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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network



RESEARCH ARTICLE

BIOSCIENCE RESEARCH, 2019 16(4): 3321-3329.

OPEN ACCESS

Impacts of phytohormones on the physiological performance and root profiles of legumes seedlings

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It is a great challenge for researchers to enhance physiological performance and root profile of seedlings. This challenge depends on the improvement of germination rate, survival rates of seedlings and seedling establishment. Thus, this experiment was conducted to improve the physiological performances and root profiles of two native legumes, *L. leucocephala* and *P. pterocarpum*, from seedling stage using growth regulating hormones. Before sowing, seeds of *L. leucocephala* and *P. pterocarpum* were soaked in Gibberellins (GA₃), Indole-3-Butyric Acid (IBA), GA₃+IBA and distilled water for germination. Then, seedlings were grown in soil under greenhouse conditions and their physiological performances and root profiles were investigated. The results showed that GA₃ hormone increased the germination percent of species studied. Additionally, IBA application resulted in higher photosynthetic rates than control. In terms of root profiles, GA₃+IBA treated seedlings of both species had the highest root length and root volume. Moreover, GA₃+IBA treated seedlings had a higher number of fine roots and root tips than control. The photosynthetic rates of the species studied were positively correlated with the stomatal conductance ($r=0.82$), chlorophyll content ($r=0.89$) and root length ($r=0.75$), implying that photosynthetic rate was enhanced by increasing stomatal conductance, chlorophyll content and root length. In conclusion, this study suggested that GA₃+IBA hormone is more biologically active than single treatment of IBA and GA₃ and the best choice for seedling establishment as it improved germination percent, physiological performances and root profiles.

Keywords: Germination rate; Hormones; Root profiles; Photosynthetic rate; Chlorophyll content

INTRODUCTION

Seed germination and seedling establishment are a crucial phase for plant propagation and a great challenge for researchers to grow up with better physiological performances and root profiles (Chen et al., 2013; Yang et al., 2012). Slow root-shoot growth of seedlings is associated to poor yield production and survival rates (Wahyuni et al., 2003). Seedling growth and survival rate related to the production of roots

especially fine roots (Saifuddin et al., 2017; Jackson et al., 2012). Additionally, seedling growth with high root profiles and fine roots will facilitate plants to get better root anchorage and improve water uptake, nutrient absorption capacity and survival rates (Khuder et al., 2007; Kollarova et al., 2012). Hence, it is essential to ensure seedlings growth with high root profiles and fine roots. Wahyuni et al. (2003) and Kollarova et al. (2012) documented that seeds

treated in different plant hormones increased germination percent, survival rate, root profiles and physiological performance. Moreover, it is well accepted that plant hormones play an important role in controlling the growth, development, metabolism and morphogenesis of seedlings (Yang et al., 2012; Pop et al., 2011). Plant growth regulators can be applied as a spray or dip prior to or at the time of planting to promote post-transplant root growth in tree species (Percival et al., 2004).

As a result, plant can receive additional nutrient and hormonal signal to stimulate new lateral root and root branching with excessive adventitious or fine roots (Giehl et al., 2012). Auxin phytohormone viz. Indole-3 Butyric Acid (IBA) is usually used to promote root initiation, adventitious root formation and early root development (Pop et al., 2011; Saifuddin et al., 2013). By soaking seeds and seedlings in IBA solution, the roots can grow in a faster rate as well (Wahyuni et al., 2003). Moreover, it increased root volume and overall root mass, altered root architecture, increased expression level of protein and biochemical content and increased tolerance or resistance to a pest or pathogen of seedlings (Strader et al., 2008). Furthermore, application of IBA increased root vigor, plant vitality and survival rate of newly planted seedlings (El-Shraiy et al., 2009). However, other phytohormones that include cytokinin and abscisic acid can also control the shoot and root growth process. But, IBA has a major role in almost all steps of lateral root initiation and development (Saifuddin et al., 2017; Walia et al., 2012). Another phytohormone, gibberellin (GA₃) regulated a wide range of developmental processes, including seed germination, leaf expansion, stem elongation and early flowering (Saifuddin et al., 2009). GA₃ normally acts by signaling the proteins which stimulate cell division rapidly. Gou et al. (2010) reported that there was a role for GA₃ in primary root elongation and it appeared to affect cell expansion in the root elongation zone when applied with auxin hormones. In addition combination of IBA and GA₃ hormones increased the function of both hormones in plant cell and improve seedling performance by stimulating root growth and preventing root desiccation during transit to the planting site. The influence of GA₃ and IBA hormones on physiological performance and root growth of legume tree species have received little study. Therefore, the aims of this study were to investigate the influence of GA₃ and IBA hormone on germination, physiological

performance and root profiles of two legume tree species under glasshouse conditions.

