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Adventitious Root Development from Stem Segments of Potato (*Solanum tuberosum* L.) by using Indole-3-Butyric Acid under *In vitro* Conditions

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Abstract – Adventitious buds derived from the adventitious roots of *in vitro* explants have the potential for mutation breeding of potato cv. granola. This study was done to find out the optimum Indole-3-Butyric Acid (IBA) strength for the *in vitro* adventitious root formation by culturing potato stem cuttings on MS media containing 0, 5, 15, 25, 40, 60 mg L^{-1} IBA concentrations. The findings showed that different strengths of IBA influenced remarkably ($p<0.05$) on days taken for root initiation, number of roots, root length and diameter and also root formation % in potato cv. granola. A high concentration (40 mg L^{-1}) of IBA produced callus quickly than the low concentration of IBA. Among six concentrations of IBA, 40 mg L^{1} concentrations showed the highest (60%) root formation with about 15 roots per explant while 25 mg L^{-1} IBA concentration exhibited 55% root formation with 12 roots per explant. Therefore, it could be stated that based on the quadratic curve, the IBA concentration of 42.69 mg L^{-1} is the favourable concentration for obtaining the highest adventitious root formation from stem segments in potato cv. Granola.

Keywords: Adventitious root, Indole-3-Butyric Acid*, In vitro* mutation

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1. Introduction

Potato (*Solanum tuberosum* L.) is a high economically valuable dicotyledonous tuber crop consumed in the world. Potato global production is over 368 million tons per annum and it ranks fourth after maize, rice and wheat (Albiski *et al,* 2012). It is mostly cultivated by farmers with limited resources and the minimal land availability (Kumlay and Ercisli, 2015). In Sri Lanka, potato is the major crop cultivated in the Nuwara-Eliya district in the upcountry wet zone above 1500 mean sea level (MSL) extensively in the Badulla district in the upcountry intermediate zone between 1000 to 1500 MSL and to a lesser extent in Jaffna and Puttalam districts in the dry zone. Potato is vegetatively propagated by using tubers for commercial cultivation. However, this method has low multiplication rates and poor yield due to susceptibility to diseases and pests (Mahfouze *et al*., 2012). Many cultivars exhibit poor yield with diminished tuber size and sunken eyes in tubers affectsthe market value of tubers in the developing countries (Bado *et al*., 2015). The number of eyes in each tuber is a vital criterion determining the number of main stems (Shirani *et al*., 2008).

The *in vitro* propagation is a suitable alternative method for potato production. In potato, the induction of mutation produces mutants for different characters such as long shelf life and an increase in tolerance of abiotic and biotic stresses (Albiski *et al*., 2012). In *vitro* cultures in mutation breeding offer several advantages over the conventional methods (Zia *et al.,* 2018). Mutagenic treatments are applied to tuber buds for rapid production of the mutants in potato. Single-node stem cuttings are irradiated with gamma rays under *in vitro* conditions for shoot propagation in potato mutation breeding (Bado *et al*., 2016). The callus induction and the regeneration of adventitious roots are mainly influenced by the kind and strength of plant growth regulators incorporated into the culture medium. According to the literature survey, high concentrations of auxin rather than cytokinin inhibit the shoot bud formation while they induce mutant cell lines for specific traits. Therefore, an experiment was done to find the suitable IBA strength, which produces the highest adventitious roots from stem segments of potato for the mutant breeding program.

2. Materials and Methods

This work was conducted at the Tissue Culture Laboratory, Agriculture Research Station, Sitaeliya, Nuwaraeliya in 2019. Stem segments without nodes were excised from five-week-old *in vitro* plantlets (Figure 1). Murashige and Skoog (MS) media containing various concentrations (0-60 mg L^{-1}) of Indole-3-Butyric acid (IBA) were used in this experiment. All the media contained 30 gL^{-1} sucrose and the pH was 5.8 before autoclaving. After autoclaving, 10 mL medium was poured into each sterilized culture bottle.

