International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **2(12),** 11-17, December **(2013)** *Int. Res. J. Biological Sci.*

Effect of Two Plant Growth Hormones and Potting Media on an Ornamental Foliage Plant, *Ophiopogon* sp.

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Available online at: www.isca.in, www.isca.me
Received 8th July 2013, revised 8th September 2013, accepted 9th October 2013

Abstract

Ophiopogon sp. is a perennial herb native to China. It has been introduced to some tropical countries as an ornamental plant species. This plant has a good local and foreign market as potted plants and cut foliage. As these plants are very slow growing, obtaining leaves with required length and quality are difficult. The aim of this study was to investigate the performance of two plant growth hormones and potting media on the growth of Ophiopogon sp. to aid in improving it. Pot experiments were carried out to test the effect of plant growth hormones (Indole acetic acid and Benzyl amino purine) and potting media (Coir dust: compost: sand 1:1:1 and leaf mould: soil: sand 1:1:1) on the above and below ground growth. Three experiments were conducted using the two potting media, experiment 1 and experiment 2 with individual application of IAA and BAP respectively and experiment 3 with IAA and BAP in combination. In experiment 1, there was a significant increase ($p \leq 0.05$) in fresh weight with potting medium 2 and leaf length with potting medium 1, at 100 mg/l IAA. In experiment 2, the highest fresh weight (in potting medium 2) was obtained at 75 mg/l BAP. Leaf length was significantly higher in all the BAP treated plants compared to the untreated control. In Experiment 3, highest fresh weight and highest leaf length was observed at IAA with BAP combination of 1:1. Out of the three experiments, the combination of BAP and IAA volume in 1:1 gave the best results than when used alone. Potting medium 2 showed a significantly higher performance in fresh weight of plant and leaf length than potting medium 1.

Key words: *Ophiopogon* sp., hormones, potting medium.

Introduction

Floriculture is the science of growing, harvesting, storing and processing of ornamental plants[1]. It is an important industry in many countries and can be a gate way for economic development[2]. Rose, chrysanthemum, carnation, lily, *Dracaena* sp., *Cordyline* sp., *Dieffenbachia* sp. and *Ophiopogon* sp. are some of the cut flowers and cut foliage used in the global floriculture industry[3,4]. Floriculture in Sri Lanka started as an industry in 1970 and has grown rapidly during the last few years to become one of the Sri Lanka's major foreign exchange generating ventures. According to Exporters Association of Sri Lanka the total floriculture exports by Sri Lanka, in 2011 was 17.37 million US $ which accounts for only 0.16% contribution to the total exports[5]. Sri Lanka being a tropical country blessed with favorable natural climatic conditions has a great potential of improvement in this sector[6].

Ophiopogon sp. (Family: Liliaceae[7]) is a small perennial herb with a short rhizomatous stem. The leaves are linear usually with whitish streaked abaxially, 15-70 cm in length and 3-15 mm in width[8]. The leaf tussocks are usually surrounded by brownish, membranous sheaths[8]. *Ophiopogon* sp. prefers fertile, moist, humus-rich soil that has good drainage[8]. These plants can withstand drought conditions, adaptable and easy to maintain[9]. However, there are some concerns associated with these plants, such as they are slow growing, proper drainage is essential and are prone to pest and fungi attack. Although this poses a serious problem in the industry very few investigations have been done to improve this plant. Experiments done with different irradiance levels (full sun and 20%, 50%, 80% shade respectively) have not been effective as they have shown no difference in the total leaf length, mean leaf area or specific leaf weight of *Ophiopogon* sp. leaves[10]. Tennekoon et al. has reported that use of Benzyl amino purine (BAP) and Indole acetic acid (IAA) has improved the growth performance and quality of *Chlorophytom comosum,* an ornamental foliage potted plant[11].

