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# Successful Grafting of Two Indonesian Clones of *Piper nigrum* L. with *P. colubrinum* Link.: Effects of IBA and NAA on Rooting and Effects of BA on Grafting

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**ABSTRACT**

This study aimed to investigate the effects of auxin types and their concentrations on rooting of the rootstock cuttings and BA on grafting. First, IBA, NAA or IBA+NAA, each at 0, 1000, 2000, and 3000 ppm were used as treatments to study rooting of *Piper colubrinum*. In the second experiment, BA (0 and 50 ppm) was applied to *P. nigrum* scion clones of Natar-1 and Petaling-2, before being grafted to the rootstocks. Results showed, that all types of auxins (IBA, NAA or IBA+NAA) induced rooting and shoot growth, and the increase of auxin concentrations led to the increase of rooting. However, their effectiveness was different. NAA and IBA+NAA were superior to IBA, and at 2000 ppm, IBA+NAA resulted in the best plant growth, as indicated by higher values of both rooting and shoot growth parameters. Compared to control, BA treatment on scions resulted in higher grafting success (80% vs. 93%) for Natar-1, and (73% vs.100%) for Petaling-2 clones. BA treatment on scions also induced more calluses in the graft union and better shoot growth. To our knowledge, this is the first report on the use of BA to increase success of grafting between *P. nigrum* and *P. colubrinum*.

## INTRODUCTION

Pepper (*Piper nigrum* L.) is currently used for food, pharmaceutical, and perfume industries. In international trade, the main commodity of pepper is black and white peppers. These two types come from the same pepper plants, but with different proccesses. The black pepper is produced from unripe fruits which are directly dried without peeling the fruit skin, while the white pepper is produced from ripe fruits which are peeled and then dried. Data from FAOSTAT (2023) showed that Indonesia is the second biggest pepper producers in the world, with the average production since 1994 to 2021 of 77,958.29 tonnes, after Vietnam (123,214.72 tonnes). The following countries rank at third to fifth in term of average pepper production (in tonnes) since 1994 to 2021: India (61,319.14), Brazil (60,024.21) and Burkina Faso (45,998.15).

One of the major problems of pepper cultivation in Indonesia is the infestation of stem rot disease (SRD) caused by *Phytophthora capsici* Leonian. The incidence of SRD was found in almost all pepper plantations in Indonesia, like Java, Sumatra, Kalimantan and Sulawesi (Wahyuno et al., 2007). The disease caused the production losses up to 10-15% per year (Harni & Amaria, 2012). Lampung Province is the second largest producer of pepper in Indonesia after Bangka Belitung Province with their respective contributions amounting to 16.65% and 38.02% of the total pepper production in Indonesia (Mahdi & Suprehatin, 2021). However, the pepper production in Lampung has decreased in recent years due to the respected SRD.

Most pepper plantations in Lampung use cuttings as planting materials. They use superior clones, but none of them are resistant to SRD (Saefudin, 2014). The use of commercial clones grafted to *P. colubrinum* Link. as rootstocks is one way out to overcome the problem. *P. colubrinum* Link. was reported to have resistancy againsts

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SRD (Chinnapappa et al., 2019). However, reports on optimum treatments for the success of grafting between various clones of *P. nigrum* as scions and *P. colubrinum* as rootstocks were limited. The grafting study between *P. nigrum* and *P. colubrinum* has been reported by several researchers. Vanaja et al. (2007) reported that the best month for higher success of grafting of various *P. nigrum* clones / *P. colubrinum* (> 90%) were February and March, whereas grafting was conducted during the other months resulted in 62%-88% success. Sarga & Sujatha (2019) reported that among *Piper* species used as rootstocks with *P. nigrum* scions, grafting of *P. nigrum / P.nigrum* resulted in highest success (96.6%), while grafting success of *P. nigrum / P. colubrinum* was 66.3%.

