RESEARCH ARTICLE

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ANTIOXIDANT DEFENSE SYSTEM OF BLACK RICE GROWN UNDER DROUGHT

ABSTRACT:
A pot experiment was conducted to study the oxidative response of drought-stressed black rice to foliar application of pyridoxine (0.03mM) and nicotinic acid (1.5mM). Drought stress was imposed by withholding water for 10 days, until leaf rolling symptoms was observed. After ten days from imposition of drought stress, water deficit resulted in a significant increase in H$_2$O$_2$ content thereby increased lipid peroxidation product; malonaldehyde (MDH) accompanied by high rate of ion leakage (IE) and consequently reduced the relative water content (RWC) compared with their corresponding controls. Drought stress resulted in a considerable increase in the activities of phenol peroxidase (POX), ascorbate oxidase (ASO), and catalase (CAT) enzymes of both shoots and roots of black rice seedlings, while the activities of glutathione reductase (GR), polyphenol oxidase (PPO) and superoxide dismutase (SOD) significantly decreased. Foliar application of pyridoxine or nicotinic acid mitigated the injurious effects of drought stress through the significant reduction in ion leakage, reducing membrane peroxidation, high ratio of chlorophylls/carotenoids thus restoring the membrane integrity and enhancing the defense system in terms of ascorbic acid, glutathione and total phenol contents as well as stimulating the activities of some antioxidant enzymes (GR, SOD, and POX). It may be suggested that the enhancement of the antioxidants in black rice seedlings is one of the mechanisms of drought tolerance under stress condition.

KEY WORDS:
Black rice, Drought, Antioxidants, Pyridoxine, Nicotinic acid

INTRODUCTION:
Rice (Oryza sativa L.) has become the most important cereal crop for the improvement of human health due to the starch, protein, oil, and the majority of micronutrients (Khush, 2005; Lee et al., 2007). Rice shows a very high antioxidant activity and proposed as important source antioxidants (Chung et al., 2001). Although white rice is widely consumed, there are developed many special rice that contain color pigments, such as black, brown, and red (Lee et al., 1988). Black rice contains health-promoting anthocyanin antioxidants and strong free radical scavenging activities (Chiang et al., 2006). Rice oil contains linolenic, linoleic, oleic unsaturated fatty acids and small quantities of vitamin E (Marchesi et al., 2002).

Drought is the main factor limiting rice production in Egypt. Most rice is grown in soils submerged with water. Rice is more susceptible to drought than other cereals (Kato, 2004). Developing drought tolerant rice cultivars is the main issue to increase rice production and to alleviate food insecurity and poverty in Egypt. Drought affects nearly all the plant growth processes; however, the stress response depends upon the intensity, rate, and duration of exposure and the stage of crop growth. Inhibition of leaf growth, decreasing the root: shoot ratio by water stress can be considered to be an adaptive response (Zhao et al., 2008). Plants partially protect themselves against drought stress by leaf rolling. Relatively little attention in physiological studies of cereal species has been thrown on leaf forms or shapes under drought condition. Leaf rolling usually linearly related to leaf water potential. Thus leaf rolling in rice may be used to estimate the other loss obvious effects of water deficit (Begg, 1980; Couturier et al., 2011). However, research showed that drought or other stress conditions caused rice leaf rolling which result in reduction in photosynthetic activities (Terzi et al., 2009; Saruhan et al., 2010).

Drought stress usually causes oxidative stress due to closure of stomata (Lei et al., 2006), disturbance in photosynthesis (Ben Ahmed et al., 2009), reduction in dry weight (Sikuku et al., 2010) and formation of reactive oxygen species (ROS) in mitochondria and

