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Rooting Response of Melada (*Piper colubrinum*) to Several Mixed Concentrations of IBA and NAA and Two Types of Commercial Root Stimulant

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Abstract

This paper aims to study the best type of auxin on *Piper colubrinum* rooting compared to commercial type stimulants and to study the effective and efficient mixed auxin concentration for *P. colubrinum* cuttings. The experiment was carried out at the Greenhouse of the Faculty of Agriculture, University of Lampung from May 2018 to December 2018. The experiment used a completely randomized design with 3 replications. The treatment applied mixed auxin (NAA and IBA) with a total concentration of 1500 ppm, 2000 ppm, 2500 ppm for both mixtures and applied two types of commercial stimulants. From the 0.05 BNT test analysis results we obtain that the combined auxin NAA + IBA with a total concentration of 1500 ppm is more effective and efficient compared to other treatment on the root formation of *P. colubrinum* cuttings. Although the effect on the variable is higher, it is considered the same in the 0.05 BNT test. In mixed auxin with a total of 1500 ppm the average number of primary roots in the node and in the cross section of the stem are 12 strands and 9.2 strands respectively. Furthermore, the average number of primary roots is 21.2 strands, the average length of primary roots is 27 cm, and the average root wet weight is 8.3 g.

Keywords: Melada, colubrinum, Auxin, Cuttings, Pepper

1. Introduction

Pepper (*Piper nigrum*) is a strategic commodity. Currently, pepper yields have decreased due to stem rot disease. Damage due to stem rot caused by the fungus *Phytophthora capsici* reaches 10-15% per year of the total pepper plants in Indonesia (Manohara *et al*, 2005). To overcome stem rot attacks, several methods have been carried out, namely technical culture, application of biological and chemical agents, and creating resistant plants (Lucas, 2011). Researchers have found another way as an alternative to overcome this disease, i.e., grafting the rootstock of plants that are resistant to stem rot disease with pepper scion, which is called the grafting technique.

The Grafting technique is a technique of grafting pepper scion with *Piper colubrinum* rootstock. This technique is used to avoid the occurrence of stem rot disease in pepper plants. *P. colubrinum* is a wild relative of pepper plants that is resistant to plant pathogens, namely *Phytophthora capsici* and nematodes such as *Meloidogyne incognita* and *Radopholus similes*. In addition, *P. colubrinum* has a strong root system and is resistant to several biotic and abiotic stress conditions. *P. colubrinum* needs to be propagated efficiently to obtain plant material as rootstock to produce large quantities of connected pepper seedlings. According to Raja *et al.* (2018), *P. colubrinum* is a species of piper with the highest percentage of rooting (82.24%) and grows faster so that it is effectively propagated by stem cuttings compared to other piper species.

Cuttings are a method of plant propagation using pieces of plant parts. According to Caplan *et al*. (2018), plant propagation by cuttings can be influenced by several factors, leaf number, leaf tip removal, position of stem cutting on the stock plant, and rooting hormone. Moreover, Indriatama (2020) stated that plant species, types, and concentrations of ZPT are factors that affect effectiveness in root induction.

Growth regulators are non-nutrient organic compounds that affect growth and development at low concentrations (Taiz and Zeiger, 2006), and become herbicides at high concentrations (Blythe *et al*, 2007). Auxin is a growth regulator used to stimulate root growth. Propagation by cuttings with auxin application can increase the initiation of adventitious roots (Taiz and Zeiger, 2006; Hopkins *et al*., 2008). The types of auxins commonly used for stem or leaf cuttings are NAA and IBA (Hopkins *et al*., 2008).

The research conducted by Yusnita *et al*. (2018) that the most effective auxin treatment on Jamaican guava was a mixture of NAA and IBA with 1000 ppm each because it produced longer roots, better roots morphologically, a higher percentage of shots, and faster time for root formation. The effect of the hormone auxin on rooting and plant development was studied in other species, such as Rosemary, Sage, and Elderberry (Gudeva *et al*., 2017), *Ficus benjamina* L. (Topacoglu *et al*., 2016), by applying IBA, NAA, and a mixture of each auxin was reported to be effective.

In pepper cuttings of Natar 1 variety, a mixture of auxin NAA and IBA with a concentration of 6000 ppm was the best to stimulate root formation (Artha *et al*., 2015), whereas in *Piper crocatum* Ruizan Pav. the concentration of NAA 4000 ppm + IBA 1000 ppm was the best to stimulate root formation (Maulida *et al*., 2014). For this reason, it is important to conduct this research to study efficient techniques on how to obtain large quantities of *P. colubrinum* plant material for rootstock to produce grafted pepper seedlings.