To increase the grafting success and growth of the grafted plants, cuttings of *P. colubrinum* as rootstocks should have extensive adventitious roots (> 10). Auxins have been well documented to be effective to induce rooting of cuttings of many plant species, including some *Piper* species. Experiments with *P. nigrum* showed that treatment with IBA (indole-butyric acid) resulted in better rooting of cuttings (Nguyen et al., 2020; Prajapati et al., 2018). In addition to IBA, NAA (naphthaleneacetic acid) was also effective for rooting in *P. nigrum* L. (Nguyen et al., 2020). A combination of IBA and NAA was reported to be as effective as (Wulandari et al., 2018) or more effective than single IBA (Prajapati et al., 2018) for rooting in *P. nigrum*. Greater effects of IBA and NAA combination than single IBA for rooting of cuttings was also demonstrated in other species such as Malay apple (Yusnita et al., 2018). In our previous experiment with *P. colubrinum* cuttings, it was found that a combination of IBA and NAA stimulated higher number of primary adventitious roots than those treated with IBA or single NAA (Hanum et al., 2022). However, the total auxin concentrations tested were only 1500 and 2000 ppm, and the number of roots produced were still in trend of increment. In this experiment, the total auxin concentrations being tested was increased and assigned in a broader range, i.e., from 1000 ppm to 3000 ppm, to confirm response reproducibility and to get more information if higher auxin concentration produced more roots in *P. colubrinum* cuttings.

The formation of graft union is also important for successful grafting. In grafting of *P. nigrum / P. colubrinum*, Sarga & Sujatha (2019) found that the 66.3% successful grafting in the first three months decreased drastically in to 30% after one year of observation. This very low survival of *P. nigrum / P. colubrinum* grafting after one year was suggested due to the peculiar arrangement of vascular bundles in *Piper* species. It was observed that one year *P. nigrum* / *P. colubrinum* grafted plants exhibited the formation of a dense necrotic area at the graft interface, and a proper cambial connection between the grafting partners was not evident. Plant growth regulators have been reported to be involved in the formation of graft union, among them being the cytokinin. In grapevine, Köse & Güleryüz (2006) reported the profuse proliferation of calluses at the graft union in response to kinetin or benzyladenine (BA) application. In the grafting of apple, Adams (2016) also reported promotive effects of BA for the formation of graft union, as indicated by increased strength of graft union. As an attempt to improve the long-lasting survival of *P. nigrum / P. colubrinum* grafting, this research was conducted with aims of investigating (1) effects of IBA, NAA, and the mixture of IBA+NAA on rooting of cuttings of *P. colubrinum* and (2) effects of BA application to scions on the success and shoot growth in grafted *P.nigrum / P.colubrinum* plants.

## MATERIALS AND METHODS

This research was conducted at greenhouse of Faculty of Agriculture, University of Lampung since April to October 2020. Two serial experiments were done to study rooting of *Piper colubrinum* cuttings, followed by the grafting of two clones of *P. nigrum* on *P. colubrinum* rootstock.

### Experiment I: Effects of NAA and IBA on Rooting of *Piper colubrinum* Cuttings

Cuttings of *P. colubrinum* were obtained from the province of West Kalimantan, Indonesia. Two-node-cuttings of 20 cm in length and approximately 1 cm in diameter from 6-8 month-old orthotropic shoots were cleaned with running tap water and soaked in solution of 2 g/l Mankozep fungicide for 10 minutes before being treated. Before planting, the cuttings were treated with auxin.The treatments consisted of two factors, i.e., types of auxins (IBA, NAA, IBA+NAA) and auxin concentrations (0, 1000, 2000, and 3000 ppm). The IBA+NAA consisted of 50% of IBA and 50% of NAA in ppm. The application of auxins was done in the form of auxin paste consisting of auxin and talcum powder with the addition of water. The equal amount of paste was put on the basal end of each cutting. Experiment I was arranged in a completely randomized design with three replicates and 8 cuttings were used in each experimental unit. Each cutting was planted in a polybag (17x25 cm) containing soil, compost, and husk charcoal (2:1:1 v/v) as the planting media and maintained in a greenhouse. Each of the planted cuttings was covered with a transparent plastic bag for three weeks to keep the humidity above 90%. After 12 weeks, number of adventitious root emerging from the stem, or primary adventitious roots, number of secondary roots (first branches of the primary adventitious roots), root length, root fresh weight and shoot length were recorded.

**Experimen II: Effects of BA on Grafting of *Piper colobrinum* as Rootstock and Two Clones of *Piper nigrum* as Scions**

Two clones of *P. nigrum* L., Natar-1 and Petaling-2, were used as scions, and grafted to *P. colubrinum* as rootstocks. The rootstocks were grown from cuttings treated with 2000 ppm of IBA+NAA. After being grown for three months, the rootstock stems were cut-off approximately at 15 cm from the base of the stems and at 1 cm above the stem nodes. The stems were then cut longitudinally to allow insertion of scions. The scions, two-node cuttings of approximately 15 cm in length with one leaf in each node from two clones of *P. nigrum* climbing (orthotropic) shoots, were cut at their base in V-form to fit the insertion of the rootstock stems. The scions were grafted by inserting the V-formed cut of the scions into the stem of the rootstocks, and then each joint was tied with a plastic tape.

