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Tissue Culture of Potato (*Solanum tuberosum* L.): A Review

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ABSTRACT

Plant tissue culture is the method of culturing the plant cells or tissues under controlled aseptic conditions on a solid or liquid medium. It has value in basic research for the production of true to type, disease free and micro-propagated plants. Besides this also has commercial application. This review work outlines the work done on pertaining to various aspects of *in vitro* micro-propagation, regeneration, rooting, hardening, in potato.

Plant tissue culture of potato (*Solanum tuberosum* L.)

Potato can be propagated sexually (by botanical seed, also called true potato seed) and asexually (vegetative) by means of tubers. Seed potato tubers are utilized for multiplication and production (Struik and Wiersema, 1999). This method has a number of disadvantages like low rate of multiplication and high risk of various diseases. Tissue culture techniques have become very popular and alternative means for vegetative propagation of plants in recent years. As an emerging technology, the plant tissue culture has a great impact on both agriculture and industry by providing plants to meet the ever increasing world demand. It has made significant contributions to the advancement of agricultural sciences in recent times and today they constitute an indispensable tool in modern agriculture. It is due to that we can create a large number of clones from a single seed or explants, select desirable traits, decrease the amount of space required for field trials and to eliminate plant diseases through careful selection and sterile techniques. It a biological tool that involves exciting prospects for crop productivity and improvement under aseptic conditions (Jain, 2001). The system is characterized by very flexible rapid multiplication giving a high rate of multiplication in a very short period.

Micro-propagation

Micro-propagation is a potential biotechnological tool that has become a commercially viable method of *in vitro* clonal

propagation of a wide range of herbaceous and woody plants (Garcia *et al.,* 2010). Micro-propagation can be defined as *in vitro* clonal propagation through tissue culture and rapidly multiplying plant material to produce a large number of progeny plants. This technique was first started by Morel in 1960 for propagation of orchids and is now applied to several plants. It has been proved to be very efficient technique to speed-up the production of high quality pathogen-free plantlets, in terms of genetic and physiological uniformities (Sathish *et al.,* 2011; Supaibulwattana *et al.,* 2011) The term tissue culture techniques covers a wide range of techniques including *in vitro* culture of organs (shoot tips, root tips, runner tips, stem segments, flowers, anthers, ovaries, ovules, embryos etc.) tissues, cells and protoplasts. In potato, various tissues can be used as explants for shoot generation directly (Anjum and Ali, 2004b). In general, the efficiency of micro-propagation depends on a source of explants and explants itself, treatment of explants while preparing them for *in vitro* culture, composition of culture media, routes of micro propagation 4 followed, and performance of regenerated plantlets. These techniques are becoming useful tools for rapid and clonal multiplication of potato. Since 1902, when Haberlandt conceived the idea of totipotency considerable success has been achieved in the field of plant tissue culture. The commercial feasibility of these techniques has been demonstrated in many diverse plant species including fruit and vegetable plants. For large scale production of uniform, identical seed material of potato, micro-propagation can be the better alternative over conventional propagation of potato. By using the technique, which involves low cost components, large-scale clonal material can be produced in short time duration. Use of micro-propagation for commercial seed production has moved potato from test tubes to field. Potato production is being seriously hampered due to certain viruses, fungus and bacterial diseases. Researchers showed that some viruses can decrease the yield by 40% singly and in combination with other viruses, the loss is 90% (Siddiqui *et al.,* 1996). Potato virus free clones with meristem culture methods were conducted by Nagib *et al.* (2003). The organ that is to serve as tissue source, depends upon the physiological or ontogenic age of the organ, the season in which the explants is obtained, the size of explants and overall quality of the parent plant from which the plant is being obtained (Murashige, 1974).

To maintain an aseptic environment, all culture vessels, media and instruments used in handling tissues, as well as explant itself must be sterilized. The importance is to keep the air, surface and floor free of dust. All operations should be carried out in laminar airflow sterile cabinet (Chawla, 2003). For micro-propagation, MS basal medium has proven to be quite effective in several studies (Gopal *et al.,* 1980; Hussey and Stacey, 1981; Aburkhes *et al.,* 1984; Rosell *et al.,* 1987; Ozkaynak and Samanci, 2005). The most important step before the inoculation of explants is surface sterilization. Different sterilization agents can be used like $HgCl_2$ (0.1%), NaOCl (5.25% v/v approx.), $CaOCl_2$ (0.8% v/v), 70% ethanol, H2O2 (3-10% v/v) etc. The explants of potato were surface sterilized by treating them with sodium hypochlorite (0.1%) for 8 minutes, followed by 5 minute wash of savlon, and 30 second wash of 70% alcohol, at last 6-7 wash of distilled water followed by every treatment (Badoni and Chauhan, 2010). Hoque (2010) have described sterilization treatment for *Solanum tuberosum*, which includes the surface sterilization by dipping in 0.5 $HgCl_2$ solution for 3-5 minute and then washed 6-7 times with autoclaved distilled water. Yasmin *et al.* (2011) used dissected segments of sprouts as the experimental plant material and were surface sterilized with 10% commercial

