

Original Research Article

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Optimization of Activated Charcoal on *in vitro* Growth and Development of Potato (*Solanum tuberosum* L.)

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ABSTRACT

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Activated charcoal is often used in plant tissue culture to improve cell growth and development by absorbing the beneficial plant growth promoting substances from the media. Thus the main aim of this study was to evaluate the influence of different concentrations of activated charcoal on the recalcitrant varieties of potato. The MS media strength (full and half) did not influence the plant growth and development of potato varieties as both the strengths did equally well in combination with lower concentration of activated charcoal (200mg/l). The use of activated charcoal could be done in a controlled manner (tested concentrations) in order to maintain the endophytes / microplant balance. However, studies in this line need further investigations for lowering the concentrations with a view to avoid the negative effects.

Introduction

Potato (*Solanum tuberosum* L.) is the fourth most important food crop of the world. It is an amenable crop which can be used in tissue culture techniques, ranging from *in vitro* propagation via shoot cultures for regeneration of whole plants from protoplasts (Buckseth *et al.*, 2016). This whole plant can be regenerated from a range of cultured tissues like roots, leaves, tubers and stems or from single cells. In general terms, these all involve

the growth of plant cells, tissues and organs in sterile conditions, supported by an appropriate culture medium (Harshal and Gautam, 2014). Murashige and Skoog medium (MS medium) is most widely used for the vegetative propagation of many plant species *in vitro* (Murashige and Skoog, 1962). Tissue culture based hi-tech seed potato production is spreading very fast in the country (Buckseth *et al.*, 2017). Micropropagation at faster rate and free from cultural contamination is an integral component for the success of *in vitro* mass

multiplication of potato (Buckseth *et al.*, 2017). Activated charcoal is often used in plant tissue culture to improve cell growth and development. It stimulates nitrogen uptake by shoots and provides dark environment, which promotes *in vitro* rooting (Sharma *et al.*, 2012). It is a porous and tasteless material which is distinguished from elementary carbon by removal of all non-carbon impurities and oxidation of carbon surface (Thomas, 2008). Several authors have reported its beneficial effect as a supplement on the media. Several authors reported that activated charcoal due to its beneficial effect has been used as a supplement on the media. It includes absorption of phenolic complex (Pan and Staden, 1998); root stimulator (George and Ravishankar, 1997); rhizome growth (Kim and Lee, 1992); development improvement (Choi and Chung, 1989); and absorption of toxic substances present in the media (Fridborg and Eriksson, 1978). Based on the performance on the standard Murashige and Skoog medium, the cultivar Kufri Jyoti has been classified as recalcitrant since the microplants during *in vitro* multiplication shows very slow growth, clumping of internodes, yellowing of basal leaves and premature senescence hence considered as recalcitrant variety (Venkataslam *et al.*, 2015).

In view of the above, the main aim of the study was to analyze and evaluate the content of activated charcoal in half and full strength Murashige and Skoog media on different growth parameters of potato varieties Kufri Sindhuri and Kufri Jyoti.

Materials and Methods

The research was carried out at Central Potato Research Institute, Shimla to study the effect of activated charcoal in full and 1/2 strength MS media on different growth parameters of micro plant of Kufri Jyoti and Kufri Sindhuri. The standard culture medium (Murashige and

Skoog, 1962) and half strength MS media was supplemented with six different concentrations of activated charcoal i.e. 50mg^l⁻¹, 100 mg^l⁻¹, 200 mg^l⁻¹, 300 mg^l⁻¹, 400 mg^l⁻¹ and 500mg^l⁻¹. Nodal segment culture in which the axillary and apical buds grow into new plants is predominantly used for initial shoot multiplication. The axillary/apical buds of these nodal segments grow into full plants within a period of about 3 weeks. These plants can be further sub-cultured on fresh medium. Excise nodal stem segments from the third and fourth nodes from the stem apex. Each nodal cutting should be 1-2 cm long, and the leaves should be detached. Such cuttings are used to initiate *in vitro* cultures (Buckseth *et al.*, 2017). The explants used in the study were double-node cuttings derived from the middle portion of microplantlets. The explants (three double nodes per test tube) were cultured on the above said activated charcoal media along with control (MS medium without Activated charcoal). Culture tubes were incubated at 22±1°C under 16 hr photoperiod (irradiance of 60µmolm⁻²s⁻¹). After twenty-one days of culturing, observations were recorded on microplant height (cm), number of leaves, nodes and roots, root length (cm) of each microplant of a test tube. In case of number of roots only primary roots were counted and root length was recorded for the longest root of each plant. The experiment was conducted in a factorial completely randomized design. Each treatment comprised four replicates, each replicate consisting of four test tubes having three plantlets. The three-way analysis of variance was done using the software AGRES and means were separated according to the least significant differences at 0.05 level of probability and the graphs were prepared with the graph pad prism 5.0 software.

