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Responses of the five shallot cultivars to salicylic acid treatment under stress drought conditions

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Abstract

Salicylic acid is a growth hormone that has been widely used to induce resistance to biotic and abiotic stresses. Plant genotypes have different responses to drought stress conditions. This study aimed to analyze the response of five shallot cultivars to drought stress mediated by salicylic acid. A total of five shallot cultivars treated with salicylic acid were tested under drought stress conditions. This study used a completely randomized design with 3 factors, namely the first was the concentration of salicylic acid (0 mM, 0.5 mM, and 1 mM), the second was the shallot cultivar (Bima Brebes, Tajuk, Bauci, Super Philip and Bima Juna), and the third was drought stress (without drought stress and with drought stress). All collected data were subjected to analysis of variance (ANOVA) and the mean differences were compared using Duncan Multiple Range test (α = 95%). Observation variables included leaf length, leaf number, stomatal density, relative water content, total chlorophyll, and leaf proline content. Based on the results of the study, drought stress significantly decreased leaf length and leaf number, reduced chlorophyll content, relative water content and stomatal density, chlorophyll content, and increased proline content of the leaves. On the other hand, exogenous application of SA to drought stressed shallot plants improved morphophysiological characters of shallot. Application of 1 mM salicylic acid was the best concentration. The results of this study also obtained two cultivars that showed fairly consistent morphophysiological performance, namely Bima Juna and Tajuk cultivars. These two cultivars can be recommended as genetic materials in the assembly of drought-tolerant varieties mediated by salicylic acid.

INTRODUCTION

Drought is a serious threat to productivity, including shallots. Shallots require sufficient water supply for their growth and development. Lack of water can cause stress to plants, inhibit growth and affect bulb production (Swasono, 2012). In addition, if drought occurs at the beginning of the plant growth phase, it results in a reduction in bulb size and a decrease in shallot bulb weight by up to 58.9% (Hadiawati et al., 2018). Drought stress can significantly reduce stomatal conductance and photosynthesis, inhibit the synthesis of photosynthetic pigments, and ultimately decrease plant growth (Hasanuzzaman et al., 2013 and Chaudhry et al., 2020) and cause damage to shallot bulbs (Ghodke et al., 2018). For this reason, it is necessary to produce superior varieties that are both draught-tolerant and high-yielding. To increase drought tolerance, resistance-inducing agents such as salicylic acid can be used (Khan et al., 2015; Indarwati et al., 2021; Yousefvand et al., 2022).

Salicylic acid (SA) is a phytohormone that plays a role in regulating plant growth and development, as well as enhancing their response to biotic and abiotic stress factors. Exogenous application of SA to stressed plants, either through seed soaking, nutrient solution

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addition via irrigation, or spraying, has been reported to activate mechanisms that enhance tolerance to biotic and abiotic stress (Raskin, 1992). Salicylic acid plays a role in increasing the capacity of plants to withstand drought by several different mechanisms (Khan et al., 2015). One of the main mechanisms is the regulation of stomatal opening and closing, which allows plants to regulate water use more efficiently (Kang et al., 2014). Salicylic acid has also been shown to regulate the expression of genes associated with drought responses, aiding in the mitigation of oxidative stress caused by drought (Jumali et al., 2011). SA is also involved in the regulation of important plant physiological processes such as photosynthesis, nitrogen metabolism, proline (Pro) metabolism, glycinebetaine (GB) production, antioxidant defense systems, thereby protecting plants from diseases and abiotic stresses (Kang et al., 2014).

Genetic variability among shallot cultivars leads to differences in the level of drought tolerance (Fischer and Maurer, 1978). Therefore, research on increasing drought tolerance in several shallot cultivars through salicylic acid mediation is necessary. This study examined the response of SA in five shallot cultivars identified for their varying resistance to drought stress: four drought-sensitive cultivars (Bima Brebes, Super philip, Bauci, and Bima Juna) and one drought-tolerant cultivar (Tajuk). This study aimed to analyze the response of five shallot cultivars to drought stress mediated by salicylic acid.

