INTRODUCTION

As an important ornamental plant, Chrysanthemum or chrysanthemums is one of the ten, or one of the four popular cut flowers in China and the world respectively. This flower also occupied an important position in the global flower industry (Wang, Zhang, Chen, Fang, & Teng, 2014). The shape of the crown, beautiful colors, and affordable prices make Chrysanthemum dominates the flower industry in Southeast Asia and European countries. In Indonesia, Chrysanthemum as potted plants are a favorite in the market, and potentially to be export. By increasing the quality of chrysanthemum plants, this flower can be a potential business.

Chrysanthemum in East Java, Indonesia provided great demand for a long period because the potted chrysanthemum is higher than other ornamental plants based on the market demand in domestic and international (Nurmalinda & Hayati, 2014). In 2018, East Java as one of the Provincial centers for ornamental plants produced a percentage of Chrysanthemum production ca. 28.25%. Based on the volume of chrysanthemum exports in 2018, the FOB value increased from 699,176 US$ to 817,208 US$. For Indonesia, Japan as the main destination export of chrysanthemum, their demand grow up 59.11 tons from 49.52 tons (BPS, 2018).

Careful and specific processing and treatment systems are required in the production of Chrysanthemum. About 500 species are planted in Indian from thousands of chrysanthemum species (Rana, 2007), but not all types are appropriate to be cultivated in pot media. Specific techniques are needed to plant chrysanthemums in media of pot with the required environments. The length of the stem segment and the length of flower freshness are variables of chrysanthemum quality. The height of chrysanthemums as one of the quality criteria for potted flowers (Crater, 1992; Yuniarto, Kurniati, Suryawati, & Meilasari, 2018), needs to reach the suitable size standard for potted plants, which is
from 1.5 to 2 times the pot height. Inhibitors can be used to decrease the length of the stem internodes and prolong flower freshness so that the plant height meets market criteria. Each type of chrysanthemum has a different response depending on the growth phase. Studies on various stages of plant growth in response to retardants need to be carried out to see the ability of plants (Sances, Flora, Patil, Spence, & Shinde, 2013).

Chrysanthemums are divided into three categories based on their growth phase. To reach the market criteria all categories should be modified. The first, varieties that have fast-growing. The fast-growing species can be stimulated by stopping prolonged exposure when the plant reaches a height of 30-35 cm (ca. 3-4 weeks). The second, varieties with moderate growth (moderate-growing). For the moderate-growing species, the long day phase could be ended when the plant has achieved a height of 35-45 cm (ca. 4-5 weeks). The third, varieties that have slow growth (Slow-growing). Cahyono & Ismail (1999) mentioned that the slow-growing species, within five weeks or more, the long day phase should be ended when the plants had reached ca. 45-50 cm height.

A common retardant is applied to chrysanthemums to adjust the height of the flowers. Rose et al. (2012) mentions that daminozide or 2,2-dimethylhydrazine as a retardant commonly applied to the potted chrysanthemums is a growth regulator (N-(dimethylamino) succinic acid). As a synthetic growth regulator, daminozide inhibits the growth of a plant. Karlović, Vršek, Šindrak, & Židovec (2004) mentioned that B-Nine or Alar as a product containing daminozide and Maleic Hydrazide can be used to manage height, branching, improve the flower freshness and the better quality of chrysanthemum. However, so far the application at four weeks after planting (WAP) and at flower initiation with 8000 ppm daminozide, does not meet the quality standard for several types of potted chrysanthemums. A one-time application of daminozide has not shown significant results, but the second and subsequent applications can reduce plant height (Abod & Yasin, 2002). Related to previous research, it is necessary to study the effect of application frequency of daminozide on the potted chrysanthemums that are commonly planted in East Java.

**MATERIALS AND METHODS**

**Research Materials, Period and Place**

The research was carried out in a greenhouse from June to October 2019 in Tutur village, Nongkojajar sub-district, Pasuruan Regency at 900 meters after sea level (masl) at 24°C of daily temperature rate. Tobaga, Time Jewel, and Cosmo Magenta were chosen as representative of fast-, moderate-, and slow-growing varieties respectively. Each used seedling was a rooted cutting with the length of cutting ca. 12 cm. The 4000 ppm daminozide, cocopeat, cow manure (2:1), AB nutrient, pesticides (i.e.: Antracol, Dursban, Samite, and Kardan), pots with 17 cm in diameter, sprayer, hose brooch, and drip installation were materials and tools in this research. The one cutting of chrysanthemum was cultivated with upright direction on the center of the pot. Plant seedlings were produced from Parent Plant (MP) shoots that have been rooted for 10-12 days. These seeds have been graded according to the criteria before being planted in pots. The other criteria were free from plant diseases and pests, 10 cm height, and no rotten, or no wilted. The long day phase was given from 0 DAP until the formation of lateral shoots from the leaf axils about 2-3 cm after the buds were pinched. Long days are applied to all varieties of chrysanthemum for 3-4 weeks. While the short day phase was conditioned when lateral shoots were formed in the leaf axils of about 2-3 cm until the plant was on harvesting age. Fertilizing and watering were conducted at two-days intervals. Daminozide was applied to plants two weeks after planting (WAP) and at flower initiation (ca. 6 WAP). Pesticide was applied three times a week, adjusted to the type of pest and disease attack that occurs. Chrysanthemum planting was conducted in a plastic house.

