Effects of Different Timings of Drought Stress and Plant Growth-Promoting Rhizobacteria Inoculation on the Photosynthetic Characteristics of Shallot (*Allium ascalonicum* L.)

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ABSTRACT

Shallots are essential vegetables in horticulture, but there is insufficient information available on the effects of drought stress on different growth stages and the inoculation of plant growth-promoting rhizobacteria (PGPR) on their photosynthetic characteristics. This study aims to investigate the effects of drought stress at different growth stages (vegetative growth phase, bulb initiation phase, bulb development phase, and maturation phase) and PGPR inoculation (*Pseudomonas* Pb04 and *Bacillus* Pb03) to mitigate the negative impact of drought stress on photosynthetic characteristics, chlorophyll content, and shallot yield. The results showed that the optimal photosynthesis rate, chlorophyll content, and yield of shallots were most tolerant when the plants experienced drought stress in the maturation phase compared to other growth phases. During the maturation phase, the reduction in photosynthesis rate at PPFD 900 µmol·m⁻²s⁻¹ is only 19.1% compared to plants without drought stress. Drought stress during the bulb growth phase takes the longest to recover conditions after stress, leading to inhibited growth when stress occurs during this phase. In the bulb growth phase, the decrease in photosynthesis rate is 34.8% compared to the treatment without drought stress. PGPR can mitigate the sensitivity of plants to drought stress. *Pseudomonas* Pb04 predominantly suppresses the impact of drought stress during the vegetative growth phase, while *Bacillus* Pb03 has a more dominant effect on drought stress occurring during bulb initiation, bulb development, and maturation phases.

Keywords: shallot, inoculation, rhizobacteria, growth stage, drought stress, photosynthetic.

INTRODUCTION

Shallot (*Allium ascalonicum* L.) is a globally significant species with economic importance. Under favorable environmental conditions, shallot leaves progressively transform into photosynthetic units, influencing the overall physiological processes of the plant (Sánchez et al., 2020). The shallot bulb is primarily regarded as an accumulation of secondary metabolites such as phenol, flavonoids, minerals, and vitamins. Since photosynthesis and water loss through transpiration share a common pathway, physiological measurements typically encompass photosynthesis, gas exchange, and internal leaf CO_2 concentration estimates, providing mechanistic insights into plant growth (Pugh and Müller., et al. 2016). Compared to other plants, shallots are more sensitive to drought stress. Water requirements and plant tolerance to drought vary at each growth stage. Different growth phases can influence photosynthetic activity, nutrient accumulation, and other biochemical processes contributing to drought tolerance (Sharon et al., 2016). Therefore, understanding how growth phases affect a plant response to drought is crucial in planning effective strategies for water management. Therefore, understanding how growth phases affect a plant response to drought is

crucial in planning effective plant protection strategies and water management. Characteristics optimal photosynthesis is the condition in which plants undergo photosynthesis with maximum efficiency. This occurs when plants receive sufficient light, water, and carbon dioxide to support the photosynthesis process efficiently. Photosynthesis provides energy and organic materials for plant growth and development, ultimately determining crop yields. Optimal photosynthesis a crucial role in understanding the mechanisms of plant adaptation to drought stress. Drought stress can lead to reduced water availability for photosynthesis, which can disrupt plant growth and development (Seleiman et al., 2021).

Plants that undergo optimal photosynthesis tend to be more efficient in water use. This mechanism can help plants continue to grow and develop even under drought conditions. Under optimal conditions, plants can produce enough energy for growth. During drought stress, optimal photosynthesis can produce protective compounds such as osmolites and antioxidants that help protect plant tissues from damage caused by oxidative stress (Chauhan et.al., 2023). To determine optimal photosynthesis conditions, we can look at several key parameters related to the photosynthesis process, such as the light compensation point (LCP), light saturation point (LSP), dark respiration (RD), and maximum net photosynthesis (PNmax). Water is a crucial factor influencing the photosynthetic process in plants. It affects the activity of carbon assimilation enzymes in photosynthesis, stomatal opening, accumulation of metabolites, and cell pigment composition (Calzadilla et al., 2022). Insufficient water availability and low irrigation intensity adversely affect plant photosynthesis, significantly reducing crop yields (Zhang et al., 2018). The light response curve depicts the relationship between the rate of photosynthesis and the density of photosynthetic photon flux (PPFD) and is widely used for the physiological characterization of leaf-level gas exchange (Rukmangada et al., 2018). These curves serve as valuable criteria for environmental control and are essential tools for simulation models designed to predict plant behavior in response to environmental stress conditions (Schuwirth et al., 2019).

PGPR (plant growth promoting rhizobacteria) are microbes that live in the rhizosphere (the area around plant roots) and provide benefits to plants, including in overcoming drought stress. *Bacillus* and *Pseudomonas* are strains of PGPR that can alleviate the effects of drought stress by regulating genes responsive to stress, producing phytohormones, osmolites, siderophores, volatile organic compounds, and exopolysaccharides, and enhancing 1-aminocyclopropane-1-carboxylate deaminase activity (Kaushal and Wani, 2016). These PGPR can enhance drought tolerance in important crops and may be used to reduce crop losses under water-limited conditions, thereby improving patterns of photosynthetic characteristics (Vurukonda et al., 2016).

In previous studies, the *Bacillus* can withstand drought stress through efficient nitrogen fixation, phosphate solubilization, ammonia production, and indole acetic acid (IAA) production (Azeem et al. (2022)). The research results confirmed that drought stress inhibits plant biomass and nutrient content in maize varieties, specifically in certain varieties. Another result by Fonseca et al. (2022) revealed that *Bacillus subtilis* can enhance the tolerance of sugarcane plants to water stress in sugarcane plants grown under drought stress, and also increase the concentration of N, P, Mg, and S in the leaves, chlorophyll concentration, net photosynthesis rate, and improves water use efficiency to a greater extent. Furthermore, there is a decrease in parameters related to stress levels. In sweet corn cultivation the shoot and root growth as well as its yield, decreased under 60% field capacity irrigation significantly (Zarei et al. (2019)). Inoculation with P. fluorescens strain not only promoted growth and yield but also ameliorated the adverse effects of water deficit stress. These two strains, with moderate ACC (1-amonicyclopropene-1-carboxylate) deaminase activity and auxin synthesis as well as the highest ability to solubilize phosphate and produce siderophores. Uzma et al. (2022) stated that these five strains are tolerant to drought and capable of producing IAA, ACC deaminase, and siderophores. *Pseudomonas* inoculation showed the potential ability to alleviate drought stress in Vigna radiata and significantly increase seed yield compared to stressed control plants.

