Volume: 2, Issue: 2, (December, 2020), pp 56-69
www.rjt.ac.lk/agri/sljae

SRI LANKAN JOURNAL OF AGRICULTURE AND ECOSYSTEMS *e*ISSN: 2673-1401

SLJAE

ORIGINAL ARTICLE

Effect of Plant Growth Regulators on Seed Germination in Walla Patta (*Gyrinops walla*)

K.P.S.S. Dharmasena and K.K.I.U. Arunakumara*

Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka

Correspondence:
*kkiuaruna@crop.ruh.ac.lk,

https://orcid.org/0000-0002-57081-0215

DOI: http://doi.org/10.4038/sljae.v2i2.38

Abstract

Walla patta (*Gyrinops walla* Gaertn.) belongs to family Thymelaeaceae, is a valuable endemic plant in Sri Lanka. Recently, this species has become a topic of wide interest due to its ability to produce highly valuable agarwood resin. Commercial cultivation of the species is yet to start, thus natural habitats are tremendously under pressure due to illegal harvest. The seeds of *G. walla* are recalcitrant and loose the viability within a shorter period of time. Due to the limited availability of information, the present study was aimed at evaluating the effect of plant growth regulators on seed germination. The experiment was conducted in a protected plant house at the Faculty of Agriculture, University of Ruhuna from September 2019 to January 2020. A completely randomized design (CRD) was used with three replicates. The influence of gibberellic acid (GA_3), indole-3-acetic acid (IAA), and indol-3-butyric acid (IBA) at 800 ppm, 1000 ppm, and 1200 ppm concentrations on seed germination of the *G. walla* were investigated. Seeds soaked in nine different hormone solutions for 24 hours were placed in disinfected sand media and germinations were recorded daily up to 60 days. Seeds treated with water served as the control. Final germination percentage, mean germination time, mean daily germination, germination rate index and average time taken to start the germination were recorded. Hormone treatments except IBA at 1000 ppm, significantly increased ($p<0.05$) the seed germination, when compared with the control. GA_3 at 1200 ppm showed the highest (65%) germination followed by IAA at 1000 ppm (53.33%) and 800 ppm (50%). GA_3 treatments showed a significantly ($p<0.05$) faster germination rate than IAA and IBA. Based on the results, GA_3 at 1200 ppm can be recommended to enhance the seed germination of *G. walla*.

Keywords: *Gibberellic acid (GA_3), Gyrinops walla, Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA), Seed germination*

Date of Submission: 03-05-2020

Date of Acceptance: 22-09-2020

1. Introduction

Walla patta (*Gyrinops walla*), belongs to family Thymelaeaceae is a valuable plant endemic to Sri Lanka. It is known as "Sri Lankan Agar". The *G. walla* tree grows up to 15 m height with small, rounded crown and distributed in the lower elevations of wet and intermediate zones in Sri Lanka (Subasinghe 2013; Alwis et al. 2016). With the discovery of highly valuable resin called "agarwood", the species has gained amplified attention (Alwis et al. 2016; Mohamed and Lee 2016). Dark colour resin agarwood (Subasinghe and Hettiarachchi 2015) is found to form in the heartwood of certain genera (*Aquilaria*, *Gyrinops*, *Aetoxylon*, and *Gonystylus*) of the Thymelaeaceae family as a self-defence mechanism (Alwis et al. 2019) in response to biotic and abiotic stresses (Singh and Sharma 2015). Agarwood is widely used as therapeutic perfumes, traditional medicine, and aromatic food ingredient and for religious purposes (Liu et al. 2013). The attractive aroma of agarwood, which receives high ritual and social significance in Asian, Indian, and Middle Eastern cultures, is released once the piece of heartwood is gently burnt (Alwis et al. 2019). *Gyrinops* genera has eight species including *G. walla*, which is the only species naturally found in Sri Lanka (Alwis et al. 2016).

