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Research Article

Growth and Physiological Attributes of Rice by the Inoculation of Osmotolerant Rhizobacteria (*Enterobacter flavescens*) under Drought Condition

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ABSTRACT

Rice (*Oryza sativa* L.) has mechanism for morphological, physiological, and biochemical self-defense in response to drought conditions. The ability of osmotolerant rhizobacteria to develop association with plants suggests that it could be used as an inoculum to support plant growth under drought stress. The purpose of this study is to determine the response of 'IR64' and 'Situ Bagendit' to the inoculation with osmotolerant rhizobacteria under drought conditions. The experiment had 3 treatment factors: 2 rice cultivars ('IR64' and 'Situ Bagendit'), 3 drought treatments (25%, 50% and 100% field capacity), and 2 types of rhizobacteria treatments (without inoculation and with inoculation using osmotolerant rhizobacteria (*Enterobacter flavescens*). Plant growth was measured in terms of plant height, number of leaves, number of tillers and panicles, and percentage of filled grain. Physiological and biochemical parameters, namely chlorophyll, carotenoids, proline, superoxide dismutase (SOD) peroxidase (POX) and ascorbate peroxidase (APX) were measured. The inoculation of osmotolerant rhizobacteria enhanced 'IR64' and 'Situ Bagendit' growth (plant height, number of leaves, tillers and panicles) and increased the percentage of grains in 'IR64' cultivar. Proline content, SOD, and APX activities were all increased by osmotolerant rhizobacteria inoculation, however, carotenoid content was decreased. Plant growth, physiological and biochemical responses of both cultivar to drought were enhanced by inoculation with osmotolerant rhizobacteria.

INTRODUCTION

Water availability is crucial in sustaining growth and development of plant. Climate change has reduced the availability of water for agriculture. Drought can affect various cellular, biochemical, and physiological attributes in plant (Bouman & Tuong 2001; Mundree et al. 2004). This stress can cause metabolic changes in plant by controlling osmotic pressure and creating free radicals as ROS (reactive oxygen species) (Bhattacharjee 2005; Halliwell 2006). Plants develop non-enzymatic or enzymatic oxidative defense system in response to free radicals (Miller 2008). The non-enzymatic defense system can take the form of antioxidant production, while enzymatically it can take form of enhanced SOD, POX and APX biosynthesis (Wang et al. 2005).

Rhizobacteria are microbes living around plant roots and playing a crucial role in plant growth. Several species of rhizobacteria have been identified as having a role in promoting plant development and crop yield (Loon 2007; Elango et al. 2013). Changes in water availability and environmental circumstances will have an impact physiologically of the soil microbial population, which will alter cell osmolarity and osmotic pressure on rhizosphere microorganisms (Miller & Janet 1996). Some rhizospheric microorganisms have developed osmotolerant systems for survival. They are also able to synthesize organic compounds in the cytoplasm that act as osmoregulators or osmoprotectants during osmotic stress or drought stress by producing proline compounds and/or glycine betaine (Csonka 1989). According to (Munro et al. 1989; Kunin & Rudi 1991), microorganisms and plants produce glycine betaine, an osmolyte that helps them endure osmotic stress. Osmoprotectants help rhizobacteria to adapt and to survive under salt and drought stress by keeping the osmotic potential of cell greater than that of their surroundings.

The ability of rhizobacteria to develop association with plants in the root system of plants opens its potential as an inoculum for plant cultivation. In a drought stressed environment, such an association, is likely to improve plant development. The addition of rhizobacteria isolates is known to stimulate plant growth by increasing P availability and nitrogen fixation (Gholami et al. 2008). It is also suggested that betaine produced by rhizobacteria reduced on the root surface is able to the root solute potential, resulting in a flow to the rhizosphere in a way that the rhizobacteria can survive under drought stress conditions. Glycine betaine is an osmolyte that is not found in all higher plants, but is produced by a variety of microbes and plants to help them withstand osmotic stress. Some bacteria, such as *Cyanobacteria*, *Eschericia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae,* and *Azospirillum sp.* generate glycinebetaine, a significant osmoprotectant (Yuwono 2005). *Enterobacter flavescens* is one of the rhizobacteria that can create an osmoprotectants, such as proline and/or glycine betaine compounds, in response to osmotic stress or drought stress (Csonka 1989). Osmoprotectants help rhizobacteria adapt and survive in environments that are stressed by salt or drought by maintaining the cell's osmotic potential higher than its surroundings. This is accomplished in *Escherichia coli* via osmoregulatory processes that result in a cytoplasm that has an appropriate osmotic pressure and is conducive to enzyme action. *E. coli* cells accumulate potassium ions and activate system for the transport and synthesis of several organic osmolytes compatible with metabolism in response to osmotic stress and a decrease in cell turgor pressure, preventing cell dehydration and stabilizing enzyme activity in high ionic strength solutions (Munro et al. 1989). Plants accumulate glycinebetaine and proline during osmotic stress suggest that these two compounds have the potential to counteract the inhibitor effects of osmotic stress in bacteria. Proline and glycinebetaine interact with proteins in a unique way that protect them from denaturation in the presence of high electrolyte concentration. These two chemicals are simply inert compatible solutes that help cells maintain turgor in high-osmolarity environment (Csonka 1989).

