

Effect of Paclobutrazol and Auxin on Growth Plantlet of Garlic Varieties in *in Vitro* Culture

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Abstract: Area of cultivating garlic in Indonesia experienced a sharp decline. In a period of 20 years (1991-2011), the decline of garlic planting area reaches 91%. The fact is causing erosion of genetic resources of several varieties of garlic. Propagated vegetatively garlic through *in vitro* culture aims to obtain quality bulblet. This study aims to get the combination treatment between paclobutrazol, indole acetic acid (IAA), indole butyric acid (IBA) and naphthalene acetic acid (NAA) in some varieties of garlic in Indonesia. This study used a completely randomized design (CRD) with two treatment factors, i.e., on varieties (Bali, Gunung Kidul, Slawi, Tawangmangu and Mataram) and 1.5 ppm paclobutrazol combination with a wide auxin (IAA 0.5 ppm, 0.5 ppm IBA, NAA 0.5 ppm). Variable observations include the current appear shoots and roots, number of shoots and roots, shoots and root length. Observations showed that the combination of paclobutrazol + NAA is able to produce plantlets that have the best vigor than paclobutrazol + IAA and paclobutrazol + IBA combination treatment.

Key words: Garlic, *in vitro*, paclobutrazol, auxin.

1. Introduction

Garlic (*Allium sativum* L.) is an important commodity in Indonesia. However, 95% garlic needs in the domestic market are filled by import. In 2012, domestic garlic production was only 17.638 tons, while the volume of imports reached 386.592 tons [1, 2]. The amount of volume of imported garlic resulted in a decrease of farmers interest on cultivate garlic. Within a period of 20 years (1991-2011), the decline garlic planting area reached 91% of the 21.130 ha planted area in 1991 and then became 1.828 ha in 2011 [3, 4].

Decreased interest of farmers in cultivating garlic will affect the erosion of garlic germplasm resources in Indonesia. Field observations also obtained information that the sources of genetic garlic in

Indonesia have been classified as endangered. For example, varieties Gunung Kidul and Mataram are now very difficult to obtain.

Efforts to cultivate the interest of farmers to cultivate garlic among others, are the provision of quality bulblet. Bulblet quality will affect the high productivity so farmers will get adequate benefit.

An effort in the provision of quality seed is through *in vitro* culture. *In vitro* propagation of garlic is very important because garlic is an apomiksis plant. Besides garlic cultivated generally form the sterile seeds so that difficult propagated through seeds [5, 6]. Garlic cultivation in Indonesia has been infected by a virus which resulted in low yield. The virus is transmitted generation to generation through the vegetative organs. *In vitro* culture garlic is able to produce bulblet that is free of the virus [7, 8].

A very important stage in the *in vitro* culture is

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acclimatization [9]. Plantlets were able to adjust the microenvironment during acclimatization needed plantlets healthy condition, sturdy and strong. The addition of paclobutrazol and auxin is expected to grow plantlets according the criteria.

2. Materials and Methods

Materials used in this study were bullets from different regions in Indonesia, namely: Gunung Kidul, Yogyakarta; Tawangmangu, Central Java; Slawi, Central Java; Singaraja, Bali and Bima, Mataram. Explants were taken from garlic bulbs discs (0.5 cm to 1 cm). Sterilization of explants conducted using 70% alcohol for 30 s so that the explants free from contamination.

The medium used was Murashige and Skoog 1962 (MS) in the form of solid medium with the addition of plant growth regulators (PGR). The addition of combination of PGR was paclobutrazol 1.5 ppm + indole acetic acid (IAA) 0.5 ppm; paclobutrazol 1.5 ppm + indole butyric acid (IBA) 0.5 ppm with paclobutrazol 1.5 ppm + naphthalene acetic acid (NAA) 0.5 ppm.

This research was conducted in Laboratory of Tissue Culture, Faculty of Agriculture, Sebelas Maret University, Central Java Province, Indonesia. This research began from February until May 2014.

Research was done with completely randomized design (CRD) with two treatments. There were varieties of local garlic (Bali, Gunung Kidul, Slawi, Tawangmangu, Mataram) and combination paclobutrazol 1.5 ppm with kind of auxin 0.5 ppm (IAA, IBA, NAA). Analysis of the data used analysis of variance (ANOVA). Test differences used Duncan's multiple range test (DMRT). The observations of variables were including days taken to shooting, days taken to rooting, root length, number of roots, number of shoots and shoot length. Root length, number of roots, number of shoots and shoot length were observed in the acclimatization (the age of two weeks).

