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EFFECT OF DROUGHT STRESS ON GROWTH, PROLINE AND ANTIOXIDANT ENZYME ACTIVITIES OF UPLAND RICE

M.S. Lum†, M.M. Hanafi*, Y. M. Rafii, and A. S. N. Akmar*

Laboratory of Food Crops, Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
*Laboratory of Plantation Crops, Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
†Corresponding author: lmmoksam@yahoo.com

ABSTRACT

Responses of eight upland rice (*Oryza sativa* L.) varieties subjected to different drought levels were investigated in laboratory to evaluate eight local upland rice varieties against five drought levels (0, -2, -4, -6, and -8 bars) at germination and early seedling growth stage of plant development. Data were analyzed statistically for growth parameters; shoot length, root length, and dry matter yield, and biochemical parameters; proline and antioxidant enzymes activity (catalase, superoxide dismutase and peroxidase), were measured. Experiment units were arranged factorial completely randomized design with four replications. The drought-tolerant variety, Pulot Wangi tolerated PEG at the highest drought level (-8 bar) and showed no significantly difference relation to control. However, drought-sensitive variety, Kusam was markedly affected even at the lowest drought level used. Concomitantly, the activity of antioxidant enzymes catalase, peroxidase and superoxide dismutase in the drought-tolerant varieties increased markedly during drought stress, while decreased by drought stress in the drought sensitive variety. Consequently, this led to a marked difference in the accumulation of proline in the upland rice varieties. It may be concluded that the activities of antioxidant enzymes and proline accumulation were associated with the dry mass production and consequently with the drought tolerance of the upland rice varieties.

Key words: upland rice, drought stress, antioxidant enzyme activities, polyethylene glycol.

INTRODUCTON

Drought is one of the major abiotic stresses that severely affect and reduce the yield and productivity of food crops worldwide up to 70% (Kaur *et al.*, 2008; Thakur *et al*., 2010; Akram *et al*., 2013). The response of plants to drought stress is complex and involves changes in their morphology, physiology and metabolism. Reduction of plant growth is the most typical symptom of drought stress (Sairam and Srivastava, 2001).

Drought stress leads to accumulation of reactive oxygen species (ROS), generated mostly in chloroplast and to some extend in mitochondria, causing oxidative stress. Major ROS molecules are singlet oxygen, superoxide anion radicals, hydroxyl radicals and hydrogen peroxide (H_2O_2). Plants under drought stress display some defense mechanisms to protect themselves from the damaging effect of oxidative stress. Plants with high induced antioxidant levels have better tolerance and resistance to oxidative damage (Parida and Das, 2005). The ROS scavenging mechanism is among the common defense responses against abiotic stresses (Vranová *et al*., 2002). To detoxify ROS, plants can intrinsically develop different types of antioxidants reducing oxidative damage and conferring drought tolerance. The ROS scavengers are antioxidant enzymes containing superoxide dismutase, peroxidase and catalase (Demiral and Turkan, 2005; Khan and Panda, 2008).

Rice grows in the tropics, subtropics, semi-arid tropics and temperate regions of the world. More than 90% of the world's rice production in Asia (Roy and Misra, 2002). However, rice often threatened by severe abiotic stresses, of which the most common is drought. Although upland rice contributes a relatively small proportion of the total rice area worldwide, it is the predominant rice cultivation method in growing area under rainfall, like Latin America and West Africa (Gupta and O'Toole, 1986). Upland rice variety developed high adaptability to drought stress when exposure to many environmental constraints (Pessoa-Filha *et al*., 2007; Rabello *et al*., 2008; Ji *et al*., 2012).

The aim of this work was to study the comparative effects of different concentrations of PEG (drought inducer) on growth, proline accumulation and antioxidant enzyme activities (e.g., CAT, POD and SOD) of eight upland rice varieties to analyze the significance of these parameters in drought stress tolerance and to assign the most tolerant of these varieties to drought stress. Comparison of these parameters in these upland rice varieties differing in drought tolerance may be helpful in developing a better understanding and provide additional information on the mechanisms of drought tolerance and develop a selection indicator for breeding program.