In Indonesia, rice is the most widely consumed food. However, rice productivity is decreasing due to problems such as salinity and drought stress. Currently, there are many superior rice cultivars with increased higher productivity compared to local cultivars. In the face of drought, the use of suitable and adaptable superior cultivars could be a viable option for rice production. In specific growing condition, superior variety seeds have a high purity, high growth percentage, and high yield potential. 'IR64' is a lowland rice cultivar that can be planted in irrigated or swampy rice fields, while 'Situ Bagendit' is a rice cultivar that can be grown on dry land or paddy fields (Suprihatno et al. 2009). 'Situ Bagendit' is known as upland rice which demonstrated resistance to drought stress compared to rice 'IR64'.

In response to decreasing availability of freshwater, alternative sources are required by improving plant adaptability in drought condition. For drought survival, several rhizospheric bacteria have developed osmotolerant system. Rhizobacteria's ability to form associations with plants in the root system of plants makes it a viable inoculum for plant cultivation. 'IR64' and 'Situ Bagendit' were used in this study to determine the plant response under drought conditions with the application of osmotolerant rhizobacteria. This study aims at establishing the response of 'IR64' and 'Situ Bagendit' to the inoculation using osmotolerant rhizobacteria under drought condition.

MATERIALS AND METHODS

Materials

In this work, osmotolerant rhizobacteria *E. flavescens* was taken from Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, while two rice cultivar (*Oryza sativa* L.) 'IR64' and 'Situ Bagendit' were collected from the Indonesian Center for Rice Research (ICRR), Indonesia.

Methods

Osmotolerant rhizobacteria inoculation and application of drought treatment

The study was carried out in the Greenhouse of the Sawitsari Research Station, Faculty of Biology, Universitas Gadjah Mada, Indonesia between July 2019-February 2020. The experiment involved three factors and was conducted in a completely randomized design (CRD). The first factor was the rice cultivar with two rice cultivar ('IR64' and 'Situ Bagendit'), the second factor was inoculation using osmotolerant rhizobacteria (*E. flavescens*) and without inoculation, and the last factor was three levels of drought at 25%, 50%, and 100% of field capacity.

Seed sterilization was accomplished by soaking the seeds in 70% (v/v) ethanol for 5 minutes, followed by submersion in 0,2 percent $HgCl_2$ for 4 minutes. The seeds were rinsed six times with sterile distilled water following surface sterilisation. Rice seeds were inoculated with osmotolerant rhizobacteria inoculants in a volume of 5 mL (10^8 cfu/mL) prior to planting. During 21 days, the soil's water-holding capacity was sustained at field capacity. Following that, the soil moisture was changed to achieve 25%, 50%, and 100% field capacity. Each treatment's field capacity is maintained by watering every three days till 12 weeks.

Plant Growth Parameters

Plant height, number of leaves, tillers, and panicles were measured weekly during treatment. The distance between the longest leaf tip and the plant above the soil surface was used to determine plant height.

Physiological Responses Measurement

Physiological characteristics were measured using the (Harborne 1984) method with a few adjustments on chlorophyll and carotenoid content. Cold acetone solution (3 mL of 80%) was used to homogenize a 0.3 grams leaf sample pulverized in a mortar. The spectrophotometer (Thermo Scientific GENESYS 10 UV Scanning) was used to analyze chlorophyll content at 470 nm, 645 nm, and 664 nm multi-wavelengths and the results were expresses in mg.g^{-1} FW (fresh weight).

Proline content analysis was measured using (Bates et al. 1973) method. Leaf samples of 0.25 grams were pulverized and homogenized in 5 ml containing 3% sulfosalicylic acid solution. The sample was mixed with ninhydrin reagent (ninhydrin, acetic acid, and phosphoric acid) and glacial acetic acid in a 1:1:1 ratio, and then heated in a water bath (Memmer GmbH + Co.KG.WNB-7) at 95 ^{o}C for 60 minutes. The solution was cooled to 25^{o} C and reacted with toluene to form two layers. The absorbance of the solution at 520 nm wavelength was compared to the standard proline curve to measure proline levels.

