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Increasing the growth and yield of shallot (*Allium cepa* L. Aggregatum group) by using Methyl Jasmonic Acid (MeJA) concentrations under drought condition

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Abstract

Drought is one of the limiting factors for plant production, and there is a continuous demand for drought tolerant plants. Shallots are considered a shallow-rooted crop and have been reported to have little tolerance to drought conditions. One of the efforts to increase plant tolerance to drought conditions is through the addition of Methyl Jasmonic Acid (MeJA). MeJA is involved in several physiological and biochemical procedures in plant growth and development. Application of MeJA can increase the plant tolerance to drought conditions through chlorophyll synthesis, stomatal conductivity, transpiration, net photosynthetic rate and biomass production. This research was conducted in a complete randomized block design with three replications to determine the response of shallot plants to the application of MeJA (0 µM, 25 µM, 50 µM and 100 µM) under drought conditions, which was simulated through the frequency of watering (once daily, every other day, once in three days). Soil water content calculated before the watering treatment was 24.45%, 20.34% and 18.45% for watering once daily, every other day, once in three days, respectively. The results showed that the addition of MeJA played a role in enhancing the growth and productivity of shallot plants under normal and drought conditions. Application of 50 µM of MeJA could increase the Water Use Efficiency, maintain the Relative Water Content, increase the width of stomatal aperture, and increase the leaf area and Leaf Area Index. This also led to increasing Net Assimilation Rate and Plant Growth Rate. Application of 50 µM of MeJA gave in increasing bulb productivity and reached 7.86 ton.ha^{-1}, which was 58.2 % higher than that of in control (without MeJA application). Application of MeJA to shallot plants exhibited avoiding type of physiological tolerance.

INTRODUCTION

Shallot (*Allium cepa* L. Aggregatum group) is a vegetable as source of high nutrients consumptions. Root of shallot is characterized shallow roots. Rao (2016) stated that shallow roots make it vulnerable to environmental stresses, such as water deficit. Significant reduction in bulb weight and size is an ultimate indication of significant reduction of yield when onion plants experience water deficit (Pelter et al., 2004; Rattin et al., 2011). It is a major stress factor for plants associated with climate change. Water deficit is a condition where the ground water availability is below evapotranspiration, thereby adversely affecting the plant growth and development. Under such condition, guard cells will be closed to reduce water loss. The impact of such water loss reduction will also reduce the nutrient absorption and efficiency.

Anatomical changes under drought stress condition include damaged and decreased size of cell in roots, stems and leaves (Lipiec et al., 2013); because when water potential outside decreases, there will also be decrease in turgor due to disturbed water flow from

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the xylem. This condition will inhibit cell elongation and limit the cell division due to mitotic disorders. Physiological activity is related to the biochemical activity in the plants. Under a drought conditions, the water potential decreases, which leads to increasing ABA (abscisic acid) levels in the cells. Plants experiencing drought stress generally produce ABA compounds as well as dehydrin protein, which functions as an osmoprotectant (Yamaguchi-Shinozaki et al., 2002). Furthermore, ABA synthesized in the roots will be transported to leaves to regulate physiological processes and increase soil water availability by inhibiting water loss through stomata aperture.

To cope with draught stress condition, plant have three mechanisms: the escape, the avoidance and the tolerance. The escape is the ability of plants to complete their life cycle before extreme drought conditions, such as early flowering or fruiting. The avoidance is the ability of plants to store much water potential in the tissues to keep high cell turgidity either by reducing water loss or increasing water absorption, such as by deepening the root system. The tolerance is the ability of plants to adjust cell osmotic that keeps high turgidity by accumulating solutes in the cells, such as proline, glycine betaine, sugar and several other organic ions (Lipiec et al., 2013). Drought has affected many aspects of plant growth and slowed germination (Shekari, 2000). Stress in vegetative phase causes the decrease in stomatal conductivity, clean photosynthesis and yields (Kerepesi & Galiba, 2000).

MeJA is involved in several physiological and biochemical procedures in plant growth, such as seed development, leaf growth and aging. Jasmonates can modify aspects of plant growth and resistance toward various abiotic stresses such as drought, salinity, thermal stress, heavy metals, and flooding. Generally, addition of jasmonate can protect plants from environmental stresses by regulating physiological and biochemical responses. (Farhangi-Abriz & Ghassemi-Golezani, 2018). The application of jasmonic acid to plants under abiotic stress can be carried out through physiological mechanisms, such as nutritional balance, modification of photosynthetic processes and electron transfer, modification of roots and shoots, increasing the stomatal conductivity, changing of water absorption and leaf expansion, remobilizing the photo assimilation of vegetative parts and increasing carbon fixation in plants. The biochemical defense mechanism is by increasing the antioxidant defense system, producing the osmolyte, stimulating chlorophyll biosynthesis and glyoxalase system, increasing ascorbate and glutathione metabolism, stimulating phenolic compound biosynthesis and crosstalk in abscisic acid signaling and ethylene production.