The purpose of this study is to find the best type of auxin on *P. colubrinum* rooting and compare it to commercial types of stimulants. Furthermore, this paper also aims to study the effective and efficient mixed auxin concentration for *P. colubrinum* cuttings.

2. Materials and Methods

2.1. Materials

This research was conducted at the Greenhouse of Faculty of Agriculture, University of Lampung from May 2018 to December 2018. The plant material used was cuttings of *P. colubrinum* obtained from farmers in Sekura village, Teluk Keramat sub-district, West Kalimantan province. The cutting material used was taken from orthotropic shoots with an average age of 2 months with the age of the parent plant more than 6 months. The diameter of the cuttings is set to be 1-1.5 cm and the length is 28-35 cm.

2.2. Methods

In this research we used Completely Randomized Design (CRD) with 6 treatments, i.e., Control, NAA 750 ppm + IBA 750 ppm, NAA 1000 ppm + IBA 1000 ppm, NAA 1250 ppm + IBA 1250 ppm, ZPT A and ZPT B. Commercial stimulants used are Rooton F (ZPT A) and Root Up (ZPT B). The content of ZPT A is NAA 670 ppm, IBA 570 ppm, 2-methyl-1 Naphthalene Acetamide 310 ppm, Thyram (Tetramithium disulfate) 4 g. ZPT B content is NAA 2000 ppm, IBA 100 ppm and Thyram 4 g. Each treatment was repeated 3 times with the total number of experimental plots was 18. Each experimental plot consisted of 10 samples. The next experiment amounted to 180 cuttings. The homogeneity of variance was tested with the Bartlett test and if the assumptions were met, the data were analyzed for variance, then continued with the separation of the mean values with the BNT test at a level of 0.05.

After the above experiments are completed, the next step is to prove related to compatibility. The technique used is grafting *P. colubrinum* as rootstock and pepper as a scion. In this experiment, the auxins used were IBA and NAA with various concentrations. The first way to make auxin is to prepare the tools, namely measuring cups, beakers, spatulas, electric scales, and covers. All the ingredients are then measured and weighed as needed, powder, fungicide, 70% ethanol, NAA, and IBA. Furthermore, the ingredients that have been weighed, i.e., powder and fungicide, are put into a beaker and are stirred until evenly distributed. The weighed NAA or IBA is put into a measuring cup then dissolved with 10 ml of 70% ethanol and stirred until dissolved. The dissolved NAA or IBA is put into a beaker containing a mixture of powder and fungicide and stir evenly until there are no lumps. The mixture is then closed and stored in a room with a temperature of ± 20ºC. Stir every day for one week.Observations were made after the plants were 12 weeks old. Then the research continued with the compatibility test of grafting *P. colubrinum* and pepper plants.

3. Results and Discussion

3.1. Results

The variance analysis of observational data on the effect of giving several concentrations of mixed auxin and two types of commercial stimulants on *P. colubrinum* roots revealed that mixed auxin had a significant effect on the number of primary roots in the node, the number of primary roots in the cross-section of the stem, primary root length, root wet weight, shoot height, and number of leaf cuttings. Meanwhile, the other variables, namely the number of primary branches, the number of secondary branches, and the height of the main stem had no effect (see Table 1).

Table 1: The results of the analysis of the effects of several mixed concentrations of IBA and NAA and two types of commercial root stimulants on the roots of *Piper colubrinum*

No	Observation Variable	Treatment
1	Number of primary roots in the node	**
2	Number of primary roots in the cross-section of the stem	**
3	Number of primary roots	**
4	Primary root length	**
5	Root wet weight	**
6	Shoot height	*
7	Number of leaves	*
8	Number of secondary branches	tn
9	Number of primary branches	tn
10	Main stem height	tn

Details: ** = significantly different at the 1% level of significance;* = significantly different at the 5% level of significance; tn = Not significantly different at the 5% level

3.1.1. Percentage of Rooted Cuttings

The experimental results showed that at the age of 8 weeks after planting *P. colubrinum* without auxin had 80% rooted percentage. The treatment of mixed auxin NAA + IBA at concentrations of 1500 ppm, 2000 ppm, and 2500 ppm resulted in a smaller root percent than control. The treatments of NAA 750 ppm + IBA 750 ppm, NAA 1000 ppm + IBA 1000 ppm, and NAA 1250 ppm + IBA 1250 ppm resulted in the percentage of rooted cuttings being 70%, 66.7%, and 73.3%. The percent rooted commercial stimulant treatment was smaller than the control and mixed auxin. The Percentage of cuttings rooted in ZPT A and ZPT B treatments were 56.7% and 40% (see Figure 1).