The activity of superoxide dismutase (SOD) was measured using the (Marklund & Marklund 1974) procedure. Fresh leaf samples (0.5 grams) were frozen in liquid nitrogen and crushed, then homogenized with 0.01 M phosphate buffer (pH 7.0), 1 mM EDTA, and 1% PVP, then centrifuged for 20 minutes at 4° C at 15000 rpm. A reaction mixture was prepared, consisting of 2 mL Tris-HCl Buffer at pH 8.2, 0.5 mL 2 mM pyrogallol, and 2 ml ddH20 were combined with 0.5 mL supernatant. The generated test mixture was compared to a blank solution) containing pyrogallol) at 325 nm at 3 minutes intervals by spectrophotometer (Thermo Scientific GENESYS 10 UV Scanning). The oxidation data for pyrogallol were gathered every minute for 3 minutes and utilized to determine auto-oxidation of 100%. The results are given in units per milligram of protein (1 unit is the amount of enzyme used to inhibits 5 percent of pyrogallol oxidation per minute).

Ascorbate peroxidase activity measurements were carried out following the (Nakano & Asada 1981) protocol with modifications. Enzyme extract (100 μL), EDTA 0.1 mM (400 μL), 0.05 mM sodium phosphate buffer at pH 7.0, 0.05 mM ascorbic acid solution (400 μL) and ddH_20 (1.5 mL) were mixed together. The mixed solution was then added with 400 µL of 3% H_2O_2 solution followed by incubation for 60 seconds. Furthermore, a spectrophotometer was used to detect the decrease in absorbance at wavelength of 290 nm with a time interval of 3 minutes (Thermo Scientific GENESYS 10 UV Scanning). APX enzyme concentrations were calculated using the exclusion coefficient (**€** = 2.8 $mM^{-1}cm^{-1}$). The amount of APX that oxidizes one nmol per mL of ascorbate per minute is defined as one unit (U). The activity of the APX enzyme is measured in $U.mg^{-1}$ protein. A mixed solution without enzyme extract is used as a control.

The determination of peroxidase (POX) activity was performed using the modified (Kar & Dinabandhu 1976) protocol. Sodium phosphate buffer solution (500 µl of 0.05 mM) at pH 7.0, pyrogallol solution (10 µL), and 250 µL of 5 mM H_2O_2 solution were put into the tube, followed by 500 µL of enzyme extract and incubated at 25^0C for 15 minutes in a water bath. The tube was filled with 5% H_2SO_4 solution (250 µL) and gently shaken. The purpurogallin absorbance was measured at 420 nm with spectrophotometer (Thermo Scientific GENESYS 10 UV Scanning). The same process was used to make a blank solution, but no enzyme extract was added. The activity of the POX enzyme was measured at 420 nm (A_{420}).

Data Analysis

Homogeneity and normality of the data were analyzed using one-way ANOVA and followed by Duncan's Multiple Range Test (at 95% confidence level) using IBM-SPSS version 16.0.

RESULTS AND DISCUSSION

Results

Growth Performance

Treatment under different water availability (100%, 50%, and 25% field capacity) resulted in significantly different plant heights in the two rice cultivars treated without and with the inoculation of osmotolerant rhizobacteria (*E. flavescens*) (Figure 1). The rise plant height during the vegetative phase, and the plant does not tend to grow taller after it enters the generative phase. According to Figure 1, plant height increased until the 5th week in rice 'IR64' and 'Situ Bagendit' grown at 100% field capacity, and it remained steady until the 12th week. The increase in plant height remained until the 6th week in the 50% field capacity treatment, whereas in the 25% field capacity treatment, plant height constant at 8th week. Plant development is more ideal at 100% field capacity because the water needs are more adequate than at 25% field capacity. This situation also permits plants to perform better in their growth phase and enter their reproductive phase more quickly. The establishment of tillers and panicles was faster with 100% and 50% field capacity than with 25% field capacity treatment.

