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Clonal Propagation of *Aegle marmelos* through IBA Treatment for Sustainable Nutritional and Medicinal Supply for the Poor People of Agrarian Bangladesh

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ABSTRACT

The study was carried out at the Agriculture research field of Patuakhali Science and Technology University (PSTU) from March to October, 2017 to explore rooting performance of Aegle marmelos (Bael) through clonal propagation by stem cutting under 3 different doses of IBA (Indole Buetaric Acid) and planted in the perforated plastic tray filled with coarse sand and gravel placed in the non-mist propagator. The experiment was laid out following a Randomized Complete Block Design (RCBD) with 4 treatments and 4 replications (blocks). The treatments were T0= control, T1 = 0.2% IBA, T2 = 0.4% IBA, T3 = 0.8% IBA. The rooting ability of cuttings was significantly influenced by the application of IBA. The highest rooting percentage (60%) was recorded in A. marmelos both with 0.2% and 0.4% IBA followed by 0.8% IBA (40%). Longest root (3 cm) was recorded with 0.4% IBA followed by 0.2% IBA (1.2 cm). The maximum root number (2.25) and root diameter (2 mm) obtained from cuttings treated with 0.8% IBA followed by 0.4% IBA (2 and 1.9 mm respectively). Survival percentage of the cutlings (the rooted cuttings) significantly enhanced by exogenous rooting hormone (IBA) application. The highest survival percentage (73.5%) was observed for the cuttings treated with 0.4% IBA followed by 0.8% IBA (68.5%). Findings of the present study reveal that the plant species is highly amenable for clonal propagation by stem cuttings using low-cost non-mist propagator. Considering rooting percentage, root number and and root length, 0.4% IBA treatment may be recommended for mass production of quality planting stocks. Farmers and nursery owners can be trained up regarding this low cost non-mist propagation system of Aegle marmelos for cultivation of the species in homestead agroforestry or in fruit orchards for sustainable nutritional and medicinal supply for the poor people of agrarian Bangladesh.

Keywords:
Aegle marmelos; non-mist propagator; clonal propagation; IBA; rooting ability; steckling performance

1. Introduction

People of developing countries are suffering from under nutrition. Bangladesh's nutrition burden is significant and the rate of mal nutrition in Bangladesh remains highest in the world (Save the children 2015). About 41% people of Bangladesh are suffering from malnutrition and resultant diseases (Save the children 2015). A continuous effort is therefore needed to improve the nutritional status and to increase food security, particularly for the rural poor.

Fruits are universally promoted as healthy. The Dietary Guidelines for Americans (2010) recommend to make one-half of someone's plate with fruits and vegetables. Fruits are rich sources of vitamins, minerals, calories and other needful nutrients. Including fruits in daily diet will have multiple health benefits and will also help in disease cure (Kazi *et al.* 2015). Fruits are sources of phytochemicals that function as antioxidants, phytoestrogens, and anti-inflammatory agents and through other protective mechanisms (Slavin, 2012). The nutritional value of fruits are essential in our daily diet and places them on the crest of our edibles. Fruits contain vitamins and minerals in large quantities. Nutrition scientists emphasis to take at least 115 grams of fruit every day for balanced diet (Kazi *et al.* 2015). Appropriate nutrition stimulates optimal growth and development of human body.

Aegle marmelos (L.) Corr. commonly known as wood apple or 'Bael' which belong to family Rutaceae (Parveen *et al.* 2015) is a multipurpose fruit crop (Fig. 1) with considerable traditional socio-cultural values. *A. marmelos* is a medium sized, armed, deciduous tree (Maity *et al.* 2009) of tropical and subtropical region (Parveen et al. 2015) with an altitude ranging from 250-1200m (Maity *et al.* 2009). The tree generally grows in most of the countries of Southeast Asia (Maity *et al.* 2009, Islam *et al.* 1995). Extensive chemical investigations on various parts of the tree have been proved that the tree is biologically active against various major and minor diseases including cancer, malaria and gastroduodenal disorders (Maity *et al.* 2009). The most important part of the plant is its fruit which has a number of uses (Dhankhar, 2011).

Bael is an important fruit with its rich nutritive value. The different parts of Bael are used for various therapeutic purposes, such as for treatment of Asthma, Anaemia, Fractures, Healing of Wounds, Swollen Joints, High Blood Pressure, Jaundice, Diarrhoea and Typhoid trouble during Pregnancy (Parichha, 2004). The fruit has been used as herbal medicine for the management of diabetes mellitus in Ayurvedic, Unani and Siddha systems of medicine in some South Asian Countries (Kar *et al.* 2003, Lampronti *et al.* 2003, Karunanayake *et al.* 1984).