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chloroplasts (Asada, 1999). Determination of pigment ratios is considered as a good marker for stressed plants. High values of the ratio chlorophylls/carotenoids (a+b/x+c) up to 5 – 6 indicate photo synthetically intact photosynthetic apparatus. Under stress, chlorophyll is broken down faster than carotenoids and the pigment ratio decrease to about 2 – 3 (Lichtenthaler and Rinderle, 1988). Water stress decreased the RWC in both drought sensitive and tolerant maize leaves (Valentović et al., 2006). Moreover, stress treatment caused a significant increase in electrolyte leakage compared with control and recovered condition of kochia plant (Masoumi et al., 2010). ROS has the ability to damage lipids, proteins, enzymes and nucleic acids in the absence of protective mechanism (Ozhur et al., 2009). Peroxidation of lipids and/or electrolyte leakage is commonly considered as indicators of oxidative stress (Raza et al., 2007; Noreen et al., 2010). The degree of these damages depends up on the balance between the formation of active oxygen species and their removal by the antioxidant scavenging systems (Asada, 1999). Plants evolve a complex system of both enzymatic and non-enzymatic antioxidants to overcome oxidative stress (Alscher et al., 2002). Many investigators reported that the generation of reactive oxygen species under abiotic stress is accompanied by an increase in the activities of some antioxidant enzymes (Sairam et al., 2005; Deneto et al., 2006; Noreen et al., 2010). Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), phenol peroxidase (POX) and glutathione reductase (GR) are the antioxidant defense system, causing oxidative stress (Shehab et al., 2010).

Alleviation of stress is one of the main issues for plant biologists. Antioxidants are organic natural source required for growth of all living organisms. The beneficial effects of vitamin B6 (pyridoxine) as antioxidant has been reported in several studies in which animal cells subjected to different stress conditions (Chiang et al., 2006; Choi et al., 2007). Vitamin B6 plays a similar role in plant cells (Wei et al., 2007). Vitamin B6 is able to quench reactive oxygen species in vitro, and exogenously applied vitamin B6 protects plant cells against cell death induced by singlet oxygen, \(^1\text{O}_2\) (Havaux et al., 2009). Additional analyses revealed that vitamin B6 is also able to quench superoxide (Jang et al., 1997), thereby, protects plant protoplasts against \(^1\text{O}_2\)-induced cell death. Moreover, exogenous application of vitamin B6 mitigated lipid peroxidation in plant cell (Wei et al., 2007).

Vitamin B6 serves as a coenzyme of approximately 100 enzymes that catalyze essential chemical reactions (Lee et al., 1988; Lekem, 1990). It plays an important role in protein, carbohydrate and lipid metabolism as well as it is involved in the sodium-potassium balance (McCormick and Greene, 1994). Furthermore, pyridoxine is involved in the synthesis of messenger RNA which determines amino acids sequence in polypeptide synthesis (McCormick, 2006).

On the other hand nicotinic acid as an obligatory member of the pyridine nucleotide cycle plays an important role in energy transfer reactions in the metabolism of glucose, fats and alcohols. Nicotinic acid are converted inside the cell to a physiologically active compound called nicotinamide adenine dinucleotide (NAD) which is involved in several metabolic processes in plant and animal cells (Elson and Hass, 2006). Exogenous application of nicotinic acid was successful in ameliorating the adverse of NaCl on growth of some plants (Shaddad and Heikal 1982; Zidan, 1991; Ali, 2002). In general, beneficial effect of vitamins on yield quantity and quality has been reported for various crops by (Samiullah et al., 1991; Ghourab and Wahdan, 2000; El Bassiouny et al., 2005; El-Tohamy and El-Gready 2007; Malhi et al., 2007; El-Tohamy et al., 2008; Emam et al., 2011). However, no information is available about the possible role of vitamins in amelioration of oxidative stress under water deficit condition. Therefore, the present work was undertaken to study the effect of nicotinic acid and pyridoxine in enhancement of the oxidative defense system of black rice plants under water deficit condition.

MATERIAL AND METHODS:

Plant material and growth condition:

Pot experiment was conducted in the green house of Faculty of Science, Ain Shams University. Pure strains of black rice grains (Oryza sativa L) were obtained from the Agriculture Research Center, Rice Research Institute in Giza, Egypt. The grains were surface sterilized by immersing in 1% sodium hypochlorite solution for 5 minutes, then rinsed thoroughly with distilled water. The sterilized rice grains were sown in pots (40x60 cm) filled with homogenous loamy soil (2 Kg). All pots were supplied with sufficient irrigation up to 20 days. The pots were divided into two groups; drought stress condition was imposed to the first group by...
withholding irrigation until leaf rolling symptoms appear as a stress indicator (for about 10 days) while the other group was irrigated and flooded with water (about 5 cm above soil surface) to serve as control.

