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ORIGINAL ARTICLE

Effect of Plant Growth Regulators on Growth of Wallapatta (*Gyrinops walla* Gaertn.) Seedlings

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Abstract

Gyrinops walla Gaertn. belongs to the family Thymelaeaceae and is the only agarwood producing plant species naturally found in Sri Lanka. With the recognition of its ability to produce highly valuable agarwood, the species is under threat due to illegal harvesting from the natural habitats. Commercial cultivation of the species is yet to be started due to lack of information and experience on relevant cultural practices. The present study was therefore aimed at evaluating the effect of plant growth regulators (PGRs) on growth of *G. walla* seedlings. The experiment was conducted at the Faculty of Agriculture, University of Ruhuna from August 2019 to May 2020. Different combinations of Gibberellic acid (GA_3) and 6-Benzylaminopurine (6-BAP) were tested as foliar applications in an experiment arranged in Randomized Complete Block Design with three replicates. Stem height, stem diameter, fresh and dry weight of shoot and root were measured as growth parameters. The combination treatment of GA_3 and 6-BAP at 50 ppm and 100 ppm respectively resulted in significantly ($p<0.05$) higher stem height, stem diameter, leaf area, fresh and dry weight of shoots and roots compared to the control. Furthermore, the treatment significantly ($p<0.05$) increased the shoot: root ratio also compared to the control. Based on the results, foliar application of GA_3 (50 ppm) along with 6-BAP (100 ppm) can be recommended to enhance the seedling growth of *G. walla*.

Keywords: *Gibberellic acid, 6-BAP, agarwood, seedling growth*

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1. Introduction

Gyrinops walla is an evergreen plant species belongs to the family Thymelaeaceae (Selvaskanthan et al. 2020). As the species was first reported in Sri Lanka, it is known as Sri Lankan agarwood (Subasinghe 2013). It is naturally found in wetland forests (Gunatilleke et al. 2013), jungles and woodlands (Hirimburegama 2015). *G walla* is increasingly popularized recently with the discovery of its ability to form agarwood (Selvaskanthan et al. 2020; Subasinghe, 2014), a highly treasured resin produced in *Aquilaria, Aetoxylon, Gyrinops* and *Gonystylus* genera of the Thymelaeaceae family. As reported by Buddhapriya and Senarath (2018), the chemical composition of the agarwood produced in *G. walla* is very much similar to that of found in *Aquilaria* species. Due to non-availability of commercial cultivations and continuous illegal harvesting from natural habitats, the species is under severe threat. Consequently, *G. walla* has been listed as an endangered plant species in Convention on International Trade in Endangered Species (CITES) of Wild Fauna and Flora (Subasinghe 2014). Furthermore, as it has been identified as a vulnerable species by the Biodiversity Secretariat of Sri Lanka, exportation of timber, tissues or any extracts from the species was banned in ensuring the conservation of the species. In order to minimize the pressure on natural habitats, it is imperative to start commercial-scale cultivations. Although seed propagation is known to be the most suitable method of propagation, seedlings must be maintained at the nursery for about 12 months before field planting (Arunakumara et al. 2022). Thus, attention has been paid on enhancing the early growth of seedlings to reduce nursery period. In this context, exogenous application of plant growth regulators (PGRs) has been widely employed to enhance seed germination and seedling growth of number of aromatic and medicinal plants, vegetables, and ornamentals (Ali et al. 2010; Da Silva Vieira et al. 2010; Gholami et al. 2013; Lee, 2003; Singh et al. 2014). Though PGRs are organic compounds naturally produced within plants, synthetic growth regulators are also used as they show similar responses when applied exogenously (Khan and Mazied 2018). PGRs are generally found in many cells and tissues altering cell activation by sending chemical signals or messengers to cells to promote or inhibit functions such as activating the genes that code for specific enzymes (Khan and Mazied 2018). There are several classes of PGRs, viz., gibberellins, auxins, cytokinins, abscisic acid and ethylene. Among the actions of gibberellic acids (GAs), cell division and stem elongation have been often documented (Lee 2003). In addition, GAs are known to display actions in determining the speed of seed germination and co-efficiency of germination, root length, shoot/root ratio, biomass and vigour index, mobilization efficiency, emergence index, parthenocarpic fruit development, leaf emergence and fruits senescence (Lee 2003; Thakare et al. 2011). Cytokinins are also reported have actions in enhancing cell division in plant roots and shoots, breaking seed dormancy, regulating nucleic acids, preventing aging, and falling down of flowers and fruits, chloroplast biogenesis, apical dominance, vascular differentiation, nutrient mobilization, shoot differentiation, anthocyanin production, and photomorphogenic development (Fahad et al. 2015; Korkutal et al. 2008). 6-Benzylpurine (6-BAP/ N6-Benzyladenine) is a first-generation synthetic cytokinin which plays an important role in plant cell division and growth (Liu et al. 2019). Considering their multiple actions, a wide range of exogenous application of PGRs is found in Agriculture and Forestry. However, the success depends on the species and growth phase of the plant (Hedden and Thomas 2012). As limited number of scientific studies and reports are available on seedling growth and nursery period, the present study was aimed at investigating the effect of PGRs on growth of *G. walla* seedlings.