Plant growth regulators and appropriate soil conditions are two of the important factors affecting the growth of plants. Cyatokinin and Auxin are two plant growth regulators that are associated with plant growth[13,12]. Cytokinin regulate cell division, retention of chlorophyll, promote light induced formation of chlorophyll, lateral bud development, cell expansion and regulation of sink/source relationships[14-18]. Auxin promote cell elongation, apical dominance in shoot, fruit drop or retention, vascular tissue differentiation, suppress the growth of side buds and stimulate root growth[14,19,20]. IAA is a natural auxin while BAP is a synthetic cyatokinin.

Soad et al. have found that BAP has significant effects on growth parameters of *Codiaeum variegatum* plants in terms of

plant height, number of branches, and leaves per plant, root length and leaf area as well as fresh and dry weights of stems, leaves and roots compared with the untreated plants. BAP at 150 mg/l has given the highest value for the number of branches per plant and stem diameter compared with the other treatments (50 and 100 mg/l) and control plants (0 mg/l)[21]. Authors suggested that this result may be due to the stimulatory effects of BAP[21]. Mazher et al. have mentioned that foliar application of kinetin (synthetic cytokinin) has significantly affected the plant height, number of branches, fresh and dry weight of herbs as well as total carbohydrates, protein and total carotenoids in plants such as *Salvia officinalis*, *Lavandula officinalis* and *Tagetes minuta*[17]. According to Victorio, plantlets of *Alpinia zerumbet* cultured *in vitro* for 4 months resulted in an increase of both, proliferation rate and number of leaves after the addition of 2 mg/l of IAA plus thidiazuron compared with control medium. IAA is widely used in tissue culture to improve rooting and increase root weight[22]. Asadi et al. have reported that *Rose* 'Morrasia' cultured on Murashige and skoog (MS) medium *in vitro* showed a highest number of shoots produced in media with 3 mg/l BAP without NAA[23]. Furthermore, Roy et al. have stated that the number of roots and longest roots per rose explants was highest in 1.0 mg/l BAP and 0.5 mg/l NAA[24].

Cresswell has observed early germination of seeds, greater size as well as uniformity of broccoli, tomato and lettuce seedlings, germinated and grown in coir dust compared to sphagnum[25]. Handreck concluded that plants in coir dust based media showed more Ca, S, Cu and Fe leakage, but less K leakage than peat based media[26]. The author also observed greater immobilization of soluble nitrogen with coir dust than peat, which was also confirmed by Cresswell[20,25]. An investigation carried out by Meerow on the growth of tropical foliage plants, showed that the growth index, shoot and root dry weights with coir dust medium was significantly higher for *Ravenea rivularis* (majesty palm) and marginally higher for *Anthurium sp.* than sedge peat medium[27]. Therefore, high quality coir dust appears to be an acceptable substitute for sphagnum or sedge peat in soilless container media[27]. Geisel and Seaver suggested that compost help the soil to hold water while reducing the water runoff, add nutrients and beneficial microbes, increase soil organic matter, assist in healthy root architecture, help balance soil pH and thereby improve plant growth[28]. Vanderlinden claimed that leaf mould is a better soil amendment than compost since it enriches soil structure, increases water retention and provides better conditions for soil life[29]. Therefore, the objectives of this study were to determine the effect of IAA and BAP plant growth hormones and two different potting media on the growth performance of *Ophiopogon* sp.

Material and Methods

Study site and plant material: The experiments were conducted in the Department of Botany, University of Peradeniya, Sri Lanka (7°15'15''N 80°35'48"E) during the period of July 2010 to March 2011. Shoots of *Ophiopogon* sp. were obtained from the Royal Botanic Gardens, Peradeniya, Sri Lanka (7°16'16"N 80°35'44"E).

Establishment of planting material: Plastic pots of 6cm in diameter and 15cm in depth were used for the cultivation, filled with two types of media. Potting medium1 containing a mixture of coir dust, compost and sand (1:1:1). Potting medium2 containing a mixture of leaf mould, soil and sand (1:1:1). Shoots of *Ophiopogon* sp. trimmed up to 2cm from the root-shoot junction were potted.

