DISCUSSION

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Surface sterilization of the explants

Explant's surface carry a wide range of microbial contaminants, which are to be taken care of by surface sterilization before transferring it onto the nutrient medium (Dhingra, 1992). Both bacterial and fungal contamination was observed during the initial establishment of potato sprout tips in the present studies. It is a known fact that loss of cultures during *in vitro* propagation is mainly due to bacterial (Wainwright and England, 1978) and fungal (Enjalric *et.al.*,, 1988) contaminants. Normally, exogenous micro-organisms can be effectively eliminated by using surface sterilants (Bonga, 1982). In the present studies Mercuric chloride ($HgCl_2$) at 0.08% concentration has been found to be effective in the initial sterilization of explants. Randhawa and Chandra (1990) also used $HgCl_2$ for surface sterilization of six Indian potato cultivars at a higher concentration and longer duration (0.1% for 8 minutes). But results of the present studies clearly suggest that higher concentration of $HgCl_2$ for longer duration causes browning and subsequent death of explants in both var DR and var HR.

Pretreatment of explants with a combination of various agents (antibiotic, fungicide and activated charcoal) before the routine surface sterilization was found to be very effective, yielding 85-95% contamination free cultures. A similar strategy of pretreatment with antioxidants, antibiotic and fungicides was used by Singh *et al*, (1992) for *in vitro* establishment of a tree species *Madhuca latifolia*. D'Silva and D'Souza (1993) used pretreatment with bavistin, followed by $HgCl_2$ and ascorbic acid to reduce contamination and phenolic exudation for establishment of Cashewnut explants.

Production of pathogen-free microshoots from shoot-tip of Potato

The Murashige and Skoog (1962) - MS and Linsmaier and Skoog (1965) - LS are the most widely used salt composition in tissue culture (Gramborg and Phillips, 1996). Thus, MS and LS media were tried to find out the best medium for the culture of potato shoots. Reduction of shoot growth was observed in LS medium, which may

be due to the omission of nicotinic acid, pyridoxine-HCl and glycine. Promotion of shoot growth observed in MS medium was due to the elongation of internodes. Shoots with elongated internodes were highly useful in obtaining single node cuttings. Major factors limiting the rate of micropropagation are short overall height of the plantlets and crowding of the nodes (Miller *et.al.*, 1985). Amiroucha *et.al.*, (1985) reported that MS medium was in general superior to Knop's medium when micropropagation was initiated from tuber sprouts. The development of potato shoot during the micropropagation stage dependent on the previous culture medium on which the shoot tips were initially grown (Amezqueta *et.al.*, 1989). Comparisons of several media were also made by Stace-Smith and Mellor (1968), Huth and Bode (1970), Christensen (1970), Tapio (1972) etc. and they all found MS medium to be superior for potato shoot growth.

Growth and multiplication rate of single node potato explants in micropropagation depends on the cultivars (Caligiri *et al.*, 1989 and Evans, 1993). Caligiri *et al*, (1989) also reported that fairly uniform micropropagation response over cultivars were obtained at 20°C and 3% sucrose.

The efficiency of micropropagation depends on high multiplication rate and the production of healthy shoots (Yeo and Reed, 1995). Growth regulators can influence the growth and development of *in vitro* cultured plants. Usually *in vitro* plants do not produce sufficient quantities of growth regulators (Kumar, 1999). Growth regulators need to be selectively added to the culture media to obtain desired growth.

Auxins are required in tissue culture media for the induction of cell division and root initiation, while cytokinins are required for cell division, shoot multiplication and axillary bud proliferation (Kumar, 1999). In the present studies, growth and development of *in vitro* shoots were strongly influenced by the presence of exogenous growth substances in the culture medium.