MATERIALS AND METHODS

Seed materials

Seeds of *L. leucocephala* (LL) and *P. pterocarpum* (PP) were collected from Forest Research Institute Malaysia (FRIM). Before commencement of the experiment, seeds were immersed in 500 ml beaker filled with distilled water. A number of the seeds which failed to sink were eliminated from beaker. Seeds were then surface sterilized in 1% sodium hypochlorite (NaOCl) solutions for 5 minutes and then rinsed with distilled water.

Seed soaking with plant growth regulators

An individual hormone solution of GA₃ and IBA was prepared at the rate of 20 mgL⁻¹. Homogenous seeds of *L. leucocephala* and *P. pterocarpum* were soaked in 50 ml of respective hormonal (GA₃, IBA and GA₃+IBA) solution. Treated seeds in distilled water were considered as control. Germination was conducted by placing twenty seeds in a petri dish lined with cotton. Each treatment was replicated three times with completely randomized design (CRD). The cotton was moistened daily with respective hormonal solution (distilled water for control treatment) and the process was preceded for 15 days. The petri dishes were placed in growth chamber in 16 h light and 8 h dark at temperature of 24±2°C conditions maintained light intensity of about 400 μE m⁻² s⁻¹.

Seed germination rate

Seed germination rate (GR) was determined following the equation: $GR = SG/TS \times 100$, where: GR = Germination Rate, SG = Seeds Germinated, TS = Total Seeds Socked.

Seedlings plantation

After 15 days of hormonal treatment, seedlings were planted in pots (10 cm in diameter). Pots were filled with slope soil and seedlings were grown up to 90 days in a glasshouse conditions (average 12-h photoperiod, maximum photosynthetically active radiation of 2100 μE m⁻² s⁻¹, temperature of 21-32°C and relative humidity of 60–90%). The pots were irrigated with tap water once at two days regular interval to avoid water stress. The pots were arranged in a completely randomized design

(CRD) and each treatment was replicated five times.

Measurement of plant height, internode length and dry matter

The plant height and internode length were measured at 90 days of growth using a measuring tape. The shoot and root dry matter (oven-dried at 80°C for 72 h) of five replicated seedlings of each treatment were determined using a balance (Model-PJ3000, Mettler Toledo, Japan).

Measurements of photosynthesis, transpiration rate and stomatal conductance

The photosynthetic rate, transpiration rate and stomatal conductance of five replicated seedlings of each treatment plants were measured using the Portable Photosynthesis System (Model LI-6400XT, USA) at 90 days of plant age.

Estimation of chlorophyll content

Chlorophyll content (five leaves per treatment) was measured using a chlorophyll meter (SPAD-502, Minolta Co. Japan). The leaf was inserted into the leaf clip and value was taken three times from different spots of a single leaf. It was measured at 90 days of plant age.

Root profiles

The roots of different species were analyzed by scanning in WinRHIZO Pro Software (WinRHIZO Version 2008a, Regent Instruments Inc., Canada) at the end of experiment. This software was used to assess the total root length, volume, tips, forks, crossing and fine roots.

Statistical analysis

The data was analyzed using SPSS software (Version 16). Two ways ANOVA was applied to evaluate significant differences among the treatments. The LSD ($p < 0.05$) was calculated using the error mean squares of the analysis of variance.

RESULTS AND DISCUSSION

The seed germination rate (GR) is an important parameter to select the suitable plant hormone which can assist to produce large quantities of seedlings for commercial purposes. Based on the present findings, it was observed that GA₃ resulted in the maximum GR and IBA+GA₃ treatments resulted in intermediate GR while IBA showed the minimum GR (Table 1). It is therefore suggested that seeds soaking in GA₃ is suitable to enhance GR of both species. Though,

different hormones have been widely used to enhance GR of different plant species and variable results were reported by researchers (Khaliq and Matloob, 2012; Hae and Funnah, 2011). It is well recommended that GA₃ treatment increased GR in most of the plant species (Devi et al., 2012). This result was similar to the findings of Jamil and Rah (2007) who observed that GA₃ treatment enhanced GR and as well as early seedling growth.