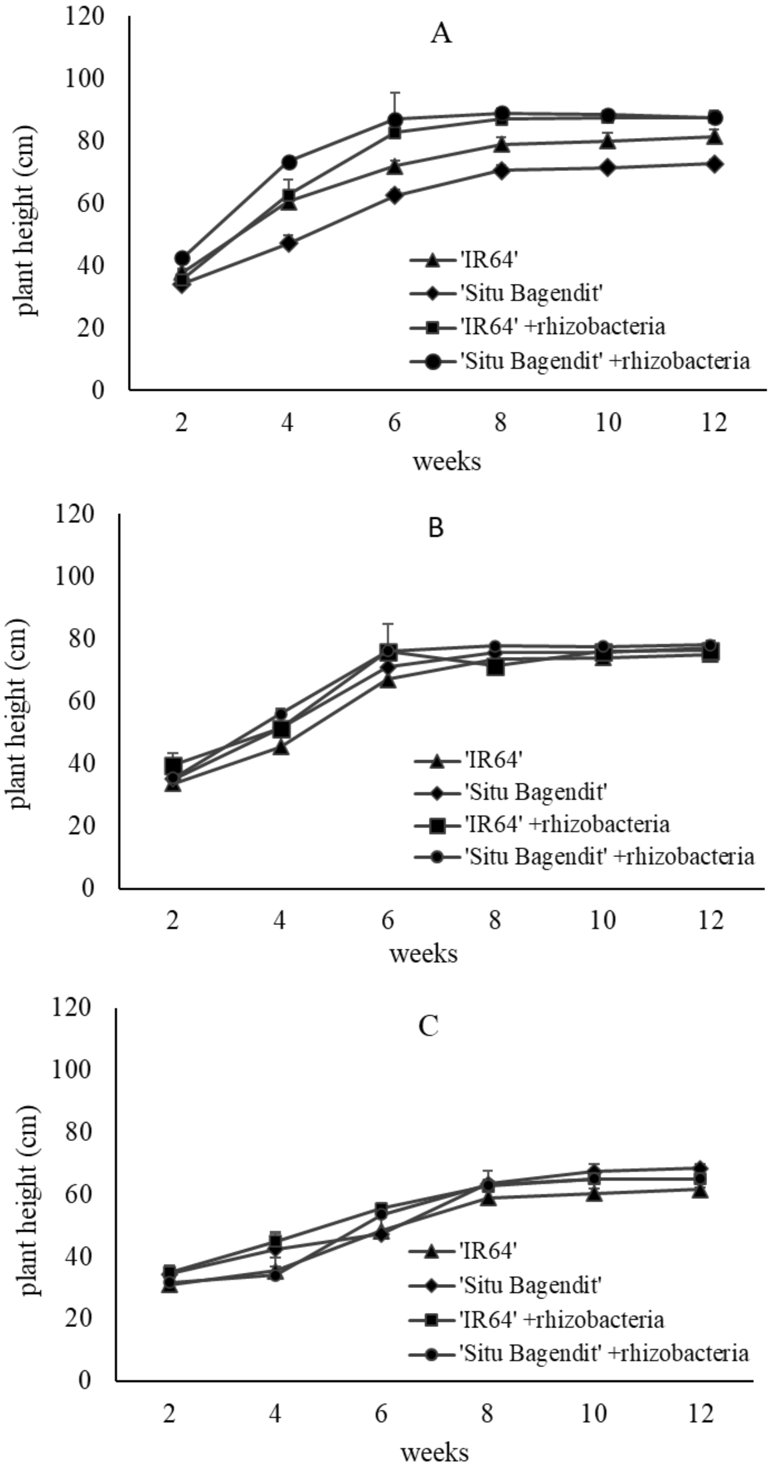

Figure 1. Effect of rhizobacteria inoculation on plant height of rice 'IR64' and 'Situ Bagendit' for 12 weeks under drought condition A: 100% field capacity, B: 50% field capacity, and C: 25% field capacity.

According to Figure 1, the highest increase in plant height was found in ‘Situ Bagendit’ with inoculation using osmotolerant rhizobacteria in the 100% field capacity treatment, while the treatment without inoculation using osmotolerant rhizobacteria resulted in the lowest plant height. In comparison to ‘IR64’ with and without inoculation using osmotolerant rhizobacteria at 50% field capacity treatment, ‘Situ Bagendit’ with osmotolerant rhizobacteria inoculation produced the maximum plant height. ‘Situ Bagendit’ produced higher plant height than rice ‘IR64’ at 25% field capacity treatment.

Based on the data obtained during 12 weeks of planting, it is observed that 'Situ Bagendit' plants produced higher plant height compared to 'IR64' (Table 1). Treatments of water availability (100%, 50% and 25% field capacity) resulted in considerably varied plant heights in two rice cultivars, both with and without rhizobacteria inoculation. Under 25% field capacity, 'Situ Bagendit' plants without rhizobacterial inoculation produced higher plant height compared to 'IR64'. This proves that ‘Situ Bagendit’ is more resistant to drought than ‘IR64’. The inoculation of rhizobacteria to each rice cultivar gave maximum results in supporting plant growth, both at 100%, 50%, and 25% field capacity.

