ABIOTIC STRESS AND BIOFERTILIZER ON THE *Pereskia bleo* (Kunth) DC. AGAINST GROWTH, PROLINE, AND ANTIOXIDANT

Intan Christin Dullah^{1*}, Sulandjari², and Supriyono²

¹Post Graduate Student of Agronomy, Sebelas Maret University, Surakarta, Central Java 57126, Indonesia ²Departement of Agrotechnology, Faculty of Agriculture, Sebelas Maret University *Submitted : 2018-11-06 Accepted : 2018-12-28*

ABSTRACT

Pereskia bleo is a spiny bush from the genus Pereskia and family Cactaceae. Its leaves are shiny green, flowering and fruitful. Some researchers were indicated that *P.bleo* leaves contain high antioxidants, anti-cancer, treat diabetes, hemorrhoids, hypertension, and gastric pain. The purpose of this study is to investigate the effects of abiotic stress and intake of biofertilizers on growth, proline, and antioxidants. The experiment was arranged using the nested design (complete random factorial) with three treatments. the first treatment of sodium chloride with three levels is; 0 ppm, 5 ppm, 10 ppm and the treatment of the two biofertilizers with two levels is; 5 ppm, 10 ppm. One and two treatments nested in treatment three were 500 ml, 300 ml, and 150 ml water volume per polybag. Each treatment was repeated three times, resulting in a total sample of 54 plants. The findings indicate that abiotic pressure suppressed growth and chlorophyll, while the proline tended to decrease. Abiotic stress can increase the antioxidant content and promote the interaction between salinity and biofertilizer against antioxidants.

Keywords: Biofertilizer, Drought stress, Pereskia bleo, Salinity, Secondary metabolites

How to Cite: Dullah, I.C., Sulandjari, and Supriyono. (2019). Abiotic Stress and Biofertilizer on the *Pereskia bleo* (Kunth) DC. Against Growth, Proline, and Antioxidant. Sains Tanah Journal of Soil Science and Agroclimatology, 16(1): 36-45 (doi: 10.20961/stjssa.v16i1.25158)

Permalink/DOI: http://dx.doi.org/10.20961/stjssa.v16i1.25158

INTRODUCTION

Pereskia, family Cactaceae originates from America and is distributed in tropical and subtropical regions. The genus has 17 species including *Pereskia bleo*, *Pereskia*. *Grandifolia* and *Pereskia*. *acuelata* (Zareisedehizadeh et al., 2014). Pereskia is a spiny, shrubby, and leafy plant. The leaves of, this plant are simple, spiral, glossy and succulent (Wiart, 2006). The flowers are yellow to red and the ripe fruit is yellow, a fleshy, cone-shaped and contains seeds (Zareisedehizadeh et al., 2014). The ingredients

* Corresponding Author: Email: intanchristin0@gmail.com of phytochemicals active Pereskia bleo include alkaloids, flavonoids, catechins, ß carotene and high antioxidants (Tan et al., 2005). In Malaysia, China and Indians in America, Pereskia bleo leaves are traditionally used for the treatment of cancer, hypertension, diabetes and other diseases. In addition to the treatment of leaves, Pereskia bleo is also used as a vegetable because it contains many minerals (Malek et al., 2009; Murillo et al., 2010). Drought can be caused by water, that- available in the soil which is reduced and or high evapotranspiration so that plants lose turgor pressure. In addition, salinity also causes drought stress, among others, because of the high salt content in soil solution where rainfall is not able to dissolve it so that the potential of water in the medium grows low and ionic poisoning due to excessive uptake of sodium and chloride ions (Jamil et al., 2007).