**The Application of Daminozide**

The spraying of retardant (daminozide) was applied to the potted chrysanthemum based on the number of treatments, Alar with 95% of active ingredient daminozide at 4000 ppm was used. The dose given at each treatment was the same at two and four weeks after transplanting (WAT) ca. 5 ml per plant, and at six WAT, 10 ml dose per plant was applied. The application frequency of retardant was amended to the amount of given treatment, i.e. (1) the zero doses (without) of daminozide application, (2) applied once (at 2 WAT), (3) applied twice (at 4
and 6 WAT), and (4) applied thrice (at 2, 4, 6 WAT). The daminozide was sprayed on the entire plant canopy consistently in the morning.

**Design and Parameters of Research Experiment**

The research adopted the Split Plot Design. The main plots were the chrysanthemum types based on the growth phase namely Tobaga as fast-growing type, Time Jewel as moderate-growing type, and Cosmo Magenta as slow-growing type. The subplots were the application frequency of daminozide: (1) without or zero daminozide application, (2) applied once, (3) applied twice, and (4) applied thrice. Thus, there were 36 experimental units. Each sample consisted of twenty treatments. The observation variables are plant height, number of internodes, flower diameter, pedicels length, number of flowers, initiation age of flower, coloring age of flower, harvest time, and vase life. The chrysanthemum pot vase life was indicated by the time the petal flower experiences senescence or 25% browning. Four plant samples for vegetative growth were observed at 21 days after planting (DAP). The interval of observation was 14 days until the time of harvest.

**Analysis of Data**

The analysis of variance (ANOVA) was used to analyze data. If there were significant differences, the Honestly Significance Difference (HSD) test is applied. Microsoft Excel and SPSS software were used to arrange data and to conduct the analysis.

**RESULTS AND DISCUSSION**

**Number of Internode**

Based on the plant height, there is an interaction between the types of chrysanthemum plants and the frequency of daminozide. Higher frequency application of daminozide resulted in shorter plants (Fig. 1). Three applications of daminozide resulted in the shortest of plant height. The frequency of application of daminozide three times to fast-, moderate-, and slow-growing plants resulted in plant heights of 16.08 cm, 7.38 cm, and 6.21 cm, respectively, compared to control plants.

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**Fig. 1.** Plant height on three chrysanthemum types on three differences of daminozide application frequency namely: Without (F1), Once (F2), Twice (F3), and Thrice (F4) application; A, B, and C are fast-growing, moderate-growing, and slow-growing types respectively.
The chrysanthemum plant height was reduced along with the rising concentration and frequency of daminozide application (Kannan, Jawaharlal, Singh, & Ganesh, 2016; Sitawati & Ni’mah, 2018). The decline in plant height caused by the daminozide application was possible due to the decreasing of cell numbers in the stem, and apical suppression which prevents cell division. The standard of plant height is 1.5-2 times the height of the pot, where the pot height is 12 cm, it is expected that the height of the chrysanthemum flower is 18-24 cm at harvest. Based on Fig. 2A, the relationship between the application frequencies of daminozide, plant height, and internode numbers on three chrysanthemum types.
on three types of potted chrysanthemums showed that Tobaga (fast-growing), Time Jewel (moderate-growing), and Cosmo Magenta (slow-growing) required daminozide application three times, once, and 0 times to produce a chrysanthemum height of approx. 18-24 cm. Generally to control plant size, daminozide is used (Papafotiou & Vagena, 2012). The inhibiting of enkaurene to enkaurene acid in the gibberellin formation pathway that inhibit the endogenous gibberellin is the action mechanism of daminozide. This is also described by Kannan, Jawaharlal, Singh, & Ganesh (2016) that daminozide inhibits the biosynthesis or action of gibberellins which play a role in cell elongation (Suhadi, Nur hidayati, & Sharon, 2017). So that plants given daminozide contain lower endogenous gibberellins which result in inhibition of plant height.

Based on the difference in the growth phase, the number of internodes in the three types of chrysanthemum showed differences in plant appearance at different frequencies of Daminozide application (Fig. 2B). The frequency of application of daminozide led to a reduction in the number of internodes. Increasing the frequency of daminozide will reduce the number of chrysanthemum segments in fast-, moderate-, and slow-growing chrysanthemums Fig. 2B.