NAA at a low concentration (0.1 mg/l) induced rooting in the nodal segments within 3-4 days of incubation. These roots must have helped in absorption of nutrients from the medium, which might have led to improved growth of shoots. Incorporation of BAP individually or in combination with auxin, in the medium did not bring about any improvement in shoot growth. Similar effect of auxin Cytokinin interaction on

meristem culture of potato was reported by Novak and Zadina (1987). They have mentioned that NAA and BAP caused expressive callus growth of the primary explant without subsequent shoot or root regeneration. However, their results showed that auxins - NAA or IAA within the concentration range of 0.1-10 μM were insufficient to induce meristem growth in the primary culture of potato, which is contradictory to the present studies. Pennazio and Vecchiati (1976) reported that additon of NAA to the medium stimulates early rooting in potato shoot culture, hence increases the number of rooted plantlets. Nirmal Babu *et.al*, (1996, 1997) reported production of multiple shoot and *in vitro* rooting in *Zingiber officinale* and *Curcuma longa* by incorporating 1 mg/l NAA in MS medium.

In the present studies, BAP alone did not show any positive effect on shoot growth of var DR or var HR However, Haque *et.al.,* (1996) reported that multiple shoot regeneration were obtained in four potato cultivars when BAP was used at a concentration of 1 mg/l in MS and 1/2 MS media. Belleti *et.al.*, (1990) reported that the presence of BAP in the medium greatly inhibited root formation in potato single node cultures and their growth which is similar to the present studies.

The Gibberellins are infrequently used in plant tissue culture to inhibit callus growth and elongation of the shoot. Novak and Zadina (1987) reported GA_3 as the most effective gibberallin to induce shoot and root development from isolated tips of potato within the range of 0.1-10 μM. In the present studies GA_3 significantly increased the growth of the shoots in both the varieties. Healthy shoots with mean length of 4.4cm and number of nodes 3.8 per shoot were produced in var DR in MS medium supplemented with 0.1 mg/l GA_3, along with 0.01 mg/l NAA and 2 mg/l Ca-pantothenate. Whereas maximum length of shoots (5.1 cm) and number of nodes (3.4) per shoot were produced in var HR in MS medium supplemented with 0.25 mg/l GA_3, 0.01 mg/l NAA and 2 mg/l CA-pantothanate. The present studies also revealed how varieties differ among them with respect to their GA requirement. Promotion of shoot growth in potato by using GA_3 has also been observed by Dhingra *et.al.* (1980); Espinoza *et.al.,* (1986), Randhawa and Chandra (1990); Kuttyamma and Maity (1998), Seabrook *et.al.,* (1993), Estrada *et al.,* (1986).

IBA (0.5 mg/l) and ADS (0.5 mg/l) in combination with BAP (1.5 mg/l) produced elongated shoots with longer internodes, when single node cuttings of potato were cultured on liquid medium supported with filter paper bridge. Callus formation was observed in the basal part of cultured nodal segments (Table-12, 13). Gill *et.al.*, (1996) reported maximum shoot bud generation of *Eucalyptus tereticormis* Sm., when cultured on MS medium supplemented with IBA (0.5 mg/l) and BA (1mg/l). Deshpande *et.al.*, (1998) reported sprouting of dormant axillary buds of nodal explants of *Ficus religiosa* L. when cultured on MS medium supplemented with IBA (0.2 mg/l) and BA (5mg/l). They have also mentioned that multiple shoots were obtained when cultured on MS medium containing BA (1.5 mg/l) and ADS (1.5 mg/l). But in the present studies, shoots produced in IBA, BAP and ADS containing medium was thin and unhealthy. When ADS alone was incorporated in the medium it produced larger dark green leaves but did not improve the growth of shoots (Table: 12 and 13).

When vitamin levels were slightly modified in the MS medium, potato shoot growth was found to be better than that in the control. Incorporation of Thiamine-HCl at a concentration of 1.0 mg/l (over and above the concentration in MS basal medium - 0.1 mg/l) resulted in better growth of shoots (Table: 15 and 16).

Heszky *et al.*, (1983) used modified MS medium for the better response of isolated meristem tips of axillary buds of potato. The modified MS medium contained no glycine but concentrations of niacine and Pyridoxine-HCl were doubled (1 mg/l) in additon to the amount of Thiamine-HCl being present hundred fold (10 mg/l). Dodds *et al.*, (1988) used modified MS medium for the better growth of single node cuttings of potato by increasing the concentration of Thiamine-HCl from 0.1 mg/l to 0.4 mg/l. Slack and Tufford (1995) prepared a separate vitamin stock for the meristem culture of potato. They have used increased concentration of Pyridoxine-HCl (1.0 mg/l), Nicotinic acid (1.0 mg/l) and Thiamine-HCl (0.8 mg/l). Several other researchers have also observed improved growth of shoots by increasing the concentration of Thiamine-HCl (Akita and Takayama, 1994; Jimenez *et al.*, 1999; Estrada *et.al.*, 1986).