Therefore, this study aims to compare the characteristic parameters of photosynthesis under different drought stress treatments during various growth stages of shallots based on PGPR inoculation. The aim is to understand the mechanisms of photosynthetic characteristics in influencing the

yield of shallot bulbs. These findings will provide a theoretical background for optimizing the timing of drought stress and PGPR application to enhance the cultivation of shallots.

MATERIALS AND METHODS

Materials used

The experiment was set up from March until July 2023 in a greenhouse at the Agricultural Development Polytechnic of Malang, Indonesia. 450 meters above sea level. The average daily temperature inside the greenhouse ranges from 24 to 29°C, while the average daily temperature outside the greenhouse ranges from 19 to 26°C. Shallots are chosen as the experimental crop. Uniform plant seedlings (2.5 grams each) are planted in experimental boxes measuring 180×180 cm, filled with soil and compost in a 1:0.25 ratio. One hundred seedlings are planted in each experimental box. Initially, the plants receive 100% field capacity irrigation, followed by drought stress (45–50% field capacity) during the respective phases according to the experimental treatments.

Microbiological material

Two strains of PGPR were used as inoculum *Bacillus* Pb03 (*Bacillus subtilis*) and *Pseudomonas* Pb04 (*Pseudomonas fluorescens*) (Figure 1) isolated from the rhizosphere soil of Shallot in central crop Shallot in Probolinggo, East Java, Indonesia, and the control to which only sterile nutrient broth was applied. The Rhizobacteria belong to the microbial collection of the Soil Microbiology area of the Soil Science Program of the Ministry of Agriculture, which isolated them and tested their rhizobacterial capacity. The inoculation of the strains or application of treatments was carried out at 5 and 30 days after transplanting, inoculum was applied with a concentration greater than $1×10^6$ mL^{-1} cells at the base of each plant.

Experimental design

The experimental design utilized is a randomized block factorial design with two factors and three replications. The first factor is the timing of drought stress, consisting of 5 treatments. Consists of, control (without drought stress), drought stress during the vegetative growth phase, bulb initiation, bulb development, and maturation. The second factor is the type of PGPR inoculation, comprising three treatments. The first treatment involves inoculation with *Bacillus* Pb03, the second with *Pseudomonas* Pb04, and the third without PGPR inoculation. Therefore, the total number of experiments in the entire study is 15, each repeated with three replications.

The research was conducted in a greenhouse. Other variables such as sunlight, soil moisture, and temperature were controlled daily by monitoring and periodically to maintain suitable conditions for the study. For sunlight intensity, periodic measurements were taken using a lux meter, and the intensity of sunlight entering the greenhouse was maintained at 80%. To control the amount of sunlight entering, researchers could use paranet to cover the roof when necessary. Soil moisture was periodically measured using a hygrometer, and soil moisture was maintained at 80% for control and 45–50% for drought stress treatment. Soil moisture was controlled using a programmed irrigation system that adjusted irrigation based on the plants needs according to the research treatment. The temperature was periodically measured using an air thermometer, and the temperature inside the greenhouse was maintained at 24–29°C.
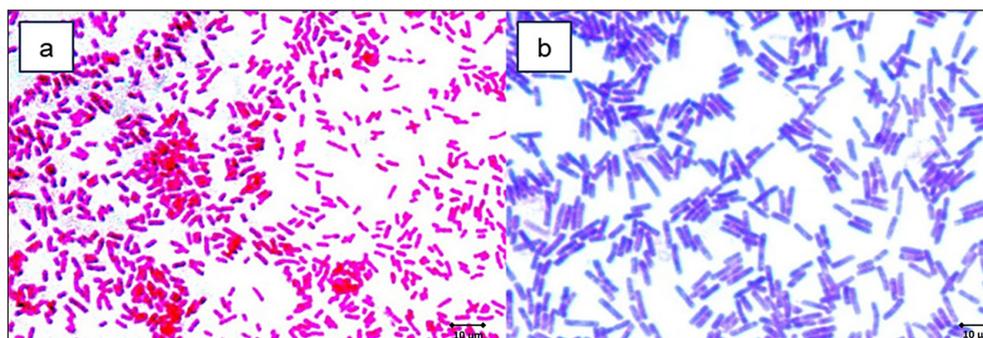
Figure 1. Plant growth promoting rhizobacteria; (a) *Pseudomonas fluorescens* Pb04; (b) *Bacillus subtilis* Pb03

The greenhouse temperature was controlled using a ventilation system that allowed fresh air to enter and hot air to exit.

Measurement of the light response curve

The light response curve was developed using the LI-6400XT portable photosynthesis system (Li-Cor Inc., Lincoln, Nebraska, USA). To mitigate the impact of environmental fluctuations on gas exchange measurements, all assessments were conducted in a greenhouse with a photosynthetic photon flux density (PPFD) at the leaf surface of 600 mol·m^{-2}·s^{-1}, relative humidity of 60–70%, and controlled temperature. The response curve was fitted using a modified rectangular hyperbolic model (Ruan et al., 2022):

$$P_N = \alpha \frac{1 - \beta \cdot PPFD}{1 + \gamma \cdot PPFD} \cdot PPFD - RD \qquad (1)$$

PN represents the net photosynthesis rate, α denotes the initial slope of the light response curve or apparent quantum efficiency (AQY), β and Υ are dimensionless coefficients independent of PPFD, and RD stands for dark respiration rate. The saturated light net photosynthesis rate (PNmax), light saturation point (LSP), and light compensation point (LCP) are determined by the following formulas (Zhou et al., 2022):

$$P_{Nmax} = \alpha \left[\frac{\sqrt{\beta + \gamma} - \sqrt{\beta}}{\gamma} \right]^2 - RD \qquad (2)$$

$$LSP = \frac{\sqrt{(\beta + \gamma) / \beta} - 1}{\gamma} \qquad (3)$$

$$LCP = \frac{\alpha - \beta\gamma - \sqrt{(\gamma RD - a)^2 - 4\alpha\beta RD}}{2\alpha\beta} \qquad (4)$$

P_{Nmax} reflects the maximum capacity of the plant to utilize light as an energy source to support photosynthetic reactions. LSP provides information about how much light the plant requires to reach the maximum photosynthetic rate. LCP, or the light compensation point, is the intensity of light at which the plant's photosynthetic rate equals the respiration rate.