The number of leaves was significantly different in rice 'IR64' and 'Situ Bagendit' between treatments with and without osmotolerant rhizobacterial inoculation. The number of leaves in two cultivars differed significantly between treatments of 100% field capacity with 50% and 25% field capacity, while there was no significant difference between treatments of 50% and 25% field capacity. The highest number of leaves was found under 25% of field capacity with osmotolerant rhizobacteria inoculation to 'IR64' and 'Situ Bagendit', while the least number of leaves was found in 'Situ Bagendit' rice at 50% and 25% field capacity without rhizobacterial inoculation (Table 1).

Different level of water availability also resulted in different number of tiller and panicle in the two rice cultivars treated without and with the inoculation of osmotolerant rhizobacteria (Table 2). ‘IR64’ at 25% field capacity without rhizobacteria inoculation had lowest average number of tillers. Both cultivars of rice generated the same number of tiller in each field capacity, regardless of whether they were inoculated with rhizobacteria or not. “Situ Bagendit” produced the most tillers at 100% field capacity without rhizobacteria inoculation treatment (with average 5.33 tillers), which was not substantially different from the treatment with inoculation. When comparing treatments with 50% and 25% field capacity, the number of tillers ‘IR64’ and ‘Situ Bagendit’ at 100% field capacity with or without rhizobacteria inoculation resulted in the highest number of tillers, while the treatment with 25% field capacity resulted the lowest number of tillers.

Table 1. Plant height and leaf number of rice (*Oryza sativa* L.) ‘IR64’ and ‘Situ Bagendit’ at week 12 at 100%, 50%, and 25% field capacity.

Parameter	Field Capacity	‘IR64’		‘Situ Bagendit’	
		Without Rhizobacteria	With Rhizobacteria	Without Rhizobacteria	With Rhizobacteria
Plant Height (cm)	100%	79.50 ^{b}	87.33 ^{a}	85.33 ^{a}	87.33 ^{a}
	50%	75.00 ^{c}	76.33 ^{cb}	77.00 ^{cb}	78.00 ^{cb}
	25%	61.67 ^{e}	65.00 ^{ed}	68.33 ^{d}	65.00 ^{ed}
Leaf Number	100%	11.00 ^{p}	8.00 ^{q}	11.00 ^{p}	8.00 ^{q}
	50%	8.67 ^{q}	11.00 ^{p}	7.67 ^{q}	8.67 ^{q}
	25%	9.00 ^{q}	11.67 ^{p}	7.67 ^{q}	11.67 ^{p}

Values having same letter (s) in a row and column of each parameter was not significantly different at ($p \leq 0.05$) level of significant by DMRT.

Table 2. Number of tiller, panicle, and percentage of filled grain of rice (*Oryza sativa* L.) 'IR64' and 'Situ Bagendit' at week 12 at 100%, 50%, and 25% field capacity.

Parameter	Field Capacity	'IR64'		'Situ Bagendit'	
		Without Rhizobacteria	With Rhizobacteria	Without Rhizobacteria	With Rhizobacteria
Number of Tiller	100%	3.67 ^{edc}	4.67 ^{cba}	5.33 ^{a}	4.33 ^{dcba}
	50%	3.33 ^{edcb}	4.00 ^{edcb}	3.00 ^{fe}	4.33 ^{dcba}
	25%	2.33 ^{f}	3.67 ^{edc}	3.00 ^{fe}	3.33 ^{fed}
Number of Panicle	100%	3.67 ^{lk}	4.67 ^{ji}	5.33 ^{i}	4.67 ^{ji}
	50%	3.33 ^{kj}	4.67 ^{ji}	3.00 ^{ml}	4.33 ^{kj}
	25%	2.33 ^{m}	3.00 ^{ml}	3.00 ^{ml}	3.00 ^{ml}
Percentage of Filled Grain (%)	100%	65.72 ^{q}	78.62 ^{p}	32.48 ^{s}	61.02 ^{q}
	50%	47.12 ^{r}	46.90 ^{r}	17.47 ^{ut}	34.80 ^{s}
	25%	19.54 ^{t}	46.25 ^{r}	10.80 ^{u}	21.32 ^{t}

Values having same letter (s) in a row and column of each parameter was not significantly different at ($p \leq 0.05$) level of significant by DMRT.

'Situ Bagendit' produced the most panicles with 100% field capacity treatment without osmotolerant rhizobacteria inoculation with average of 5.33 panicles. The results were similar to those obtained with rice 'IR64' at 100% and 50% field capacity by inoculation with rhizobacteria, as well as with 'IR64' at 100% and 50% field capacity by inoculation with rhizobacteria. The lowest average number of panicles was found at 25% field capacity (2.33 panicles) was recorded in 'IR64', which was not significantly different from the inoculation treatment with rhizobacteria, and with 'Situ Bagendit' at 25% and 50% field capacity without rhizobacteria inoculation and 25% field capacity with rhizobacteria inoculation. The average number of tillers and panicles produced by both rice cultivars was about the same. The number of panicles produced by 'Situ Bagendit' at 100% field capacity treatment with rhizobacteria inoculation was higher than the number of tillers, whereas the number of tillers was higher in 'IR64' and 'Situ Bagendit' at 25% field capacity with rhizobacteria osmotolerant in comparison to number of panicles.