MATERIALS AND METHODS

Research design

This study was a controlled study conducted in a greenhouse. The experimental design used was a completely randomized design (CRD) with 3 factors. The first factor was the concentration of salicylic acid, which included 0 mM (S0), 0.5 mM (S1) and 1 mM (S2). The second factor was the shallot cultivar, which included five varieties: Bima Brebes (V1), Tajuk (V2), Bauci (V3), Super Philip (V4) and Bima Juna (V5). The third factor was drought stress, with two levels: without drought stress (WDS) and with drought stress (DS). Each treatment combination consisted of 10 plants and was repeated 3 times.

Preparation of plant material

The research material used 2–3-month-old shallot seedlings, MERCK salicylic acid. Planting media, consisting of a 1:1 mixture of soil and compost, was weighed 5 kg and placed into polybags measuring 25 cm x 30 cm. Planting 1 bulb polybag^{-1} for each treatment combination was done with 3 replications. Salicylic acid was applied one day before the onset of drought stress by spraying 25mL of SA on the leaves twice in the morning and evening as much as 25 ml plant^{-1} (Purbajanti, 2023). Drought stress treatment was given to plants aged 14 days after planting (DAP) until harvest. When the plants entered the age of 13 DAP, some plants were watered in soil moisture conditions at field capacity, and others were maintained in drought stress conditions (60% FC) (Swasono, 2012). Plants experiencing drought stress were watered with water up to 60% FC every 2 days (after 70% wilting symptoms on the leaves). Wilting symptoms began to occur when the soil water content reached <60–70% of field capacity. Shallot fertilizer consists of primary and supplementary fertilizer. The primary fertilizer was applied three days before planting, consisting of NPK fertilizer (16:16:16) at 250 kg ha^{-1}, KCL at 30 kg ha^{-1}, and SP-36 at 50 kg ha^{-1}. The first supplementary fertilizer was applied at 15 days after planting (DAP), consisting of ZA at 400 kg ha^{-1}. The second supplementary fertilizer was applied at 30 DAP, consisting of urea at 180 kg ha^{-1} (PUSLITBANGHORTI, 2015).

Variable of observation

Morphological measurements

All morphological measurements were taken from one plant from each polybag at the age of 28 DAP. Leaf length (cm) of shallot cultivars was measured using a measuring tape. The leaf number was averaged by counting three plants from each polybag.

Physiological measurements

Physiological parameters were measured from all two groups (Control and Drought Stress) from three different plants in three replicates from each group. All the physiological measurements were taken at 72 hours after the drought stress treatment, between 09:00–13:00 (Chaudhry et al., 2020).

Stomatal density

A small piece of the lower epidermis was cut off the leaf (leaf 3 or 4), placed on a glass object, added with a little water, and covered with a cover glass.

The number of stomata was then observed and counted from different fields of view.

Relative water content (RWC)

Shallot leaf segments were about 5 cm long (the 3rd or 4th leaf). The fresh weight of shallot leaves was measured and the turgid weight of the leaves was determined after keeping them in distilled water overnight. The leaves were dried in oven drying at 95°C for 2–3 h to ensure complete drying of the leaves before determining the dry weight. Relative water content (RWC) values of plants were calculated according to the following equation (Larcher, 1995):

RWC (%) = [(Fresh weight - dry weight)/(Turgor weight - dry weight)] x 100

Chlorophyl content

Leaf Chlorophyl (Chl) a and b content and carotenoids were determined by 0.5 g of fresh shallot leaves homogenized in 80% of 10 mL acetone. The homogenized solution was kept at 4°C overnight for complete extraction. The mixture was centrifuged at 10,000 rpm for 5 min at 4°C. The absorption of the supernatant was measured using a UV-Vis spectrophotometer (UV-1800, Shimadzu) at 470 nm, 646 nm, and 663 nm. Calculation of Chl a, Chl b, and carotenoids contents were determined by formula defined by Arnon (1949).

