Section A-Research paper

Effect of GA3 on seed germination and seedling growth of threatened *Meliantha Suavis* Pierre species in Thai Nguyen province,Vietnam

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ABSTRACT

An experiment was conducted to evaluate the effects of gibberellic acid (GA_3) on seed germination and seedling growth of threatened *Meliantha suavis* Pierre species (*M. suavis*) in Thai Nguyen province. The effect of all treatments on seed gemination parameters and seedling parameters of plant were recorded. The result showed that application of 50 ppm GA_3 had positive impact of increasing germination percentage, germination index, germination energy and seed vigor index for all treatment as compared to untreated control. However, GA_3 50 ppm application produced the lowest germination time, but significantly increased plant height, stem diameter, number of leaves compared to the untreated control. The highest seedling parameters were recorded from the application of 50 ppm GA3 at optimum transplanting time. It was concluded that GA_3 application can be an effective growth hormone to enhance seed germination performance, reduce germination time, and improve seedling growth of threatened *M. suavis* species.

Keywords: GA_3, *meliantha suavis*, seed germination, seedling growth, threatened species.

INTRODUCTION

Meliantha suavis Pierre *(M.suavis)* has been distributed in provinces of Vietnam including Lao Cai, Cao Bang, Bac Kan, Lang Son, Quang Ninh, Bac Giang, Ninh Binh, Thanh Hoa, Nghe An, Ha Tinh, Hue and Thai Nguyen. The species is commonly known as Ngot rung, Phac Van, Rau ngot nui (Abdul-Baki and Anderson 1973) with high nutrients and commercial value (Charoenchai et al 2013; Le et al. 2018; Tianpech et al. 2008). Recently, in Viet Nam the *M. suavis* is identified to be vulnerable locally with the rating "threatened in Red List of Threatened Species" including Thai Nguyen province by habitat loss by deforestation and overexploiting of flowers. Habitats destruction decreased number of individual plants (Ban et al. 2007). Therefore, it is necessary to conduct researches on seed propagation to provide *M. suavis* seedling in various ex situ and in situ conservation at the local level.

Seed vigor, which is one of the role components of seed quality and refers to the potential of a seed to germinate rapidly and uniformly under a wide range of field conditions, has been regarded as the essential requirement for agricultural production (Bewley et al.2013; Finch-savage and Bassel 2015). According to Chavagnat (1977) the germination rate depends on the quality of the seeds, degree of dormancy of each seed and their dormancy percentage. Moreover, seed dormancy and the development of these seed derived plants can be effected by the use of hormones promoters (Giannoulis et al.2020; Liopa-Tsakalidi et al. 2011).

GA3 is plays important role in regulating the seed germination and plant growth (Cavusoglu and Sulusoglu 2015) releasing dormancy and improving seed germination (Kucerna et al. 2005), significantly reduces the stratification requirement of slow germinating seed (Kucerna et al. 2005). According to Liopa-Tsakalidi *et al* (2011) seed germination rate increased in *lavender* species when GA3 solutions were added. Exogenous application of GA3 is present to promote the release of dormancy leading to seed germination. Moreover, GA3 can also stimulate the expression of hydrolytic enzymes involved in the conversion of starch to sugar. GA can affect plant growth by controlling starch accumulation and use (Skubacz and Daszkowska-Golec 2017). Therefore, the study was carried to evaluate the effects different concentrations of GA3 on seed germination, and seedling development as well as transplatning time in *M. suavis* species.

MATERIALS AND METHODS

Plant materials and experiment treatments: The experiment was carried out in Na Khao's garden at Trung Hoi communue, Dinh Hoa district, Thai Nguyen province from July 2022 to February 2023, with the *M. suavis*'seesds were harvested in July 2022 in the forest region of Thai Nguyen province. The experiment consists of 5 treatments including untreated control, GA_3 25 ppm, GA_3 50 ppm, GA_3 100 ppm and GA_3 150 ppm designed by Randomized Complete Block Design (RCBD) with three replicated, with 30 seeds each one was taken as an experiment unit.