Chlorophyll measurement

The materials utilized in chlorophyll content analysis comprised shallot leaves, and the James method (Smith dan Benitez, 2013) was employed under drought stress conditions. Absorption readings with a UV spectrophotometer were conducted for drought stress resistance determination at wavelengths (λ) of 649 nm and 665 nm, with three replicates per sample. The chlorophyll content was calculated using the following formula:

$$\text{Chlorophyll a} = 12.25\,\lambda_{663} - 2.79\,\lambda_{649} \qquad (5)$$

$$\text{Chlorophyll b} = 21.50\,\lambda_{649} - 5.10\,\lambda_{663} \qquad (6)$$

Measurement of yield

The measurement of shallot production yield is based on the weight of harvested bulbs. The fresh bulb weight is determined by weighing the harvested bulbs per plant using an electronic analytical balance with a precision of 0.01. Similarly, for the dry bulb weight, the bulbs are considered three weeks after being stored in the bulb storage facility under the same conditions.

Statistical analysis

The results are expressed as the mean ± standard deviation from three replicates in each individual. Data were analyzed using a two-way analysis of variance (ANOVA) with DSAASTAT software. Multiple treatments were compared using the Tukey Honestly Significant Difference (Turkey) test. Graphical representations were generated using SigmaPlot 14.5 software.

RESULTS

Light response curve (LRC)

The LRC is a graph illustrating how the photosynthetic rate of plants varies with light intensity. The light response curve was characterized by synchronous changes in treatments involving drought stress and the inoculation of PGPR types (Figure 2). The result shows within the PPFD range of 0–300 µmol·m^{-2}s^{-1}, there were no significant differences in the net photosynthesis (PN) values among PGPR inoculation treatments and drought stress timing. Beyond 300 µmol·m^{-2}s^{-1}, distinct separations in PN curves occurred across all drought stress timing treatments except for the control (Figure 2a). Plants experiencing drought stress may undergo a reduction in growth and photosynthetic capacity, reflected in the light response curve as an overall decrease in photosynthetic rates and a shift towards lower light intensities. PN decreased during drought stress in the

vegetative growth phase by approximately 30.5% (Figure 2b), bulb initiation by 34.8% (Figure 2c), bulb development by 34.5% (Figure 2d), and maturation by 19.1% (Figure 2e), compared to plants without drought stress.

Among all drought stress duration treatments, PGPR assistance helped enhance net photosynthesis, approaching the control. In the vegetative growth phase (Figure 2b), *Pseudomonas* Pb04 demonstrated a higher increase in net photosynthesis than *Bacillus* Pb03, with a 16.3% increase in PN compared to the control. However, in subsequent phases (bulb initiation, bulb development, and maturation), *Bacillus* Pb03 dominated the increase in net photosynthesis compared to *Pseudomonas* Pb04 (Figure 2c, 2d, 2e). Inoculation with *Bacillus* bacteria (Figure 2d) increased net photosynthesis by 37.7% compared to PN values without PGPR inoculation during drought stress in the bulb development phase. Although the initial light response curve may indicate a decline in photosynthetic activity, PGPR can enhance plant adaptation and contribute to the partial or complete recovery of photosynthetic functions.

Apparent quantum yield (AQY)

The AQY value indicates the extent to which a plant can convert received light into chemical energy, particularly in producing oxygen (Wang and Domen, 2019). The AQY values show significance in treatments involving different timing of drought stress. Drought stress can decrease AQY values because stressful conditions affect a plant ability to capture and use light efficiently (Figure 3a). This is evident in treatments without inoculation, where drought stress in all phases resulted in a significant decrease compared to the control. The reduction in AQY values compared to the control, respectively, in the vegetative phase was 30.8%, bulb initiation 16.2%, bulb development 23.1%, and maturation 30.8%.

PGPR can help mitigate the impact of drought on the reduction of AQY values (Figure 3a). In the vegetative growth phase, *Pseudomonas* Pb04 more dominantly increased AQY values compared to *Bacillus* Pb03, with a 32.8% increase in AQY compared to without PGPR. However, during drought stress in bulb initiation, bulb development, and maturation, *Bacillus* Pb03 became
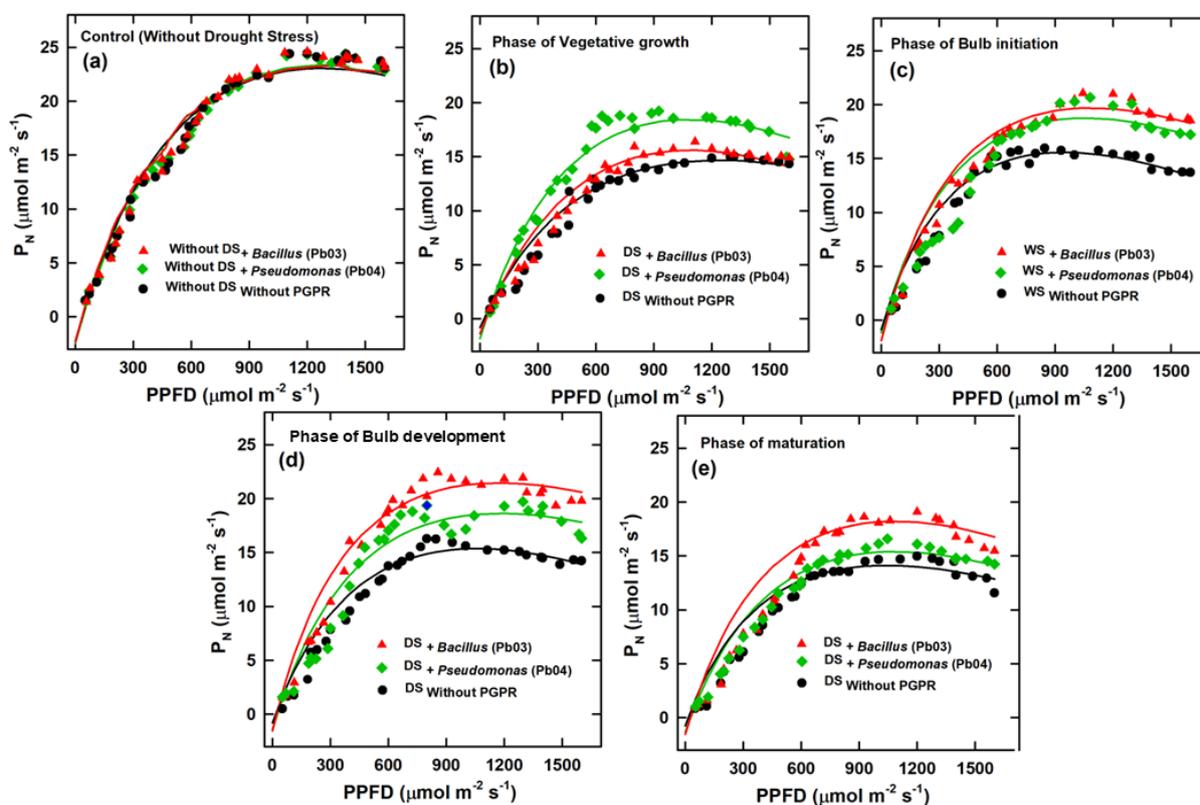
Figure 2. Net photosynthesis at different drought stress timing and inoculation PGPR conditions that is, (a) without drought stress, (b) vegetative growth phase, (c) bulb initiation phase, (d) bulb development phase, and (e) maturation phase