Figure 1. *In vitro stock plantlets.*

Six treatments (Table 1) were arranged in a complete randomized design with three replications. *In vitro* plantlets (8-10 cm height) were used to obtain stem segments (1 cm) under

laminar airflow conditions and they were placed vertically in the culture media. After culturing, culture bottles were covered with lids and labeled. All the cultures were placed in a growth cabinet and exposed to 23±1°C temperature, 75% relative humidity and 3000 Lux of light intensity. The cultures were observed regularly and the time required for callus formation (days), callus formation percentage, the time required for first root formation (days), root formation percentage, root number per explant as well as root length and diameter (mm) were recorded. Data were analyzed using the SAS statistical software.

Table 1
IBA concentrations used in the experiment.

Treatments	T1	T2	T3	T4	T5	T6
IBA concentrations (mg L^{-1})	0	5	15	25	40	60

3. Results and Discussion

Days taken for callus formation

The number of days taken for callus induction was reduced with the increase (10- 25 mg L^{-1}) in IBA concentration and further increase of IBA has not reduced the time required for callus initiation (Figure 2). None of the explants cultured on MS medium without IBA produced calli while explants cultured on medium with the lowest IBA concentration (5 mg L^{1}) took 9 days to produce calli while this was 6 days for IBA concentrations above 25 mg L^{1}. Moreover, there was no linear tendency between the number of days required for callus formation with the different IBA concentrations.

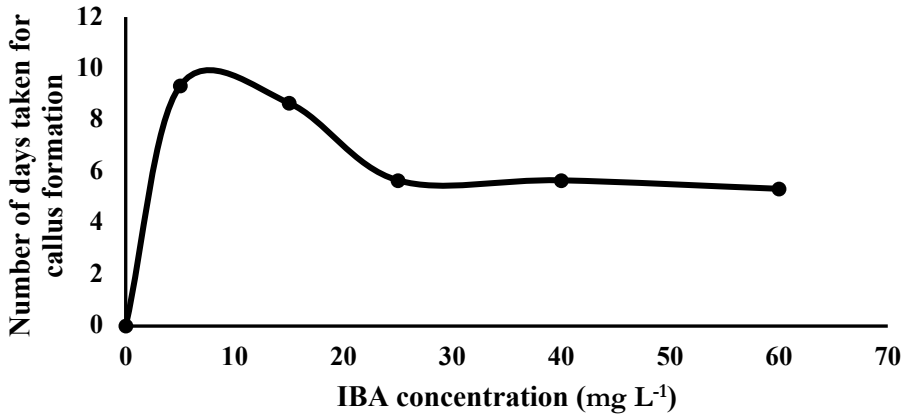

Figure 2. *Effect of IBA concentrations on number of days taken for callus formation*.

Callus formation percentage from stem segments

The highest percentage of callus formation was noted on The MS medium with 40 mg L^{1} IBA concentration (Figure 3) while at the lowest IBA strength (5 mg L^{-1}), 10% of the callus formation (percentage of explants exhibited calli) was recorded. Based on the quadratic curve, IBA concentration of 36.55 mg L^{-1} showed highest percentage (72.9%) of callus production compared to all the other concentrations. Further, an increase in IBA concentration reduced the callus formation percentage and 42.2% of callus initiation derived 60 mg L^{-1} according to

the quadratic equation (Table 2). It was noted that there was linear quadratic relationship between IBA concentration and the percentage of callus formation.