The seeds were soaking in a range of GA3 concentrations (25, 50, 100, 150 ppm), whereas the untreated control was soaking in water for 10 hours. Seeds were sowed in germination trays, then 30 seeds for each treatment were taken by the emergence of a 2 cm root length and sowed individually in polyethylene bags with one seed per polyethylene bags.

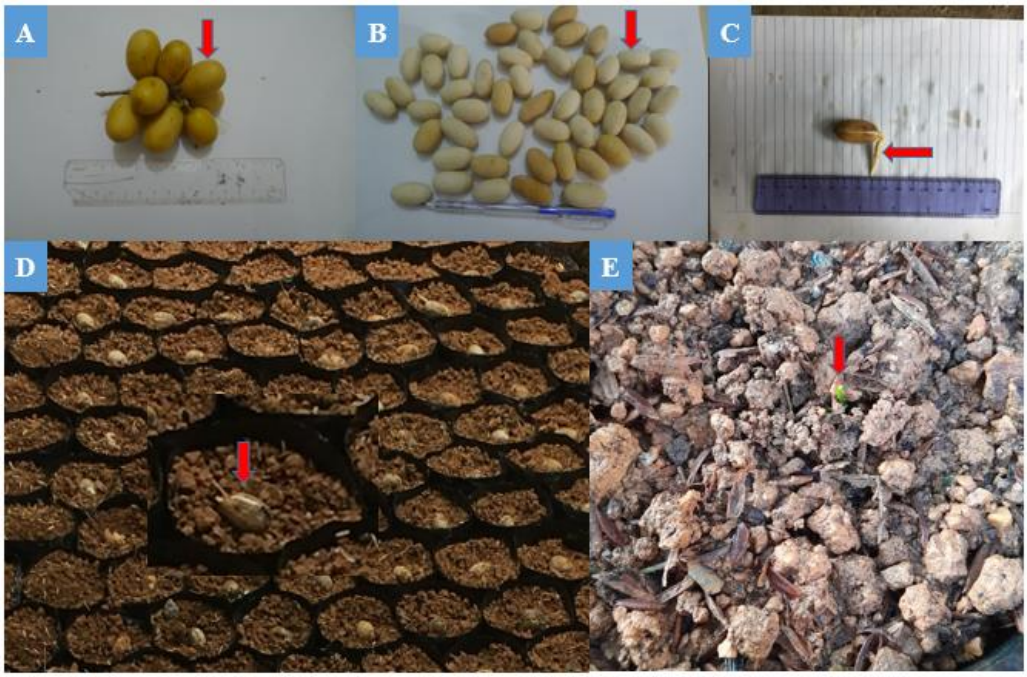


Figure. 1. *A: Matured fruit; B: Seed after remove seed coat; C: Radicle root; D: Seed sowed in polyethylene bags; E: Radicle length*

Data collection:

For the seed germination percentage (%), the total number of seed germination was determined when the radicle length size was 5 mm. Seed germination percentage (GP) was calculated according to the following formula by Liopa-Tsakalidi et al (2011).

$$GP\% = \frac{\text{No. of seeds germination}}{\text{Total seeds}} \times 100$$

The germination index (GI) was calculated using the following formula by Bewley et al (2013), where *Gt* is the number of germinated seeds on day *t.*

$$GI = \sum (Gt/t)$$

The germination energy (GE) was calculated using the following formula by Finch-savage and Bassel (2015).

$$GE = \sum (G1+G2+G3)/\text{the total number of seed} \times 100\%$$

Mean germination time (MGT- day) was calculated according to the following formula by Kandil et al (2012), where n is the number of seeds germinated at time d; d is days from the beginning of the germination test.

$$MGT = \sum \frac{dn}{n}$$

The Seedling vigour index (SVI) was calculated using the following formula by Al-Ansari and Ksiksi (2016), where *GP* is the germination percentage, *Mr* is the mean

root length (mm) and *Mh* is the mean hypocotyl length (mm), as described by Abdul-Baki & Anderson (1973).

$$SVI = GP \times (Mr + Mh)$$

Growth parameter: Randomly selected fifteen plants were tagged for following observations: Seedling growth include height of seedling, diameter of stem, leaf size, number of leaves per seedling were measured weekly. At transplanting period height of seedling, diameter of stem, leaf size, number of leaves per seedling and root weight were determined. In which, height of seedling was measured from ground level to the tip of opened leaf. Diameter of stem was measured with the help of digital verneer calipers just above the ground surface and the average was calculated. Number of leaves per seedling was counted and the average was calculated. Leaf size (length and width) was determined with the help of Vernier caliper. Average root weight was determined by weighing and the average was calculated.

