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NITRIC OXIDE PREVENTS RICE CALLUS *(Oryza sativa)* FROM DROUGHT STRESS

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ABSTRACT

Rice (*Oryza sativa*) is the major food crop in Asia and North Africa, but stress conditions such as drought often cause severe yield loss in the majority of the agricultural regions of the world. In the present study, we evaluated the protective effect of nitric oxide (NO) against drought stress in rice callus. The drought stress was induced using different concentrations (5%, 10%, 15% and 20%) of polyethylene glycol (PEG). The increased contents of hydrogen peroxide (H_2O_2) (~ five folds) indicated that drought induced oxidative stress. The increased contents of malondialdehyde (MDA) (~thirteen folds) indicated the induction of lipids peroxidation. Also, drought increased the contents of the total soluble amino acids, carbohydrates, phenols, ascorbic acids (AsA), glutathione (GSH). In addition, drought induced the activity of the antioxidant defense enzymes: superoxide distumase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR). The increase of the mentioned constituents as well as the enzymes activity was in a drought dose-dependent manner. The addition of sodium nitroprusside (SNP) as nitric oxide donor to a final concentration of 100 micromole in the culture media prevented the callus from the drought-induced oxidative stress indicated by the decrease of H_2O_2 and malondialdehyde contents and the increase of the antioxidant defense enzymes activity as well as the non-enzymatic antioxidant constituents. These results suggest that the exogenous application of NO could be advantageous against oxidative stress, and could confer tolerance to drought stress in plants.

Key words: *antioxidant, drought, lipid peroxidation, nitric oxide, Oryza sativa, oxidative stress, rice, sodium nitroprusside.*

Abbreviations: AsA, ascorbic acid; ASC, ascorbate; APX, ascorbate peroxidase; DHA, dehydroascorbic acid; EDTA, ethylenediamine-*N,N,N,N*-tetraacetic acid; FAA, free amino acids; FW, fresh weight; GSH, reduced glutathione; GSSG, oxidized glutathione; GR, glutathione reductase; ROS, reactive oxygen species; CAT, catalase; MDA, malondialdehyde; NAA Naphthalenea cetic acid., NBT, nitroblue tetrazolium; PAL, Phenylalanine ammonia-lyase; PEG, polyethylene glycol; POD, peroxidase; SNP, sodium nitroprusside; and SOD, superoxide dismutase.

1. INTRODUCTION

As generally known, water is a key environmental factor, which is a major limiting factor for plant growth, development and production. Drought stress inhibits photosynthesis of plants, causes changes in chlorophyll contents and components and damages in photosynthetic apparatus (Iturbe-Ormaetxe *et al.,* 1998). It also inhibits the photochemical activities and decreases the activities of enzymes in Calvin cycle (Monakhova and Chernyad'ev, 2002). Drought induces several physiological, biochemical and molecular responses in plants, which may help them to adapt to such limiting environmental conditions (Bajaj *et al.,* 1999; Arora *et al.,* 2002).

A common effect of drought stress is the disturbance between the generation and quenching of reactive oxygen species (ROS) (Smirnoff, 1998). ROS includes singlet oxygen (O_2^1), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^·$). They are considered to be toxic byproducts of many degenerative reactions in plants because they can react with various cellular components to induce oxidative damage (Mittler, 2002). Tissue damage occurs when the capacity of their antioxidative systems is insufficient to counteract the amount of

ROS being generated. Malondialdehyde (MDA) is a marker commonly used for assessing membrane lipid peroxidation (Sgherri *et al.*, 2003). In plants, ROS detoxification is carried out by a network of reactions involving enzymes such as superoxide dismutase (SOD: EC 1.15.1.1), catalase (CAT: EC 1.11.1.6), peroxidase (POD: EC 1.11.1.7), ascorbate peroxidase (APX: EC 1.11.1.11), glutathione reductase (GR: EC 1.6.4.2) and low-molecular weight non-enzymatic antioxidants such as ascorbate (ASC), reduced glutathione (GSH), carotinoids, α-tocopherol and flavonoids (Mittler, 2002). SOD removes superoxide anion free radicals accompanying with the formation of hydrogen peroxide (H_2O_2), which is then detoxified by CAT and POD. In the ascorbate-glutathione cycle, APX reduces H_2O_2 using ascorbate as an electron donor. Oxidized ascorbate is then reduced by GSH generated from oxidized glutathione (GSSG) catalyzed by GR at the expense of NADPH. In environmental stress conditions such as drought, high activities of antioxidant enzymes and high contents of nonenzymatic constituents are important for plants to tolerate stresses (Gong *et al.*, 2005).