The agarwood resin produced in *G. walla* is chemically similar to that of produced by *Aquilaria* species (Subasinghe et al. 2012; Mohamed and Lee 2016), thus the species gained high recognition among traders and consumers. The best quality agarwood is generally guaranteed a price over US$ 30,000 per kg, thus placed among the most expensive natural raw materials in the world (Subasinghe 2013; Abdin 2014). The annual global market for agarwood is estimated to be increased steadily (Akter et al. 2013). Illegal harvesting of *G. walla* from natural habitats is frequently reported as commercial plantations are yet to be established. Due to limited natural distribution of the species and overexploitation, there is a high threat on natural stock of *G. walla*. Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) has listed *Gyrinops* and *Aquilaria* species under Appendix II for the conservation (Mohamed and Lee 2016).

G. walla mainly propagated by seeds which, in fact, is the most reliable method of propagation. However, the seeds of *G. walla* show low natural germination as observed by the present study, which might be due to rapid decline of viability after shedding from the trees. As stated by Alwis et al. (2016), who studied the effect of storage time on

germination and concluded that *G. walla* seeds cannot be stored for a long period. Plant growth regulators (PGRs) have been widely employed in enhancing seed germination of many crops (Unal 2013; Han and Yan 2015; Vishal and Kumar 2018). External application of PGRs to seeds could enhance seed germination and seedling establishment of many aromatic and medicinal plants (Ali et al. 2010; Gholami et al. 2013; Singh et al. 2014). The present study was carried out to investigate the effect of gibberellic acid (GA_3), indole-3-acetic acid (IAA), and indol-3-butyric acid (IBA) on seed germination of *G. walla.*

2. Materials and Methods

Seed collection

Mature fruits were collected from fifteen well grown *G. walla* plants in Yagirala forest located in the low country wet zone of Sri Lanka. Seeds collection was done in September 2019. The length and width of fruits of five samples having randomly selected 100 fresh fruits were measured using a vernier caliper. The fresh weight of selected fruits was also measured using an electronic balance (model BS 1100H+).

Collected fruits were placed on trays and kept under room temperature until rupturing. Prior to treatment application, the length and diameter of seeds of five samples each contained randomly selected 100 seeds were measured for using a vernier caliper. Similarly, the seed weight was also measured using an electrical balance. Uniform seeds were screened and randomly selected five samples each contained 20 seeds were tested for viability using Tetrazolium test.

Treatments and experimental design

The experiment was conducted in a protected plant house at the Faculty of Agriculture, University of Ruhuna, from September 2019 to February 2020. A Completely Randomized Design (CRD) with three replicates was used.

Analytical grade gibberellic acid (GA_3), indole-3-acetic acid (IAA), and indol-3-butyric acid (IBA) were used in preparing 800 ppm, 1000 ppm and 1200 ppm concentrations. Which were based on similar studies (Rojas-Are´chiga et al. 2011; Guney et al. 2017). Seeds were soaked in relevant hormone solution (T_1- IAA at 800 ppm, T_2- IAA at 1000 ppm, T_3- IAA at 1200 ppm, T_4- IBA at 800 ppm, T_5- IBA at 1000 ppm, T_6- IBA at 1200 ppm, T_7- GA_3 at 800 ppm, T_8- GA_3 at 1000 ppm, T_9- GA_3 at 1200 ppm), containing a fungicide (Captan 75% WP @ 1.5 g a.i L^{-1}) for 24 hours. Similarly, seeds soaked in water with added fungicide were used as the control (T_{10}).

Preparation of seed trays and seed sowing

Germination trays were filled with disinfected sand (Thiram 75% WP @ 1.5 g a.i Kg^{-1} sand) and seeds were sown by placing tail-like structure above the medium following the method described by Alwis et al. (2016). Seeds were allowed to germinate under 50% shade. Trays were irrigated twice a day and a fungicide (Captan 75% WP @ 1.5 g a.i L^{-1}) was sprayed as necessary to avoid fungal contamination.

Data collection

Germinations were recorded daily up to 60 days. Seeds with about 2 mm protruding radicle were considered as germinated.