'IR64' under 100% of field capacity inoculated with rhizobacteria resulted in the highest percentage of filled grain (78,62%), while the lowest percentage was found in 'Situ Bagendit' under 25% of field capacity without the inoculation of rhizobacteria. Water availability at 25% of field capacity resulted in the lowest percentage of grain compared to 100% treatment and 50% of field capacity. The osmotolerant rhizobacteria inoculation also gave a significant difference in the resulting grain compared to the treatment without rhizobacteria, both in rice 'IR64' and 'Situ Bagendit' on treatment of 100%, 50% and 25% of field capacity.

According to Salsinha et al. (2021), with the increase in drought stress level, the proportion of growth parameters (plant height, and number of tillers) decrease significantly ($p<0,05$). Salsinha et al. (2021) also shows that drought-tolerant cultivar (Boawae 100 Malam and Padi Merah Kuatnana) was significantly different from Ciherang cultivar. In this study, the analysis of growth shows that 'Situ Bagendit' is drought-tolerant cultivar with high susceptibility to drought stress. Drought stress affects food crop development and yield. Due to a lack of water, the development process from vegetative to reproductive phase has also been slowed that also damage cell membranes, perhaps leading to cell death. Plant morphological growth, like height, number of tillers, panicles and grains was reduced in the presence of drought stress (Farooq et al. 2009). Drought during vegetative phase can inhibit the growth of leaves and roots. Drought stress during flowering and grain filling reduced grain yield considerably as compared to the control. The decrease in yield at the flowering stage is largely due to the decrease in the number of grains per panicle. Stress during the growth stage can decrease assimilation translocation to seed which decreases seed weight and increases the percentage of empty seeds. The success of seed formation depends on the availability of assimilates translocated to the seeds. The source of assimilates for seed formation comes from the flag leaves. Flag leaves contribute 45% assimilation for the formation of rice seeds (Abou-Khalifa et al. 2008).

Physiological responses

Plant uses photosynthetic pigments primarily for light gathering and the creation of reducing power. Although chlorophyll a and b are susceptible to soil drying, carotenoids play a complementary role in helping plants endure drought (Farooq et al. 2009). The highest chlorophyll content was found in rice 'IR64' under 50% field capacity with rhizobacterial osmotolerant inoculation. 'Situ Bagendit' under 100% field capacity resulted in the lowest chlorophyll content compared to the 50% and 25% field capacity treatment, as well as with the 'IR64' plant treatment (Table 3). Drought stress altered the ratios of chlorophyll a and b, as well as carotenoids. Drought stressed cotton (Massacci et al. 2008) and sunflower plants (Kiani et al. 2008) were found to have reduced chlorophyll content. As a relative water content and leaf water potential diminish, the foliar photosynthesis rate of higher plants is known to decrease. According Salsinha et al. (2021), drought tolerance is higher in rice cultivars with lower chlorophyll reduction percentage (Hare Tora and Boawaw 100 Malam with 9.37% and 7.56 % respectively), however Pak Mutin, Gogo Jak and Padi Putih Maumere had the biggest fall in chlorophyll levels indicating a high drought sensitivity. As water content and leaf water potential fell in higher plants, the photosynthetic rate is decreased. The measurement of chlorophyll a and b shows that when the amount of water in treatment was lowered, the amount of photosynthetic pigments was similarly reduced, affecting the morphological and physiological processes of the plants (Usman et al. 2013).

Drought can cause carotenoid damage due to free radical activity. According to Martinez-Ferri et al. (2004), Jaleel et al. (2009); Du et al. (2010) and Anjum et al. (2011), enhanced biosynthesis and free radical destructive activity, as well as increased conversion of carotenoid pigment into other chemicals such as ABA, might cause a decrease in pigment content, which is essential to create plant adaptation strategies in response to drought stress.

Table 3. Chlorophyll, Carotenoid and Proline Content of rice (*Oryza sativa* L 'IR64' and 'Situ Bagendit') at week 12 under 100%, 50%, and 25% field capacity.