Drought stress also induces various biochemical and physiological responses in plants. Physiological studies have shown that sugars (such as raffinose family oligosaccharides (RFO), sucrose, and trehalose), sugar alcohols (such as mannitol and sorbitol), amino acids (such as proline), and amines (such as glycine betaine and polyamines) accumulate under drought stress conditions in different plant species. These metabolites accumulate under drought stress and function as osmolytes, antioxidants or scavengers that help the plants to avoid and/or tolerate stresses. The changes in these metabolites at the cellular level are thought to be associated with protecting cellular function or with maintaining the structure of cellular components (Bartels and Sunkar, 2005).

Phenolics are the most widely distributed secondary plant products (30–45% of plant organic matter) and are important antioxidants. Phenolic compounds are derived mainly from L-phenylalanine *via* nitrogen-free skeletons of t-cinnamate. This initial step of phenylpropanoid synthesis is mediated by phenylalanine ammonia-lyase (PAL, EC 4.3.1.5). The increase of PAL activity is a common response of plant cells to biotic and abiotic stresses. Studies with several different species of plants have shown that PAL is activated by many environmental factors (Jozef *et al.*, 2007; Mikal, 2000).

Nitric oxide (NO) is a signaling molecule that has been implicated in the activation of plant defenses. Nitric oxide is a bioactive free radical which plays important roles in many physiological processes in plants, such as growth, development, senescence and adaptive responses to multiple stresses (Zhao *et al.*, 2004; Graziano and Lamattina, 2005). Under ROS-related toxicity, NO may act as a chain breaker and thus limits the oxidative damage. Recently, a function of nitric oxide in the protection of plants against oxidative stress under various adverse conditions was reported (Beligni and Lamattina, 2002; Shi *et al.*, 2005). Many previous studies have reported the presence of NO in the plant kingdom and its involvement in growth, development and defense responses (Beligni and Lamattina 1999; Zhang *et al.*, 2003).

The aim of the present study was to investigate the effects of sodium nitroprusside (SNP; a nitric oxide donor) treatment on the rice enzymatic and non-enzymatic antioxidant systems during drought stress mediated by polyethylene glycol (PEG).

2. MATERIALS AND METHODS

Chemicals used in the experiment were mainly obtained from Sigma, Aldrich and Fluka.

2.1. Plant callus culture

The plant cell line for the study was initiated from callus induced from young stems of rice (*Oryza sativa*) plants Sakha 102. The callus was initiated in a MS-Medium supplemented with 4.0 mg/L of Naphthalene acetic acid (NAA), 4.0 mg/L of kinetin, 20 g/L sucrose and 8 g/L agar. The medium was adjusted to pH 5.8 and then sterilized at 121°C for 20 min, incubated in the dark at 25°C. The callus was propagated for 5 months by the time of this study. The subcultures were performed every 4 weeks.

2.2. Experimental design and culture treatment

The experimental design consisted of: (I) a control callus growing in MS-Medium free of polyethylene glycol (PEG) and sodium nitroprusside (SNP); (II) a callus growing in drought condition induced by addition of different PEG concentrations (5%,10%,15% and 20%); and (III) a callus growing in a drought condition but in the presence of 100 μM SNP (as a Nitric Oxide donor). This NO donor and its dosage used in the experiments were chosen based on previous studies (Laspina *et al.*, 2005; Wang and Higgins, 2006). The SNP and PEG were pre-dissolved in distilled water and sterilized by filtration then added to the culture medium to the indicated final

concentrations. Callus cells were harvested after four weeks, washed twice with 100 ml distilled water on a porous – glass funnel with filter paper (Whatman No.1) and the excess water was removed by filter paper, then frozen in liquid nitrogen and stored in the deep freezer for further analysis.