A comparison of growth of shoots raised from nodal explants on solid and liquid media clearly revealed the better efficiency of liquid medium to support the growth. The shoots inoculated onto liquid medium grow very fast. Nodal explants after

21 days of incubation in liquid medium produced 7.1 cm and 7.2 cm long shoots with 5.4 and 5.6 mean number of nodes per shoot in var DR and var HR respectively. However, solid medium produced only 4.3 cm and 5.1 cm long shoots with 3.6 and 3.4 mean number of nodes per shoot in var DR and var HR respectively (Table-16, 17). Schilde-Renlscher and Schmiediche (1984) and Rosell *et.al.*, (1987) have also reported that the rate of growth and multiplication is faster in liquid medium as compared to the agar based medium. Mellor and Stace-Smith (1987) observed that roots develop more readily in liquid than in solid medium. Watad *et.al.*, (1995) reported that, in *Aconitum napellus*, rate of shoot multiplication was 47% higher in the liquid media than in solid media. The close contact of tissues with the liquid medium might facilitate better uptake of nutrients. The shoots formed in liquid medium were sometimes vitrified.

Production of healthy potato shoots by shake culture

Shake cultures or liquid agitated cultures are known to stimulate the growth of various cultured cells and have been used advantageously for the large scale produciton of cell masses or economically important secondary plant metabolites. The technique, however, remains relatively unused in organ cultures (Takayama and Misawa, 1981). A direct positive correlation between agitated liquid media with shoot production has been established in different genera (Hammerschlag, 1982; Harris and Mason, 1983; Mujib *et.al.*, 1995). Direct oxygenation stimulates higher metabolic rates, which in turn perhaps dictates improved proliferation process (Mijib *et.al.*, 1995). The response of shoots in shake culture or liquid agitated culture, however, differ from those that take place in semisolid culture or liquid stationary culture. The closer contact of a greater surface area of the explant, with the liquid medium may greatly facilitate the uptake of nutrients, which must be the reason of the faster growth of the culture in this technique.

A large number of shoots (10.6 in var DR and 8.6 in var HR) were obtained in this present studies when 5 segments of *in vitro* shoot with three nodes were cultured on liquid MS medium agitated in a gyratory shaker. Medium was supplemented with

GA_3, NAA Ca-pantothanate and Thiamine-HCl. The only problem in liquid shake cultures was vitrification.

Higher rate of multiplication and production of elongated shoots were reported in *Catharanthus roseus* in liquid agitated culture compared to semisolid medium (Mujib *et al*, 1995). A 15-fold increase in fresh weight per month was also reported in shake cultures of *Stevia rebondiana* (Akita *et.al.*, 1994). Reghunath and Bajaj (1992) reported that shoot explants cultured in liquid medium under gyratory shaking produced 57% more axillary branches than those cultured in semisolid medium in the case of Cardamom.

Silver nitrate ($AgNO_3$), a potent inhibitor of ethylene (Beyer, 1976), when used in the propagation medium at a low concentration, markedly enhanced the production of healthy shoots in potato shoot culture. The involvement of ethylene in plant tissue growth and differentiation has been widely investigated. Application of ethylene precursors and/or inhibitors has shown that ethylene may often have diverse effects in tissue culture systems (Sandra *et.al*, 2000).

Ethylene, though it promotes callus growth (Songstad *et.al*,1991), it generally appears to inhibit somatic embryogenesis and shoot regeneration (Biddington, 1992). $AgNO_3$ was shown to promote regeneration in *Brassica campestris* (Palmer, 1992) and *Helianthus annuus* (Chraibi *et.al.*,1991). Similarly, $AgNO_3$ improved somatic embryogenesis in *Hevea brasiliensis* (Auboiron *et.al.*,1990), *Solanum tuberosum* (Tiainen, 1992) and *Hordeum vulgare* (Evans and Bttery, 1994).

