Agronomic Characteristics Enhancement on Genotypes of Chrysanthemum Polyploidy with Different Planting Media

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Abstract. Since variations in chrysanthemums are still limited, Indonesia still imports. An effort to expand the variation can be done through polyploidy techniques using colchicine. Pasopati varieties of the chrysanthemum were propagated in vitro and treated with colchicine and planted in different media compositions to observe performance and obtain superior genotype. Experiment with a factorial pattern that consist of two factors: the composition of the planting medium (m1 = top soil, m2 = cocopeat, m3 = husk charcoal, m4 = top soil + cocopeat m6 = cocopeat + chaff charcoal) and polyploidy chrysanthemum genotypes (g1 = KAR0, g2 = KAR1, g3 = KAR2, g4 = KAR3, and g5 = KAR4) with two replications was designed. The results showed there were variations in plant height, stem diameter, number of nodes, number of floret, and stalk length on polyploidy Chrysanthemum genotypes due to different planting media. The m4= top soil + cocopeat can increase plant height, stem diameter, number of nodes, stalk length and number of floret KAR4 genotype.

Keyword: chrysanthemum, colchicine, media composition, polyploidy

1. Introduction
Chrysanthemum is an ornamental plant which is very popular both as cut flowers for flower arrangements and for decoration materials. Indonesian chrysanthemum production does not meet the consumers desired in quality standards, namely large size, stalk length up to ± 70 cm high, clean flowers and no spots, and flower crowns do not fall off easily [1-2]. Polyploidy is one of the breeding techniques to increase the character diversity of a species and obtain plants with superior character by using colchicine. Research by [3], namely soaking chrysanthemum seeds with colchicine in in vitro cultivation, resulted in morphological characteristics variations of chrysanthemum plants and 1 hour colchicine soaking gave a better affect on the number of leaves, number of nodes, root length, number of roots and height of plantlet. The research of [4], chrysanthemum which was treated with colchicine had a larger size of plant parts compared to controls. The best phenotype character was produced by colchicine soaking treatment for 12 hours [5]. The sizes of seeds and stomata were primarily affected by cytotype, while the plant performance differed between natural and synthetic polyploids. Most of the performance characteristics are also determined by the application of colchicine to the parent, and most of these results are specific populations.

Growing media as a place to grow rooted and developing plants is an important environmental factor. Ornamental plants generally require a loose, porous, fertile media, containing organic material, free from plant-disturbing organisms and having good aeration and drainage [6]. Fuel husk has a high...
carbon content (C), making the planting media loose. Husk charcoal contains N 0.32%, PO 15%, KO 31%, Ca 0.95%, and Fe 180 ppm, Mn 80 ppm, Zn 14.1 ppm and pH 6.8. Another characteristic of husk charcoal is light weight (specific gravity of 0.2 kg L⁻¹), Air circulation and capacity to hold high water, black so that it can absorb sunlight affectively, has properties that easily bind water, not easily clot or condense so that plant roots can grow perfectly [7].

According to [8] for potted chrysanthemums, planting media are obtained with consideration of being easily obtained, relatively inexpensive, lightweight and must have physical and chemical properties that can support root growth and nutrient uptake optimally. The ideal growing media conditions are obtained from a combination of organic and inorganic materials. Organic materials can include chopped ferns, compost, humus, sawdust, top soil, charcoal husks, and cocopeat. Inorganic materials can be in the form of clay, sand, poor sand, gravel, and gel. The use of fuel husk in the planting media does not need to be sterilized anymore because pathogenic microbes have died during the combustion process. The media of rice husk charcoal and drip irrigation system (drip irrigation) showed the highest results significantly against seedling height, number of leaves, leaf area, fresh and dry weight of seeds, and root volume [9]. Experiment by [10] showed that significantly influenced by the different potting growing media used in this study.

