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GROWTH RESPONSE OF RED GALANGAL TOWARDS DIFFERENT WATER LEVELS AND MYCORRHIZAL INOCULATION

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ABSTRACT: The aim of this research was to observe the responses of red galangal (*Alpinia purpurata* (Vieill.) K. Schum.) growth on treatment of water levels and mycorrhizal inoculation.This research used experimental method with randomized complete block design. Treatments included two factors: (1) the inoculation of mycorrhizal consisting of two levels i.e. non-mycorrhiza and mycorrhizal plants, and (2) the water content consisting of five levels i.e. 0%, 20%, 40%, 60%, and 80%. Each treatment was repeated three times. Observations were conducted at 10 weeks after planting on the parameters of plant height increase, number of buds, stomatal density, dry weight, chlorophyll content, and percentage of mycorrhizal infection degree. The data were analyzed using Analysis of Variance (ANOVA) and posthoc with Duncan Multiple Range Test (DMRT) at α 5%. The results showed that in the water content of 40%, the red galangal growth still showed normal growth, which can be seen in the parameters of plant height increase, number of shoot, stomatal density, dry weight, chlorophyll content, and percentage of mycorrhizal infection degree.

Keywords : growth, red galangal, water content, mycorrhiza

INTRODUCTION:

Red galangal (*Alpinia purpurata* (Vieill.) K. Schum.) is one type of galangal that has been widely used as a drug ingredient. This plant is one of the spice plants of the zingiberaceae family which has long been exploited by the community as food, beverages and traditional medicine. *Alpinia purpurata* (Vieill.) K. Schum. which is known by the local name red galangal, laos or lazza, is one of the potential medicinal plants to be developed its use (Itokawa and Takeya, 1993). The red galangal rizome can be used as a medicine for some diseases such as anti-inflammatory, analgesic, anti-hyperglycemic, anti-allergy, antimicrobial, antifungal, anticancer, antibacterial and antioxidant (Bermawie et al., 2012). The active compounds contained in the red galangal rhizome of *Alpinia purpurata* K. Schum include flavonoids, kaempferol-3-routoside and kaempferol-3-oliucronide (Victorio et al., 2009).

The productivity and quality of red galangal is influenced by many factors, including soil fertility, planting techniques, climatic conditions and soil water status. Water deficit or drought stress is one of the inhibiting factors plant growth and development (Liu et al., 2011; Fernandez-Lizarazo and Moreno-Fonseca, 2016). Drought stress may result in changes on plant morphology, physiology, and biochemistry, which will adversely affect on plant growth and productivity. The response of plants to water shortages can be seen in the metabolic activities, morphology, growth rate, or their productivity. Drought affects the photosynthetic biochemical reactions, resulting in a decreased rate of photosynthesis (Fitter and Hay, 2001).

One of the ways to reduce the effect of drought stress and increase tolerance to the stress of water shortage in plants is the utilization of Arbuscula Mycorrhiza Fungi (AMF). Arbuscular mycorrhiza fungi (AMF) have been previously reported capable of providing greater resistance to drought stress in some plant species (Liu et al., 2015). This mycorrhiza has the ability to associate with nearly 90% of the plant species (Bonfante and Genre, 2010). Mycelia of AMF expands the root surface resulting in increased nutrient and water uptake by plant roots (Bethlenfalvay et al., 1988) thereby improving water status and increasing drought resistance of the host plant (Allen and Allen, 1986). As according to Fernández-Lizarazo and Moreno-Fonseca (2016) that AMF colonization of plant roots can modify the response of plants on water deficits by improving water status, stomatal conductance, and nutrition status. This modification will increase CO_2 assimilation and photoasimilate production that improve the host plant growth during drought.

MATERIALS AND METHODS:

Planting medium and biological materials

The seedlings of red galangal (*Alpinia purpurata* (Vieill.) K. Schum.) were provided by Hall of Horticulture Seed Development and Various Plants, Pasir Banteng. Planting medium used sterilized at 70 ° C for 2 h on three consecutive days. The medium used consisted of a mixture of soil and manure (2:1). Indigenous mycorrhizal was obtained from Rancabuaya District, Cianjur Regency, West Java, Indonesia.