In the present studies, it was observed that, low concentration of $AgNO_3$ (0.5 mg/l) greatly influences the health of the shoots of potato, produced in liquid agitated culture. $AgNO_3$ at 0.5 mg/l level significantly increased the number of shoots per flask in var HR, whereas in var DR number of shoots per flask was not affected. (Table:20 and 21). Dark green leaves with wider leaf lamina was observed in both the varieties under the influence of $AgNO_3$. However, higher concentration of $AgNO_3$ significantly reduced the length of the shoots as well as number of nodes per shoot.

Mader-Johanna (1999) also reported that $AgNO_3$ increased the leaf size, elongation and greening of roots and reduced shoot height and hairiness of shoots and roots of *in vitro* shoots of *Solanum tuberosum*. However, in the present studies, it was

observed that vitrification, which is a problem in liquid culture, was comparatively less, when medium was supplemented with $AgNO_3$. It was also noticed that contamination by microorganisms was minimum in $AgNO_3$ containing medium. It may be because of the toxic effect of $AgNO_3$. Addition of Silver nitrate caused oxidative browning of the medium after light exposure and at high concentration leaves which were in contact with the medium turned black. Mori *et al.*, (1999) also reported the effect of $AgNO_3$ on elimination of endogenous contamination of *Zantedeschia hybrida* Black magic. Their results showed that 24 hours treatment of the explants in 0.2 mg/l $AgNO_3$ prior to culture on MS medium reduced the outbreak of endogenous contamination by 14%. Medros-Molina *et.al*,(1999) reported that $AgNO_3$ (15-40 μM) showed a very strong anti-browning effect on the medium and explants of *Pistacia atlantica*. They have also mentioned that the developing sturdy shoots produced in $AgNO_3$ containing medium had large and green leaves. Positive effect of $AgNO_3$ on culture media were also reported in *Brassica oleracea* L. (Zobayed *et.al*,1999); *Coffea canephora* (Sandra *et.al*,2000) *Picea glauca* (Meskaoui EL *et al.*,2000) etc.

Optimization of microtuber production in potato

Formation of microtubers are a complicated developmental process controlled by many factors. These factors include cultivar, growth regulator, sucrose, temperature, light etc. Perl *et al.*,(1991) reported that potato tuberization was triggered by high concentration of sucrose. Present results also confirm the importance of high sucrose concentration for the formation of microtubers. 8% sucrose included in the media was found to be the best for the induction of *in vitro* tubers. Importance of high sucrose concentration was also described by Wang and Hu (1982), Hussey and Stacey (1984) Abbott and Belcher (1986) and Garner and Blake (1989). The use of 8% (w/v) sucrose advanced the initiation of tuberization compared to 6% (w/v) and at harvest gave more and larger microtubers in both the varieties (Table:22 and 23). According to Khuri and Moorby (1995), the increased concentration of sucrose could supply more carbon to the plantlets, but probably also affected the initiation of microtubers by changing the osmotic potential of the medium. However, sucrose concentration more than 8% (w/v) significantly decreased the number as well as size of the microtubers.

The development of potato tuber is dependent on the ability of source organ (leaf) to produce and export photo assimilates. Under *in vitro* conditions, the photo assimilates are substituted by the sucrose in the medium (Zsofia *et al.,* 1997). Experiments with antisense RNA inhibition showed that blocking the sucrose transport or the enzymes involved in starch synthesis highly influence the rate of tuberization as well as the number, size and shape of the tubers formed (Mullar-Rober *et.al.,* 1992; Riesmeir *et.al.,* 1994; Zrenner, *et.al.,* 1995).