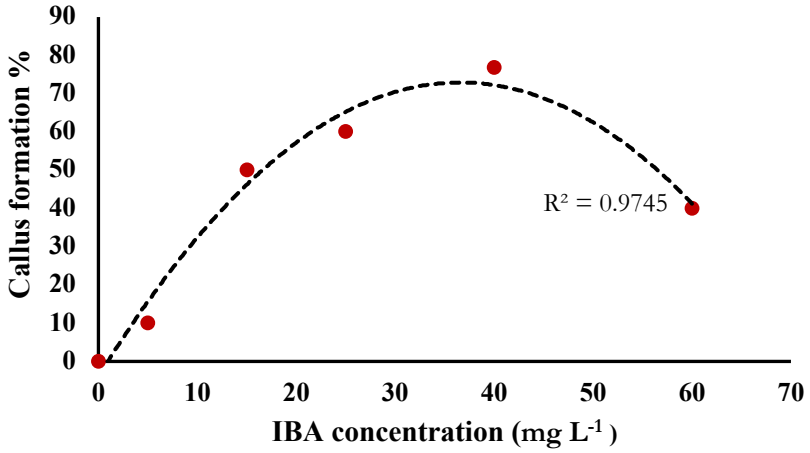

Figure 3: *Effect of IBA concentrations on percentage of callus formation in stem segments*.

Table 2
Quadratic equation and optimum concentration for attaining high value in each paprameter

Parameter	Quadratic Equation	Optimum IBA Concentration (mgL^{-1})
Callus formation %	$y = -0.0575x^2 + 4.2039x - 3.9135$	36.55
Root formation %	$y = -0.0372x^2 + 3.1796x - 6.6822$	42.69
Number of roots	$y = -0.0099x^2 + 0.8078x - 1.3354$	40.79
Root length	$y = -0.0087x^2 + 0.7282x - 1.1034$	41.85
Root diameter	$y = -0.0013x^2 + 0.1006x - 0.1549$	38.69

Root formation from stem callus

Similar to callus formation, root initiation was also increased with the increase in IBA concentration upto 40 mg L^{-1}. Highest percentage of root formation has occurred at 42.69 mg L^{-1} IBA concentration resulting from quadratic equation (Table 2). Interestingly calli did not produce roots below 5 mgL^{-1} IBA concentrations (Figure 4). Seran and Thinesh (2015) suggested that high IBA concentration (6000 ppm) is better for establishing dragon fruit cuttings under *ex vitro* conditions. Typically roots produced in the culture are not functional (Seran and Ahmad, 2018). Kumlay (2014) stated that root number increased when culturing potato nodal explants in the medium containing 0.25 mgL^{-1} GA3 with 1 mg L^{-1} IBA.

IBA concentrations of 25 and 40 mg L^{-1} formed first root from callus within a second week, and roots were produced quickly in this medium than the other treatments. Concentrations at 15 and 60 mg L^{-1} IBA took somewhat same days for root formation. As a result, the low (15 mg L^{-}) and high IBA concentration (60 mg L^{-1}) were unsuitable for quick root formation. They took a longer period (3 weeks) for root formation from the day of culturing. All the roots were initiated from the callus produced by explants. Roots elongated after 4 weeks, and it indicated that root growth was enough for shoot bud formation from the adventitious roots. Elaleem *et al.* (2009) reported that root system was indispensable for

axillary bud's release of potato. Park *et al*. (2017) reported that roots provide water and mineral to bud formation in *in vitro* grown peach shoots.