The decrease in the number of stem internodes has an impact on the decrease in plant height, by the use of growth inhibitors. Application of growth inhibitors provides some physiological effects on plants such as inhibiting cell elongation in subapical meristems, shortening plant segments, thickening stems, inhibiting etiolation, inhibiting aging, extending shelf life, increasing fertilization, helping germination, and sprouting (Wattimena, 1987). Suhadi, Nurhidayati, & Sharon (2017) reported that daminozide as a growth inhibitor plays a role in inhibiting cell elongation, and the addition of stem segments by reducing biosynthesis of gibberellin.

In inhibiting biosynthesis of gibberellin, daminozide will ultimately reduce cell proliferation, and this cell proliferation will result in the reducing of leaf expansion and stem extension (Sumadi, Suminar, Murgayanti, & Nuraini, 2015). The mechanism of decreasing height by daminozide initiates by inhibition of the active GA formation, which plays a role in stimulating cell elongation. The application of daminozide causes the active GA formation in the form of GA8 to be inhibited and results in the slowing of extension and cell division in the subapical meristem (Rademacher, 2010; Schmieder, Grausgruber-Gröger, Grassi, Steinborn, & Novak, 2010). The application of daminozide can reduce the segment length of chrysanthemum by enhancing the concentration and frequency of application of daminozide (Papafotiou & Vagena, 2012). Banko & Stefani (1988) explained in more detail that the application of Daminozide reduced plant length and height in Yellow Marvel Z. elegans and liliput plants.

### Palisade Length

Based on the cross-section of the leaves, it was shown that the leaves of the three types of chrysanthemum had mesophyll tissue which could be distinguished based on palisade and sponge tissue. Fig. 3 describes the morphological structure of the leaf tissue on the three chrysanthemum types. Based on the palisade length, the three types of plants that were given Daminozide experienced an increase in the length of the palisade tissue. The frequency of application of Daminozide will increase palisade parenchyma cells and can further increase leaf thickness in plants, to stimulate plant photosynthetic activity.

The palisade thickness as a plant modification, it can enhance the photosynthesis efficiency because there are chloroplasts in the palisade tissue (Fahn, 1990). The enhancing of palisade length and thickness of leaf were triggered by a decrease in leaf area due to the daminozide application. According to Kasele, Shanahan, & Nielsen (1995), the growth inhibitor alter the plant morphology and vegetative growth by creating smaller plants and thicker leaves through the increasing of stomata, chlorophyll, and protein content. The thicker leaves were consistent to the longer palisade tissue. The application of retardant can inhibit the growth and internode growth, and shoot inhibition. Inhibition of shoots can stimulate the results of photosynthesis used for the formation of other tissues. This mechanism can explain that the inhibition of plant height growth due to the application of the retardant causes the assimilation results to be distributed to other tissues such as being used for thickening of leaf tissue.

### Long Pedicellus

Plants with fast growth phase have a higher pedicellus than plants with medium and slow growth phase (Fig. 4). The difference in the length of the pedicellus is influenced by the difference in
the growth phase of each type of chrysanthemum used in the research. Red plants have a fast growth phase, while white bone plants have a medium growth phase and pink plants have a slow growth phase. So the plants will respond to the length of the pedicellus according to the growth phase. Meanwhile, if it is reviewed based on the influence of the application frequency of daminozide that the without daminozide application provide the higher pedicellus length than to the applied daminozide one. In the flowering initiation, the daminozide application provides the shorter pedicellus and stimulates the blooming synchronously (Cahyono & Ismail, 1999). In addition, the daminozide application in the vegetative phase, produces the shorter internode stems than the none (Cahyono & Ismail, 1999). So plants that are applied Daminozide when entering the phase of flower formation will reduce the length of the pedicellus on the plants.

**Amount of Flowers**

The increasing number of flowers are produced by the daminozide application (Fig. 4). The greater number of flowers in plants with Daminozide application is supported by increased chlorophyll content and stomata density in chrysanthemums with the increasing frequency of Daminozide. The application of daminozide to chrysanthemum caused the dark green leaf color. This is related to the accumulation of chlorophyll per leaf area, where a reduction in leaf area leads to higher chlorophyll accumulation in smaller leaves. Dark green leaves indicate that the chlorophyll content is higher and has a higher yield potential (Taiz & Zeiger, 2002; Yudiwanti, 2007). The darker leaves increase the efficiency in the light capturing to support the higher photosynthetic activity, and then the photosynthates are distributed to the other tissues (Taiz & Zeiger, 2002).