Application of fertilizer and watering: Solid fertilizer with high phosphorous (10:52:10) was added to every pot as a basal dressing (i. e 10 pellets per pot). All the plants were supplied with a foliar spray of high nitrogen fertilizer (30:10:10). The foliar applications were carried out in two weeks intervals. Watering was done as required by the plants.

Experiment 1 and 2: Potted plants were arranged in complete randomized design with 5 hormone (Sigma-Aldrich) treatments (IAA and BAP respectively in the two experiments) and 8 replicates per treatment using two potting media (potting medium 1 and 2). Application of different concentrations (25, 50, 75 and 100 mg/l) was carried out twice as foliar spray while the control was sprayed with distilled water.

Experiment 3: Potted plants were arranged in complete randomized design with 5 hormone treatments and 10 replicates for each treatment using two potting media (potting medium 1 and 2). After analyzing the results of experiment 1, 100 mg/l IAA showed the best performance. Therefore, experiment 3 was conducted to find the best BAP concentration which works well with 100 mg/l IAA. Application of BAP (25, 50, 75 and100 mg/l) with IAA (100 mg/l) was carried out twice as foliar spray while the control was sprayed with distilled water.

Application of growth hormones: IAA and BAP growth hormone solutions were freshly prepared for every application. The first application of growth hormones was done 3 weeks after the plant establishment and the second was done 10 days after the first application. In the third experiment, first BAP application was done 3 weeks after the plant establishment, the first IAA application was done 10 days after the first BAP application, the second BAP application was done 10 day after the first IAA application and the second IAA application was done 10 days after the second BAP application.

Measurements: Postharvest measurements were taken after the harvest which was done 5 months after plant establishment by uprooting the plants carefully and weighing them on a digital measuring scale. Fresh weight was measured immediately and the dry weight was taken by drying them in an oven at 60°C until a constant weight was obtained. Then three leaves from each pot were randomly harvested and each of their leaf length was measured using a ruler.

Analysis of data: All the experiments were performed using a completely randomized design. Results of the treatments were analysed using the SAS statistical software (version 6.12, SAS institute Inc.cary, NC, USA). Duncan mean separation test was used to identify the differences between treatments**.**

Results and Discussion

Experiment 1: The most effective treatments which gave significantly (P<0.05) higher mean fresh weight (11.56g) and leaf length (21.6cm) were at 100 and 75 mg/l IAA treatments respectively for potting medium 2. Significantly higher mean leaf length (17.3cm) was observed at 100 mg/l IAA treatments for potting medium 1 (figure-1). For both potting media, mean number of new leaves (9.15) was highest in 100mg/l treated plants compared to other treatments (5.23) including the control. Potting medium 2 performed better compared to potting medium 1 for the parameters leaf length (14.34cm and 18.94cm for potting media 1 and 2 respectively) and fresh weight (6.55g and 9.39g for potting media 1 and 2 respectively). However results showed no significant difference between the potting media for the parameter number of new leaves (5.86 and 4.9 for potting media 1 and 2 respectively).

Experiment 2: The most effective BAP treatment which had the highest mean plant fresh weight (6.52g) was 75 mg/l for potting medium 2. Leaf length (18.69cm) was significantly (P<0.05) higher in all BAP treated plants compared to the control (11.4cm) for both potting media (figure-2). Potting medium 2 performed better compared to potting medium1 for the parameters leaf length (12.9cm and 15.56cmfor potting media 1 and 2 respectively) and fresh weight (3.81g and 5.3g for potting media 1 and 2 respectively).

Table-1
Concentrations of hormones used for each treatment in the three experiments with *Ophiopogon* sp.