In this study, it was found that plant height and internode length were increased by GA₃ and GA₃+IBA treatment compared to the IBA and control (Table 2). It might be due to the cell differentiation and expansion induced by GA₃ and GA₃+IBA treatments (Afzal et al., 2007; Gou et al., 2010; Saifuddin et al., 2017). Conversely, single application of IBA showed a positive effect on adventitious root initiation and this is the potential activity of IBA as a rooting hormone. However, IBA did not affect on the increment of plant height and internode length. Mir et al. (2010) reported that plant height responded positively when IBA was treated along with GA₃, while IBA alone was prohibitive. However, during the investigation, GA₃+IBA application resulted in maximum increase in root dry weight, while GA₃ favored maximum increase in shoot dry weight over the control of *L. leucocephala* (Table 3). Additionally, the combined treatment of GA₃ and IBA increased root biomass of *P. pterocarpum*. However, GA₃ treatment provided a significant reduction of leaf chlorophyll content in both species (Table 4). These results were in agreement with findings of Saifuddin et al. (2009) who observed that GA₃ application decreased chlorophyll content and other photosynthetic pigments in bougainvillea plants. The role of GA₃ in reducing chlorophyll content was also confirmed by Turkyilmaz et al. (Turkyilmaz et al., 2005), who found that application of GA₃ increased leaf area, consequently decreased chlorophyll concentration per leaf unit area. While El-Wahed et al. (2006) reported that chlorophyll content increased by application of IBA. The effect of IBA on chlorophyll content has been mentioned by several researchers (Ludwing-Muller, 2000; El-Shraiy et al., 2009) and they reported that the increment of chlorophyll content was associated with total soluble sugars and soluble proteins of leaf.

The present study has shown that GA₃ treatment reduced the photosynthetic rate as compared to the control (Fig. 1).

Table 1: Effects of hormonal treatments on germination rate of *L. leucocephala* and *P. pterocarpum* seeds

Species	Hormone	Germination rate (%)
<i>L. leucocephala</i>	Control	73.33±1.6f
	GA ₃	96.66±1.6a
	IBA	83.33±1.6d
	GA ₃ +IBA	90±0bc
<i>P. pterocarpum</i>	Control	65±2.8g
	GA ₃	91.66±1.6ab
	IBA	76.66±1.6ef
	GA ₃ +IBA	81.66±1.66de

Means (means ± standard error) with different letters were significantly different (p<0.05).

Table 2: The effects of different plant hormones on seedling growth

Species	Hormone	Plant height (cm)	Internode length (cm)	Leaf number
<i>L. leucocephala</i>	Control	17.5±0.2ef	2.6±0.06c	6.6±0.2g
	GA ₃	40.8±0.8a	4.3±0.06a	9.4±0.3b
	IBA	17.8±0.2e	2.4±0.04c	7.2±0.2f
	GA ₃ +IBA	31.2±0.5b	3.9±0.08ab	7.8±0.1e
<i>P. pterocarpum</i>	Control	10±0.3g	1.6±0.04d	5.6±0.5h
	GA ₃	31±0.4bc	3.1±0.1c	10±0.7a
	IBA	10.4±0.2g	1.1±0.03d	8.2±0.3cd
	GA ₃ +IBA	21.8±0.3d	2.5±0.09c	8.4±0.3c

Means (means ± standard error) with different letters within the same column were significantly different (p<0.05)

Table 3: The effects of different plant hormones on shoot dry weight (SDW) and root dry weight (RDW) of *L. leucocephala* and *P. pterocarpum*

Species	Hormone	SDW	RDW
<i>L. leucocephala</i>	Control	3.56±0.11e	1.11±0.03ef
	GA ₃	8.76±0.18a	1.27±0.03e
	IBA	4.04±0.1e	2.41±0.03b
	GA ₃ +IBA	7.27±0.1b	2.9±0.06a
<i>P. pterocarpum</i>	Control	2.98±0.06e	0.93±0.02f
	GA ₃	7.08±0.6bc	1.06±0.03ef
	IBA	3.38±0.1e	2.07±0.03d
	GA ₃ +IBA	6.24±0.1d	2.34±0.2bc