Proline contents

A total of 100 mg of leaves (young leaves) were harvested. Furthermore, the leaves were placed in a mortar and ground with the addition of 3% sulfosalicylic acid (fresh weight 5 μL mg^{-1}). The experiment was carried out on ice. The samples were centrifuged at maximum speed for 5 minutes at room temperature. Meanwhile, other reagents were prepared by mixing 100 μL of supernatant with 100 μL of 3% sulfosalicylic acid, 200 μL of glacial acetic acid, 200 μL of acid ninhydrin. The reaction mixture was incubated for 1 hour at 96°C. The next step was to make a sample extract by adding 1 mL of toluene to the reaction mixture. The sample extract was rotated for 30 seconds and left for 5 minutes to allow the separation of the organic and water phases. The toluene-containing chromophore was transferred into a new tube to measure the absorbance at 520 nm using toluene as a standard concentration (concentration in mg g^{-1} FW or micromoles g^{-1} FW). To determine the proline concentration, a standard concentration curve was used (Bates et al., 1973).

Statistical analysis

The collected data of leaf length, leaf number, stomatal density and WRC were subjected to analysis of variance (ANOVA) using SPSS software version 23. If a significant effect existed, further testing would be needed to determine the difference between treatments using Duncan Multiple Range Test (DMRT) with alpha 5%. Meanwhile, data of chlorophyll and proline content were expressed as mean ± SE for three samples in each combination treatment.

RESULTS AND DISCUSSION

Morphological performance

Shallots require sufficient water throughout the growth period, as drought stress can lead to stunted growth. In this study, drought stress was given from the vegetative phase to harvest. Drought stress caused significant decrease in leaf length of up to 10.46% (Table 1). This is line with the report by Poudel et al. (2020); Indarwati et al. (2021); Purbajanti (2023), that plants experience a decrease in leaf length under drought stress conditions. According to Salam et al. (2022), the decrease in leaf length is caused by protoplasm dehydration which ultimately reduces cell division, cell expansion, and eventually the loss of cell turgidity. Similar to leaf length, the leaf number has also decreased due to drought stress. The decrease in the leaf number was 12.16% under drought stress (Table 1). Drought may inhibit the rate of photosynthesis, potentially hindering the formation of new leaves. This is in line with the results of research by Ginting et al. (2024), that drought stress causes leaf length, leaf number, and yield components of Batu Ijo variety of red onion to decrease. Drought stress inhibits cell division, enlargement, differentiation which hinders the formation of new organs and results in decreased plant growth.

The study's results demonstrated that salicylic acid at both concentrations (0.5 mM and 1 mM) enhanced leaf length and leaf number under drought stress conditions (Table 1). The shallot treated with 0.5 mM salicylic acid exhibited a considerable increase in leaf length and leaf count, but 1 mM salicylic acid had no notable impact. The leaf length and leaf count of plants treated with 0.5 mM SA rose by 12.56% and 28.18%, respectively. Salicylic

Table 1. Leaf length, leaf number, stomatal density and relative water content results of salicylic acid treatment on 5 shallot cultivars under drought stress

Treatments	Leaf length (cm)	Leaf number (blades)	Stomatal density (mm)	RWC (%)
Drought stress level				
WDS	26.00 a	11.43 a	58.92 a	24.07 a
DS	23.28 b	10.04 b	56.47 a	16.02 b
Salicylic acid concentration				
0 mM	23.17 b	9.58 b	59.94 ab	13.17 c
0.5 mM	26.08 a	12.28 a	57.20 a	21.19 b
1 mM	24.87 b	10.34 b	55.95 b	24.77 a
Cultivars				
Bima Brebes (V1)	23.93 b	9.46 b	65.50 a	16.56 c
Tajuk (V2)	25.28 b	13.40 a	53.42 b	22.61 a
Bauji (V3)	23.09 b	8.57 b	68.62 a	20.78 ab
Super philip (V4)	24.46 b	9.82 b	49.54 b	19.07 bc
Bima Juna (V5)	26.77 a	12.42 a	51.41 b	21.20 ab
CV (%)	13.36	19.44	17.41	23.03
Interaction	(ns)	(ns)	(ns)	(ns)

Remarks: RWC= Relative water content, WDS = without drought stress condition, DS = with drought stress condition). Means followed by the same letters in the same column show no significant difference based on the Duncan Multiple Range Test with an alpha of 0.05. Ns shows no interaction between drought stress, salicylic acid concentration and shallot cultivars.

acid is believed to play a role in plant growth metabolism. Khan et al. (2022) asserted that salicylic acid is a phytohormone that enhances plant growth and drought stress tolerance. Raskin (1992) stated that salicylic acid has a role in regulating plant growth and development, as well as in responding to biotic and abiotic stressors.