Experiment II was arranged in a completely randomized design and the treatments were arranged as combinations of two factors, i.e., clones of *P. nigrum* scions (Natar-1 and Petaling-2) and concentrations of BA (0 and 50 ppm). Each treatment consisted of three replications, and each experimental unit consisted of seven grafted plants. The application of BA was done by soaking the scions for 15 minutes in a solution of 50 ppm BA, and letting them dry out, right before being grafted to the rootstocks. In addition, the nodes of the scions were sprayed with 50 ppm of the BA solution once a week and this treatment was carried out until the fourth week after grafting. After 12 weeks, percent of grafting success, shoot length, node number, shoot number, and leaf number were recorded. In addition, visual observation was done on callus formation at graft union, or the joint of scion-rootstock representing each treatment by slicing the joint, and taking photos.

**Data Analysis**

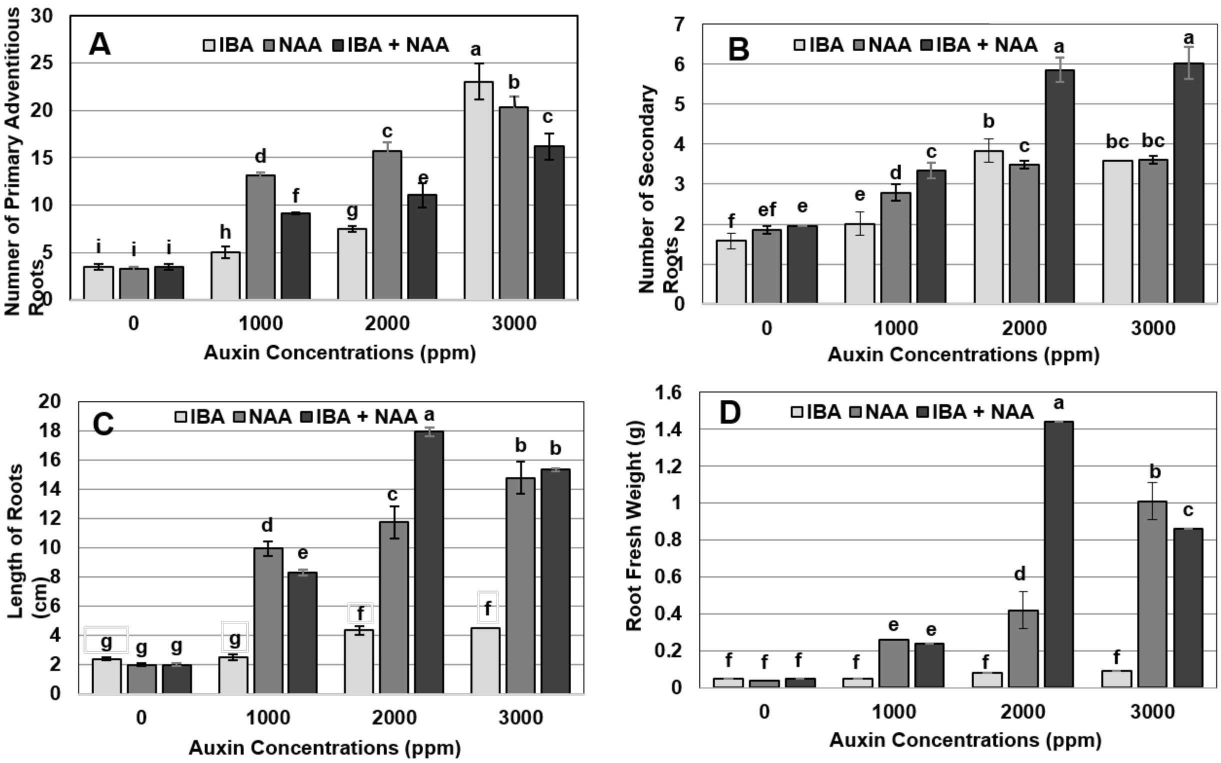
All of the data taken from the two experiments were subjected to analysis of variance, and if there was any significant difference among treatments, the mean comparison was tested using LSD test at 5%.