At the end of the germination period, the final germination percentage (FGP), mean germination time (MGT), germination rate index (GRI), mean daily germination (MDG) were calculated using equations below (Aravind et al. 2019).

$$FGP = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds\ sown} \times 100 \quad \ldots\ldots (1)$$

$$MGT = \frac{\sum_{i=1}^{k} N_i T_i}{\sum_{i=1}^{k} N_i} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where;

T_i = Time from the start of the experiment to the i^{th} observation,

N_i = Number of seeds germinated in the i^{th} time (not the accumulated number, but the number corresponding to the i^{th} observation),

k = last time of germination.

$$GRI = \frac{N_1}{T_1} + \frac{N_2}{T_2} + \frac{N_3}{T_3} + \cdots + \frac{N_n}{T_n} \quad \ldots\ldots\ldots\ldots\ldots (3)$$

Where;

N1, N2, N3, ..., Nn are the number of germinated seeds observed at a time (days) T1, T2, T3, ..., Tn after sowing (not accumulated/cumulative number, but the number of seeds that germinated at the specific time).

$$MDG = \frac{Final\ germination\ percentage}{Total\ \ number\ of\ days} \quad \ldots\ldots\ldots\ldots (4)$$

Statistical analysis

Data were subjected to Analysis of Variance (ANOVA) using SAS Package version 9.1. Percentage values were arcsine transformed as appropriate before subjecting them to ANOVA. The Least Significant Difference Test (LSD) at a probability of 5 % was used to compare means.

3. Results and Discussion

As shown in Table 1, the average fresh weight of fruit and seed of *G. walla* varied within a range of 648–822 mg and 65–95 mg, respectively. The average size of a fruit was about 30.34 mm in length and 10.60 mm width (Table 2). Yellowish green colour fruit contained two tadpoles-like seeds as stated by Mohamed and Lee (2016).

Table 1: Average length, width and fresh weight of fruits and seeds

	Length (mm)	Width (mm)	Weight (mg)
Fruit	30.34 ± 5.46	10.60 ± 2.21	735 ± 87
Seed	16.45 ± 2.34	5.01 ± 1.24	80 ± 15

Final germination percentage

Table 2: Effect of different concentrations of PGRs on final germination percentage

Treatments	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
FGP	50.00^{b}	53.33^{b}	40.00^{c}	36.67^{c}	26.67^{de}	33.33^{cd}	36.67^{c}	35.00^{c}	65.00^{a}	21.67^{e}

Means with the same letters are not significantly different at α=0.05 (n=3). CV%=11.22
For brevity; T1- IAA at 800 ppm, T2- IAA at 1000 ppm, T3- IAA at 1200 ppm, T4- IBA at 800 ppm, T5- IBA at 1000 ppm, T6- IBA at 1200 ppm, T7- GA_3 at 800 ppm, T8- GA_3 at 1000 ppm, T9- GA_3 at 1200 ppm, T10- Control

