The agronomic performance and artemisinin content of colchicine-induced polyploid genotypes *Artemisia cina*

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**Abstract.** Artemisinin is a secondary metabolite contained in the potential genus Artemisia as an antimalarial. *Artemisia cina* Berg ex Polyakov is a species from Artemisia that grows as a weed in Indonesia and contains artemisinin. The problem is that the artemisinin content in *A. cina* is minimal. The efforts must be made to increase the content. Clone improvement through breeding through genetic manipulation and enhancement of technical culture are the strategies that can use to improve the artemisinin content of *A. cina*. The purpose of this study was to determine the agronomic performance of nine *A. cina* polyploid genotypes (genotypes A, B, C, D, E, F, G, H, I) obtained from polyploidy induction using colchicines. The results of this study showed that leaf area and chlorophyll content were not significantly different among genotypes. In contrast, shoot dry weight, and plant height showed that I genotype was significantly higher than other genotypes. Glandular trichomes density of genotype was more elevated than different genotypes. Genotypes F, H, I had high artemisinin content compared to other genotypes. The genotype I showed higher artemisinin weight than other genotypes.

Key Word: Genotype, colchicines, agronomic character, artemisinin.

1. **Introduction**

Artemisia is a plant that can use as medicine. Treatment exploration on Artemisia has primarily used artemisinin as a source of potential antimalarial. There was much research regarding this topic. However, one species of Artemisia, which grows a weed on highlands of Indonesia and contains artemisinin, is *Artemisia cina* Berg ex Polyakov. Artemisinin is a product from the secondary metabolism of *A. cina*. The artemisinin content on *A. cina* is minimal, ranging from 0.0075% to 0.066% [1–5]. The effort to increase artemisinin content on *A. cina* has been made through tissue culture or agronomic practices. However, that technique has not improved the artemisinin content of *A. cina* plants, because *A. cina* is a wild plant that has not cultivated.

The presence of artemisinin potential contained in *A. cina*, genetically engineered. The Improvements in technical culture are a great alternative to increase the content of artemisinin in the production of varieties of *A. cina*. Improvements to varieties through genetic engineering through artificial polyploidy is one method in improving biomass and secondary metabolites contents in plants. Colchicine is one of the chemicals commonly used to induce polyploidy in plants with a high success rate. Colchicine is an anti-mitotic agent is used for chromosome multiplication under in vitro conditions. Colchicine plays a role in inhibiting chromosome segregation during cell division in chromosome multiplication. The effect of colchicine for in vitro chromosome multiplication differs in terms of concentration, method, and duration of treatment, as well as plant genetic factors [6–7]. In
addition to the different chromosomes, polyploid plants have different morphological characteristics compared to diploid plants [8]. Colchicine treatment in A. annua plants produces the highest tetraploid plants [9]. Polyploidy induction in A. annua plants to polyploid through colchicine treatment has 38% higher artemisinin content than diploid plants [10]. The artemisinin content in tetraploid A. annua is 1.5 times greater than that of diploid plants [9]. The artemisinin content in plants A. annua tetraploid average increased from 39% to 56% compared to diploid plants A. annua [11]. The content of artemisinin in A. cina tetraploid is higher (0.488%) versus diploid plant (0.04%) [12].

Agronomics performance is the growth and development of the plant-related field. Agronomic appearance is observed in A. cina polyploids and associated with artemisinin. They are broadleaves, shoot dry weight, root dry weight, density and size of trichomes glands, density and size of stomata, and leaf chlorophyll content. found that Artificial polyploidy induction using a 2.4-D plant growth regulator combined with BA was able to improve agronomic characters of A. cina polyploids compared that diploid i.e., on broadleaf, trichome glands density, the size of stomata, and chlorophyll content of leaves [13].

Artemisia spp. has been known as the plant that has a high economic value. The economic benefit is evident from the International Association of plant breeders’ rights. The International Union for the Protection of New Varieties of Plants (UPOV) already enters the genus Artemisia spp. in a list of plants that need a protected creation results of new varieties in 2009. However, A. cina is not yet explicitly listed. Besides, there is no guideline what characters that must be observed for the development of new crop varieties/assemblers of Artemisia spp (The International Union for the Protection of New Varieties of Plants [14]. The purpose of this study was to determine the agronomic performance of nine A. cina polyploids (genotypes A, B, C, D, E, F, G, H, I) obtained from polyploidy induction using colchicines.

2. Material and method

A. cina used in this research was a putative polyploid. A. cina putative 1 (P1) is the result of the colchicine induction. A cina polyploid plants were planted in polybags with a 50 cm diameter filled with garden soil and manure ratio of 1: 1. After two weeks, he moved to the shading house with 50% shade. One week after moving to the shading house, the first fertilization was carried out, namely Urea 152 kg/ha, SP36 233 kg/ha, and KCl 250 kg/ha. At the first fertilization, given ½ dose for urea while the other one dose. The second urea fertilization is carried out when the plants are one month after the first fertilization. GA3 growth regulators were applied to all genotypes when the plants were 30 days after moving to the shading house.

