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The Effectiveness of Bio-catharanthine on Peanut (*Arachis hypogea* L.) Lurik Cultivar

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ABSTRACT
The productivity of peanut (*Arachis hypogea* L.) in Indonesia from 2017 until 2021 is estimated to have average production growth minus 11,82% per year and cannot meet the domestic need of the peanut. According to that statistic, Indonesia is the world's second largest importer of peanuts. Looking for undeveloped peanut varieties or cultivars is a key to a major solution for increasing productivity. Lurik is a peanut cultivar with 3-4 seeds in each pod that has been reported to be productive. To maximize its productivity there is a way called polyploidization to double the plant genome. Madagascar periwinkle (*Catharanthus roseus* L.) contains alkaloids of vincristine and vinblastine which have ability to bind to the microtubular proteins of the mitotic spindle and prevent the cell from making the spindles so it will be no cell division. Bio-catharanthine, such as *C. roseus* extract, can be a less expensive and promising polyploidy agent. Peanut (*A. hypogea* L.) Lurik cultivar seeds were soaked in bio-catharanthine liquid at seven different concentration levels for 24 hours. This study found that this treatment has a normal data distribution, but no significant effect on harvest traits such as main stem height (MSH), pod wet weight (PW), and number of pods (NP), with the best concentration of bio-catharanthine being 5% for MSH and PW traits, and 4% for NP. This study requires molecular research to provide possibilityof bio-catharanthine to increase productivity and to meet the qualification of peanut (A. *hypogea* L.) Lurik cultivar as a promising quality seed for the best yield of peanut crop.

Keywords: *Bio-catharanthine, Lurik cultivar, Peanut, Ploidy, Productivity*.

1. INTRODUCTION

The productivity of peanut (*Arachis hypogea* L.) in Indonesia is estimated to be minus 11,82 percent per year from 2017 to 2021, and it cannot meet the domestic demand for peanut [1]. Despite the fact that peanuts are one of the most important foods in some locations, there is not enough acreage dedicated to peanut production. Peanut is a rotation crop, not the primary crop. The harvested area is insufficient to meet national demands for peanuts [2]. These facts placed Indonesia to be the second country as the peanut importer in the world [3]. To increase productivity, exploring a peanut variety or cultivar that has not been fully developed can be a solution. Lurik is one of the peanut cultivars that has 3-4 seeds in each pod and has been reported to have high productivity, with the average weight of Lurik seed being nearly two and a half times that of common peanut [4].

Polyploidization is a process that can double the plant genome and can be used to boost plant productivity [5]. This treatment is not a new thing in agriculture, and rapid progress has been made in crops, such as wheat, cotton, oat, banana, and potato, meanwhile, the yields of them were doubled when the genomes were duplicated [6,7]. Polyploidization treatment also increased plant height, thickness of fruit flesh, and the number of fruits per plant of Katokkon pepper [8].

Bio-catharantine such as Madagascar periwinkle (*Catharantus roseus* L.) extract can be used as an alternative and less expensive polyploidy agent. *C. roseus* produces more than 130 terpenoid indole alkaloids (TIAs) making this plant a chemical factory. There are vinca-alkaloids, one of TIA in C. *roseus*, that can bind to free tubulin, thereby preventing microtubule assembly and concomitant mitotic spindle formation, they cannot associate with polymerized tubulin present in microtubules [9] and stand a chance to double the chromosome making a promising polyploidy agent. C. *roseus* extract can induce polyploidization in peanut var. Talam [10], *Zephyranthes rosea* Lindl. [11], *Eucalyptus pellita* F. Muell [12], and soybean [13].

In this context, the purpose of the present study aimed (1) to know the effect of bio-catharanthine induction on peanut Lurik cultivar and (2) to determine the precise concentration of bio-catharanthine required for the best peanut result of the peanut Lurik cultivar.

2. METHODS

2.1. Plant Materials

This study was conducted using a collection of peanuts (A. *hypogea* L.) of Litbang Garuda 5 variety (as control negative) and Lurik cultivar that provided by Kacang Lurik Team, the Laboratory of Genetics and Breeding, Faculty of Biology, UGM. The bio-catharanthine for this study was obtained from the Laboratory of Genetics and Breeding, Faculty of Biology, UGM as *C. roseus* extract product in powder form. The solvent used to dissolve bio-catharanthine powder was aquadest. The selected seeds were induced with seven (7) concentration levels of bio-catharanthine in seven (7) treatments namely 0% bio-catharanthine in Litbang Garuda 5 variety for control negative (A); 0% bio-catharanthine in Lurik cultivar as control positive (B); 1% bio-catharanthine in Lurik cultivar (C); 2% bio-catharanthine in Lurik cultivar (D); 3% bio-catharanthine in Lurik cultivar (E); 4% bio-catharanthine in Lurik cultivar (F); and 5% bio-catharanthine in Lurik cultivar (G). All treatments were soaked for 24 hours.