Experimental design and data analysis

The experiment was conducted in the greenhouse. Pots were arranged in a randomized complete block design with three replications. Treatments included two factors: (1) the inoculation of mycorrhizal (non-inoculated and inoculated mycorrhizal) and (2) the water content (0%, 20%, 40%, 60% and 80%). Observations were conducted on 10 weeks after planting of growth parameters (plant height increase, number of buds, stomatal density, dry weight,

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chlorophyll content, and percentage of mycorrhizal infection degree. The data were analyzed using Analysis of Variance (ANOVA) and if it showed significant difference followed by Duncan Multiple Range Test (DMRT) at α=5%.

Propagation of mycorrhizal

Mycorrhizal were propagated using corn crops (*Zea mays*). Mycorrhizal propagules consisting of spores, hyphae, mycelium, infected soil and roots were cultured on corn crops (*Zea mays*) in polybags. The sterile soil samples of 10 kg were put into polybags, then on each polybag was planted with corn seedling. Ten grams of mycorrhizal propagules were added in the planting medium. Mycorrhizal propagation was carried out until the end of the vegetative period. Furthermore, the soil at a depth of 0-20 cm or around the zone of rhizosphere was used as a source of inoculum.

Water level and mycorrhizal inoculation treatment

Three months old red galangal plants were selected homogeneous and then planted in polybags that have contained planting media. Mycorrhizal inoculants in the form of propagules are given as much as 15 grams for each polybag. Treatment of watering with different water content was done at two weeks after planting. Watering was done every day with different field capacity, i.e. 0%, 20%, 40%, 60%, and 80% until the end of observation. To maintain water content in the media, weighing the soil was done daily and adds water according to the reduced water volume.

Parameters measurement

Observations were made at 10 weeks after planting, which included plant height increase, leaf number, leaf area, bud number, dry weight, stomatal density, chlorophyll content and degree of mycorrhizal infection. The plant height increase was done by calculating the final height difference with the height initial. Leaf area measurements were made using a leaf area meter CI-202 portable laser. Measurements of dry weight were carried out by drying the plant material into the oven at a temperature of 70^0 C to obtain a constant weight. Chlorophyll content was measured with chlorophyll meter Opti-Science CCM-20. Observations of stomata density using a replica method. Transparent nail polish applied to the leaf surface, allowed to dry, then spread transparent nail polish peeled away slowly. Replica of stomata placed on a glass slide and examined under a light microscope with a magnification of 400 x (Shrestha and Kang, 2016).

Mycorrhizal infection

Roots from each treatment were collected and washed gently under running tap water and cut into 1 cm long segments, bleached in 10% KOH 10% for 10-30 minutes, acidified in 1% HCl solution for 30 min, soaked in dye solution of Fuchsin for 10 min. Ten root segments arranged on a glass object, and then observed under a microscope. Calculation of root infections by AMF was done by the slide method (Giovannetti and Mosse, 1980).

RESULTS AND DISCUSSION:

The results showed that water levels significantly effects on plant height increase, leaves number, buds number, stomatal density, dry weight dan chlorophyll content in P<0.05. Also, the results showed that mycorrhiza significantly on plant height increase, leaves number, wet and dry weight, and chlorophyll content in P<0.05. Where as the interaction of water levels and mycorrhizal significantly effects on dry weight in P<0.05.

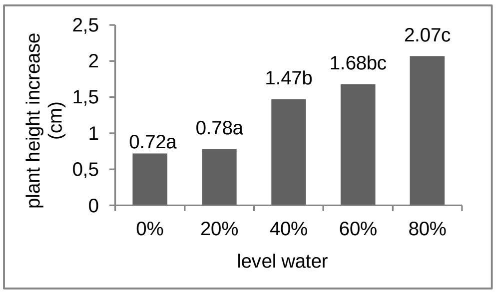

Fig 1. Average plant height increase on different water content. (Means followed by the same letter are not significantly different (P < 0.05)