3. Results and Discussion

3.1 Days Taken to Shooting and Rooting

Gunung Kidul variety has ability of shoots induction faster than the other varieties, while the faster rooting induction was shown by varieties of Tawangmangu and Mataram. Induction of sprouting and rooting was strongly influenced by genetic factors. Gunung Kidul variety was variety of garlic lowlands, while the other varieties were variety of garlic plateau. Different growth would affect embryo growth which would further affect the induction of sprouting and rooting.

Paclobutrazol 1.5 ppm + IAA 0.5 ppm was able to induce the emergence of shoots and roots more quickly, followed by paclobutrazol 1.5 ppm + IBA 0.5 ppm and paclobutrazol 1.5 ppm + NAA 0.5 ppm. Paclobutrazol was one of the compounds that have function in inhibiting growth. This growth inhibition occurs because paclobutrazol inhibits gibberellin biosynthesis resulting in inhibition of cell elongation [10]. Paclobutrazol reduced the number of stomata holes, improved epicuticular and shortened the stems and thicken roots [11]. Paclobutrazol is effective in inhibiting the growth of shoots [12]. Growth inhibition by paclobutrazol would increase the vigor and resilience of plantlets [13]. Vigorous plantlets are plantlets with roots and shoots growing strong and healthy Paclobutrazol was expected to play a role in the inhibition of shoot elongation, so that shoots can grow strong and healthy. Plantlets with high vigor would influence the success rate of acclimatization.

The roles of IAA, NAA and IBA on sprouting and rooting were already proven in many studies where IAA, NAA or IBA were able to stimulate germination and rooting [14-16]. IBA is one of the effective auxins which stimulate root formation on cuttings of sweet orange [15]. IAA is significantly able to induce rooting in cuttings of *Accasia nicotiana* [14]. IAA on plant *Azadirachta indica* is able to stimulate rooting [16]. NAA played a role in increasing the number of

roots but did not play a role in increasing the length of the root [17].

3.2 Number of Shoots and Roots

Mataram variety has different number of roots and less than varieties Bali, Gunung Kidul and Tawangmangu. But for variable number of shoots, Bali variety has the fewest number of shoots as compared to other varieties (Table 1). Paclobutrazol + NAA combination was able to induce the most number of roots compared to the combination of Paclobutrazol + IAA or Paclobutrazol + IBA combination (Fig. 1). NAA more effectively increase the number of roots [17, 18]. NAA also give real effect on grape cuttings to increase rooting [19]. NAA was also able to increase the number of regenerated

bulb on garlic [7]. Paclobutrazol plays a role in the inhibition of germination while NAA stimulates root growth, so the combination of paclobutrazol and NAA is able to provide growth of plantlets with good vigor (Fig. 1). Plantlets with good vigor has a high growth potential during acclimatization.

3.3 The Length of Shoots and Roots

Root length in Mataram varieties showed the lowest value followed by Bali variety, while varieties Tawangmangu, Slawi and Gunung Kidul have an average higher root length than the varieties Bali and Mataram. But all three varieties were not significantly different from each other (Table 1). The combination of paclobutrazol + NAA showed higher root length than the combination of paclobutrazol + IAA as well

Table 1 Effect of different auxin and varieties for shoot-root growth in garlic.

Treatment	Variable					
	Days taken to shooting	Days taken to rooing	Number of root	Number of shoot	Root length (cm)	Shoot length (cm)
Varieties						
Bali	4.26 ^d	8.2 ^d	4.93 ^b	1.2 ^a	1.13 ^b	11.2 ^b
Gunung Kidul	2.13 ^a	7.53 ^{bc}	5.06 ^b	1.67 ^b	2.14 ^c	11.41 ^b
Slawi	3.4 ^b	8.06 ^{cd}	6.53 ^b	1.8 ^b	1.9 ^c	15.01 ^c
Tawangmangu	4 ^c	6.86 ^a	5.2 ^b	1.73 ^b	1.8 ^c	11.93 ^b
Mataram	3.46 ^b	7.4 ^{ab}	2.6 ^a	1.53 ^b	0.53 ^a	4.73 ^a
Plant growth regulator						
Paclobutrasol + IAA	2.2 ^a	6.1 ^a	4.28 ^a	1.64 ^a	1.21 ^a	10.77 ^a
Paclobutrasol + IBA	3.4 ^b	7.08 ^b	4.6 ^{ab}	1.56 ^a	1.54 ^{ab}	10.24 ^a
Paclobutrasol + NAA	4.76 ^c	9.64 ^c	5.72 ^c	1.56 ^a	1.74 ^c	11.56 ^a