Statistical analysis:

The data obtained from the study was analyzed by using SAS 9.1 statistical software. Duncan's multiple range tests was used to compare significant differences among treatment at $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of GA3 application on seed germination parameters of the Melientha suavis Pierre:

GA3 needed for seed germination because GA3 plays a fundamental role in enhancement of the germination due to acts on the embryo and causes synthesis of hydrolyzing enzymes particularly amylase and protease and this hydrolyzed food is utilized for growth of embryo (Urbanova and Leubner-Metzger 2018), significantly increase the seed vigor index (Mihaiela et al. 2020). As the results displayed in Table 1 showded that there were significant differences among treatments concerning GP (Germination percentage), MGT (Mean germination time), GI (Germination index), GE (germination energy) and SVI (seed vigro index) at $p < 0.05$. In term, applying with 50 ppm GA3 were found to the highest GP (91.1%), GI (2,3%), GE (62.0%) and SVI (133), whereas the lowest GP, GI, GE, SVI were recorded in untreated control with value of (58.9%, 1.2%, 36.1% and 76.2 respectively). Enhancement of GP, GI, GE and SVI seems to be quantitatively correlated with GA3 application, which is similar to results reported by other authors (GI 2016; Mihaiela et al. 2019; Urbanova and Leubner-Metzger 2018). Hence, the result indicated that GP, GI, GE, SVI were considerably enhanced by GA3 application compared with the untreated control. On the other hand, the result revealed that application of 50 ppm GA_3 produced the lowest mean germination time (5.4 days), while the untreated control showed the longest mean germination time (12.3 days). GA_3 application resulted a decrease in MGT, an increase in GP due to it took one less day to complete the germination. Similar finding was reported by Cornea-Cipcigan et al (2020).

Table 1. Effect of GA3 on the seed gemination parameters of the *Melientha suavis* Pierre

Treartment	GP (%)	MGT (days)	GI (%)	GE (%)	SVI
GA3 0 ppm (untreated	58.9[d]	12.3[a]	1.2[e]	36.1[d]	76.2[d]

control)					
GA3 25 ppm	80.0^{b}	7.1^{b}	1.9^{b}	52.6^{b}	112.9^{b}
GA3 50 ppm	91.1^{a}	5.4^{b}	2.3^{a}	62.0^{a}	133.8^{a}
GA3 100 ppm	76.7^{b}	7.5^{b}	1.7^{c}	48.9^{b}	102.8^{bc}
GA3 150 ppm	70.0^{c}	11.0^{a}	1.5^{d}	43.0^{c}	99.9^{c}
P	<0.05	<0.05	<0.05	<0.05	<0.05
LSD 0.05	4.93	2.6	0.16	4.04	11.1

GP, MGT, GI, GE and SVI indicate germination percentage, mean germination time, germination index, germination energy, coefficient of the velocity of germination, Seedling vigour index respectively. Mean in each column followed by the same letters are not significantly different at P ≤ 0.05 according to Duncan's multiple range test.

Moveover, to identify the effect of main factors on seed germination, the pearson correlation between GI, GE, SVI, MGT on GP was applied. The result was indicated in Table 2 the highest significant correlations were obtained between GE and GP (0.97287; p < .0001), MGT and GP (-0.84803; p < .0001). It seem that the enhancement of GE has been associated with an increase of GP. Similar finding was reported by Gi (2016). However, the decrease of GP had a relationship with increasing MGT, which is in accordant with reported by Mihaiela et al (2020).