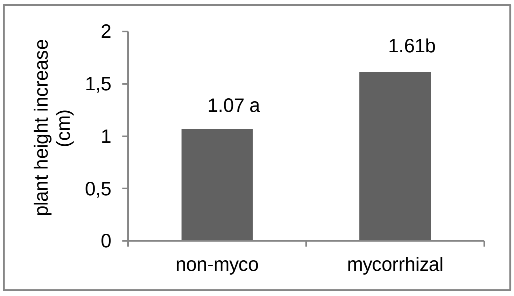

Fig 2. Average plant height increase on mycorrhiza inoculation treatment. (Means followed by the same letter are not significantly different (P < 0.05)

The average plant height increases following the increase in water content in the media. At 80% water content, the average of plant height showed the highest result of 2.07 cm. The decrease of water supply up to 40% in planting medium still showed the average of high plant which is not significantly different with the water content of 60% (**Figure 1**). This showed that water supply up to 40% in planting medium, red galangal plants still have a relatively good tolerance to plant height parameters. Plant height growth is closely related to cell division and enlargement of meristem tissue at the tip of the stem. Under conditions of drought stress, cell elongation in plants is inhibited by reduced turgor pressure. Reduced water absorption leads to a decrease in the water content of the tissues resulting in loss of turgor. Drought stress also reduces

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the photoassimilation and metabolites needed for cell division, the consequences of mitosis are impaired then the extension and expansion of cells decreases (Farooq et al., 2009). Under drought stress, the endogenous auxin and cytokinin content usually decreased (Nielsen and Orcutte, 1996) resulting in inhibition of cell division and elongation.

Figure 2 showed that the average plant height was higher on mycorrhizal red galangal plant (1.61 cm) compared non-mycorrhizal plant. Increased growth in mycorrhizal plants may be due to increased efficiency of P by hyphae of AM Fungi. Phosphorus is needed in the process of cell division in meristem tissue. If the P element increases, it will increase the activity of cleavage, thus affecting the plant height (Mosse, 1981). As reported by Balemi (2009) that the most frequently P stress effect in most crops is the stunted plant growth.

Phosphorus uptake is regulated by phosphatases enzymes (Yan et al., 2001). Mycorrhizal produces phosphatase enzymes that catalyze the hydrolysis reaction of insoluble phosphate compounds in the soil. Bhromsiri and Bhromsiri (2010) that the ability of AMF to absorb phosphorus from organic materials and minerals through the mechanism of increasing the diffusion zone around the roots and the production of phosphatase enzymes. In addition, according to Garg (2013) that AMF can improve the efficiency of N assimilation in plant, increases the activity of the nitrate reductase. Increased status of N in mycorrhizal is due to increased N transport through hyphae, which also increases P concentration required for nitrate reductase phosphorylation when low N concentrations (Caravaca et al., 2005).

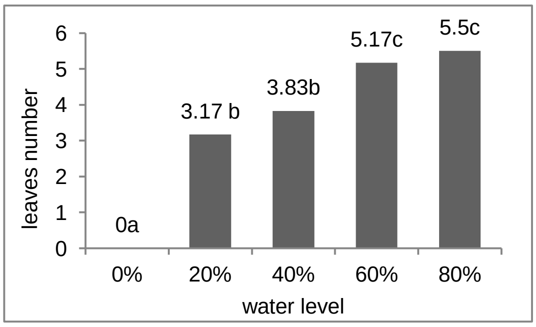

Fig. 3. Average leaves number on different water content. (Means followed by the same letter are not significantly different (P < 0.05)

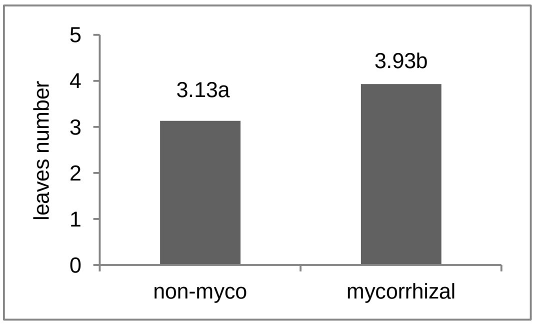

Fig. 4. Average leaves number on mycorrhizal inoculation treatment. (Means followed by the same letter are not significantly different (P < 0.05)

Figure 3 revealed that the highest average number of leaves were in 60% and 80% water content, respectively ie. 5.17 and 5.5. The decrease of water supply to 20% in planting medium still showed the average number of leaf that is not significantly different with the water content of 40%. This showed that at water content up to 60% in medium, red galangal plant still had a relatively good tolerance to leaf number parameter. Leaf growth is the first physiological process affected by drought stress. Leaf growth stops rapidly at the beginning of water deficit and it also stimulates leaf senesence (Frank et al., 1996).