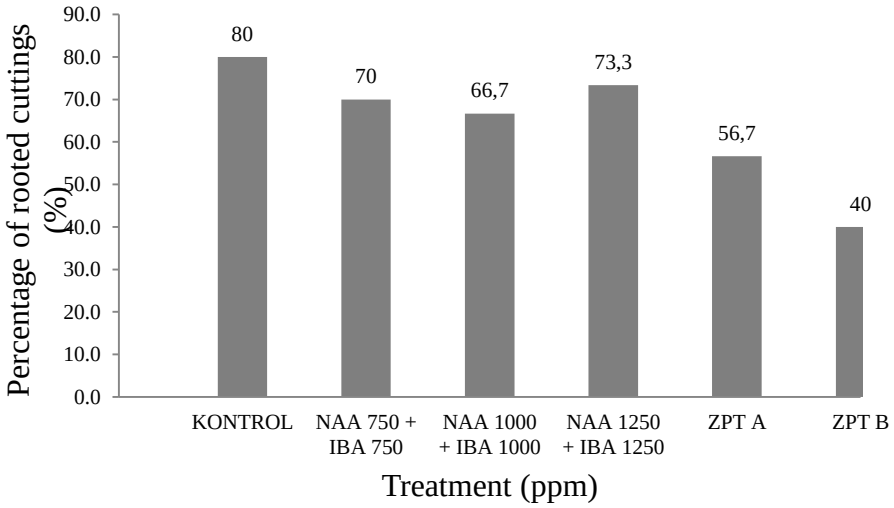

Figure 1: Effect of a mixture of IBA and NAA and two types of commercial root stimulants on thepercentage of rooted cuttings of *P. colubrinum* 8 weeks after planting

The administration of a mixture of NAA and IBA as well as two types of commercial stimulants, namely ZPT A and ZPT B could increase the number of primary roots in the nodes on *P. colubrinum* cuttings. Based on the results of the separation of the mean value with the BNT test of 0.05 (see Table 2).

Table 2: Mean values of the effects of several mixed concentrations of IBA and NAA and two types of commercial root stimulants on *Piper colubrinum*

Treatment	Primary roots in the node	Primary roots in the cross-section of the stem	Number of primary roots	Primary root length	Root wet weight	Shoot height	Number of leaves
Control	4,2c	6.9c	11.12d	17.1bc	4.2e	43.3ab	11.5a
NAA 750 + IBA 750	12a	9.2b	21.19b	27a	8.3a	43.7ab	10.7a
NAA 1000 + IBA 1000	13.4a	14a	27.42a	20.1b	6.9b	43.5ab	10.9a
NAA 1250 + IBA 1250	13.4a	14a	27.43a	19.6bc	6.2c	35.9bc	8.5ab
ZPT A	9.7b	7c	16.92c	17.4bc	5.7c	45.8a	11.6a
ZPT B	9.4b	7.5c	16.92c	14.7c	4.3d	33.7c	4.7b
BNT (5%)	1.53	1.08	1.75	3.83	0.65	6.1	2.55

3.1.2. Number of Primary Roots in Nodes

The average value of the number of primary roots in the highest node was in the treatment of NAA 1250 ppm + IBA 1250 ppm, which was 13.4 the same value as the treatment of NAA 1000 ppm + IBA 1000 ppm. Then the average value followed by treatment, NAA 750 ppm + IBA 750 ppm, and ZPT A and ZPT B was11.95>9.71>9.42. And the average number of primary roots in the smallest node in the control was 4.2 strands.

3.1.3. Number of Primary Roots in Stem Cross

The administration of a mixture of NAA and IBA as well as two types of commercial stimulants, namely ZPT A and ZPT B could increase the number of primary roots in the cross-section of the stem on *P. colubrinum*. Based on the results of the separation of the mean value with the 0.05 BNT test in Table 2.

The average number of primary roots in the cross-section of the stem, the highest average value of NAA 1000 ppm + IBA 1000 ppm, namely 14 strands, the value is the same as NAA 1250 ppm + IBA 1250 ppm, then the average value of the number of primary roots in the cross-section is followed by treatment NAA 750 ppm + IBA 750 ppm and ZPT B and ZPT A were 9.2>7.5>7 strands. And the average number of primary roots in the smallest cross-section of the stem in the control treatment was 6.9 strands.