more dominant in increasing AQY values. *Bacillus* bacteria increased AQY values by up to 24.7%, 30.1%, and 37.8% respectively, in the bulb initiation, bulb development, and maturation phase compared to without PGPR.

Maximum photosynthetic rate (PNmax)

Drought stress can significantly impact the PNmax, representing the maximum net photosynthetic rate achievable by plants under ideal conditions (Bencze, Bamberger and Janda, 2014). In this study, all treatments without the addition of PGPR showed decreased Pnmax values (Figure 3b). The reduction in Pnmax values compared to without drought stress, respectively, vegetative phase was 42.9%, bulb initiation 39.4%, bulb development 44.7%, and maturation 20.3%.

The utilization of PGPR can help alleviate the impact of drought stress, with drought stress during the maturation phase being the most stable compared to other stress phases. PGPR
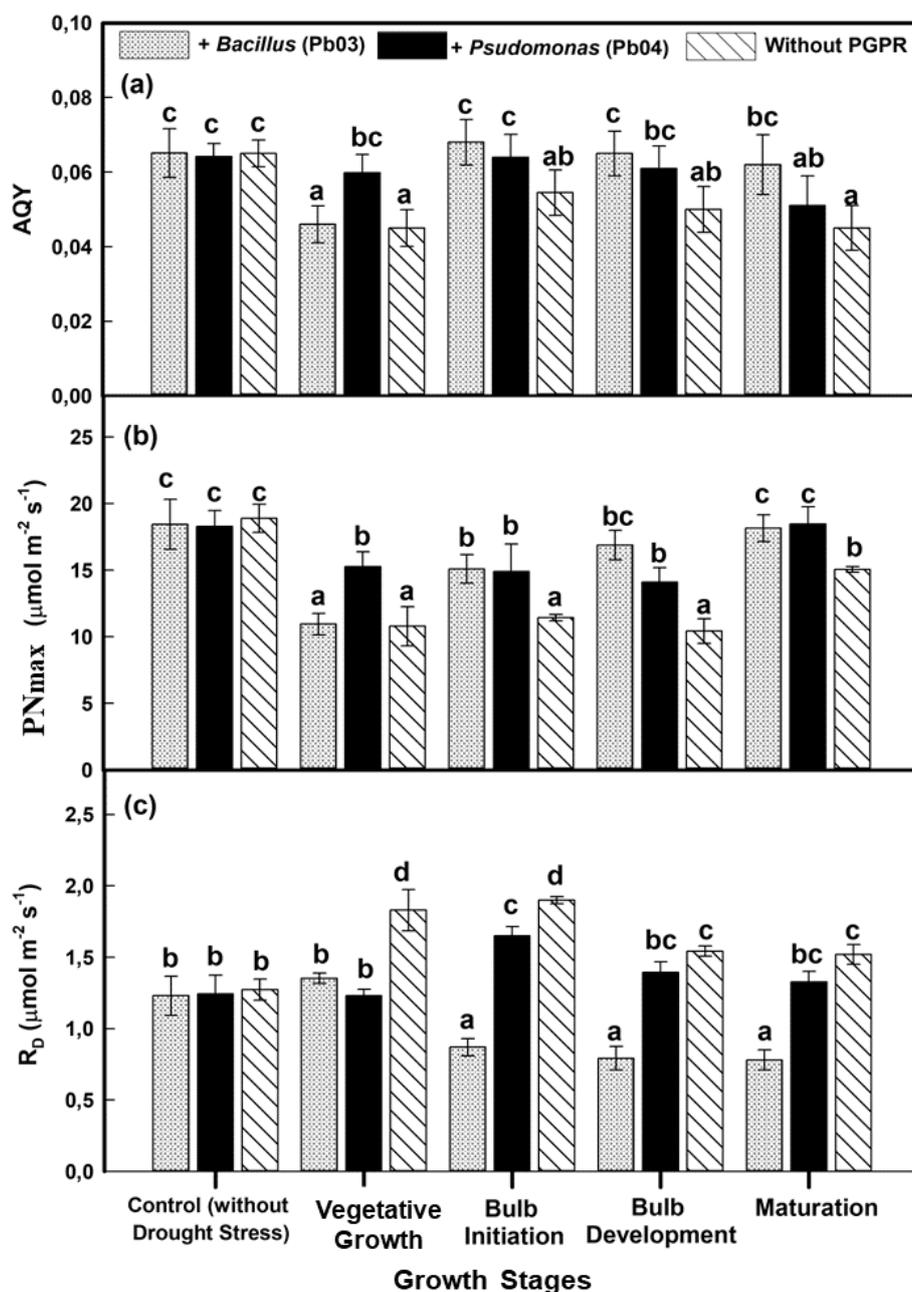
Figure 3. (a) Apparent quantum yield, (b) maximum photosynthetic rate, and (c) dark respiration rate under different timings drought stress and inoculation PGPR conditions. Mean values with standard error of the mean (n = 3). Letters indicate significant differences at $p < 0.05$ according to the Turkey test

inoculation treatments did not significantly better than without stress treatment. However, drought stress and *Bacillus* Pb03 inoculation given during the bulb initiation and bulb development phases, when compared to the control, only reduced PNmax values by 8.5% and increased PNmax by 61.7%, respectively, compared to treatments without PGPR inoculation. In the vegetative phase, *Pseudomonas* Pb04 inoculation increased PNmax by 41.5% compared to without PGPR, while *Bacillus* Pb03 inoculation did not significantly differ from without PGPR (Figure 3b).