Table 2. Pearson correlation coefficients and probability values of the relationships GP measures (N=15)[1]

	GP	**GI**	**GE**	**SVI**	**MGT**
GP	1.00000	0.96911*	0.97287*	0.96730*	-0.84803*
		(0.0001)	(0.0001)	(0.0001)	(0.0001)
GI	0.96911*	1.00000	0.99557*	0.92971*	-0.88799*
	(0.0001)		(0.0001)	(0.0001)	(0.0001)
GE	0.97287*	0.99557*	1.00000	0.92386*	-0.87401*
	(0.0001)	(0.0001)		(0.0001)	(0.0001)
SVI	0.96730*	0.92971*	0.92386*	1.00000	-0.79579*
	(0.0001)	(0.0001)	(0.0001)		(0.0004)
MGT	-0.84803*	-0.88799*	-0.87401*	-0.79579*	1.00000
	(0.0001)	(0.0001)	(0.0001)	(0.0004)	

[1]Values given in parenthesis are P-value. Correlations coefficients followed by asterisks indicated a statistical significant probability of correlations between variables.

According to the Pearson correlation in Table 2, the linear regression models between the MGT, GE with GP were analyzed to measure relationship between MGT, GE and GP. The Figure 2A and 2B indicated that GP was positively related to the GE and MGT, negatively related to MGT (R^2 = 0.962* and R^2 = 0.991*, respectively).

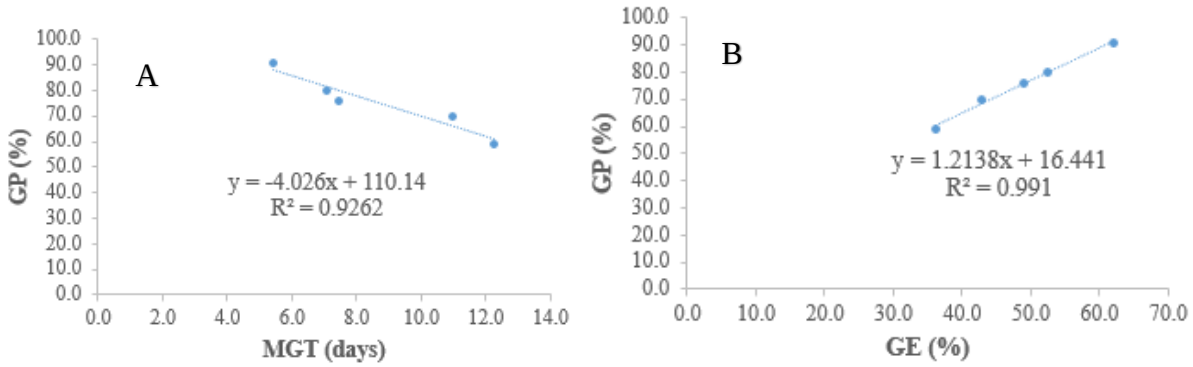
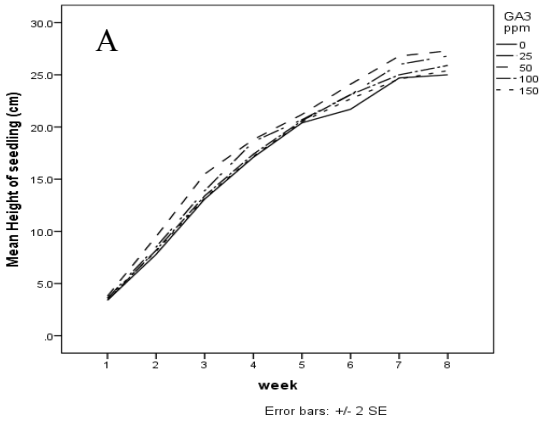
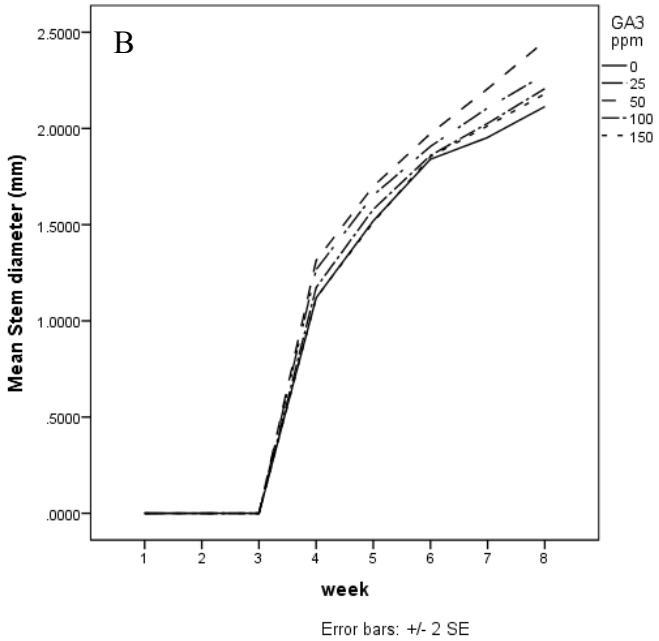