In the flowering stage, the highest photosynthesis rates (8.612 μmol CO₂ m⁻² s⁻¹) as well as leaf area (1.766 dm²) of petunias were obtained on growing media with 60% biolan peat, 30% acid peat and 10% perlite (BP60-AP30-P10). Flowering responses to growing conditions vary greatly among plants and the biggest number of ornamental tobacco flowers (22 flowers plant⁻¹) was registered as an affect of BP60-AP30-P10 media. Growing media with the BP60-AP30-P10 formula seem to be the most adequate growth substrate to develop profitable crops for petunias and ornamental tobacco with high decorative value. Growing media compositions of soil+compost+cocopeat (2:1:1) and soil+compost+husk charcoal+cocopeat (2:1:1:1) treated by the watering interval once in 2 days increased the percentage of tuberization and the weight of tubers per plant of potatoes. The combination of farmyard manure, silt, coconut compost, peat moss and leaf compost (1:1:1:1) exhibited best results for the growth and production of potted Ravena plants. The substrate was considered excellent due to its density, saturation percentage, structure, texture, consistency and Organic Matter (OM) as well as nitrogen, phosphorus and potassium concentration [11].

Chrysanthemum genotypes KAR₀(Pasopati varieties), and KAR₁, KAR₂, KAR₃, and KAR₄ are the results of early generation polyploidy which have unstable character because they are still experiencing physiological disorders and require optimal planting media to support their growth. Information about the optimal planting media for polyploid chrysanthemum plant growth is not widely known. The research aims to study the affect of planting media on polyploid genotype performance and to obtain superior chrysanthemum genotype.

2. Methods
Experiments conducted in a greenhouse of Faculty of Agriculture, University of Winaya Mukti at TanjungsariSumedang, Indonesia with a height of 874 m above sea level. The experiment started in June until August 2018. The materials and tools used in the experiment were chrysanthemum plants resulting from polyploidy (KAR₀Pasopati, KAR₁, KAR₂, KAR₃, KAR₄), husk charcoal, cocopeat, soil type Andosol, Root Up, PPC (Liquid Complement Fertilizer) Biosugih, clean water, fungicide and bactericide Bactosin, insecticide Curacron 250 EC, insecticide Abacel, Altimeter, polybag size 20 cm × 20 cm, ray, tweezers, seedbed tray, markers, sticks, label paper, insecticide Curacron 250 EC, insecticide Abacel, Altimeter, polybag size 20 cm × 20 cm, ray, tweezers, seedbed tray, markers, sticks, label paper, masking tape, tape wire, spatula, razor blade, thermometer, ruler, bucket, plastic lid, machete, marker, hand sprayer, measuring cup, stationery, and camera.

In factorial pattern experimental design, the first factor is the composition of the planting medium with a volume ratio consisting of 6 levels m₁ = top soil, m₂ = cocopeat, m₃ = husk charcoal, m₄ = soil + cocopeat, m₅ = soil + husk charcoal, m₆ = cocopeat + husk charcoal with volume ratio of 1: 1, and the second factor of chrysanthemum genotypes resulting from polyploidy consists of 5 types namely g₁ =
KAR_0, g_2 = KAR_1, g_3 = KAR_2, g_4 = KAR_3, g_5 = KAR_4 so that there are 30 treatment combinations with each treatment repeated about 2 times. Each treatment consists of 4 polybags so that the total is 240 polybags. Observation variables: plant height, stem diameter, number of leaves, number of nodes, stalk length, number of florets, and flower diameter. The analysis technique to prove the hypothesis using F-test and Duncan’s multiple range test about 5% significance levels, respectively, were applied.

3. Results and discussions

3.1 Plant Height
Based on Table 1, chrysanthemum genotypes of KAR_2 polyploidy planted on top soil + charcoal husk media at 12 weeks after planting (WAP) were higher than other treatments. The average number that marked with lowercase letters (vertical direction) and the same capital letters (horizontal direction) was not significantly different according to the Duncan’s Multiple Range Test about 5% significance level. Growing media is one of the determining factors whether plants will well grow or not. Planting media provide water, air, and mineral elements to be absorbed by plants through their roots [9]. Growing media affect on plant height, flower appearance, and harvest age, respectively [4]. The chrysanthemum genotypes showed higher plant height at 12 WAP, suspected due to the genetic differences between the chrysanthemum genotypes by colchicine treatment. The colchicine can increase cell size so that it affects plant height as a results of the study of [2]. The colchicine treatment was more affective in seedling treatment when the length of hypocotyls ranged from 5 to 8 mm.