Wu et al. (2012) reported that spray application of 10 µM Methyl Jasmonic Acid (MeJA) on cauliflower (*Brassica oleracea* L.) was able to increase the plant tolerance to drought conditions through chlorophyll synthesis, stomatal conductivity, transpiration, net photosynthetic rate and biomass production. In addition, Ilyas et al. (2017) reported that the application of 100 µM of jasmonic acid to wheat seeds (*Triticum aestivum* L.) priming could increase the percentage of germination, accumulation of proline and dissolved carbohydrates, and shoot growth of wheat plants under drought conditions. Several studies indicate the beneficial role of the applying MeJA for regulating the physiological processes, plant water relations and induction of antioxidant defense mechanisms that are important for plant tolerance toward water deficit. In shallot plantation, farmers give watering through field canal irrigation, which consumes much water from irrigation facilities (Saleh and Atmaja, 2017). Water irrigation becomes problem in dry season when debit water is low, and shallot plantation is in drought condition. Solichah and Rangga (2018) reported that shallot production in Brebes was highly dependent on the availability of water supplied through pump irrigation during the dry season. Shallot plant tolerance to drought conditions may be induced by MeJA application, and this research was conducted to determine the response of the growth and yield of shallots towards the application of MeJA under drought conditions.

MATERIALS AND METHODS

Location and time

This research was carried out at the Tri Dharma experimental field (07^{o}48' 17"S and 110^{o}24' 45"E) of Faculty of Agriculture, Universitas Gadjah Mada located at an altitude of 107 m above sea level from April to June 2020. The average air temperature was 32.48˚ C, and the air humidity was 68.14% in average. The soil type of regosol (sandy loam) was used.

Materials

The materials used in this research included shallot bulbs of Bima Brebes variety. It is suitable for lowlands with dry bulbs production reaching up to 9.9 tons.ha^{-1} and good adaptability to dry land agro-ecosystems (PUSLITBANGHORTI, 2015).

Research design

The research was conducted in a plastic house with a factorial arrangement on the basis of Randomized Complete Block Design (RCBD) with three replications. Treatments were different levels of MeJA concentrations (0 µM, 25 µM, 50 µM and 100 µM) and drought condition that was simulated by frequency of watering, consisting of once daily (normal water availability), every other day (moderate) and once in three days (drought condition).

Watering treatment

Watering was applied with same volume of 583,3 ml per plant per week in all plants for two weeks. Then, watering was applied following the treatments by giving water to soil media carefully until water coming out from base of planting box. The calculation of the watering volume is presented in Table 1. Soil moisture content was analyzed by sampling soil in 20 cm depth before watering. Soil sample treated with watering once daily showed field capacity moisture content of 24.45%; Soil samples with watering every other day and once in three days showed moisture content of 20.34% and 18.82%, respectively.

Application of Methyl Jasmonic Acid (MeJA) and drought stress treatments

MeJA solution was made by dissolving MeJA ($C_{12}H_{20}O_3$) according to the treatment concentration into distilled water, namely 0 µM, 25 µM, 50 µM and 100 µM. Then, MeJA solution was used for soaking the bulbs during 30 minutes before planting and spraying the leaves two weeks after planting by 9 ml/tray. As a control, plants without MeJA application were administered. Regular watering was carried out every day until day-14, then watering treatments were applied on each experimental until day-50.

Preparation of the planting and the planting media

Twelve bulbs were planted on each tray measuring 45 cm × 32 cm × 16 cm with a spacing of 10 cm × 10 cm. The planting medium was soil (Regosol) mixed with 20 tons.ha^{-1} of manure and 250 kg.ha^{-1} of NPK fertilizer (16:16:16), 50 kg.ha^{-1} of SP-36 and 30 kg.ha^{-1} of KCl three days before planting. ZA was given as much as 400 kg.ha^{-1} as the second fertilization two weeks after planting, followed by 180 kg.ha^{-1} Urea as the third fertilization four weeks after planting (Agency for Horticulture Research and Development, 2016).