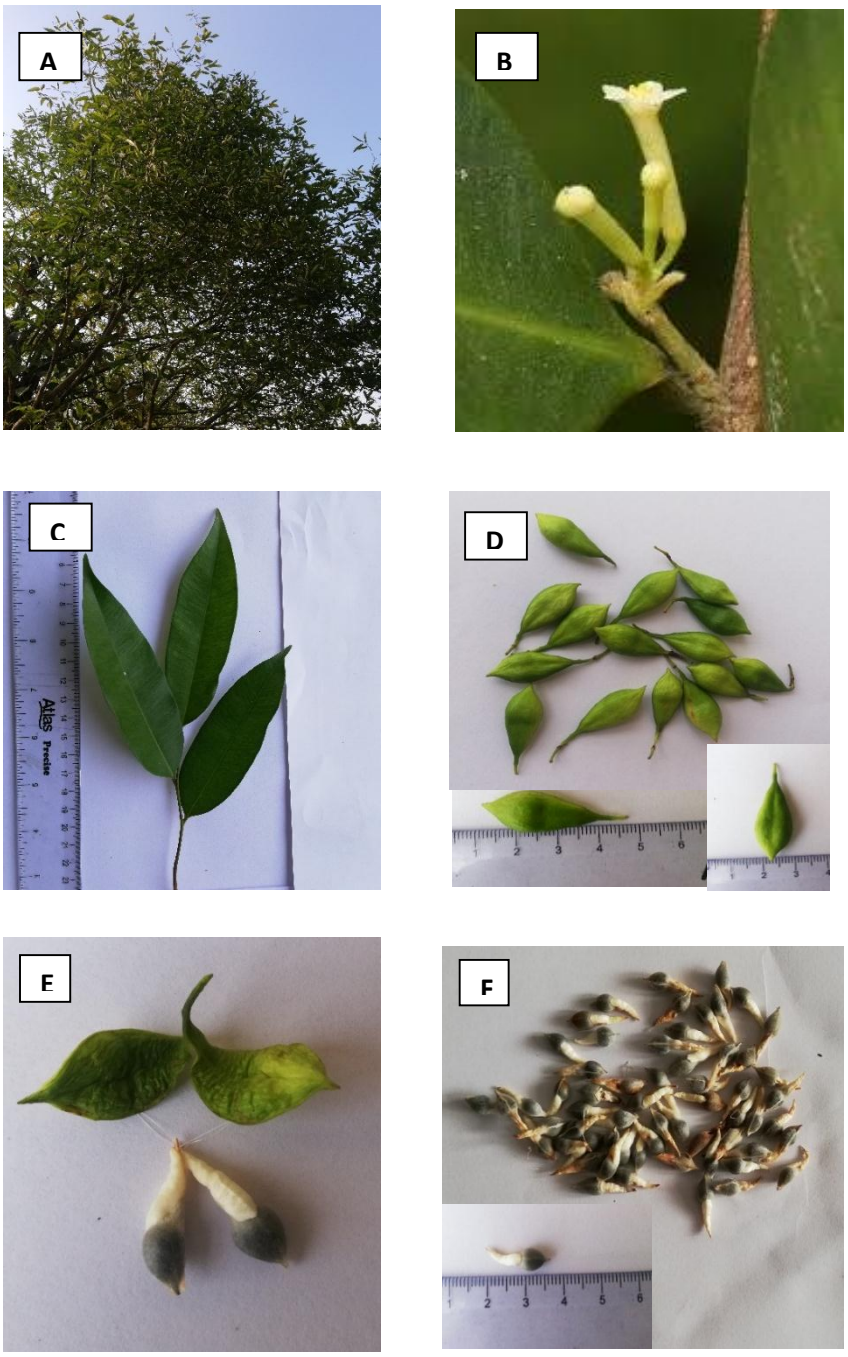

Plate1: Botanical features of G. walla. (A) Tree canopy, (B) Flower, (C) Leaves, (D) Fruits, (E) Ruptured fruit, (F) Seeds.

highest final germination (65%) was observed in GA_3 at 1200 ppm, which was significantly higher than the rest and was 43.33% higher than the control (Table 2). Previous studies have also highlighted the germination promotive effect of GA_3 on seed germination of *Mentha piperita*, *Ocimum basilicum* and *Coriandrum sativum* (Elhindi et al. 2016), *Asparagus sprengeri* (Dhoran and Gudadhe 2012), and *Withania somnifera* (Khanna et al. 2013) than IAA and IBA. In same studies, GA_3 treatments reported an increase of seed germination by 52 %, 32.6%, 25.3%, 45% and 43% respectively, when compared with respective controls.

GA3 increased seed germination in concentration-dependent manner as reported by Koyuncu (2005) for *Morus nigra*, Kumar et al (2014) for *Coriandrum sativum* and Demirsoy et al (2010) for *Arbutus unedo*, which was in agreement with present results as GA_3 at 800 ppm and 1000 ppm showed significantly low germination rates of 35% and 36.67%, respectively compared to 1200 ppm (Table 2). However, Das (2015) reported that GA_3 at 500 ppm could result 77% of germination in *Aquillaria agallocha* seeds, where control showed a germination of 49.5%.

IAA at 800 ppm and 1000 ppm resulted in the second highest germination 53.33 % and 50 %, respectively; though, the values were not significantly different. However, these two germination values respectively 31.66 % and 28.33 % were higher than the control. According to Maku et al (2014), IAA was the most effective plant hormone for enhancing germination in *Tetrapleura tetraptera* seeds.

The PGR IBA showed comparatively higher FGP (36.67 %) in seeds treated with 800 ppm followed by 1200 ppm (33.33 %) and with the lowest germination of 26.67 % was recorded in 1000 ppm. However, the values of FGP of all IBA concentrations were higher than that of the control (21.67 %). According to Elhindi et al (2016), IBA treatments were less effective than IAA and other plant growth regulators such as NAA, and GA3 for *Mentha piperita*, *Ocimum basilicum*, *Coriandrum sativum* seeds. Dhoran and Gudadhe (2012) also reported similar results with *Asparagus sprengeri*.