MATERIALS AND METHODS

Plant materials, growth conditions and drought treatments: Seeds of 8 local upland rice varieties (Becor, Kusam, Nabawan, Bertih, Pulot wangi, Hita, Tenom and Sintok), collected from Malaysia were used in the study. The seeds were allowed to germinate on a filter paper in 9 cm diameter petri dishes, moistened with 4 mL of distilled water (control) or PEG (-2, -4, -6 and -8 bars PEG test solutions). The petri dishes were arranged in completely randomized design (CRD) with four replicates for each treatment and stored at room temperature (24 ± 2 °C) under dark conditions. The petri dishes were covered to prevent the loss of moisture by evaporation under laboratory condition. The ten-day old rice seedlings were subjected to drought stress. The growth parameters of seedlings were shoot length, root length, and dry matter yield. Three seedlings from each replicate were randomly sampled from the petri dishes. The root and shoot lengths were measured while the freshly harvested organs were oven-dried (80 °C) to constant weight.

Proline determination: Free proline was extracted from 200 mg of leaf sample in 3% (w/v) aqueous sulfosalicylic acid and estimated by using ninhydrin reagent according to the method of Bates *et al*. (1973). The organic toluene phase containing the chromophore was separated and absorbance of red colour developed was read at 520 nm. Proline concentration was determined using calibration curve and expressed as mg g^{-1} fresh weight.

Enzyme extraction: Leaf sample (500 mg) was frozen in liquid nitrogen and finely ground by pestle in a chilled motor, the frozen powder was added to 10 mL of phosphate buffer (pH 7.0). The homogenate was centrifuged at 15000 × *g* for 10 min at 4 ºC and supernatant was used as enzyme source for catalase (CAT; EC 1. 11. 1. 6), superoxide dismutase (SOD; EC 1. 15. 1. 1) and peroxidase (POD; EC 1. 11. 1. 7) assays.

Assays of Antioxidant Enzyme Activities

Assay of CAT activity: The assay mixture in total volume of 3 mL contained 0.5 mL of 0.2 M phosphate buffer (pH 7.0), 0.3 mL of (v/v) H_2O_2 and 0.1 ml of enzyme. The final volume was made 3 ml by adding distilled water. The reaction was started by adding enzyme and change in optical density was measured at 240 nm at 0 min and 3 min on UV-Vis spectrophotometer. The molar extinction coefficient of H_2O_2 at 240 nm was taken as 36 $\mu mol^{-1} cm^{-1}$ and the results were expressed as μmol H_2O_2 min^{-1} g^{-1} protein (Luck, 1974; Aebi and Bergmeyer, 1983).

Assay of SOD activity: Three ml of reaction mixture containing 0.1 ml of 1.5 M Na_2CO_3, 0.2 ml of 200 mM methionine, 0.1 ml of 3 mM EDTA, 0.1 ml of 2.25 mM *p*-nitroblue tetrazolium chloride (NBT), 1.5 ml of 100 mM potassium phosphate buffer (pH 7.5), 1 ml of distilled water and 0.05 ml of enzyme samples. The tube without enzyme was taken as control. Reaction was started by adding 0.1 ml 60 µM riboflavin and placing the tubes below a light source of two 15 W fluorescent lamps for 15 min. The reaction was stopped by switching off the light and covering the tubes with black cloth. Absorbance was recorded at 560 nm. An illuminated blank without protein gave the maximum reduction of NBT, and therefore, the maximum absorbance at 560 nm. SOD activity is presented as absorbance of blank minus absorbance of sample, giving the total inhibition, calculated per microgram protein. The activity of SOD was expressed as U mg $^{-1}$ protein. One unit of activity is the amount of protein required to inhibit 50 % initial reduction of NBT under light (Beauchamp and Fridovich, 1971; Dhindsa *et al.*, 1981).

Assay of POD activity: The assay mixture of 3 ml contained 1.5 ml of 0.1 M phosphate buffer (pH 7.0), 1 ml freshly prepared 10 mM guaiacol, 0.1 ml enzyme extract and 0.1 ml of 12.3 mM H_2O_2. Initial absorbance was read at 436 nm and then increase in the absorbance was noted at the interval of 30 s on UV-Vis spectrophotometer. Activity was calculated using the extinction coefficient 26.6 mM^{-1} cm^{-1} for the oxidized tetraguaiacol polymer. Enzyme activity was expressed as μmol guaiacol oxidized min^{-1} g^{-1} protein (Putter, 1974; Jebara *et al.*, 2005).