Treatments	Experiment 1	Experiment 2	Experiment 3
	IAA only	**BAP only**	**IAA & BAP**
T0	Control (distilled water)	Control (distilled water)	100 mg/l IAA
T1	25 mg/l IAA	25 mg/l BAP	100 mg/l IAA + 25 mg/l BAP
T2	50 mg/l IAA	50 mg/l BAP	100 mg/l IAA + 50 mg/l BAP
T3	75 mg/l IAA	75 mg/l IAA	100 mg/l IAA + 75 mg/l BAP
T4	100 mg/l IAA	100 mg/l BAP	100 mg/l IAA + 100 mg/l BAP

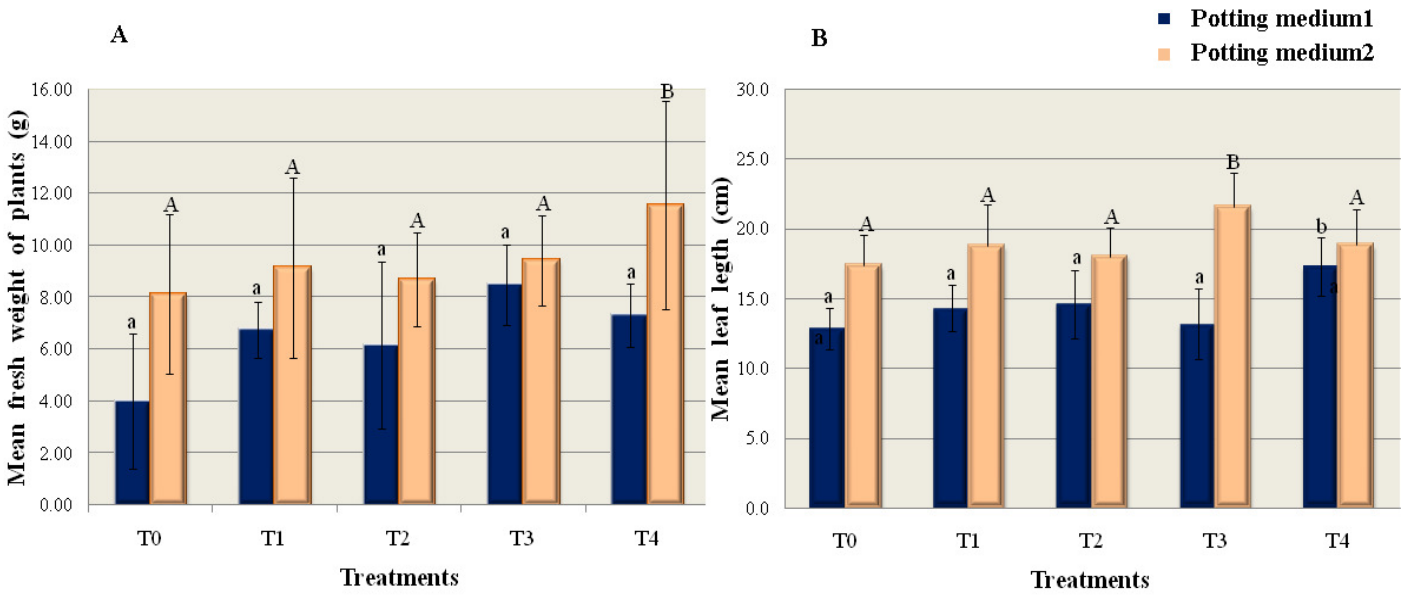

Figure-1
Effect of different IAA treatments of 0mg/l (T0), 25mg/l (T1), 50mg/l (T2), 75mg/l (T3) and 100mg/l (T4) in potting medium 1(coir dust: compost: sand 1:1:1) and potting medium 2 (leaf mould: soil: sand 1:1:1) on mean fresh weight of plants (A) and mean leaf length (B). Error bars=±Standard Deviation (SD). Means with the same letter are not significantly different at P≤0.05

Experiment 3: The most effective BAP treatment with 100 mg/l IAA which had a higher mean plant fresh weight for potting medium 1 (5.71g) and potting medium 2 (8.95g) was at 100 mg/l compared to other treatments (5.11g). Leaf length was significantly higher for both potting media (17cm and 22.9cm for potting media 1 and 2 respectively) at 100mg/l BAP in combination with 100 mg/l IAA compared to other treatments (11.4cm) (figure-3). Potting medium 2 performed better compared to potting medium 1 for the parameters leaf length (12.9cm and 15.56cm for potting media 1 and 2 respectively) and fresh weight (3.81g and 5.30g for potting media 1 and 2 respectively) agreeing with the results of experiment 1 and 2.