2.3. Preparation of enzyme extracts

The callus samples (1.0 g) were crushed into fine powder using liquid nitrogen. Soluble protein was extracted by homogenizing the powder in 5mL 50mM phosphate buffer (pH 7.8) containing 1mM EDTA and 1% PVP, with the addition of 1mM ASC in the case of APX assay. The homogenate was centrifuged at 15,000xg for 20 min, and the supernatant was used for the following enzyme activity assays. Protein content was determined according to the Bradford's method (Bradford, 1976) with bovine serum albumin (BSA) as standard.

2.4. Enzyme activity assays

Super oxide dismutase (SOD) (EC 1.15.1.1) was assayed by measuring its ability to inhibit the photochemical reduction of NBT using the method of Beauchamp and Fridovich (1971). The reaction mixture (3 ml) contained 50 mM phosphate buffer pH 7.8, 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 1.0 mM EDTA and 20 µl enzyme extract. Riboflavin was added last and the reaction was initiated by placing the tubes 30 cm below 15 W fluorescent lamps. The reaction was started by switching on the light and was allowed to run for 10 min. Switching off the light stopped the reaction and the tubes were covered with black cloth. Non-illuminated tubes served as control. The absorbance at 560 nm was recorded. One unit of SOD was defined as that being contained in the volume of extract that caused a 50% inhibition of the SOD-inhibitable fraction of the NBT reduction.

Catalase (CA) (EC 1.11.1.6) activity was determined by following the consumption of H_2O_2 using the method of (Aebi, 1984). The reaction mixture (3ml) contained 50 mM potassium phosphate buffer pH 7.0, 15 mM H_2O_2 and 50 µl enzyme extract. The reaction was initiated by adding the H_2O_2. The consumption of H_2O_2 was monitored spectrophotometrically at 240 nm (E = 39.4 mM^{-1} cm^{-1}) for 3min. Enzyme activity was expressed in µM H_2O_2 min^{-1}.

Ascorbate peroxidase (APX) (EC 1.11.1.11) activity was determined spectrophotometrically by following the decrease in the absorbance at 290 nm (E = 2.8 mM^{-1} cm^{-1}) using the method of (Nakano and Asada, 1981). The reaction mixture (3 ml) contained 50 mM potassium phosphate buffer pH 7.0, 0.3 mM ascorbate, 0.1 mM H_2O_2 and 50 µL enzyme extract. The rates were corrected for non-enzymatic oxidation of ascorbate by the inclusion of reaction mixture without enzyme extract. Absorbance decreased 0.01 at 290 nm was defined as an activity unit during 1 min/ mg protein.

Glutathione reductase (GR) (EC 1.6.4.2) activity was determined based on the decrease in absorbance at 340 nm due to the oxidation of NADPH to NADP according to the method of Fryer *et al.* (1998). The reaction mixture in a total volume of 1ml contained 80 mM potassium phosphate, pH 7.8, 200µM DTPA, 500µM oxidized glutathione (GSSG), 150µM NADPH and 100µl enzyme extract. Correction was made for the non-GTR-dependent oxidation of NADPH by excluding GSSG from the reaction mixture.

Phenylalanine ammonium-lyase (PAL) (EC 4.3.1.5) activity was determined based on the rate of cinnamic acid production as described by Ochoa-Alejo and Gòmez-Peralta (1993). Briefly, 1ml of 50mM Tris–HCl buffer pH 8.8 containing 15mM of β-mercaptoethanol , 0.5ml of 10mM L-phenylalanine, 0.4ml of double distilled water and 0.1ml of enzyme extract were incubated at 37°C for 1 hr. The reaction was terminated by the addition of 0.5ml of 6M HCl, and the product was extracted with 15ml ethyl acetate, followed by evaporation to remove the extracting solvent. The solid residue was suspended in 3ml of 0.05M NaOH and the cinnamic acid concentration wherein was quantified with the absorbance measured at 290nm. One unit of PAL activity is equal to 1µmol of cinnamic acid produced per min.

2.5. Analysis of lipid peroxidation

Lipid peroxidation was measured in terms of MDA content following the method of Heath and Packer (1968) modified by Song *et al.* (2006). Callus (0.5 g) was homogenized using mortar and pestle in 10% trichloroacetic acid and then the homogenate was centrifuged at 4000 xg for 30 min. A 2 mL aliquot of the supernatant was mixed with 2 mL of 10% trichloroacetic acid containing 0.5% thiobarbituric acid. The mixture was heated at 100 °C for 30 min. The absorbance of the supernatant was measured at 532 nm, with a reading at 600 nm subtracted from it to account for non-specific turbidity. The amount of malonaldehyde was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.6. Hydrogen peroxide determination

The hydrogen peroxide (H_2O_2) contents in the callus were assayed according to the method of Velikova *et al.* (2000). Callus was crushed into fine powder using liquid nitrogen then homogenized with 0.1% (w/v) TCA. The extract was centrifuged at 12,000 xg for 15 min, after which to 0.5 ml of the supernatant was added 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI, and the absorbance was read at 390 nm. The content of H_2O_2 was given on a standard curve.