Results and Discussion

The experiment showed that the explants grown in activated charcoal full strength and

half strength media produced desirable results as compared to the activated charcoal free media. However, the media strength (full and half) did not influence the plant growth and development of both the varieties as the strengths did equally well in combination with lower concentration of activated charcoal. Kufri Jyoti showed significant results (28.5% increase) for microplant height and root length (15.89% increase) with treatment T₂ (MS+100mg^l⁻¹ activated charcoal) and T₃ (MS+200 mg^l⁻¹ activated charcoal) when compared to the control (MS Media without activated charcoal) (Fig. 1A) Whereas, in Kufri Sindhuri, an increase of 50% in microplant height was found with T₃ (MS+200 mg^l⁻¹ activated charcoal) and other growth parameters with different treatments (T₁, T₂,

T₃, T₄, T₅ and T₆) of activated charcoal showed significant increase as compared to control (Fig. 1B). In case of number of leaves and nodes the results were on a higher side in Kufri Jyoti with treatment T₅ (MS+400 mg^l⁻¹ activated charcoal) when compared to the control (Fig. 1A). However, Bansal and Gokhale (2012) reported that in *Oroxylum indicum* the shoots formed in the presence of the activated charcoal were rather weak with small leaves. They observed that only low and moderate concentrations of activated charcoal were impelling, while high concentrations failed to induce rooting. It is possible that the addition of high concentrations of AC may induce nutrient deficiencies in culture media. Such a deficiency will affect explant growth and rooting (Pan and Staden, 1998).

Fig.1 Effect of activated charcoal full strength MS Media on *in vitro* growth of (A) Kufri Jyoti; and (B) Kufri Sindhuri; C, control; T₁, (MS+50mg^l⁻¹ AC); T₂, (MS+100mg^l⁻¹ AC); T₃, (MS+200mg^l⁻¹ AC); T₄, (MS+300mg^l⁻¹ AC); T₅, (MS+400 mg^l⁻¹AC); T₆, (MS+500 mg^l⁻¹ AC)

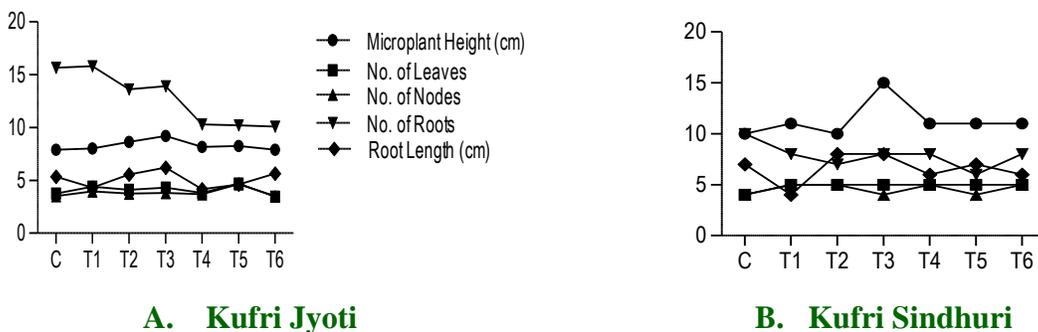
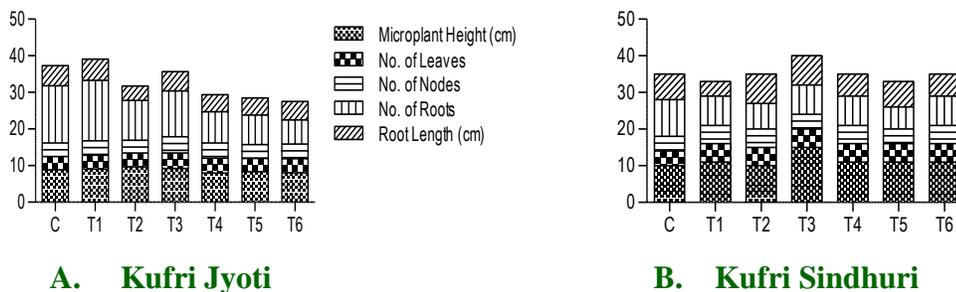