3.1.4. Number of Primary Roots

The administration of a mixture of NAA and IBA as well as two types of commercial stimulants, namely ZPT A and ZPT B could increase the number of primary roots in *P. colubrinum*. Based on the results of the separation of the mean value with the BNT test of 0.05 (see Table 2).

The average number of primary roots, the highest average value of NAA 1250 ppm + IBA 1250 ppm is 27.4 strands, the value is the same as NAA 1000 ppm + IBA 1000 ppm, then the average value of the number of primary roots in the cross-section of the stem is followed by NAA treatment 750 ppm + IBA 750 ppm and ZPT B and ZPT A were 921.2>16.9>16.9 strands. And the average number of primary roots in the smallest stem cross-section in the control treatment was 11.1 strands.

3.1.5. Primary Root Length

The administration of a mixture of NAA + IBA and two types of commercial stimulants namely ZPT A and ZPT B could increase the primary root length in *P. colubrinum*.

The average primary root length, the highest average value of NAA 750 ppm + IBA 750 ppm is 27cm. then followed by treatment with 1000 ppm NAA + 1000 ppm IBA, 1250 ppm NAA + 1250 ppm IBA, ZPT A, and control, namely 20>19.6>17.4>17.1 cm. And the smallest average primary root length with ZPT B treatment was 14.7 cm.

3.1.6. Root Wet Weight

The administration of a mixture of NAA + IBA and two types of commercial stimulants, namely ZPT A and ZPT could increase the wet weight of roots in *P. colubrinum*.

The average wet weight of roots, the highest average value of root wet weight was in the treatment of NAA 750 ppm + IBA 750 ppm, namely 8.3 g. Then followed by treatment with 1000 ppm NAA + 1000 ppm IBA, 1250 ppm NAA + 1250 ppm IBA, ZPT A, and ZPT B which were 6.9>5.9>5.7>4.3 g. And the lowest average wet weight of roots with control treatment was 4.2 g. Root performance can be seen in Figure 2.

Figure 2: Performance of root cuttings of *P. colubrinum* with mixed auxin treatment compared to commercial stimulants (ZPT A and ZPT B)

3.2. Discussions

The results of observations in Table 2 shows that the combination treatment of NAA and IBA had different effects on the observed variables of *P. colubrinum* cuttings. Mixed combination treatment increased the value of the observed variable and decreased the value of the observation on *P. colubrinum* cuttings. The treatment of NAA 1250 ppm + IBA 1250 ppm, NAA 1000 ppm + IBA 1000 ppm, and NAA 750 ppm + IBA 750 ppm did not affect the increase in the percentage of rooted cuttings of *P. colubrinum*. The combination treatment of NAA 1250 ppm + 1250 ppm affected increasing the number of primary roots in the book, the number of primary roots in the cross-section of the stem, the number of primary roots, root length, and root wet weight. In the treatment of NAA 1250 ppm + IBA 1250 ppm, the number of primary roots in *P. colubrinum* cuttings was 13.4 strands compared to 4.2 strands in the control.

The effect of the treatment of NAA 1250 ppm + IBA 1250 ppm could increase the number of roots in the cross-section of the stem, namely 14 strands compared to 6.9 strands in the control. The effect of the treatment of NAA 1250 ppm + IBA 1250 ppm was able to increase the number of primary roots, namely 27.4 strands compared to 11.1 strands in the control. In the treatment of NAA 1250 ppm + IBA 1250 ppm, the primary root length was 19.6 cm compared to the control, which was 17.09 cm. At 1250 ppm NAA + 1250 ppm IBA can increase the wet weight of the roots which is 6.2 g compared to the control which is 4.2 g. The treatment of 1000 ppm NAA + 1000 ppm IBA can increase the number of primary roots in the node, namely 13.4 strands compared to the control, which is 4.2 strands. The treatment of 1000 ppm NAA + 1000 ppm IBA can increase the number of roots in the cross-section of the stem, which is 14 strands compared to the control, which is 6.9 strands.

The treatment of 1000 ppm NAA + 1000 ppm IBA can increase the number of primary roots which is 27.4 strands compared to the control which is 11.1 strands. The treatment of 1000 ppm NAA + 1000 ppm IBA can increase the primary root length by 20.1 cm compared to the control which is 17.1 cm. The treatment of 1000 ppm NAA + 1000 ppm IBA can increase the wet weight of the roots which is 6.9 g compared to the control of 4.2 g.