Table 1. Plant water demand per week (ml)

Watering frequency	Concen-tration of MeJA (µM)	Amount of water given per plant per week (ml)									Amount of water during growth (L)
		Early vegetative phase				Amount of water per plant per day	Maximum vegetative phase			Amount of water per plant per day	
		1	2	3	4		5	6	7		
Once daily	0	583.33	437.5	437.5	354.17	60,42	520.83	479.17	666.67	79,36	3.5
	25	583.33	437.5	437.5	354.17	60,42	520.83	479.17	666.67	79,36	3.5
	50	583.33	437.5	437.5	354.17	60,42	520.83	479.17	666.67	79,36	3.5
	100	583.33	437.5	437.5	354.17	60,42	520.83	479.17	666.67	79,36	3.5
Every second day	0	583.33	437.5	250	375	54,86	312.5	500	541.67	64,5	3.0
	25	583.33	437.5	250	375	54,86	312.5	500	541.67	64,5	3.0
	50	583.33	437.5	250	375	54,86	312.5	500	541.67	64,5	3.0
	100	583.33	437.5	250	375	54,86	312.5	500	541.67	64,5	3.0
Every third day	0	583.33	437.5	250	250	50,7	437.5	291.67	479.17	57,54	2.7
	25	583.33	437.5	250	250	50,7	437.5	291.67	479.17	57,54	2.7
	50	583.33	437.5	250	250	50,7	437.5	291.67	479.17	57,54	2.7
	100	583.33	437.5	250	250	50,7	437.5	291.67	479.17	57,54	2.7

Soil moisture content analysis

Soil moisture content for shallot plants aged 14 days after planting was measured by taking the soil samples given with both normal (field capacity), moderate, and drought-stressed treatments before and after treatments. The sample was weighed to obtain fresh weight. Then, it was put into the oven at a temperature of 120^{o}C for 48 hours and weighed to obtain the dried weight. Then, the difference between the initial and final weights were calculated to obtain the value of soil moisture content of each soil condition and treatment by using the following formula:

$$\text{Moisture content (\%)} = \frac{\text{a-b}}{\text{b}} \times 100\%$$

Remarks:
a = fresh weight of soil
b = dried weight of soil

Measuring water requirement for plant

Water demand of shallot plants aged 0 to 50 days after planting (harvest) was measured by recording the daily water requirement of each planting media tray.

Water Use Efficiency (WUE)

Water Use Efficiency was calculated based on the amount of water needed by plants in one life cycle, which was up to 50 days after planting for shallot. The amount of watering was adjusted to the value of the field capacity. Plant dry weight was measured by measuring plant weight (g.plant^{-1}) that had been dried in an oven at 80^{o}C to reach a constant weight value, then the calculation formula for Anyia & Herzog (2004) was applied as the following:

$$\text{WUE} = \frac{\text{dry weight of the plant (g)}}{\text{water requirement of each plant (Liter)}}$$

Relative Water Content (RWC)

Relative Water Content was carried out by taking leaf samples and measuring the fresh weight (fw). Then, the samples were then put into plastic filled with water and let sit for 24 hours. After the soaked leaves were taken out, water attached to the surface was cleaned using a tissue. Next, the turgid weight (tw) was weighed. The leaves were then dried in an oven at a temperature of 80^{o}C to reach a constant weight as the dry weight (dw). Relative Water Content (RWC) was calculated under the formula by Jensen et al. (1996) as follows:

$$\text{Relative water content} = \frac{\text{fw-dw}}{\text{tw-dw}} \times 100\%$$

Remarks:
fw = fresh weight
dw = dry weight
tw = turgid weight (saturated)

Analysis of proline

Analysis of proline content was carried out based on Bates et al. (1973) by taking 0.5 g of finely mortar -pounded leaves as the sample in 10 ml of 3% sulfosalicylic acid solution. The pounded result was filtered using the Whatman 2 filter paper. Two ml of the filtrate was reacted with 2 ml of ninhydrin acid (made by heating 1.25 g of ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 µM phosphoric acid until dissolved) and 2 ml of glacial acetic acid in a test tube at 100^{o}C for one hour. The reaction was ended by inserting the test tube into a beaker containing ice. The mixture was extracted with 4 ml of toluene then shaken with a stirrer for 15–20 seconds. The red toluene containing proline at the top was taken using a pipette. The solution absorbance solution was read by using the CARY 50 CONC Variant Spectrophotometer at 520 nm wavelength with a toluene blank.

$$\text{Proline content} = (64.3649 \times \text{absorbance reading} + (-5.2987)) \times 0.347$$

The width of stomatal aperture

Observation of the width of the stomatal aperture was done by making a print on the underside of the leaf using transparent nail polish. Then, the section was pasted with transparent insulation before carefully peeled off to obtain the stomata prints. The isolation was attached to the object glass for further observation at 40 × 10 magnification. The observation results were documented using the Optilab viewer and measured by using the Image Raster software in µm units (Rahayuningsih, 2017).

Leaf area

The leaf area was observed on shallot plants aged 7 weeks after planting by using a leaf area measuring device and applying the scanner method (Smika and Klute, 1982). The number shown on the monitor was the size of the leaf area and multiplied by two (the leaves on the shallot plant are cylindrical) to obtain the actual leaf area meter (AT Area Meter MK2) in cm^2.

