The application of phosphorus and potassium to increase drought tolerance in *Pereskia bleo* (Kunt) DC with proline and antioxidant indicators

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Abstract. The El Nino anomaly caused a decrease in rainfall and the occurrence of irrigation water drought. *Pereskia bleo* leaves contain high antioxidants. Proline in leaf tissue plays a role in reducing osmotic potential and maintaining cell turgor. Potassium can regulate the osmotic pressure and turgor, whereas Phosphate deficiency and drought will reduce the result of photosynthesis. This study aims to assess the role of Potassium and Phosphate in the tolerance and dynamics of secondary metabolites in *P. bleo* under drought conditions. The study was conducted from April to July 2018. The drought was artificially induced by controlling watering treatment for 30% and 60% field capacity once every 3 days after the plants are two months old. Using a Nested Randomized Complete Block Design. Fertilizing of KCl and SP36 are nested into 3 doses of each (0; 1.5 and 3) g/polybag at the beginning of planting. The results showed that the drought reducing plant growth but in contrast raising the levels of proline and antioxidants. There was an interaction between Potassium and Phosphate in the treatment of drought. Applying fertilizer Phosphate and Potassium could improve *P. bleo* tolerance to drought.

1. Introduction

Global climate change in the last ten years brought significant changes to the surface of the earth. In Indonesia, climate change markedly increases the frequency of El Nino events. The El Nino anomaly caused a decrease in rainfall and the occurrence of irrigation water drought, which adversely affected plant growth, physiological and biochemical activities which cause a decrease in crop yield [1].

The responses of medicinal plants to drought stress are different on the growth and productivity of secondary metabolites. The results of several experiments in the study showed that some medicinal plants increase the productivity of secondary metabolites when exposed to drought stress [2]. Anthocyanins are reported to accumulate under stress, and plant tissues containing anthocyanin are usually more resistant to drought [3]. Drought stress in *Papaver somniferum* has induced three types of alkaloids (narcotine, morpnume, and codeine) [4].

*Pereskia bleo* (Kunt) is classified as a primitive cactus that has leaves, flowers, and fruit. People in Malaysia and China use the leaves for salad and vegetable cuisine [5,6]. Studies showed that *P Bleo* leaves could potentially cure inflammation, digestive disorders, hypertension, and cancer due to its...
antioxidants content such as alkaloids, carotenoids, phenolic, minerals, and vitamins [7]. Although this plant has not been cultivated, it can grow well in Indonesia. 

*P. bleo* belongs to the family Cactaceae, comes from South America and grows in other countries with a tropical climate. In Malay, it is known as 'Jarum Tujuh Bilah' or in Chinese, it is called 'Cak Sing Cam'. This plant is a thorny bush that grows as high as 2 to 8 m, has orange flowers. The leaves of this plant are simple, spiral, shiny, and fresh [8]. *P. bleo* leaves contain antioxidants from the main flavonoids, namely catechins, quercetin, epicatein and myricetin besides that the concentration of β-carotene and α-tocopherol is quite high. In some studies have the potential to be anti-cancer, anti-tumor and anti-inflammatory [9].

Potassium impacts on osmotic potential and contributes to the maintenance of turgor [10]. Mengel and Kirkby [11] reported that the physiological process of Potassium by regulating turgor pressure and photosynthesis; cation translocation and enzyme activation, meanwhile, Cakmak [12] states that plants affected by drought stress require more internal potassium. While the effects of water deficits can be reduced by increasing potassium intake [13].

Most types of soil, including fertile soil, indicate that the availability of phosphorus elements is low [14], P solidity and drought will reduce photosynthesis results, reduce ion mobility, and nutrient availability [15]. Phosphorus application significantly increases the resistance to the negative effects of drought and proven fertilization P in legumes under limited water conditions can increase plant resistance and yield [16]. A common response that occurs in plants that experience abiotic stress is the accumulation of proline to protect the plant by functioning as a cellular osmotic regulator. While the enzymatic process that occurs is the increased Reactive Oxygen Species (ROS) and as a plant defense enhances the formation of antioxidants superoxide dismutase (SOD) [17] so that it can be an indicator of tolerance to drought. The purpose of this study was to examine the role of potassium and phosphate tolerance and the dynamics of secondary metabolites of *P bleo* in drought conditions with indicators of proline and antioxidants.