The 100g bael fruit can supply 137K calorie energy, 30.6gm carbohydrates, 2.2gm dietary fiber, 0.2gm fat, 1.8 gm protein, 1.5gm mineral, 186 gm IU vitamin A, 0.01 gm Thiamine (vitamin B1), 1.2 mg Riboflavin (vitamin B2), 0.9 mg Nicotinic acid, 0.01 mg vitamin C, 0.09 mg Calcium, 0.3 mg Iron, 0.05 mg Phosphorus and 0.6 mg Potassium (Shankar 1969, Paricha 2004). However, the most important ingredients present in bael plants are alkaloids, terpenoids, steriods, phenols glycosides and tannins (Venkatesan et al., 2009). In spite of possessing a good amount of nutritional and medicinal properties for mankind, surprisingly the fruit crop has always been paid little attention.

A. *marmelos* is generally propagated by seeds for cultivation (Singh *et al.* 1976). But viability of seeds is short and generally prone to insect attack (Purohit and Vyas 2004). Seedlings germinated from this type of seeds show great variation in morphological and biochemical characters due to heterozygous nature of plant. Propagation through budding and soft wood grafting is season dependent, slow and labor intensive (Pati *et al.* 2008). Vegetative propagation through root sucker is slow, difficult and cumbersome (Ajeethkumar and Seeni 1998). Another method for conservation of bael is micro-propagation. But it is expensive and often problematic because of great genotypic variation in the regeneration responses, presence of phenolic compounds, long complex cycles and the process of aging (Tantos *et al.* 2001). Thus, even though it's medicinal and high nutritional value, commercial orcharding is not expanding at a faster rate due to severe shortage of panting material (Pati *et al.* 2008). Hence, propagation of bael through stem cuttings by IBA treatment can be a convenient and cheap method to obtain fully developed trees at considerably low cost. Unfortunately, information about the artificial regeneration of bael through clonal propagation by stem cutting is very scarce and no research work has been done yet on this aspect in Bangladesh. So, the present study attempted to investigate the rooting ability of small leafy branch cuttings of bael in low-cost, non-mist propagator with or without rooting hormone (IBA) for artificial regeneration of the plants for sustainable nutritional and medicinal supply for the poor people of Bangladesh.

Figure 1. Full grown tree bearing fruit (A), ripe fruit of *Aegle marmelos (B)*, sapling of of *Aegle marmelos* with juvenile shoots at germplasm centre

2. Materials and Method

2.1. Study location, duration and climate

The study was conducted during March, 2016 to May, 2017 at the Agriculture research field of Patuakhali Science and Technology University, Bangladesh (lies between 21º48' and 22º36' north latitudes and between 90º08' and 90º41' east longitudes) (Fig. 2) which enjoys typical tropical climate characterized by hot humid summer and cool dry winter. The average temperature ranged between 14.3° to 27.4°C in winter and between 24.3° to 33.6°C during summer from 2010 to 2014 (Fig. 3). The average annual rainfall was 2657 mm and varied from 1877 mm to 3120 mm and rainfall usually takes place between June and September (Fig. 3). The day length varies from 10 h 45 min in December to 13 h 25 min in June. Relative humidity is minimum (64%) in February and maximum (95%) in June to September.