## RESULTS AND DISCUSSION

Results of the first experiments showed that all types of auxins (either IBA, NAA alone or IBA+NAA), and their concentrations at 2000 ppm and 3000 ppm applied to *P. colubrinum* cuttings significantly increased adventitious root formation and promoted shoot growth as shown by the rooting and shoot growth parameters observed (Fig. 1 A, B, C, D and Fig. 2). Cuttings of *P. colubrinum* without application of auxin produced the lowest values of all rooting and shoot growth parameters compared to those all types of auxins treatments. This indicated that application of auxin was needed to produce better growth of *P. colubrinum* cuttings for rootstock. The formation of adventitious roots in cuttings is an effective method for clonal propagation and has been used for large scale production of various forestry and horticultural plants (Steffens & Rasmussen, 2016). It has been well documented that auxin played as central regulator in root formation in plants (Li, 2021). Among the auxin types mostly used for rooting in cuttings were IBA and NAA, either applied alone or in combination (Hartmann et al., 2011).

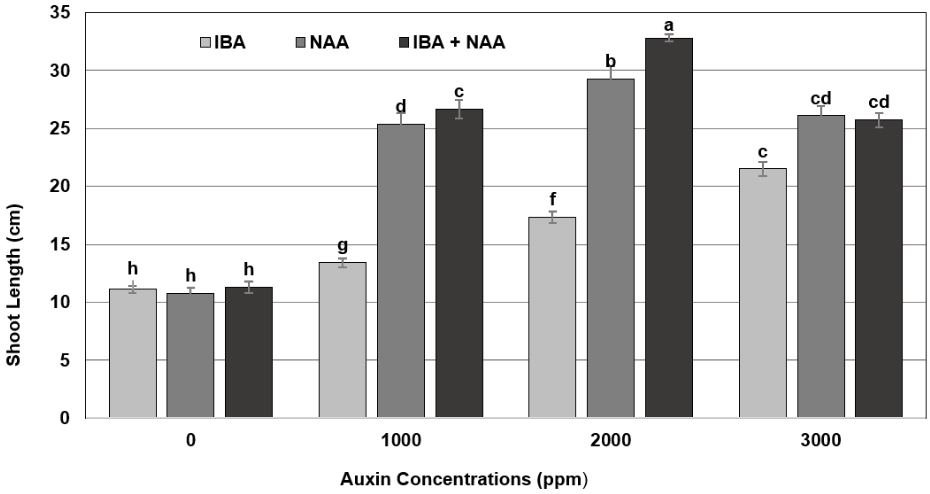
In the first experiment, it was observed that IBA at 1000 and 2000 ppm increased number of primary and secondary adventitious roots of *P. colubrinum* cuttings. There was a further increase in primary adventitious root number with the increase concentration up to 3000 ppm (Fig. 1 A, B). Similar results of the effect of IBA on root formation of cuttings in several *Piper* species were also reported, such as in *Piper nigrum* (Nguyen et al., 2020; Prajapati et al., 2018; Freire et al., 2017; Sannidhi et al., 2018; Akshay et al., 2018; Wulandari et al., 2018); *P. longum* (Ravindran et al., 2016), *P. betle* (Muttaleb et al., 2017), and *P. sarmentosum* Roxb. (Waman et al., 2019). The case also occurred with the effects of NAA applied at 1000, 2000 and 3000 ppm. Nguyen et al. (2020) also reported the effectiveness of NAA on root formation of cuttings in *P. nigrum*. However, when the effects of IBA and NAA were compared at 1000 and 2000 ppm, NAA was more effective than IBA for root formation, as indicated by more primary root numbers affected by NAA than by IBA. Application of IBA or NAA alone at 3000 ppm gave the higher numbers of primary and secondary roots compared to those treated with 2000 ppm. Effects of IBA+NAA mixture at 2000 ppm and 3000 ppm resulted in less primary root numbers than those of NAA alone (Fig. 1A). It is understandable since IBA was less effective than NAA for root formation. Interestingly, these treatments gave higher value of secondary adventitious roots (Fig. 1B).