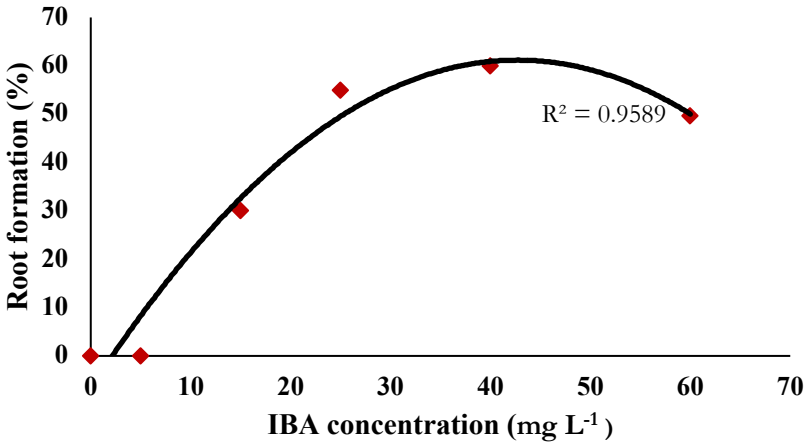

Figure 4: *Association between IBA concentration and root formation percentage.*

Number of roots formed from stem callus

There was no linear tendency to the number of root formation with IBA concentrations. Number of roots increased with the increase in IBA concentrations (15-40 mg L^{-1}). Highest root number (15) has resulted from stem segment on MS medium containing 40 mg L^{1} IBA concentration (Figure 5). Moreover, 25 mg L^{-1} IBA concentration produced 12 roots. No roots were observed at the 5 mg L^{-1} IBA concentration medium. Franklin *et al*. (2004) reported that IBA has the ability to produce roots and proved its practical usage in inducing root formation on eggplant stem cuttings.

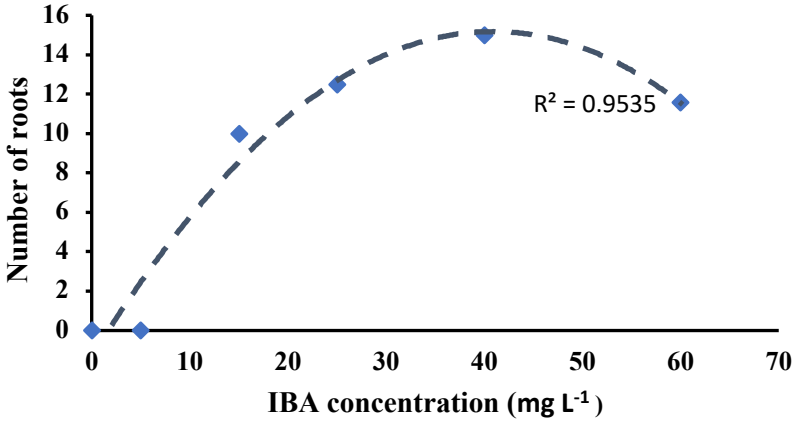

Figure 5: *Relationship between IBA concentration and number of roots formed from stem callus.*

Al-Taleb *et al.* (2011) stated the root number per explant was high (10) in medium containing IBA. Kumlay (2014) indicated that high (16) root number was formed in IBA-containing media than other auxins. All these research findings indicated that roots were produced after shoot formation at a low concentration of IBA. Nevertheless, in the present study, roots developed directly from the callus; therefore, it needs a high IBA concentration to

form the adventitious root (Figure 6 A-C). In this study, reason for the adventitious root formation is to initiate the adventitious buds from the adventitious roots.

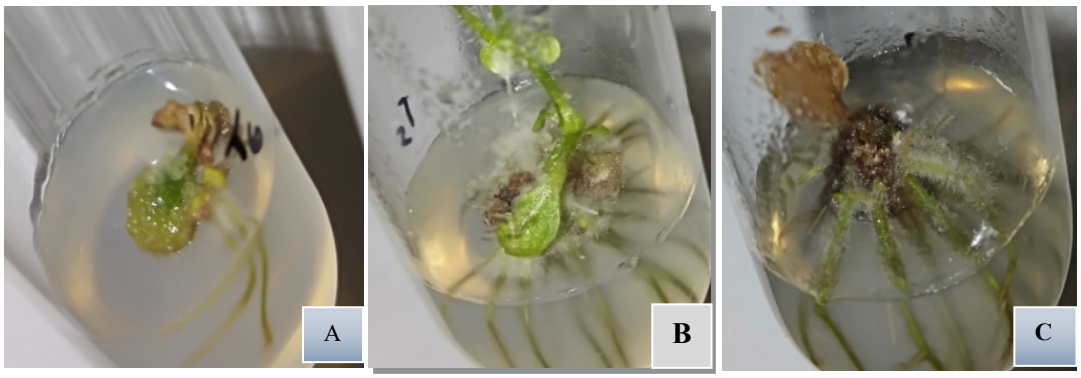

Figure 6: *Callus formation at initial stage (A) and root formations in 15 mg L^{-1} (B) and 40 mg L^{-1} concentrations (C).*