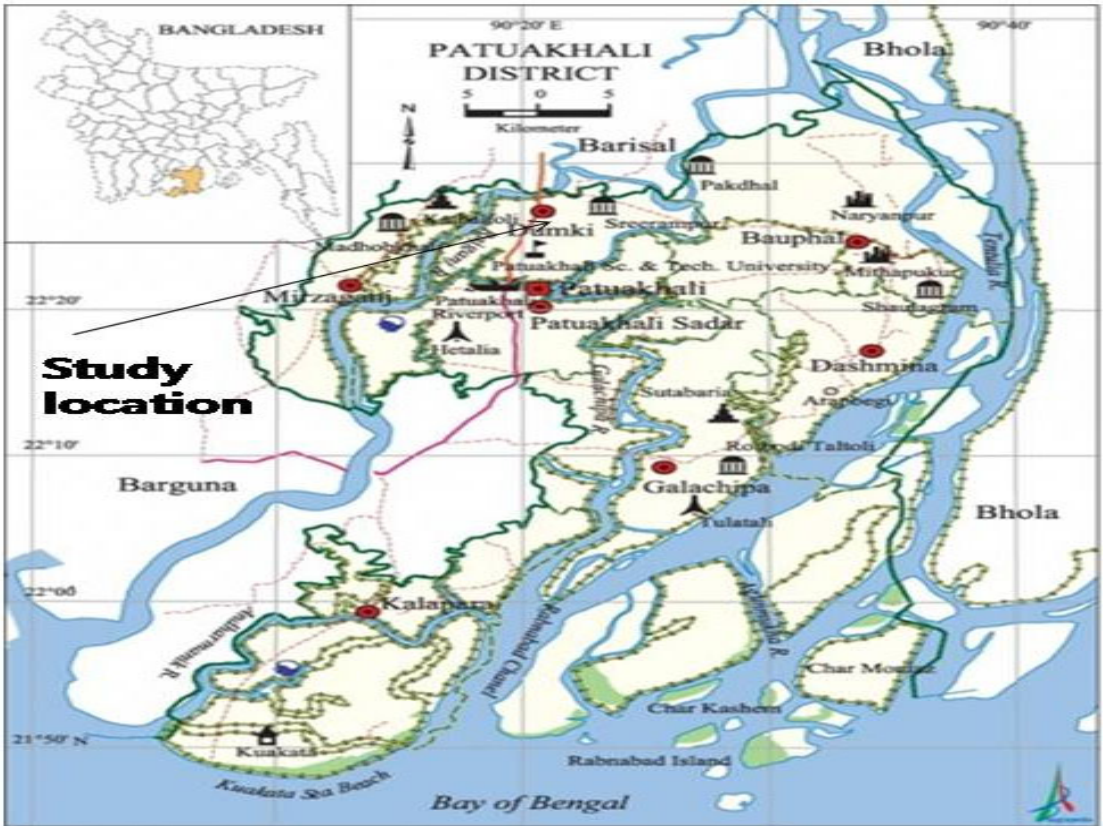

Figure 2. Geographical location of PSTU at Dumki Upazila

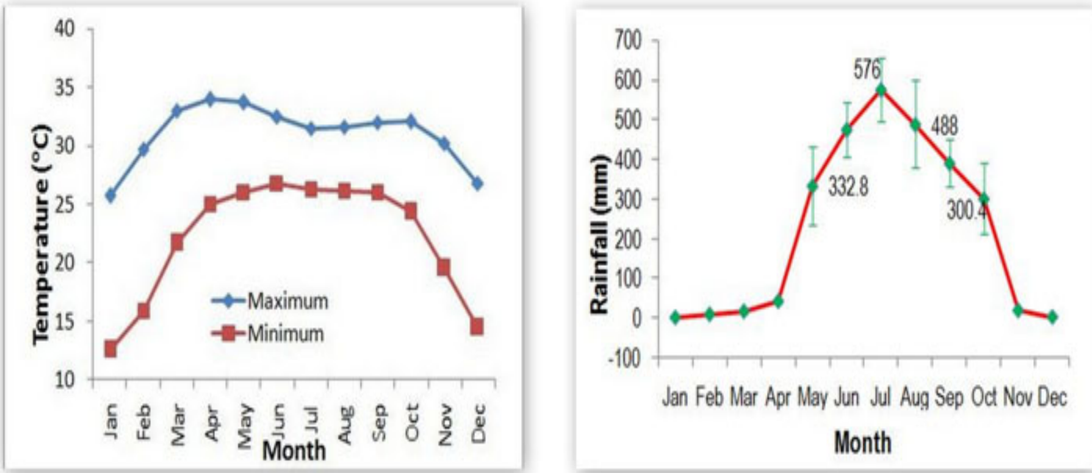

Figure 3. Temperature and rainfall at Patuakhali region from data of conjugative 5 years (2010-2014) (Source: Khepupara meteorological station)

2.2. Preparation of stem cuttings and setting up the experiment

The study on vegetative propagation was carried out in a low cost non-mist propagator (Fig. 4). The non-mist propagator was constructed following the design described by Leakey *et al.* (1990) modified by Kamaluddin (1996) and was covered with sheet of transparent polythene such that the base was completely watertight and the lid was also airtight. The polythene base of the propagator was covered with a 10 cm thick layer of moist coarse sand mixed with successive layers of fine gravels and small stones. This layer supported rooting media kept in perforated plastic trays.

Juvenile shoots of *A. marmelos* were collected from 4-years old stock plants raised in the Germplasm Centre of PSTU to get quality propagules. Two-node stem cuttings were prepared keeping two leaves intact and one leaf was trimmed to 50% leaf area and then immersed briefly in a solution of fungicide Diathane M45 (Rohm and Co Ltd, France; 2 g/L in water) to avoid fungal infection. The cuttings were then rinsed and kept under shade for 10 minutes in open air for drying.

As growth regulators are organic compounds that needed in the least amount which can support, inhibit, and may alter physiological processes of plants (Ulfa et al. 2015), stem cuttings were then treated with 0% (control), 0.2%, 0.4% and 0.8% IBA (Indole 3-Butyric Acid) solutions by dipping the cutting base into IBA solution to test effect of IBA on rooting ability and finally treated cuttings were planted into perforated plastic trays (12 cm depth) filled with coarse sand (*Sylhet* sand) mixed with fine gravel following a Randomized Complete Block Design (RCBD).

