3% Na-alginate showed better performance in MS based medium than soil-peat mixture.
11. During the formation of microtubers, four developmental stages were observed, (a) bud initiation, (b) bud growth, (c) cessation of bud or axillary shoot growth and (d) tuber initiation.