Table 4: The effects of different plant hormones on leaf Chlorophyll content

Species	Hormone	Chlorophyll
<i>L. leucocephala</i>	Control	45.9±0.8c
	GA ₃	41.9±0.31d
	IBA	55.5±0.73a
	GA ₃ +IBA	50±0.74b
<i>P. pterocarpum</i>	Control	26.3±0.28g
	GA ₃	23.2±0.26h
	IBA	33±0.36e
	GA ₃ +IBA	30.2±0.37f

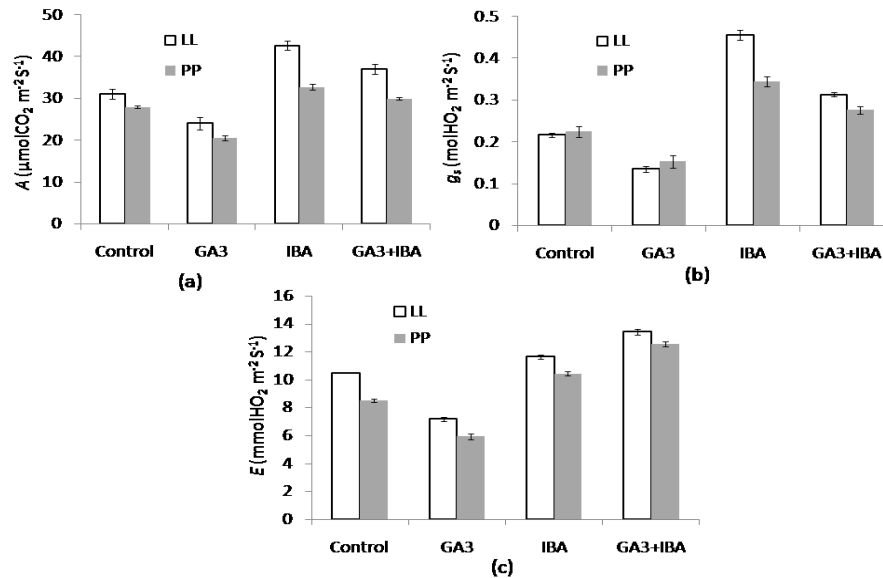


Figure 1: Effects of different hormones on photosynthetic characteristics of the species studied. Error bar denotes SE (±)

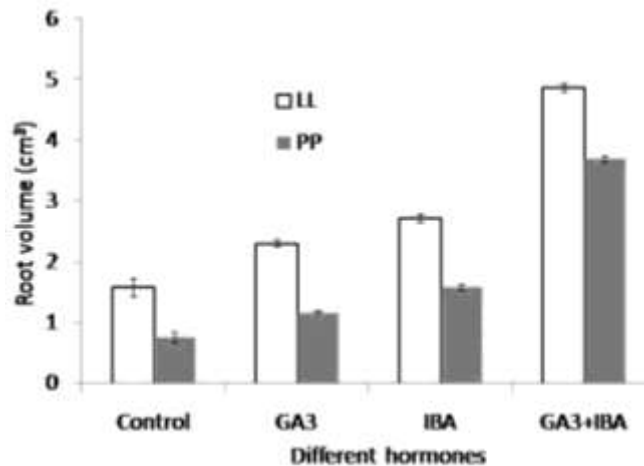


Figure 2: Effects of different hormone on root volume of the species studied. Error bar denotes SE (±)

The results are in agreement with the earlier findings of Dijkstra et al. (1990) and they observed that photosynthetic rate per unit leaf was decreased by the application of GA₃ hormone, while it stimulated shoot growth very fast. Additionally, Wahyuni et al. (2003) referred that the leaves of GA₃ treated seedlings become thin and chlorophyll content per unit area was decreased as well. Therefore, this decrease in photosynthetic rates might be caused by chlorophyll content stress or lower chlorophyll content per unit leaf area (Saifuddin et al., 2009). In this study, IBA treatment increased photosynthetic rates by prompting stomatal opening or deleteriously affecting the photosynthetic apparatus, chlorophyll content.

Percival and Barnes (2004) showed that IBA regulates photosynthetic rates through the increment of chlorophyll levels and enzymatic process. Additionally, the increase in photosynthetic rate might also be attributed to the presence of more advantageous roots in IBA treated seedlings (2006). In case of GA₃+IBA, a higher photosynthetic rate was also observed which was due to the presence of higher chlorophyll content and adventitious roots of seedlings. Additionally, the transpiration rates and stomatal conductance were higher in GA₃+IBA treated seedlings than the control seedlings.