2. Materials and methods

The implementation of this study was from April to July 2018. The drought was artificially induced by controlling water treatment for 30% and 60% field capacity (fc) once every 3 days after the plants were two months old. Using the Complete Random Nested Block Design. KCl (K) and SP36 (P) fertilization were nested into 3 doses (0, 1.5 and 3) g / polybag respectively at the beginning of planting. Analysis of the parameter variables for growth, chlorophyll, proline, and antioxidants.

2.1. An assessment of leaf chlorophyll concentration

Leaf chlorophyll content was determined using the spectrophotometer method [18]. Leaf tissue weighing 1 g was crushed in a mortar and 80% acetone added to it in sufficient quantities to allow the tissue to be thoroughly homogenized. The supernatant was decanted through a filter paper into a 100 ml volumetric flask. The resulting solution was thoroughly mixed and 5 ml pipetted into a 50 ml volumetric flask and made up to volume with 80% acetone. The absorbance of the extract was then measured at 645 nm wavelengths for chlorophyll a, b and total chlorophyll respectively, using spectrophotometer with 80% acetone been used as Blank.

2.2. Proline analysis

Proline analysis was performed according to Abrahám et al. [19]. Briefly, 50 mg of extract was homogenized in 10 ml sulphasalicylic acid (3% w/v) and filtrated through filter paper. Two milliliters of the filtrate was mixed with 2 ml of acid ninhydrin solution (1.25 g ninhydrin + 30 ml glacial acetic acid + 20 ml 6 M H3PO4) and 2 ml of glacial acetic acid and kept at +100°C for 1 h. Then the reaction was stopped by transferring the mixture to an ice bath. Four milliliters of toluene was added to the
mixture and vortexed for 15–20 s. The toluene phase was aspirated and absorbance at 520 nm was measured using pure toluene as reference. A calibration curve was prepared with pure proline. Results were expressed as µg proline/gram extract.

2.3. DPPH radical scavenging assay
Radical scavenging activity of plant extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined spectrophotometrically [20, 21]. The principle of the assay is based on the color change of the DPPH solution from purple to yellow as the radical is quenched by the antioxidant. Briefly, 1 ml of 0.1 mM DPPH in methanol was mixed with 3 ml of extract solution with differing concentrations (5–250 µg ml⁻¹) and the mixture was vortexed. The samples were kept in the dark for 30 min at room temperature and then the decrease in absorbance at 517 nm was measured. The absorbance of DPPH solution in the absence of plant extract was measured as the control. Ascorbic acid, BHT and rutin were used as positive controls. DPPH radical scavenging activity was expressed using the formula: % DPPH radical scavenging activity = [(A₀ - A₁)/A₀] x 100 where A₀ was the absorbance of the control and A₁ was the absorbance of the sample.

3. Result and discussion

3.1. Growth and component
Drought has a significant influence on growth. Phosphate and potassium applications have no effect on growth components except total chlorophyll and the interaction of the combination of Phosphate and potassium on root shoot ratio. Analysis of variance is presented in table 1.

| Source       | Plant High (cm) | Leaf number | Root volume | Root shoot ratio | Chlorophyll a mg g⁻¹ | Chlorophyll b mg g⁻¹ | Chlorophyll total mg g⁻¹ |
|--------------|-----------------|-------------|-------------|------------------|-----------------------|-----------------------|------------------------|
| Drought (d)  | *               | **          | *           | ns               | ns                    | ns                    | ns                     |
| Potassium (Kd) | ns             | ns          | ns          | ns               | ns                    | ns                    | ns                     |
| Phosphorus (Pd) | ns             | ns          | ns          | ns               | ns                    | ns                    | *                      |
| Interaction Kd*Pd | ns             | ns          | *           | *                | ns                    | ns                    | ns                     |

Note: ns= not significantly; * or** = significant or very significant in 5% or 1%. Kd and Pd are nested in drought

Table 1. Shows that drought has a significant or very significant effect on the growth component. Drought reduces growth by affecting photosynthesis, respiration, membrane stability index and nutritional metabolism. While morphological and physiological changes in response to drought can help identify plants that are tolerant of drought. Drought inhibits plant height and decreases the number of leaves (22). In this study, watering 30% field capacity (fc) reduced plant height 18% and reducing the number of leaves 60% compared to watering 60% field capacity (fc).

In drought stress conditions, plants close the stomata to avoid further water loss. Drought stress resulting in loss of turgor and lower plant nutrient uptake. Furthermore, drought also decreases the leaf’s internal CO2 concentration and also the activity of enzymes and ATP synthesis, which results in a decrease in the rate of net photosynthesis under drought stress. Reduced inhibition of photosynthesis under stress is of great importance for drought tolerance [23].