Low temperature and continuous darkness during tuberization stage exerted a positive effect on the number of microtubers. In the present studies, maximum number of tubers were produced at 15° ± 2°C under continuous dark condition (Table:24 and 25). Microtubers produced under light and dark condition showed almost same average fresh weight of tubers in var DR, whereas in var HR average fresh weight was comparatively high when produced under continuous dark condition in both the varieties compared to that in light condition. According to Wang and Hu (1982), the optimum temperature for *in vitro* tuberization is 20°C and a higher temperature (above 28°C) is strongly inhibitory to tuberization. (Okazawa 1967; Wang and Hu,1982). However, some workers obtained microtubers at lower temperatures too viz. 8-10°C (Thieme and Pett, 1982; Wattimene, 1983). According to Wang and Hu (1985), this lower temperature might be making up for the absence of growth regulating substances, especially cytokinin, in the microtuber induction medium. Photoperiod also plays an important role in the tuberization process (Gregory, 1956) There are contradictory reports on light/photoperiod requirements for *in vitro* microtuberization. Wang and Hu (1982), found that 8 h photoperiod was better than a 16 h. photoperiod. On the contrary, other worker reported that microtuber production was faster under continuous darkness (Lawrence and Barker, 1963; Stallknecht and Fransworth, 1982; Estrada *et.al.,*1986), which is in accordance with the present observations. Garner and Blake (1989) observed that incubation of cultures for a period of one month under 16 h. days followed by transfer to 8 h. photoperiod gave the most rapid microtuber development. Whereas, Slimman *et.al*, (1989), found that microtubers from all the cultivars tested had a higher mean fresh weight when placed under an 8 h. photoperiod compared to those placed under total darkness. According to Naik and Sarkar (2000),

optimum tuberization occurs under continuous darkness during cytokinin-induced tuberization, but a longer photoperiod with higher light intensity is required when cytokinin is not used. Zarrabeitia *et.al.* (1997) also reported that, microtuberization of cv. Jaerla was earlier in darkness than under short days regardless of the propagation media used.

Plant hormones like cytokinins enhance tuber formation while gibberellins inhibit this developmental process (Cutter, G.E. 1978). The promotion of microtuberization on cultured shoots by cytokinins has been demonstrated by many workers (Palmer and Smith, 1969; Wang and Hu, 1982; Hussey and Stacey, 1984; Estrada *et.al.*, 1986; Ortiz-Montiel and Lozoya-Saldana, 1987). Palmer and Smith (1969) found that the promotive effect of cytokinin on tuber formation was optimal if isolated stolons were cultured on medium containing 6% sucrose. However, Hussey and Stacey (1984) reported that, without BAP, higher sucrose favoured tuberization in 8 h. day but not in continuous light and the promotive effect of BAP was enhanced in both day lengths by higher sucrose levels. The inhibitory effect of GA_3 was demonstrated by many workers (Lovell and Booth, 1967; Tizio, 1971; Hussey and Stacey, 1984). Vrengdenhil *et.al.*, (1998) found that, when GA was present in medium containing 8% sucrose, tuber formation was rarely observed during the 10 day observation period and the buds developed into stolon-like structures, having a hook at the tip. However, CCC, which prevents the biosynthesis of GA_3, enhances tuberization and reinforces the promoting effect of cytokinin (Yamamoto and Nakata, 1997). In the present studies, microtuber formation was observed visually, 10-12 days after culturing them under continuous darkness on a medium supplemented with 8% sucrose and various levels of BAP and CCC. The increase in the number of microtubers under the influence of BAP and CCC was more rapid than that on the medium with BAP alone. This indicates that CCC accelerates tuberization at the early stage of culture. The number and fresh weight of microtubers of var DR was maximum on the medium supplemented with 5 mg/l BAP and 500 mg/l CCC, which was in accordance with the results of Yamamoto and Nakata (1997) and many other workers (Hussey and Stacey, 1984; Estrada *et.al.*, 1986). However, maximum number and fresh weight of tubers

were observed in var HR under the influence of 7.5 mg/l BAP and 750 mg/l CCC, which may be due to the varietal difference.