The pots of each group were divided into three equal sets, the shoots of the first set (reference control), were sprayed with distilled water, while those of the second and third sets were sprayed with aqueous solution of nicotinic acid and pyridoxine at concentrations 0.03 mM and 1.5mM respectively. Spraying was conducted three times at 2 days intervals (the soil was covered during spraying).

This experiment was carried out in a Complete Randomized Design (CRD) with 3 replicates. Seedlings were exposed to normal day length with natural temperature (about 22/13 ± 2°C and 11 h photoperiod). Seedlings were collected after 30 days from sowing; the harvested plants were partitioned into shoots and roots. Some seedlings were collected for measuring pigment content (chlorophylls a-b, carotenoids x+c) in leaves, relative water content (RWC), leaf membrane leakage (EL), lipid peroxidation product (MDA), hydrogen peroxide and proline content, total phenols as well as enzymatic and non enzymatic antioxidants.

Leaf rolling was assessed visually in each pot, in all the treatments, several plants were assessed and the pots were given a mean leaf rolling score, ranging from 1 to 5, with 1 being flat and 5 a tightly rolled leaf (O’Toole and Moya, 1978).

Chemical analysis:

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were extracted in 80% acetone and determined spectrophotometrically as recommended by Lichtenthaler (1987). The absorbance was determined using Spekol Spectrocolourimeter (VEB Carl Zeiss) in the wavelengths of 663 nm (chlorophyll a - Chl a), 644 nm (chlorophyll b - Chl b) and 480 nm (carotenoids -x+c).

Relative water content was determined by the method of Basnayake et al. (1993). However, Leaf membrane damage was determined by recording electrolyte leakage (EL) as described by Valentonic et al. (2006). The product of lipid peroxidation, a membrane damage indicator was estimated by the thiobarbituric acid reaction to measure the amount of malondialdehyde, as described by Heath and Packer (1968). The hydrogen peroxide (H$_2$O$_2$) content in the leaves was assayed according to the method of Velikova et al. (2000).

The antioxidant substances such as total phenols, glutathione and ascorbic acid were extracted and estimated following the methods described by Malik and Singh (1980), Hissin and Hilf (1976), and Mukherjee and Choudhuri (1983), respectively. However, the antioxidant enzymes were extracted from rice seedlings by using a known volume of phosphate buffer (pH 7). The crude extracts were used for enzyme assay. Superoxide dismutase activity (C u-Zn SOD EC 1.15.1.1) was determined by measuring the inhibition of the auto-oxidation of pyrogallol using a modified method of Marklund and Marklund (1974). Catalase (CAT EC 1.11.1.6) activity was assayed following the method of Chen et al. (2000). Polyphenol Peroxidase (POX EC 1.11.1.7) and oxidase (PPO EC 1.10.3.1) activities were determined according to the method described by Kar and Mishra (1976). Ascorbate peroxidase (APX EC 1.11.1.1) and oxidase (ASO EC 1.10.3.3) activities were assayed by the method reported by Koricheva et al. (1997) and Diallinas et al. (1997) respectively. Glutathione reductase (GR EC 1.6.4.2) activity was assayed as described by Schaedle and Bassham (1977).

Statistical analysis:

Analysis of variance was conducted using ANOVA one way variance test using Microsoft Excel 2000. Statistical probability values were calculated to quantity levels of significance for each treatment type. The values of analysis of seedlings grown under drought condition were used as a reference controls for stressed seedlings treated with pyridoxine or nicotinic acid, as well as they compared also, with each other.

RESULTS AND DISCUSSION:

Knowledge of pigment content and pigment ratios of leaves is a prerequisite to indicate the physiological status of plants. Drought stress is known to reduce chlorophyll content, causes chlorosis and full leaf rolling at sever water deficit (Jaleel et al., 2008).