The data in **Figure 4** showed that red galangal with mycorrhizal has a significantly higher average of leaf number than plant without mycorrhiza (3.93). Increased P absorption by mycorrhizal hypha affects leaf formation in plants. The study conducted by Lynch et al. (1991) showed that the leaves number of the P-untreated plant was lower than the P treated plant.

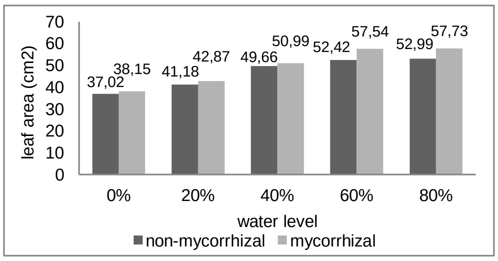

Fig. 5. Average leaf area (cm^2) of red galangal on different water content and mycorrhiza treatments

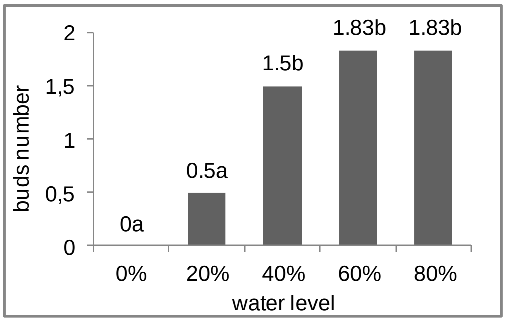

Fig. 6. Average buds number on different water content. (Means followed by the same letter are not significantly different (P < 0.05)

Figure 5 showed that the average leaf area decreases with decreasing levels of water content in planting medium on all water content treatments. The inoculated plant of mycorrhiza has a higher average leaf area than a plant without mycorrhiza. The lowest average leaf area (37.02 cm^2) in non-mycorrhizal plants and water content of 0%, while the highest average (57.73 cm^2) in mycorrhizal plants with 80% water content.

According to Songsri et al. (2009) the mechanism of plants to avoid drought stress, is to modify their root system to absorb more water. In mycorrhizal plants, AM fungi hyphae expands the absorption area of host plant roots (Muthukumar and Udaiyan 2010). Mycorrhizal-plant symbiosis increases the P uptake required by plants to support overall growth including increased leaf area. Plenet et al. (2000) reported that low P concentrations will lead to lower leaf area which negatively impacts to low light interception and growth. P deficiency can suppress the rate of cell division (Assuero et al., 2004) and the extension of epidermal cells (Radin and Eidenbock, 1984) which may eventually lead to a decrease in the number and extent of leaves. Chiera et al. (2002) stated that the apical meristem and leaf initiation activity was also inhibited by low P levels.

The result given in **Figure 6** revealed that the average number of buds decreases by decreasing water content in the media. At the lowest water content of 20% where the plant can still live obtained the lowest average number of buds was 0.5 buds. These results indicated that for bud formation required adequate water availability. Drought stress inhibits the production of endogenous auxin and cytokinin (Nilsen and Orcutte, 1996). Cytokinin along with auxins, stimulate cell division and control of morphogenesis. Cytokinin is very effective in encouraging bud initiation. The balance between auxin and cytokinin usually gives the effective organogenesis (Van Staden et al., 2008). Thus, the drought stress may inhibit shoot formation.