Drought stress resulted in the loss of turgor and lower plant nutrient uptake [24]. *Pereskia* has a CAM metabolism pathways that can maintain the water potential of leaves high enough and efficient for the
photosynthesis [25]. In general, drought suppresses chlorophyll content, but in this study, only total chlorophyll is affected. Plant height and number of leaves decreased because the cell enlargement will be depressed. Photosynthesis decrease because stomata close and decrease cell turgor which is reflected in a decrease in the number of leaves [26].

Figure 1. The effect of KCL (K) and SP36 (P) fertilizer dose on the level of drought on (A) Root Volume and (B) Root Shoot Ratio

Drought up to 30% of field capacity (fc) (Fig. 1.A) significantly suppresses root volume. The response of plant roots to water stress depends on the type of plant and the amount of drought pressure, but in general, drought will reduce root volume [27]. Besides, Hsiao and Xu [28] stated that vegetative growth is more sensitive to water pressure, root growth is more resistant than leaf growth. This is also shown in the root shoot ratio (Fig. 1B). The mechanism of avoiding drought by maintaining water absorption through increased root growth at the beginning of drought to absorb water in deep soils [29]. There are interactions in the combination of potassium and phosphorus applications. Potassium enhances physiological processes by regulating turgor pressure and photosynthesis, translocation, and activation of cations and enzymes [30]. Because nutritional and water requirements are closely related, fertilizer applications tend to increase plant efficiency in using available water. This shows a significant interaction between soil moisture deficit and nutrient acquisition.

The application of phosphorus plays an important role in root development thereby increasing accessibility to other nutrients in the rhizosphere [31]. In addition, the root tip is responsible for sensing and signaling the availability of phosphorus, and initializing a reduction in root growth in environments that lack phosphorus [32]. Higher root biomass also increases the ability of roots to regulate soil moisture, contributing to drought tolerance [33]. Therefore, phosphorus application can increase plant tolerance to drought through the promotion of root biomass. This higher root biomass will be a further advantage in drought conditions where water is available in deeper soil profiles [34]. In contrast to the study reported by Graciano et al. [35] that there was an increase in the rate of growth and accumulation of biomass in plants fertilized with phosphor, but in this study, the application of SP36 was not significant to the growth of P bleo. High variability in the response of different plant species to phosphorus application and phosphor absorption occurs at the soil surface, and lower diffusion rates and slower movements towards the roots, compared to other nutrients, will affect the efficiency of their use [36].
3.2. Proline and antioxidant content

The level of drought stress and fertilization have a significant effect on proline levels (Figure 2A). There is an interaction between KCl and SP36 fertilization at the several levels of watering for antioxidants (Figure 2B).

Potassium applications in plants exposed to drought will increase the ability to regulate turgor pressure and photosynthetic activity. So the effect of drought can be helped by potassium fertilization. While the contribution of phosphorus in plants affected by drought is to expand the field of phosphorus absorption [14]. The use of P fertilizer reduces its deficiency in soils, increases the ability of plants that are tolerant of stress and results in adjustments to physiological, morphological, and biochemical processes that increase plant growth [37].

When severe water shortages occur, the amino acid, especially proline, accumulate in plant tissues. Under drought pressure the proline content is significantly increased, related to metabolic rate, decreased catabolism and reduced enzyme activity and subsequently proline can alter the activity of antioxidant enzymes [38]. Drought-tolerant varieties show that an increase in the enzyme catalase, peroxidase and superoxide dismutase. Mittler [31] states that antioxidant enzymes and proline accumulation are related to plant tolerance to drought stress.

In this study, drought and fertilization have a significant effect and there are interactions on the dose of KCl and SP36 on proline levels (Figure 1A). Proline metabolism is coordinated in membrane and protein against the effect of abiotic stress [28]. A significant increase in proline content under stress is associated with increased rates of synthesis, reduced catabolism levels and loss of enzyme inhibitors, which have shown that proline can also alter the activities of antioxidant enzymes. The antioxidant activity of catalase enzyme, peroxidase, and superoxide dismutase in drought-tolerant varieties increased significantly. It can be concluded that antioxidant enzymes and accumulation of proline associated plant tolerance to drought stress [31].

4. Conclusion
Climate change produces climate anomalies that impact drought stress and it has a negative effect on plant growth but in contrast not always on secondary metabolism. In this study, the application of
potassium and phosphorus increases the tolerance potential of *Pereskia bleo* by increasing the root biomass, and the dynamics of the proline associated with antioxidant enzymes.

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