| Treatments                      | g_1   | g_2   | g_3   | g_4   | g_5   |
|--------------------------------|-------|-------|-------|-------|-------|
| m_1 (top soil)                 | 21.63 | a     | 14.43 b| 18.35 bc| 8.70 abc| 13.33 a|
| m_2 (cocopeat)                 | 2.20  a| 1.80  a| 3.38 a| 5.23 ab| 2.13 a|
| m_3 (husk charcoal)            | 5.50  a| 7.68  a| 7.95 a| 12.63 c| 16.23 a|
| m_4 (top soil + cocopeat)      | 4.25  a| 4.20  a| 12.83 b| 5.50 ab| 18.60 b|
| m_5 (top soil + husk charcoal) | 17.40 b| 21.13 b| 23.75 c| 12.25 bc| 20.73 b|
| m_6 (cocopeat + husk charcoal) | 2.28  a| 7.88  a| 2.43 a| 2.65 a| 4.50 a|

3.2 Stem Diameters
Based on Table 2, it known that the growing media influences the stem diameter of each polyploidy chrysanthemum genotype. The KRA_2 genotype planted on top soil + husk charcoal had the widest stem diameter. One external factor that affects plant growth is the media. In principle, planting media must be able to provide nutrients, water and oxygen for plants and the selection of the right media will provide maximum growth. Cholchicine reduction in plant height, number of leaves per branch, but increase in number of branches.

Similar with, the research showed that control group exhibit higher value in terms of plant height, number of leaves, dry, and fresh weight of bulbs compared to treatment groups with colchicine.
Concentration of 0% (control) within 12 hr of immersion duration (d_0t2) showed the highest plant of height about 22.38 cm, producing 5.05 leaves and dry weight of 1.57 g. The heaviest weight of fresh bulbs was observed from treatment group, then with concentration of 0.3% colchicine within 0 hr of immersion duration (d_3t0) yielding 4.58 g. In general, high concentrations of colchicine will reduce the number of leaves for almost all types of plants. Colchicine can affect cell division in plant tissue regions and spread through cells, disrupting cellular mechanisms and causing toxicity at high concentrations, which causes slow growth and tissue damage.

Stomatal size was negatively correlated with stomatal frequency. Stomatal size and stomatal frequency can be used as indirect methods for identification of ploidy level of Phlox. This finding demonstrates the existence of genetic variation for the morphological response to ploidy change in _P. drummondii_[12]. There were differences characters in plant height and stem diameter between genotypes which planted in different media (namely the interaction between genotypes of Chrysanthemum Polyploidy and the composition of the planting media). This interaction is caused by genetic composition between different polyploidy genotypes and nutrients, oxygen, and nutrients in each different media composition. The interaction between genotypes and the environment is also shown by [13]and [14].

**Table 2. Stem Diameters at the age of 12 WAP**

| Treatments                        | Average Stem Diameter at the age of 12 WAP (mm) |
|-----------------------------------|-----------------------------------------------|
|                                   | g1 (KAR0) | g2 (KAR1) | g3 (KAR2) | g4 (KAR3) | g5 (KAR4) |
| m1 (top soil)                    | 1.08      | 0.89      | 1.07      | 0.58      | 0.68      |
| m2 (cocopeat)                    | 0.23      | 0.14      | 0.21      | 0.82      | 0.38      |
| m3 (husk charcoal)               | 1.07      | 0.79      | 0.86      | 0.57      | 0.58      |
| m4 (top soil + cocopeat)         | 0.66      | 0.64      | 0.92      | 0.48      | 0.69      |
| m5 (top soil + husk charcoal)    | 1.44      | 1.37      | 1.64      | 0.95      | 1.15      |
| m6 (cocopeat + husk charcoal)    | 0.25      | 0.36      | 0.16      | 0.33      | 0.43      |

