



The Effect of NAA and Ga₃ on The Growth Performance of *Ceriops tagal* Seedling

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Abstract

Plant Growth Regulator (PGR) had been widely utilized in improving plant quality and productivity. The mechanism includes the manipulation of plant adaptability over environmental stress. This research aimed to study the effect of PGR on the growth of *C. tagal* seedling and to formulate the applicable treatment to improve its growth performance. The research was conducted through laboratory experiment, including the combination of NAA and GA₃ with the designed concentration of 0 ppm, 150 ppm and 300 ppm. The experiment lasted for one month and involved weekly observation. The plants were treated by spraying once every three days. Observations were conducted to the survival rate, height increment and leaf number development. ANOVA was conducted as statistical analysis method. The result showed that there were no significant effect of PGRs on the height increment of *C. tagal* seedling. The survival rate was lowest in the control and 150 ppm NAA treatments, while treatment with 300 ppm GA₃ was highest. The application of 300 ppm GA₃ was the best treatment for the livelihood of *C. tagal* seedling, while the application of 150 ppm GA₃ was considerable alternative due to its growth performance. This research provide a novel finding concerning the application of PGRs on the seeding process of *C. tagal*. Thus, it could be applied in the seeding activities by the mangrove farmers to improve the productivity and quality of mangrove seedling.

How to Cite

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INTRODUCTION

The improvement of mangrove seedling quality is required in order to enhance its adaptation capability. Increasing upland and coastal area development had caused the severe damage of the ecosystem (Shahbudin, *et al.*, 2012). Thus, mangrove could not establish and grow optimally. Plantation efforts had been conducted to hinder further coastal degradation and to initiate the ecosystem recovery (Andradi-Brown, *et al.*, 2013). Even though, the diversity of mangrove which had been decreased needs to be restored as well.

Rehabilitation of mangrove ecosystem is generally conducted through plantation of pioneer species such as *Avicennia marina* and *Rhizophora mucronata* (Munandar, *et al.*, 2014). The utilization of any other species usually resulted in low survival and growth rate. Unsuitable environmental condition had been considered as the main affecting factor (Sofawi, *et al.*, 2017). Coastal degradation lead to the change of erosion-sedimentation pattern, soil structure, tidal area, inundation periods and etc (Lageweg & Slangen, 2017). Thus, the environmental condition can no longer support the establishment of several mangrove species, while the existing plants might get disturbed as well.

The development of mangrove ecosystem including the species enrichment occurs through the succession which requires a very long period. Thus, the environmental suitability for the establishment of particular mangrove species might be achieved after decades (Cunha-Lignon *et al.*, 2009) and; T4. The consequences would be the low mangrove diversity in the degraded coastal area, or worse, it may only consisted of single mangrove species due to environmental limitation.

In order to overcome this problem, several efforts could be conducted to improve the adaptability of particular mangrove species to the existing environmental condition. These efforts could be the modification of plantation technique (Andradi-Brown *et al.*, 2013), site (Bashan, *et al.*, 2013) a hurricane moved a large sand dune, blocking the sole outlet channel of a mangrove. In the absence of daily tidal flow, the two ponds containing the mangrove vegetation evaporated, the secondary drainage channels were lost, and a salt crust formed on the bed of the ponds. The mangrove lost most of its trees and the remaining suffered from osmotic shock that led to defoliation. Restoration involved creating a knickpoint retreat (waterfall retreat effect, or improvement of seedling quality (Cortina, *et al.*, 2013). The first

and second are generally applied in the field application. Furthermore, the application of the third method is not common to the farmers.

Plant growth regulators (PGR) are the important components of plant which define the growth pattern. The substances play the role in stimulating or inhibiting the development of particular plant organs (Giron, *et al.*, 2013). The application of PGR includes the genetical modification of plants for certain purposes, such as increasing growth and crop production (Sari, *et al.*, 2014), shorten the productive age, improving the adaptability (Saputro, *et al.*, 2016), etc. In the last few decades, genetical engineering had been taking the responsibility on the development of new plant varieties (Ren, *et al.*, 2011).

Naphtalene Acetic Acid (NAA) and Gibberellic Acid (GA₃) are some of the generally utilized PGR for plant genetic modifications. The NAA belongs to the auxin group compounds which has the function in stimulating callus and organ (Zayova, *et al.*, 2012). The NAA is frequently used to increase crop production (Abbasi, *et al.*, 2013; Bakhsh, *et al.*, 2011; Basuchaudhuri, 2016) and plant quality (Awan, *et al.*, 2015). The GA₃ belongs to the gibberellin which has the function in accelerating germination (Sheykhbaglou, *et al.*, 2014). GA₃ is mostly utilized to increase germination percentage (Chetouani *et al.*, 2017). The applied combination of both compounds is expected to provide better development of mangrove seedling.