Environmental stress affects the growth of plants such as drought and high salinity. These environmental pressure have the potential to reduce crop productivity. High salinity causes a decrease in water potential and affects the availability of water and also reduces nutrient uptake (Hussain et al., 2009). Water deficit and salinity of the plant can be seen the signs on the growth and physiological responses. So, we can consider the tolerance ability of a plant to drought and salinity. Drought results in germination inhibited, vegetative and generative growth is depressed. Salinity also affects photosynthesis, further suppress leaf area, chlorophyll content and stomatal closure. For example, in Mentha piperita L (Tabatabaie & Nazari, 2007), Catharanthus (Jaleel et al., 2008) and Thymus vulgaris L (Aziz et al., 2008). The challenge of environmental stress is not only for food crops but also for medicinal plants. But with secondary metabolite productivity, abiotic stress is not always the result of decrease, as evidenced by some studies proving that abiotic stress conditions raise the secondarv metabolite levels of medicinal plants.

Damage to plants due to salinity mainly through the effects of osmosis, certain ionic toxicity, and nutritional stress. Proline is one of the amino acids that play a role in the tolerance of plants against abiotic stress. In *Echium* amoeum medicinal plants increased salinity has increased proline levels even though the dry weight of the root's decreases (Ashraf & Orooj, 2006). Salinity stress was shown to increase proline levels and antioxidant (Munns & Tester, 2008). Biochemical and physiological responses to drought and salinity stress in plants include inhibition of growth and reduced activity and biochemical enzymatic constituents (Jaleel & Gopi, 2007). One of the plant's biochemical responses to salt stress is the production of reactive oxygen forms (ROS) that disrupt the balance between ROS production and loss of effect by antioxidant enzymes which cause oxidative damage to lipids, proteins, and nucleic acids (Navyar & Gupta, 2006). Antioxidants consist of enzymes including catalase, ascorbate peroxidase, glutathione, ascorbate, total carotenoids, phenolics and flavonoids (Taïbi et al., 2016). Increased antioxidant activity is in line with the increase in enzymes. Some researchers stated that drained stress resistance has worked with increased antioxidant activity (Reddy et al., 2004). Yazici et al. (2007) reported that there has been an increase in enzyme antioxidant activity and proline accumulation in saline conditions.

Biofertilizer is a natural fertilizer that contains many microorganisms that can help improve plant growth and yield. Thus, the biological fertilizer is also believed to increase soil fertility. Therefore, the volume of watering, Sodium Chloride (NaCl), and biological fertilizer in experimental plants in Biofertilizer is a natural fertilizer that contains many microorganisms that can help improve plant growth and yield (Bhardwaj et al., 2014). Thus, the biological fertilizer is also believed to increase soil fertility. Therefore, the volume of watering, salinity, and biological fertilizer in experimental plants in this study is intended to investigate the effect of abiotic pressure and biological fertilizers on growth, proline, and antioxidant of Pereskia bleo.

MATERIALS AND METHODS

The research was conducted in August 2017–February 2018 in the greenhouse of the Faculty of Agriculture at Sebelas Maret University, Surakarta. *Pereskia bleo* seedlings that have been propagated by means of stem cuttings were used as planting materials. *Pereskia bleo* was grown in polybags that contained 5 kg of media consisting 2 : 1 soil and manure ratio.

The experimental was arranged using Nested design (completely randomized factorial) with NaCl treatment; 0 ppm, 5 ppm, 10 ppm, and biofertilizer; 5 ppm, 10 ppm, each nested in a watering volume (below field capacity) of 500 ml, 300 ml and 150 ml per polybag. Watering was done according to treatment watering once every three days according to the watering volume. Each treatment was repeated three times, resulting in a total sample of 54 plants. Parameter variables to plant height, number of leaves, leave area root and shoot; chlorophyll a, b and total; proline and antioxidants. Chlorophyll analysis by spectrophotometer method; Proline with the ninhydrin method (Bates et al., 1973), and antioxidants by Diphenylpicrylhydrazyl method.