Under drought condition, chlorophylls a and b in the rice leaves were significantly reduced by 39.8% and 50.2% respectively compared with those of the well-watered control (Table 1). Lichtenthaler and Rinderle, (1988) reported that, under normal condition, the (a/b) ratio amounts to value of 2.6 to 3.1. In stressed leaves, however, the (a/b) ratio eventually decline to one or even less than one. They added that, high values of the ratio chlorophylls/carotenoids (a+b/x+c) up to 5 – 6 indicate the full efficiency of photosynthetic apparatus. In this study, the same result was obtained, the ratios (a/b) sharply decrease from 2.6 to1.6 in stressed rice leaves compared to the well watered control. In addition, data presented in table 1 revealed that, the ratio (a+b/x+c) significantly decreased in stressed leaves to about 2.5. The low value of the ratio (a/b/x+c) indicated that the chlorophyll is faster broken than carotenoids. Foliar spraying of rice seedlings by either pyridoxine or nicotinic acid induced
significance increases in chl a, chl b, restoring the ratio of (a+b/x+c) to reach about 5.4 and 4.5, respectively indicating complete recovery and intact photosynthetic apparatus.

Table 1. Effect of foliar application of pyridoxine or nicotinic acid on pigment content (chlorophylls (a+b), carotenoids (x+c) in μg/g FW) and pigment ratios (chlor/a, chlorophyll/carotenoids (a+b)/(x+c)) in leaves of black rice grown under drought conditions.

<table>
<thead>
<tr>
<th>Leaf rolling score</th>
<th>Chl.a</th>
<th>Chl.b</th>
<th>Carot. Chl.(a/b)</th>
<th>(a+b/x+c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well watered</td>
<td>65.8</td>
<td>23.7</td>
<td>17.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Drought</td>
<td>26.2*</td>
<td>12.9*</td>
<td>15.5*</td>
<td>1.6*</td>
</tr>
<tr>
<td>Drought + Pyridoxine</td>
<td>50.0*</td>
<td>24.8*</td>
<td>16.1*</td>
<td>2.1*</td>
</tr>
<tr>
<td>Drought + nicotinic acid</td>
<td>50.9*</td>
<td>20.7*</td>
<td>16.0*</td>
<td>1.67*</td>
</tr>
</tbody>
</table>

Values with a superscript are significant different from the control. Letter a =* at P > 0.05, b =** at P < 0.01, c =*** at P < 0.001, and absence of letter = non significant.

Leaf rolling as a concomitant response of reduced chlorophyll content was one of the first visual symptoms. Leaf rolling in rice is one way to reduce transpiration and used as an important criterion during screening of genotypes for drought tolerance (Lilley and Fukai, 1994). Leaf rolling and leaflet closure during water deficit might be attributed to the interception of solar radiation and, thus, decrease leaf temperature and water loss by transpiration; which considered as one of the drought avoidance mechanisms evolved in plants. Rolling leaf with moderate rate were found helpful in keeping leaf erect and reduce water transpiration; this can increase efficiency of light utilization rate (Couturier et al., 2011). Our results indicated that, the healthy rice leaves (score=0) start to fold (shallow V-shape) after 3 days of withholding water (score = 1). Leaves became tightly rolled after 10 days under drought conditions. Consequently, leaf rolling may be an indicator of water content in rice and is used by breeders for selection of tolerant cultivars in rice (Chang et al., 1972; ÔToole and Moya, 1978; ÔToole and Chang, 1979). Foliar spraying of rice seedlings by either pyridoxine or nicotinic acid showed an amazing capacity for recovery and induced decreases of leaf rolling scores (Table 1).

Data presented in table 2 and 3, clearly indicate that, proline content was higher in roots than shoots. Under drought condition, proline content in shoots and roots of black rice seedlings were significantly increased up to about (9 and 3 fold, respectively) as compared with those of the well-watered control. Proline was reported to improve tolerance of water stressed plants (Deivanai et al., 2011). The accumulation of proline might be involved in cellular osmotic adjustment, detoxification of ROS, protection of membrane integrity and stabilization of protein (Trovato et al., 2008; Ashraf and Foolad, 2007; Liu et al., 2011). However, vitamin treatments significantly decreased the proline content to a value lower than that of well watered seedlings. The greatest reduction in proline accumulation was recorded in both shoot and root of stressed rice seedlings exposed to pyridoxine. However, nicotinic acid increased the proline level in shoot while the reverse was recorded in root (Tables 2 & 3).