Ceriops tagal is among the threatened mangrove species due to coastal degradation. The habitat of *C. tagal* is in the inner mangrove area approaching the dry land (Liao, *et al.*, 2009; Wibisono, 2016). Suitable substrate for *C. tagal* growth is sandy mud with the salinity range of 0-15 ppt (Duke *et al.*, 2010). The intensive upland development and increasing coastal degradation had caused the distribution of *C. tagal* to be limited. Plantation of *C. tagal* had showed lower establishment level compared to its natural germination (Mukhlisi & Gunawan, 2016). Thus, *C. tagal* needs to be protected from further loss and should be bred to increase its abundance.

The application of plant growth regulator is rarely done on the improvement of plant quality, especially for mangrove. Even though, the impact of this method needs to be understood to achieve alternate solution on plantation problems. In order to formulate the appropriate solution for *C. tagal* adaptability improvement, a research needs to be conducted. This research aimed to study the effect of plant growth regulator on the growth of *C. tagal* seedling and to formulate the best appli-

cable treatment to improve its growth performance. This research is expected to provide technical recommendation on the application of PGRs to improve the growth of *C. tagal*. Thus, the seeding could be conducted more effectively.

METHODS

Time and location

This research was conducted in the greenhouse laboratory of Faculty of Science and Mathematics Diponegoro University from August to September 2017.

Experiment design

Experimental design involved the application of plant growth regulator through the combination of NAA and GA₃. The treatments included the concentration of 0 ppm, 150 ppm, and 300 ppm of the regulator. The treatment without NAA and GA₃ (0 ppm) acted as the control. The complete experiment design is described as follows:

- N0G0 (control) = without NAA and GA₃
- N0G1 = only 150 ppm GA₃
- N0G2 = only 300 ppm GA₃
- N1G0 = only 150 ppm NAA
- N1G1 = 150 ppm NAA + 150 ppm GA₃
- N1G2 = 150 ppm NAA + 300 ppm GA₃
- N2G0 = only 300 ppm NAA
- N2G1 = 300 ppm NAA + 150 ppm GA₃
- N2G2 = 300 ppm NAA + 300 ppm GA₃

Treatment activities

The mangrove species utilized in this research was *Ceriops tagal* seedling which had a couple of leaf. The seedling was planted in the polybag under the greenhouse. The plant growth regulator was given to the mangrove seedling during the experiment by spraying on its plant surface (leaf and trunk) every three days with three sprays. The experiment was conducted for one month which involved 4 observation periods with one week interval.

Data collection and analysis

Data collected were included the survival rate, height increment, and leaf development. Height increment was analyzed with factorial ANOVA to understand the both partial and simultaneous effect of plant growth regulators. Analysis factors included the concentration of NAA and GA₃.

RESULTS AND DISCUSSIONS

The experiment indicated that *C. tagal* seedlings responded the PGR application. The variations of survival rate, height increment and leaf development were observed during the research. These parameters showed the most appropriate treatment to improve the seeding application. Each parameter plays important role in the development of mangrove plants. The survival rate indicates the capability of mangrove seedling to establish and become the most important factor in the seed production. The height increment plays important role in defining its growth, which then determine its ecological value. The leaf development is an important aspect of plants metabolism which plays important role in supporting plant growth.

The survival rate indicates the environmental suitability for seedling growth. Unsuitable condition would increase plant stress and mortality. The application of PGR was expected to favor mangrove seedling due to unsuitable environmental condition. The survival rate of *C. tagal* seedling during the research was noticeable. The observation showed the gradually decrease of seedling survival rate among weeks. Some treatments showed extreme survival decrease after one month of experiment. Detailed survival rate of *C. tagal* during the research is presented in Table 1.

Table 1. Cummulative survival rate of *C. tagal* treated with plant growth regulator

Treatment	Week of Observation (%)			
	I	II	III	IV
N0G0	100	60	40	20
N1G0	100	80	40	20
N2G0	100	80	60	60
N0G1	100	80	60	40
N0G2	100	80	80	80
N1G1	100	80	80	60
N2G1	100	100	80	60
N1G2	80	60	40	40
N2G2	100	60	60	40
Min	80	60	40	20
Max	100	100	80	80
Average	98	78	62	49

Table 1 showed that the mortality of mangrove seedling had began since the first week, while in the third week seedling mortality was observed at all treatments. Among the applied treatments, the control (N0G0) and N1G0(150 ppm NAA) had the lowest final survival rate compared to any other treatments. The survival of *C. tagal* in treatment N2G1 (300 ppm NAA + 150 ppm GA₃) was great during the early period, then gradually decreased. The maximum survival was achieved from treatment N0G2 (300 ppm GA₃), which could maintain long term survival. This result indicated that the application of 300 ppm GA₃ could improve the adaptability of *C. tagal* seedling.