The first experiment also showed that while the auxin treatments promoted root initiation, their effects on root growth, as indicated by number of secondary roots, root length and root fresh weight, were depended on the auxin types. IBA had no significant effects on root fresh weight (Fig. 1D), even though it caused an increase in the number of primary and secondary adventitious roots (Fig. 1A, 1B) and root length (Fig.1C). This, seems due to the thinner and shorter morphological characteristics of roots induced by IBA compared to those induced by NAA or IBA+NAA (Fig. 3). On the other hand, NAA or IBA+NAA asserted more growth-promoting effects as indicated by higher root lengths and root fresh weights (Fig. 1C,D) than those treated with IBA alone. When we compare the effect of NAA with IBA+NAA, it is interesting to note that the mixture of the two auxins at 2000 ppm resulted in higher root growth than NAA alone as indicated by higher root length and root fresh weight. This fenomenon was also reported by Prajapati et al. (2018) on cuttings of *Piper nigrum*; Yusnita et al. (2018) on cuttings of Malay apple, and in our previous study using *P. colubrinum cuttings* (Hanum et al., 2022).



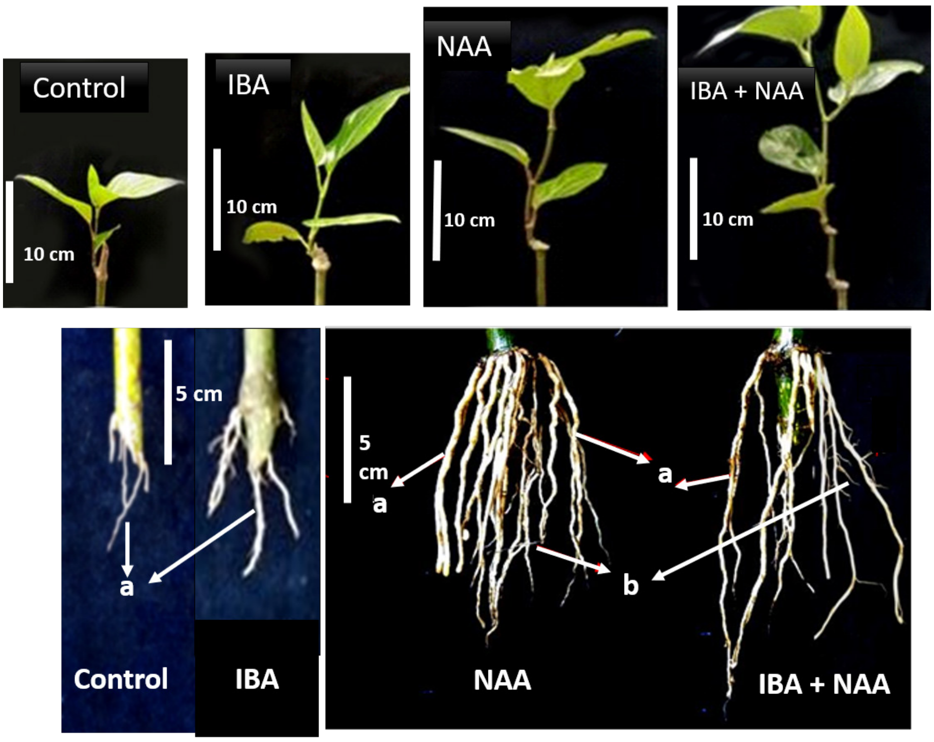

Remarks: Bars represent SD. Mean values followed by the same letter shows no significant difference based on LSD at 5% significance level

**Fig. 1.** Effects of different types and concentrations of auxins on: number of primary adventitious roots (A), number of secondary roots (B), root length (C), and root fresh weight (D) of *Piper colubrinum* cuttings at 12 weeks after planting




Remarks: Bars represent SD. Mean values followed by the same letter shows no significant difference based on LSD at 5% significance level

**Fig. 2.** Effects of different types and concentrations of auxins on shoot length of of *Piper colubrinum* cuttings at 12 weeks after planting




Remarks: a. Primary adventitious roots, representing adventitious roots directly emerged from the stem cuttings; b. Secondary adventitious roots are branched of the primary adventitious roots

**Fig. 3.** Shoot and root performances of *Piper colubrinum* cuttings at 12 weeks after planting without auxin treatment (control) and with different types of auxins at 2000 ppm