2. Materials and Methods

Experimental location and duration

The experiment was conducted in a greenhouse at the Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka from August 2019 to May 2020. The area receives an average annual precipitation of 3500 mm with the annual temperature of 28^{o}C.

Raising seedlings and preparation for experiment

Matured fruits were collected from well-grown *G. walla* plants in the Yagirala forest located in the low country wet zone of Sri Lanka. They were placed on trays and kept at room temperature until rupturing. Uniform seeds were screened and randomly selected five samples each containing twenty seeds were employed in Tetrazolium test for checking the viability. Seeds were then soaked in a fungicide (Captan 75% WP @ 1.5 g a.i/l) solution for 15 minutes and placed in germination trays filled with disinfected sand (Thiram 75% WP @ 1.5 g a.i/Kg sand). Seeds were allowed to germinate under shade (50%). Watering was done twice a day and a fungicide (Captan 75% WP @ 1.5 g a.i/l) was sprayed as necessary to avoid fungal attacks. Once germinated, seedlings were transferred to poly bags filled with a disinfected media (Thiram 75% WP @ 1.5 g a.i/Kg media) containing compost: sand: topsoil (1:1:1: by volume).

Preparation of PGRs solutions

Gibberellic acid (GA_3) 1000 ppm solution

One gram of analytical graded (99.98% purity) Gibberellic acid (GA_3) was measured using an analytical balance (model BS 1100H+) and dissolved with a few drops of 100% ethanol in a 1 L flat bottom flask. After the solution becomes transparent, 500 ml of distilled water was added and stirred well. The solution was then volume upped to 1000 ml with distilled water and kept in a refrigerator until use.

Cytokinin (6-BAP) 1000 ppm solution

One gram of analytical graded (>99% purity) cytokinin (6-BAP) was measured using an analytical balance (model BS 1100H+) and dissolved with few drops of 1N sodium hydroxide (NaOH) solution in a 1 L flat bottom flask. Once the solution becomes transparent, 500 ml of distilled water was added stirred well. The solution was then volume upped to 1000 ml mark with distilled water and kept in a refrigerator until use.

Treatment applications and aftercare

Two months old, healthy, uniform seedlings in poly bags (20 x 12.5 cm) were selected for the study. They were kept on a black polythene layer with 30-40 cm spacing. Following treatments were used as foliar applications (Table 1).

Seedlings which received the same amount of water without any PGRs were served as the control. Randomized Complete Block Design (RCBD) was used with three replicates. A fertilizer mixture (Nitrogen: Phosphorous: Potassium at the ratio of 16:16:16) was added at the rate of 5g/plant at the beginning and after 8 weeks of the treatments. Irrigation was done daily and if the climate was dry, the seedlings were watered two times a day. Daily inspection and handpicking of weeds were done.

Data collection and analysis

Stem height, stem diameter, total leaf area, fresh and dry weights of shoots and roots were measured as growth parameters. Seedling height was measured from the collar region to the tip of the main stem using a meter scale in centimeters (cm). The seedling girth was measured one ½ inches from the collar region using a Vernier calliper in millimetres (mm). The total leaf area was measured using a leaf area meter (model: Delta T) in square centimetres (cm^2). Shoot and root fresh weight was measured after uprooting and cleaning the seedlings. Shoot and root dry weight was measured after drying them in an oven at 60°C until a constant weight. The increment of growth parameters was calculated by computing the difference between two consecutive values of the particular interval. Data collection was done at 2 weeks interval for continuously 16 weeks after applying treatments. The data were statistically analyzed using SAS 9.3.1 software and Microsoft excels 2007. Duncan Multiple Range Test (DMRT) was used to separate the means.

3. Results and Discussion

Effect of PGRs on stem height

The highest stem height (42.78 cm) was observed in seedlings treated with GA_3 @ 100 ppm + 6-BAP @ 50 ppm which is significantly higher than all the other treatments and also 13.2 cm higher than that of the control (Fig.1) which reported the lowest stem height (29.58 cm).