The extraction and estimation of the chlorophyll were done by grounding one gram of finely cut leaf sample with 80 % acetone, then centrifuged. The supernatant is transferred. The absorbance of the solution was read at 645 nm against the solvent (80% acetone) as blank. The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were then calculated using the equation as follows:

Chlorophyll a (mg/g) = 12.72(OD663) - 2.59(OD645)

Chlorophyll b (mg/g) = 22.9(OD 645) - (OD 663) Total Chlorophyll (mg/g) = 20.31(OD 645) + 8.05(OD 663)

Proline analysis was performed according to Bates et al. (1973; Marin et al., 2009), where diethyl ether extract was exempted from this test since proline is practically insoluble in diethyl ether. 50 mg of extract were homogenized in 10 ml sulphosalicylic 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^o C for 1 h. 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 was measured using pure toluene as a reference (Marin et al., 2009). A calibration curve was prepared with pure proline.

Radical scavenging activity of plant extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined bv spectrophotometrically (Molyneux, 2004). 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 ethanol was mixed with 3 ml of extract solution with differing concentrations (5–250 g/ml) 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 and BHT were used as positive controls. DPPH radical scavenging activity was expressed using the formula: % DPPH radical scavenging activity = $[(A_0 - A_1)/A_0] - 100$ where A_0 was the absorbance of the control and A_1 was the absorbance of the sample.

RESULTS

Soil fertility level on planting medium in this research has low level indicated by organic content 1.89% (low); pH H₂O 7.4 (Neutral); Nitrogen available 0.26% (intermediate); available Phosphorus 8.38% (intermediate); available Potassium 0.24 me% (Low); and cation exchange capacity 16 me% (low).

Table 1 shows that the watering volume has a significant or very significant effect on the growth components, namely plant height, leaf number, leaf area, and root-shoot ratio. Meanwhile, the salinity in the watering volume has a very significant effect on the growth component except for plant height. All treatments have no significant effect on chlorophyll and proline, even though it has a very significant effect on antioxidants. There is interaction between salinity and an biofertilizer in the watering volume to antioxidants.

Table 2 shows that in all treatments there was pressure on plant height even though it was not statistically significant. The treatment of 10 ppm NaCl at 150 ml watering volume produced the lowest plant height compared to the above watering volume. Salinity and drought stress results in a reduced number of leaves and leaf area. The treatment of 10 ppm NaCl at the 150 ml watering volume produced the least number of leaves and the lowest leaf area. Salinity and drought stress suppress the ratio of shoot roots, treatment of 10 ppm NaCl at 150 ml watering volume produces the lowest root canopy ratio. Salinity and drought stress suppress the components of chlorophyll a, chlorophyll b and total chlorophyll, although they are not statistically significant.

In this research, all treatments did not significantly affect the proline content (Table 1). <u>Table 3</u> shows that the treatment of 10 ppm biofertilizer and 10 ppm NaCl decreases the proline content in each watering volume, even though it is not statistically significant. On the other hand, Figure 1 depicts the higher level of stress promoted the higher antioxidant contents.

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Source	Plant high	Leaf number	Leaf area	Root- shoot ratio	Chlorophyll a	Chlorophyll b	Total chlorophyll	Proline	Antioxidant
W	**	**	**	*	ns	ns	ns	ns	**
S	ns	**	**	**	ns	ns	ns	ns	**
В	ns	ns	ns	ns	ns	ns	ns	ns	**
S*B	ns	ns	ns	ns	ns	ns	ns	ns	**

Table 1. Analysis of variance in the effect of salinity and biofertilizer in watering volume on growth, proline, and antioxidants

Note: W= Watering volume; S= Salinity; B= Biofertilizer; S*B= Interaction; ** = very significant ; * = significant; ns= not significant