Table 2. Effects of foliar application of pyridoxine or nicotinic acid on RWC, EL, MDA, H$_2$O$_2$, proline content, and antioxidant compounds (GSH, ASA, and total phenols) of black rice shoots grown under drought conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RWC (%)</th>
<th>EL %</th>
<th>MDA nmol/g F.W.</th>
<th>H$_2$O$_2$ nmol/g F.W.</th>
<th>GSH nmol/g F.W.</th>
<th>ASA nmol/g F.W.</th>
<th>Phenols mg/g F.W.</th>
<th>Proline μg/g FW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well-watered</td>
<td>76.3</td>
<td>9.3</td>
<td>3.57</td>
<td>8.66</td>
<td>18.1</td>
<td>72.3</td>
<td>7.7</td>
<td>12.5</td>
</tr>
<tr>
<td>Drought</td>
<td>560.4*</td>
<td>3.2*</td>
<td>5.06*</td>
<td>1.66*</td>
<td>50.3*</td>
<td>30.4*</td>
<td>87.9*</td>
<td>53.10*</td>
</tr>
<tr>
<td>Drought + Pyridoxine</td>
<td>68.4*</td>
<td>7.8*</td>
<td>3.88*</td>
<td>1.95*</td>
<td>20.2*</td>
<td>69.5*</td>
<td>7.3*</td>
<td>15.7*</td>
</tr>
<tr>
<td>Drought + nicotinic acid</td>
<td>171.1*</td>
<td>6.5*</td>
<td>4.03*</td>
<td>8.11*</td>
<td>19.1*</td>
<td>65.7*</td>
<td>9.5*</td>
<td>13.7*</td>
</tr>
</tbody>
</table>

Values with a superscript are significant different from the control. Letter a =* at P > 0.05, b =** at P < 0.01, c =*** at P < 0.001, and absence of letter = non significant.

Table 3. Effects of foliar application of pyridoxine or nicotinic acid on EL, MDA, H$_2$O$_2$, proline contents, and antioxidant compounds (GSH, ASA, and total phenols) of black rice roots grown under drought conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RWC (%)</th>
<th>EL %</th>
<th>MDA nmol/g F.W.</th>
<th>H$_2$O$_2$ nmol/g F.W.</th>
<th>GSH nmol/g F.W.</th>
<th>ASA nmol/g F.W.</th>
<th>Phenols mg/g F.W.</th>
<th>Proline μg/g FW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well-watered</td>
<td>230.3</td>
<td>11.5</td>
<td>2.57</td>
<td>0.44</td>
<td>16.1</td>
<td>8.8</td>
<td>11.5</td>
<td>7.7*</td>
</tr>
<tr>
<td>Drought</td>
<td>2145.8*</td>
<td>5.2*</td>
<td>6.9*</td>
<td>0.50*</td>
<td>30.3*</td>
<td>57.9*</td>
<td>33.1*</td>
<td>11.7*</td>
</tr>
<tr>
<td>Drought + Pyridoxine</td>
<td>102.4</td>
<td>10.6</td>
<td>3.8*</td>
<td>0.85*</td>
<td>19.2*</td>
<td>7.4</td>
<td>11.7*</td>
<td>8.5</td>
</tr>
<tr>
<td>Drought + nicotinic acid</td>
<td>344.5*</td>
<td>6.9*</td>
<td>4.2*</td>
<td>0.81*</td>
<td>20.1*</td>
<td>8.5</td>
<td>19.1*</td>
<td>19.1*</td>
</tr>
</tbody>
</table>

Values with a superscript are significant different from the control. Letter a =* at P > 0.05, b =** at P < 0.01, c =*** at P < 0.001, and absence of letter = non significant.