2.2. Experiment Site

This research was conducted from April 15th until August 6th, 2021. The induction was carried out at the Laboratory of Genetics and Breeding, Faculty of Biology, UGM. The seed was planted in The Greenhouse at Mutihan Research Station, Madurejo Village, Prambanan District, Sleman Regency, Special Region of Yogyakarta. The location is 119 meters above sea level and the pH of the soil that had been recorded during cultivation was 7,2.

2.3. Experimental Design

The experimental design was a completely randomized design (RAL) with seven (7) treatments and four (4) replications for each, separated by 30 cm and planted in 5 cm depth of soil. Fertilization using NPK was done 24 days after planting (DAP). The weeding was carried out twice during cultivation until harvest time.

2.4. Quantitative Traits Measured

The quantitative traits for this study are main stem height (MSH), pod wet weight (PW), and the number of pods (NP). All traits were measured at harvest time as average result number on four plants for each treatment on the 100th DAP for Litbang Garuda 5 variety and on the 114th DAP for Lurik cultivar. The main stem height was measured from the base of the main stem until its ends using a tape measure in centimeters. Pod wet weigh was weighed after separating all pods from their pegs. The number of pods was counted manually. For pod wet weight and the number of pods traits, only pods that have harvestable seeds were weighed and counted.

2.5. Data Analysis

The data were analysed using statistical analysis of IBM SPSS Statistics 25 to show its normality and one-way ANOVA then DMRT for further tests to show the signification between treatments.

3. RESULTS AND DISCUSSION

The first flowering of the plant occurred on 23rd DAP and then, on 34th DAP, the gynophores already had their first pegging. The data distribution of the average of MSH, PW, and NP traits that had been tested using statistical analysis Shapiro-Wilk test were normal with every significant value of the treatments greater than 0,05. Further testing using ANOVA showed that every significant value of the treatments had greater than 0,05 which revealed not significant result in all traits.

Descriptively, the highest MSH trait is treatment G which was induced by 5% bio-catharanthine with 115 cm and the shortest is treatment D which was induced by 2% bio-catharanthine, with 85,5 cm. Moreover, for this trait, treatment A also has MSH over 100 cm, like treatment G, with 103,25 cm, while the result for other treatments is below 100 cm, like treatment D, namely treatment B with 90,75 cm, treatment C with 96 cm, treatment E with 87 cm, and treatment F with 90,5 cm. The results in this trait are unique because the difference inducing concentration of bio-catharanthine on peanut seed turns out can initiate higher main stem height and shorter main stem height than the height of the control plants, both in control negative and positive plants.

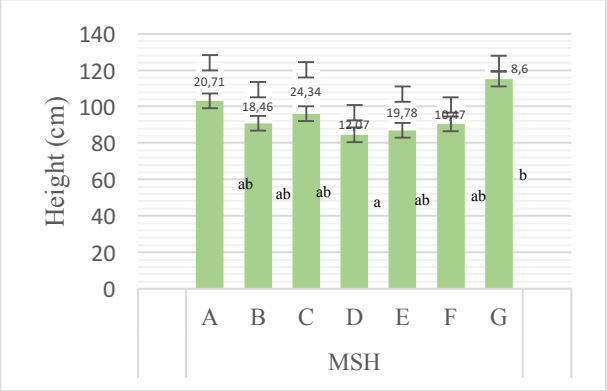

Figure 1 The result of the average main stem height (MSH).

For PW trait, treatment G has the best average weight of four plant replicants with 68,25 grams, following by treatment F with 64,75 grams, treatment B with 58 grams, treatment E with 57,5 grams, treatment C with 54,25 grams, treatment D with 48 grams and treatment A that using Litbang Garuda 5 variety in 0% bio-catharanthine, as the lightest weight with 38,25 grams. The range number between the highest result and the lowest result in this trait is 30 grams, almost 50% difference.