Mean germination time

Table 3: Effect of different concentrations of PGRs on mean germination time

Treatments	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
MGT	40.05^{cd}	38.27^{d}	39.41^{cd}	42.35^{ab}	38.63^{d}	43.20^{a}	35.53^{e}	34.96^{e}	34.94^{e}	40.82^{bc}

Means with the same letters are not significantly different at α=0.05 (n=3). CV%=11.22 For brevity; T1- IAA at 800 ppm, T2- IAA at 1000 ppm, T3- IAA at 1200 ppm, T4- IBA at 800 ppm, T5- IBA at 1000 ppm, T6- IBA at 1200 ppm, T7- GA_3 at 800 ppm, T8- GA_3 at 1000 ppm, T9- GA_3 at 1200 ppm, T10- Control

Generally, the lower mean of germination time indicate a faster germination of seeds. The lowest MGT value (34.94 days) was recorded in GA_3 at 1200 ppm followed by 1000 ppm (34.96 days) and 800 ppm (35.53 days) (Table 3). However, three MGT values were not significantly different. According to the Elhindi et al. (2016), when compared with auxins (IAA and IBA), GA_3 boosted early germination of *Mentha piperita* and *Ocimum basilicum* seeds. *Asparagus sprengeri* seeds also showed early germination with GA_3 (Dhoran and Gudadhe 2012). The highest MGT value of 43.20 days was recorded with IBA at 1200 ppm, while IBA at 800 ppm and 1000 ppm resulted in MGT of 42.35 and 38.63 days respectively and MGT values of all IBA concentrations were significant to MGT values of GA_3 (Table 3).

Mean daily germination

Table 4: Effect of different concentrations of PGRs on mean daily germination

Treatments	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
MDG	0.94^{b}	1.01^{b}	0.75^{c}	0.69^{c}	0.50^{de}	0.63^{cd}	0.70^{c}	0.66^{c}	1.23^{a}	0.41^{e}

Means with the same letters are not significantly different at α=0.05 (n=3). CV%=1.91 For brevity; T1- IAA at 800 ppm, T2- IAA at 1000 ppm, T3- IAA at 1200 ppm, T4- IBA at 800 ppm, T5- IBA at 1000 ppm, T6- IBA at 1200 ppm, T7- GA_3 at 800 ppm, T8- GA_3 at 1000 ppm, T9- GA_3 at 1200 ppm, T10- Control

The highest MDG value of 1.23 % was recorded in GA_3 at 1200 ppm, which was significantly different from all the other treatments. The second highest MDG value of 1.01 % was recorded in IAA at 1000 ppm followed by IAA at 800 ppm (0.94 %) (Table 4).

Germination rate index

Table 5: Effect of different concentrations of PGRs on germination rate index

Treatments	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
GRI	0.26^{b}	0.29^{b}	0.21^{c}	0.18^{cd}	0.15^{de}	0.16^{d}	0.21^{c}	0.20^{c}	0.39^{a}	0.11^{e}

Means with the same letters are not significantly different at α=0.05 (n=3). CV%=10.54
For brevity; T1- IAA at 800 ppm, T2- IAA at 1000 ppm, T3- IAA at 1200 ppm, T4- IBA at 800 ppm, T5- IBA at 1000 ppm, T6- IBA at 1200 ppm, T7- GA_3 at 800 ppm, T8- GA_3 at 1000 ppm, T9- GA_3 at 1200 ppm, T10- Control

Germination rate index values indicate the number of seeds germinated per day, thus higher GRI values indicate higher and faster germination. The GRI value of 0.39 observed in GA_3 at 1200 ppm was significantly higher than any of the other treatments. The second highest GRI value of 0.29 was recorded in IAA at 1000 ppm followed by IAA at 800 ppm (0.26) (Table 5).