The morphological performance, as measured by leaf length and leaf count, varies among five shallot cultivars examined under dry circumstances (Table 1). Variations in leaf length and leaf count across shallot cultivars exhibit distinct phenotypic characteristics between cultivars. This is controlled by several variables, including genetic and environmental elements (Akmal, 2022). The study results indicated that the Bima Juna cultivar (V5) had the greatest leaf length (26.77 cm) and leaf count (12.22 blades) relative to other cultivars. Additionally, the Tajuk (V2) and Super Philip (V4) cultivars exhibited commendable leaf length and leaf count. Consequently, the Bima Juna, Tajuk, and Super Philip cultivars showed effective adaptation to drought stress conditions. Conversely, the Bauci cultivar had the lowest plant height and leaf count.

Physiological performance

Stomatal density

In general, plants cultivated in water deficit conditions tend to reduce water evaporation. One of the mechanisms of shallots in overcoming water deficit is by reducing stomata density (Indarwati et al., 2021). The results of the study showed that drought stress causes a decrease in stomata density. However, there was a significant decrease in stomata density after being treated with 0.5 mM salicylic acid, which was 4.57%, while the decrease in stomata density in the 1 mM treatment was 6.66% (Table 1). It is suspected that the presence of exogenous salicylic acid given to shallot plants has shown an effect in reducing the density of leaf stomata. This is in line with the results of research by Indarwati et al., (2021) which showed that the application of 0.5 mM salicylic acid can reduce transpiration by reducing stomata density, allowing plants to avoid water deficit conditions and enabling them to grow and develop better compared to plants without salicylic acid. According to Swasono (2012), one of the adaptations of drought-resistant plants is by reducing stomatal density to suppress water evaporation through leaves (transpiration). Adaptation of drought-resistant plants, one of which is by reducing the density of stomata to suppress water evaporation through leaves (transpiration).

Shallot cultivars planted under drought stress conditions showed differences in response between cultivars. Cultivars Tajuk (V2), Super Philip (V4) and Bima Juna (V5) were able to reduce water deficit better by reducing the density of stomata under drought stress conditions (Table 1). This is suspected because the three cultivars were tolerant to drought. In contrast to the Bima Brebes (V1) and Bauci (V3) cultivars which showed a large number of stomata, it is possible that the two cultivars were unable to maintain the reduction of water in the leaves so that they could not adapt well to drought stress conditions.

Relative water content (RWC)

Relative water content (RWC) is considered as a measure of plant water status, which reflects metabolic activity in tissues and is used as an important index for drought tolerance (Ahmad et al., 2014). In addition, RWC is considered as an indicator of stress tolerance (Purbajanti, 2023). Based on the results presented in table 1, the RWC of plants under drought stress conditions was significantly lower (16.02%) than plants under non-drought stress conditions (24.07%). Negative (low) RWC causes oxidative stress in plants (Chaudhry et al., 2020), which is due to inhibition of root growth during drought (Swasono, 2012).

Salicylic acid, as an exogenous agent, contributes to the mechanism of plant resistance to drought stress (Purbajanti, 2023 and Yousefvand et al., 2022). The study results demonstrated that RWC in SA treatment at both doses (0.5 mM and 1 mM) was considerably elevated compared to the absence of SA (Table 1). Consistent with the findings of Semida et al. (2016), the foliar application of salicylic acid enhanced drought stress tolerance in plants by improving photosynthetic efficiency and plant water status, as assessed by the membrane stability index and relative water content (RWC). Salicylic acid at 1 mM was the most efficacious in enhancing relative water content during drought stress, resulting in an increase of 88.08%.

Drought-resistant cultivars can save water inside plant tissues. Cultivars with elevated water content often demonstrate favorable tolerance to drought circumstances (Maghsoudi et al., 2018 and Purbajanti, 2023). This study identified Tajuk (V2), Bauci (V3), and Bima Juna (V5) as the cultivars with the highest relative water content (RWC). These three cultivars demonstrated resilience to drought stress. Swasono's research (2012) indicates that relative water content (RWC) in tolerant variety exceeds that of sensitive cultivars under drought stress circumstances. Semida et al. (2016) posited that enhancing plant tolerance via improved water usage efficiency is mostly attributed to the modulation of sugar metabolism inside the guard cells.

