Effect aquadest-extracted *Gloriosa superba* seed as mutagen on morphology of *Artemisia annua*

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Abstract. *Gloriosa superba* is a plant that contains colchicine in all parts of organs, especially in the seeds. Its extract is as a mutagen to produce plants with polyploid cells. *Artemisia annua* is a plant that produces active ingredients artemisinin as malarial drugs, hemorrhoids therapy, aromatherapy, antiviral, anticancer, and anti-bacterial. The aims of this research was to determine the effect aquadest-extracted *Gloriosa superba* seed as a mutagen to *Artemisia annua* morphology. Extraction of *Gloriosa superba* seeds obtained from Sukoharjo using maceration method with aquadest solvent (1:1). The extracts were diluted (0, 25, 50, 75 and 100%) for *Artemisia annua* sprinkling with different times (0, 30, 60 and 90 minutes). Observations of morphology *Artemisia annua* included height, stem circumference, number of branches, number of leaves, leaf width and leaf length. The treatments did not affect plant morphology observation included height, stem circumference, number of branches, number of leaves, leaf width, and leaf length. The EB treatment (100%, 30 minutes) was higher (120 cm) than other. In all treatments stem circumference about 2.5 cm, number of branches ranged between 40-50, leaves width ranged 9 -16 cm, and leaf length ranged 8 -15 cm.

1. Introduction

*Gloriosa superba* is an herbaceous vine and consisted of tubers, stems, leaves, flowers and seeds containing colchicine. Previous research has shown that the *Gloriosa superba* seed has a higher content of colchicine than other organs of this plant. Colchicine is a chemical used as a polyploid induction in plants because it is effective and easily soluble in water [1]. *Gloriosa superba* seed extract can be utilized as a mutagen in diploid plants into polyploid plants. *Artemisia annua* is a medicinal plant from China and cultivated in Indonesia which produces artemisinin as malaria drug. Artemisinin is sesquiterpene lactone endoperoxide extracted and isolated from leaves *Artemisia annua* as anti malarial drug [2]. Pathogen *P. falciparum* has been resistant to quinine as a malaria drug [3], thus attempting another alternative plant as a malaria drug. Polyploid plants often exhibit new phenotypes, such as broader leaves, excellent quality, high yield and increased resistance to environmental stresses and disease, and increase the secondary metabolite content of a plant [4]. The aim of this research is to know the effect aquadest-extracted *Gloriosa superba* seed obtained from Mulur, Sukoharjo on morphology of *Artemisia annua*. 

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2. Methods
This research used sampling method and experimental laboratory method. *Gloriosa superba* obtained by sampling method in Mulur, Sukoharjo during January-February 2017. Samples used in the form of seeds which are then dried using oven with temperature 70˚C for 24 hours. Dry seeds extraction using maceration method with aquadest solvent (1:1) for 24 hours with 3 replications. Analysis of colchicine content and level on extract of *Gloriosa superba* seeds using TLC-Densitometry method. The aquaest-extracted *Gloriosa superba* seeds was divided into several concentrations (0, 25, 50, 75 and 100%) for the treatments. *Artemisia annua* seeds were obtained from B2P2TOOT, Tawangmangu. Seedling was done in B2P2TOOT Karangpandan garden, Karanganyar at the beginning of the rainy season. The seeding process of *Artemisia annua* seeds to be sprouts ± 2 weeks. At 2-week-old strong sprouts were then treated with aquaest-extracted *Gloriosa superba* seeds. Treatment of *Artemisia annua* sprouts was performed with above mentioned extract concentrations and soaking sprouts times (0, 30, 60 and 90 minutes) (Table 1). Sprouts of treatment were grown on polybags. Observations of morphology *Artemisia annua* included height, stem circumference, number of branches, number of leaves, leaf width and leaf length. The parameters of morphological observation were done once every week until 50% of the plant population forms primordial flowers. Observation data of height, stem circumference, number of branches, number of leaves, leaf width and leaf length were recorded every week. Data were used as an analysis to compare the morphology between the treated plants.

Table 1. Design of the treatments

| Concentration | A (0%) | B (30) | C (60) | D (90) |
|---------------|--------|--------|--------|--------|
| AA            | AB     | AC     | AD     |
| BA            | BB     | BC     | BD     |
| CA            | CB     | CC     | CD     |
| DA            | DB     | DC     | DD     |
| EA            | EB     | EC     | ED     |

3. Results and Discussion
*Artemisia annua* is a medicinal plant that produces artemisinin as a malaria drug. Increased biomass and artemisinin levels in these plants were performed by induction of ploidies using colchicine. Utilization of *Gloriosa superba* as a plant containing colchicine is used for induction of *Artemisia annua* sprouts. Ploid-induced plants produce not only high artemisinin, but also morphologically superior to diploid plants. In this research conducted a study on the effects of morphology *Artemisia annua* that the soaking spouts by aquaest-extract *Gloriosa superba* seeds. The results of this study show the morphology of *Artemisia annua* treatment results include height, stem circumference, number of branches, number of leaves, leaf width and leaf length (Table 1).