2.7. Total glutathione determination

The level of total glutathione (GSH) was determined with Ellman's reagent according to De Vos *et al.*, 1992. The buffer was mixed with 630 µl of 0.5 M K_2HPO_4 and 25 µl of mM 5, 5'–dithiobis (2-nitrobenzoic acid) (final pH 7). The absorbance at 412 nm was read after 2 min. GSH was used as a standard.

2.8. Phenolic compounds determination

Total phenols in the callus methanolic extract were determined by Folin-Ciocalteau reagent according to McDoland *et al.* (2001). The samples were homogenized in 80% methanol (1:10 g ml^{-1}) and were kept in a water bath at 70°C for 15 min with frequent agitation. 0.5 ml of the methanolic extract or gallic acid (standard phenolic compound) was mixed with Folin-Ciocalteau reagent (5 ml; 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml; 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by measuring the absorbance at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg L^{-1} solutions of gallic acid in methanol:water (50:50, v/v). Total phenol values were expressed in terms of gallic acid equivalents (mg g^{-1} fresh weight of callus tissues).

2.9. Determination of ascorbic acid, total amino acids and total soluble sugars

The ascorbate content was measured using 2, 4-dinitrophenolindophenol. The absorbance was measured at 520 nm according to Omaye *et al.* (1979). Total amino acids were determined according to the method of Rosein (1957). Total soluble sugars were determined in the callus ethanolic extract using the phenol-sulfuric acid method according to Dubois *et al.* (1956).

2.10. Statistical analysis

The data were analyzed using SPSS (version 10) program. Mean and standard error were descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples. P-values <0.05 were considered significant.

3. RESULTS AND DISCUSSION

3.1. Effects of PEG and SNP treatments on H_2O_2 concentrations and lipid peroxidation

Since oxidative damage is generated under drought stress through the formation of ROS in plants (Beligni and Lamattina, 2002), H_2O_2 was measured. Compared to control, drought stress significantly increased H_2O_2 contents in rice callus (Table 1). The H_2O_2 contents tended to increase with the intensity of the drought stress, resulting in a significantly higher value under 20% PEG treatment. The H_2O_2 contents increased 3, 4, and about 5 folds when 5%, 15% and 20% PEG were used. Addition of sodium nitroprusside (SNP) as nitric oxide donor to a final concentration of 100 µM in the culture media decreased the accumulation of H_2O_2 in rice callus under drought stress (Table1).

As lipid peroxidation is one of the consequences of oxidative damage, we monitored changes of lipid peroxidation by measuring the

Table (1): Effects of PEG and SNP treatments on the H_2O_2 contents and lipid peroxidation (MDA) in rice callus.

Treatments	H_2O_2 (µ mol/g FW)	MDA (µ mol/ g FW)
Negative control	80.86 ± 5.76^{i}	0.89 ± 0.06^{e}
PEG (5%)	243.54 ± 13.76^{f}	5.75 ± 0.23^{d}
PEG (10%)	264.98 ± 18.32^{e}	8.43 ± 0.37^{b}
PEG (15%)	327.86 ± 15.76^{c}	9.43 ± 0.49^{b}
PEG (20%)	387.90 ± 23.46^{a}	11.98 ± 0.78^{a}
PEG (5%) +SNP	189.24 ± 12.14^{h}	4.85 ± 0.36^{d}
PEG (10%) +SNP	212.95 ± 17.56^{g}	6.27 ± 0.58^{cd}
PEG (15%) +SNP	289.67 ± 22.11^{d}	7.93 ± 0.62^{bc}
PEG (20%) +SNP	349.47 ± 23.71^{b}	8.00 ± 0.73^{bc}
LSD	16.183	1.514

Means in the same column with the same letter are not significantly at P< 0.05

amounts of MDA. As shown in Table (1), similar to H_2O_2 change, drought stress significantly increased MDA concentration in rice callus compared to the control. The MDA was 13 folds in the callus treated with 20% PEG compared to the control. The application of NO donor significantly reduced the accumulation of MDA, especially at high drought stress conditions.