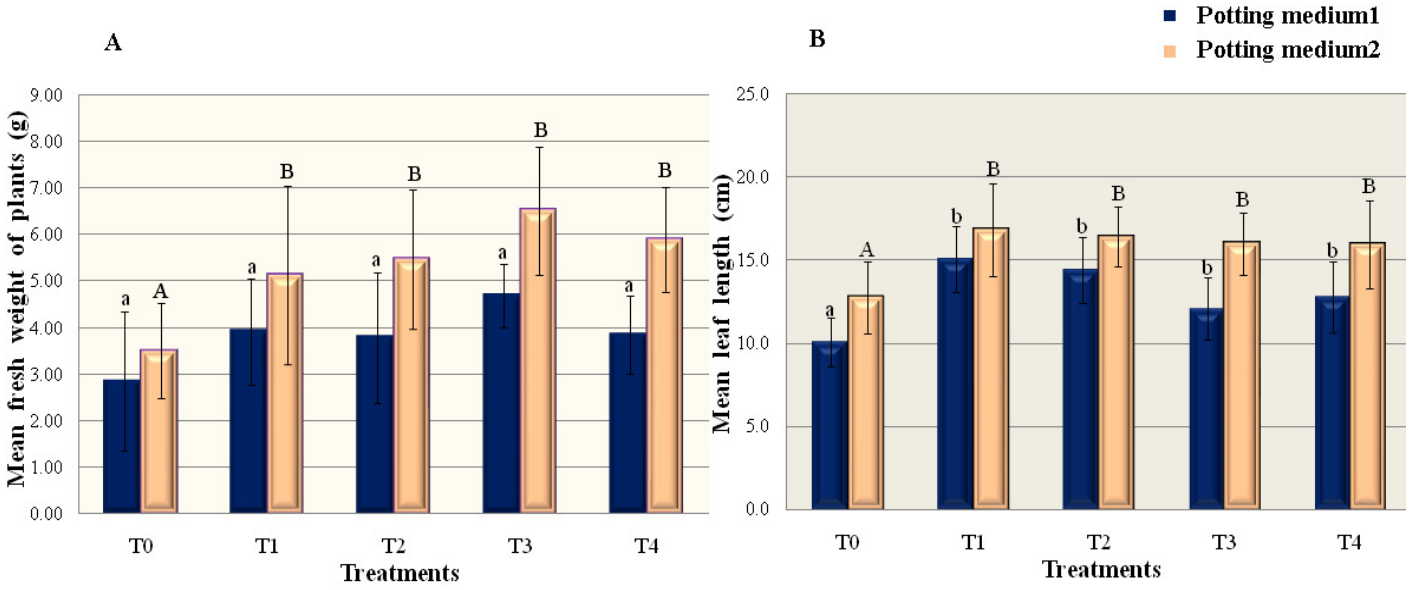

Figure-2

Effect of different BAP treatments of 0mg/l (T0), 25mg/l (T1), 50mg/l (T2), 75mg/l (T3) and 100mg/l (T4) in potting medium 1 (coir dust: compost: sand 1:1:1) and potting medium 2 (leaf mould: soil: sand 1:1:1) on mean fresh weight of plants (A) and mean leaf length (B). Error bars=± Standard Deviation (SD). Means with the same letter are not significantly different at P≤0.05