A total of 160 cuttings were placed under four different treatments with four replications per treatment. Ten cuttings treated with same concentration of IBA were placed in each plastic tray and each tray served as an experimental plot. Thus, the number of replication of cuttings per treatment was 40. The cuttings were watered once only just after setting inside the propagator.

2.3. Propagator environment, aftercare and transplanting

The propagator was kept under bamboo made shed to avoid excessive heat accumulation. The propagator was opened briefly in the morning and in the late afternoon to facilitate gaseous exchange. Whenever the propagator lid was opened for observation, a fine jet of water spraying was applied to cuttings to maintain a low vapor pressure deficit inside the propagator. This resulted in a permanently humid environment throughout the propagation period (around 85% relative humidity).

The assessments of rooting success were carried out weekly after the first two weeks of cutting placement in the propagator. A cutting was considered as rooted when it had at least one root exceeding 1 cm long. The rooting of cuttings in the propagator completed within six weeks after putting the cuttings into the rooting media in propagator.

After four weeks in the rooting medium, the rooted cuttings in the propagator were weaned before transferring them in to poly bags, particularly towards the end of rooting period during root lignifications. For weaning, the propagator was kept open at night for three days and then at day and night for another three days. The weaned rooted cuttings were then transferred into poly bags filled with soil and decomposed cow dung at a ratio of 3:1. Before planting into the poly bags, rooted cuttings were measured for rooting percentage, number of roots developed per cutting and the length of longest root.

2.4. Data analysis

The experiment was laid out following a Randomized Complete Block Design (RCBD) with 4 treatments and 4 replications (blocks). Mean values for root number, root length and root diameter were calculated on experimental plot basis. All data were analyzed through computer based statistical program MSTAT-C (Michigan State University, East Lansing, MI, USA) following the basic principles, as outlined by Gomez and Gomez (1984). Significant effects of treatments were determined by analysis of variance (ANOVA) and treatment means were compared at 5% level of significance by Duncan's Multiple Range Test (DMRT). Rooting percentage was calculated and values were adjusted accordingly using arc sign transformation formula before putting the data into Analysis of Variance since the percentages of cuttings rooted were distributed between the range of 30 to 100 and proportions were based on equal denominator.

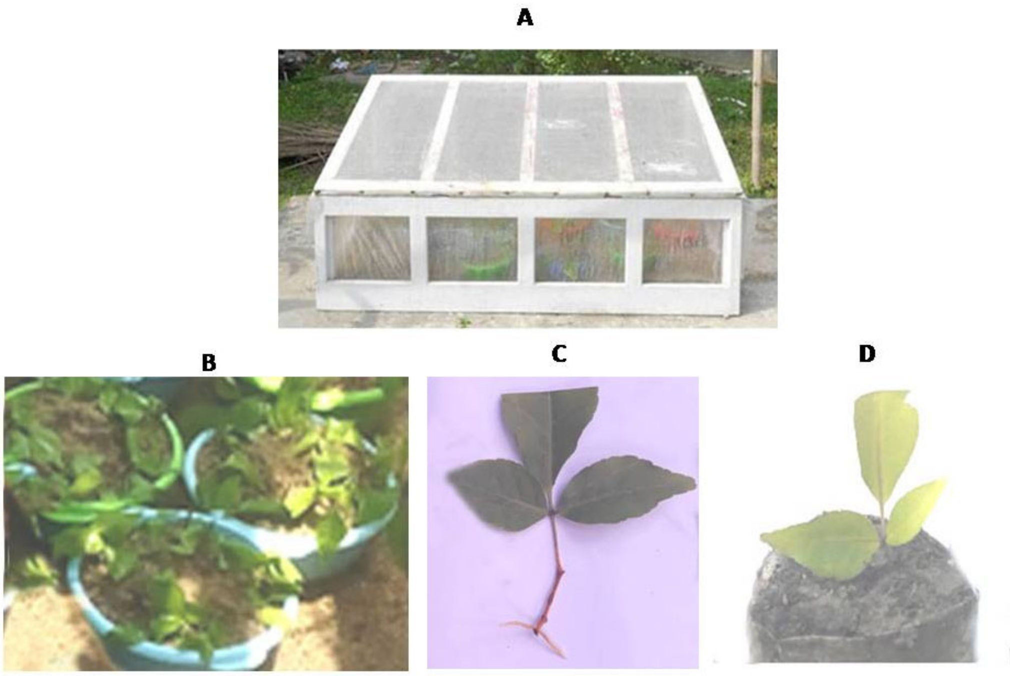

Figure 4. Non-mist wooden frame polythene propagator containing perforated plastic trays after setting of the experiment (A), Plastic trays with rooted cuttings (B), Rooted cutting (cutling) {C}, Transplanted cutling (D)

3. Results and Discussion

3.1. Rooting ability

Rooting percentage of A. *marmelos* cuttings was significantly enhanced by application of exogenous rooting hormone (IBA) (Fig. 6). The highest rooting percentage (60%) was obtained simultaneously from the cuttings treated with 0.2% and 0.4% IBA solution followed by the cuttings treated with 0.8% IBA (40%). The lowest rooting percentage (20%) was in cuttings without IBA treatment (control).