2.1. Leaf area analysis

Measurement of leaf area using the scanner method with iDaun software. Broadleaf measurements were done when the plant was four weeks old after it moved into the shading house (aged eight weeks from start to grafting). The leaves were measured when it opened up correctly in the fourth position.

2.2. Trichome glandular density analysis

The method used to measure the glandular density of trichomes is the replication method [15]. The observation of trichome glands was done by spreading top coat nail polish on the surface leaf's epidermis. Then, using affixed duct tape to peel the green area and placed it above the glass objects of the microscope, and then calculated the density of the trichomes gland. The measurement of the density of trichomes glands was done using USB Optilab camera and raster image program.

2.3. Chlorophyll analysis

Analysis of chlorophyll content using a modified method [16]. The leaves that were used were the leaves that had opened up correctly. Weighing the leaves to 0.04 grams, then cut into small pieces, placed it on a ceramic bowl, added 5 mL of DMSO, and then incubated in the darkroom at room temperature for 48 hours. After 48 hours, the leaves filtered with a filter paper. The absorption value
was measured using a spectrophotometer (UV-mini 1240, UV VIS Spectrophotometer, Shimadzu) at a wavelength of 649 nm (A665) and 665 nm (A649). Chlorophyll content expressed in mg g⁻¹ fresh weight.

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\text{Chlorophyll a} = (12.19 \times A665) - (3.45 \times A649) \tag{1}
\]

\[
\text{Chlorophyll b} = (21.99 \times A649) - (5.32 \times A665) \tag{2}
\]

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\text{Total Chlorophyll} = (18.54 \times A649) + (6.87 \times A665) \tag{3}
\]

2.4. Analysis of dry weight shoots
The shoot dry weight was measured using the oven drying method [17]. Observation of dry weight of shoots was done at the end of the study. The dry weight of shoots was measured by drying the leaves in the oven at a temperature of 40°C until constant weight. Then, it weighed with the units of grams (g).

2.5. Artemisinin content analysis by high performance liquid chromatography
Analysis of artemisinin content was performed 12 weeks after plantlets were transferred to the soil media. Shoots (the top of the plant) was cut then dried in an oven at a temperature of 40°C until dry. Furthermore, artemisinin content was analyzed using HPLC [18]. 100 mg dry weight of the sample was then extracted with 2 ml of Toluene, and filtered using filter paper and then inserted into the flakon. Analysis of artemisinin was quantitatively by the use of HPLC [18]. First, artemisinin was hydrolyzed in the alkaline solution. The hydrolysis product called Q260 was then measured at the wavelength of 260 nm. The procedure of quantitative analysis was as follows: 500 μL of the extraction with toluene was taken then evaporated / dried, the residue that remained was dissolved in 200 mL of methanol again. Next, 800 mL of NaOH solution (0.2% w / v) was added and the mixture was agitated with a vortex formed and heated in water bath for 30 minutes at the temperature of 50°C. After it got cold, 200 μL of methanol and 800 mL 0.2 M acetic acid were added, then artemisinin was measured by HPLC, at a wavelength of 260 nm, using a RP-18 column Licrospher length of 10 cm. The mobile phase used was methanol: 0:05 Kaliumdihidrogenphosphat mM (55: 45), a flow rate of 1.3 mL / min. Retention time was 40 minutes. Q260 product was stable for at least 4 days at 4°C storage temperature.

2.6. Data analysis
The experimental design used in this study was a random Groups Design with three replicates. It was analyzed using statistic analysis (ANOVA) and a variety of prints with advanced test DMRT on levels 5%. The data were analyzed using the program SAS 9.1.3.

3. Results and discussion
Based on the results of the analysis of the prints range (table 1) shows that the characteristics of agronomic crop for plant height on A. cina high-yield crops polyploids on genotype I differ markedly higher than those of the other genotype. The height of plants is the result of the Division and the unfolding of the cells. Application of Gibberellin (GA₃) to all observed genotypes also affected the increase in plant height. The sensitivity of each genotype to the administration of GA₃ is different. The application of growth regulators to plants depends on the plant's age, the dose of growth regulators given, and the part of the plant that is the target. Gibberellin has a role in supporting cell elongation, cambium activity, and supporting new RNA and protein synthesis. Plants respond to GA₃ with increased stem length. The GA₃ effect is mainly in the elongation of plant internodes caused by an increase in the expansion of cells in these segments. The role of Gibberellin in stem elongation is the result of 3 processes. The first process is cleavage at the end of the stem. The results of [19] show that cell division is caused by the gibberellin stimulus to cells in the G1 phase to enter the S phase and shorten the S phase immediately. The second process is that Gibberellin stimulates cell growth by
increasing the hydrolysis of starch, fructans, and sucrose into glucose and fructose to be used for energy-producing respiration. This energy will then be used to form cell walls and other cell components so that the cell formation process can take place quickly. The third process is increasing cell wall plasticity by Gibberellin. Gibberellins also reduce water potential to enter the cell more quickly, and cell expansion occurs. Agronomic perform for wide leaves, chlorophyll and the density of glandular trichome on Nine genotype A. cina observed do not differ markedly between genotype.

