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Tissue Culture Protocol for Production of Planting Materials of Apple by Using Shoot Tip Culture

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Abstract*—Shoot tip explants were taken from in vitro germinated apple seedlings using imported apple (Red Delicious). Apple seeds were germinated in hormone free Murashige and Skoog (MS) medium. In vitro raised shoot tips were transferred to different concentrations (0.0, 0.5, 1.0 1.5, 2.0, 2.5, 3.0, 3.5, 4, 4.5, 5 mgL^{-1}) of Benzyl Amino Purine (BAP) in MS medium for shoot proliferation. The highest rate of shoot multiplication was determined by number of uniform sized shoots per propagule. Significantly highest mean number of shoots was recorded in 4 mgL^{-1}BAP in MS medium. Subsequently, shoots were transferred into rooting media. Five replicates consisting two levels as full and half strength MS media and six levels of Indole Butric Acid (IBA) concentrations (0.0, 1.0, 2.0, 3.0, 4.0, 5.0 mgL^{-1}) were tested. Significantly highest mean number of roots was observed in half strength MS medium with 2 mgL^{-1} IBA for root induction.*

***Keywords*—** *Indole Butric Acid (IBA), Benzyl Amino Purine (BAP), Micro propagation, Root induction of apple*.

I. INTRODUCTION

Apple (*Malus pumila*), fruit of the genus *Malus* belongs to family Rosaceae. It is a pomaceous fruit and is the most widely cultivated fruit crop in the world. The major production areas are temperate regions. When consider about the propagation methods, such as budding and grafting are used successfully by many nursery growers. Though it is successful, these methods are slow, labor intensive and may require large amounts of land. Alternatively, successful *in vitro* clonal propagation methods are reported in apple for commercial applications in the fruit industry (Bommineni *et al.*, 2001).

Sri Lanka has a potential to grow apple in some climatic areas. Nowadays, apple cultivation is developing at Kundasala and Bandarawela areas by the Department of Agriculture, Sri Lanka. However, unavailability of true to type planting material is a major barrier to promote apple cultivation in Sri Lanka. In this regard, plant tissue culture plays a vital role to overcome this problem and it is fast and dependable method for the production of a large quantity of uniform plantlets in a short time throughout the year.

II. MATERIALS AND METHODS

Apple seeds were taken from imported apple (Red Delicious) fruits and germinated in hormone free MS (Murashige and Skoog, 1962) medium under growth room condition. Shoot tip explants were taken from *in vitro* germinated apple seedlings and transferred to different concentration (0.0, 0.5, 1.0 1.5, 2.0, 2.5, 3.0, 3.5, 4, 4.5, 5 mgL^{-1}) of Benzyl Amino Purine (BAP) in full strength MS medium for shoot proliferation. The culture bottles were arranged in CRD and each treatment was consisted with three replicates. At the end of the 4th week, all treatments were statistically evaluated for highest rate of shoot multiplication by ANOVA procedure using SAS statistical software. The highest rate of shoot multiplication was determined by number of uniform sized shoots per propagule. After selecting the best BAP concentration, shoots were multiplied in that selected medium by sub culturing shoots at 4 weeks interval to get large number of apple shoots. Same size apple shoots were selected from apple cultures with 4 leaves and 2cm length. After that, those shoots were transferred to different concentration (0, 1.0, 2.0, 3.0, 4.0, 5.0 mgL^{-1}) of Indole Butyric Acid (IBA) in full and half strengths MS media for root formation. The culture bottles were arranged in CRD with 5 replicates for each treatment. At the end of the 10th week, all treatments were statistically evaluated for rooting performance (length of root and number of roots) by ANOVA procedure using SAS statistical software. Parametric data was analyzed using ANOVA procedure and non-parametric (count) data was analyzed using ANOVA procedure after square root transformation. Mean separation was done using Tukey's grouping test. All media and equipment were autoclaved under 15 min at 121°C and 15psi. The pH of the medium was adjusted up to 5.8 before autoclaving. Cultures were grown at 26°C with 16 h photoperiods provided by white fluorescent lights with an intensity of 5000 lux.

III. RESULTS AND DISCUSSION

The highest shoot growth of apple was observed in 4 mgL^{-1} BAP. In addition, shoots and leaves in 4 mgL^{-1} showed green color appearance and healthy growth compared to other treatments. Lowest shoot growth was performed in BAP 0 mgL^{-1}. The figure 1 shows apple shoots initiation in full strength MS medium with different BAP concentrations and effect of different BAP concentration in shoot multiplication shows in figure 2.