Statistical analysis: The mean values were taken from measurements of four replicates and standard error (SE) of the means was calculated. Data collected was analyzed statistically using Analysis of Variance (ANOVA) method using SAS 9.2 software to identify significant differences among upland rice varieties and among treatments. Least significant difference (LSD) test was applied to comparisons among means. The correlation between physiological and biochemical parameters and drought levels were evaluated using Pearson's correlation coefficients.

RESULTS

The dry matter production of the eight upland rice varieties differed in their response to drought stress (Table I). Upland rice var Pulot Wangi tolerated all studied drought levels (0 to -8 bar PEG) and showed no significant differences among all treatments applied. However, all upland rice varieties displayed a significant reduction in dry matter of different organs as compared with the control. Generally, the growth of the drought-sensitive variety, Kusam was extensively inhibited at lower PEG concentration (-2 bar). In contrast, the drought-tolerant variety, Pulot Wangi developed up to -8 bar PEG concentration. On the other hand, upland rice varieties, Becor, Nabawan, Bertih, Hita, Tenom and Sintok showed moderate shoot length (> 30%) and root length (> 48%) at highest PEG concentration (-8 bar).

Proline content in the leaves of upland rice varieties increased gradually for all the tested varieties (Table II). There was a significant increase of proline content in drought-tolerant variety, Pulot Wangi and exhibited highest proline content at all drought levels relative to the control. In drought-sensitive variety, Kusam showed lowest proline content at highest drought level compared to other varieties. Besides, the upland rice varieties, Hita, Sintok, Bertih and Becor showed higher proline content than Nabawan and Tanom at the -8 bar PEG concentration.

In the drought-tolerant variety, Pulot Wangi, CAT activity increased sharply in relative to the control (Table III). This enzyme activity increased gradually up to the level of -8 bar PEG, and higher than the control. Furthermore, all drought levels induced a significant increase in CAT activity in all the upland rice varieties. Moreover, upland rice varieties, Sintok, Nabawan and Kusam showed higher CAT activity than Becor, Hita, Bertih and Tenom at the highest PEG concentration (-8 bar).

The data indicated a highly significant increase in SOD activity in the drought-tolerant variety, Pulot Wangi, while in the drought-sensitive variety, Kusam, there is little change in the activity of this enzyme at all the drought levels (Table IV). Generally, all upland rice varieties displayed a significant increased in SOD as compared with the control. In addition, all upland rice varieties showed highest SOD activity at the highest PEG concentration (-8 bar). The SOD activity of Pulot Wangi exhibited more than 10 times higher than other upland rice varieties. The Kusam showed lowest SOD activity as compared to other upland rice varieties.

Table 1. Effect of drought stress on growth parameters of seedling of local upland rice varieties