The highest root volume was observed for both species in combined treatment of GA₃ and IBA (Fig. 2). IBA stimulated root elongation and had a higher root volume than control. Whereas,

root volume was not affected significantly by GA₃ treatment. Therefore, it can be said that the effect of GA₃ is increased in presence of IBA hormone.

A positive correlation (r=0.82) was observed between stomatal conductance and the photosynthetic rate of seedlings (Fig. 3).

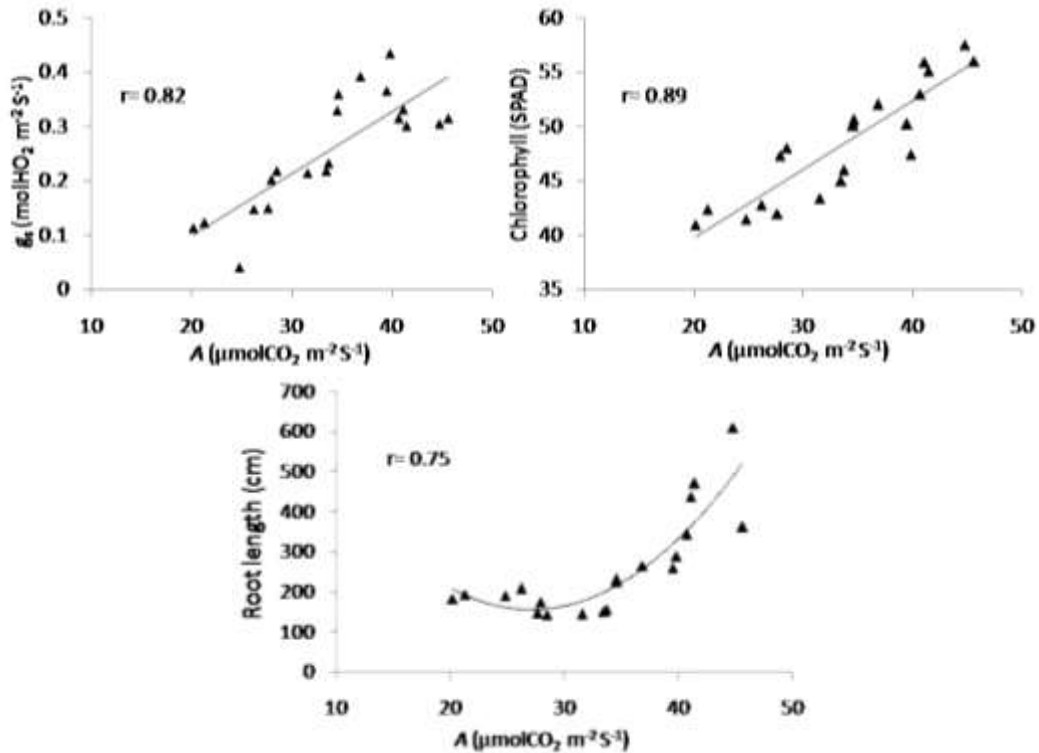


Figure 3: Some correlation among the parameters studied

Table 5: Effects of different hormone on root length of species studied

Species	Hormone	Fine roots (0-2 mm) length (cm) *(%)	Thin roots (2-4.5 mm) length (cm) *(%)	Total root length (cm) *(%)
<i>L. leucocephala</i>	Control	138.6 (90%)	15.6 (10%)	154.2 (100%)
	GA ₃	162.8 (88%)	22.3 (12%)	185.1 (100%)
	IBA	226.5 (89%)	27 (11%)	253.5 (100%)
	GA ₃ +IBA	464.6 (92%)	41.9 (8%)	506.5 (100%)
<i>P. pterocarpum</i>	Control	81.2 (90%)	9.4 (10%)	90.6 (100%)
	GA ₃	114.8 (91%)	11.9 (9%)	126.7 (100%)
	IBA	161.5 (91%)	16.8 (9%)	178.3 (100%)
	GA ₃ +IBA	355.2 (91%)	33.6 (9%)	388.8 (100%)

* Percentage of root length in bracket

Table 6: Root tips, forks and crossing are affected by hormones.