Dark respiration rate (R_D)

Drought stress can influence the R_D in plants, which is associated with the energy requirements during periods when plants cannot perform photosynthesis caused by drought (Seleiman et al., 2021). This study shows increased R_D values in shallot plants subjected to drought stress. The rise in R_D values compared to the control during drought stress, respectively, in the vegetative phase was 44.1%, bulb initiation 49.6%, bulb development 21.3%, and maturation 19.7% (Figure 3c). In response to drought stress, plants elevate respiration rates to meet the increased energy demands associated with adapting to drought.

The inoculation of PGPR treatment can reduce R_D values under drought stress during the vegetative growth and bulb initiation phases; however, there is no significant difference during the bulb development and maturation phases. Inoculation with *Bacillus* Pb03 during the bulb initiation phase can decrease R_D values by up to 54.2% compared to without PGPR. Meanwhile, *Pseudomonas* Pb04 reduces R_D values by 27.9% compared to without PGPR during the vegetative growth phase (Figure 3c).

Light saturation point

The light saturation point (LSP) is the light intensity level at which the photosynthetic rate reaches saturation (Zhang et al., 2021). At this point, further increases in light intensity will not enhance the photosynthetic rate, as the plant has reached its maximum capacity to absorb light (Yufeng et al., 2024). The research results indicate that drought stress can decrease LSP values (Figure 4a). In treatments without PGPR inoculation, respectively, LSP decreased by 20.6% in the

vegetative phase, bulb initiation by 32.2%, bulb development by 27.3%, and maturation by 17.3% compared to treatments without drought stress.

Insignificant LSP values were observed during the maturation phase of all plants subjected to drought stress and PGPR inoculation. However, in other phases, PGPR inoculation increased LSP values compared to treatments without PGPR inoculation. During the bulb initiation and development phases, *Bacillus* Pb03 inoculation was more dominant than *Pseudomonas* Pb04 inoculation. The increase in LSP during the bulb initiation phase was 29.1% with *Bacillus* Pb03 inoculation compared to without inoculation PGPR and increased by 16.5% compared to *Pseudomonas* Pb04 inoculation. Conversely, during the vegetative growth phase, *Pseudomonas* Pb04 inoculation was more dominant, showing a 9.1% increase compared to *Bacillus* Pb03, and was not significantly different from without PGPR (Figure 4a).

Light compensation point

The light compensation point (LCP) is the light intensity level at which a plant respiration rate equals its photosynthetic rate (Sales, et al. 2023). At this point, plants begin producing oxygen in sufficient quantities through photosynthesis to meet their oxygen needs through respiration. Below this stress, plants consume more oxygen through respiration than they produce through photosynthesis (Li et al., 2021).

In this study, as plants experienced increased drought stress, LCP values also increased. The highest increase in LCP values was observed during drought stress in the bulb development phase. The rise in LCP was 29.3%, 35.5%, 40.9%, and 32.4%, during the vegetative, bulb initiation, bulb development, and maturation phases, respectively, compared to plants without drought stress(Figure 4b).

Figure 4b shows that adding PGPR inoculation can decrease LCP values compared to treatments without PGPR inoculation. The most significant decrease in LCP, with *Pseudomonas* Pb04 inoculation, was during the vegetative growth phase, approximately 24.5% compared to the drought stress treatment without PGPR inoculation. Meanwhile, with *Bacillus* Pb03 bacterial inoculation, the highest decrease occurred during the bulb initiation phase, around 16.2%, compared to the drought stress treatment without PGPR inoculation.
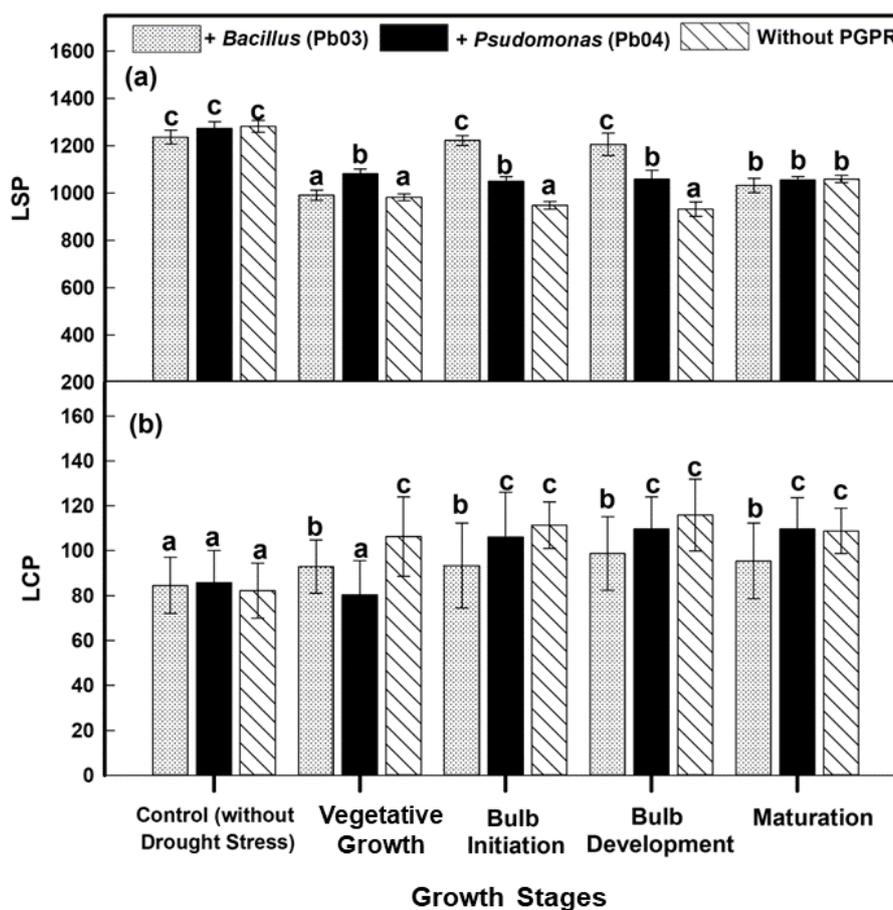
Figure 4. (a) Light-saturation point and (b) light-compensation point under different drought stress timings and inoculation PGPR conditions. Mean values with standard error of the mean (n = 3). Letters indicate significant differences at p < 0.05 according to the Turkey test

Chlorophyll content

Chlorophyll is a light-capturing pigment, and its molecules have a structure that allows them to absorb light energy (Mandal and Dutta, 2020). Drought leads to decreased plant water availability, crucial for various biochemical processes within plant cells, including those involved in chlorophyll synthesis (Mehravi et al., 2023). The research results also indicate a shift in the graph curve when plants are subjected to drought stress.