Varieties	PEG (Bar)	Shoot length (cm)	% of reduction	Root length (cm)	% of reduction	Dry matter (mg plant^{-1})	% of reduction
Becor	0	11.6 ^{a}	100.00	10.8 ^{a}	100.00	31.5 ^{a}	100.00
	-2	7.5 ^{b}	64.66	8.8 ^{ab}	81.48	28.9 ^{a}	91.75
	-4	6.1 ^{bc}	52.59	7.7 ^{ab}	71.30	27.2 ^{a}	86.35
	-6	5.2 ^{cd}	44.83	6.9 ^{b}	63.89	26.3 ^{a}	83.49
	-8	3.6 ^{d}	31.03	5.6 ^{b}	51.85	16.2 ^{b}	51.43
LSD 5%		2.0		3.4		8.4	
Kusam	0	9.5 ^{a}	100.00	11.6 ^{a}	100.00	29.1 ^{a}	100.00
	-2	5.7 ^{b}	60.00	8.2 ^{ab}	70.69	26.2 ^{a}	90.03
	-4	3.4 ^{bc}	35.79	7.6 ^{ab}	65.52	25.8 ^{a}	88.66
	-6	2.4 ^{c}	25.26	7.0 ^{b}	60.34	25.4 ^{a}	87.29
	-8	2.2 ^{c}	23.16	5.0 ^{b}	43.10	14.4 ^{b}	49.48
LSD 5%		2.6		4.0		6.3	
Nabawan	0	8.7 ^{a}	100.00	9.2 ^{a}	100.00	28.0 ^{a}	100.00
	-2	7.7 ^{a}	88.51	9.0 ^{a}	97.83	27.8 ^{a}	99.29
	-4	7.3 ^{a}	83.91	8.8 ^{ab}	95.65	23.8 ^{b}	85.00
	-6	4.6 ^{b}	52.87	6.7 ^{ab}	72.83	23.7 ^{b}	84.64
	-8	4.2 ^{b}	48.28	5.6 ^{b}	60.87	12.0 ^{c}	42.86
LSD 5%		1.5		3.3		3.3	
Bertih	0	9.9 ^{a}	100.00	9.8	100.00	24.2 ^{a}	100.00
	-2	9.3 ^{ab}	93.94	9.2	93.88	22.9 ^{a}	94.63
	-4	7.6 ^{b}	76.77	8.9	90.82	22.3 ^{a}	92.15
	-6	5.3 ^{c}	53.54	8.2	83.67	20.9 ^{a}	86.36
	-8	4.4 ^{c}	44.44	8.1	82.65	10.8 ^{b}	44.63
LSD 5%		2.2		NS		5.0	

Values are mean of four replications. Different letters indicate significant differences between means of four replications according to LSD test (probability level of 5%). NS – No significant

POD activity in Pulot Wangi increased markedly at the most drought levels as shown in Table V. Moreover, POD activity increased with increasing drought stress in all upland rice varieties. However, in Becor, POD activity significantly increased by the rise of drought level but shows lowest activity at highest drought level compared to other varieties. On the other hand, the upland rice varieties, Hita and Tenom exhibited higher POD activity than Bertih, Nabawan, Sintok and Kusam at the highest PEG concentration (-8 bar).

The result of the correlation analysis under water stress condition showed that physiological (shoots length and root length) and biochemical (proline content, SOD and POD) parameters had significant correlation (Table VI). There were positive and significant correlation among shoot length and root length ($p<0.05$), proline content ($p<0.01$) and SOD ($p<0.05$). In addition, there were positive and significant correlation between root length and proline content ($p<0.01$) and POD ($p<0.05$). Moreover, the proline content and POD was positively correlated at $p<0.01$. In contrast, the CAT and dry matter was negatively correlated in this study (Table VI).

Table 2. Effect of drought stress on proline content (mg g^{-1} fresh weight) of seedling of local upland rice varieties

PEG	**Proline Content (mg g^{-1} fresh weight)**							
Bar	Becor	Kusam	Nabawan	Bertih	Pulot Wangi	Hita	Tenom	Sintok
0	19.34 ^{d}	26.68 ^{d}	32.80 ^{e}	36.14 ^{e}	55.07 ^{d}	25.15 ^{e}	43.88 ^{d}	48.29 ^{e}
-2	53.95 ^{cd}	29.00 ^{d}	59.76 ^{d}	93.48 ^{d}	97.92 ^{d}	79.93 ^{d}	87.57 ^{c}	64.97 ^{d}
-4	103.86 ^{bc}	59.07 ^{c}	125.32 ^{c}	144.28 ^{c}	246.44 ^{c}	178.89 ^{c}	124.43 ^{b}	77.06 ^{c}
-6	145.52 ^{b}	76.21 ^{b}	296.65 ^{b}	334.62 ^{b}	389.69 ^{b}	313.00 ^{b}	248.88 ^{a}	206.01 ^{b}
-8	416.20 ^{a}	249.61 ^{a}	309.94 ^{a}	417.51 ^{a}	581.78 ^{a}	538.53 ^{a}	259.89 ^{a}	417.58 ^{a}
LSD 5%	62.51	12.85	8.96	18.31	50.65	10.38	11.05	9.76