In vitro grown apple shoots were cultured in two levels of MS (full and half strengths) and six concentrations of IBA to study the effect on root induction. In this experiment average number of roots per plant and average length of main roots were recorded. Root emergence commenced in T2 (half MS + 2 mgL^{-1} IBA), T1 (half MS + 1 mgL^{-1} IBA) and T3 (half MS + 3 mgL^{-1} IBA) treatments after 6th weeks of establishment, while other treatments had delayed root emergence.

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Fig. 1. Figure a and b show *in vitro* grown apple shoot tips in full strength MS medium with different concentrations of BAP

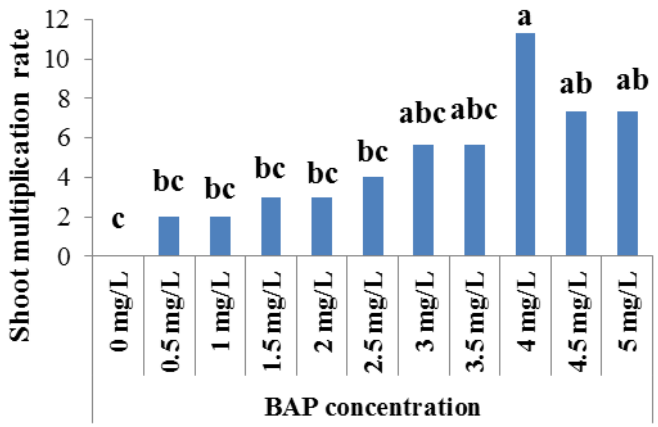

Fig. 2. Shoot multiplication rate performed in different concentrations of BAP

Where T2 (full and half strengths MS + 2 mgL^{-1} IBA) showed the highest growth rate including shoot height and number of leaves compared to other treatments. In addition, shoots and leaves in full strength MS series showed green color appearance compared to half strength MS medium (figure 3). Shoots on half strength MS series showed short and thick roots. While shoot on full strength MS series produced long and thin roots. Further, number of callus formation was observed at the base of shoots within experimental period when IBA level was increased.

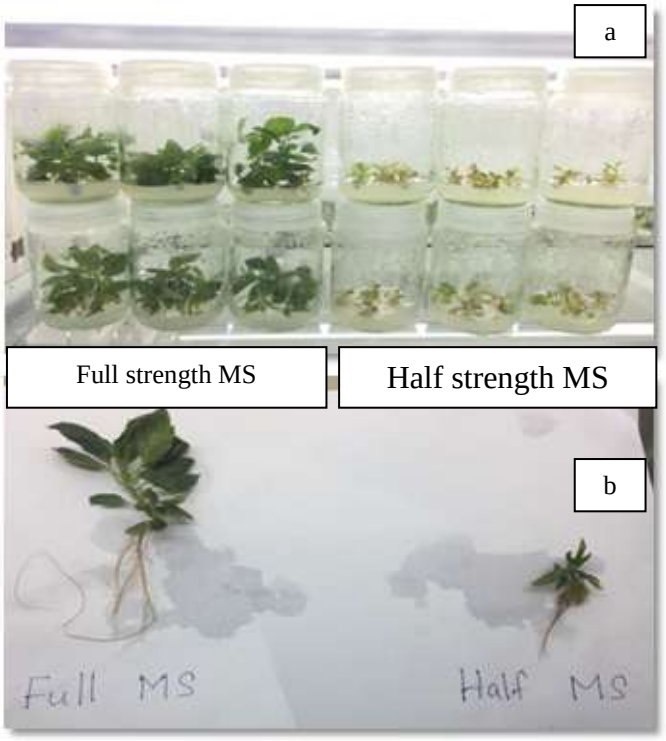

Fig. 3. a- Appearance of shoot growth and root growth in full and half strengths MS media. b- In vitro root induction in full and half strengths MS media.

According to statistical analysis, there was no significant difference on combine effect of MS media and IBA concentration, but those separately affected the average root length. When consider about strength of the MS media, the highest root length was performed in full strength MS medium. But, when considered IBA concentrations, the highest root length was performed in IBA 2 mgL^{-1} and the lowest root length was performed in IBA 0 mgL^{-1} (figure 4).