Leaf Area Index (LAI)

Leaf area index (LAI) was a description of leaf area on a field. It was measured on shallot plants aged 7 weeks after planting. The LAI value was calculated using the equation by Gardner et al. (1991):

$$\text{Leaf area index} = \frac{\text{leaf area (cm}^2\text{)}}{\text{field area (10 cm x 10 cm)}}$$

Net Assimilation Rate (NAR)

Net Assimilation Rate (NAR) was the ability of plants to produce dry weight per unit leaf area per time. NAR was calculated based on the equations by Gardner et al. (1991):

$$NAR = \frac{W2\text{-}W1}{T2\text{-}T1} \times \frac{\ln La1\text{-}\ln La2}{La2\text{-}La1}\ g.cm^{-2}.weeks^{-1}$$

Plant Growth Rate (PGR)

Plant growth rate was the ability of a plant to produce assimilated dry material per leaf area unit per time unit ($g.dm^{-2}.weeks^{-1}$). According to Gardner et al. (1991), Plant Growth Rate (PGR) was calculated under the following equation:

$$PGR = \frac{1}{Ga} \times \frac{W2\text{-}W1}{T2\text{-}T1}\ g.dm^{-2}.weeks^{-1}$$

Remarks:
LAI = Leaf Area Index
La = Leaf area (cm^2)
Ga = Planting distance (10 × 10 cm^2)
NAR = Net Assimilation Rate ($g.cm^{-2}.week^{-1}$)
PGR = Plant Growth Rate ($g.cm^{-2}.week^{-1}$)
W1 = dry weight per planting time 1 (g)
W2 = dry weight per planting time 2 (g)
T1 = Observation time 1 (4 weeks)
T2 = Observation time 2 (7 weeks)

Plant dry weight

The plant dry weight of samples was determined after dried in an oven at 80°C temperature for 2 × 24 hours or until constant dry weight (grams) was reached when the plant were 7 weeks after planting. Then, each part of the roots, leaves and bulbs was weighed.

Bulb weight

The selected bulbs were then cleaned from the soil and dried without direct sunlight for approximately one week, then the weight of the bulbs was weighed in grams.

Harvest index and productivity

Harvest index was calculated as the ratio of economic weight (bulbs) to biological weight (plants) in percent. Productivity was calculated as the ratio of yielded bulb to the harvested field (tonnes/h).

Data analysis

Data were analyzed using the SAS 9.4 software with analysis of variance followed by the HSD/Tukey test at 95% of confidence level.

RESULTS AND DISCUSSION

Water demand of the plants and soil moisture content

Water demand of the shallot plants varied on each growth phase (Table 1). During the early vegetative phase, when the shallot plants aged 1–4 weeks after planting, water demand was low, which was 60.42 ml, 54.86 ml, and 50.7 ml per plant per day on watering once daily, every other day and once in three days, respectively. It began to increase to maximum vegetative phase, which were 79.36 ml, 64.5 ml and 57.54 ml per plant per day on watering once daily, every other day and once in three days, respectively, when the shallot plants aged 5-7 weeks after planting. As the plant entered 5 weeks after planting due to the beginning phase of bulbs formation, water consumption increased.

The experiment of watering frequency was an approach to drought stress condition within a certain period. Once daily watering frequency was the optimal water content condition, where the water content was approximate to the field capacity of 24.45%–28.86 %. The two-day and three-day watering frequency showed a decrease in soil moisture by 16.8% and 24.54% from the initial condition, respectively. However, this value had not reached the permanent wilting point. Table 2 shows that longer watering frequency leads to low soil moisture content. The three-day watering frequency significantly reduced

Table 2. Soil moisture content of shallot plants aged 21 days after planting (%)

Watering frequency	Soil moisture content (%)	
	Before watering	After watering
once daily	24.45 ± 0.53	28.86 ± 1.33
every 2 days	20.34 ±0.96	24.01 ± 0.24
every 3 days	18.45±0.87	21.31 ± 0.21

Table 3. Water-use efficiency (g/L) of shallots plants aged 7 weeks after planting

Watering frequency	MeJA concentrations				
	0 µM	25 µM	50 µM	100 µM	Average
once daily	0.88	0.99	1.03	0.96	0.96 a
every 2 days	0.57	0.91	1.21	0.83	0.88 a
every 3 days	0.55	0.75	1.02	0.59	0.73 a
Average	0.67 q	0.88 pq	1.09 p	0.80 pq	(–)
CV (%)	7.89				

Remarks: Numbers followed by the same letter in the same column or row show no significant difference based on 5% Tukey test. The sign (–) indicates there is no interaction between factors.