Production of microtuber was also affected by the culture conditions. When single node cuttings were cultured directly onto the tuberization medium, plant height was inhibited. Only a single tuber was produced from the axil of the nodal explants. The size and fresh weight of the tubers were not satisfactory. This may be because of the less reserve materials in the single node cuttings and less absorption of nutrient from the medium by single nodes. Rooting was not observed in this case. When cultures were grown in solid propagation media and were supplemented with liquid tuberization medium, large numbers of small microtubers were produced. This may be due to the good growth of the plant as well as availability of nutrients and carbon source. Similar use of bilayer medium has also been reported for the anther culture or regeneration of embryos in *Capsicum annum* (Dolect-Sanjvan *et.al.*, 1997) and *Solanum tuberosum* (Johanson, 1988). Shoots produced in liquid agitated culture when supplied with liquid tuberization medium produced less number but larger microtubers. According to Sarkar and Naik (1997) in commercial potato production microtuber size is more important than microtuber number.

Ethylene accumulation in sealed culture vessels appeared to be a major factor in the inhibition of microtuberization. Many investigators concluded that ethylene inhibits tuber initiation (Mingo-Castel *et.al.*, 1974;1976; Hussey and Stacey, 1984; Vrengdentil and Straik, 1989; Belleti, 1993). Hussey and Stacey (1984) also reported that the addition of $KMnO_4$ to the culture vessels to absorb ethylene markedly increased tuberization of potato. No starch accumulation was observed in the studies of Palmer and Barkar (1973) when cultures were treated with CEPA (2-Chloroethyl phosphonic acid – a source of ethylene).

In the present studies $AgNO_3$ was used as an ethylene inhibitor in the culture medium and it showed an adverse effect on microtuber production. Shoots derived from liquid propagation medium without any $AgNO_3$ when exposed to tuberization medium containing $AgNO_3$ produced microtubers with better size and large number (Table:30 and 31). This may be due to the inhibition of ethylene production by $AgNO_3$.

Shoots produced in liquid propagation medium supplemented with 0.5 mg/l $AgNO_3$, were healthy, green and had large green leaves. These shoots when exposed to tuberization medium containing $AgNO_3$, markedly increased the size of the tubers in both the varieties (Table:32 and 33). The number of A grade tuber were higher (5-6/ flask in both varieties) in comparison to that in the previous experiment (2-3/flask) where $AgNO_3$ was incorporated only in the tuberization medium. Production of greater photosynthates by healthy shoots having larger leaf area might have an important role in increasing the size of the microtubers.

Shoots produced in liquid propagation medium supplemented with high concentration of $AgNO_3$ (5mg/l) were stunted and had less number of nodes. These shoots when exposed to tuberization medium containing $AgNO_3$ showed no significant difference in the yield of microtubers between the control and treated one (Table: 34 and 35). Number of A grade tubers produced in this experiment (3/flask) was comparable to that observed in the previous experiment where propagation medium was devoid of $AgNO_3$ (Table:30 and 31), whereas total number of tubers has drastically reduced when propagation medium was supplemented with 5 mg/l $AgNO_3$ (Table: 34 and 35)

Shoots derived from liquid propagation medium containing 10 mg/l $AgNO_3$ were dwarf, having large green leaves and less number of nodes per shoot. When high levels of GA_3 were incorporated with 10 mg/l $AgNO_3$ in propagation medium stolon like shoots were produced which were thin and etiolated. Incorporation of high levels of GA_3 showed a carryover effect on tuberization stage. No sign of tuberization was observed in these shoots. Vrengdenhil *et al.*, (1998), reported that when GA_3 was present in medium containing 8% sucrose, tuber formation was rarely observed. They have also mentioned that Gibberillin was able to prevent tuber formation even when all other factors (high sucrose, low N, BAP, short days) were favourable for tuber induction. Struik and Wiersema (1999), also reported inhibition of tuberization by GA. Present results suggest that 0.25 mg/l GA_3 was the best for shoot propagation stage to achieve best results with respect to microtuber production (Table:36 and 37).

Storage and shelf life of potato microtubers

Shelf life of potato microtubers is influenced by several factors, such as, tuber weight, storage temperature and storage duration. Difference in the storage capacity between the two varieties were also noticed during the present studies. Percentage germination of microtubers of A and B grades were higher than C grades after storage for a period of three months (Table: 38 and 39). A heavy loss of microtubers was observed due to the shrinkage and drying after 5 months of storage. Excessive biomass loss results in deterioration of seed quality and poor sprouting (Singh and Naik, 1993). To avoid this problem Naik and Sarkar (1997) suggested a method of greening the microtubers before harvesting by exposing them to a 16 h. photoperiod at 25°C for 10 days.

