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Influence of indole butyric acid on root induction in daughter plants of strawberry

M.M. Hossain* and O. Gony

Department of Horticulture, Bangladesh Agricultural University, Mymensingh-2202. Bangladesh.
**E-mail*: *mokter.agr@bau.edu.bd*

Abstract

Strawberry is a promising small fruit crop in Bangladesh. Farmers are giving priority to grow this short duration fruit in their field due to early fruiting and high market demand. The main obstacle is shortage of quality planting materials during planting time. Moreover, plants produced from tissue culture techniques are very costly, as a result many of interested growers are unable to cultivate strawberry. Therefore, this study was undertaken to find out the impact of indole butyric acid (IBA) on root induction in strawberry daughter plants. Four concentrations of IBA (0, 100, 200 and 300 ppm) were applied at the base of newly born daughter plants of strawberry and inserted them in a polybag (10 ×5 cm) with loamy soil for rooting. Thereafter, rooting performance of daughter plants was checked at 10, 15, 20 and 25 days after application (DAA) of IBA. The experiment was conducted following randomized complete block design with three replications. Results showed that different concentrations of IBA exhibited differently on root and shoot development of newly grown strawberry plants. All the studied parameters were significantly influenced by IBA solutions except root length of plants. Among the concentrations, 100 and 200 ppm IBA showed successful results in respect of all traits studied while 300 ppm IBA performed better in fresh weight of leaves of new plants. However, it can be concluded that grower can use 100 and/or 200 ppm IBA solution at the base of newly grown daughter plants to expedite rooting and shoot development of strawberry plants.

Key words: Strawberry, runners, root-shoot growth, PGRs, indole butyric acid

Introduction

Cultivated strawberry (*Fragaria×ananassa* Duch.) is one of the most popular soft and nutritious fruit among the small fruits grown in the world. It is an important fruit crop showing wide adaptability to various environmental conditions around the world (Hancock, 1999). It is widely cultivated under protected and open condition in temperate and sub-tropical countries with temperature range of 22-25 °C during day and 7-13 °C at night (Lyngdoh, 2014). It has shown worldwide popularity and reaching the regular diets of millions of people as a fresh table fruit. It is used as garnishing for salads, cakes, also used as jams, pastes, juice, flavoring of ice creams, milk shakes and many other food items. It is an excellent source of several food ingredients (Moraga *et al.,* 2006). This fruit is widely accepted, mainly for its multiple flavor and nutritious properties and also for its bright red color and juicy texture (Schieberle and Hofmann, 1997; Ulrich *et al.,* 1997). Strawberry is packed with antioxidants which are essential in decreasing cardiovascular diseases, maintaining blood sugar levels. Strawberry supplementation reduced oxidative damage to low density lipoprotein while maintaining reductions in blood lipids and enhancing diet palatability (Jenkins *et al.*, 2008). The leading strawberry producing countries of the world are China, USA, Mexico, Turkey, Spain, Egypt, Koran, Japan, Russia and Germany (FAO STAT, 2016). Strawberry is being grown in Bangladesh for more than a decade, meanwhile a number of varieties have been developed from Bangladesh Agricultural Research Institute (BARI), Bangladesh Agricultural University (BAU) and Rajshahi University. According to Bangladesh Strawberry Association, in 2016 total of 867 hectares of lands have been brought under strawberry cultivation (The Daily Observer, February 10, 2016). Many researchers have tried to enhance growth, yield, quality of strawberry using various means at home and abroad (Shiow *et al.,* 1998; Sharma *et al.,* 2013; Hossain *et al.,* 2015; Chowhan *et al.,* 2016; Adnan *et al.,* 2017; Paul *et al.,* 2017; Ahmed *et al.,* 2019). This delicate fruit has high market demand therefore many of promising growers of this country are growing strawberry using tissue cultured plants as well as daughter plants from runners. Micropropagation of strawberry through axillary buds has been studied for a long time and this technique provides more runners than conventional method (Boxus, 1989). Although micropropagation of strawberry has several advantages such as the ability to produce virus free plants, rapid multiplication ability (George, 1996; Lopez-Aranda *et al.,* 1994) but it could cost 4 to 5 times higher than conventional propagation (George, 1996). Farmers of our country are cultivating strawberry spending a lion's share of production cost for plant. On the other hand, tissue cultured plants are needed to be acclimatized with the field environment before transplanting, as a result date of planting is delayed thus plant growth and fruit yield become inferior. Therefore, it is important to get a low cost, easy and rapid propagation technique to produce strawberry plants from runners. In this study we examined the impact of indole butyric acid (IBA) on rooting in strawberry daughter plants which might assist timely transplanting of this crop.