Table 4. The relative water content (%) of shallots plants aged 6 weeks after planting

Watering frequency	MeJA concentrations				
	0 µM	25 µM	50 µM	100 µM	Average
once daily	86.84	89.84	87.44	88.93	88.26 a
every 2 days	85.37	86.74	87.95	88.58	87.16 a
every 3 days	82.13	85.28	85.45	82.13	83.89 b
Average	84.97 p	87.28 p	86.95 p	86.55 p	(–)
CV (%)	3.46				

Remarks: Numbers followed by the same letter in the same column or row show no significant difference based on 5% Tukey test. The sign (–) indicates there is no interaction between factors.

the soil moisture, both before and after watering. However, after watering, soil moisture returned to normal at field capacity conditions.

Water-Use Efficiency (WUE)

Under drought conditions, plant can reduce water use by increasing its water use efficiency (Blum, 2005). Water Use Efficiency describes the amount of carbon assimilated as biomass produced per unit of water used by a plant within its life cycle. Water deficit leads to low ability of the plants to use water to produce plant biomass. The three-day watering frequency causes decreasing WUE, there by producing low biomass. Meanwhile, the MeJA at 50 µM significantly increased WUE in compare to the control. MeJA at 100µM concentration can reduce WUE (Table 3). Meanwhile, application of MeJA at 50µM can increase plant growth through the increased WUE. Barati and Ghadiri (2017) reported that prolonged water stress over a certain period could affect early fruit development, CO_2 assimilation, and dry mass accumulation leading to serious yield reductions, thereby lowering plant WUE. This is consistent with other studies in tomatoes, potatoes and eggplant, reporting that water stress can affect early fruit development, CO_2 assimilation, and biomass accumulation leading to serious yield reductions, and thus lower WUE (Kang et al., 2004; Ertek et al, 2006b; Cantore et al., 2016).

Relative water content

Drought condition can decrease cell turgor, increase macromolecular concentration and affect cell membranes and the potential for chemical activity of water in plants (Mubiyanto, 1997). Three days watering frequency showed the lowest relative water content value compared to one-day and two-day watering frequency. This indicated that drought stress could reduce water content in plant cells. Meanwhile, the implementation of MeJA indicated the same relative water content value (Table 4).

Such similar relative water content valuesindicated that plants tended to maintain turgidity for cell elongation and division.

Proline

Under drought condition, the water potential was reduced and ABA levels in the cells increased. Thus, plants responded by adjusting cell osmosis to maintain high turgidity by accumulating solutes, include proline, glycine and betaine. According to Todaka et al. (2015), drought conditions cause a decrease in cell water potential and turgor, which increases the concentration of inter- or intracellular solutes and other adverse effects on plant growth. Based on Table 5, there was a tendency of increasing proline levels by the two-day and three-day watering frequency, while there was a decrease in proline levels in the MeJA application, although statistically it was not significantly different. The cause can be attributed to the modification of gene expression due to the addition of MeJA. According to Wager (2012) and Boex-Fontvieille et al. (2016), jasmonic acid and its derivatives induce the expression of defense coding genes such as proteinases, thionins and proteinase inhibitors. The enzymes that play a role in the synthesis of proline are pyrroline-5-carboxylate synthetases (P5CS) and pyrroline-5-carboxylate reductases (P5CR). Possibly, the presence of JA can inhibit the performance of these enzymes so that the proline content decreases. This indicated that the plant was not experiencing drought stress.

Width of the stomatal aperture

Table 6 indicates that one-day watering frequency without MeJA application obtained the largest width of stomatal aperture compared to other treatments. It decreased in the two-day and three-day watering frequency. This was due to the inhibitory effect of stomata closure due to drought stress (Sarabi et al., 2019). This response results in a reduction in the rate of transpiration so that water loss can be limited, and CO_2 diffusion is reduced. This results in a decrease in the photosynthesis process in plants (Shao et al., 2018).; Zhu et al., 2020; Dawood and Abeed, 2020). Stomata are the guard cells, the entry and exit of water and air used by plants for metabolic processes (photosynthesis, transpiration and respiration). Large stomatal aperture width can increase CO_2 diffusion in plants. Adequate supply of water will enable the plants to carry out optimal photosynthesis because water is one of the main ingredients in the photosynthesis process, where the process of reducing carbon dioxide into carbohydrates or glucose occurs. However, MeJA at 50 µM could increase the width of stomata aperture

Table 5. Proline content (µmol/g)of shallot plants at the age of 7 weeks after planting

Watering frequency	MeJA concentrations				
	0 µM	25 µM	50 µM	100 µM	Average
once daily	1.98	1.83	2.05	1.34	1.80 a
every 2 days	3.45	2.05	0.60	2.46	2.14 a
every 3 days	2.26	1.90	2.73	2.17	2.26 a
Average	2.56 p	1.92 p	1.79 p	1.99 p	(–)
CV (%)	18.34				

Remarks: Numbers followed by the same letter in the same column or row show no significant difference based on 5% Tukey test. The sign (–) indicates there is no interaction between factors.

