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Extended Abstracts

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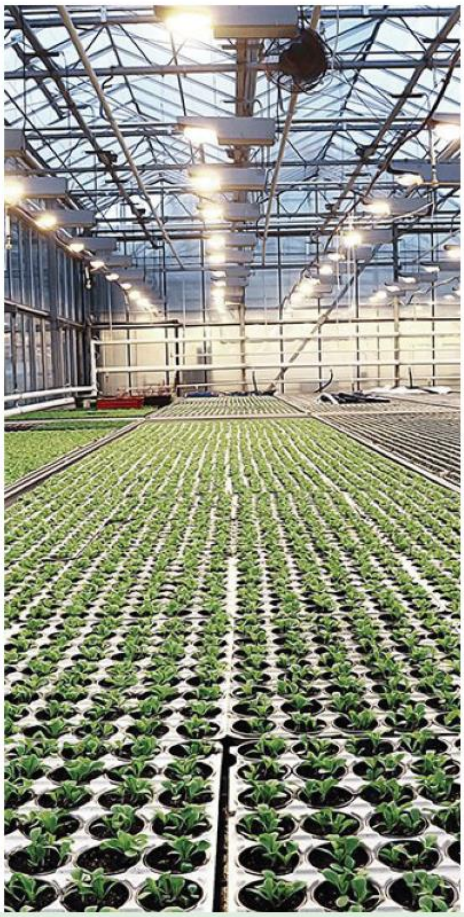


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Effect of BAP and hypocotyl explants of tomato (*Lycopersicon esculentum* Mill.) var. KC1 for *in vitro* plant regeneration

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1. Introduction

Tomato is considered an important vegetable crop that belongs to the family Solanaceae. The tomato contains vitamins, minerals, fibre, protein, carotenoids, polyphenols, amino acids, fatty acids etc. (Chaudhary et al., 2018). It is highly popularized due to its anticancer and antioxidant characteristic (Khuong et al., 2013). Also, it is consumed as fresh fruit and processed in multiple forms such as salads, cooked vegetables, sauce, and pickle (Lenucci et al., 2006). Several kinds of explants have been used specifically in tomatoes to regenerate plants (Raziuddin et al., 2004). The types and positions of explants and also plant growth regulators used in the culture medium mainly affect the success of *in vitro* response of tomato (Yildiz, 2012). The different concentrations of plant growth regulators and various sources of explants have been used for the plant regeneration of tomatoes. Several studies show that the hypocotyl explant is an effective explant source for the induction of callus for plant regeneration (Setiaji, 2020). Park et al. (2003) reported that MS medium with 1.0 mg/l BAP and 0.1 mg/l NAA enhanced callus formation and shoot regeneration in tomatoes. Further, MS medium with BAP and NAA gave the best responses in terms of callus induction for hypocotyl explants of tomato cultivars (Chandel & Katiyar, 2000). Accordingly, there is a need to find optimum concentrations of plant growth regulators and position of explants for the shoot and root induction from hypocotyl explants of tomato. Therefore, this study was done to select the suitable concentration of BAP for *in vitro* response of hypocotyl explants and to evaluate the effect of different positions of hypocotyl explants of tomato variety KC1 for the plant regeneration process.

2. Materials and Methods

This experiment was conducted to study the effects of BAP on *in vitro* regeneration of tomato plants from hypocotyls explants at the Tissue Culture Laboratory, the Eastern University of Sri Lanka in 2017. The experiment was laid out in complete randomized design (CRD). Mature seeds of tomato cv. KC-1 was obtained from the Horticultural Crops Research and Development Institute, Department of Agriculture, Gannoruwa, Sri Lanka and used as a source of explants in this experiment. Surface sterilization of tomato seeds was done by spraying with 70% ethanol for 3 min followed by 5.23% sodium hypochlorite (Clorox^{TM}) at 20% (v/v) treatment with two drops of Tween-20 for 20 min. The seeds were then washed with sterilized distilled water four times until washed out the detergent. Seeds were then kept in a sterilized Whatman No. 1 paper for germination. *In vitro* 12 days old seedlings were used to excise the hypocotyl explants for the regeneration process. In the present study, Murashige and Skoog (MS) basal medium (1962) along with various concentrations of different growth hormones were used. A quantity of 30 g/l of sucrose and 0.8% w/v agar was added to the medium. The pH of the medium was adjusted to 5.8. Media containing culture vessels were then autoclaved at 15 psi at 121º C temperature for 20 min.

Hypocotyl explants (1.0 cm long) were collected from 12 days old *in vitro* grown seedlings and surface sterilized. Then they were inoculated onto MS medium containing 0, 0.5, 1.0 and 1.5 mg/l BAP with 0.2 mg/l NAA. Subsequently, hypocotyl explants were excised in three different positions, such as top portion (near to cotyledonary node), middle portion and bottom portion (near to root base) from *in vitro* germinated seeds under aseptic conditions and inoculated onto

MS medium containing 1.5 mg/l BAP with 0.2 mg/l NAA. The culture vessels containing explants were incubated at 25±0.5ºC under white fluorescent light. A photoperiod of 16 hours light with the intensity of 2000 lux and 70% humidity was maintained. The observation was made at regular intervals and this experiment was laid out in complete randomized design and repeated. The collected data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of Statistical Analysis Software (SAS). The mean comparisons between treatments were done by using Tukey's (HSD) test at 5% significant level.