Strawberry plants can be propagated both by seeds and vegetative means (runners, stolons/divisions) but vegetative propagation using runners is commonly practiced (Saito *et al.,* 2009; Murti and Yeoung, 2013). Research is being continuined in different parts of the world to increase the number of strawberry runners as well as daughter plants in different growing systems (Savini *et al.,* 2008; Mohamed *et al.,* 2018; Liu *et al.*, 2019). GA_3 was effective in increasing number of runners in strawberry (Luangprasert, 1994; Asadi *et al.,* 2013). Rooting in daughter plants depends on growth media, air humidity and quality of plantlets (Treder *et al.,* 2014). The growth media plays a critical role in the rooting process of runners. It helps to provide moisture, support, nutrients and aeration for growing plants. Wei *et al.* (2020) reported that different propagation media had influenced the rooting and early growth of daughter plant. Various root media have significant effect on the growth of daughter plant (Soon *et al.,* 2015). In our field condition, runners or stolons took 3 to 4 weeks to produce roots in daughter plants thus hampered timely planting in the field. Since early planting is preferably better to get higher and longtime fruiting in Bangladesh condition, therefore, an initiative was taken to enhance rooting in daughter plant using PGRs.

It is reported that PGRs such as indole acetic acid (IAA), IBA, naphthalene acetic acid (NAA) plays a significant influence on rooting of hard wood and semi hard wood cuttings (Aminah *et al.,* 1995; Stefancic *et al.,* 2005; Anyasi, 2011; Murti and Yeoung, 2013; Galavi *et al.,* 2013). Rooting of stem cuttings of chrysanthemum and carnation was increased by IBA and naphtaleneacetic acid (NAA) (van der Pol and Vogelegang, 1983; Yonekura *et al.,* 1999). It was also noticed that abscisic acid (ABA) increased rooting in bean runner cuttings (Hartung *et al.,* 1980) and pea cuttings (Rasmussen and Andersen, 1980). PGRs are commonly used in tissue culture techniques for producing plantlets of strawberry (Anderson *et al.*, 1982; Mohamed, 2007; Sim *et al.,* 2007; Murti and Yeoung, 2013) but its application in rooting of strawberry runners is very limited. However, phytohormonal stimulation of rooting in cuttings of strawberry runners has been reported by Saito *et al.* (2009).

Application of PGRs may be an important alternative to initiate roots in strawberry runners quicker than normal process which might facilitate early production of plants and also early/timely planting of strawberry in the field. Thus growth and yield of strawberry will be increased. Therefore, this study was undertaken in order to find out a suitable concentration of IBA for rooting in strawberry runners/stolons. In this experiment intact daughter plants were used to speedup rooting using different concentrations of IBA at the base in a soil media.

Materials and methods

Experimental site: The study was conducted at Horticulture Farm, Department of Horticulture, Bangladesh Agricultural University, Mymensingh during September 2016 to March 2018. The experimental site is located at 24°44´N latitude and 90°23´ E longitude having an altitude of 18 m from sea level. The climate is sub-tropical in nature having plenty of sun shine with moderate low temperature during October to March.

Plant materials: Strawberry plants were used as plating materials for this study. From our previous results we found that the runner production tendency was maximum in BARI strawberry 1, therefore, plants of BARI Strawberry 1 were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. The mother strawberry plants were planted in the experimental field of Horticulture Farm, Bangladesh Agricultural University for further runner production in the month of September-October 2017. Necessary intercultural operations like weeding, irrigation, manuring, fertilization, control of insects pests were done properly during the experimental period.

Chemical: IBA was used as a rooting hormone for induction of roots in the newly born runners of strawberry for early preparation of strawberry plants.