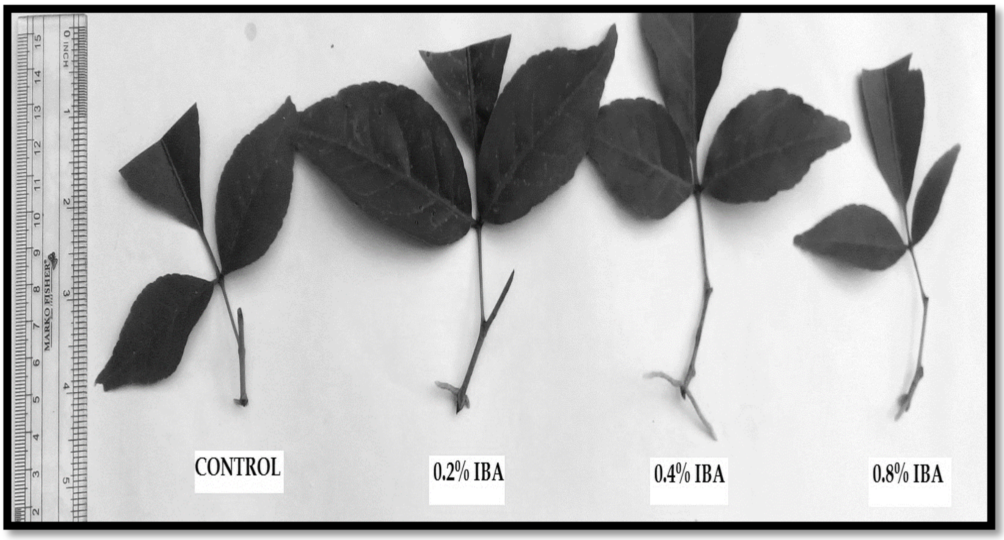

Figure 5. Rooting ability of cuttings of *A. marmelos* under various IBA treatments

Applied rooting hormone IBA is known to intensify the rooting percentage of cuttings as explained by many authors.

The effect of IBA on the rooting of *Punica granatum* was studied by Kabir et al. (2017b) and 0.2% IBA solution found best that showed 70% rooting percentage where as in control it was 35.5%. Effect of IBA was applied on *Milicia excelsa* by Ofori *et al.* (1996) and observed that the cuttings treated with 0.2% (2000 ppm) IBA rooted best and values declined gradually with successive increases in IBA concentration above 0.2%.

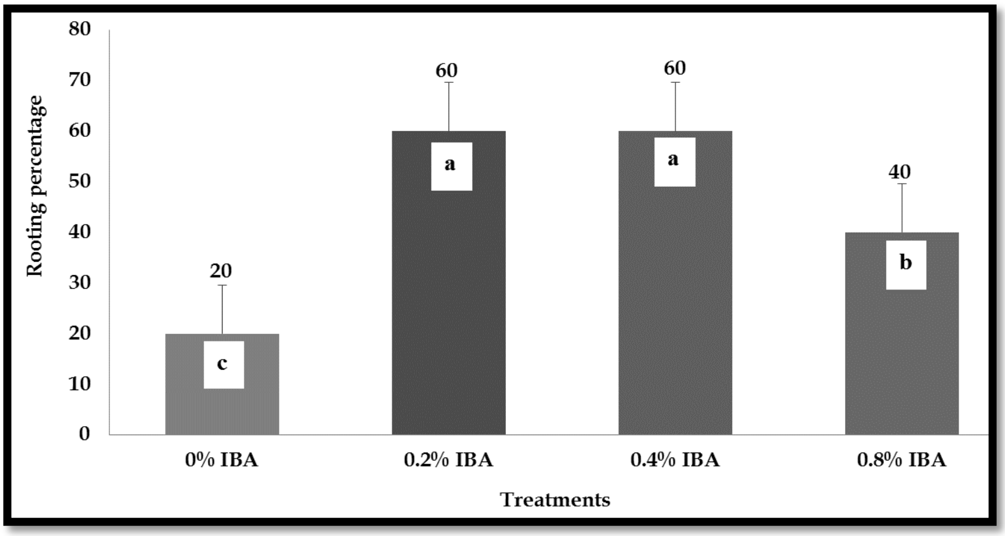

Figure 6. Rooting percentages of *A. marmelos* stem cuttings under different IBA concentrations (vertical bar represents standard error)

3.2. Root number

IBA treatment had a significant influence on root number of cuttings (Fig. 7). The highest number of root (2.25) was observed in 0.8% IBA treated cuttings, followed by 0.4% IBA (2) and 0.2% IBA (1.5) treated cuttings. Root number was lowest (1) in cuttings without IBA treatment. This result is in accordance with the findings of Abdullah *et al.* (2005) for *Baccaurea sapida*, where highest root number was observed in 0.8% IBA treated cuttings and lowest was in cuttings without (0%) IBA treatment.