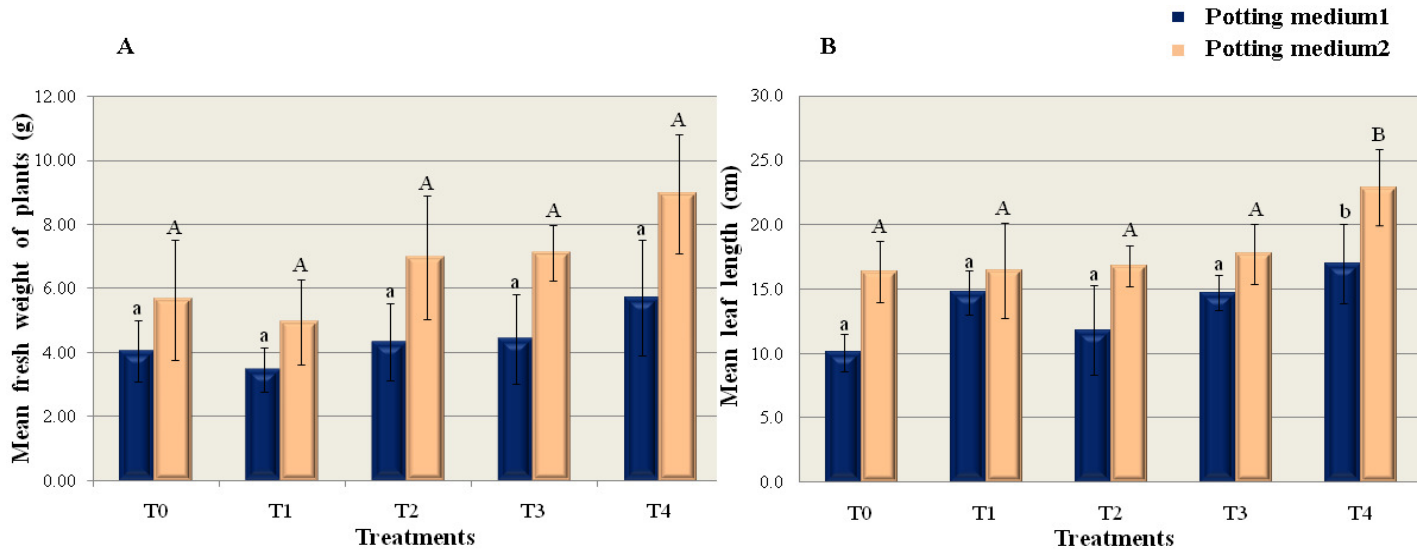

Figure-3

Effect of different BAP treatments 0mg/l (T0), 25mg/l (T1), 50mg/l (T2), 75mg/l (T3) and 100mg/l (T4) with the combination of 100mg/l IAA in potting medium 1 (coir dust: compost: sand 1:1:1) and potting medium 2 (leaf mould: soil: sand 1:1:1) on mean fresh weight of plants (A) and mean leaf length (B). Error bars=± Standard Deviation (SD). Means with the same letter are not significantly different at P≤0.05

Comparison of mean leaf length (main parameter) among the three experiments: Highest leaf length increase (13.8cm and 18.2cm for potting media 1 and 2 respectively) was observed in experiment 3 (combination of BAP and IAA), compared to experiment 1 and 2 (application of BAP only and IAA only). The highest increase in leaf length (17.0cm and 22.9cm for potting media 1 and 2 respectively) in experiment 3 was with 100mg/l BAP and 100mg/l IAA consisting of a combination of BAP and IAA in 1:1 ratio compared to the best treatment in experiment 1 of 100mg/l IAA only and experiment 2 of 75mg/l BAP only (figure-4).

Comparison of the two potting media: Potting medium 2 showed a significantly ($P<0.05$) higher performance in plant fresh weight (7.54g) and leaf length (17.25cm) than in potting medium 1 (5.93g and 13.68cm respectively) regardless of the hormone applications (figures-1 and 2). All plants grown in potting medium 2 had many new longer adventitious roots compared to potting medium 1 (figure-3). However, in potting medium 1 there were very few or no new adventitious root development and the new roots formed were from the existing roots which were shorter and thinner compared to potting medium 2 (figure-5).