Effect of IBA on root length

Root is an important plant part which is used to absorb the nutrient and water. Hence, increment in root length causes to increase the surface area for the nutrient absorption and bud formation. When considering the six treatments, 40 mg L^{-1} IBA concentration had highest root length (14.06 mm) and length (9.82 mm) was low in 15 mg L-1 IBA (Figure 7). The average root length (12.94 mm) was observed at 25 mg L^{-1} IBA. However, the root length (11.34 mm) at 60 mg L^{-1} of IBA was lower than that at 25 mg L^{-1} of IBA. The medium with IBA concentration (41.85 mg L^{-1}) produced 14.16 mm length based on quadratic equation. No any root was noted in low concentration (5 mg L^{-1}), and also hormone free medium (Figure 7). There was no linear relationship between the root length and IBA concentration. Skalicky *et al*. (2018) reported the root length was significantly affected by IBA in stem cuttings.

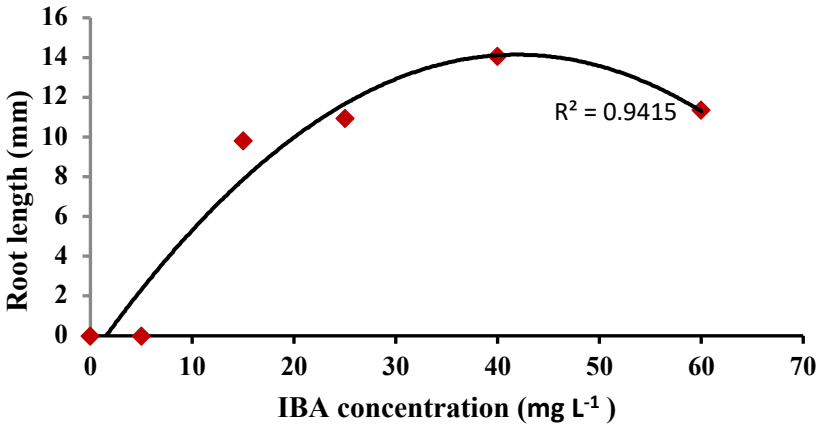

Figure 7. *The Association between IBA concentration and root length.*

Effect of IBA on root diameter

Figure 8 reveals that there was no linear association between IBA concentration and root diameter. Highest root diameter (1.73 mm) was obtained in 25 mg L^{-1} of IBA concentration.

The concentration at 15 mg L^{-1} IBA formed thin roots with diameter of 1.18 mm) and 60 mg L^{-1} IBA concentration formed thick roots (1.21 mm diameter) than roots in 15 mg L^{-1} concentration. Frick and Strader (2017) found additional effect of high concentration of auxin increased callus formation and thickening of the roots. Kumlay and Ercisli (2015) stated that IBA has significant effect on rooting, especially cuttings which are considered hard to root. Based on quadratic curves, the quadratic equations for the percentage of callus formation, root formation %, root number, root length and root diameter were presented in Table 2. The optimum concentration of IBA for obtaining high value in each parameter was derived from the quadratic eqoation and also given in the Table 2.