Table 6. Width of stomata aperture in shallot leaves aged 6 weeks after planting

Watering frequency	MeJA concentrations				
	0 µM	25 µM	50 µM	100 µM	Average
once daily	7.60 ab	7.73 a	6.27 bc	5.83 dc	6.86
every 2 days	5.47 dc	5.33 dc	6.10 c	5.57 dc	5.62
every 3 days	4.63 d	5.57 dc	7.87 a	6.23 bc	6.08
Average	5.90	6.21	6.74	5.88	(+)
CV (%)	7.98				

Remarks: Numbers followed by the same letter in the same column or row show no significant difference based on 5% Tukey test. The sign (–) indicates there is no interaction between factors.

in water deficit condition (three-day watering frequency). This was the impact of the turgor maintenance effect on plant cells associated with stomatal aperture. Wu et al. (2012) stated that the spray application of MeJA to cauliflower (*Brassica oleracea* L.) was able to increase the plant tolerance under drought conditions through chlorophyll synthesis, the stomatal conductance, the transpiration, the net photosynthetic rate and the biomass production.

Leaf Area and Leaf Area Index (LAI)

Glucose from photosynthesis is then broken down into protein molecules that are useful in the formation of plant vegetative organs, one of which is leaf. Watering frequency had a significant effect on leaf area and leaf area index. One-day watering frequency showed the highest leaf area and leaf area index, while plants with water deficit (three days watering interval) showed the lowest leaf area and leaf area index values. Applying MeJA in various concentrations significantly affected the value of leaf area and leaf area index. Application of MeJA at 50 µM significantly increased leaf area and leaf area index. Shallots without application of MeJA showed the lowest leaf area and leaf area index values (Table 7).

The leaf area index ranged from 1-2. This indicates that the plant leaves do not shade each other because the leaf morphology is like a pointed pipe that grows upwards with little chance that the leaves shade each other. Large leaf area in shallots illustrates the high ability to capture sunlight radiation, which plays a role in the photosynthesis process. The formation of a larger leaf area can maximize the process of photosynthesis, thus enabling the production of greater assimilation. However, under drought stress conditions, the leaf area produced was narrower when compared to conditions without stress. High MeJA concentration actually inhibited the shallots expansion. Leaf area and LAI continued to increase by MeJA at 50 µM concentration, then decreased at 100 µM. Its application could inhibit morphological and physiological changes in plants. The pleiotropic action depended on the given concentration. Zalewski et al. (2010) stated that the application of MeJA at 100 µM could inhibit germination, growth and leaf expansion in some plants. Similar to this study, the application of MeJA at 25 µM and 50 µM could increase the leaves expansion, then decrease as the concentration of MeJA was increased. An increase in jasmonic acid on one hand and a decrease in gibberellin levels on the other caused inhibition of plant leaf growth. Low levels of gibberellins cause a decrease in the rate of cell division and cell differentiation so that vegetative growth decreases (Weaver, 1972).

Net Assimilation Rate (NAR) and Plant Growth Rate (PGR)

The inhibition of leaf expansion would have an impact in the form of the decreasing ability of the leaves to absorb light. High leaf area increased NAR and PGR values. As in the case of MeJA at 50 µM, it significantly increased NAR and PGR compared to other treatments (Table 8). Several components

Table 7. Leaf area (cm^2) and leaf area Index of shallot plants aged 6 weeks after planting

Treatment	Leaf area (cm^2)	Leaf area index
Watering frequency		
once daily	212.22 a	2.12 a
every 2 days	183.87 ab	1.84 ab
every 3 days	137.35 b	1.40 b
MeJA concentration		
0 µM	128.89 q	1.29 q
25µM	183.65 pq	1.84 pq
50 µM	225.61 p	2.26 p
100 M	173.11 pq	1.73 pq
Interaction	(–)	(–)
CV (%)	6.94	17.63

Remarks: Numbers followed by the same letter in the same column or row show no significant difference based on 5% Tukey test. The sign (–) indicates there is no interaction between factors.

Table 8. Net assimilation rate ($mg.cm^{-2}.week^{-1}$) and plant growth rate ($mg.dm^{-2}.week^{-1}$) of shallot plants aged 3–6 weeks after planting

Treatment	Net assimilation rate ($mg.cm^{-2}.week^{-1}$)	Plant growth rate ($mg.dm^{-2}.week^{-1}$)
Watering frequency		
once daily	9.79 a	1608.0 a
every 2 days	9.25 a	1476.7 a
every 3 days	10.07 a	1202.8 a
MeJA concentration		
0 µM	8.10 q	973.7 q
25µM	7.31 q	1233.0 q
50 µM	13.27 p	2136.3 p
100 M	10.13 q	1374.8 q
Interaction	(–)	(–)
CV (%)	14.92	17.04

Remarks: Numbers followed by the same letter in the same column or row show no significant difference based on 5% Tukey test. The sign (–) indicates there is no interaction between factors.