Average time taken to start germination

Table 6: Average time taken to start germination

Treatments	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Average time (days)	30	28	28	31	28	34	27	27	24	32

Means with the same letters within each column are not significantly different at α=0.05 (n=3) For brevity; T1- IAA at 800 ppm, T2- IAA at 1000 ppm, T3- IAA at 1200 ppm, T4- IBA at 800 ppm, T5- IBA at 1000 ppm, T6- IBA at 1200 ppm, T7- GA_3 at 800 ppm, T8- GA_3 at 1000 ppm, T9- GA_3 at 1200 ppm, T10- Control

The Table 7 presents the average time taken to start germination of *G. walla* seeds. The lowest time (24 days) was recorded with GA_3 at 1200 ppm followed by GA_3 at 800 ppm and 1000 ppm (27 days). The highest time (34 days) was taken by IBA at 1200 ppm, while IBA at 800 ppm and 1000 ppm showed 31 and

28 days, respectively. In the case of IAA, the lowest time (28 days) was observed at 1000 ppm and 1200 ppm, while IAA at 800 ppm it was observed in 30 days.

The seed germination capacity of a species is dependent upon many factors including abiotic and reproductive such as phenology, pollination, and fertilization (He et al. 2005). Tabin and Shrivastava (2014) stated that despite the recalcitrant nature, the species of Aquilaria could regenerate freely under natural conditions in the forest probably due to availability of necessary moisture and light under the shaded canopy, which enhance seed germination. Accordingly, the fleshy covering of Aquilaria fruits also provides some moisture ensuring the survival of seeds until commencing the germination. According to Soehartono and Newton (2001), natural regeneration of Aquilaria is moderately higher though seed productions and dispersion under natural habitats are poor as stated by He et al. (2005). The present study also noticed that despite the recalcitrant nature, *G. walla* was enabling to regenerate through seeds under natural conditions, if ample moisture and shade is available as in rain forests. Due to continuous metabolism of recalcitrant seeds (Tabin and Shrivastava 2014), viability is lost when the moisture content drops below a certain critical level before the initiation of germination. Therefore, in the present study, measures were taken to use fresh seeds for the PGRs treatments to ensure adequate germinability of seeds.

A wide range of applications of PGRs is found in Agriculture and Forestry. GA_3 is known for its ability to break dormancy and enhance seed germination (Bentsink and Koornneef 2008; Finkelstein et al. 2008). GA_3 is involved in increasing the expansion of embryonic cell and inducing the hydrolytic enzymes that weaken the tissues surrounding the radicle to overcome the mechanical resistance due to the seed coat. In addition, GA_3, mobilizes the seed storage reservoirs (Miransari and Smith 2014; Rami and Patel 2014). In the present study, GA_3 at 1200 ppm showed significantly higher germination percentage as well as fastest germination (Table 2, 4 and 5). This might be attributed to the functions of GA_3 that leads to gradual softening of seed coat facilitating the respiration of seeds, uptake oxygen and water easily, and to protrude the radicle easily into the ground.

The hormone auxin is important in regulating several aspects of growth and development of plants through the transport inhibitor response1 (TIR1)/Additional F box protein (AFB)-Aux/indole-3-acetic acid (IAA) –AUXIN RESPONSE FACTOR (ARF) signalling system

(Chapman and Estelle 2009; Vanneste and Friml 2009). Auxins enhance seed germination by involving the activities of different enzymes and may increase the cell growth and development. Furthermore, auxins enhance the rate of metabolism during germination and enhance the cell elongation. The promoting effect of auxins on germination may be attributed to their indirect effect through changes in the membrane permeability and solubilization of carbohydrates through the synthesis of different enzymes responsible for promoting effects and production of some precursors needed for germination (Venkatesh et al. 2000). In the present study, higher germination percentage showed in IAA at 1000 ppm (53.33 %) followed by IAA at 800 ppm (50 %) might be due to the above activities of auxin (Table 2).

4. Conclusion

GA_3 at 1200 ppm was found to be the most effective hormone treatment for enhancing seed germination of *G. walla*. The treatment also showed the highest germination and the fastest germination. Hence, this can be recommended to enhance the germination of *G. walla* seeds in mass scale establishment protocols

5. Acknowledgements

Authors acknowledge the academic and non-academic staff of the Department of Crop Science, Faculty of Agriculture, University of Ruhuna to make this research a success.

Conflicts of Interest: The authors declare that there are no conflicts of interest regarding the publication of this paper.