*Artemisia annua* sprouts was used in soaking treatment aquaest-extracted in 2 weeks old with an average height of ± 1cm. Treatment of soaking sprouts using variable concentration of aquaest-extract *Gloriosa superba* seed and soaking time. Sprouts result of treatment planting on polybag with soil media, husk and manure. Morphological observation data were observed a week after sprouting soaking treatment until 50% of the plant population formed primordial flowers. The problems experienced during the growth process of *Artemisia annua* sprouts are the weather and caterpillar pests. These conditions affect the growth of sprouts with one another which is a repeat of the treatment. The observed data each week varies due to the condition until the plants form primordial flowers.
Table 2. Morphological data of Artemisia annua

| Sample | Height (cm) | Stem Circumference (cm) | Number of branches | Number of leaves | Leaf width (cm) | Leaf length (cm) |
|--------|-------------|------------------------|--------------------|-----------------|----------------|-----------------|
| AA     | 100         | 2                      | 55                 | 45              | 10             | 11              |
| BA     | 108         | 2.5                    | 53                 | 56              | 10             | 11              |
| CA     | 110         | 2.5                    | 47                 | 43              | 11             | 10              |
| DA     | 98          | 2                      | 43                 | 39              | 11             | 10              |
| EA     | 98          | 2                      | 23                 | 28              | 9              | 11.5            |
| AB     | 97          | 2                      | 49                 | 39              | 9              | 8               |
| BB     | 110         | 2.5                    | 40                 | 38              | 12             | 11              |
| CB     | 98          | 2                      | 42                 | 30              | 11             | 9               |
| DB     | 104         | 2.5                    | 53                 | 33              | 11             | 11.5            |
| EB     | 120         | 2.5                    | 40                 | 30              | 10             | 11              |
| AC     | 105         | 2.5                    | 44                 | 37              | 10.5           | 9               |
| BC     | 100         | 2.5                    | 57                 | 35              | 12             | 11              |
| CC     | 100         | 2                      | 51                 | 42              | 13             | 12              |
| DC     | 103         | 2.5                    | 50                 | 37              | 14             | 13              |
| EC     | 100         | 2.5                    | 50                 | 36              | 12             | 12.5            |
| AD     | 108         | 2.5                    | 54                 | 39              | 9              | 10              |
| BD     | 98          | 2                      | 48                 | 41              | 11             | 10              |
| CD     | 93          | 2                      | 38                 | 30              | 12.5           | 11              |
| DD     | 106         | 2.5                    | 51                 | 46              | 13             | 12              |
| ED     | 100         | 2.5                    | 54                 | 50              | 16             | 15              |

The plant height morphology was not different between treatments. The presence of caterpillar pests that feed on leaves early in Artemisia annua growth inhibits plant growth. In addition, entering the rainy season with a little more sunlight causes the plants easily die because it is too wet. The ability of plants with each other on the same treatment is also different is shown by the adaptation to seasonal changes. Plant height at the end of the observation is not much different from each other. This may be due to environmental factors during the growth of Artemisia annua which suffered some disruption.

Other morphological observations include circumference stem, number of branches, number of leaves, leaf width and leaf length no significant difference. The morphological measurements observed and recorded for all plants are similar. In this study cannot be concluded diploid and polyploid plants because morphologically all the plants were difference between each other. The leaves of Artemisia annua look greener and thicker than the control plants.

The morphological effect of Artemisia annua on treatment with aquadest-extracted Gloriosa superba seeds in this study cannot be clearly presented. The growth of this plant is strongly influenced by many environmental factors because basically this plant is still being studied for cultivation in Indonesia. In addition, plant morphology is also strongly influenced by growing environmental conditions, so morphological data is only used as supporting data to infer the effect of the treatment that has been done. To be able to know more clearly and precisely needs to be observed cytology such as stomata and plant chromosomes of plants treatment.

4. Conclusion
The aquadest-extracted Gloriosa superba seed showed no significant difference between the treatments. Height, stem circumference, number of branches, number of leaves, leaf width and leaf length in treated plants showed no significant different data. In other observation, Artemisia annua by treatment aquadest extract of Gloriosa superba seeds has thicker leaf and look greener than control.
plant. The morphology of treated plants is superior when compared to diploid plants cannot be proven in this study.

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