Increased H_2O_2 and lipid peroxidation have been observed in plants growing under different stress conditions (Gómez *et al.*, 1999; Mittova *et al.*, 2004; Song *et al.*, 2006; Sun *et al.*, 2007). Osmotic stress induces the accumulation of ROS and causes oxidative damage to the plant (Beligni and Lamattina, 1999). Results of this experiment also revealed that PEG treatment induced oxidative stress in rice callus as indicated by the increased accumulation of H_2O_2 and MAD. The increased H_2O_2 concentration in the callus growing under drought stress probably resulted from a drought-induced increase in the rate of O_2^{-} production, which is considered to be the main precursor of mitochondrial H_2O_2 (Møller, 2001). Zottini *et al.* (2002) reported previously that NO can affect plant mitochondrial functionality. This experiment showed that oxidative stress of rice induced by drought was effectively alleviated by the application of SNP as a NO donor.

3.2. Responses of antioxidant enzymes to PEG and SNP treatments

To establish whether the decrease in H_2O_2 amount and lipid peroxidation in PEG+SNP treatments was due to an activation of cellular antioxidant enzymes, the activities of key antioxidant enzymes were tested. As shown in Table (2) the activity of the antioxidant enzymes increased under the drought stress condition and the increase in the activity was dose-dependent. The results indicated that the growing of callus under drought condition (20% PEG) led to an increase in CAT, GR, APX and SOD activity to about 3, 2.5, 2, 1.5 folds compared to control, respectively. The addition of sodium nitroprusside (SNP) as nitric oxide donor to a final concentration of 100 μM in the culture media significantly enhanced the activity to 3.7, 3.1, 3.4, and 1.77 fold, respectively. These activity changes are strong hints that the drought treatment actually led to oxidative stress. The treatment by low concentration of NO improved the activity of antioxidant enzymes including SOD, APX, GR and CAT.

As key elements in the defense mechanisms, changes in ROS-scavenging enzymes activity in plants have been observed under different environmental stresses. For example, it has been reported that ROS-scavenging enzymes increased under saline conditions in the shoot cultures of rice (Fadzilla, *et al.*, 1997), wheat (Meneguzzo, *et al.*, 1999), under iron deficiency in maize (Sun, *et al.*, 2007), and under heat stress in the callus of reed (Song *et al.*, 2006). In the present experiment, activities of SOD, CAT, APX and GR in rice callus were greatly induced under the drought stress and the addition of sodium nitroprusside (SNP) as nitric oxide donor significantly enhanced the activity of these ROS-scavenging enzymes. These results indicated that SNP can play an important role in protecting the plants from the oxidative damage caused by drought stress by inducing the antioxidant enzymes activities. Our results are in agreement with Sun *et al.* (2007), who suggested that NO

Table (2): Effects of PEG and SNP treatments on the activities of superoxide dismutase (SOD), Ascorbate Peroxidase (APX), glutathione reductase (GR) and catalase (CAT) activity in rice callus.

Treatments	SOD activity (Unit/mg protein)	APX activity (Unit/mg protein)	GR activity (μmol/mg protein/min)	CAT activity (n mol/mg protein/min)
Negative control	170.2 ± 10.65^{e}	12.61 ± 1.01^{f}	2.88 ± 0.18^{i}	19.26 ± 1.18^{f}
PEG (5%)	173.4 ± 12.43^{e}	13.65 ± 1.34^{f}	4.28 ± 0.24^{h}	21.35 ± 1.67^{f}
PEG (10%)	183.7 ± 14.54^{de}	20.12 ± 1.87^{e}	5.64 ± 0.47^{g}	27.84 ± 1.99^{ef}
PEG (15%)	211.3 ± 17.83^{cd}	22.95 ± 2.01^{d}	5.98 ± 0.39^{f}	41.37 ± 2.37^{d}
PEG (20%)	256.5 ± 18.65^{b}	25.65 ± 2.33^{c}	6.96 ± 0.52^{d}	58.97 ± 3.23^{b}
PEG (5%) +SNP	190.5 ± 15.38^{de}	18.54 ± 1.46^{e}	6.43 ± 0.52^{e}	24.95 ± 1.96^{f}
PEG(10%)+SNP	210.5 ± 17.67^{cd}	25.43 ± 2.91^{c}	7.94 ± 0.49^{c}	33.96 ± 2.37^{e}
PEG(15%)+SNP	230.9 ± 18.56^{c}	31.37 ± 2.74^{b}	8.43 ± 0.67^{b}	50.59 ± 3.87^{c}
PEG(20%)+SNP	300.5 ± 22.69^{a}	42.56 ± 3.02^{a}	9.03 ± 0.49^{a}	71.29 ± 4.07^{a}
LSD	24.232	1.651	0.225	6.66