The chlorophyll content is significantly influenced (p<0.05) by bacterial inoculation in four specified growth phases under drought stress conditions (Figures 5 and 6). Chlorophyll values (a and b) demonstrate that early exposure to drought stress affects chlorophyll content over time, influencing the plant's quality. Inoculation treatments show a noticeable shift in the chlorophyll content curve for stressed plants compared to without-stressed plants. When applied during drought stress in the vegetative phase, *Pseudomonas* Pb04

dominantly enhances chlorophyll content. On the other hand, drought stress during the bulb initiation and development phases is more dominantly influenced by *Bacillus* Pb03 bacterial inoculation, resulting in increased chlorophyll content. However, the results are insignificant when drought stress is applied during the maturation phase. The findings underscore the impact of bacterial inoculation on mitigating the effects of drought stress on chlorophyll content in different growth phases.

Yield

As depicted in Figure 7a, a difference is evident in the outcomes of shallot cultivation under drought stress and PGPR inoculation conditions. In the absence of PGPR inoculation under drought stress conditions, a substantial decrease in the fresh weight of shallot bulbs is observed, in the vegetative, bulb initiation, bulb development, and maturation phases, with reductions of 45.2, 52.85%, 49.8%, and 13.8%, respectively,
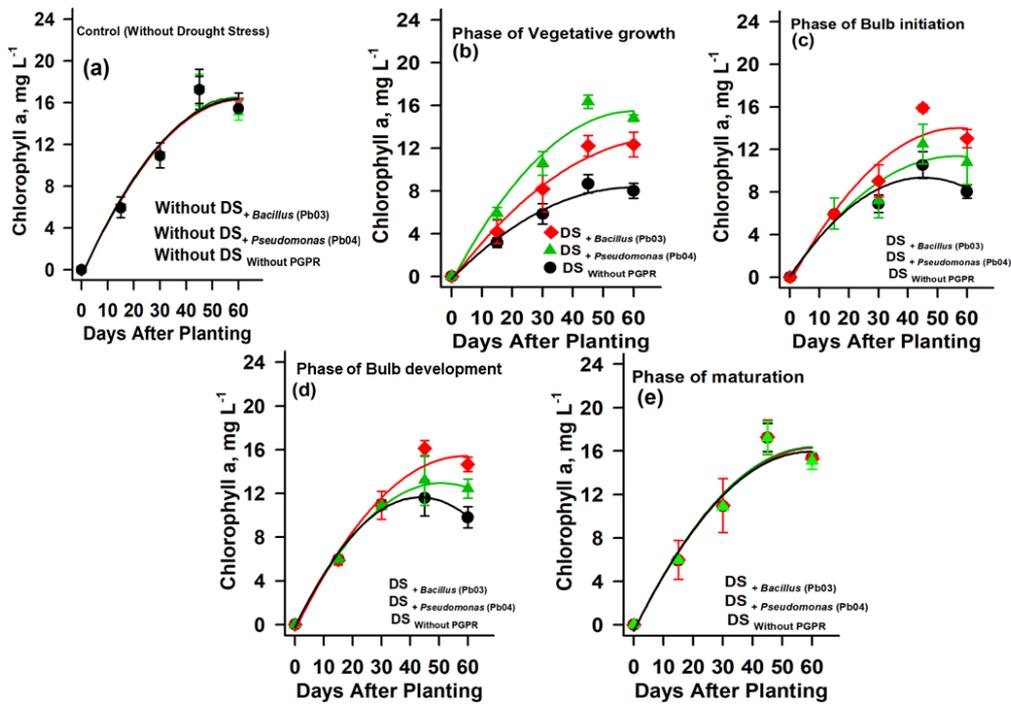
Figure 5. Chlorophyll a under different drought stress timings and inoculation PGPR conditions of (a) without drought stress, (b) vegetative growth phase, (c) bulb initiation phase, (d) bulb development phase, and (e) maturation phase
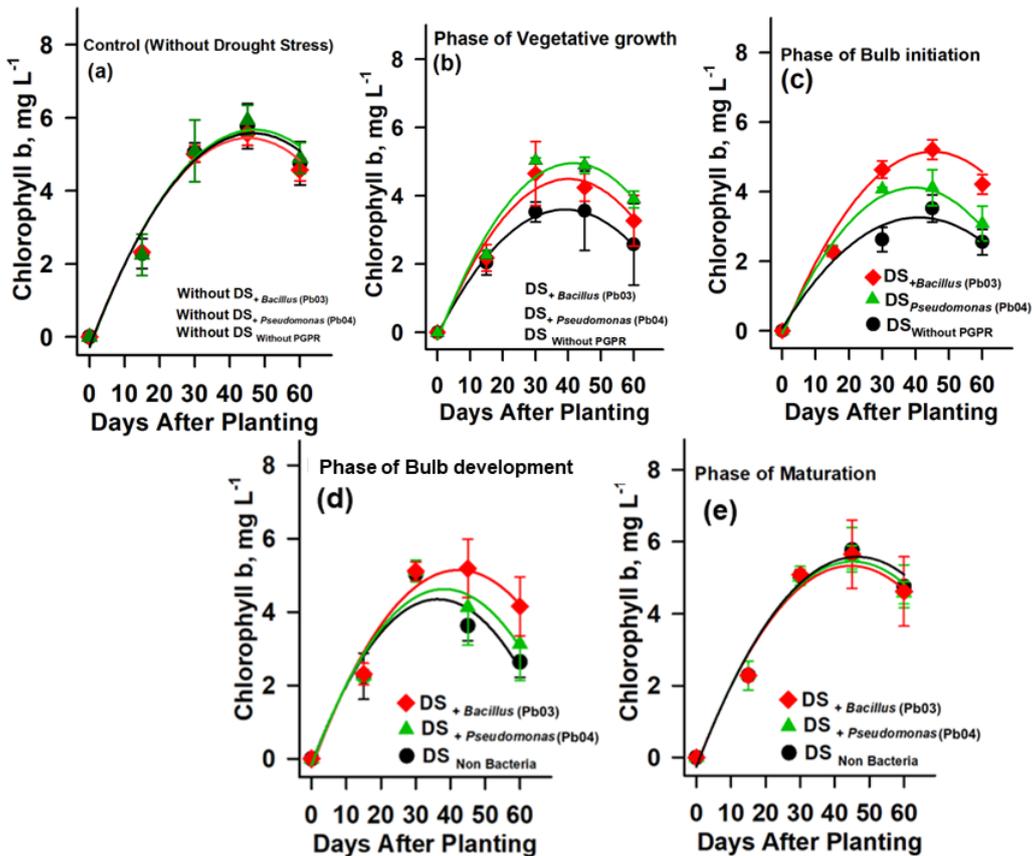
Figure 6. Chlorophyll b under different drought stress timings and inoculation PGPR conditions of (a) without drought stress, (b) vegetative growth phase, (c) bulb initiation phase, (d) bulb development phase, and (e) maturation phase