6. References

Abdin M J (2014) The agarwood industry: yet to utilize in Bangladesh. Int. J Econ. Manag. Sci. 3: 163–166. 10.2139/ssrn.2430055

Akter S, Islam M T, Zulkefeli M, Khan S I (2013) Agarwood production: a multidisciplinary field to be explored in Bangladesh. Int. J Pharm. Life Sci. 2: 22–32. 10.3329/ijpls.v2i1.15132.

Ali T, Hossein P, Asghar F, Salman Z, Ali Z C M (2010) The effect of different treatments on improving seed germination characteristics in medicinal species of *Descurainia sophia* and *Plantago ovata.* Afr. J Biotechnol. 965886593.

Alwis H N, Subasinghe S M C U P D, Hettiarachchi D S (2016) Effect of storage time and temperature on *Gyrinops walla* Gaertn. seed germination. Environmental Professionals Sri Lanka 5(2): 16–24.

Alwis W N, Subasinghe S M C U P D, Hettiarachchi D S (2019) Characterisation and variation of agarwood resins from *Gyrinops walla*. Tropical Forest Science 31(2): 222–229.

Aravind J, Devi S V, Radhamani J, Jacob S R, Srinivasan K (2019) The germination metrics package: a brief introduction. ICAR-National Bureau of Plant Genetic Resources, New Delhi: 46.

Bentsink L, Koornneef M (2008) Seed dormancy and germination. In: Somerville C R, Meyerowitz E M, editors. The Arabidopsis Book. Rockville, MD: American Society of Plant Biologists.

Chapman E J, Estelle M (2009) Mechanism of auxin-regulated gene expression in plants. Annu Rev Genet 43: 265–285.

Das N (2015) The effect of different pre-sowing treatments on the germination of *Aquilaria agallocha* and *Shorea robusta* seeds in the nursery. Indian Forester 141(3): 285–292. doi: 10.1156/2015.

Demirsoy L, Demirsoy H, Celikel G, Macit I, Ersoy B (2010) Seed treatment with GA_3 or stratification enhances emergence of some strawberry tree genotypes: short communication. Horticultural Science 37(1): 34–37. doi: 10.17221/28/2009-hortsci.

Dhoran V S, Gudadhe S P (2012) Effect of plant growth regulators on seed germination and seedling vigour in *Asparagus sprengeri*. International Research Journal of Biological Sciences 1(7): 6–10.

Elhindi K M, Dewir Y H, Asrar A W, Abdel-Salam E, El-Din A S, Ali M (2016) Improvement of seed germination in three medicinal plant species by plant growth regulators. Horticultural Science 51(7): 887–891. doi: 10.21273/hortsci.51.7.887.

Finkelstein R, Reeves W, Ariizumi T, Steber C (2008) Molecular aspects of seed dormancy. Annu Rev Plant Biol 59: 387–415.

Gholami H, Farhadi R, Rahimi M, Zeinalikharaji A, Askari A (2013) Effect of growth hormones on physiology characteristics and essential oil of basil under drought stress condition. J Amer Sci 96163.

Guney K, Cetin M, Guney K B, Melekoglu A (2017) The Effects of some hormone applications on *Lilium martagon* L. germination and morphological characters. Pol J Environ Stud 26(6): 2533-2538. DOI: 10.15244/pjoes/70895.

Han C, Yang P (2015). Studies on the molecular mechanisms of seed germination. Proteomics 15: 1671–1679. doi: 10.1002/pmic.201400375.

He M L, Qi S Y, Hu L J (2005) Rapid in vitro propagation of medicinally important

Aquilaria agallocha. Journal of Zhejiang University SCIENCE 6B (8): 849-852.

Khanna P K, Kumar A, Chandra R, Verma V (2013) Germination behaviour of seeds of *Withania somnifera* (L.) dunal: a high value medicinal plant. Physiology and Molecular Biology of Plants 19(3): 449–454. doi: 10.1007/s12298-013-0169-3.

Koyuncu F (2005) Breaking seed dormancy in black mulberry (*Morus nigra* L.) by cold stratification and exogenous application of gibberellic acid. ACTA Biologica Cracoviensia 2: 23–26.