Experimental design: The experiment was conducted following randomized complete block design with three replications and each replication comprised with ten daughter plants. The following doses of IBA were applied to induce early rooting in daughter plants:

T_0: 0 ppm IBA solution *i.e.*, Control (tap water), T_1: 100 ppm IBA solution, T_2: 200 ppm IBA solution, and T_3: 300 ppm IBA solution

Preparation and application of IBA: Laboratory grade IBA was used for preparation of different concentrations of IBA solution for this study. Since, IBA is water insoluble therefore, the required amount of IBA powder was initially dissolved in few milliliters of 75 % ethyl alcohol thereafter this solution was diluted with water to make desired concentration of IBA solution. Newly born daughter strawberry plants were put in polybag with sufficient amount of loamy soil in it then different concentrations of IBA solution was applied directly to the base of the daughter plants using hand sprayer. Thereafter, polybags with intact daughter plants were partially inserted into soil for holding rigidly. The rooting and shoot performance of strawberry daughter plants was investigated at 10, 15, 20 and 25 days after application of IBA solution.

Data recording: It was a destructive process where treated and untreated strawberry daughter plants were carefully picked up with polybag from soil and immediately removed polybag. Adhering soil and other debris were separated by washing the roots with tap water. Thereafter, number and length of roots per plant were recorded. Root fresh weight was taken after wipe off water from root surface using Kimwipe tissue paper. After measuring fresh weight of roots it was transferred to electric oven for drying at 70±2 ºC until the constant weight reached. Dry weight of the sample was measured using EJ-250 NEWTON SERIES compact balance. Thereafter, percent dry matter content of root sample was calculated according to the following formula-

Dry matter content (%) = (dry weight/fresh weight) × 100

At the same time number of leaves, leaf length (including leaf petiole and leaf blade) and fresh weight of leaves were recorded from each treated and untreated strawberry plants.

Leaf area (LA) of strawberry plants was measured by using LI-3100C Leaf Area Meter (LI-COR, Bioscience, USA). It was measured from matured fresh leaves of five treated and untreated plants in each time course.

Statistical analysis: Data obtained from different parameters were statistically analyzed to find out the difference among the treatments. The analysis of variance (ANOVA) for all parameters was performed by *F*-test. The significance of the difference between pairs of treatment means was compared by Duncun's Multiple Range Test (DMRT) at 5 % levels of probability (Gomez and Gomez, 1984).

Results

Effect of IBA on root number: To enhance rooting in strawberry runners, various concentrations (0, 100, 200 and 300 ppm) of IBA were applied at the base of runners and the effects of hormone were investigated at 10, 15, 20 and 25 days after application of IBA solution. The results showed that the number roots per plant increased progressively with time and there was a significant difference among the hormone treatments and time after hormone application (Fig. 1). At 15 and 20 days after application (DAA), it was observed that the number of roots was maximum (244, 271) with the application of 200 ppm IBA solution followed by 100 ppm (176.67, 159.67) and 300 ppm (113, 152.67) and the lowest root number (61.67, 64.33) was found in control treatment (0 ppm IBA).

On the other hand, at 25 DAA, it was noticed that 100 ppm and 200 ppm IBA performed better to increase the number of roots per strawberry plant and there was no significant difference between these two treatments on production of roots in strawberry runner. However, the maximum number of roots (350) was recorded in 100 ppm IBA treatment followed by 200 ppm IBA (342.33), 300 ppm (254.33) and the minimum number of roots (159.67) was recorded in untreated control (0 ppm IBA) (Fig. 1). However, from the findings of this study it can be stated that a highly encouraging result was observed regarding roots initiation and number of root per plant of strawberry.