Drought usually leads to oxidative stress, when the production of ROS exceeds the capacity of antioxidant defense system the membranes lose their stability, increasing ion leakage (Blokhina et al., 2003). Data presented in table 2 and 3 showed significant decrease in relative water content (RWC) and increase in ion leakage in shoots and roots of the stressed rice seedlings compared with their corresponding controls. The reduction in the relative water content in response to drought stress has been reported in a wide variety of plants (Valentović et al., 2006; Masoumi et al., 2010). Drought stress disturbs
plant water relations (Anjum et al., 2011). The observed decrease in RWC was concomitant with an increase in ion leakage and loss of membrane stability (Tables 2 & 3). Plant membranes are subjected to changes often associated with the increases in permeability and loss of integrity under environmental stresses (Blokhina et al., 2003). Therefore, the ability of cell membranes to control the rate of ion exchange in and out the cell is used as an indicator of membrane damage. Plants under water deficit condition accumulate superoxide radical and hydrogen peroxide which can directly attack membrane lipids (Menconi et al., 1995). Drought stress induced a high significant increase in lipid peroxidation product (MDA) in both shoots and roots of stressed rice seedlings (Tables 2 & 3). The increase in MDA content was accompanied by an increase ion leakage resulting in enhanced membrane permeability. Similar results have been reached by Zhou et al. (2007). Foliar spraying of pyridoxine or nicotinic acid alleviated the harmful effects of drought stress through the significant reduction in both ion leakage and lipid peroxidation product (MDA). Therefore, vitamin B6 can be considered as a new member of the network of protective compounds involved in the management of \( \text{O}_2 \) in plants, mitigated lipid peroxidation (Wei et al., 2007). The vitamin effects might be attributed to the high activity of antioxidant enzymes in shoots and roots of the stressed rice cultivars (Gunes et al., 2007).

The present data revealed that, the total phenol content significantly increased in shoots and roots of drought stressed black rice seedlings. Such increase reaches about 93% and 13%, respectively compared to the well watered seedlings (Tables 2 & 3). So, it was observed that the total phenols were markedly accumulated in shoots compared to roots in rice seedlings under drought condition. Such effect was concomitant with a pronounced decrease in PPO activity. Phenols play a role in cell acclimation against stress (Lee et al., 2007). In addition, phenolic compounds exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Pokorny et al., 2001). Phenolic compounds can also, decrease membrane fluidity (Gaballah et al., 2006). Application of either pyridoxine or nicotinic acid induced further increase of total phenols in shoots and roots of stressed rice seedlings (Tables 2 & 3). Such increase might be attributed to the decreased PPO activity (El-Shalakany et al., 2010).

Imposition of drought stress significantly decreased glutathione (GSH) concentration in shoots and roots of stressed rice seedlings (Tables 2 & 3). This decrease was concomitant with a significant decrease in GR activity. The reduction in GSH level might be attributed to decrease of GSH biosynthesis and/or increase of its degradation (Noctor and Foyer, 1998). Moreover, such effect might be also attributed to the role of GSH in the regeneration of another potent antioxidant as ASA. It is possible that under water deficit conditions, ASA levels increased in ascorbate-glutathione cycle, whereas GSH levels decrease. The decreased activities of ASO have been reflected by the increased levels of ASA (Tables 2 & 4) these enzymes withdraw ascorbate as substrate. ASA can directly scavenge superoxide, hydroxyl radicals and singlet oxygen and reduce hydrogen peroxide to water via ascorbate peroxidase reduction (Noctor and Foyer, 1998).

**Table 4. Effects of foliar application of pyridoxine or nicotinic acid on the activities of antioxidant enzymes of black rice shoot**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GR mM NADPH/mg protein</th>
<th>SOD Unit/mg protein</th>
<th>ASO mM oxidized/g quinon/min</th>
<th>POX Amount of quinon/g FW./min</th>
<th>CAT mM of H2O2/g FW./min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well-watered</td>
<td>1.54</td>
<td>0.272</td>
<td>24.5</td>
<td>81.5</td>
<td>117.6</td>
</tr>
<tr>
<td>Drought</td>
<td>0.30a</td>
<td>0.102b</td>
<td>21.5a</td>
<td>24.8b</td>
<td>144.1b</td>
</tr>
<tr>
<td>Drought +Pyridoxine</td>
<td>1.22c</td>
<td>0.193c</td>
<td>29.8c</td>
<td>58.5c</td>
<td>164.2d</td>
</tr>
<tr>
<td>Drought +nicotinic acid</td>
<td>0.98c</td>
<td>0.170b</td>
<td>25.3c</td>
<td>49.9c</td>
<td>141.3b</td>
</tr>
</tbody>
</table>

Values with a superscript are significant different from the control. \( a =* at P > 0.05, b =** at P < 0.01, c =*** at P < 0.001, \) and absence of letter = non significant.