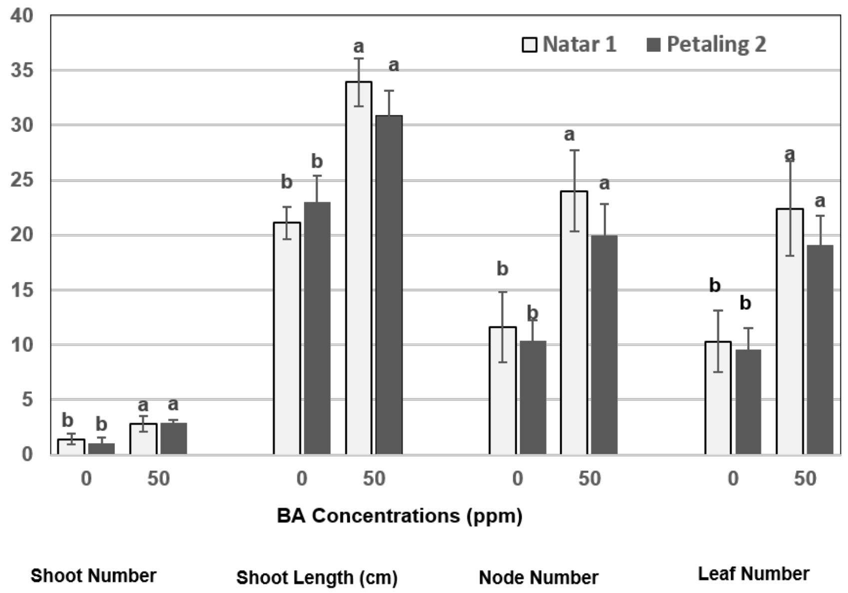
In the shoot growth parameters (Fig.2), results of the experiment showed that in general the higher concentrations of auxins were employed the longer shoots were obtained, except for NAA and IBA+NAA at higher concentrations than 2000 ppm. The results also showed that the longest shoot was obtained at IBA + NAA 2000 ppm (Fig. 2) and this result corresponded with the highest secondary root number (Fig.1B), the longest root (Fig.1C), and the highest root fresh weight (Fig.1D). This suggested that a good root system would lead to a good shoot system (Fig.3). Thus, it was concluded from this experiment that IBA + NAA at 2000 ppm was the best treatment for rooting as well as shoot growth on *P. colubrinum* cuttings. Accordingly, it is expected that cuttings of *P. colubrinum* treated with IBA + NAA at 2000 ppm would result in more successful grafting with *Piper nigrum* as scions.

Results of the experiment showed that the control produced significantly lowest roots (Fig. 1, Fig. 3) as well as shoot growth (Fig. 2, Fig. 3), compared to all types of auxins applied on the basal of *P. colubrinum* cuttings, at concentrations of 2000 ppm or 3000 ppm. These results indicated that the *P. colubrinum* cuttings might lack of endogenous auxin needed for root formation during the auxin-active stage. As discussed by Hartmann et al. (2011), adventitious root formation in cuttings consisted of two stages, namely root initiation stage and root elongation stage. During root initiation stage, the cells in the basal of cuttings underwent de-differentiation, formation of root initials and development of root primordia. In the root elongation stage, the root primordia elongated, root tips grew outward through cortex and finally emerged from the epidermis of the stem. The root initiation stage consisted of the auxin-active and the auxin in-active stage. The auxin-active stage, lasted about several days, during which continuous existence of auxin was required for the formation of root initials. This auxin-active stage was followed by auxin-in active stage, during which the root formation was not significantly affected by the absence of auxin. It appeared that exogenous application of auxins at the basal parts of cuttings has ensured the availability of auxin required for the formation of root initials in the auxin-active stage.

It was also showed from the first experiment, in *P. colubrinum* cuttings, NAA was more effective than IBA alone, and the mixture of IBA+NAA at 2000 ppm gave the best root formation in accordance with the best shoot growth. It was well documented, that beside exogenous application of auxin, adventitious root formation and subsequent shoot growth were affected by several factors, including some internal factors such as genotypes, age of cuttings, types of stem cuttings, carbohydrate content, endogenous hormone content, existence of root inhibitors and some external factors such as substrates and suitable micro-environment of cuttings (Hartmann et al., 2011). The better results of adventitious root formation in *P. colubrinum* as affected by NAA or IBA+NAA compared to those treated with IBA alone, was most likely to be genetic dependent factor. Among *Piper* species, *P. colubrinum* cuttings was shown to be the easiest to root (Raja et al., 2018). Consistence with results of this study, it was also reported that NAA+IBA mixture resulted in better rooting of *P. guineense* cuttings, compared to the application of NAA or IBA alone (Alaje et al., 2022). This research finding is important because the mixture of IBA+NAA treatment not only produced the highest secondary adventitious roots, root length, and root fresh weight, but also resulted in better shoot growth as indicated by the increased of shoot length, suggesting the existence of supporting effects of roots on shoot growth.