Study on other fruit species by several authors also suggested that application of IBA solution at different concentrations effect on root number development. Kabir *et al.* (2017b) reported for *Punica granatum* that application of 0.4% of IBA solution enhanced root number from 6 (control) to 32. For *Flacourtia indica* the same trend was found that application of IBA increased root number from 2.5 (control) to 7.5 (0.4% IBA) (Kabir *et al.* 2017a).

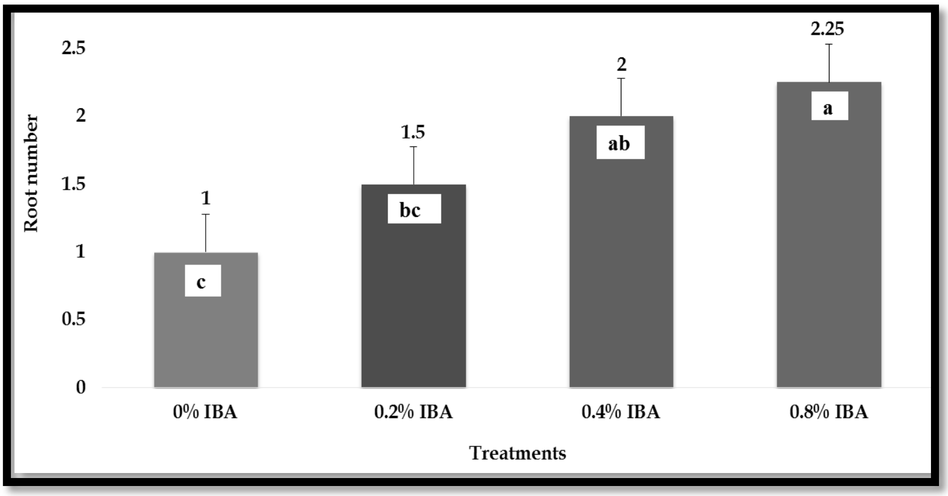

Figure 7. Root numbers of *A. marmelos* cuttings developed under various IBA concentrations (vertical bar represents standard error)

3.3. Root length

Root length was significantly influenced with the increasing IBA concentrations (Fig. 8 and 9). The longest root (3 cm) was found in cuttings treated with 0.4% IBA, whereas the shortest root (0.7) was recorded from the cuttings without IBA treatment. Similar result was reported by Abdullah *et al.* (2005) for *Baccaurea sapida*. The result of the present study is also similar to those of the reports of apple rootstock cuttings (Badshah *et al.* 1995) and 'GiSelA 5' cuttings (Gulen *et al.* 2004), in which the IBA treatments commenced the root length.

Figure 8. Longest root length recorded for different concentrations of IBA

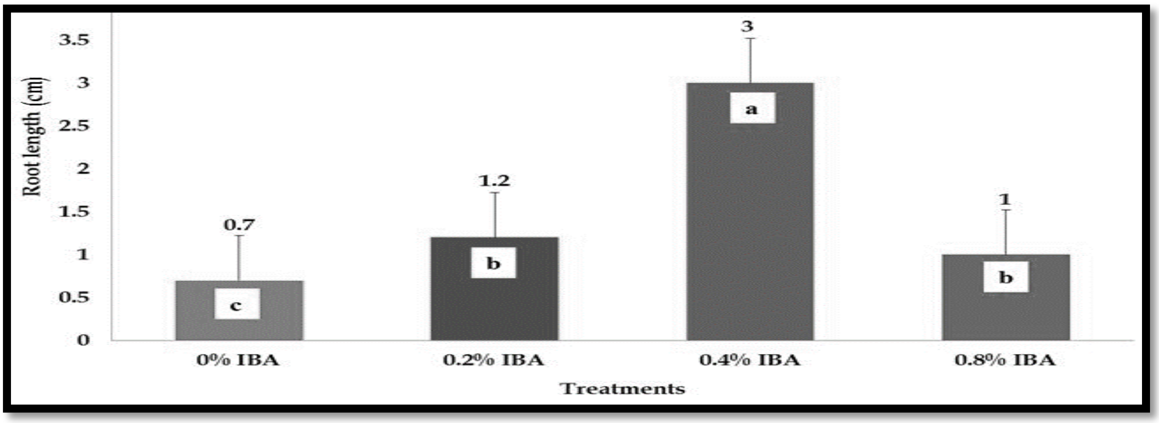

Figure 9. Root lengths of *A. marmelos* cuttings developed under various IBA concentrations after six weeks from establishing the cuttings (vertical bar represents standard error)