compared to plants without stress. Under drought stress conditions, the highest increase in bulb fresh weight due to PGPR inoculation is observed during the *Bacillus* Pb03 inoculation in the bulb initiation phase, showing a 70.5% improvement compared to the without PGPR. Meanwhile, for the *pseudomonas* Pb04 inoculation, the most significant yield enhancement occurs during drought stress in the vegetative growth phase, with a weight increase of 53.5% compared to the without PGPR. Notably, during the maturation phase, insignificant is observed across all treatments.

The dry bulb weight results closely mirror the trends observed in fresh bulb weight. Under drought stress conditions without PGPR inoculation, a substantial reduction in the dry weight of shallot bulbs, in the vegetative, bulb initiation, bulb development, and maturation phases, with declines of 49.1%, 60.8%, 69.2%, and 16.4%, respectively, compared to plants without stress. (Figure 7b). The inoculation of *Bacillus* Pb03 proves influential in enhancing

the dry bulb weight, particularly during the bulb initiation phase, resulting in a remarkable 77.4% increase compared to plants without inoculated PGPR. Meanwhile, the highest increase in dry bulb weight due to *pseudomonas* Pb04 inoculation occurs under drought stress during the vegetative growth phase, reaching 59.5%. In contrast, drought stress during the maturation phase yields dry bulb weights that are not statistically significant.

DISCUSSION

This research indicates that the timing of drought stress and the inoculation of different bacterial strains, along with their interactions, significantly affect the photosynthetic characteristics, chlorophyll content, and yield of shallots. Shifts in AQY, LSP, and LCP suggest an adaptation of shallot plants to drought conditions. It is conceivable that plants optimize light utilization
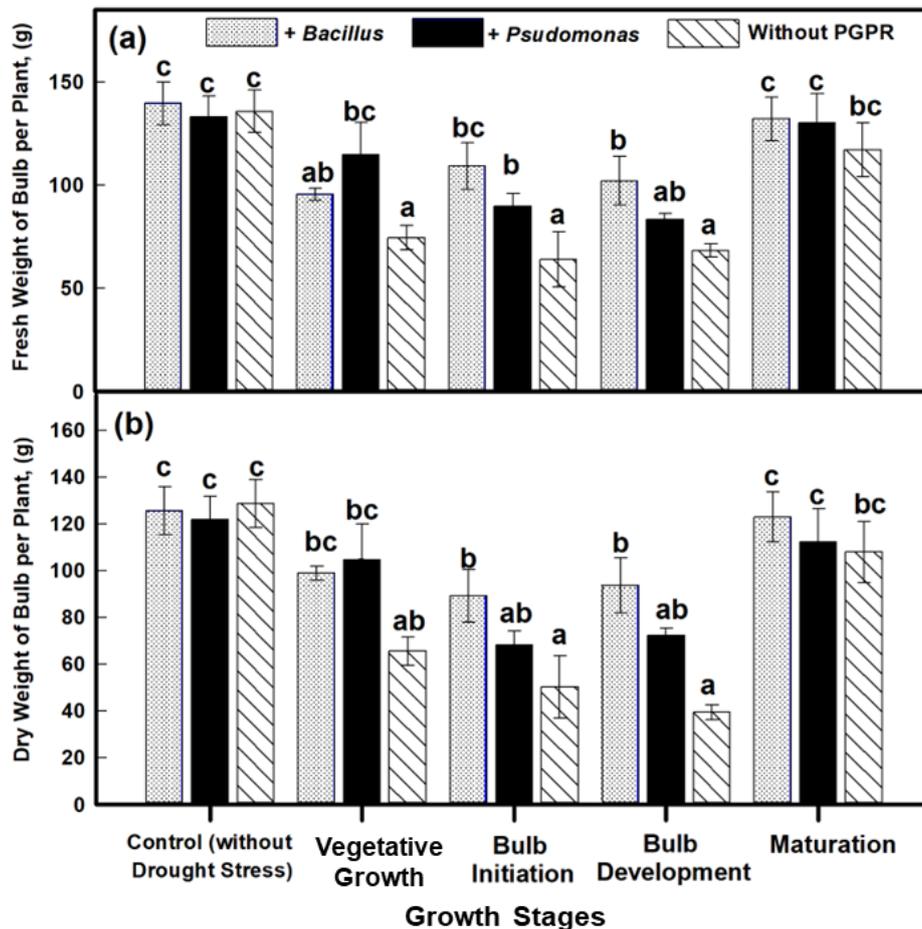
Figure 7. (a) Fresh bulb and (b) dry bulb weight per plant under different drought stress timings and inoculation PGPR. Mean values with standard error of the mean (n = 3). Letters indicate significant differences at p < 0.05 according to Turkey test

at lower light levels to cope with limited water availability (Vialet et al., 2017).