The height increment of mangrove seedling indicates the environmental support for plant growth. Height increment data processing was conducted for periodic growth and cumulative growth. Periodic height increment indicated the effect of the PGR on the growth rate of mangrove seedling. Observation on the periodic height increment of *C. tagal* during the research is presented in Table 2.

The periodic growth increment as shown in Table 2 indicated that the growth rate of *C. tagal* seedling was fluctuative. The optimum growth rate dynamically changed among periods. This result showed that there was no particular effect

of the treatment on the growth rate of *C. tagal* seedling. Data analysis which was conducted with ANOVA showed that there was no significant neither particular nor simultaneous effect of PGRs on the growth rate of *C. tagal*. Analysis on the effect of NAA showed the calculated F value of 0.584 with the probability of 0.559, while the effect of GA₃ showed the F value of 0.637 and probability of 0.530. Simultaneous effect of both PGR resulted the F value of 0.338 and probability of 0.852.

Cumulative height increment among periods indicated the total growth of plants in relative to its initial height. The difference of periodic growth rate of mangrove might be so low, thus it was not significant. However, the cumulative growth might show different pattern. The significant difference could be of the growth might be noticeable after a several periods. Table 3 shows the cumulative growth of *C. tagal* seedling during the research.

Table 3 shows that there is a noticeable difference of final height increment of *C. tagal* among treatments. Highest increment is achieved from treatment N0G1 (150 ppm GA₃), while the lowest is achieved from treatment N1G2 (150 ppm NAA + 300 ppm GA₃). This indicates that increasing PGR concentration does not always lead to the higher plant growth. Data analysis

Table 2. Periodic height increment of *C. tagal* treated with plant growth regulator

Treatment	Value	Week of Observation											
		I			II			III			IV		
N0G0	Range	0.1	-	2.5	0.6	-	1.8	0.6	-	2.1	0.9	-	1.6
	Average	1.06	±	1.19	1.10	±	0.62	1.15	±	0.66	1.20	±	0.32
N1G0	Range	0.3	-	2.2	0.7	-	1.2	0.7	-	1.3	0.9	-	1.4
	Average	0.82	±	0.79	0.93	±	0.26	0.93	±	0.32	1.05	±	0.24
N2G0	Range	0.3	-	2.2	0.1	-	3	0.1	-	2	0.2	-	2
	Average	1.02	±	0.79	1.28	±	1.25	0.88	±	0.84	0.94	±	0.68
N0G1	Range	0.5	-	2	0.2	-	1	0.2	-	1.9	0.5	-	1.2
	Average	1.00	±	0.71	0.80	±	0.40	1.08	±	0.70	0.93	±	0.34
N0G2	Range	0.1	-	1.3	0.5	-	1.7	0.9	-	1.1	0.7	-	1.9
	Average	0.58	±	0.58	0.85	±	0.57	1.03	±	0.10	1.32	±	0.51
N1G1	Range	0.6	-	2.3	0.9	-	1.3	0.6	-	1.8	0.2	-	1.1
	Average	1.06	±	0.70	1.10	±	0.18	1.06	±	0.45	0.65	±	0.47
N2G1	Range	0.1	-	1.9	0.3	-	1.6	0.2	-	1.5	0.7	-	2.6
	Average	0.82	±	0.69	0.88	±	0.48	0.83	±	0.54	1.43	±	0.85
N1G2	Range	0.1	-	1	0.1	-	0.8	0.5	-	1.4	0.2	-	2.2
	Average	0.38	±	0.42	0.48	±	0.30	0.98	±	0.49	0.94	±	0.78
N2G2	Range	0.2	-	0.6	1	-	1.5	0.5	-	1.9	0.4	-	2.6
	Average	0.40	±	0.16	1.30	±	0.26	0.92	±	0.60	1.18	±	0.98

Table 3. Cummulative height increment of *C. tagal* treated with plant growth regulator