bleach containing three drops of polyoxyethylene sorbitan monolaurate (Tween-20) for 10 minutes. Explant is of prime importance in their response to tissue culture system for callus initiation and regeneration. The purpose was to compare a range of media and explants to select highly efficient reliable and reproducible regeneration system to maximize the 5 production of regenerates for transformation of new genes to potato plant. Le (1991) also found that when stem section of varieties Bintje and Desiree were cultured, then best growth was shown by explants taken from central portion of the stem in both varieties. The use of single-node cuttings excised from tissue cultured plantlets is more common and avoids the influence of tuber tissue from which sprout sections originate (Mohamed and Alsadon, 2010). Nodal cuttings were also used for auxiliary shoot development and suggested to be the best explants source by several researchers (Roca *et al.,* 1978; Hussey and Stacey, 1981) on either liquid or agar solidified medium. Vanaei *et al. (*2008) studied the propagation of two commercial cultivars of potato (Marfona and Agria) using meristem tip as explants source. Mohamed *et al.,* (2009) used potato single node as an explant for his experiment. Potato tubers were also used as an explants source (Mutasim *et al.,* 2010). Calli were induced from 1.0 cm^2 tuber segment of potato cultivar Almera on MS medium supplemented with different levels of 2, 4-D (1.0-5.0 mg/l). Disease free healthy tubers were propagated on MS media with different concentrations of kinetin (Hoque, 2010). The sprouts were rinsed 3-times with sterile distilled water under the clean bench. Kong *et al., (*1998) cultured apical meristems of four potato cultivars in modified MS solid medium with BAP, NAA and GA_3. They obtained 68.1 - 86.6% plantlets in 46 - 64 days. They showed that modified medium with NAA is the best for rapidly producing virus free plantlets. In another study, Shibli *et al., (*2001) sub-cultured *in vitro* shoots of *Solanum tuberosum* L cv. spunta in liquid MS medium containing 0.0, 0.5, 1.0, 1.5 and 2.0 mg/l benzyladenine or kinetin. They observed a significant reduction in stem and internodal length by increasing BA and kinetin concentration in MS medium. BA up to 1.0 and 1.5mg/l resulted in an increase in number of proliferating shoots and nodes per culture flask. The use of gibberellins (gibberellic acid, GA_3), on the other hand have also shown positive effect on micro-propagation in potato as indicated by the study of (Pereira and Fortes, 2003). They developed a protocol for *in vitro* multiplication of potato in liquid culture medium. The explants of potato (cv. Eliza) with an auxiliary bud were cultured in six different levels of GA_3. They observed that full-strength MS medium supplemented with 0.25 mg/l gibberellic acid, 5.0 mg/l pantothenic acid, 1.0 mg/l thiamine and 20 g/l sucrose under constant agitation was the most suitable one. In another study, In addition to BA, NAA, 2, 4-D, KIN and GA_3, several other plant growth regulators (IBA, IAA, picloram, TDZ) and compounds like chloride were also reported in literature for micro-propagation of potato. Incorporation of cytokinin affected the level of endogenous auxin by inhibiting the oxidation of additional IAA, maintaining the optimum level of this auxin for shoot morphogenetic response (Manjula and Nair, 2002). Benzylaminopurine (BAP), zeatin or kinetin added individually to the nutrient medium might stimulate shoot formation. In terms of effectiveness in promoting shoot initiation, reports were contradictory, showing zeatin to be less, equal, or often superior to BA as the cytokinin component (Anjum and Ali, 2004a). GA_3 activates the cell division 6 cycle by regulating the transition from G1 to S phase, and G2 to M phase by the expression of several cyclin-dependent protein kinases (CDKs), which lead to an increase in mitotic