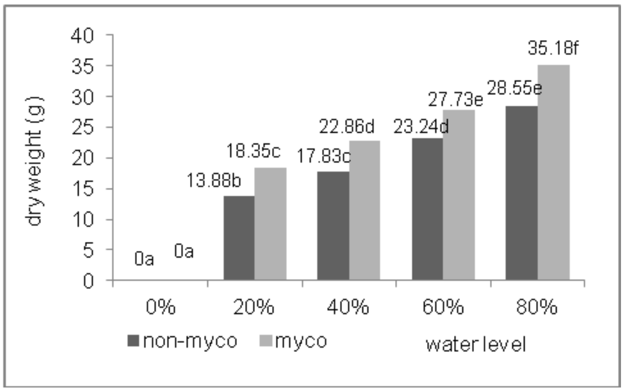

Figure 7. Average dry weight on interaction water content and mycorrhiza inoculation treatments. (Means followed by the same letter are not significantly different (P < 0.05)

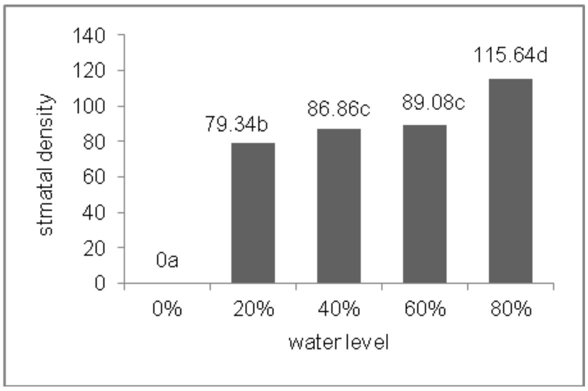

Figure 8. Average stomatal density on different water content. (Means followed by the same letter are not significantly different (P < 0.05)

Figure 7 revealed that the average dry weight of the experimental red galangal plant showed significantly difference in varying water content and mycorrhizal inoculation treatment. Dry weight increased by increasing water content in media, so it was the highest in the treatment of 80% water content i.e. 11.46 grams. The decreased of water content up to 40% in medium showed average dry weight which was not significantly different with 60% water content. It was showed that at the water content of 40%, red galangal plants had a relatively good tolerance to the parameter of dry weight. According to Taiz and Zeiger (2010) dry weight is one indicator of plant metabolism process. If the metabolic process increases, the resulting dry matter also increases, in contrast, decreased metabolic activity can lead to decreased dry matter of the plant. Dry matter of plant is the product of the photosynthesis process. Water is needed in the process of photosynthesis where the process is an important physiological process for biomass accumulation. Drought stress damages the structure and function of the PSII reaction center and disrupts the transport of electrons in the photosynthesis apparatus whereas symbiosis AM reduces the adverse effects of drought stress on the PSII reaction center so that photosynthesis is efficient (Baker, 2008). Zhu et al. (2012) reported that symbiosis of AM can improve PSII photochemical efficiency in both well watered and drought conditions. Decreased soil water levels can inhibit the diffusion of CO_2 through stomata so that the photosynthesis process is disrupted. As disclosed Goicoechea et al. (2005) that decrease in soil water content causes stomatal closure that reduces net photosynthesis in plants. Improvement of water status in mycorrhizal plants may increase net photosynthesis due to increased stomatal conductance resulting in greater CO_2 diffusion within the mesophyll (Boldt et al., 2011). As disclosed by Ruiz-Lozano et al. (1995) that generally, the decrease in CO_2 assimilation rate is related to the reduction of water status in the leaves caused mainly by stomatal closure and increased epidermal resistance of the leaf. Transpiration is usually suppressed by water stress along with photosynthesis emphasis. Thereby decreasing the water content can reduce the photoasimilate which will eventually inhibit the growth of the whole plant and decrease the dry weight.

Figure 8 indicated that the decreased water content caused significantly decrease in the average stomatal density. The lowest average stomatal density was 79.34 cells/mm^2 at the 20% water content and the highest was 115.64 cells /mm^2 at 80% water content which significantly different from other treatments. Differences of water content in the medium cause the difference in average stomatal density of red galangal plant. Some plants adapt to drought stress by reducing the size of stomata and the number of stomata (Price and Courtois, 1991) so that the density of stomata will be reduced in water-deficient plants.