An important factor that limits the use of microtubers is dormancy which depends on the method used for their production (Naik and Sarkar, 2000). Microtubers produced under light have shorter dormancy whereas under complete darkness they have longer dormancy period. Dormancy is linearly related to the length of storage and inversely correlated with microtuber size (Ranelli *et.al.*, 1994). Leclerc *et al.*, (1995) observed that dormancy in microtubers was cultivar specific. Naik and Sarkar (2000) observed that small microtubers have more dormancy period than the bigger ones. But in the present studies it was observed that C grade tubers start germinating even under storage condition at 4°C from 90 days onwards.

Production of Minitubers

The performance of plants raised from different grades of microtubers in nursery bed with respect to average number of minitubers per plant and average minituber weight, varied between the two varieties. Plants derived from microtubers were normal and yielded seed sized minitubers within 100 days. Better performance was observed in var DR with respect to all the parameters observed than in var HR Maximum number of minitubers was formed from plants raised from A grade microtubers. Their average fresh weight was also significantly higher than those produced in plants raised from B and C grade microtubers (Table:40 and 41). Powell

et al., (1989) also found large differences between the performance of microtubers of different cultivars depending on the methods used, to grow the seed tubers. Distance between the rows in the field also affected the performance of microtubers. Closer spacing gave a significant increase in tuber yield per hectare.

Encapsulation of potato nodal segments

Synthetic seed or artificial seed technology is referred, in a narrow sense, to encapsulated somatic embryos and in a broader sense, it also refers to encapsulated buds, bulbs or any forms of meristems which can develop into plantlets (Rao *et.al.*, 2000). Initially synseed production was limited to using somatic embryos. Recently it has been focussed on the use of vegetative propagates like axillary buds, adventitious buds, shoot-tips, cormlets, bulbs and protocorms for production of synthetic seeds (Rao *et al.*, 1996). Encapsulated shoot buds of a number of plant species shown to have the potential as an alternative source of artificial seeds (Mathur *et al.*, 1988; 1989; Bapat *et al.*, 1987; Bapat and Rao, 1990). Encapsulation of shoot-tips of cardamom var Malabar was reported by Rao *et.al.*, (2000). Sharma *et.al.*, (1994) encapsulated shoot buds derived from healthy rhizomes of ginger in 4% sodium-alginate. Rao *et.al.*, (2000) encapsulated shoot-tips of Banana in 3% sodium alginate. In the present study nodal segments of *in vitro* shoot of potato was encapsulated in 3% sodium alginate. It was found that encapsulated nodes showed better germination performance in MS medium than in soil-peat mixture (Table: 42 and 43). It was also observed that germination percentage was highest when they were transferred immediately to the medium. Nutrient-encapsulation of nodal segments of potato was also reported by Sarkar and Naik (1998).

Encapsulated nodes of potato can be a useful tool for propagation, delivery of tissue cultured plants, germplasm storage and exchange if they can be stored for a longer period.

Developmental studies of microtubers

Microtubers produced in *in vitro* condition have the same developmental and structural features as tubers grown *in vivo* (Peterson and Barker, 1979). The parts

which can be distinguished in a mature microtuber are the skin or periderm, the cortex, vascular elements and the pith.

As soon as the stolon starts swelling, formation of periderm starts. Periderm consists of 10-12 layered suberized phellem cells, which is called periderm files. Both anticlinal and periclinal divisions occur during the formation of periderm. Artschwager (1918) also reported anticlinal divisions in the epidermis followed by periclinal divisions, which forms periderm. However, this number of suberized cell layers (periderm files) and the total thickness of the skin vary substantially, partly dependent on variety (Artschwager, 1924) and also on growing conditions (Peterson *et.al.*, 1985). According to Reeve *et.al*, (1969) periderm formation usually begins at the stem end of a young tuber.