Values are mean of four replications. Different letters indicate significant differences between means of four replications according to LSD test (probability level of 5%)

Table3. Effect of drought stress on catalase activity (µmol min^{-1} g^{-1} protein) of seedling of local upland rice varieties

PEG	**Catalase (CAT) (µmol min^{-1} g^{-1} protein)**							
Bar	**Becor**	**Kusam**	**Nabawan**	**Bertih**	**Pulot Wangi**	**Hita**	**Tenom**	**Sintok**
0	0.02 ^{b}	0.04 ^{b}	0.08 ^{b}	0.08 ^{c}	0.05 ^{b}	0.08 ^{b}	0.02 ^{c}	0.08 ^{d}
-2	0.08 ^{b}	0.13 ^{b}	0.11 ^{b}	0.10 ^{c}	0.13 ^{b}	0.10 ^{b}	0.07 ^{bc}	0.38 ^{cd}
-4	0.13 ^{b}	0.26 ^{b}	0.11 ^{b}	0.16 ^{b}	0.18 ^{b}	0.13 ^{b}	0.10 ^{abc}	0.63 ^{bc}
-6	0.16 ^{b}	0.87 ^{a}	0.13 ^{b}	0.20 ^{b}	0.18 ^{b}	0.26 ^{b}	0.16 ^{ab}	0.80 ^{b}
-8	0.95 ^{a}	1.10 ^{a}	1.85 ^{a}	0.34 ^{a}	3.48 ^{a}	0.65 ^{a}	0.20 ^{a}	2.47 ^{a}
LSD 5%	0.14	0.58	0.10	0.05	0.93	0.29	0.10	0.42

Values are mean of four replications. Different letters indicate significant differences between means of four replications according to LSD test (probability level of 5%)

Table 4. Effect of drought stress on superoxide dismutase activity (U g^{-1} protein) of seedling of local upland rice varieties

PEG	**Superoxide dismutase (SOD) (U g^{-1} protein)**							
Bar	**Becor**	**Kusam**	**Nabawan**	**Bertih**	**Pulot wangi**	**Hita**	**Tenom**	**Sintok**
0	15.01 e	14.28 e	15.13 e	7.92 e	75.04 e	17.11 e	26.47 e	13.34 e
-2	47.03 d	62.71 d	91.71 d	124.65 d	115.72 d	106.71 d	64.92 d	114.12 d
-4	75.17 c	116.30 c	111.04 c	147.89 c	126.36 c	122.70 c	108.58 c	150.31 c
-6	292.44 b	123.08 b	152.95 b	174.16 b	146.17 b	335.09 b	129.93 b	193.70 b
-8	893.91 a	129.16 a	170.84 a	196.42 a	1092.57 a	553.49 a	235.15 a	338.08 a
LSD 5%	3.64	5.42	1.56	3.91	5.31	8.66	17.46	15.68

Values are mean of four replications. Different letters indicate significant differences between means of four replications according to LSD test (probability level of 5%)

Table 5. Effect of drought stress on peroxidase activity (μmol min^{-1} g^{-1} protein) of seedling of local upland rice varieties

PEG	Peroxidase (POD) (μmol min^{-1} g^{-1} protein)							
Bar	Becor	Kusam	Nabawan	Bertih	Pulot Wangi	Hita	Tenom	Sintok
0	2.63 ^{b}	2.73 ^{b}	21.38 ^{c}	22.58 ^{d}	5.14 ^{d}	23.41 ^{d}	13.93 ^{e}	12.29 ^{d}
-2	3.08 ^{b}	4.22 ^{b}	21.97 ^{c}	30.71 ^{c}	62.81 ^{c}	45.59 ^{c}	28.54 ^{d}	26.90 ^{c}
-4	3.53 ^{b}	4.80 ^{b}	23.35 ^{c}	35.83 ^{c}	69.90 ^{c}	53.40 ^{c}	44.83 ^{c}	29.94 ^{c}
-6	6.71 ^{b}	6.74 ^{b}	33.92 ^{b}	46.02 ^{b}	95.60 ^{b}	155.31 ^{b}	56.67 ^{b}	34.92 ^{b}
-8	28.78 ^{a}	32.20 ^{a}	81.32 ^{a}	89.91 ^{a}	312.57 ^{a}	208.16 ^{a}	168.62 ^{a}	64.54 ^{a}
LSD 5%	7.66	10.73	7.62	7.24	14.29	18.48	7.40	3.82