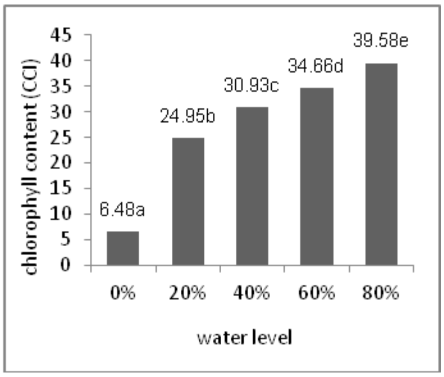

Fig. 9. Average chlorophyll content on different water content. (Means followed by the same letter are not significantly different (P < 0.05)

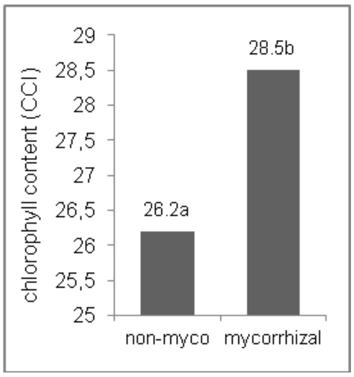

Fig. 10. Average chlorophyll content on mycorrhiza inoculation treatment (Means followed by the same letter are not significantly different (P < 0.05)

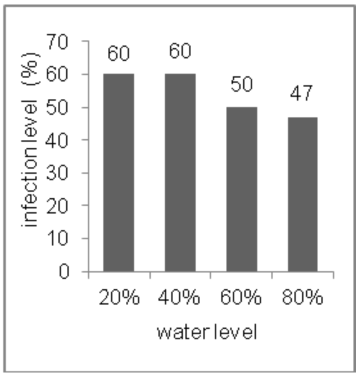

Fig. 11. Percentage of mycorrhizal infection degrees in red galangal roots

The data recorded in **Figure 9** revealed that the chlorophyll content of red galangal plant was significantly decreased by reducing the water content levels. The maximum decreasing of chlorophyll content was obtained in plants with 0% water content treatment. The lowest average chlorophyll content of 6.48 CCI was found in the water content treatment 0% and the highest was 39.58 CCI at 80%. Drought stress from the mild to the most severe level affects the biochemical processes taking place in cells. Drought affects the photosynthetic biochemical reactions, resulting in a decreased rate of photosynthesis. One aspect of photosynthesis that is particularly sensitive to drought stress, including mild stress level, is chlorophyll biosynthesis (Fitter and Hay, 2001). Khayatnezhad and Gholamin (2012) reported that chlorophyll content in ten genotypes of maize under drought stress is lower than those treated with normal levels of water. This condition indicates a reduction in chlorophyll levels caused by drought stress.

The result in **Figure 10** showed that mycorrhizal inoculation was able to significantly increase the mean chlorophyll content in red galangal. The mean chlorophyll content in mycorrhizal plant (28.5 CCI) was higher than in non mycorrhizal plant (26.2 CCI). This is due to the inoculated plants of mycorrhiza have larger and more numerous bundle sheat chloroplasts (Krishna and Bagyaraj, 1984). According to Arya and Buch (2013) plants inoculated with AM fungi brought about significant changes in chlorophyll a, b and total chlorophyll content (Arya and Buch, 2013). Manoharan et al. (2008) reported that the increase in the net photosynthesis in mycorrhizal plants is due to increased chlorophyll and carotenoid content. These facts prove that there is an increase in chlorophyll levels in the association mycorrhiza-host plant.

The results given in **Figure 11** showed that the mycorrhizal infection was influenced by water content in media. The mycorrhizal infection was reduced from 60% at 20% and 40% water content to 47% at the highest water content of 80%. It was indicated that the highest mycorrhizal infection was recorded at low and moderate levels of water content. At water content of 0%, mycorrhizal infections can not be observed because the red galangal plant was dying due to the condition of severe drought stress. The roots of non-mycorrhizal red galangal plants were not injected by AM fungi. The red galangal plants inoculated with mycorrhizal were infected in all water content treatments. The increasing water content decreased mycorrhizal infection. These results are similar to those reported by Donko et al. (2014) that mycorrhizal colonization rates were found to be higher in the drier regions that support the water and nutrients absorption of the host plant. In conditions of high soil water content occurs a reduction in the degree of colonization of arbuscules. Wolfe et al. (2007) explains that water-saturated soil causes soil microorganisms such as mycorrhiza lack of oxygen.

CONCLUSION:

The mycorrhizal red galangal plants still showed normal growth at water content of 40% which can be seen from the parameters of plant height increase, the number of leaves, the number of buds, the stomatal density, the dry weight, the chlorophyll content, and the percentage of mycorrhizal infection degree.

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