Means in the same column with the same letter are not significantly at $P< 0.05$.

can protect maize plants from iron deficiency-induced oxidative stress by reacting with ROS directly or by changing activities of ROS-scavenging enzymes. Also, Song *et al.* (2006) suggested that NO can effectively protect calluses of two ecotypes of reed from oxidative stress induced by heat and that NO might act as a signal in activating active oxygen scavenging enzymes under heat stress and thus confer reed callus thermotolerance. Moreover, Laspina, *et al.*, (2005) suggest that the exogenous application of NO could be advantageous against Cd-toxicity, and could confer tolerance to heavy metal stress in plants. Recently, Zhao *et al.* (2007) suggested that NO can effectively protect against oxidative damage and confer an increased tolerance to osmotic stress in reed suspension culture.

3.3. Glutathione and Ascorbate contents

Since ROS are generated in plants under drought stress leading to oxidative damage, the most abundant soluble non-enzymatic antioxidants, glutathione and ascorbate, were analyzed in order to establish the antioxidant soluble status of the rice callus, after 4 weeks of drought stress. Glutathione content increased under drought stress (Table 3). It reached more than 2.3 folds in respect to the control in callus growing under drought stress (20% PEG). The treatment by low concentration of SNP led to an increase of Glutathione content (3.4 folds in respect to the control). As shown in Table (3), similar to glutathione change, drought stress significantly increased the ascorbate concentration in rice callus compared to control, and the SNP treatment increased the ascorbate contents under drought stress. These results indicate the protective role of NO against the oxidative stress. Our results were in agreement with Sircelj *et al.* (2005), who found that moderate drought stress increased the concentrations of ascorbic acid, total GSH, ß-carotene, and ∝-tocopherol, indicating the adaptation to oxidative stress in apple trees. Manivannan *et al.* (2007) demonstrated that drought stress and the treatments with propiconazole (PCZ) as stress protector increased ascorbic acid (AA) and other antioxidant contents as well as the activities of antioxidant enzymes.

3.4. Total carbohydrates and amino acids

Drought is also known to affect the metabolism of soluble carbohydrates, a group of compounds that may act as compatible solutes as well as antioxidants. These compounds usually increase as a result of water deficit (Hanson *et al.*, 1982; Sircelj *et al.*, 2005; Smirnoff, 1993). Another group of compounds, which may be affected by water deficit are free amino acids. Proline and total free amino acids are often increased in water-stressed leaves (Pinheiro *et al.*, 2004; van Heerden and Krüger, 2002). The adaptive significance of amino acid accumulation during stress is still uncertain, but it appears that their major role is most likely in osmotic adjustment (Hanson *et al.*, 1982). Proline may also act as antioxidant (Ramachandra Reddy *et al.*, 2004).

As shown in Table (3) drought stress and SNP treatments increased the soluble sugars and amino acids. Total soluble sugar contents increased rapidly and reached its maximum (2 folds) at high treatments of PEG (20%) without or with the

Table (3): Effects of PEG and SNP treatments on the contents of reduced glutathione, Total ascorbic acid, total soluble carbohydrates and total amino acids in rice callus.