Values are mean of four replications. Different letters indicate significant differences between means of four replications according to LSD test (probability level of 5%)

Table 6. Correlation matrix between studied traits in upland rice varieties under drought stress

Parameters	Shoot length	Root length	Dry matter	Proline	Catalase	Superoxide dismutase	Peroxidase
Shoot length	-						
Root length	0.7574*	-					
Dry matter	0.1121	0.3440	-				
Proline	0.8943**	0.8394**	0.3268	-			
Catalase	0.3239	0.3310	-0.1353	0.1889	-		
Superoxide dismutase	0.7361*	0.6575	0.6250	0.6788	0.2825	-	
Peroxidase	0.5976	0.8169*	0.4702	0.8715**	0.1049	0.5078	-

*significant ($p<0.05$); **significant ($p<0.01$)

DISCUSSION

The results obtained from the present work clearly demonstrated that the upland rice varieties displayed distinct variation in drought tolerance during vegetative growth stage. Accordingly, we can identified the most drought-tolerant variety is Pulot Wangi while the drought-sensitive variety is Kusam based on the data obtained (Table I – V).All the upland rice varieties displayed significant reduction in shoot length at the most drought levels as compared with control (Table I). This reduction in growth might be due to low osmotic potential as well as a decrease in wall extensibility and cellular expansion (Mohammadkhani and Heidari, 2008). Furthermore, the root length of all upland rice varieties exhibited significant reduction at highest drought level as compare to control (Table I). Reduction of root length under stress conditions may due to an impediment of cell division and elongation leading kinds of tuberization (Fraser *et al*., 1990). In addition, the proline content of all upland rice varieties increases sharply up to highest drought level (Table II). The proline accumulated in plants under water stress can protects the cell by balancing the osmotic potential of cytosol with that of vacuole and external environment (Pireivatloum *et al*., 2010).

Tolerance to drought-stress in higher plants correlates to the levels of antioxidant systems and substrates (Athar *et al*., 2008). To combat the effects of drought–induced oxidative stress, plants develop a complex mechanism of antioxidant system. Relatively higher activities of ROS-scavenging enzymes have been reported in tolerant genotypes when compared to susceptible ones, suggesting that the antioxidant system plays an important role in plant tolerance against environmental stress. Generally, all varieties exhibited lowest enzymatic activity under normal condition (without PEG). This indicated plants will produce more CAT, SOD and POD under drought conditions to remove the extra ROS in cells. In this study, CAT SOD and POD activities increased markedly in the drought tolerant varieties, while they reduced in the sensitive one. This showed that drought-tolerant varieties were efficient scavenger of H_2O_2, which may result in better protection against H_2O_2.

The CAT is one of the highest turnover rates for all enzymes with the potential to directly dismutate H_2O_2 into H_2O and O_2 and is indispensible for ROS detoxification in peroxisomes during stress condition (Sairam and Srivastava, 2001). The SOD detoxifies superoxide anion free radicals (O_2^-) by forming H_2O_2, and then the H_2O_2 can be eliminated by CAT and POD (Hasheminasab *et al*., 2012). Moreover, POD also involved in various plant processes, including lignification (Hendriks *et al*., 1991), oxidation of phenolics (Largrimini, 1991), regulation of cell elongation (Mohammadkhani and Heidari, 2008) and detoxification of toxic compounds such as H_2O_2 (Chaparzadeh *et al*., 2004). The tolerance of some genotypes to environmental stresses has been associated with higher activities of antioxidant enzymes. For example, the drought-tolerant species of pigeon pea (*Cajanus cajan*) (Kumar *et al*., 2011), wheat (*Triticum aestivum*) (Hasheminasab *et al*., 2012; Omar, 2012) and black gram (*Phaseolus mungo*) (Pratap and Sharma, 2010) had higher activities of SOD, POD and CAT than the drought-sensitive species.