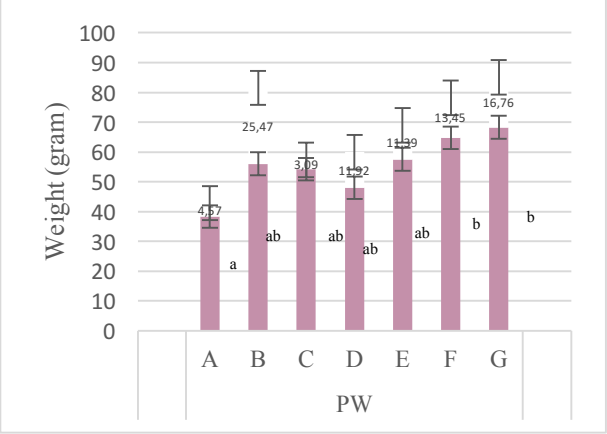

Figure 2 The result of the average pod wet weight (PW).

Unlike MSH and PW that showed treatment G as the best result, the result of NP trait showed a different result. Treatment F with the seed induced by 4% bio-catharanthine has the greatest average number of pods with 24,25 pods. The second highest result of NP trait is treatment G with 22,75 pods, a difference of 1,5 pods with treatment F. On the other hand, other treatments have the result of NP trait below 20, namely treatment E with 19 pods, treatment A with 18,75 pods, treatment B with 16,75 pods, treatment C with 17,5 pods. Treatment D has the least result of NP trait with 15,25 pods in average number.

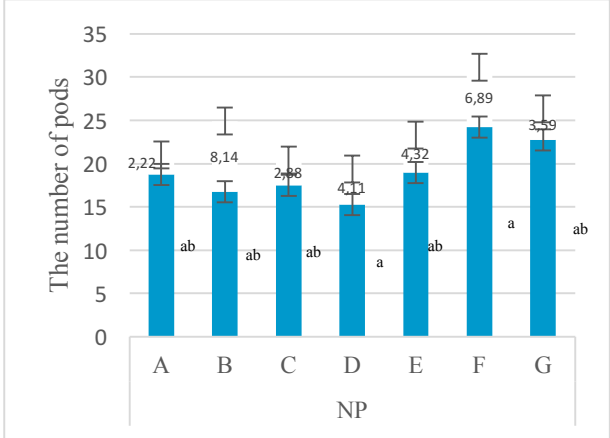

Figure 3 The result of the average number of pods (NP).

Even though in this study the result did not give a significant result in phenotype traits, it still has possibility that bio-catharantine could have an effect at the molecular level because Bio-catharanthine such as *C. roseus* extract contains alkaloids of vincristine and vinblastine which have ability to bind to the microtubular proteins of the mitotic spindle and prevent the cell from making the spindles so it will be no cell division during the anaphase of mitosis [14, 15]. If the cell cannot divide, the cell genome will be doubled. In *Arabidopsis*, compared to diploid plant, the plants with higher ploidy levels clearly differed from diploids based on other phenotypic characteristics with significant increase in stem height and dry weight [16].

Moreover, despite of the homogeneous result of MSH, PW, and NP as phenotype traits, the vincristine and vinblastine in bio-catharanthine may bring deleterious effect in the cell of te peanut plan. This can be caused by genomic stress experience due to incompatibilities and genetic redundancy of the cell [17]. On the other hand, the molecular study approach is necessary to get the specific result.

In summary, the result of this study showed that seed induction using bio-catharanthine has normal distribution data but not significant effect in phenotype traits such as main stem height (MSH), pod wet weight (PW), and the number of pods (NP) in peanut (*A. hypogea* L.) Lurik cultivar with G as the highest result of main stem height and pod wet weight in 115 cm and 68,25 grams, and F as the best result of the number of pods in 24,25 pods. Based on the result, the best bio-catharanthine concentration of MSH and PW traits is 5%, and for NP trait is 4%. In spite of the better impact of bio-catharanthine on Lurik cultivar that had been harvested, it is indispensable to understand that the differences of sea level of cultivation location, type of the soil, type of fertilizer used, and pH of the soil may affect the result of the peanut crop. However, research in molecular level should be conducted to confirm the

possibility of bio-catharanthine as a polyploidy agent for peanut (*A. hypogea* L.) Lurik cultivar.

AUTHORS' CONTRIBUTIONS

The authors confirm contribution to the paper as follow: laboratory and data collection: D. I. Rohmah, M. Mulyani, L. N. Janah; SPSS: M. Mulyani, D. I. Rohmah; analysis and interpretation of results: D. I. Rohmah; manuscript: D. I. Rohmah, B. S. Daryono, A. Pancoro, Miftahudin, A. T. Wibowo; manuscript review: B. S. Daryono, A. Pancoro, Miftahudin, A. T. Wibowo. All authors approved the final version of the manuscript.

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