Treatment	Value	Week of Observation											
		I			II			III			IV		
N0G0	Range	0.1	-	2.5	0.7	-	2.2	2	-	2.8	3.3	-	3.3
	Average	1.06	±	1.19	1.30	±	0.79	2.40	±	0.57	3.30	±	--
N1G0	Range	0.3	-	2.2	1	-	1.7	2.3	-	2.7	4.1	-	4.1
	Average	0.82	±	0.79	1.40	±	0.29	2.50	±	0.28	4.10	±	--
N2G0	Range	0.3	-	2.2	1.6	-	4.1	2	-	3.9	3	-	4.4
	Average	1.02	±	0.79	2.48	±	1.12	3.07	±	0.97	3.90	±	0.78
N0G1	Range	0.5	-	2	1.5	-	3	2.9	-	3.4	4.1	-	4.4
	Average	1.00	±	0.71	1.93	±	0.72	3.17	±	0.25	4.25	±	0.21
N0G2	Range	0.1	-	1.3	0.6	-	3	1.7	-	3.9	2.6	-	5.8
	Average	0.58	±	0.58	1.50	±	1.12	2.53	±	1.05	4.00	±	1.44
N1G1	Range	0.6	-	2.3	1.5	-	2.1	2.1	-	3.9	3.2	-	4.1
	Average	1.06	±	0.70	1.85	±	0.26	2.95	±	0.75	3.57	±	0.47
N2G1	Range	0.1	-	1.9	0.9	-	3.5	1.1	-	2.9	3.2	-	3.7
	Average	0.82	±	0.69	1.70	±	1.03	2.08	±	0.75	3.47	±	0.25
N1G2	Range	0.1	-	1	0.6	-	0.9	2	-	2.3	2.2	-	3.4
	Average	0.38	±	0.42	0.77	±	0.15	2.15	±	0.21	2.80	±	0.85
N2G2	Range	0.2	-	0.6	1.3	-	2	2.3	-	3.9	2.7	-	5
	Average	0.40	±	0.16	1.67	±	0.35	2.87	±	0.90	3.85	±	1.63

by using anova showed that cummulative height increment was significantly different at the third and fourth observation compared to the first observation. However, there was no significant different between neither the second to the third and fourth, nor third to fourth observations. The NAA and GA₃ on the cummulative height increment are not significant neither partially nor simultaneously.

The analysis of the periodic difference showed that F value of 4.173 and probability 0.007. Factorial analysis on the effect of NAA partially resulted the F value of 1.172 and probability of 0.313, while the GA₃ resulted the F value of 1.837 and probability of 0.163. The simultaneous analysis showed the F value of 2.363 and probability of 0.056. Thus, the addition of PGR on seedling for 1 month did not give significant effect on the height increment of *C. tagal* seedling. However, the cumulative growth was noticeable.

The last parameter observed in this research was the leaf development. Faster leaf development should improve seedling establishment through metabolism process. Thus, the application of PGR was expected to stimulate the leaf development of *C. tagal* seedling during the experiment. The observation result of cummulative leaf development during the research is pre-

sented in Table 4.

The observation result of leaf development as presented in Table 4 shows that the maximum leaf development during 1 month experiment is 2 leaves. Early leaf development is observed from treatment N0G1 (150 ppm GA₃) and N1G1 (150 ppm NAA + 150 ppm GA₃) in the first period. However, the final observation shows that highest average of leaf development is achieved from treatment N0G2 (300 ppm GA₃). The high leaf development average value in the treatment N0G0 (control) and N1G0 (150 ppm NAA) was due to its low survival which was only one seedling stand remain in the last observation.

The combination of the analysis result showed that the best treatment to improve the seedling growth quality of *C. tagal* was N0G2 (300 ppm GA₃). Instead of its best survival compared to the other treatments, it also provided a good final growth. The final observation of this treatment showed the survival of 80% and height increment of 4 cm. The second potential treatment was N2G0 (300 ppm NAA) which had lower survival and growth with the level of 60% and 3.9 cm respectively.

In order to grow optimally, a balanced nutrient supply is required by plants as well as hormonal stimulation in the PGR application.