Under water stress conditions, the proline content showed highest and positively correlated with shoot length ($r = 0.8943$, $p<0.01$), root length ($r = 0.8394$, $p<0.01$) and POD ($r = 0.8715$, $p<0.01$). Accumulation of proline content under water stress indicates accumulated proline might act as a compatible solute regulating and reducing water loss from the plant cell during water deficit (Yokota *et al*., 2006) and play important role in osmosis balance (Fedina *et al*., 2002). Proline accumulates under stress also supplies energy for survivor and growth and thereby helps the plants to tolerate stress condition (Kumar *et al*., 2011).Thus, the ptoline content is a good indicator for screening drought tolerant varieties in water stress condition (Bayoumi *et al*., 2008; Kumar *et al*., 2011; Rahdari *et al*., 2012).

Conclusion: The upland rice varieties in this study showed differential responses for growth, proline accumulation and enzymatic activities measured. The scavenging system in drought-tolerant variety, Pulot Wangi exhibited higher CAT, POD and SOD activities, than in the drought-sensitive variety (Kusam). Thus, the drought tolerance of these upland rice varieties seems to be linked to the activities of these antioxidant enzymes. The drought tolerance of upland rice varieties could induce antioxidative enzyme system more efficiently, resulting in growth suppression and higher proline content under drought stress.

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REFERENCES

Aebi, H.E. and H.U. Bergmeyer (1983). Methods of Enzymatic Analysis, 3, Verlag Chemic, Deerfield Beach, FL.p273-286.

Akram, H. M., A. Ali, A. Sattar, H. S.U. Rehman and A. Bibi (2013). Impact of water deficit stress on various physiological and agronomic traits of three Basmati rice (*Oryza sativa* L) cultivars. J. Anim. Plant Sci. 23(5): 1415-1423.

Athar, H., A. Khan and M. Ashraf (2008). Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. Environ. Exp. Bot. 63: 224–231.

Bates, L.S., R.P. Walden and I.D. Teare (1973). Rapid determination of free proline for water studies. Plant and Soil39: 205-208.

Bayoumi, T.Y., M.H. Eid and E.M. Metwali (2008). Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. Afr. J. Biotech. 7(14): 2341-2352.

Beauchamp, C. and I. Fridovich (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44: 276-287.

Chaparzadeh, N., M.L. D'Amico, R.A. Khavari-Nejad, R. Izzo and F. Navari-Izzo (2004). Antioxidative responses of *Calendula officinalis* under salinity conditions. Plant Physiol. Biochem. 42: 695–701.

Demiral, T. and I. Turkan (2005). Comparative lipid peroxidation, antioxidant defense system and proline content in roots of two rice cultivars differing in salt tolerance. Environ. Exp. Bot. 53: 247–257.

Dhindsa, R.S., P. Plumb-Dhindsa and T.A. Thorne (1981). Leaf Senescence: Correlated with increased levels of membrane permeability and lipid peroxidation and decreased level of superoxide dismutase. J. Exp. Bot. 32: 93-101.

Fedina, I.S., K. Georgieva and I. Grigorova (2002). Light-dark changes in proline content of barley leaves under salt stress. Inst. Plant Physiol. 45(1): 59-63.

Fraser, T., W. Silk and T. Rosr (1990). Effect of low water potential on cortical cell length in growing region on maize roots. Plant Physiol.93: 648-651.

Gupta, P.C. and J.C. O'Toole (1986). Upland rice: a global perspective. Los Banos, Laguna, IRRI.

Hasheminasab, H., M.T. Assad, A. Aliakbari and R. Sahhafi (2012). Influence of drought stress on oxidative damage and antioxidant defense systems in tolerant and susceptible wheat genotypes. J. Agric. Sci. 4(8): 20-30.

Hendriks, T., H.J. Wijsman and L.C. Van Loon (1991). Petunia peroxidase a: Isolation, purification and characteristics. European J. Biochem. 199: 139–146.