			Treatment				
Parameter	Watering _	Salinity NaCl (ppm)					
		0	5	10	Average		
Plant high (cm ²)	500 ml	62.40	62.23	63.37	62.67 ^P		
	300 ml	56.75	54.62	59.93	57.09 ^Q		
	150 ml	53.78	56.08	57.08	55.65 ^R		
Number of leaves	500 ml	62.67ª	83.67ª	58.50 ^b	68.28 ^P		
	300 ml	58.50 ^b	51.33 ^b	77.67 ^{ab}	62.50 ^Q		
	150 ml	40.33 ^{bc}	36.67 ^c	35.00 ^c	37.33 ^R		
Leaf area (cm ²)	500 ml	0.43ª	0.31 ^b	0.29 ^{bc}	0.24 ^P		
	300 ml	0.34 ^b	0.26 ^{bc}	0.25 ^{bc}	0.28 ^P		
	150 ml	0.09 ^c	0.07 ^{bc}	0.06 ^c	0.08 ^Q		
Root Shoot Ratio	500 ml	0.23ª	0.17 ^b	0.21ª	0.20 ^P		
	300 ml	0.22ª	0.18 ^b	0.13 ^c	0.18 ^Q		
	150 ml	0.19 ^c	0.17 ^b	0.17 ^b	0.17 ^Q		
Chlorophyll a	500 ml	13.94	10.85	11.70	12.16		
	300 ml	12.31	11.42	10.32	11.35		
	150 ml	11.40	9.61	9.55	10.19		
Chlorophyll b	500 ml	4.53	4.17	4.66	4.45		
	300 ml	4.52	5.74	3.78	4.68		
	150 ml	5.09	3.70	3.91	4.23		
Total Chlorophyll	500 ml	19.12	14.61	16.09	16.61		
	300 ml	17.11	15.49	14.11	15.57		
	150 ml	16.50	13.22	13.47	14.40		

Table 2. Effect of salinity and biofertilizer in watering volumes on growth component

Notes: Numbers followed by the same letter (a-c) in columns and rows or (P-R) in the column, each parameter shows no significant difference in 1% or 5% DMRT test

Table 5. Effects of samily and proferences nested in the watering volume on profile content	Table 3. Effects of salinit	y and biofertilizer nested in the watering volume on proline co	ontent
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Watering	Biofertilizer	0 ppm NaCl	5 ppm NaCl	10 ppm NaCl
500 ml	5 ppm bio	2.45	0.35	1.05
	10 ppm bio	0.6	0.53	0.73
300 ml	5 ppm bio	1.04	0.38	0.06
	10 ppm bio	0.64	0.78	0.45
150 ml	5 ppm bio	1.19	0.85	1.35
	10 ppm bio	0.02	0.44	0.46

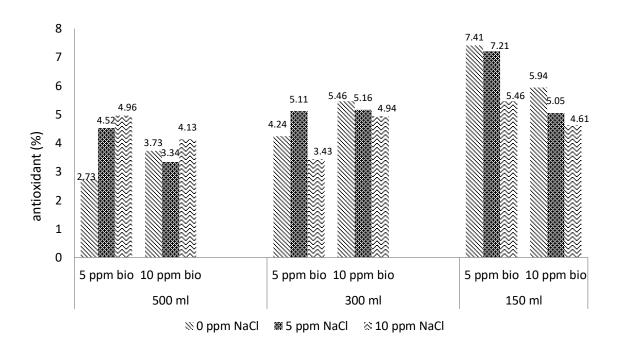


Figure 1. Histogram of biofertilizer and salinity interaction at several watering volume levels of antioxidant content

DISCUSSION

Abiotic stress includes drought and salinity is one of the important factors that affect every aspect of growth and crop production. These stresses cause water to be unavailable in the root area so that the absorption of water and nutrients for the metabolism of the plant becomes disrupted. Kannan & Kulandaivelu (2011) stated that the availability of nutrients in growing media influence on plant growth. However, drought stress from lack of water or productivity can affect the active ingredients of the plant.

Table 2 shows that drought has negatively affected growth. The watering volume of 300 - 150 ml was significantly different from the adequacy of water as much as 500ml. Water potential is lower due to drought stress, hence the cell enlargement and generative process will be depressed. Cell growth decreases in response to plants facing drought stress due to inhibited cell development and cell turgor pressure. Turgor cells decrease resulting in stunted growth and enlargement of leaves. Therefore, in this study drought caused the number of leaves and leaf area to be depressed, plant biomass also decreased so that the root-shoot ratio decreased as the plant response mechanism avoided the magnitude of transpiration and water absorption activities. The research of (Bhardwaj et al., 2014) showed that drought stress suppressed plant height, number of leaves and root dry weight. Drought also inhibits plant growth and development due to the inhibition of cell division and closing of the stomata.