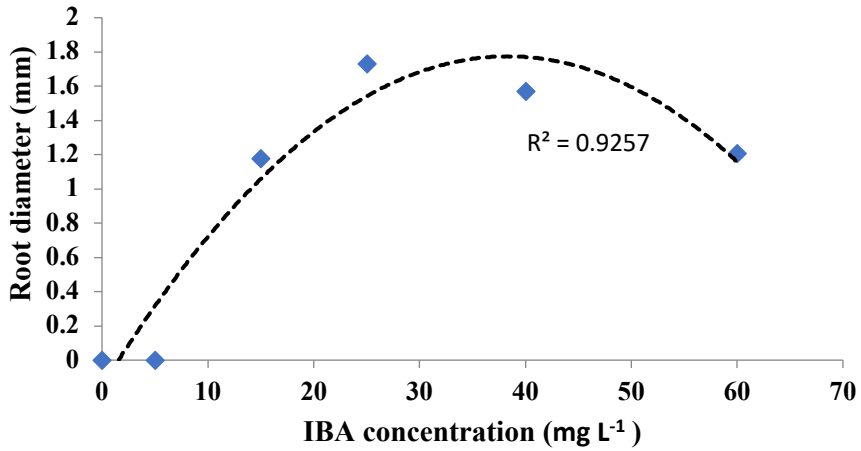

Figure 8. *Relationship between IBA concentration and root diameter.*

Shoot formation from stem callus

The high percentage of shoot formation occurred at 15 mg L^{-1} IBA and reduction in shoot formation occurred with the increase in IBA concentrations (Figure 9). Hence, the overall results revealed the reducing in the IBA concentration cause increased in the shoot formation. Because cytokinin is a hormone responsible for shoot formation and auxin is a responsible for root formation therefore when the IBA concentration was increased then shoots formation was reduced. Olatunji *et al.* (2017) stated the callus formation from which embryos and shoots regenerated on potato explant on a complex MS medium containing 0.2 mg L^{-1} NAA and 0.5 mg L^{-1} BA. Mullar (2011) indicated high level of auxin inhibits shoot formation and optimum fresh weight of callus was obtained on medium with 2 mg L^{-1} auxin and 0.02 mg L^{-1} kinetin.

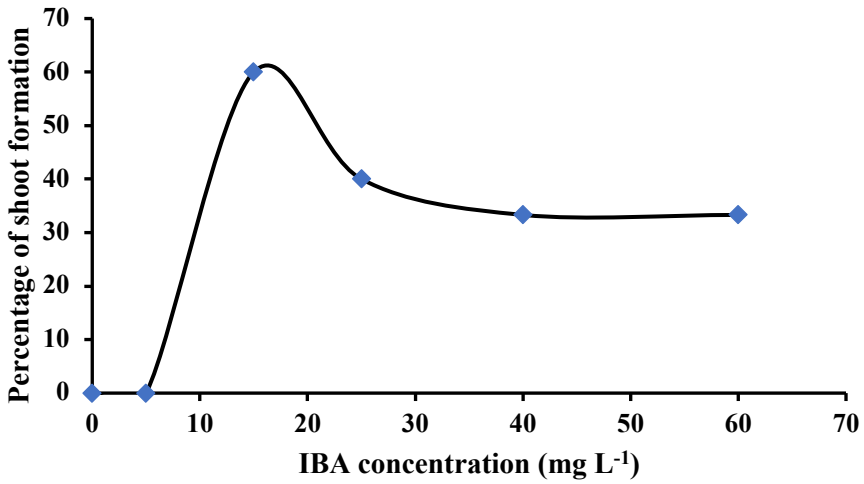

Figure 9. *Effect of IBA concentration on percentage of shoot formation*.

4. Conclusion

This study showed that the adventitious root formation was influenced by IBA concentration in the medium. The IBA strength remarkably ($P<0.05$) influenced the days taken for the root formation, root formation percentage, length, diameter and number of roots in granola potato variety. When the IBA concentration increased, the growth attributes also increased. According to the results, it could be concluded that IBA concentration of 42.69 mg L^{-1} is more suitable for highest adventitious root formation from stem segments under *in vitro* conditions.

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