Species	Hormone	Tips	Forks	Crossings
<i>L. leucocephala</i>	Control	282±6f	571±9f	172±7f
	GA ₃	339±7d	723±6d	280±11d
	IBA	490±13bc	881±7c	309±8c
	GA ₃ +IBA	585±7a	1747±31a	746±10a
<i>P. pterocarpum</i>	Control	222±6h	275±19h	74±9h
	GA ₃	268±7fg	465±9g	160±5fg
	IBA	308±4e	712±16de	245±5e
	GA ₃ +IBA	566±6ab	1579±9b	612±8b

Means (means ± standard error) with different letters within the same column were significantly different ($p < 0.05$)

Additionally, there was a strong correlation ($r=0.89$) between photosynthetic rate and chlorophyll content, implying that high chlorophyll content could help in improving the photosynthetic rate. Saifuddin et al. (2009) also observed that photosynthetic activities were improved by the enhanced chlorophyll content of a leaf. Therefore, the GA₃+IBA treated seedlings could cause stomatal opening, as indicated by a higher chlorophyll content and subsequent increment in the photosynthetic rate. But the variations in transpiration, stomatal conductance and photosynthetic rates of *L. leucocephala* and *P. pterocarpum* can be attributed to species. Furthermore, for the both species, photosynthetic rate was positively correlated ($r=0.75$) with root length, implying that high root length could help to increase photosynthetic rate by enhancing water and nutrient uptake from soil. The result of this present study is in agreement with the earlier reports by Misson et al. (2006) who documented that root growth occurred simultaneously with the increase of canopy photosynthesis. Additionally, Srivastava and Srivastava (2007) discussed that the biomass accumulation depends on photosynthetic efficiency which is decreased by GA₃ treatment.

Fine root (<2 mm in diameter) length was increased significantly by GA₃+IBA. Fine root length was also increased by IBA treatment, while fine root length was not increased significantly by application of GA₃ (Table 5). Additionally, the results correspond with Figure 2 showed the trend of total root volume was significantly increased in GA₃+IBA. In this observation, fine root length of both species represents more than 90% of total root length. The data showed that relative value (%) of fine root length in each treatment is similar when compared with both species root system. It was documented that the fine root production is a major sign for successful performance of

seedlings especially in forest area as it secured the essential nutrition flow (Saifuddin et al., 2017; Skrzyszowski and Kupka, 2008). Moreover, plant with a higher number of fine roots is beneficial in resisting surface erosion (Saifuddin and Normaniza, 2012). It is well known that the fine roots assist to keep the surface soil together increasing root-soil interaction. The results of the present observations suggested that IBA+GA₃ are the best hormone for a higher number of fine root production, root length and root volume than IBA or GA₃. Consequently, these produced roots with high root length and volume will be able to maximize the root-soil interaction or matrix.

Additionally, a higher number of root tips, forks and crossing were observed in GA₃+IBA treatment of both species (Table 6). These characterized roots are reflected more to fine roots and contained about 92%. It can be assumed that produced root tips and forks by GA₃+IBA and IBA will allow in nailing a higher volume of soil. Therefore, fine roots with more root tips are presumably attributed to a higher root-soil interaction than control. Moreover, increased root volume would lead in absorbing enough water and nutrients to allow the plants to grow healthy with better physiology.

CONCLUSION

In conclusion, results showed that GA₃ improved germination rate and shoot length of the species studied. However, GA₃ had negative effects on chlorophyll content and photosynthetic rate. IBA+GA₃ improved germination rate, shoot length and had a positive effect on chlorophyll content, photosynthetic rate and root initiation. IBA improved adventitious root initiation, chlorophyll content and photosynthetic rate as well. The improvement of photosynthesis was resulted for chlorophyll content and stomatal conductance ascending. This study indicated a

hypothesis that better physiological performance such as photosynthetic rate, stomatal conductance and chlorophyll content resulted in enhancement of root initiation of both seedlings. And in order to maximize the physiological processes (such as photosynthetic rate and shoot elongation) and root profiles (such as root length and root volume), IBA+GA₃ is recommended to apply on seeds as it is more biologically active than single treatment of IBA or GA₃.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT

Financial support from the Postgraduate Research Fund (Project No. PV052-2011A), University of Malaya, Malaysia is gratefully acknowledged.

AUTHOR CONTRIBUTIONS

Field experiment was carried out by MS and NO. Wrote the paper by MS and Reviewed and edited the paper MMK. All authors read and approved the final version.

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