Salinity suppresses growth components except for plant height. The higher the concentration of NaCl, the more suppressed the growth will be. So that plant height, leaf number, leaf area and root-shoot ratio at 150 ml watering volume were significantly lower than larger watering volumes. NaCl can cause a decrease in osmosis pressure which causes plants to have difficulty absorbing water. This will reduce the rate of photosynthesis because the stomata close and decrease cell turgor which is reflected in a decrease in the number of leaves and leaf area (Xu & Zhou, 2008).

Chlorophyll content is one of the indicators used to determine the response of plants to drought and salinity stress (Ai & Banyo, 2011). Table 1 shows that drought stress has no significant effect on chlorophyll a, chlorophyll b and total chlorophyll. However, the analysis of chlorophyll content was different between treatments, the higher the stress level the lower the chlorophyll content produced (Table 2). Water availability is an important factor in the process of chlorophyll formation (Alonso et al., 2002). In this study, drought and salinity produced a decreased chlorophyll content in Pereskia bleo plants according to several studies including Ambede et al. (2012) and Keyvan (2010), who examined the effect of salinity and dryness on chlorophyll content in ginger and wheat plants which showed a decrease in chlorophyll content. The stress of drought and salinity stress increases osmotic pressure and Reactive Oxygen Species (ROS) content in plants that can cause cell damage so that the biochemical processes of photosynthesis in plants will be hampered (Ambede et al., 2012).

Proline accumulation is an indicator of the plant's response to abiotic stresses, especially drought and salinity. Some studies report that proline accumulation is lower in tolerant plants than in plants that are sensitive to salinity stress and drought (Jungklang et al., 2017). Proline is a compound that includes amino acids that can be used to show the plant's response to stress in drought and salinity (Li et al., 2018). Each plant produces a different proline content, this is because the characteristics indicate that drought stress, salinity, and biological fertilizers do not significantly affect the proline content (Table 1). However, the analysis of the proline content was different between treatments, the higher the stress level the lower the proline content produced. This is due to the provision of biofertilizers to plants that provide nutrients for plants to keep plants remain tolerant. Plant defense mechanisms against stress are carried out with morphological and physiological adaptations (Mathius et al., 2004)

Antioxidants consist of enzymes including catalase, ascorbic peroxidase, glutathione, ascorbate, total carotenoids, phenolics and flavonoids (Taïbi et al., 2016). Increased formation and activity of the antioxidants indicates stressful plants (Agati et al., 2012). A higher level of stress accompanied with a higher the antioxidant observed in Figure 1 is in line with the research of Abdillah et al. (2015) regarding the effect drought Stress on antioxidant content in sorghum plants showing that increased antioxidant content as a mechanism of defense and tolerance to drought. This increase in antioxidant activity is consistent with the increase in enzymes. Stressful plants respond with the production of a form of reactive oxygen (ROS) that disrupts the balance between the production of ROS and the loss of effects by antioxidant enzymes that cause oxidative damage to lipids, proteins and nucleic acids with a consequence of increase antioxidants. Plant growth booster Rhizobacteria (PGPR) contained in biofertilizers can increase plant resistance to drought and salinity (Yang et al., 2009). The bacteria contained in biofertilizers help root development by increasing root surface area and the number of root tips, to provide nutrients for plants during stressful conditions (Yang et al., 2009).

CONCLUSION

This study shows that abiotic pressure suppressed growth, whereas chlorophyll and proline decreased. There was an interaction between salinity and biofertilizer towards antioxidants. 10 ppm NaCl in 150 ml water volume produced the lowest growth, while 0 ppm NaCl and 5 ppm biofertilizer in 150 ml water resulted in the highest antioxidant. Further research by examining the balanced salinity, drought, and biofertilizer concentrations to get high yields with high antioxidant content is necessary.

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