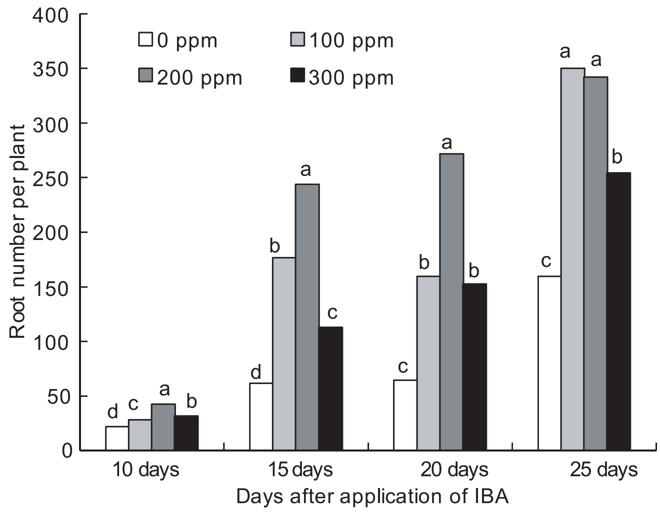

Fig. 1. Effect of different concentrations of IBA solution on number roots in strawberry plant at different days after application of IBA. Bars with different letters are significantly different (*P*=0.05).

Effect of IBA on root length: Root length of strawberry plants was not influenced by different concentrations of IBA at the early stage of application but it was influenced after 25 days of application of IBA solution. However, it was observed that untreated control plants produced the longest roots as compared to 100, 200 and 300 ppm IBA treated plants at 10, 15, 20 and 25 days after application of IBA solution (Fig. 2). This result indicates that auxins like IBA may take slightly longer period to influence the length of roots in strawberry plants. However, at 25 days after application of IBA, maximum length of root was recorded in control plant (10.60 cm) followed by 200 ppm IBA (9.50 cm), 100 ppm IBA (9.07 cm) and the minimum length of roots (8.17 cm) was found in 300 ppm IBA treatment (Fig. 2).

Effect of IBA on root fresh weight: Fresh weight of roots per

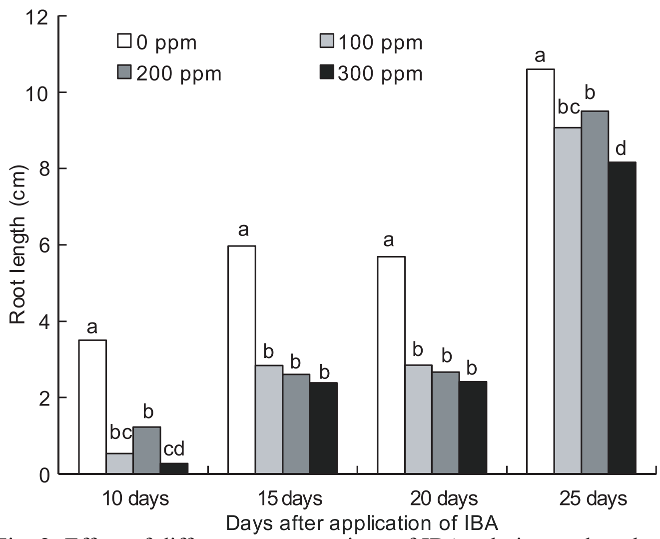

Fig. 2. Effect of different concentrations of IBA solution on length of roots in strawberry plants at different days after application of IBA. Bars with different letters are significantly different (*P*=0.05).

plant was significantly influenced by the application of different concentration of IBA solution. Root fresh weight increased with time after application of IBA. The effects of IBA solution on fresh weight of strawberry roots was much apparent after 20 days after application. From the results it was observed that at 25 days after application, 200 ppm IBA solution produced maximum fresh weight of roots (2.32 g) followed by 100 ppm (1.86 g), 300 ppm (1.81 g) and the minimum root fresh weight (1.15 g) was obtained from untreated control plant (Fig. 3). There were no significant difference between the effects of 100 ppm and 300 ppm IBA solution.