Treatments	GSH (µmol/g FW)	Total ascorbate (mg/gFW)	Total soluble sugars (mg/g FW)	Total amino acids (mg/g FW)
Negative control	3.67 ± 0.276[e]	7.61 ± 0.48[f]	12.76 ± 0.96[d]	2.01 ± 0.19[h]
PEG (5%)	5.87 ± 0.36[d]	11.43 ± 0.97[e]	13.87 ± 0.89[d]	3.03 ± 0.16[g]
PEG (10%)	6.19 ± 0.43[d]	13.21 ± 0.99[de]	17.64 ± 1.23[c]	3.54 ± 0.28[e]
PEG (15%)	7.98 ± 0.65[c]	14.34 ± 1.18[cd]	21.44 ± 1.74[ab]	4.39 ± 0.26[d]
PEG (20%)	8.54 ± 0.75c	15.98 ± 1.38[bc]	23.44 ± 1.78[a]	4.76 ± 0.29[c]
PEG (5%) + SNP	7.24 ± 0.39[cd]	12.25 ± 0.98[e]	16.54 ± 1.58[c]	3.21 ± 0.23[f]
PEG (10%) + SNP	8.32 ± 0.59[c]	14.57 ± 1.38[cd]	20.12 ± 1.79[b]	4.25 ± 0.32[d]
PEG (15%) + SNP	9.87 ± 0.78[b]	16.84 ± 1.45[b]	22.12 ± 1.59[ab]	4.98 ± 0.31[b]
PEG (20%) + SNP	12.64 ± 0.99[a]	21.95 ± 1.98[a]	23.54 ± 1.49[a]	5.64 ± 0.39[a]
LSD	1.184	1.618	1.715	0.173

Means in the same column with the same letter are not significantly at $P < 0.05$.

addition of SNP. Also, as in the case of total carbohydrates, drought stress increased total amino acid content 2.1 and 2.8 folds when 20% PEG was used without the addition of SNP and in the presence of SPN, respectively (Table 3). The present results indicated that the total amino acids and soluble sugars are significant contributors to metabolism under stress as mentioned before by (Harding *et al.*, 2003). It has been suggested that under water stress soluble sugars and amino acids function as osmolytes to maintain and stabilize cell proteins and structures during drought stress (Bartels and Sunkar, 2005; Bohnert, *et al.*, 1995). Several genes that are involved in the metabolism of these osmolytes have been found to increase the tolerance of abiotic stress in plants (Bartels and Sunkar, 2005).

3.5.Total phenolics and PAL activity

Among the approximately 50,000"secondary" plant metabolites, phenolics represent the largest group. Phenolic compounds play an important role in plant resistance and defense against microbial infections which are intimately connected with ROS. Phenolics are derived mainly from L-phenylalanine *via* nitrogen-free skeletons of *t*-cinnamate. This initial step of phenylpropanoid synthesis is mediated by phenylalanine ammonia-lyase. Under stress conditions, plant phenolic content and PAL activity may increase (Grassmann *et al.*, 2002).

Effect of NO treatment on the activity of phenylalanine ammonia lyase (PAL) and total phenols in drought-stressed rice callus are represented in Table (4). Compared to the control, drought stress significantly increased PAL activity and phenolic contents in rice callus. The PAL activity and phenolic contents tended to increase with the intensity of the drought stress, resulting in significantly higher value under 20% PEG treatment. The phenolic contents increased 2.5, 4.5, and 5.3 folds when 5%, 15% and 20% PEG were used. Addition of sodium nitroprusside (SNP) in the culture media increased the accumulation of phenolics in rice callus under drought stress to reach 6.3 folds under 20% PEG treatment Table (4). The increase of the phenolics was correlated with the increase in PAL activity. PAL activity increased to 6.5 and 11 folds under 20% PEG treatment without and with the addition of SNP, respectively. Studies with several different species of plants have shown that PAL is activated by many environmental factors (Jozef *et al.*, 2007; Mikal, 2000). These results illustrate the important role of PAL induction for the synthesis of phenolics, which have been reported as important antioxidant (Grassmann *et al.*, 2002; Laughton *et al.*, 1989; Stadler *et al.*, 1995).

Table (4): Effects of PEG and SNP treatments on phenylalanine ammonia-lyase (PAL) and total phenol content in rice callus.

