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Comparative study on the effect of different substrate compositions on performance of tissue cultured *Dendrobium bigibbum* during the pre-hardening process

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Development of planting material using tissue culture is an established and widely used mass propagation method in the floriculture industry. However, considerable percentage of tissue culture raised plants may not survive during acclimatization process, due to inability to compete with soil microbes and adverse environmental conditions. The present study aimed to establish a successful pre-acclimatization procedure for *in vitro* produced *Dendrobium bigibbum* plants. Plants of 2.5 cm average height, with at least 3 leaves and 2-3 roots were selected for pre-acclimatization practices. Plants were planted in vessels with different compositions of substrates denoted as A and B series (A series-Sand:Compost:Charcoal Powder; A1-1:2:0.5, A2-2:2:0.5, A3-1:1:0.5, A4-1:2:1 and B series-Sand:Compost: Charcoal Chips (4-5 mm size); B1-1:2:0.5, B2-2:2:0.5, B3-1:1:0.5, B4-1:2:1) with Murashige and Skoog (MS) basal medium supplemented with benzylaminopurine (BAP) (0.1 mg/L) and naphthaleneacetic acid (NAA) (2 mg/L). Plants were grown under laboratory conditions at 22 ± 1 ^{0}C, and 16/8 hr photoperiod for three months before the acclimatization process. As the control, plants were grown in MS basal medium without any substrates. One-way ANOVA was used to determine whether there is a significant difference between tested samples using Minitab 16. According to the results obtained, there was no significant difference ($p>0.05$) in the shoot lengths of "A series" compared to the control. However, "B series" has shown a significant difference in the shoot lengths compared to the control ($p<0.05$). The highest shoot length of 3.87 ± 0.25 was observed in B1 medium. When fresh weights were compared, in "A series", only A1 showed a higher significant difference (1.30 ± 0.08) with the control while "B series" performed better compared to the control. Dry weight in both A and B series have shown a significant difference compared to the control. Plants grown in B1 medium has the highest number of roots (16.67 ± 1.53) compared to the other treatments. After acclimatization, the highest survival rate of plants were observed in "B series" (90%) whereas "A series" and control showed survival rates of 60% and 30% respectively. Hence, B series is suitable for shoot multiplication, root generation as well as pre-hardening to survive *in vivo*.

Keywords: Dendrobium, *In vitro* propagation, pre-acclimatization, substrate composition