It is evident from the present study that the cutting of *A. marmelos* did not rooted well without any IBA treatment. The rooting ability of cutting in respect to rooting percentage, root number and root length was significantly enhanced with the applications of 0.2% to 0.8% IBA solution. Application of rooting hormone (IBA) increased the rooting ability of cutting also reported by many authors including Hossain (1999), Hosaain *et al.* (2002), Hossain *et al.* (2004), Abdullah *et al.* (2005), Dias *et al.* (1999), Rosa *et al.* (1997), Kamaluddin *et al.* (1996), Kamaluddin and Ali (1996) and Kamaluddin *et al.* (1998), Navjot and Kahlon (2002), Scaloppi and Martins (2004), Tripathi and Shukla (2004), Ram *et al.* (2005). Hosaain *et al.* (2011).

Applied auxin is known to intensify root-forming process in cutting for instance, polysaccharide hydrolysis is activated under the influence of applied IBA, as a result the contents of physiologically active sugar increases providing materials for meristamatic tissues and later for root primordia and roots. Hassig (1983) examined the function of endogenous root forming components of plants, which had auxin components and non-auxin components. He demonstrated the auxin components was required for development of callus in which root primordia initiated, but for subsequent primordial development both auxin and non-auxin components were needed. It may be possible that in cutting with optimum amount of endogenous auxin content and increasing of root number reflected the effect of applied auxin.

3.4. Root diameter

IBA at 0.8% concentrations resulted significantly highest root diameter (2 mm) followed by 1.9 mm for 0.4% IBA treatment (Fig. 10). The lowest root diameter (1.8 mm) was recorded for both the control and 0.2% IBA treatment. It supports the investigation by Pati *et al. (*2008) that application of IBA enhanced root diameter of *A. marmelos*.

These findings were similar to the findings of Singh (2017). Auxin application has been found to enhance the histological features like formation of callus and tissue and differentiation of vascular tissue (Mitra and Bose, 1954). Applied auxin is known to intensify root-forming process in cutting for instance, polysaccharide hydrolysis is activated under the influence of applied IBA, as a result the contents of physiologically active sugar increases providing materials for meristamatic tissues and later for root primordia and roots. Hassig (1983) examined the function of endogenous root forming components of plants, which had auxin components and non-auxin components. He demonstrated the auxin components was required for development of callus in which root primordia initiated, but for subsequent primordial development both auxin and non-auxin components were needed. It may be possible that in cutting with optimum amount of endogenous auxin content and increasing of root number reflected the effect of applied auxin.

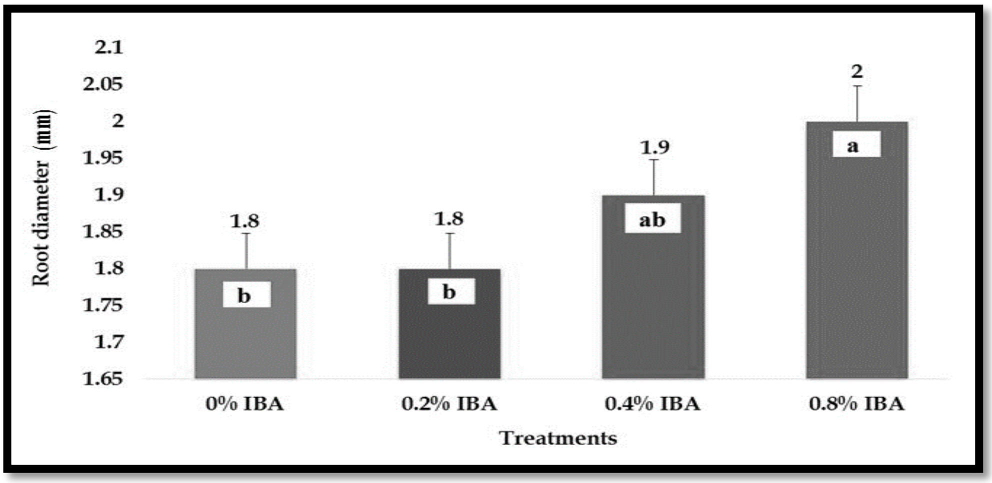

Figure 10. Root diameter of *A. marmelos* cuttings developed under various IBA concentrations after six weeks from establishing the cuttings (vertical bar represents standard error).

3.5. Steckling capacity

Survival percentage of the cutlings (the rooted cuttings) significantly enhanced by exogenous rooting hormone (IBA) application (Fig. 4 and 11). The highest survival percentage (73.5%) was observed for the cuttings treated with 0.4% IBA followed by 0.8% IBA (68.5%). The result is in accordance with the result for *Flacourtia indica* by Kabir *et al.* (2017a), *Flacourtia jangomas* by Hossain *et al.* (2011), and for *Baccaurea sapida* by Abdullah *et al.* (2005) applying rooting hormone IBA using in similar type of non-mist plant propagator.