PGRs are reported to play a crucial role in plant developmental process (Khan et al. 2020). As stated by Gupta and Chakrabarty (2013), GA_3 could play an important role in stem or intermodal elongation by stimulating cell division and expansion. Therefore, long internodes are generally reported when plants are rich in GA_3 (Korkutal et al. 2008). Present results of plant heights are in agreement with Sharafzadeh and Zare (2011), who studied the effect of exogenously applied BAP on two medicinal plants *Mentha piperita* and *Ocimum basilicum*. Furthermore, studies with *Pisum sativum* (Larson and Berg 1973), *Lycopersicum esculentum* (Bakrim et al. 2007), *Phaseolus mungo* (Chauhan et al. 2010) and chrysanthemum plants (Gupta et al.

Table 1: Treatment allocation for the foliar applications.

Treatment No.	**GA_3 concentration (ppm)**	**6-BAP concentration (ppm)**
T1 (Control)	-	-
T2	50	-
T3	100	-
T4	-	50
T5	-	100
T6	50	50
T7	50	100
T8	100	50
T9	100	100

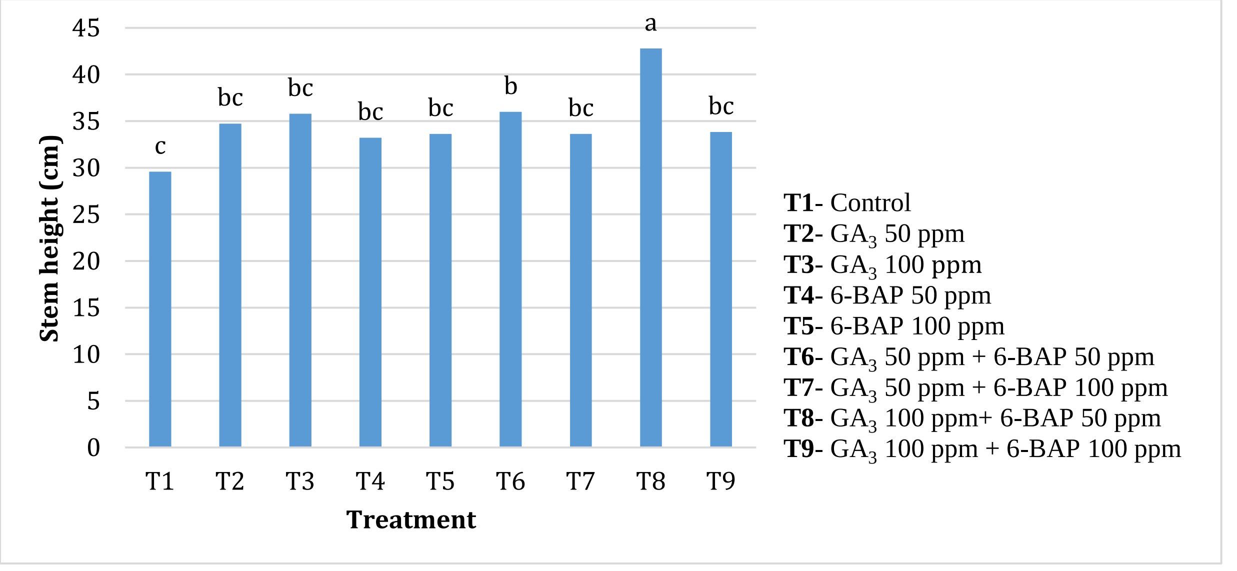

Figure 1: Effect of PGRs on stem height of *G. walla* seedlings grown in poly bags

Means with the same letters are not significantly different from each other at $\alpha=0.05$ (n=3)

2001) have proved enhanced stem elongation after application GA_3 exogenously. Similar to present results, combine application of GA_3 and BAP has enhanced shoot height of *Solanum macrocarpon* L (Opabode and Owojori 2018) and *Dizigotheeca elegantissima* (Salehi 2014) than the control.

Present findings are also in agreement with the reports of Leite et al (2003) with soya bean and Kour et al (2017) with strawberry where the application of a combination of gibberellic acid and cytokine has enhanced the growth and the stem height of the plant. As revealed by the present results, 6-BAP at higher concentration (100 ppm) showed lower stem height than that of at 50 ppm which might be due to the inhibition reaction of Cytokinin (6-BAP) on stem elongation at a higher concentration as reported by George et al (2008) who also witnessed an inhibition of shoot elongation with the increasing concentration of 6-BAP.