Among the four drought stress conditions in various growth phases, shallots inoculated with PGPR exhibit higher PNmax and LSP, indicating strong photosynthetic capacity and adaptability to drought-stressed environments. The results of this study indicate that *Pseudomonas* Pb04 is more effective when inoculated during the early growth to vegetative phase of the plant under drought stress, based on parameters such as photosynthesis, chlorophyll, and yield. Meanwhile, *Bacillus* Pb03 is effective when applied to the plants during the bulb initiation and bulb development phase (generative phase) under the same parameters and drought stress conditions. Previous studies have confirmed the sensitivity of photosynthesis to environmental stress. Shallots experiencing drought stress during the bulb maturation phase exhibit a higher open Photosystem ratio and greater photochemical efficiency (Sánchez and Sánchez, 2019). This contributes to better maintenance of photosynthetic rates compared to plants experiencing drought stress without PGPR during the vegetative phase, bulb initiation, and bulb development. Consequently, this leads to higher yields.

Plants cease accumulating organic matter when light intensity falls below the LCP. The RD value reflects the plant's consumption of photosynthesis products. The research findings indicate an increase in the respiration rate (RD) in shallot plants experiencing drought stress. This could be attributed to the plant efforts to ensure survival by enhancing energy production through respiration under stressful conditions (Czarnocka and Karpiński, 2018). LCP and RD values are elevated under drought stress conditions compared to the control treatment, suggesting that increased consumption of photosynthesis products accompanies a substantial photosynthetic capacity. This results in resource wastage, prompting PGPR to play a role in suppressing dark respiration values to reduce the consumption of photosynthesis products and mitigate resource wastage from photosynthetic yields (Khatoon et al., 2020). PGPR inoculation maintains LSP, LCP, and RD at more stable levels under drought stress. This signifies the positive role of PGPR in enhancing plant tolerance to environmental stress (Yagoubi et al., 2023). PGPR plays a crucial role in the photosynthetic performance of shallots grown under drought-stress conditions during various growth phases. Drought can diminish the activity of

carbon assimilation enzymes in photosynthesis, limiting the carbon assimilation of plants and consequently reducing the effective quantum yield of the Photosystem (Sun et al., 2023). Therefore, plants grown under drought conditions typically exhibit lower photosynthetic efficiency and biomass. This pattern is observed in this study when shallots are cultivated under drought stress during the bulb initiation and bulb development phases, showing lower average values of Pnmax and LSP compared to other treatments, with light intensity maintained below the critical level under all stressed conditions. Furthermore, shallots grown under vegetative and maturation treatments exhibit relatively lower values of LCP and RD, suggesting a survival mechanism based on resource conservation by reducing carbon loss to respiration (Wang et al., 2022).

The AQY estimates a plant utilization capacity under stressed conditions. An increase in AQY is observed in shallot plants inoculated with PGPR, indicating that PGPR can enhance the efficiency of light energy utilization in the plant's photosynthetic process, even under drought conditions (Pereira et al., 2020). In our study, PGPR inoculation, particularly with *Bacillus* Pb03, increased AQY and decreased RD in shallots during most phases of drought stress. This suggests that shallots under drought-stress conditions maintain a balance in material and energy metabolism by enhancing the utilization capacity of photosynthates and employing resources relatively efficiently (Chauhan et al., 2023).

The chlorophyll content significantly influences the photosynthetic capability and overall plant yield. Chlorophyll a and b values in leaves have been utilized as valuable indicators to assess the photosynthetic capacity. The relative chlorophyll content correlates positively with the photosynthetic rate (Tang et al., 2023). Chlorophyll serves three primary functions in the photosynthetic process: harnessing solar energy, initiating CO_2 fixation to produce carbohydrates, and providing energy for the entire ecosystem (Singh and Pandey, 2020). Chlorophyll can capture light absorbed by other pigments through photosynthesis, making it the central pigment designation in the photosynthetic reaction (Kumar et al., 2021). Chlorophyll b results from the biosynthesis of chlorophyll a and plays a crucial role in the reorganization of photosystems during adaptation to changes in light quality and intensity (Rogowski et al., 2019). Therefore, the loss of chlorophyll a

and b hurts photosynthetic efficiency. Research indicates that PGPR inoculation can positively impact chlorophyll content in plants undergoing drought stress. The mechanisms through which PGPR enhances plant resilience to drought may involve the production of compounds such as IAA (indole-3-acetic acid) and ACC deaminase, which help mitigate oxidative stress in plants (Hassan dan Maheshwari, 2018). This can protect chlorophyll pigments from damage caused by free radicals generated during drought conditions. These findings indicate that the timing of drought stress and PGPR inoculation contributes to photosynthetic efficiency. The combination of PGPR roles and the timing of drought stress plays a crucial role in assisting plants in overcoming drought.

Some specific mechanisms of action of PGPR may occur at various stages of plant development. On Vegetative Growth, *Pseudomonas* Pb04 is generally known to produce growth hormones such as IAA that can stimulate germination and root growth, and often influence plant vegetative growth by stimulating the production of additional growth hormones, such as cytokinin, which can increase branch and leaf formation. On the other hand, *Bacillus* Pb03 plays a role in enhancing plant tolerance to biotic and abiotic stress through the production of compounds that enhance the plant defense system. Generative Growth: *Bacillus* can influence the processes of flowering and fertilization in plants through its interaction with plant growth hormones. *Bacillus* can increase nutrient availability to plants by breaking down complex organic compounds into forms that can be absorbed by plants. Meanwhile, *Pseudomonas* can help improve fertilization success by enhancing the balance of plant hormones. Whereas for Generative Growth, *Bacillus* can influence the processes of flowering and fertilization in plants through its interaction with plant growth hormones. *Bacillus* can increase nutrient availability to plants by breaking down complex organic compounds into forms that can be absorbed by plants. Meanwhile, *Pseudomonas* can help improve fertilization success by enhancing the balance of plant hormones.

CONCLUSIONS

This study underscores that the effective combination of drought stress timing and PGPR inoculation can be employed to achieve optimal photosynthetic performance and yield in greenhouse conditions. Given the varying leaf photosynthetic capacities at different plant positions during development, particularly the notable differences between young and mature leaves. The choice of PGPR is critical for optimizing photosynthesis and yield under varying drought stress conditions. Drought stress during the vegetative phase is recommended for the inoculation of *Pseudomonas* Pb04, while stress during bulb initiation and bulb development phases is advised for *Bacillus* Pb03 inoculation. Moreover, during the final growth stage, the utilization of specific PGPR types becomes less significant as the plants exhibit resilience to drought stress.

Acknowledgments

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