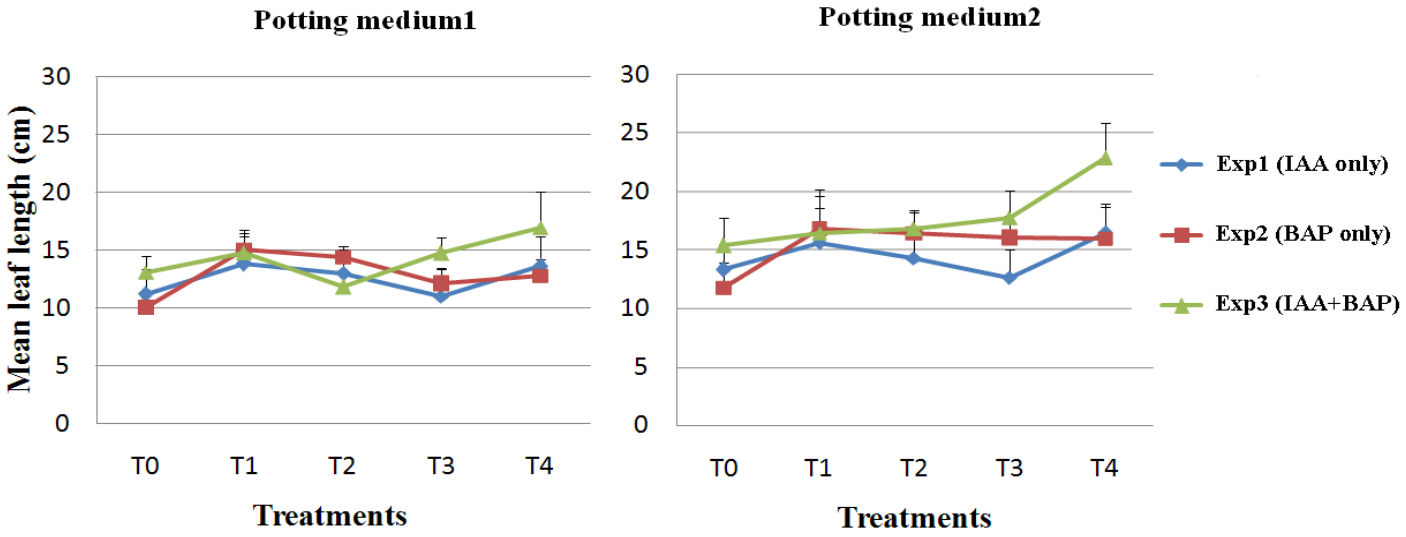

Figure-4

Effect of the experiment 1 (with five IAA treatments), experiment 2 (with five BAP treatments) and experiment 3 (100mg/l IAA with five BAP treatments) on mean leaf length in potting medium1 (Coir dust: compost: sand 1:1:1) (A) and potting medium 2 (leaf mould: soil: sand 1:1:1) (B). Error bars=± Standard Deviation (SD)

Figure-5

Root morphology of *Ophiopogon* sp. in potting medium 1 (coir dust: compost: sand 1:1:1) (A) and potting medium 2 (leaf mould: soil: sand 1:1:1) (B)