Kumar M, Agnihotri R K, Vamil R, Sharma R (2014) Effect of phytohormones on seed germination and seedling growth of *Coriandrum sativum* L. Pakistan Journal of Biological Sciences 17(4): 594–596. doi: 10.3923/pjbs.2014.594.596.

Liu Y, Chen H, Yang Y, Zhang Z, Wei J, Meng H (2013) Whole-tree agarwood inducing technique: an efficient novel technique for producing high-quality agarwood in cultivated *Aquilaria sinensis* trees. Molecules 18: 3086–3106. 10.3390/molecules18033086.

Maku J O, Gbadamosi A E, Oke S A (2014) Effect of some growth hormones on seed germination and seedling growth of *Tetrapleura tetraptera* (Thaub). International Journal of Plant Research 4(1): 36–42. doi: 10.5923/j.plant.20140401.07.

Miransari M, Smith D L (2014) Plant hormones and seed germination. Environmental and Experimental Botany 99: 110–121. doi: 10.1016/j.envexpbot.2013.11.005.

Mohamed R, Lee S Y (2016) Agarwood: science behind the fragrance. Agarwood, Tropical Forestry 1: 167. doi: 10.1007/978-981-10-0833-7.

Rami E, Patel I (2014) Effect of plant growth regulators on seed germination and seedling vigour index of *Oroxylum indicum* (L.) vent: an endangered medicinal plant. Plant Archives 14(1): 579–582.

Rojas-Are´chiga M, Aguilar K M, Golubov J, Mandujano M C (2011) Effect of gibberellic acid on germination of seeds of five species of cacti from the Chihuahuan Desert, Northern Mexico. Southwest Nat 56: 393–435. doi:10.1894/N01-DW-126.1.

Singh B, Sharma R A (2015). Plant terpenes: defence responses, phylogenetic analysis, regulation and clinical applications. 3 Biotech 5:129–151. 10.1007/s13205-014-0220-2.

Singh M, Singh K K, Badola H K (2014) Effect of temperature and plant growth regulators on seed germination response of *Oroxylum*

*indicum*a - high value threatened medicinal plant of Sikkim Himalaya. J Plant Sci Res 1115.

Soehartono T, Newton A C (2001) Conservation and sustainable use of tropical trees in the genus Aquilaria. II. The impact of gaharu harvesting in Indonesia. Biological Conservation 97:29–41.

Subasinghe S M C U P D, Hettiarachchi D S (2015) Characterisation of agarwood type resin of *Gyrinops walla* Gaertn growing in selected populations in Sri Lanka. Industrial Crops and Products 69: 76–79. doi: 10.1016/j.indcrop. 2015.01.060.

Subasinghe S M C U P D, Hettiarachchi D S, Rathnamalala E (2012) Agarwood-type resin from *Gyrinops walla* Gaertn.: a new discovery. J Tropical Forestry and Environment 2(2): 43–48. doi: 10.31357/jtfe.v2i2.594.

Subasinghe U (2013) Agarwood production of walla patta (*Gyrinops walla*): the tree of future. International Journal of Agricultural Science 2: 357–362. doi: 10.13140/2.1.2024. 2566.

Tabin T, Shrivastava K (2014) Factors affecting seed germination and establishment of critically endangered *Aquilaria malaccensis* (Thymelaeaceae). Asian Journal of Plant Science and Research 4(6): 41-46.

Unal B T, (2013) Effects of growth regulators on seed germination, seedling growth and some aspects of the metabolism of wheat under allelochemical stress. Bangladesh Journal of Botany. 42(1): 65-71.

Vanneste S, Friml J. (2009) Auxin: A trigger for change in plant development. Cell 136(6):1005–1016.

Venkatesh A, Vanangamudi M, Vanangamudi K, Parthiban K T, Ravichandran V, Vinaya Rai R S (2000) Effect of growth stimulants on seed germination and morpho-physiological attributes in pungam (*Pongamia pinnata*). J Tropical Forest Science 12(4): 643–649.

Vishal B, Kumar P P (2018) Regulation of seed germination and abiotic stresses by gibberallins and abscisic acids. Front. Plant Sci. 20 June 2018. https://doi.org/10.3389/fpls.2018.00838.