The periderm is a specialized protective layer to prevent rapid water loss from the thin walled parenchyma of the tuber and to impede the ingress of various soil pathogen (Peterson *et.al.*,1985).

Lenticels were observed in the periderm of newly developed microtubers. Young tubers recently formed from stolons have an epidermal covering with widely scattered stomata (Artschwager, 1924; Adams, 1975) and since the phellem cell walls are resistant to water loss and presumably to gaseous exchange, lenticels develop to replace stomata in the outer covering (Peterson *et.al.*, 1985). Lenticels, the only sites of gaseous exchange through the suberized periderm (Wigginton, 1974) are initiated by periclinal divisions in cortical cells subtending the substomatal cavity (Artschwager, 1918; 1924; Fellows, 1926; Adams, 1975). Well developed lenticels have considerable volume of filling tissue and have an opening to the external environment. (Peterson *et.al.*, 1985).

During microtuber formation when the elongation of stolon stops and gradual radial growth begins, pith and cortical cells start dividing longitudinally, resulting in the swelling of the tubers. However, according to Peterson *et.al.*, (1985), very little increase in number of cortical and pith cells from the initial stage to microtuber maturation and radial expansion is primarily due to an increase in volumes of cortical and pith cells with the addition of only a few cells by the vascular cambium and

phellogen. The pith is derived from ground meristem and contributes to the early enlargement of the tuber (Peterson *et al*, 1985).

Starch is the most important reserve material in the potato tuber (Artschwager, 1924) and the size of starch grains is extremely variable and is partly dependent on the age of the tuber and position within the tuber (Peterson *et.al.*, 1985). Kozlina and Berljak, (1997), reported that, the starch accumulation in basal part of axillary buds are directly correlated with microtuber development and was the first sign of tuber initiation which preceded to any visible bud changes. They have also mentioned that starch accumulation in the basal parts of potato axillary buds could be used as an indicator for microtuber induction and initiation. Hannapel (1991), monitored fresh weight and starch content to establish a developmental framework for morphological changes of microtubers, where fresh weight and starch content began to increase in axillary buds after 2 days and the first visible changes in bud morphology could be detected 4 days after the start of incubation. However, in the present studies, starch began to accumulate on the day one itself when cultures were incubated at low temperature (15° ± 2°C).

According to Durcan and Ewing (1984), starch deposition and cell mitosis were the earliest detectable anatomical changes associated with tuber initiation. Jameson *et.al*, (1985) also mentioned, starch accumulation as a precise indicator of tuber initiation.

Obata-Sasamoto and Suzuki (1979) studied changes in starch and protein contents and activities of enzymes involved in starch synthesis during tuberization of stolon tips. They have mentioned that changes in starch content during tuberization process is biphasic and increased almost proportionally to the fresh weight. Observations of the present investigation also show that starch content increase according to the size and fresh weight of the microtubers. Similarly, Vissar *et al*, (1994) also reported that starch content of the stolons and small tubers in *Solanum tuberasum* cv Bentje increase gradually with increasing fresh weight. According to Kozlina and Berljak (1997), during tuberization, leucoplasts transformed into amyloplasts, plastids which are specialized for starch synthesis and accumulation. Changes of endogenous

hormonal level caused by photoperiod can stop bud elongation which is leading to radial growth and enhanced starch deposition (Hannapal, 1991).

Present studies showed that cell division start at the early stage of tuber development and initially occur mainly in the meristemetic zone of the bud apex. Cells were dividing transversely at the early stage, resulting in the elongation of the bud and at later stage longitudinal cell division was observed in the subapical parts of the bud, leading to the swelling of the developing bud. Similar observation was reported by Duncan and Ewing (1984) and Vreugdenhil *et.al.,* (1999). Duncan and Ewing reported that starch deposition and the percentage frequency of cells in mitosis increased in the medullary region of the bud after 1 day of cutting, whereas increase in average cell size was not detected until 4 day after cutting. Vrengdenhil (1999) also observed that, cell division occurs from the first day after cutting, in both LD and SD cuttings, in the apical zone of the buds. Both the workers used single node cuttings to study the anatomical changes associated with tuberization.