Mean of different samples labeled with different letters are significantly different in each row (0.05 level by Duncan's test).

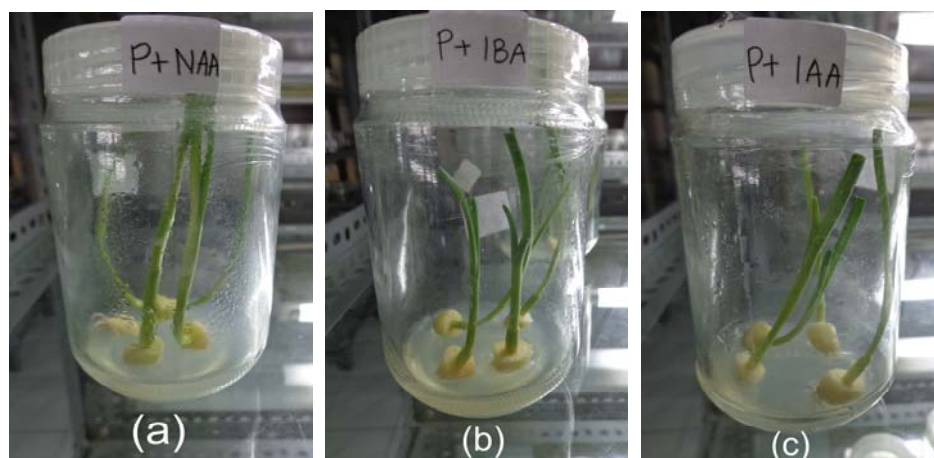


Fig. 1 Fourteen days shoots length and number of shoots in MS medium supplemented with (a) paclobutrazol 1.5 ppm + NAA 0.5 ppm, (b) paclobutrazol 1.5 ppm + IBA 0.5 ppm and (c) paclobutrazol 1.5 ppm + IAA 0.5 ppm.



Fig. 2 Fourteen days root length and number of root in MS medium supplemented with (a) paclobutrazol 1.5 ppm + NAA 0.5 ppm, (b) paclobutrazol 1.5 ppm + IBA 0.5 ppm and (c) paclobutrazol 1.5 ppm + IAA 0.5 ppm.

as combination of paclobutrazol + IBA. In oil palm *in vitro* research, paclobutrazol + NAA combination is capable of forming a better root number [20]. Plantlets on MS medium supplemented paclobutrazol + NAA combination have best level of vigor, this is shown by the number and length of roots (Fig. 1). Acclimatization stage is an important stage in the *in vitro* culture. Acclimatization is done to reduce stress, because the difference between *in vitro* environmental conditions and field environmental condition. Therefore, in order to succeed, the plantlets acclimatization should have a high vigorily characterized by solid growth, many root, and healthy. Technology of acclimatization process is a step considered the most expensive during the process of *in vitro* culture [21].

4. Conclusions

The growth of plantlets from different garlic varieties showed significant differences. Paclobutrazol + NAA produce plantlets with better vigor than paclobutrazol + IAA or paclobutrazol + IBA treatment on all varieties.

In days taken to shooting and rooting, Gunung Kidul variety has ability of shoots induction faster than the other varieties, while the faster rooting induction was shown by varieties Tawangmangu and Mataram. In number of shoots and roots, Mataram variety has different number of roots and less than varieties Bali, Gunung Kidul and Tawangmangu. But for variable number of shoots, Bali variety has the fewest number of shoots as compared to other varieties. In length of shoots and roots Mataram

varieties showed the lowest value followed by Bali variety, while varieties Tawangmangu, Slawi and Gunung Kidul have an average higher root length than the varieties Bali and Mataram.

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