3.3 _Number of Leaves and Diameter of Flower_

Based on Table 3, the composition of the planting medium affects the number of leaves at the age of 12 WAP plants. Top soil media + husk charcoal produced more leaves than the other treatments. The chrysanthemum genotypes from polyploidy showed no difference in the number of leaves. The increasing number of leaves will increase photosynthesis results. Furthermore, the results of photosynthate can be transplanted to plant tissue so that the biomass content in plants will be higher. In polyploid plants treated with colchicine, physiological disorders occur that inhibit plant growth. According to [15] the affect of colchicine slows down cell division causing slow leaf primordial development.

The composition of the planting medium affects the diameter of the flower. The largest flower diameter was produced from top soil + rice husk charcoal. Soil media and husk charcoal were oftenly used for plant growing media. They have several advantages, such as easy binding of water, not easily weathered, good source of potassium, and not easy to clot so that the roots of plants grow well. According to [16], leaf manure significantly produced the maximum vase life and diameter of flower.
while the maximum vase life and diameter of flower was obtained with mix (coconut compost+soil loam; 1:1). The largest flower diameter of polyploid chrysanthemum genotypes were obtained from KAR2 and KAR; in the Table 3. According to the results showed that the polyploid plants had gigantic characteristics such as thicker wider leaves with greater stomata size and larger flowers.

### Table 3. Number of Leaves and Diameter of Flower at the age of 12 WAP

| Treatment                  | Number of leaves | Diameter of flower (cm) |
|----------------------------|------------------|-------------------------|
| m1 (top soil)              | 8.75 bc          | 5.48 bc                 |
| m2 (cocopeat)              | 2.95 a           | 2.60 a                  |
| m3 (husk charcoal)         | 10.40 cd         | 3.47 ab                 |
| m4 (top soil + cocopeat)   | 7.85 b           | 3.35 b                  |
| m5 (top soil + husk charcoal) | 14.75 d     | 5.63 d                  |
| m6 (cocopeat + husk charcoal) | 4.50 a          | 2.35 a                  |

| Chrysanthemum Genotype Results
|----------------------------|
| Polyploidy (G) :           |
| g1 (KAR0)                  | 7.29 b           | 3.63 a                  |
| g2 (KAR1)                  | 10.58 b          | 3.04 a                  |
| g3 (KAR2)                  | 9.92 b           | 5.18 b                  |
| g4 (KAR3)                  | 9.04 b           | 3.24 a                  |
| g5 (KAR4)                  | 4.17 a           | 5.48 b                  |

### 3.4 Number of Nodes

Based on Table 4, the composition of the planting media influences the number of nodes of the chrysanthemum genotypes. The KAR1 genotype planted in the composition of top soil + charcoal husk media had the highest number of joints compared to other treatments. This is thought to be increasing plant height and number of leaves, then the number of joints is likely to increase. The top soil mixed media + charcoal husks provide sufficient nutrient food reserves to be stored in a joint that allows plants to regenerate faster. According to plants need sufficient food reserves to be stored in joints which will then be overhauled and used to bring up roots and shoots.

### Table 4. Number of Nodes at the age of 12 WAP

| Treatments                  | g1 (KAR0) | g2 (KAR1) | g3 (KAR2) | g4 (KAR3) | g5 (KAR4) |
|-----------------------------|-----------|-----------|-----------|-----------|-----------|
| m1 (top soil)               | 18.25 c   | 14.75 cd  | 17.75 c   | 13.00 b   | 12.25 b   |
| m2 (cocopeat)               | 5.15 a    | 4.25 a    | 5.00 a    | 9.25 b    | 6.50 a    |
| m3 (arangsekom)             | 12.25 c   | 12.00 bc  | 18.00 c   | 12.50 b   | 13.75 bc  |
| m4 (husk charcoal)          | 9.00 b    | 8.75 b    | 10.00 b   | 10.00 b   | 14.50 bc  |
| m5 (top soil + cocopeat)    | 18.50 c   | 19.25 e   | 15.50 c   | 11.75 b   | 19.00 c   |
| m6 (top soil + husk charcoal)| B         | B         | AB        | B         | B         |
Based on Table 5, chrysanthemum genotypes of KAR4 polyploidy results in the composition of the top soil + charcoal-growing media has the longest flower stals compared to other treatments.