Agronomic appearance for the dry weight of shoots on genotype I showed real difference results is higher than the other genotype. The dry weight of a plant are affected biomass plant itself. On table 1. Biomass from Genotype I A. cina polyploids are indicated by high crop and broad leaves. Height of the plant determines the number of nodes owned by the plant and the number of leaves produced, whereas the broad-leaf plants capture light capability, so the effect on the dry weight of the plant. Koester et al. (2014) stated that broad leaf determines the amount of light that is captured and is an important parameter to determine the productivity of plants [20].

Table 1. Agronomic perform of Artemisia cina polyploid genotype

| Genotype | Plant Height (cm) | Leaf area (cm) | Chlorophyll (mg/g) | Glandular Trichoma Density/µm | Shoot dry weigh (g) | Artemisinin content (%) |
|----------|------------------|----------------|-------------------|-------------------------------|-------------------|------------------------|
| A        | 33.153 d         | 24.967 ab      | 2.960 a           | 60.000 a                      | 6.690 cd          | 0.0145 f               |
| B        | 52.053 bc        | 28.733 ab      | 2.897 a           | 58.750 a                      | 5.163 b           | 0.0125 f               |
| C        | 54.970 bc        | 31.930 ab      | 2.800 a           | 59.500 a                      | 9.230 cb          | 0.0225 e               |
| D        | 60.037 ab        | 37.083 a       | 2.647 a           | 61.250 a                      | 10.010 cb         | 0.0355 b               |
| E        | 51.127 bc        | 30300 ab       | 2.880 a           | 62.417 a                      | 7.270 bcd         | 0.0320 c               |
| F        | 56.247 abc       | 29.507 ab      | 2.643 a           | 65.140 a                      | 7.310 bcd         | 0.0405 a               |
| G        | 61.070 ab        | 28.990 ab      | 2.257 a           | 61.750 a                      | 10.840 b          | 0.0270 d               |
| H        | 43.987 cd        | 35.230 a       | 2.290 a           | 64.110 a                      | 8.163 bcd         | 0.0400 a               |
| I        | 70.833 a         | 35.990 a       | 2.120 a           | 70.250 a                      | 16.347 a          | 0.0390 a               |

The mean value followed by different letters showed significant (α = 0.05) in the DMRT test.

Table 1 shows that the levels of artemisinin on genotype F, H, and I differ markedly higher than those of the other genotypes. Artemisinin is a secondary metabolite produced by A. cina. The availability of precursor compound as starting material for the synthesis of artemisinin is to affect levels of artemisinin in A. cina. Acetyl CoA is a precursor compound for the synthesis of artemisinin. Acetyl CoA synthesized from the sugars derived from the results of photosynthesis. It allegedly produced the dry ingredients used for the formation of artemisinin [12]. Figure 1 dry weight indicates that the highest-weight of artemisinin produced by I genotype. A dry weight of artemisinin is affected by the heading and levels of artemisinin. Besides, it is also affected by the number of glandular trichomes per unit area. From the observations, I genotype had a higher dry weight of shoots and a more elevated amount of glandular trichomes compared to other genotypes. Artemisinin biosynthesis and storage are present in glandular trichomes. The density of the glandular trichomes correlates with the artemisinin content. Artemisinin levels have a positive correlation; the correlation coefficient is 0.51 and significant with glandular trichome density. Thus, the higher the glandular density of trichomes, the artemisinin content will increase [12]. Correlation between trichome density and artemisinin content is in line with the results of research by [21], who stated that glandular trichome density correlates with artemisinin content.
Figure 1. Artemisinin production on A. cina polyploidy genotype.

4. Conclusion
Agronomic appearance is the character of the growth and development of plants. The glandular trichomes, leaf area, and chlorophyll content are not significant for genotypes A, B, C, D, E, F, G, H, I. Agronomic appearances of plant height, dry weight shoot on genotype I significantly higher compared to the other genotype. Levels of artemisinin on genotype F, genotype H, and genotype I differ markedly higher than those of the different genotypes. The weight of artemisinin on genotype I is higher than most other genotypes.

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