The success of rooting in cuttings depends upon the species and cultivar, condition of cutting wood, type of cuttings (hardwood, semi-hard wood cuttings, softwood and herbal cuttings), season, amount of internal auxin and many other factors (Hartmann *et al.* 2002, Daneh-loueipour *et al.* 2006). Auxin is essential for rooting commencement. It has been reported that auxin existence is necessary for induction of the root starter cells (Hartman *et al.* 2002). Cuttings are failed to root initiation when internal auxin amount is not enough to accelerate. Hence, external auxin is widely used on the stem cuttings for accelerating the formation of adventitious roots (Galavi *et al.* 2013). Auxin has an effect on speed and increases the percentage of rooting of the stem cuttings (Kasim and Rayya 2009). Auxin that has been found most reliable in stimulating adventitious root production in cuttings is indole butyric acid (IBA) (Randhawa and Mukhopadhyay 1994). IBA is the most effective on promoting root-initiation and adventitious root production in stem cuttings (Waisel 1991).

The effects of different IBA concentrations on rooting ability of stem cuttings were also investigated previously by Puri and Verma 1996, Aminah 2003, Majeed *et al.,* 2009, Hoque 2016, Manokari *et al.*, 2016, Shekhawat and Manokari 2016, Ercisli *et al.* (2001), Akhtar *et al.* (2002), Khan *et al.* (2004), Kazankaya *et al.* (2005), Dawa *et al.* (2013) and it has been indicated that IBA had a significant effect on the rooting. According to Hartmann et al. (2002), IBA is the best root promoter due to its fast auxin activity and an enzymatic system of fairly slow destruction. Strydom and Hartman (1959) found the positive effect of auxin on the increase of respiration rate and a high level of amino acid storage at the base of cuttings 24 hours after treatment with auxin. Results from the present study showed that the rooting ability such as number of roots, root length, root diameter, and steckling percentage were significantly affected by IBA treatment.

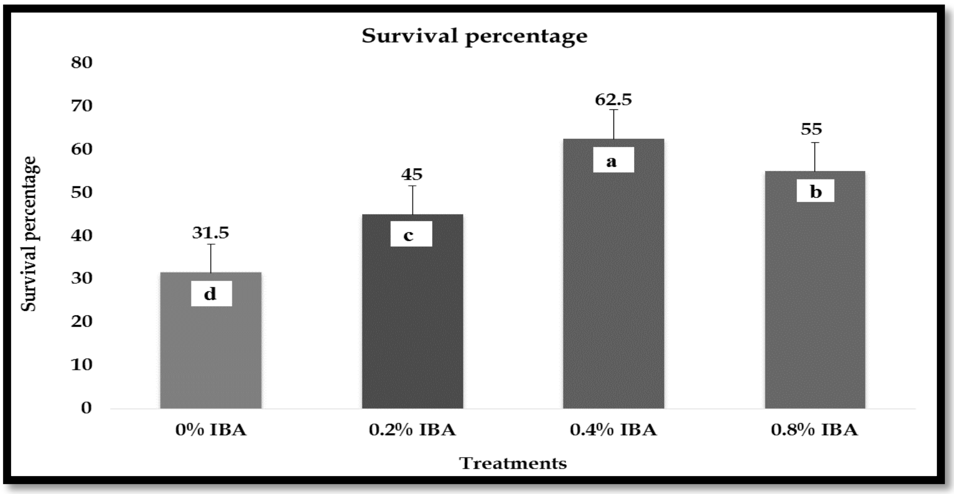

Figure 11. Survival percentage of *A. marmelos* cutlings developed under various IBA concentrations three months after transferring into plastic pots (vertical bar represents standard error)

4. Conclusion

Inadequacies of quality planting materials remain the major constraints for establishing large scale commercial plantation of *A. marmelos* in Bangladesh and other tropical countries. Conventional propagation like seed germination, budding, grafting are season dependent, slow and much laborious. Moreover, seeds of *A. marmelos* are generally short viable and prone to pest or insect attack. To provide higher yield of quality fruit to increasing poor people, vegetative propagation of *A. marmelos* through stem cutting can be a better and helpful option for multiplication. In the present study, *A. marmelos* was found amenable for clonal propagation through stem cutting with application of rooting hormone IBA using low-cost non-mist propagator. Considering rooting percentage, root number and root length, 0.4% IBA treatment of stem cuttings may be recommended for mass production of quality planting stocks for the cultivation of the species in homestead agroforestry or in fruit orchards for sustainable nutritional and medicinal supply for the poor people of agrarian Bangladesh.

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