The second experiment used rooted *P. colubrinum* cuttings treated with 2000 ppm of IBA+NAA as rootstocks, with *P. nigrum* of clone Natar-1 and Petaling-2 as scions. Results of the second experiment showed that the application of 50 ppm BA on scions led to higher success of grafting. It occurred for both grafting of *P. nigrum* clone Natar-1 and Petaling-2 scions on *P. colubrinum* rootstock. Grafting of *P. nigrum* scions from clone Natar-1 resulted in 93% of success with BA application, compared to 80% of success without BA application, while grafting of *P. nigrum* scions from clone Petaling-2 led to 100% of success with BA application, compared to 73% of success without BA application. Analysis of variance of the data as well as the LSD test on shoot number, shoot length, node number, and leaf number showed that the application of BA had significant effects, yet not significant on the clone treatment. There was no interaction effect of the two factors on those parameters. Fig. 4 showed that the application of 50 ppm BA significantly resulted in more shoots, longer shoots, more nodes, and more leaves both in clone Natar-1 and Petaling-2. However, there was no significant difference between *P. nigrum* clone Natar-1 and Petaling-2 on all shoot parameters observed. Fig. 5 showed the performance of shoots of the two scions of *P. nigrum* clones as affected by the application of BA. It was shown that BA treated scions grew significantly better than those of the untreated ones.

It also can be seen in Fig. 5, that the application of BA also led to better graft union as indicated by more calluses formed in the BA treated scions compared to non-treated control, which was evident both in the intact joint (Fig. 5a) and in the longitudinally sliced joint (Fig. 5b). This callus-promoting effects of BA on graft union was also reported by other researchers, for examples in grafting of apples (Adams, 2016) and grafting of grapevine (Köse & Güleryüz, 2006).




Remarks: Bar represent SD. Mean values for each parameter followed by the same letter shows no significant difference based on LSD at 5% significance level

**Fig. 4.** Effects of 50 ppm BA on shoot number, shoot length, node number, and leaf number of *Piper nigrum* scions clone Natar-1 and Petaling-2 grafted on *Piper colubrinum* as rootstocks at 12 weeks after grafting

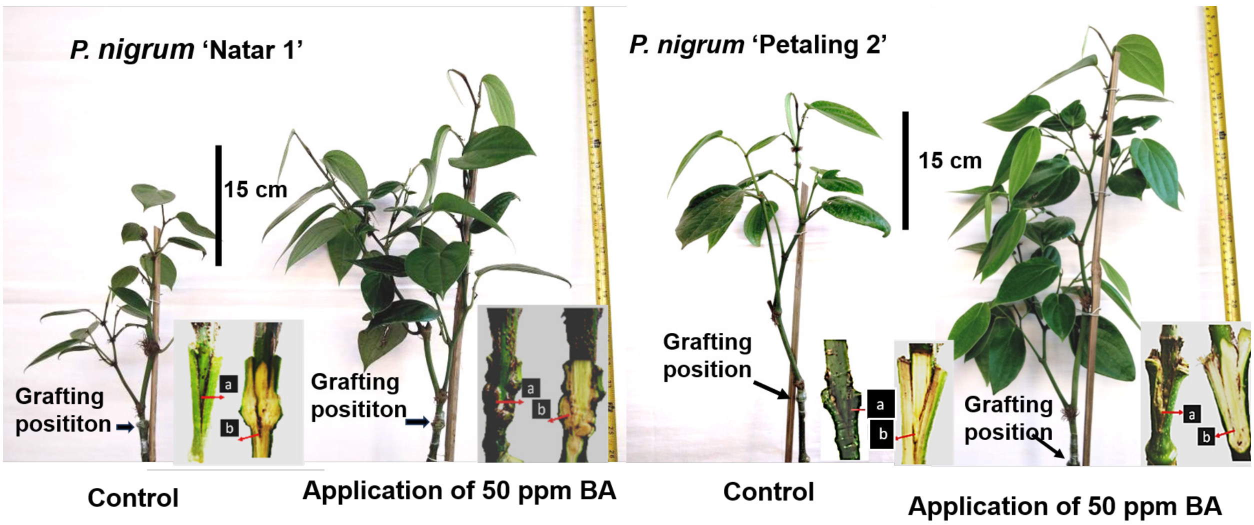



**Fig. 5.** Effects of application of 50 ppm BA on the growth of scion shoots in *Piper nigrum* clones Natar-1 and Petaling-2 with *Piper colubrinum* as rootstocks at 12 weeks after grafting. Bar = 15 cm. The closed-up figures showed the graft-unions: a. intact joints, and b. longitudinally sliced joints, showing more callus formation in the BA-treated graft unions compared to the non-treated control