Effect of PGRs on stem diameter

The highest stem diameter (5.91 mm) was observed in seedlings treated with GA_3 @ 100 ppm + 6-BAP @ 50 ppm which is significantly higher than that of the control (Fig.2). Results of the present study are similar to the previous studies soya bean where application of gibberellin and cytokinin as a combination has resulted in higher stem diameter (Leite et al. 2003). According to Çavuşoğlu et al (2008), PGRs pre-treatments have enhanced the stem diameter, epidermis cell width, cortex zone thickness, vascular bundle width, xylem width, trachea diameter and phloem width in comparison with the control radish seedlings.

Effect of PGRs on total leaf area

The highest leaf area (457.78 cm^2) was recorded from the treatment contained GA_3 @ 100 ppm + 6-BAP @ 50 ppm which is significantly higher than that of the control (150 cm^2) (Fig.3).

Plant leaf area is a crucial factor which determines the light interception thereby rate of photosynthesis, dry matter accumulation and finally the plant productivity (Wahdan et al. 2011). Exogenous application of PGRs has shown increased leaf area through cell division and elongation (Harris et al. 2004; Hopkins and Huner 2004). According to Adiga et al (2017), foliar application of GA_3 @ 50 ppm has recorded the highest leaf area in *Anacardium occidentale* where unsprayed (control) recorded the lowest leaf area. According to Mehraj et al (2015), exogenous application of GA_3 @ 50 ppm has resulted in the tallest plant, longest petiole, the highest number of leaves, largest leaf area, the maximum number of branches, highest fresh weight and dry weight of okra compared to other treatments.

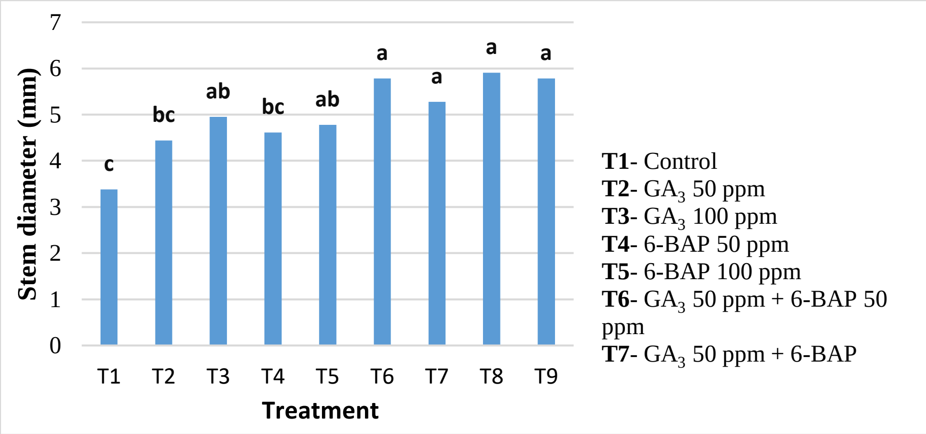

Figure 2: Effect of PGRs on stem diameter of *G. walla* seedlings grown in poly bags

Means with the same letters are not significantly different from each other α=0.05 (n=3)

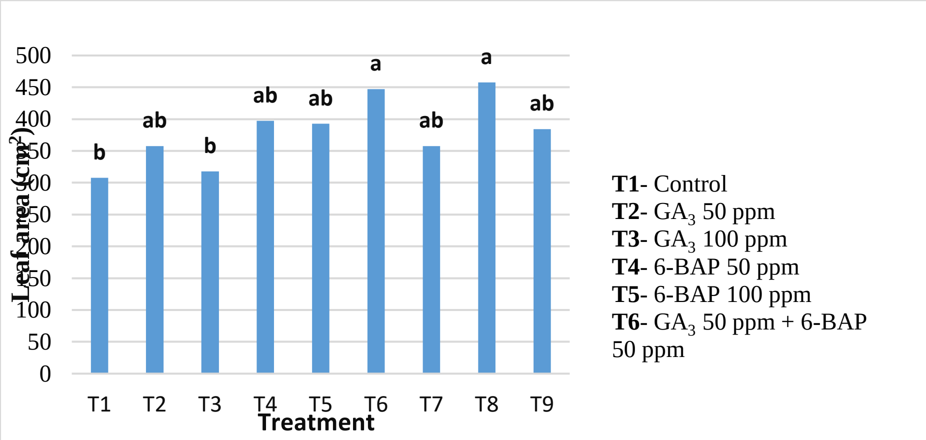

Figure 3: Effect of PGRs on stem total leaf area of *G. walla* seedlings grown in poly bags

Means with the same letters are not significantly different from each other α=0.05 (n=3)

According to Sable et al. (2015), GA_3 @ 200 ppm has produced the maximum number of leaves and leaf area in Gladiolus (*Gladiolus grandiflora* L.) where untreated plants recorded twelve leaves with a leaf area of 75 cm^2. As reported by Opabode and Owojori (2018), combine application of BAP and GA_3 has resulted in highest number of leaves and leaf area in *Solanum macrocarpon* L. The combine application of PGRs on *Vitis spp* has enhanced leaf area (Bhat et al. 2011). GA_3 application on *Solanum tuberosum* (Kumar et al. 1981), *Withania somnifera* (Kukreti et al. 2013) and BAP application on *Helianthus annuus* (Hernández 1996) have resulted in increased leaf area similar to the findings of present study with *G. walla*.