Chrysanthemum plants that were given daminozide more often had a stronger and more durable plant morphology. It’s because of the higher number of stomata increases the flow of carbon dioxide into the mesophyll tissue that can reduce the photosynthates. This process finally, distributes the photosynthates into the plant tissue (Lakshmipathi, Adiga, Kalaivanan, & Halesh, 2017). So that the plants that were applied with daminozide had a higher number of flowers.

**Flower Diameter**

Flower crown diameter that has bloomed > 75% indicates that the frequency of daminozide has no significant effect on flower diameter for each type of chrysanthemum based on growth rate (Fig. 4). Daminozide does not affect flower diameter on chrysanthemum plants (Papafotiou & Vagena, 2012). The size of flowers that were treated by B-nine application had the same size with without application. Differences in flower diameter are influenced by types of chrysanthemum. The moderate-growth plant type provides the larger diameter of flower than the others (Burnett, Keever, Kessler Jr., & Gilliam, 2000). The flower diameter are related to the genetic impacts on the chrysanthemum types (Fig. 5).

![Fig. 3. Cross-section of chrysanthemum leaf tissues that treated by once, twice, thrice, and zero applications of daminozide](image-url)
Fig. 4. Morphological indicators based on the three plant types (A) and the application frequency of daminozide (B). Different letters in the same morphological indicators describes the differences based on Tukey HSD (at α < 5 %); DAP, DAH, and HSD are days after planting, days after harvesting and, Honestly Significance Differences respectively.
Flowering Initiation

When the plant initiates to create flower buds, the initiation time of flower was observed (the physiological changes are followed by the changes on morphological characters). The type of chrysanthemum and the application frequency of daminozide affect the age at which flowers appear. Plants with application frequency three times, namely at the age of 2, 4, and 6 WAP had the slowest period of flowering (Fig. 4). The flowering time of Achillea plants on B-Nine application was slower than the none and the other species (Burnett, Keever, Kessler Jr., & Gilliam, 2000). This may be due to the daminozide role as an anti-gibberellin which inhibits biosynthesis. Paclobutrazol inhibits gibberellins in the sub-apical meristem which then causes a decrease in the speed of cell division, thereby inhibiting vegetative growth which is required for flower, fruit and fruit development (Wattimena, 1987). Paclobutrazol and daminozide are anti-gibberellins that role to inhibit plant growth.

Harvesting and Coloring of Chrysants

Coloring of flower was observed when the primordial flower had ca. 25% of color. When the flower primordia have shown flower color or the flower petals have seen 2-3 cm, the harvest age is observed. Coloration age and harvest age were faster in plants that were not given daminozide (Fig. 4). The application of daminozide will inhibit the gibberellin in the terpenoid pathway by constraining enzymes that catalyze the metabolic reaction. Inhibition of cell division due to inhibition of gibberellin function will result in inhibition of vegetative growth needed to form flowers. GA deficiency in Arabidopsis and rice plants will interfere with other development and pollen formation (Zhang et al., 2014). The application of daminozide causes the delay of flower formation, and the initiation, coloring, and harvesting processes will be slower than the without application one.

Flower Vase Life

Flower freshness was shorter in plants that did not apply daminozide. The treated plants by
Daminozide at once, twice, and three times resulted in longer flower freshness compared to the untreated plants (Fig. 4). Krisantini & Tjia (2011) mentioned that the controlling of the plant height makes the plant size more proportional and stable in size for the potted plants. The shorter and stronger plant postures also make the plant more resistant, which can expand its durability indoors.

Plants that were given daminozide had higher chlorophyll content, stomata density, and leaf thickness. Chrysanthemum that are treated by daminozide frequently will have a darker green leaf color. In addition, application of daminozide on plant stimulates the strong plant morphology and the long freshness. The increasing of stomata number increases CO$_2$ flow to the mesophyll tissue, and then reduced to produce much more photosynthates to be distributed into all tissues (Lakshmipathi, Adiga, Kalaivanan, & Halesh, 2017). In addition, parenchyma palisade cells increases the leaf thickness that can also increase the photosynthesis. Application of daminozide inhibits the growth and the increasing of stem segments (Sambeka, Runtunuwu, & Rogi, 2012).

CONCLUSION AND SUGGESTION

There are four conclusions in this research namely: (1) the increasing of daminozide application frequency on three types of chrysanthemum is effective to control the height and flower vase life; (2) three application frequency of daminozide on plants reduced the flower senescence at 40.49% for Tobaga (fast-growing type), 31.06% for Time Jewel (moderate-growing type), and 31.64% for Cosmo Magenta (slow-growing type); (3) the application of daminozide enlarged the palisade thickness, and reduced the number of plant internodes; and (4) based on application of daminozide, fast-growing, medium-growing and slow-growing types requires thrice (at 2, 4, 6 WAT), twice (at 2 and 4 WAT), and zero application respectively to produce potted chrysanthemum about 18-24 cm height.

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