Table 9. Total fresh weight (g) and dry weight (g) of shallot plants at 7 weeks after planting

Treatment	Fresh weight (g)	Dry weight (g)
Watering frequency		
once daily	5.12 a	0.56 a
every 2 days	4.47 ab	0.53 a
every 3 days	3.50 a	0.44 a
MeJA concentration		
0 µM	3.20 q	0.36 q
25µM	4.51 pq	0.50 q
50 µM	5.46 p	0.69 p
100 M	4.25 pq	0.48 q
Interaction	(–)	(–)
CV (%)	13.22	5.21

Remarks: Numbers followed by the same letter in the same column or row show no significant difference based on 5% Tukey test. The sign (–) indicates there is no interaction between factors.

that can affect the rate of plant growth are leaf area and rate of assimilation (Li et al,2016). As reported by Wu et al. (2012), the application of MeJA on cauliflower seeds significantly increases photosynthesis and chlorophyll content and increases biomass production during water deficit condition. In this study, the application of MeJA could significantly increase WUE, leaf area, NAR and PGR, which in turn increased the production and results of biomass.

Total fresh and dry weight

The total dry weight of the plant is the result of accumulated assimilation of the photosynthesis process, one of which is influenced by the availability of water. Based on Table 9, watering once daily resulted in the highest fresh weight and dry weight compared to the two and three-day watering frequency. The application of MeJA at 50 µM significantly increased the fresh weight and dry weight of plants, while at 100 µM, MeJA tended to decrease both (Table 9). This shows that the addition of MeJA up to 50 µM can increase assimilate yields because plant organs (leaves) grow optimally so that the photosynthesis process can run optimally. Meanwhile, under limited water conditions, the photosynthesis process is inhibited so that the assimilate produced is lower.

Bulb weight

The weight of bulb depends on the process of photosynthesis during the growth process. The one and three-day watering frequency had a significant effect on the bulb weight. MeJA concentration also significantly affected bulb weight. The application of MeJA at 50 µM significantly increased bulb weight. However, the application of MeJA at 100 µM reduced bulb weight (Table 10). This is indicated by the appearance of plant morphology, in which the leaf area formed was better than plants without MeJA application. Shallot plants with better leaf area will capture more optimal sunlight so that the photosynthesis process runs more optimally. It can also be seen from the net assimilation rate and higher plant growth rate than plants without MeJA application, which leads to high assimilate formation.

However, the application of MeJA at 100 µM reduced bulb weight. In accordance with Triana and Kumala (2019), high concentration of MeJA (at 100 µM) could reduce the number and weight of bulbs in potato plants. This may be due to the less effective MeJA application in responding to the number and weight of the tubers. This is related to the presence but reduced of gibberellins that can interact with MeJA. The presence of gibberellins can stimulate cell elongation and stolons differentiation into tubers in potato plants (Hasibuan et al, 2015; Rodríguez-Falcón et al., 2006; Muthanna et al., 2017).

Harvest index

Based on Table 11, it can be seen that watering frequency and the application of MeJA exhibit the same harvest index value. A harvest index value of more than 75% indicated that most of the assimilated results from photosynthesis were stored in the form

Table 10. Bulb weight per shallot plant aged 7 weeks after planting

Treatment	Bulb weight (g)
Watering frequency	
once daily	12.01 a
every 2 days	9.41 ab
every 3 days	7.39 a
MeJA concentration	
0 µM	7.45 q
25 µM	10.55 p
50 µM	11.80 p
100 M	8.60 pq
Interaction	(-)
CV (%)	13.82

Remarks: Numbers followed by the same letter in the same column or row show no significant difference based on 5% Tukey test. The sign (–) indicates there is no interaction between factors.

Table 11. Harvest index (%) of shallot plants at 7 weeks after planting

Treatment	Harvest index (%)
Watering frequency	
once daily	78.80 a
every 2 days	76.44 a
every 3 days	77.46 a
MeJA concentration	
0 µM	77.55 p
25 µM	78.88 p
50 µM	78.66 p
100 M	75.18 p
Interaction	(–)
CV (%)	5.15

Remarks: Numbers followed by the same letter in the same column or row show no significant difference based on 5% Tukey test. The sign (–) indicates there is no interaction between factors.