activity in the intercalary meristem (Fabian *et al.,* 2000). Kong *et al. (*1998) used apical meristems of potato to initiate *in vitro* cultures on modified MS solid medium supplemented with BAP, NAA and GA_3. Murashige and Skoog (1962) medium supplemented with different combinations of growth regulators has been used for inoculation of multiple shoots in potato. Rout *et al. (*2001) found that BAP, kinetin and ascorbic acid give best results for regeneration or multiple shoot formation from apical shoots. Shah Zaman *et al. (*2001) found that the highest stem length and the largest single nodes in potato can be achieved in MS medium containing 0.5 mg/l NAA. Boston and Demirel (2004) indicated that the best medium for single node culture of potato is MS medium without any growth regulators. Effect of different concentrations of GA_3 and BAP on *in vitro* multiplication of potato variety Desiree was studied by Asma *et al. (*2001) they found that maximum shoot length was 8.96 cm when 4 mg/l GA_3 was used. Maximum number of shoots (14) was obtained when 2 mg/l BAP was used. Everson and Renan (2003) establish a protocol for propagation of potato in liquid culture with different hormone combination. The MS medium supplemented with GA_3 (0.25 mg/l), panthothenic acid (5.0 mg/l), thiamine (1.0 mg/l) and sucrose (20 g/l), and under constant agitation, showed high efficiency of the *in vitro* potato multiplication. Badoni and Chauhan (2010) used low cost alternative to MS salts for shoot proliferation in potato. MS and low cost media with different hormonal combination of KIN (0.04, 0.06 and 0.08 mg/l) and IAA (0.50 mg/l) were used. Hoque (2010) observed that MS medium supplemented with 4mg/l of KIN showed best performance in respect of multiple shoot regeneration. Raza (2011) studied the effect of different growth regulators (BAP, NAA, PA and GA_3) on meristem tip culture of potato and he found that the best regeneration of meristem tips was obtained when MS medium was supplemented with 1.0 mg/l pantothenic acid + 0.5 mg/l gibberellic acid. Molla (2011) studied the effect of BAP, TDZ and ZR on direct regeneration of potato. The MS medium supplemented with 3mg/l of BAP, 0.3 mg/l of TDZ and 5mg/l of ZR showed very good shoot induction. Farhatullah *et al. (*2007) reported the affects of different concentrations of GA_3 on micropropagation and suggested that the dosage of 0.248 mg/l of GA_3 boosted all the morphological characters over control and other treatments. They suggested that this level (0.248 mg/l) could be used as standard dose for micro-propagation of potato. Badoni and Chauhan (2009) studied the effect of various growth regulators on meristem tip development and *in vitro* multiplication of potato cultivar Kufri Himalini. They tried different combinations of growth regulators, e.g., GA_3, NAA, and KIN. Results showed that lower concentration of auxin (0.01 mg/l NAA) with gibberellic acid (0.25 mg/l) was the best one for the development of complete plantlets from meristem tips avoiding callus formation and with satisfactory root formation. Cheaper agar alternatives which include various types of starch and gums have been investigated in commercial 7 micropropagation (Nagamori and Kobayashi, 2001). Other options include white flour, laundry starch, semolina, potato starch, rice powder and sago (Prakash *et al.,* 2003). Some researchers used other low cost alternative medium component for *in vitro* micro-propagation of potato (*Solanum tuberosum* L.). Nyende *et al. (*2008) used table sugar as a low cost alternative medium component for commercial propagation of potato. Results also showed that table sugar not only enhanced micro-propagation but also significantly lowered the production input costs by 51% when compared with the analytical grade sucrose. Naik and Sarkar (2001) substituted agar on potato micro-propagated medium with 13% of sago and

found that the number of shoots and leaves and root length were significantly higher compared to the agar medium. Mohamed *et al.* (2009) used corn and potato starch as an agar alternative for *Solanum tuberosum* micro-propagation. The highest number of shoots (6.8) was achieved in medium with 50 or 60 g/l of PS + 1 g/l of agar. The results suggest that the combination of agar and PS or CS could offer a firm support for plant tissues and could be successfully used for potato micro-propagation. Moeini and Modarres (2003) used different concentration of NAA and BAP for root induction and studied that application of BAP and NAA decreased shooting and rooting of single nodes. Seabrook (2005) reported that in a medium lacking growth regulator, roots form readily on potato plantlets *in vitro*. Badoni and Chauhan (2010) used MS medium with different concentration of KIN and IAA for root proliferation. All the media used in this study were supplemented with 3% (w/v) sucrose, solidified with 0.8% (w/v) agar and the pH was adjusted to 5.8 ± 0.1 with 1 M NaOH before autoclaving at 121°C and 15 lb psi for 15 min. Acclimatization were done when *In vitro* rooted plants of potato were removed from rooting medium and washed to remove adhering gel and transplanted to plastic pots containing autoclaved garden soil and sand at 3:1 ratio and covered with bottle. Plants were kept under culture room conditions for 15 days then transferred to green house and placed under shade until growth was observed.

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