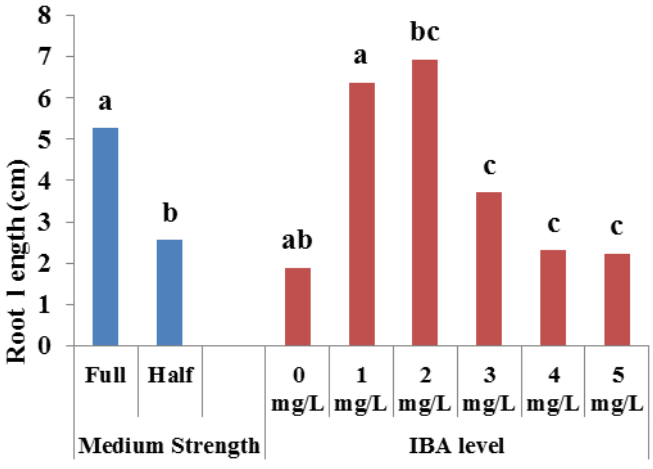

Fig. 4. Root length performed in full and half strengths of MS media in different concentrations of IBA

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There was a significant difference in number of roots when considered the strengths of the media and IBA levels. Therefore, combined effect of strengths of MS and IBA concentration was significantly affected on the average roots per plant. The highest root number was performed in IBA 2 mgL^{-1} in half strength MS medium followed by IBA 1 mgL^{-1} in half strength MS medium (figure 5).

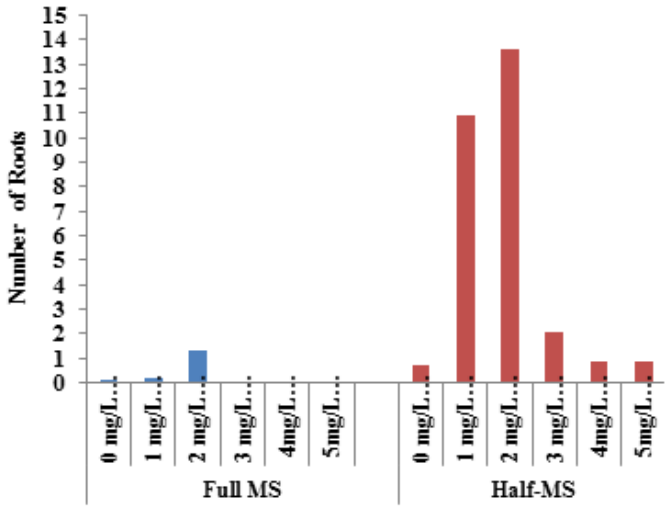

Fig. 5. Number of root performed in full and half strengths of MS media in different concentrations of IBA

Similar to above experiment Kumar *et al.* (2016) indicated that highest multiplication and elongation rate (9-10 cm long shoots) was obtained on medium having 0.04 mgL^{-1} GA3, 2.0 mgL^{-1} BAP, and 0.02 mgL^{-1} NAA. As well as according to the same authors, percentage of *in vitro* shoots of apple that rooted significantly varied with different IBA concentration in the medium. The best rooting percentage (96.66 %) obtained with half strength MS medium 1.0 mgL^{-1} IBA concentration.

Sharma *et al.* (2007) reported that the maximum rooting in apple (M7 rootstock) shoots (89.63%) was observed with 2.5 mgL^{-1} of IBA which is almost similar to above experiment. IBA at 2.0 and 3.0 mgL^{-1} concentrations also resulted in good rooting percentage of 86.63% and 79.00% respectively. As well as maximum rooting in apple (MM 106 rootstock) was observed in IBA 1mgL^{-1} (62.00%).

However in contrast to the above experiment, Meneguzzi *et al.* (2017) reported that *1 mg* L^{-1}*BAP was the best hormone concentration for successful multiplication of apple rootstock (G. 814) and w*hen concentrations above 1.5 mgL^{-1}IBA provided a decrease in rooting percentage, number and length of roots. The Maximum percentage of rooted plants (94%) was at 1.73 mgL^{-1}concentration of IBA. The highest number of roots (2.1) and highest root length (6cm) were recorded at 1.5 mgL^{-1} and 1.0 mgL^{-1} concentrations respectively.

Also in contrast to our experiment Boudabous *et al.* (2010) reported that greatest multiple shoot formation on MS medium with BAP of 1.0 and 2.0 mgL^{-1}. But concentration that exceeded 4.0 mgL^{-1} decreased the shoot growth. The best rooting was observed on half strength MS medium, supplemented with 3.0 mgL^{-1} of IBA and 2.0 gL^{-1} of activated charcoal.

IV. Conclusion

In the present study, BAP 4 mgL^{-1} was effective for highest rate of shoot multiplication. When increasing BAP than 4mgL^{-1}, rate of shoot multiplication was decreased. The highest numbers of roots were obtained on the half strength MS medium supplemented with 2 mgL^{-1} IBA. As the concentration of IBA increased the percentage of rooting decreased. However when consider about all treatments, plants which were in full strength medium had the highest shoot growth and the highest root length than half strength MS medium. Since root number is the most important factor than root length in plant growth we can recommend that, half strength MS as the best rooting medium and IBA 2 $mgL^{-1}$is the best hormone concentration for *in vitro* apple cultures.

Acknowledgment

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