Chlorophyll content

Drought stress significantly decreases photosynthesis and stomatal conductance, inhibits the synthesis of photosynthetic pigments, and ultimately leads to decreased plant growth (Semida et al., 2017 and Maghsoudi et al., 2018). Based on research results, drought experienced by shallot plants damages photosynthetic pigments. The content of chlorophyll a, chlorophyll b, and total chlorophyll decreased in stress conditions compared to optimal conditions, except for the Tajuk cultivar (Figure 1, Figure 2, and Figure 3). On the other hand, the chlorophyll content, both chlorophyll a, chlorophyll b and total chlorophyll

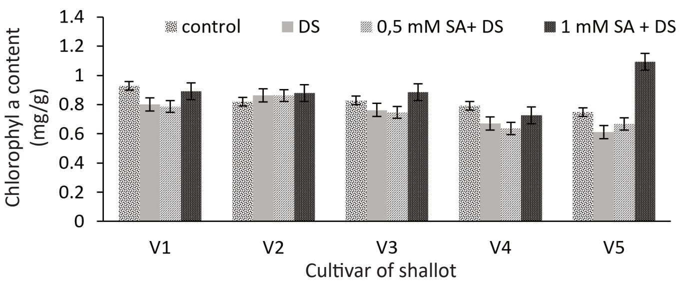

Figure 1. Chlorophyll a content in shallot mediated by SA under stress drought

Remarks: control = without SA and normal conditions, DS = SA and drought stress, SA = salicylic acid.

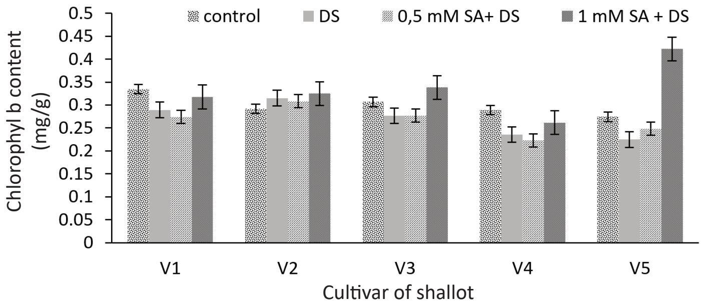

Figure 2. Chlorophyll b content in shallot mediated by SA under stress drought

Remarks: control = without SA and normal conditions, DS = SA and drought stress, SA = salicylic acid.

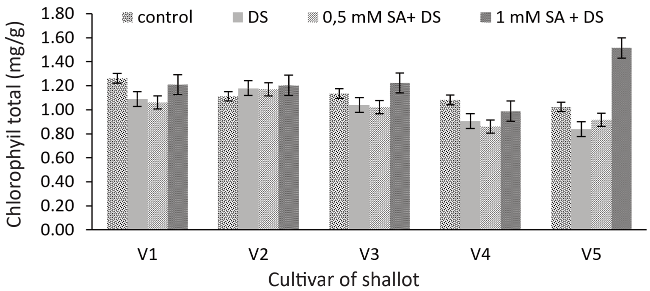

Figure 3. Total chlorophyll content in shallot mediated by SA under stress drought

Remarks: control = without SA and normal conditions, DS = SA and drought stress, SA = salicylic acid.

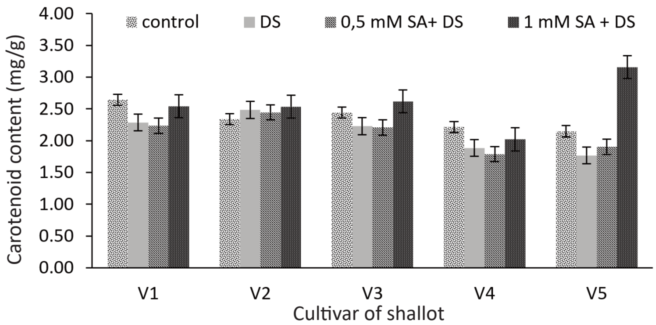

Figure 4. Carotenoid content in shallot mediated by SA under stress drought

Remarks: control = without SA and normal conditions, DS = SA and drought stress, SA = salicylic acid.