The formation of callus in the graft union played a crucial role to bridge the rootstock and scion cambial tissues. Hartmann et al. (2011) explained that the formation of graft union between rootstock and scion occurs in a series of complex processes, starting with a tight-physical contact between the scion and the rootstock cambial tissues. There is a wound healing response on the two cut surfaces of the scion and the rootstock. The outermost cell layer at the cut surfaces undergoes necrosis, and as a response to wound healing process, callus tissue (a collection of disorganized parenchyma cells that divide rapidly) forms and grows behind the necrotic cell layers. This callus bridges the scion and rootstock cambial tissues. After that, the wound healing response is followed by dissolving of the necrotic layer, then new cambium differentiation occurs in the callus. Finally, these series of graft union formation are completed by the formation of xylem vessels and new phloem, which will connect the scion to the rootstock. So, the formation of more calluses appeared to be very important to connect the joint between scion-rootstock cambial tissues. Results of the second experiment showed that there was more callus formation in the graft joint of the BA-treated scions compared to the untreated control, which was evidently occurred in the two *P. nigrum* clones tested (Fig. 5, the closed-up photos). Apparently, the application of BA on scions induced better bud-breaks and subsequently faster shoot growth. Accordingly, the better and the faster shoot growth might produce more endogenous auxin in the meristem portions of the shoots, and following basipetal transport of more endogenous auxin in scion shoots, more calluses are formed which contribute to strengthening the graft union.

It is interesting to note, that although there was no significant difference on shoot growth between the two clones of *P. nigrum* scions grafted to *P. colubrinum*, the data showed that BA treatment could enhance the grafting success between *P. colubrinum* and *P. nigrum* more effectively in Petaling-2 clone with the increase of nearly 30%, compared to Natar-1 clone, with the increase of 10% grafting success. Since the grafting was conducted in the same season and environment, this difference of grafting success might have been caused by genotype effect. Various factors have been reported to influence grafting success, such as incompatibility, plant species or genotypes, environmental condition during and following grafting, growth activity of rootstock, the craftmanship of grafting and application of growth regulators (Hartmann et al., 2011). Vanaja et al. (2007) reported that beside the months of grafting, clones of *P. nigrum* grafted on *P. colubrinum* resulted in different grafting success, i.e., ‘Panniyur 1’, ‘Panniyur 2’, ‘Panniyur 3’, Panniyur 4’ and ‘Panniyur 5’ produced average grafting success of 79%, 85%, 85%, 85% and 94% respectively, while the others, ‘Panniyur 6’, Panniyur 7’ and ‘Karimunda’ produced 65%, 74% and 6% grafting success, respectively.

This current research demonstrated the high success of grafting of two clones of *P. nigrum* (Natar-1 and Petaling-2) scions to *P. colubrinum* rootstocks, through the application of 50 ppm BA on the scions (93-100%). The application of auxins (IBA+NAA) at 2000 ppm on cuttings of *P. colubrinum* produced better rooting for rootstocks, and with the application of BA on *P. nigrum* scions, the two species of *Piper* could form better graft unions as indicated by more calluses formed in the graft joints, which evidently resulted in better shoot growth than those without the application of BA. To the best of our knowledge, this is the first report on the use of BA to improve the success of grafting between *P. nigrum* scions on *P. colubrinum* rootstocks.

## CONCLUSION

The best treatment for rooting of cuttings in *P.colubrinum* was the application of 2000 ppm of IBA and NAA (1:1) and the best treatment for grafting between *P. nigrum* clone Natar-1 and Petaling-2 as scions and *P. colubrinum* as rootstocks was the application of 50 ppm of BA on the scions. The success rate of the grafting was 93-100%. It is necessary to see the performance of the grafted plants in the field, i.e., their growth, yield, and their resistance to stem rot disease.

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