The treatment of NAA 750 ppm + IBA 750 ppm could increase the number of primary roots in the book by 12 compared to the control, which was 4.2 strands. The treatment of NAA 750 ppm + IBA 750 ppm could increase the number of primary roots in the cross-section of the stem, namely 9.2 strands compared to 6.9 strands in the control. The treatment of NAA 750 ppm + IBA 750 ppm could increase the number of primary roots, namely 21.2 strands compared to 11.1 strands in the control.

The treatment of NAA 750 ppm + IBA 750 ppm could increase the wet weight of the roots by 10.7 compared to the control, which was 4.2 g. The treatment of NAA 750 ppm + IBA 750 ppm could increase shoot height by 43.7 cm compared to 43.3 cm in control.

Based on this experiment, commercial stimulants of ZPT A and ZPT B had different effects on the treatment. ZPT A treatment did not affect the percentage of rooted cuttings compared to the control. ZPT A treatment could increase the number of primary roots in the node by 9.7 compared to 4.2 strands in the control. ZPT A Treatment An increase in the number of primary roots in the cross-section of the stem was 7 strands compared to 6.9 strands in the control. ZPT A treatment An increase in the number of primary roots in the cross-section of the stem was 16.9 strands compared to 11.1 strands in the control. ZPT A treatment could increase the primary root length by 17.4 cm compared to the control of 17.1. The ZPT A treatment increased the wet weight of the roots by 5.7 g compared to the control, which was 4.2 g. ZPT A treatment could increase the number of leaves by 11.6 compared to the control 11.54. ZPT A treatment could increase shoot height by 45.8 compared to 43.35 cm in control.

The ZPT B treatment increased the number of primary roots in the node, which was 9.4 strands compared to the control, which was 4.2 strands. ZPT B treatment can increase the number of primary roots in the cross-section of the stem, which is 7.5 compared to the control, which is 6.9 strands. ZPT B treatment can increase the number of primary roots in the cross-section of the stem, which is 16.9 strands compared to the control, which is 11.1 strands. The ZPT B treatment increased the wet weight of the roots, which was 4.3 g compared to the control 4.2 g.

The use of a combination of NAA and IBA auxin was more effective than commercial stimulants, namely ZPT A and ZPT B. This could be seen from the observations of the percentage of rooted cuttings, the number of primary roots in the node, the number of primary roots in the cross-section of the stem, the number of primary roots, the length of primary roots, root wet weight. The commercial stimulant of ZPT A and ZPT B is a combination of auxin. The composition of ZPT A is NAA 670 ppm, IBA 570 ppm, 2-methyl-1 Naphthalene Acetamide 310 ppm, Thyram (Tetramithium disulfate) 4%. The composition of ZPT B was 2000 ppm NAA, 100 ppm IBA and 4% Thyram while the mixed auxin NAA+ IBA had a total concentration of 1500 ppm, 2000 ppm, and 2500 ppm.

The experimental results above prove that the type of auxin and its concentration are one of the factors that affect rooting in the setting. These observations are corroborated by the statement of Hopkins *et al*. (2008) and Overvoorde *et al*. (2010) that the assumption of auxin concentration is an important variable in some differential developmental and growth responses to light and gravity. Another statement that confirms the above is the writing of Agustiansyah *et al*. (2018) that the factors that affect effectiveness in rooting induction are plant species, type, and concentration of ZPT. ZPT at low concentrations that affect growth and development (Taiz and Zeiger, 2006), is a type of NAA and IBA auxin (Hopkins *et al*., 2008).

From the results of the 0.05 BNT test analysis (Table 2), it can be concluded that the auxin combination of NAA + IBA at a total concentration of 1500 ppm is the best auxin in the formation of *P. colubrinum* cuttings, although the effect on the variables is different or higher but is considered the same in the BNT test. 0.05.

The conclusion is also taken based on cost considerations, if the concentration increases then the use of NAA and IBA and the required costs also increase. The prices for IBA 5 g and 25 g in December 2017 were USD 29.00 and USD 87.00, while the prices for NAA 25g and 500g were USD 20.00 and USD 121.00, respectively.

4. Conclussion

Based on the experiment that has been carried out, we can conclude that a mixture of NAA and IBA is more effective than commercial stimulants in stimulating the rooting of *P. colubrinum* cuttings. Furthermore, auxin mixture of NAA750 ppm + IBA 750 ppm was more effective and efficient than a mixture of NAA and IBA with a total of 2000 ppm and 2500 ppm in stimulating rooting of *P. colubrinum* cuttings.

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