Figure 2. Linear regression between MGT, GE on GP at the P⩽ 0.05 (A: Linear regression between MGT on GP; B: Linear regression between GE on GP)

Effect of GA3 application on seedling growth rate of Melientha suavis:

According to Mihaiela et al *(*2016), GA_3 plays an important key in enhancing plant development rate due to its stimulating cell division and expansion. Therefore, in the case of the study showed that at the 7th week of observation, GA_3 50 ppm treatment obtained the highest plant height (26.8 cm) and stem diameter (2.21 mm) development, whereas the untreated control had the lowest plant height and stem diameter development which recorded 24.7 cm and 1.95 mm, respectively (Fig. 3A, 3B). It seems that, the plant height and stem diameter growth rate were increased due to gibberellin acts through the stimulation of the division of inter-meristem between the nodes of the stems. Therefore, the study speculated that application of GA3 significantly improve plant height, stem diameter in comparison with the untreated control, which is similar with the finding reported by Chetouani (2017). Moreover, for number of leaves, the Fig. 3C showed that number of leaves growth rate was greatly enhanced by applying 50 ppm GA_3 treatment (5.4 leaves/plant), whereas the lowest number of leaves growth rate was found in untreated control, only 4.1 leaves/plant at the 7th with of observation. It seems that the leaves number growth rate with GA_3 application was considerably faster than untreated control, which is similar with the finding reported by Mihaiela et al (2020).





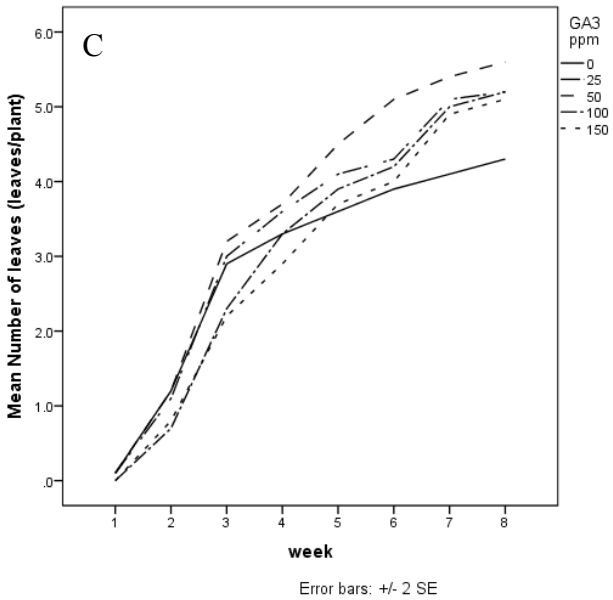


Figure.3. Effect of GA_3 on plant growth of *M.suavis (A:*Height of seedling/week; B:Stem diameter/week; D: Leaves number/week).Values error bars are mean ± SE according to Duncan's Multiple Range Test, $p < 0.05$.

Effect of GA3 application on seedling parameters of Melientha suavis Pierre at transplanting period:

GA_3 is a growth hormone of stimulating plant growth and development and plays an essential key in stem elongation by increasing cell division and expansion (Oh et al. 2015). Moreover, Salisbury and Ross (1995) states that giberelin not only spur the stem extension but also the development of the whole plant, including the roots. Hence, in the case of the study, there were significant differences in seedling parameters among treatments ($p < 0.05$) by GA3 application. In contracts, applying GA3 with 50 ppm reseponse to increase of plant height (28.2 cm), stem diamerter (2.45 mm), number of leaves (5.8 leaf/plant), leaf length (11.5 cm), leaf width (2.58 cm), and root weight (2.69), whereas the lowest values of plant height (25.5 cm), stem diamerter (2.14 mm), number of leaves (4.5 leaf/plant), leaf length (10.1 cm), leaf width (2.40 cm), and root weight (2.06 g) was recorded for the untreated control. Previous reports indicated that an increase of seedling parameters may be due to its role in stimulating cell elongation and expansion (Rasyid et al.2020; Bultynck and Lambers 2004). Therefore, it was determined that GA_3 application relatively increased the seedling parameters in comparison with the untreated control as the case in this study (Table 3). Similar finding was reported by Rasyid et al (2020).

Table 3. Effect of GA3 on seedling parameters of *M. suavis at transplanting period*

Treatment	Height of seedling (cm)	Stem diamerter (mm)	Number of leaves (leaves/plant)	Leaf length (cm)	Leaf width (cm)	Root weight (g)
GA3 0 ppm (untreated control)	25.5^{b}	2.14^{c}	4.5^{b}	10.1^{b}	2.40^{b}	2.06^{c}
GA3 25 ppm	27.9^{a}	2.29^{ab}	5.5^{a}	10.6^{ab}	2.57^{a}	2.52^{ab}
GA3 50 ppm	28.2^{a}	2.45^{a}	5.8^{a}	11.5^{a}	2.58^{a}	2.69^{a}
GA3 100 ppm	27.5^{a}	2.21^{bc}	5.4^{a}	10.4^{b}	2.43^{ab}	2.34^{abc}
GA3 150 ppm	26.8^{ab}	2.18^{bc}	5.2^{ab}	10.2^{b}	2.41^{ab}	2.15^{bc}

P	<0.05	<0.05	<0.05	<0.05	<0.05	<0,05
LSD 0.05	1.78	0.19	0.82	0.94	0.17	0.43

Mean in each column followed by the same letters are not significantly different at P≤ 0.05 according to Duncan's multiple range test.

Effect of GA3 application on transplanting rate of Melientha suavis Pierre

The result of Figure 4 showed that there were significant differences in transplanting rate among treatments. The highest amount of transplanting rate (93.9%) was recorded in 50 ppm GA_3 treatment, whereas the untreated control had the lowest amount of transplanting rate with a value of 69.4 %. It seems to be that, GA3 has a role in enhancing the seedling parameters of the plant, hence, leads to improving transplanting rate. Therefore, the study indicated that GA_3 application relatively increased the transplanting rate than the untreated control. The same finding has been reported by Salisbury and Ross (1995).

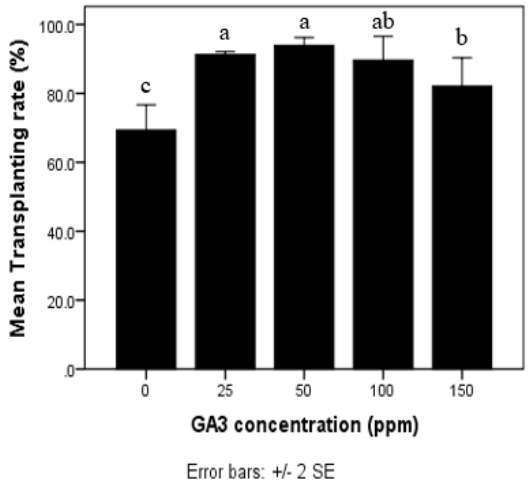


Figure.4. Effect of GA_3 on transplanting rate of *M. suavis*. Values error bars are mean ± SE according to Duncan's Multiple Range Test, $p < 0.05$.

CONCLUSIONS

Application of GA_3 at the rate of 50 ppm greatly increased GP, GI, GE, SVI as well as improved seedling height, stem diameter, number of leaves and leaf size development. Moreover, application of GA_3 with 50 ppm considerably reduced MGT. The results also suggested that 50 ppm of GA_3 improved significantly the agronomic characteristics of the seedling at the trasplanting time as well as increased transplanting rate. Application of 50 ppm GA_3 in seed propagation may be recommended as a practical technique for improving seed germination and seedling growth of *M. suavis* species.

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