Fig.2 Effect of activated charcoal half strength MS Media on *in vitro* growth of (A) Kufri Jyoti; and (B) Kufri Sindhuri; C, control; T₁, (1/2MS+50mg^l⁻¹ AC); T₂, (1/2MS+100mg^l⁻¹ AC); T₃, (1/2MS+200mg^l⁻¹ AC); T₄, (1/2MS+300mg^l⁻¹ AC); T₅: (1/2MS+400mg^l⁻¹AC); T₆: (1/2MS+50mg^l⁻¹ AC)



Roots developed in *in-vitro* regenerated shoots on this rooting media were long, thin, unbranched and too weak to be transferred for hardening process. For the number of roots in Kufri Jyoti as the concentration of activated charcoal increased there was a decrease in root formation. However, treatment T₁ (MS+50mg l⁻¹ activated charcoal) produced better results but on comparing with control no significant difference was observed. Treatment T₂ (MS+100mg l⁻¹ activated charcoal) and T₃ (MS+200mg l⁻¹ activated charcoal) resulted in higher rate of root length in Kufri Sindhuri whereas, for the number of roots the results were more desirable in control when compared to the other treatments which were in agreement with the findings of Nisyawati and Kusuma (2013) and Das and Srivastav (2015) where the explants of banana cultivar Barangan (*Musa acuminata* L.) produced higher number of shoots on activated charcoal-free media than those on media with addition of different concentrations of activated charcoal. Same results of both the varieties in half strength media were observed wherein the microplant height was 12.8% more with treatment T₂ (1/2MS+100mg l⁻¹ activated charcoal) in comparison to the other treatments as well as control (Fig. 2A, B). According to Moraes *et al.*, (2005) and Agata *et al.*, (2010), media supplemented with activated charcoal improved the *in vitro* propagation of Brazilian native orchids and *Zygostates grandiflora*. Activated charcoal also plays an important role in orchid seed germination (Kull and Arditti, 2002; Moraes *et al.*, 2005). In case of number of roots and root length, the results were significantly higher with treatment T₁ (1/2MS+50mg l⁻¹ activated charcoal). However, as the concentration of activated charcoal increased there was a decrease in the number of roots. For number of leaves and number of nodes no significant difference was found between different treatments and the control. It has been observed that activated

charcoal stimulate growth and differentiation during culture regeneration (Nayanakantha *et al.*, 2010) whereas its addition to the regeneration media may have adverse effects on growth and development, as activated charcoal is able to absorb high concentration of growth regulators (Fernando *et al.*, 2010). The non-selective adsorption of growth regulators may result in inhibitory effects of growth *in vitro* (Thomas, 2008). Activated charcoal has a significant effect on the potato microtuber induction. The positive effect of Activated charcoal on microtuberization might be due to adsorption of ethylene (a strong inhibitor for tuberization) and reduction of light in root medium. Reduction of light at the base of a shoot can provide a conducive environment to the accumulation of photosensitive auxin or co-factors (Hassan *et al.*, 2011). Therefore, it can increase tuber yield and greatly shorten the time of microtuber induction (Xu, 2003 and Liu, 2001). Thomas (2008) reported that the concentration of activated charcoal differed widely in plant tissue culture from a range of 0.002 g l⁻¹ to 150 g l⁻¹. The concentration may be related to different plant species, medium, explants, purpose etc.

Thus, it could be concluded that the genotype is solely responsible to confer the microplants sensitivity as well as its tolerance limit against different supplements in MS media. It is therefore considered of utmost importance that the use of these supplements (Activated charcoal) could be done in a controlled manner (concentrations) in order to maintain the endophytes / microplant balance.

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