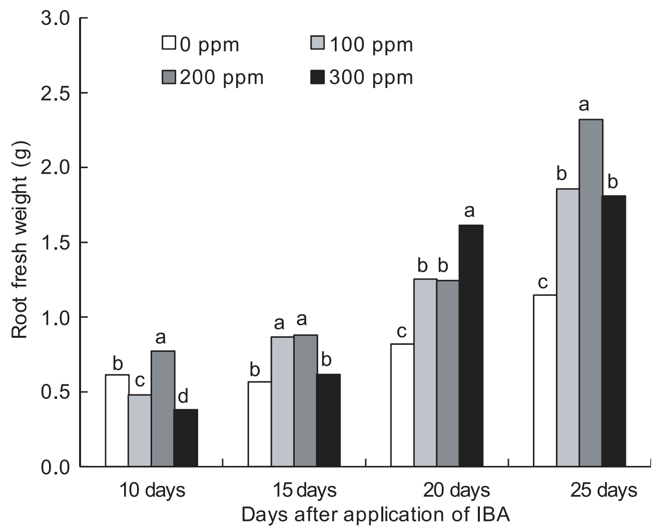

Fig. 3. Effect of different concentrations of IBA solution on fresh weight of roots in strawberry plants at different days after application of IBA. Bars with different letters are significantly different (*P*=0.05).

Effect of IBA on root dry weight: The role of IBA on root dry weight was found to be significant. At the initial stage, root dry weight increased slowly up to 20 days after application of IBA solution. The effects were significant at 25 days after application of IBA solution. At 25 days after application, the highest dry matter of roots (0.88 g plant^{-1}) was obtained from 100 ppm IBA followed by 200 ppm (0.79 g plant^{-1}), 300 ppm (0.76 g plant^{-1}) and the lowest dry weight of roots was recorded in untreated control plants (0.68 g plant^{-1}) (Fig. 4).

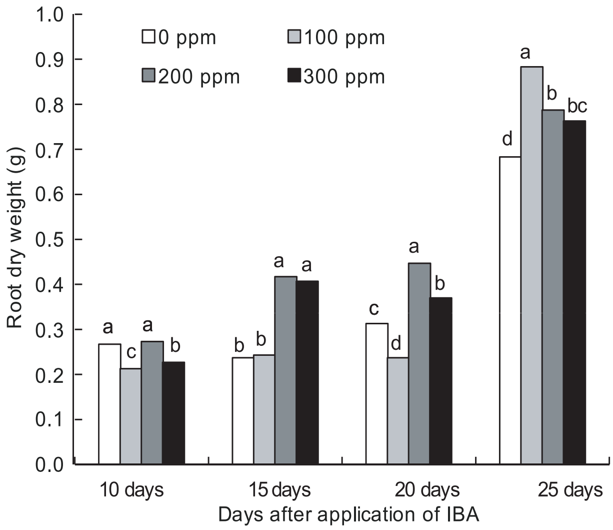

Fig. 4. Effect of different concentrations of IBA solution on root dry weight of strawberry plant at different days after application of IBA. Bars with different letters are significantly different (P=0.05).

Effect of IBA on number of leaves: Statistically significant difference was observed among the different days after application of IBA solution. As days progressed with time the number of leaves per strawberry plant increased. At each time significant differences were noticed among different levels of IBA solution. At 25 days after application of IBA, the maximum number of leaves per plant (4.33) was obtained from 300 ppm IBA which was statistically identical with 100 ppm IBA (4.33) followed by 200 ppm IBA (4.00) and the lowest number of leaves per plant was obtained from untreated control plant (3.55) (Fig. 5).

Effect of IBA on leaf area: Leaf area of strawberry plants was measured using LI-3100C Leaf Area Meter at 10, 15, 20 and 25 days after application of IBA solution. It was observed that different concentrations of IBA significantly influenced leaf area of strawberry plants. As days progressed, leaf area increased significantly by different concentration of IBA (Fig. 6). At 15 days after application of IBA, leaf area was maximum (81.66 cm^2) with the application 100 ppm IBA, followed by 200 ppm IBA (67.66 cm^2), 300 ppm IBA (62.54 cm^2) and the minimum