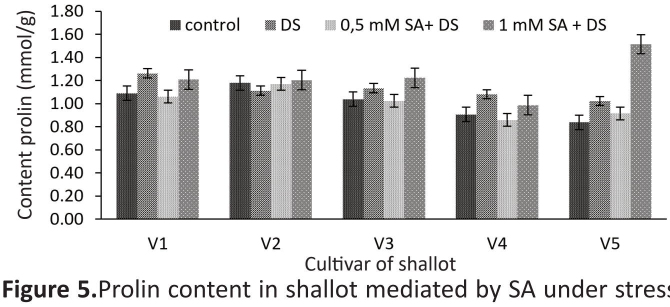

Figure 5. Prolin content in shallot mediated by SA under stress drought

Remarks: control = without SA and normal conditions, DS = SA and drought stress, SA = salicylic acid.

in 1 mM SA treatment increased in drought conditions (respectively by 20.5%, 24.1%, and 21.8%). Meanwhile, SA 0.5 mM was less effective in increasing both chlorophyll a, chlorophyll b and total chlorophyll in the Bima Brebes, Tajuk, Bauci, and Super philip cultivars. However, the Bima Juna cultivar showed different results, in the SA 0.5 mM treatment, the content of chlorophyll a, chlorophyll b and total chlorophyll increased under drought stress (Figure 1, Figure 2, and Figure 3). This allows salicylic acid to play a role in the mechanism of plant resistance to stress. According to Chaudhry et al. (2020), a higher overall chlorophyll content indicates increased light utilization and efficiency in the thylakoid membrane, thus facilitating more photosynthesis.

The negative impact of drought stress caused damage to carotenoid content in almost all tested cultivars, except for Tajuk cultivar (Figure 4). Carotenoids are antioxidants that can reduce the detrimental effects of reactive oxygen species (ROS) on plants. Damaged carotenoids can cause photoinhibition (Chaudhry et al., 2020).

The Bima Juna cultivar had the highest levels of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, followed by the Tajuk and Bauci cultivars. According to Maghsoudi et al. (2018), drought tolerant cultivars showed superior chlorophyll production capacity (higher chlorophyll levels) compared to sensitive cultivars.

Proline content

Proline levels rise in plants as a reaction to drought stress. The augmentation of proline content is crucial for stress tolerance due to proline's active involvement in osmotic adjustment, enzyme structural protection, membrane stability, and defense of enviroment stress (Maghsoudi et al., 2018). Plants react to drought stress by increasing leaf proline levels. The cultivars Bima Brebes (V1), Bauci (V3), Super Philip (V4), and Bima Juna (V5) exhibit proline buildup as a response to drought stress tolerance. Nonetheless, proline concentrations differ across each shallot cultivar (Figure 5).

The use of 1 mM salicylic acid can elevate proline levels in the Tajuk (V2), Bauci (V3), and Bima Juna (V5) cultivars under drought circumstances (Figure 5). According to Misra and Saxena (2009), salicylic acid can elevate proline levels in lentil seedling shoots, potentially via enhancing PSCR activity and reducing PDH (pyruvate dehydrogenase complex) activity. The accumulation of proline in the Tajuk, Bauci, and Bima Juna cultivars signifies their drought tolerance. This aligns with the findings of Maghsoudi et al. (2018), which indicated that drought-tolerant wheat cultivars exhibited more proline accumulation than drought-sensitive cultivars.

Conversely, in the Bima Brebes (V1) and Super Philip (V4) cultivars, the application of salicylic acid (0.5 mM and 1 mM) was less efficacious in enhancing leaf proline (Figure 5). Semida et al. (2016) published same findings, indicating that the use of SA on the shallot variety Giza 20 under drought circumstances decreased free proline and total free amino acids. It is hypothesized that SA does not participate in the control of proline or amino acids in shallot plants under drought circumstances.

CONCLUSIONS

Drought impedes the development and physiological metabolic functions of plants. Exogenous salicylic acid at 1 mM can positively enhance the characteristics of shallot cultivars. The shallot cultivars that regularly demonstrate superior morphophysiological performance include Bima Juna (V5) and Tajuk (V2). These two cultivars are proposed as chosen genotypes for the development of drought-tolerant variants facilitated by salicylic acid.

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