Treatments	PAL activity (Unit/mg protein)	Total phenols (mg/g FW)
Negative control	6.78 ± 0.37^{h}	1.06 ± 0.09^{f}
PEG (5%)	12.54± 1.07^{g}	2.62 ± 0.12^{e}
PEG (10%)	19.63 ± 1.15^{ef}	3.86 ± 0.16^{d}
PEG (15%)	23.63 ± 1.34^{e}	4.76 ± 0.31^{c}
PEG (20%)	43.87 ± 2.67^{c}	5.57 ± 0.49^{b}
PEG (5%) +SNP	17.54 ± 1.27^{f}	3.87 ± 0.20^{d}
PEG (10%) +SNP	32.58 ± 2.02^{d}	5.32 ± 0.37^{b}
PEG (15%) +SNP	51.64 ± 3.62^{b}	5.58 ± 0.41^{b}
PEG (20%) +SNP	74.95 ± 4.03^{a}	6.65 ± 0.59^{a}
LSD	4.752	0.357

Means in the same column with the same letter are not significantly at $P < 0.05$.

In conclusion, drought stress could cause oxidative damage to plant cells through excessive generation of ROS and proper concentrations of exogenous NO could improve the dehydration tolerance through enhancing the enzymatic and non-enzymatic antioxidant systems. Our results provide some evidences to the important functions of NO in plant kingdom, which need further research.

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أكسيد النيتريك يحمي كالس الأرز من أضرار الجفاف

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الملخص

يعتبر الأرز من المحاصيل الغذائية الرئيسية في أسيا و شمال أفريقيا و لكن ظروف الإجهاد المختلفة مثل الجفاف تتسبب في فقد كمية كبيرة منه. فالجفاف واحد من أهم الأسباب الرئيسية في نقص إنتاج محصول الأرز بالرقعة الزراعية في العالم. وفي هذا البحث الذي تم في كلية الزراعة – جامعة القاهرة في شهر مايو ٢٠٠٥، تمت دراسة تأثير الحماية ضد الجفاف لنيتروبروسيد الصوديوم (كبادىء لأكسيد النيتريك) علي خلايا كالس الأرز النامية تحت ظروف الجفاف بإستخدام تقنية زراعة الأنسجة. تم إحداث الجفاف بإستخدام تركيزات مختلفة من مبلمر الإيثلين جليكول (٥، ١٠، ١٥، ٢٠%). أظهرت النتائج أن الجفاف يؤدي إلي حدوث ضرر تأكسدي بدليل زيادة تركيز فوق أكسيد الهيدروجين إلي حوالي خمسة أضعاف مقارنة بالكنترول، وكذلك محتوي المالونالدهيد الدال علي حدوث أكسدة لللييبدات. وقد أوضحت النتائج أيضاً أن الجفاف يؤدي إلي زيادة محتوي الكالس من الأحماض الأمينية الكلية، الكربوهيدرات الذائبة ، حمض الأسكوربيك، الفينولات الكلية ، الجلوتاثيون المختزل، وكذلك أدي إلي زيادة نشاط انزيم الفينيل الانين امونيا لاييز ، ونشاط بعض الانزيمات المضادة للأكسدة (الأسكوربات بيروكسيديز ، السوبر أكسيد ديسميوتيز ، الجلوتاثيون ريدكتيز ، الكتاليز) وذلك بعد أربعة أسابيع من المعاملة. أوضحت التجارب أن إضافة ١٠٠ ميكرومولر نيتروبروسيد الصوديوم إلي البيئة قللت التأثير التأكسدي الضار للجفاف وذلك بتقليل تركيزكلا من فوق أكسيد الهيدروجين والمالونالدهيد، وأدت أيضاً إلي إحداث زيادة معنوية في محتوي الخلايا من المواد التي تحمي من الضغط الإسموزي والمواد المضادة للأكسدة غير الإنزيمية مثل: الأحماض الأمينية الكلية، الكربوهيدرات الذائبة ، حمض الأسكوربيك، الفينولات الكلية ، الجلوتاثيون المختزل. وكذلك أدت إلي إحداث زيادة معنوية في نشاط انزيم الفينيل الانين امونيا لاييز ، ونشاط بعض الأنزيمات المضادة للأكسدة مثل: الأسكوربات بيروكسيديز ، السوبر أكسيد ديسميوتيز ، الجلوتاثيون ريدكتيز والكتاليز والتى تمثل خطوط الدفاع التى تواجه الضرر التأكسدى الحادث تحت ظروف الجفاف. توضح هذة النتائج أهمية المعاملة بتركيزات قليلة من مصدر لأكسيد النيتريك لحماية النباتات من أضرار الجفاف وجعل النباتات أكثر مقاومة لظروف الإجهاد البيئي.

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