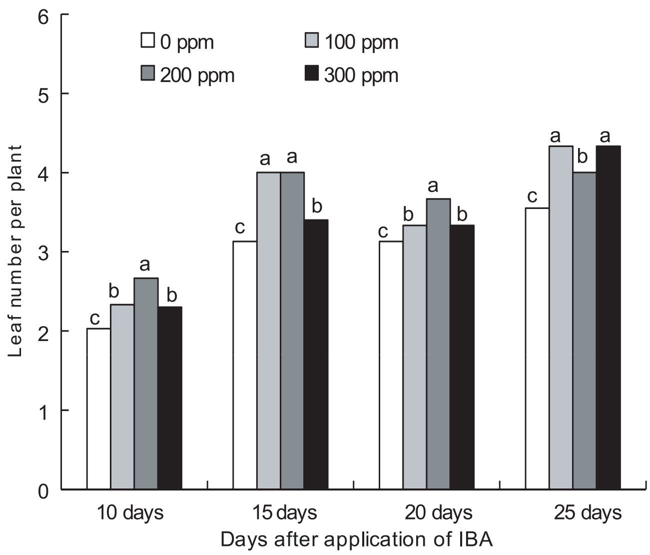

Fig. 5. Effect of different concentrations of IBA solution on number of leaves per sapling in strawberry at different days after application of IBA. Bars with different letters are significantly different (P=0.05).

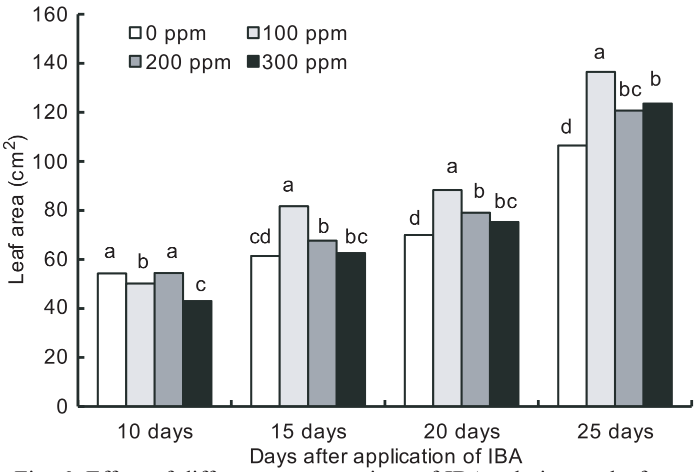

Fig. 6. Effect of different concentrations of IBA solution on leaf area of strawberry plant at different days after application of IBA. Bars with different letters are significantly different (P=0.05).

leaf area (61.40 cm^2) was recorded from the untreated control plants (Fig. 6).

Effect of IBA on leaf length: Results showed that leaf length of strawberry plant increased significantly with increasing time and concentration of IBA. At 25 days after application of IBA, maximum leaf length (13.97 cm) was in 100 ppm IBA treatment followed by 300 ppm (12.10 cm) and 200 ppm (11.60 cm) and the minimum leaf length (11.14 cm) was in untreated control (Fig. 7).

Effect of IBA on leaf fresh weight: The effects of IBA solution on leaf fresh weight of strawberry plant was found to be significant. Leaf fresh weight increased with time and concentrations of IBA. At 25 days after treatment of IBA, it was noticed that leaf fresh weight increased with increasing concentration of IBA. The maximum fresh weight of leaves was achieved from treatment of 300 ppm IBA (1.03 g) followed by 100 ppm IBA (0.79 g), 200 ppm IBA (0.70 g) and the minimum fresh weight was recorded from untreated control (0.61 g) (Fig. 8).

Discussion

A mother strawberry plant can produce 10-15 runners chain per year (Hancock, 1999) depending on variety and culture conditions

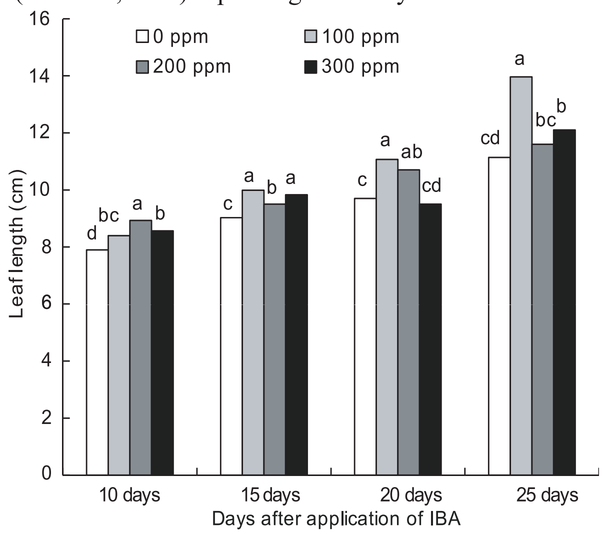

Fig. 7. Effect of different concentrations of IBA solution on leaf length of strawberry saplings at different days after application of IBA. Bars with different letters are significantly different (P=0.05).