Results indicated that the best IAA concentration for growth of *Ophiopogon* sp. was 100mg/l. This treatment increased the fresh weight, leaf length and number of new leaves of these plants. Experiments done by Victorio et al. with IAA on *Alpinia zerumbet* has also increased general growth, number of leaves and root mass compared with the untreated control plants[22]. IAA is widely used in tissue culture to improve rooting[22]. These results agree with the conclusion by Taiz and Zeiger that Auxin has the ability to stimulate cell elongation, promote apical dominance in shoot and stimulate root growth[14]. The best BAP concentration for increasing leaf length of *Ophiopogon* sp. was at 75 mg/l. Similarly Faudi observed that BAP significantly affected the growth and plant quality in terms of photosynthesis rate, plant height and plant grade of *Dracaena sanderiana* and *Codiaeum variegatum*. The best BAP concentration for *Dracaena sanderiana* and *Codiaeum variegatum* was found to be 300 mg/l and 150 mg/l respectively[30]. This suggests that the optimum levels of the hormones are plant specific[11,14,30,]. Rawia and Bedour have found that BAP has the ability to increase general growth of Croton (*Codiaeum variegatum*) compared to untreated control plants[31] and Soad et al. have observed the foliar application of different BAP treatments (50, 100 and 150 mg/l) have significantly stimulated the above and below ground plant growth compared with the untreated plants[21]. According to Taiz and Zeiger, Cytokinin regulate cell division and cell expansion[14,31]. The most effective treatment for enhanced growth of *Ophiopogon* sp. was the combination of 100mg/l IAA and 100mg/l BAP (1:1). According to Tennakoon et al. experiments done with *Chlorophytum comosom* has revealed that the number of leaves per sucker and number of suckers has increased significantly in 75mg/l IAA with combination with 75mg/l BAP in 1:1[11]. Likewise many researchers have observed variable effects of cytokinin and auxin on shoot and root regeneration in different varieties of rose[32]. This has been proven to be effective in tissue culture experiments done by Pati et al. on *Rosa damascene* and *R*osa *bourboniana*, Khosh_khui and Sink on *Rosa* 'Bridal Veil' and Roy on *Chrysanthemum morifolium*[23,32,33,34].

According to the results obtained from the three experiments, experiment 3 consisting of IAA and BAP in combination has performed better compared to experiment 1 and experiment 2 where the hormones IAA and BAP were used individually. This may be mainly due to a synergistic effect of both growth hormones on shoot and root growth[35]. IAA generally promotes cell elongation while BAP encourages cell division and cell expansion[14,18,20,36]. The succession of cell division, elongation and expansion may have led to increase the length of leaves in the third experiment. Therefore, the right balance of these hormones is required to fulfill the objectives of the research. Results indicate that the efficiency of growth hormones is greater when applied in combination of IAA and BAP than when applied as a single hormone. Tennakoon et al. reported a combination of IAA and BAP in a ratio of 1:1 (75mg/l BAP and 75mg/l IAA) on *Chlorophytum comosom* plants has improved sucker formation, quality and flower initiation compared to individual application of BAP[11]. However, the optimal concentrations and proper ratio of hormones depend on the species, variety and the environmental conditions[14,11]. In all three experiments best performance in fresh weight and leaf length was observed in plants grown on potting medium 2 compared to potting medium 1. This may be due to the differences in the texture, nutrient content and aeration of the two potting media. Handreck has claimed that plants in coir dust media require more Ca, S, Cu and Fe, but less K[26]. Furthermore, Handreck and Cresswel also confirmed a greater immobilization of soluble nitrogen with coir dust[25,26]. This may be the reason for the poor performance of potting medium 1 consisted of 1/3 coir dust. The Potting medium 2 may have performed better due to the presence of leaf mould as concluded by Vanderlinden which possesses the ability to improve soil structure, help water retention, provide a better habitat for soil life and it is a better soil amendment than compost in potting medium 1[29].

Conclusion

The growth performance of *Ophiopogon* sp. was affected by plant growth hormones and growth media used**.** The best IAA concentration for improving the growth of *Ophiopogon* sp. when used alone was 100mg/l and the best BAP concentration when used alone was 75 mg/l. The best BAP concentration in combination with the best IAA concentration of 100 mg/l was 100mg/l BAP. The most effective treatment for improving the growth of *Ophiopogon* sp. was IAA and BAP in combination of 1: 1 ratio than when used as a single hormone. The growth of *Ophiopogon* sp. can be improved by using growth regulators externally in proper ratios and concentrations[14,11]. However, the optimal concentrations and proper ratio of hormones depend on the species, variety and the environmental conditions[11]**.** Potting medium 2 (leaf mould: soil: sand in 1:1:1) showed a significantly higher performance in fresh weight of plants and length of leaves compared to potting medium 1 (coir dust: compost: sand in 1:1:1) irrespective of the hormone treatments.

Acknowledgment

Authors acknowledge Dr. K.M.G.G. Jayasuriya at the Department of Botany, University of Peradeniya, Sri Lanka for his valuable assistance and support given during data analysis process.

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