### Table 5. Length of Stalks Flower

| Treatments                      | \( g_1 \) (KAR\(_0\)) | \( g_2 \) (KAR\(_1\)) | \( g_3 \) (KAR\(_2\)) | \( g_4 \) (KAR\(_3\)) | \( g_5 \) (KAR\(_4\)) |
|--------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| m\(_1\) (top soil)             | 4.25 b           | 3.63 a           | 5.38 b           | 1.00 a           | 1.50 ab         |
| m\(_2\) (cocopeat)             | 0.00 a           | 0.00 a           | 0.00 a           | 0.00 a           | 0.00 a          |
| m\(_3\) (arangsekam)           | 0.00 a           | 1.13 a           | 0.00 a           | 2.63 a           | 3.75 b          |
| m\(_4\) (husk charcoal)        | 3.13 ab          | 7.00 b           | 5.75 b           | 1.63 a           | 7.63 c          |
| m\(_5\) (top soil + cocopeat)  | AB               | C               | BC               | A               | C               |
| m\(_6\) (top soil + husk charcoal) | A               | A               | A               | A               | A               |

3.6 Floret

Based on Table 6, the chrysanthemum genotypes of KAR\(_4\) polyploidy planted in the composition of the top soil + rice husk charcoal had a higher number of florets than the other treatments [7]. Number of leaves per plant, leaf area, number of flowers per plant and leaf area / flowers ratio. These measurements were significantly influenced by the different potting growing media used. The colchicine affects on plants were linked to strong direct affects of colchicine on the production of various plant hormones and thus on cell functioning [19], leading to strong differences in plant physiology and morphology [20]. KAR\(_4\) was adaptive to topsoil+rice husk charcoal medium and each variety had different response to the environment.

### Table 6. Number of Floret

| Treatment                     | \( g_1 \) (KAR\(_0\)) | \( g_2 \) (KAR\(_1\)) | \( g_3 \) (KAR\(_2\)) | \( g_4 \) (KAR\(_3\)) | \( g_5 \) (KAR\(_4\)) |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| m\(_1\) (top soil)            | 3.50 b           | 3.75 b           | 3.50 c           | 0.50 b           | 2.00 b          |
| m\(_2\) (cocopeat)            | 0.00 a           | 0.00 a           | 0.00 a           | 0.00 a           | 0.00 a          |
| m\(_3\) (husk charcoal)       | 0.00 a           | 0.00 a           | 0.50 a           | 0.75 b           | 2.25 b          |
There were differences in the appearance of leaves seen from the shape and color as in Figure 1. Appearance of flower shapes and colors from the top or bottom as in Figure 2 on the polyploidy chrysanthemum genotypes. These differences occur due to genetic differences due to the treatment of colchicine. The plants treated with Colchicine also had thicker and coarse leaves which were greener in colour in comparison to the Control. The development of the light green color of gladiolus leaves
in plants that were given colchicine was associated with a reduction in the synthesis of green pigments, namely chlorophyll [21]. Previous studies have shown that the reduced chlorophyll content in colchicine induced by plant tetraploids from mat rush (Juncus effusus) occurs due to structural modifications such as disintegration in the chloroplast lamellar / thylakoid membrane that affect chlorophyll synthesis. Reduction of tuber diameter and the rate of multiplication of treated plant tubers was also one of the negative affects of colchicine treatment which can be the result of polyploidy induction.

4. Conclusions
Variations in plant height, age, stem diameter, number of nodes, number of florets, length of stalks, and diameter of flower on the polyploidy Chrysanthemum genotypes due to different planting media were found. m4 planting media in KAR4 genotype can increase plant height, stem diameter, number of nodes, length of stalks and number of chrysanthemum florets.

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