Parameter	Field Capacity	'IR64'		'Situ Bagendit'	
		Without Rhizobacteria	With Rhizobacteria	Without Rhizobacteria	With Rhizobacteria
Chlorophyll (mg.g^{-1} FW)	100%	3.55 ^{cba}	3.40 ^{cba}	2.56 ^{c}	2.67 ^{cb}
	50%	3.34 ^{cba}	3.98 ^{a}	3.00 ^{cba}	3.86 ^{ba}
	25%	3.15 ^{cba}	3.13 ^{cba}	3.15 ^{cba}	3.42 ^{cba}
Carotenoid (mg.g^{-1} FW)	100%	0.85 ^{i}	0.16 ^{kj}	0.14 ^{k}	0.32 ^{kj}
	50%	0.59 ^{kji}	0.23 ^{kj}	0.52 ^{kji}	0.33 ^{kj}
	25%	0.41 ^{kji}	0.31 ^{kj}	0.62 ^{ji}	0.33 ^{kj}
Proline (μmol g^{-1} FW)	100%	0.105 ^{r}	0.109 ^{r}	0.137 ^{rq}	0.107 ^{r}
	50%	0.117 ^{r}	0.123 ^{r}	0.147 ^{rq}	0.161 ^{rq}
	25%	0.125 ^{r}	0.199 ^{rq}	0.223 ^{q}	0.344 ^{p}

Values having same letter (s) in a row and column of each parameter was not significantly different at ($p \leq 0.05$) level of significant by DMRT.

The highest carotenoid content was found in 'IR64' with 100% field capacity treatment without rhizobacterial osmotolerant inoculation. Rhizobacterial osmotolerant inoculation treatment resulted in lower carotenoid content compared to the treatment without rhizobacteria osmotolerant inoculation, both in 'IR64' and 'Situ Bagendit in under the three field capacity treatments (Table 3).

Drought has also been linked to changes the metabolism of soluble carbohydrates, a group of molecules that can act as suitable solutes and anti-oxidants. When there is a lack of water, there are chemicals which are tend to rise. Free amino acids are another type of molecules that may be impacted by lack of water. Water stressed leaves had higher levels of proline and total free amino acids (Pinheiro et al. 2004; Van Heerden 2002). A distinctive plant response to environmental challenges, notably drought stress, is the accumulation of defensive solutes like proline and soluble sugar in the leaf (Sakamoto 2002). During stress, proline and soluble sugar, both operate as osmoprotectans (HongBo et al. 2005; Reddy et al. 2004). Proline may also operate as an antioxidant. Proline content in 'Situ Bagendit' rice at 25% field capacity treatment resulted in the highest proline content. The inoculation using rhizobacteria also increases the proline content produced. The increase of the proline content in stressed plants is an adaptation to deal with stressful conditions (Table 3). Under severe situations, proline builds up and provides energy for growth and survival (Chandrashekar & Sandhyarani 1996). Proline, sucrose, glycinebetaine, and other substances accumulation in the cytoplasm have a function in osmotic adjustment, which can improve the rate of water absorption (Shehab et al. 2010; Usman et al. 2013). Proline biosynthesis is stimulated directly during stress as a drought resistance strategy. Proline has been shown to scavenge ROS and other free radicals in studies. However, exogenous proline at high concentrations (40-50 mM) has little effect on rice plants under abiotic stress (Hayat et al. 2012). As a result of the induced drought stress in the roots, plants' proline levels rise. Drought tolerant rice (such as Padi Hitam Mumere, Shintara, Padi Merah Noemuti, and Gogo Sikka) showed the highest levels of proline from control to severe drought curcumtances (Salsinha et al. 2021).

Biochemical properties

Drought stress causes a plant's reaction to be complex, involving the synthesis of polyamines and the emergence of a new group of proteins whose function is unknown. Abscisic acid is crucial to the reaction because it causes closing stomata, limiting water loss while also reducing CO_2 available for photosynthesis, which can lead to electron production in the photosystem (Arora et al. 2002). Plants have highly efficient scavenging systems for reactive oxygen species, which protect them from oxidative processes that harmful to them. These defenses are not only prevalent within the cell, but also in the apoplast to a lesser extent. Plants evolved cellular adaptive responses as a result, such as up-regulation of oxidative stress protectors and the accumulation of protective solutes. Superoxide dismutase (SOD), ascorbate peroxidase (APX), and peroxidase (POX) are antioxidant defense enzymes that help to reduce superoxide and hydrogen peroxide concentrations. The dismutation of superoxide into oxygen and hydrogen peroxide is catalyzed by superoxide-dismutase (SOD). Peroxidases which comprise both enzymic and non-enzymic H_2O_2 degradation, remove H_2O_2 (Peltzer et al. 2002). The activity of ascorbate peroxidase has mostly been found in chloroplasts and cytosols. SOD and Ascorbate Peroxidase (APX) enzymes are found in both soluble and thylakoid-bound forms in chloroplasts. The activity of APX had higher activity drought stress at 25% field capacity and 50% field capacity compared to 100% field capacity in both rice cultivars. Ascorbate peroxidase is another key antioxidant enzyme. APX participates in the oxidative chain reaction that transforms H_2O_2 into O_2 and H_2O with absorbic acid as one of the electron suppliers (Refli & Yekti 2016).