Effect of PGRs on fresh and dry weight shoots and roots

According to the Table.2, the highest fresh weights of root (07.12 g) and shoot (14.57 g) were recorded in the treatment contained GA_3 @ 100 ppm + 6-BAP @ 50 ppm which are significantly higher than those of the control. Similarly, the highest dry weights of root (2.98 g) and shoot (5.99 g) were recorded in the same treatment showing the highest shoot: root ratio.

As stated by Miceli et al. (2019), PGRs are known to play important roles in many metabolic pathways such as chlorophyll production and degradation, translocation of assimilates, nitrogen metabolism and redistribution all which ultimately contribute towards dry matter accumulation of plants though the responses may vary depending on the type of PGRs and their relative concentrations, plant species and the growth stage. Miceli et al. (2019) reported the total dry biomass of plants increased by 59.9% and 59.0%, and the epigeal biomass by 67.8% and 62.0% for lettuce and rocket, respectively, in GA_3-treated plants as compared to controls. According to them, exogenous application of GA_3 has enhanced resource repartition thereby promoting the growth of the aerial parts as witnessed by the increased shoot: root ratio. Sugiura et al. (2016) reported that plant species with relatively high levels of endogenous GAs are shown to have higher shoot: root ratios than those with low levels of endogenous GAs confirming the early reports on higher dry matter partitioning in shoots than roots in Cowpea (*Vigna unguiculata* L) in response to exogenous applications of GAs (Emongor 2007). Therefore, the exogenous application of GA_3 may promote dry matter accumulation in above ground components than in roots. Higher dry matter accumulation in vegetative parts might be due to the promotion of protein synthesis, improved enzyme activity, improved membrane permeability, enhanced uptake and use of mineral nutrients and transport of photosynthates all of which are

Table 2: Fresh and dry weights of shoots and roots of *G. walla* seedlings treated with PGRs.

Treatment	Fresh weight (g)		Dry weight (g)		Shoot/Root Ratio
	Root	Shoot	Root	Shoot	
Control	03.42 c	08.13 c	1.41 c	2.77 d	1.965
GA_3 50 ppm	05.86 ab	13.44 ab	2.46 ab	4.17 c	1.695
GA_3 100 ppm	06.45 ab	13.57 ab	2.72 a	4.34 bc	1.595
6-BAP 50 ppm	04.33 bc	09.91 c	1.74 b c	3.38 cd	1.942
6-BAP 100 ppm	05.41 b	11.91 c	2.24 b	4.13 bc	1.844
GA_3 50 ppm + 6-BAP 50 ppm	06.66 ab	14.47 a	2.74 a	4.77 b	1.741
GA_3 50 ppm + 6-BAP 100 ppm	05.12 b	10.54 c	2.17 b	3.57 cd	1.645
GA_3 100 ppm + 6-BAP 50 ppm	07.12 a	14.57 a	2.98 a	5.99 a	2.010
GA_3 100 ppm + 6-BAP 100 ppm	05.34 b	11.84 b	2.16 b	4.26 bc	1.972

Means with the same letters are not significantly different from each other $\alpha=0.05$ (n=3) known functions of PGRs. Similar to present findings, combine application of PGRs on common beans (*Phaseolus vulgaris*) has resulted in higher dry matter accumulation than other treatments Emongor (2002). According to Tanimoto (2005), auxin and gibberellin are strong accelerators of shoot growth, though their impact on root growth is not always positive. He further stated that concentration-dependent deceleration of root growth is a key to understanding their action on roots. As stated by Korkutal et al. (2008), growth performances of plants vary with the concentration of GPRs applied exogenously and with the stage of growth which also explained the present results.

4. Conclusions

Combine application of GA_3 and 6-BAP at the rate of 100 ppm + 50 ppm respectively was found to be the most effective treatment for enhancing seedling growth as measured by stem height, stem diameter, leaf area, fresh and dry weights of shoots and roots. Therefore, it can be recommended to enhance the seedling growth of *Gyrinops walla*.

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