Jebara, C., M. Jebara, F. Limam and M.E. Aouani (2005). Changes in ascorbate peroxidase, catalase, guaiacol peroxidase and superoxide dismutase activities in common bean (*Phaseolus vulgaris*) nodules under salt stress. J. Plant Physiol. 162: 929-936

Ji, K.X., Y.Y. Wang, W.N. Sun, Q.J. Lou, H.W. Mei, S.H. Shen and H. Chen (2012). Drought-responsive mechanisms in rice genotypes with contrasting drought tolerance during reproductive stage. J. Plant Physiol. 169: 336-344.

Kaur, G., S. Kumar, H. Nayyar and H.D. Upadhyaya (2008). Cold stress injury during the pod-filling phase in chickpea (*Cicer arietinum* L.): effects on quantitative and qualitative components of seeds. J. Agron. Crop Sci. 194(6): 457–464.

Khan, M.H. and S.K. Panda (2008). Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. Acta Physiol. Plant 30: 81–89.

Kumar, R.R., K. Karajol and G.R. Naik (2011). Effect of polyethylene glycol induced water stress on physiological and biochemical responses in pigeon pea (*Cajanus cajan* L. Mill sp.). Recent Res. Sci. Tech. 3(1): 148-152.

Largrimini, L.M. (1991). Wound-induced deposition of polyphenols in transgenic plants over expressing peroxidase. Plant Physiol. 96: 509–516.

Luck, H. (1974). Methods of Enzymatic Analysis. 2, Academia Press, New York, p885-894.

Mohammadkhani, N. and R. Heidari (2008). Drought-induced accumulation of soluble sugars and proline in two maize varieties. World Appl. Sci. J. 3(3): 448-453.

Omar, A.A. (2012). Impact of drought stress on germination and seedling growth parameters of some wheat cultivars. Life Sci. J. 9(1): 590-598.

Parida, A.K. and A.B. Das (2005). Salt tolerance and salinity effects on plants. Ecotoxicol. Environ. Safety 60: 324–349.

Pessoa-Filho, M., A. Belo, A.A.N. Alcochete, P.H.N. Rangel and M.E. Ferreira (2007). A set of multiplex panels of microsatellite markers for rapid molecular characterization of rice accessions. BMC Plant Biol. 7: 23.

Pireivatloum, J., N. Qasimov and H. Maralian (2010). Effect of soil water stress on yield and proline content of four wheat lines. Afr. J. Biotech.9: 36-40.

Pratap, V. and Y.K. Sharma (2010). Impact of osmotic stress on seed germination and seedling growth in black gram (*Phaseolus mungo*). J. Environ. Biol. 31(5): 721-726.

Putter, J. (1974). Methods of Enzymatic Analysis, 2, Academia Press, New York, p865.

Rabello, A.R., C.M. Guimaraes, P.H.N. Rangel, F.R. da Silva, D. Seixas, E. de Souza, A.C.M. Brasileiro, C.R. Spehar, M.E. Ferreira and A. Mehta (2008). Identification of drought-responsive genes in roots of upland rice (*Oryza sativa* L). BMC Genomics 9: 485-497.

Rahdari, P., S.M. Hosseini and S. Tavakoli (2012). The study effect of drought stress on germination, proline, sugar, lipid,protein and chlorophyll content in purslane (*Portulaca oleracea* L.) leaves. J. Med. Plants Res. 6(9): 1539-1547.

Roy, R.N. and R.V. Misra (2002). Economic and environmental impact of improved nitrogen management in Asian rice-farming system. In: Proceedings of the 20th Session of the International Rice Commission pg 23-26.

Sairam, R.K. and G.C. Srivastava (2001). Water stress tolerance of wheat (*Triticum aestivum* L.): variations in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotypes. J. Agron. Crop Sci. 186: 63-70.

Thakur, P., S. Kumar, J.A. Malik, J.D. Berger and H. Nayyar (2010). Cold stress effects on reproductive development in grain crops: an overview. Environ. Exp. Bot. 67(3): 429–443.

Vranová, V., D. Inzé and F. Van Breusegem (2002). Signal transduction during oxidative stress. J. Exp. Bot. 53: 1227–1236.

Yokota, A., K. Takahara and K. Akashi. Physiology and molecular biology of stress tolerance in plants. In: Madhavarao, K. Raghavendra and K. Janardhanreddy (eds), pp15-40, Springer.