The catalytic alterations in the detoxification of peroxide radicals into water and oxygen are catalyzed by peroxidase (POX) enzymes (Hiraga et al. 2000). This enzyme is also involved in plant adaptations such as lignification, suberization, and auxin metabolism simulation (Lagrimini et al. 1997). POX activity in rice 'IR64' and 'Situ Bagendit' experienced a decrease in rice drought rate of 50% and 25% in field capacity compared to 100% (Table 4). Peroxidase are important for scavenging H_2O_2 toxicity. Under adverse situation such as drought stress, the combined activity CAT and SOD transforms the deadly superoxide radical (O_2) and hydrogen peroxide (H_2O_2) to water and molecular oxygen, preventing cellular damage (Noctor et al. 2000; Chaitanya et al. 2002). Drought-tolerant wheat, coffee, rice, and caper cultivars had higher antioxidant system than susceptible cultivars, according to (Guo et al. 2006; Lascano et al., 2001; Lima et al. 2002; Ozkur et al. 2009).

The results of this study indicate that drought stress can simulate changes in antioxidant enzymes activity (SOD, APX, and POX) of rice leaves in both rice cultivars, 'IR64' and 'Situ Bagendit'. The change in enzyme activ-

Table 4. Oxidative Enzyme Activity of rice plant (*Oryza sativa* L 'IR64' and 'Situ Bagendit') at 12 weeks under 100%, 50%, and 25% field capacity.

Parameter	Field Capacity	'IR64'		'Situ Bagendit'	
		Without Rhizobacteria	With Rhizobacteria	Without Rhizobacteria	With Rhizobacteria
SOD (U/L)	100%	0.640 ^{a}	0.914 ^{a}	1.145 ^{a}	1.527 ^{a}
	50%	0.868 ^{a}	1.295 ^{a}	1.091 ^{a}	1.517 ^{a}
	25%	0.985 ^{a}	1.100 ^{a}	0.997 ^{a}	0.969 ^{a}
APX (U/L)	100%	2.336 ^{j}	4.403 ^{ji}	2.166 ^{j}	3.182 ^{ji}
	50%	3.734 ^{ji}	5.041 ^{ji}	3.760 ^{ji}	3.272 ^{ji}
	25%	3.801 ^{ji}	5.764 ^{i}	5.092 ^{ji}	4.990 ^{ji}
POX (A_{420})	100%	0.210 ^{p}	0.361 ^{p}	0.318 ^{p}	0.300 ^{p}
	50%	0.202 ^{p}	0.239 ^{p}	0.283 ^{p}	0.298 ^{p}
	25%	0.164 ^{p}	0.228 ^{p}	0.228 ^{p}	0.174 ^{p}

Values having same letter (s) in a row and column of each parameter was not significantly different at ($p \leq 0.05$) level of significant by DMRT.

ity plays a role in suppressing the destructive activity of free radicals. The activity of these oxidative enzymes tends to be more active in 'Situ Bagendit' (drought resistant) rice plants than in 'IR64' (Table 4). The high activity of oxidative enzymes indicates the development of a better oxidative defense system compared to drought rice.

CONCLUSION

Under stress condition, the mechanism plants defense was increased to ensure the tolerance of plant in responding to stress. The rice plants (*Oryza sativa* L.) 'IR64' and 'Situ Bagendit' are becoming increasingly stunted as drought stress increases. Inoculating osmotolerant rhizobacteria help accelerate plant growth, as indicated by increased plant height, number of leaves, tillers, and panicles in both rice cultivars, as well as a higher percentage of filled-grain in 'IR64'. The biochemical and physiological response of 'IR64' and 'Situ Bagendit' to drought were enhanced by inoculation with osmotolerant rhizobacteria, as evidenced by an increase in proline content, SOD and APX enzyme activity, while the carotenoid level reduced. The rhizobacteria osmotolerant inoculation showed the increased POX activity at IR64 cultivar. Inoculation of osmotolerant rhizobacteria can be used to increase the cultivation of rice plants under drought stress.

AUTHORS CONTRIBUTION

TY and DR designed the research and supervised all the process, HDK collected and analyzed the data and prepared the publication. All authors read and approved the final version of the manuscript.

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CONFLICT OF INTEREST

There are no conflicts of interest declared by the authors.

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