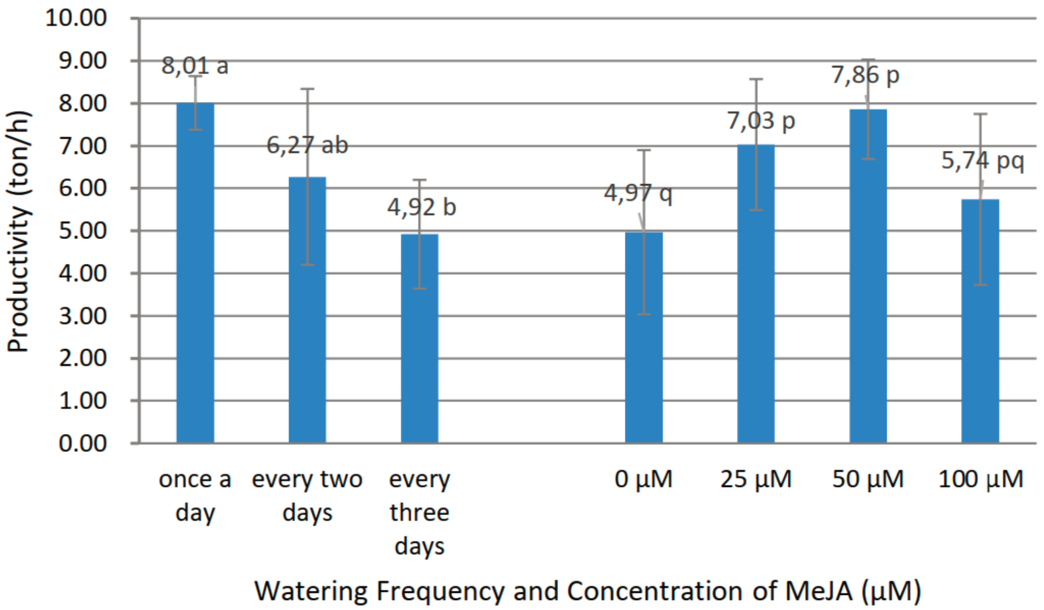

Figure 1. Productivity (tons/h) of shallots at different levels of watering frequency and concentration of Methyl Jasmonic Acid (MeJA). Bars associated with different letters in the frequency of watering and the concentration of Methyl Jasmonic Acid (MeJA) signify difference according at Tukey's test 5%.

of bulbs. Harvest Index value at one-day watering frequency and the addition of MeJA (at 25µM and 50 µM) showed higher value, although it was not significantly different statistically.

Productivity

Watering frequency and MeJA concentration had a significant effect on productivity (Figure 1). The highest productivity was obtained at one-day watering frequency and the application of MeJA at 50 µM. Based on Figure 1, water deficit in shallot cultivation can cause a decrease in productivity, and the addition of MeJA up to at 50 µM was required to increase productivity.

Jasmonic acid (JA) has various effects on plant species, both enhancing and inhibiting physiological processes. In this case, the application of MeJA had a broad impact on shallot plants, not only specific to drought conditions. Under water deficit, MeJA 50 µM can increase plant tolerance through maintenance of relative water content, increase in the width of stomatal aperture and WUE to maintain cell turgidity, resulting in increased growth components such as ILD, NAR, PGR and in yield and productivity. It is called the avoidance mechanism.

The results showed that under normal and stressed conditions, plants would experience a modified gene expression control mechanism, which causes changes in phytohormonal content in plant cells (Per et al., 2018). Linoleic acid, which is a precursor for JA biosynthesis, is oxidized by lipoxygenase. Therefore, stress conditions can increase JA biosynthesis by increasing substrate availability.

Furthermore, the application of MeJA inhibited the morphological and physiological changes of the plants. The pleiotropic action of MeJA is concentration dependent. For example, MeJA in a concentration of 100 µM can inhibit germination, growth, and leaf expansion in some plants (Zalewski et al., 2010). While the application of MeJA at 50 µM can stimulate growth, dry matter accumulation and soybean seed yield (Mabood et al., 2006). The application of MeJA inhibits plant growth because the metabolic process of JA activity is related to the disruption of the biosynthesis and bioactivity of endogenous gibberellins in the plant growth process. Gibberellins in plants can interact synergistically or antigenically with MeJA and be reduced. Gibberellins have the potential to inhibit growth (Pang et al., 2006; Kazan et al., 2012). In this study, the application of MeJA at 50 µM was able to stimulate growth, dry matter accumulation, shallot yield and productivity, while application of MeJA at 100 µM actually inhibited growth and reduced the yield of shallot bulbs.

CONCLUSIONS

The results of this research on the physiological activity of shallots under drought-stressed conditions showed that the application of MeJA at 50 µM could increase plant tolerance exhibiting avoiding type through maintenance of relative water content, increase in the width of stomatal aperture and WUE to maintain cell turgidity. The application of MeJA at 50 µM was able to stimulate growth, dry matter accumulation, yield (11.80 g.plant^{-1}) and productivity (7.86 ton.ha^{-1}), but the application of MeJA at 100 µM actually inhibited growth, thereby reducing yields.

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