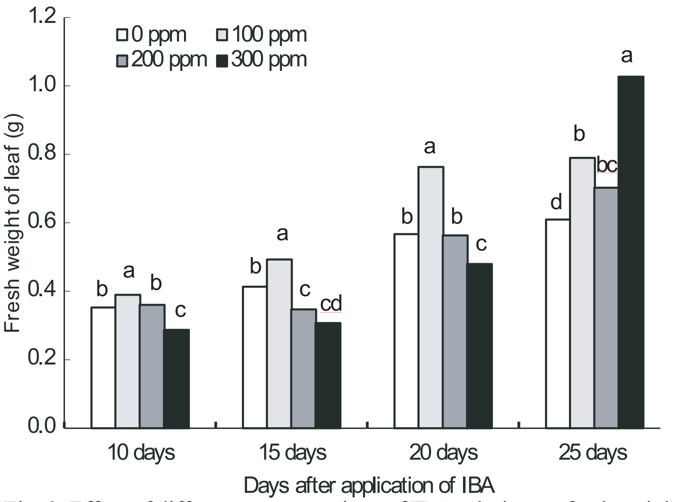

Fig. 8. Effect of different concentrations of IBA solution on fresh weight of leaf of strawberry saplings at different days after application of IBA. Bars with different letters are significantly different (P=0.05).

with 3-5 ramets/daughter plants per chain, thus it generates 40-50 clonal ramets per season. Usually couple of leaves are formed in a daughter plant before producing roots while attached with stolon. This study was carried out to explore the possibility of enhancing rooting in daughter plants of strawberry runners by imposition of various concentrations IBA at the base of daughter plants. This kind of approach for rooting in strawberry runners is not reported earlier so far, however, rooting in strawberry runners through *in vitro* techniques are reported in numerous studies around the world (James and Newton, 1977; Anderson *et al.,* 1982; Borkowska, 2001; Biswas *et al.,* 2008; Murti and Yeoung, 2013; Ashrafuzzaman *et al.,* 2013; Al-Khateeb *et al.,* 2014). Seker *et al.* (2010) noticed that plant growth regulators play a tremendous role to initiate roots in strawberry tree quicker than normal process.

In this study, we observed that root initiation, number of roots, root fresh and dry weight increased significantly with the application of IBA at the base of intact strawberry runners. Our intention was to expedite rooting in strawberry runners so that growers can get early plants for planting. In this study, we applied four concentrations of IBA (0, 100, 200 and 300 ppm) at the base of runners while it was packed in small polybag with loamy soil. The most notable result was greater in root counts as compared to untreated daughter plants, it was found that root count increased with progress of time of application while root length does not respond quickly at early time but it responded relatively higher at the end of time although length was smaller than control plant. In other crops, IBA reportedly increased rooting in cutting. Galavi *et al.* (2013) noticed that auxin (IBA) significantly influenced rooting in grape vine cuttings. They also reported that application of 4000 mg/L IBA in stem cutting produced maximum roots with highest root length, fresh and dry weight. Root fresh and dry weight of strawberry plants in this study increased significantly with application of IBA. Stefancic *et al.* (2005) reported that IBA proved as the most efficient treatment and induced earlier root formation in leaf cutting of *Prunus* 'GiSelA 5'. They also noticed that IBA induced stronger shoot growth and higher number of roots. Seker *et al.* (2010) reported that the best rooting performance was obtained from 6000 ppm IBA application. They also noted that rooting rate, viability rate and rooting quality were improved due to application IBA solutions in cutting.

Present investigation revealed that strawberry growers can use 100-200 ppm IBA solution at the base of newly grown daughter strawberry plants/plants to expedite rooting as well as shoot development of strawberry plants. This process will assist early planting of strawberry plants in the field and also help to get early and extended fruiting.

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