3. Results and Discussion

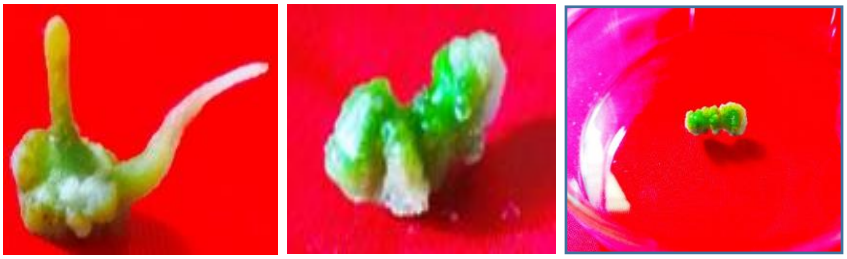
Results showed significant influence ($P<0.01$) on callus induction at the 4th week of culture (Table 01) and higher callus induction % was obtained in 1.5 mg/l BAP with 0.2 mg/l NAA medium. The least callus induction % was remarkably ($P<0.05$) obtained from the control treatment among the treatments. There was no any significant variation in callus induction % ($P<0.05$) between 1.0 mg/l BAP and 1.5 mg/l BAP in combination with 0.2 mg/l NAA. The different concentrations of BAP with 0.2 mg/l NAA in the culture media significantly influenced ($P<0.05$) the shoot bud formation percentage at the 8th week of culture (Table 01). Higher shoot bud formation was recorded in 1.5 mg/l BAP + 0.2 mg/l NAA medium than the other media. These findings are confirmed by Sarker (2013) reporting that the highest number of shoots in Tomato was noted from MS medium containing 2.0 mg/l BAP. Osman et al. (2010) reported the highest callus formation on hypocotyl explants from MS medium supplemented with 0.5 mg/l BAP and 0.1 mg/l NAA.

Table 01. Callus induction % and shoot bud formation % of hypocotyl explants cultured on MS medium with BAP and 0.2 mg/l NAA

BAP with 0.2 NAA (mg/l)	Callus induction % at 4th week	Shoot bud formation % at 8th week
0	33.3±2.0c	0.0±0.0 c
0.5	60.0±4.6b	0.0±0.0 c
1.0	80.0±2.0ab	61.3±0.6 b
1.5	93.3±1.2a	75.0±2.9 a
F test	P<0.01	P<0.05

Data are based on the availability of surviving explants cultured in the medium. Values represent the means ± standard error of the replicates. Means followed by the same letter in each column are not significantly different according to Tukey's HSD Test at a 5% significant level.

Higher root formation of 60% was noted in 0.5 mg/l BAP + 0.2 mg/l NAA medium. Further, there was no shoot bud formation on the control medium and also medium supplemented with 0.5 mg/l BAP + 0.2 mg/l NAA. Rooting is the final step of the regeneration protocol in plant tissue cultures. However, in most cases, root formation would be achieved with auxins alone with concentrations ranging from 0.1 to 1.0 mg/l (Sherkar & Chavan, 2014).



a b c

Figure 1. Callus formation in MS media containing a) 0.5 mg/l BAP and b) 1.0 mg/l BAP and c) 1.5 mg/l BAP with NAA at 3rd week

Further, different positions of hypocotyls as explants cultured in MS medium containing 1.5 mg/l BAP and 0.2 mg/l NAA also showed diverse morphogenic responses. After four weeks of culture, micro shoots were formed directly in some cultured top positions of hypocotyls on the edge of the hypocotyls while the bottom positions of hypocotyls exhibited green calli on the surface of the cultured explants. Compacted callus formed from the top portion of the hypocotyl explants near to cotyledonary node and callus colour was ranged greenish-yellow colour to green colour (Figure 2a). Further, shooty friable callus was observed from the middle portion of the cultured hypocotyl explants (Figure 2b). The bottom portion of hypocotyl explants showed thick and yellowish-white compact callus formation. Yildiz (2012) reported that shoot-regeneration efficiency is rapid in the upper part of hypocotyl explants.

a b

Figure 2. Induction of shoot regeneration from the top (a) and middle (b) hypocotyl explants after 2-3 weeks of culture

4. Conclusions

The results showed that higher callus induction % was obtained from the hypocotyl explants cultured in MS medium with 1.5 mg/l BAP and 0.2 mg/l NAA at the 4th week of culture. Similarly, higher shoot bud formation was recorded in 1.5 mg/l BAP + 0.2 mg/l NAA incorporated medium at the 8th week of culture. And also, it was concluded the top position explants of hypocotyls tend to produce direct shoots from the cultured explants whereas the bottom portion of hypocotyl explants formed compact callus.

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