Table 4. Cummulative leaf development of *C. tagal* treated with plant growth regulator

Treatment	Value	Week of Observation											
		I			II			III			IV		
N0G0	Range	0.0	-	1.0	0.0	-	2.0	0.0	-	2.0	2.0	-	2.0
	Average	0.2			0.7			1.0			2.0		
N1G0	Range	0.0	-	0.0	0.0	-	2.0	0.0	-	2.0	2.0	-	2.0
	Average	0.0			1.0			1.0			2.0		
N2G0	Range	0.0	-	0.0	0.0	-	2.0	0.0	-	2.0	0.0	-	2.0
	Average	0.0			0.5			0.7			0.5		
N0G1	Range	0.0	-	2.0	0.0	-	2.0	0.0	-	2.0	0.0	-	2.0
	Average	0.4			0.5			1.3			1.3		
N0G2	Range	0.0	-	0.0	0.0	-	2.0	0.0	-	2.0	0.0	-	2.0
	Average	0.0			1.0			1.0			1.5		
N1G1	Range	0.0	-	2.0	0.0	-	0.0	0.0	-	0.0	0.0	-	2.0
	Average	0.4			0.0			0.0			1.0		
N2G1	Range	0.0	-	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-	2.0
	Average	0.0			0.0			0.0			0.7		
N1G2	Range	0.0	-	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-	2.0
	Average	0.0			0.0			0.0			0.5		
N2G2	Range	0.0	-	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-	2.0
	Average	0.0			0.0			0.0			0.5		

Imbalanced hormone might cause plant stress or unexpected growth pattern. In order to achieve expected plant growth, the existing growth media should be considered. Plants might show different growth pattern on the different soil condition due to its nutrient availability. Particular application of PGR was also used to increase the efficiency of certain fertilizer application (Otie, *et al.*, 2016). Thus, the utilization of PGR treated seed are usually followed by strict fertilization treatment. The application of PGR was also utilized to improve plants adaptability in certain environment condition (Schiavon, *et al.*, 2014).

This research proved that the application of PGR could increase the survival and growth of *C. tagal* seedling. Observation on the control treatment (without PGRs) resulted the survival of only 20% and final height increment of 3.3 cm for 1 month. This result was extremely low. Thus, in order to improve the seeding activity of *C. tagal*, the application of PGR is required. However, the combination of both PGRs seemed to be ineffective. The survival and height increment of all mixed PGRs were lower than the application of particular GA₃ and NAA. The analysis also indicated that the effective concentration of PGRs application on *C. tagal* seedling was 300 ppm.

In optimum growth of *C. tagal* seedling in

the mixed PGR treatment might be caused by its inappropriate planting media. Lack of nutrient caused the poor organ formation and growth of the plant (José, *et al.*, 2012). In this research, plants which were grown in the polybags had limited nutrient sources. Thus, the growth of *C. tagal* seedling in the PGR applied treatments were also limited. Plant stress also affect the distribution of hormone into particular organs (Rowe, *et al.*, 2016). Particular hormone should be accumulated in the particular organs in order to stimulate its growth and optimize its function (Krouk *et al.*, 2010). Thus, the growth could not be optimized.

Auxin and gibberellin are utilized to control the quality of fruit production (Suman, *et al.*, 2017). Even though, the application of PGR does not always resulted in better plant growth. The application of NAA could even decrease plant's height, but increase its fruit productivity, shorten fruit maturity and increase the yield (Abbasi *et al.*, 2013). The gibberellin plays important role in the germination of dormant seed (Chen, *et al.*, 2008). In this research, 300 ppm GA₃ application showed the best growth survival and good but not best final height increment of *C. tagal* seedling. The role of GA₃ included the minimization of salinity stress on plants (Sahil, 2016), thus *C. tagal* could grow better than the other plants in

the salinity stressed environment such as coastal area. This result showed that there was a possibility of improving *C. tagal* survival and growth during the plantation in the coastal area through the treatment of GA₃.

Even though the PGR could be applied to improve the productivity of plants, there are limitation on the utilization. Excessive concentration of particular PGR might bring up phytotoxicity effect (Almeida, *et al.*, 2016). Thus, the concentration should be properly understood to achieve optimum result.

This research provide a novel finding concerning the application of PGRs on the in vivo seedling plantation of *C. tagal*. The utilization of GA₃ or NAA is preferred than the combination of both PGRs in order to improve seedling growth. This research also found that the higher concentration of PGRs would lead to the better seedling growth. However, the optimum limit is not yet known due to data limitation.

This result implies that the growth *C. tagal* seedling could be improved by periodic spraying of GA₃ or NAA to the plant's surface during the seeding process. Current suggested concentration is 300 ppm every 3 days. This method might help mangrove seed farmers to improve the productivity and quality of mangrove seedling, especially *C. tagal*. However, neither higher concentration nor more frequent spraying is not recommended since the impact is not known yet.

CONCLUSIONS

The application of PGRs did not effect the survival, height increment and leaf number development of *C. tagal* significantly. The best growth performance was achieved from the treatment with 300 ppm of GA₃.

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