A significant increase of GSH in stressed rice seedlings treated with either pyridoxine or nicotinic acid might nullify hazards caused by drought stress. GSH plays an important role in expression of defense genes and it may be also involved in control of cell division (Shao et al., 2008). GSH can protect the –SH group enzymes and structural protein against oxidation (Liang et al., 2006).

Considerable changes in antioxidant enzymes of black rice plants grown under water deficit condition and treated with either pyridoxine or nicotinic acid were observed in Tables 4 & 5. Drought stress resulted in a considerable increase in the activities of POX, ASO, and CAT enzymes of both shoots and roots of black rice seedlings, while the activities of GR & PPO & SOD significantly decreased. The activity of SOD of stressed black rice shoots was drastically diminished about 81% in shoots and about 51.5% in roots as being compared with the untreated control. The observed decrease in SOD activity under drought stress was concomitant with a significant increase in \( H_2O_2 \) in shoots and roots of rice seedlings despite the high activity of CAT and POX which were not
efficient to eliminate all \( \text{H}_2\text{O}_2 \) formed (Bray et al., 2000).

Table 5. Effects of foliar application of pyridoxine or nicotinic acid on the activities of antioxidant enzymes of black rice roots grown under drought conditions.

<table>
<thead>
<tr>
<th></th>
<th>GR mM NADPH / Unit/mg Protein</th>
<th>ASCO mM of ascorbate</th>
<th>PPO Amount of Oxidized quinone / g FW. / min</th>
<th>POX Amount of quinone / g FW. / min</th>
<th>CAT mM of H (_2\text{O}_2) / g FW. / min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well-watered</td>
<td>0.66</td>
<td>0.370</td>
<td>14.8</td>
<td>31.5</td>
<td>117.6</td>
</tr>
<tr>
<td>Drought</td>
<td>0.32a</td>
<td>0.166b</td>
<td>11.5b</td>
<td>21.8b</td>
<td>144.1b</td>
</tr>
<tr>
<td>Drought + Pyridoxine</td>
<td>0.64b</td>
<td>0.273a</td>
<td>24.5b</td>
<td>36.5b</td>
<td>164.2b</td>
</tr>
<tr>
<td>Drought + nicotine acid</td>
<td>0.88b</td>
<td>0.170a</td>
<td>27.3a</td>
<td>52.3a</td>
<td>141.3b</td>
</tr>
</tbody>
</table>

Values with a superscript are significant different from the control. Letter a =* at \( P > 0.05 \), b =** at \( P < 0.01 \), c =*** at \( P < 0.001 \), and absence of letter = non significant.

Foliar application of pyridoxine and nicotinic acid under drought stress resulted in further increase in the activities of SOD and POX in rice shoots and roots which was concomitant with a significant decrease in \( \text{H}_2\text{O}_2 \) level (Tables 2-4). Vitamin B6 is also able to quench superoxide from plant cell (Jang et al., 1997; Havaux et al., 2009). Vitamin B6 plays essential roles as a cofactor in a wide range of biochemical reactions, predominantly in amino acid metabolism (Lee et al., 1988). Vitamin B6 serves as a coenzyme of approximately 100 enzymes that catalyze essential chemical reactions (Lekem, 1990).

Moreover, POX showed high activity in water stressed rice seedlings (Tables 4 & 5). POX plays an important role in the formation of suberin and lignin, thus increase the rigidity of plant cell walls under drought stress (Quigora et al., 2000). An increase in the activity of antioxidant enzymes under drought stress could be indicative of an increased production of ROS and build up of protective mechanism to reduce oxidative damage triggered by drought stress (Noreen et al., 2010).

In conclusion, this study demonstrates that pyridoxine and nicotinic acid play an important role in the in vivo antioxidant defense system of black rice seedlings grown under water deficit condition.

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