EDELWEISS PUBLICATIONS
OPEN ACCESS

Volume 2 Issue 1 | PDF 118 | Pages 5

Edelweiss Applied Science and Technology

Research Article ISSN: 2576-8484

Antioxidative Potential of Garlic on Lead-Induced Oxidative Stress and Effect on Enzyme Activity in Rice Plants

Tugbobo Oladimeji S[1*], Idowu Kayode S[1], Ajao Oluwaseyi I[2]

Affiliation

[1]Department of Science Technology, Federal Polytechnic, Ado-Ekiti, Nigeria

[2]Department of Mathematics and Statistics, Federal Polytechnic, Ado-Ekiti, Nigeria

***Corresponding author:** Tugbobo Oladimeji S, Associate Professor of Biochemistry, Toxicology and Plant Biochemistry Unit, Department of Science Technology, Federal Polytechnic, Ado-Ekiti, Nigeria, Tel: +2348035303701, Email: tugbobooladimeji1@gmail.com

Citation: Tugbobo OS, Idowu KS, Oluwaseyi AI. Antioxidative Potential of Garlic on Lead-Induced Oxidative Stress and Effect on Enzyme Activity in Rice Plants (2018) Edelweiss Appli Sci Tech 2: 79-83

Received: Dec 04, 2017

Accepted: Jan 25, 2018

Published: Jan 31, 2018

Abstract

Seedling of Ofada rice (*Oryza sativum L*) were raised in sand (swampy) and clay (upland) cultures under 500mg/kg lead acetate and 500mg/kg garlic aqueous extract for 40-days. The uptake and distribution pattern of lead with possible induction of oxidative stress and likely alteration in the inherent antioxidant defense systems of the rice plants were determined. The inhibitory potential of garlic against lead-induced oxidative stress in rice seedling was also assessed. From the results, rice seedlings grown for 10-40 days under 500mg/kg lead acetate showed significant ($P<0.05$) increase in level of lipid peroxides in roots, indicating enhanced lipid peroxidation compared to control. However, incubation of garlic extract with supernatants of rice root and shoot caused a significant ($P<0.05$) reduction in the accumulation of lipid peroxides in a concentration dependent manner. In addition, there was a marked increase in antioxidant enzymes activities in lead acetate treated seedlings, where the shoot maintained higher defensive enzyme activity than roots. The results suggest that lead-induced oxidative stress could be salvaged by garlic extract and antioxidant enzymes are biomarkers for lead-induced oxidative injury in rice plants.

Keywords: Rice, Lead acetate, Garlic, Antioxidant enzymes, Lipid peroxides.

Introduction

Lead occurs naturally in soil and water. Plants absorb soil lead through their roots and thus, all plants contain small amount of this metal [1]. The relationship between plants and soil lead varies with factors ranging from chemical forms of the element in soil, soil properties, climate, plant species, etc. [2]. Lead, a toxic heavy metal and pollutant of the environment that originates from various sources like mining, pottery, casting and soldering, metallurgy, aerosols and dust from smelters, ammunition and automobile-exhaust, gasoline, etc. [3]. However, increased lead levels in the soil environment inhibits seed germination, stunts seedling growth and threatens plant metabolic reactions for proper growth and development resulting in low yields (4). High levels of heavy metals in the soil normally result in oxidative damage to plants either directly or indirectly by triggering an increased level of reactive oxygen species generation that generally cause damage to the biological molecules such as proteins, membrane lipids, chloroplast pigments, enzymes, nucleic acids, etc.[5]. These free radicals include superoxide radical (O_2^{*-}) hydroxyl radical (OH^{*-}) and hydrogen peroxide (H_2O_2) that are produced as by products during membrane linked electron transport reaction and by associated metabolic pathways [6]. Oxidative stress occurs where there is imbalance between the production of reactive oxygen species and the biological system's ability to readily detoxify the reactive intermediates produced or failure to easily repair the damage [7]. Also several studies have reported that organosulphur and polyphenolic compounds in plants protect against oxidative stress [8]. To salvage the rice plants from lead-induced oxidative stress, this study was designed to assess the uptake and distribution of lead in root and shoot of rice plants, determine the inhibitory potential of garlic as well as possible alteration in the activity of some defensive enzymes of rice plants.

Materials and Methods

Collection of Soil Sample

Soil samples were collected from five states in South-West region of Nigeria. Two locations (Toga and Owode) in Ogun state were considered for the research due to their appreciable lead levels after a comprehensive soil test.

Preparation of Garlic Extract

500g of the powdered sample (garlic cloves) was extracted via maceration for 48hrs using the method of Aguawa and Mittal [9].

Experimental Design

In both locations (Toga and Owode areas), rice seeds were surface sterilized with 0.1% sodium hypochlorite solution for 10mins and then rinsed with distilled water. After 24hrs imbibitions of seeds in water, the seedlings were raised in clay (Owode area) and sand (Toga area)

cultures respectively in lead free plastic pots. The pots received respective treatment solutions and were carefully maintained under control for seedling growth in a Biological Oxygen Demand (B.O.D) and optimum relative humidity with 12hrs photoperiod. In each location, the pots were grouped into four groups A, B, C, and D. 'A' contained seedlings treated with distilled water only and served as positive control. 'B' seedlings were treated with 500mg/kg lead acetate only, and seedling in 'C' received 500mg/kg lead acetate with 500mg/kg garlic extract simultaneously while seedling in 'D' were treated with 500mg/kg garlic extract only.

Determination of Lead in Rice Seedlings

Fresh root and shoot samples were surface sterilized with 1M HCl and then with 1mM Na_2EDTA for the surface bound lead and then dried in oven at 70^0C for 5-days. Dried samples were ground to a fine powder in mortar and pestle and digested with concentrated H_2SO_4. Digested samples were dissolved in de-ionized distilled water and lead content was estimated using atomic absorption spectrophotometer (AAS).

Oxidative Stress Assay

The level of lipid peroxidation products was determined using the method of Heath and Packer [10]. Fresh root and shoot samples were ground in 0.25% thiobarbituric acid (TBA) in 10% TCA using mortar and pestle. The mixture was heated at 95^0C for 30min then cooled in an ice bath and centrifuged at 10 000 x g for 10min. the absorbance of the supernatant was read at 532nm while total of 0.25% TBA in 10% TCA served as blank. The concentration of lipid peroxides together with the oxidative-modified proteins of plants were quantified and expressed as total TBARS as nmol g^{-1} fresh weight using an extinction coefficient of $155mM^{-1}$ cm^{-1}.

Evaluation of Garlic Inhibitory Potential

The production and inhibition of lipid peroxides from rice roots and shoots was determined using method described by [11]. The roots and shoots were ground in cold saline (1/10 w/v) with 10 up-and-down strokes in mortar and pestle. The homogenate was centrifuged for 10min at 10 000xg to obtain the supernatant and also incubated with lead acetate and garlic extract at varied concentrations together with de-ionized water at total volume 300µl at 37^0C for 1hr. The color reaction was monitored by adding 200, 250 and 500µl each of 8.1% Sodium Dodecylsulphate (SDS), acetic acid at pH 3.4 and 0.6% TBA respectively. The solution was incubated at 97^0C for 1hr and absorbance was read at 532nm.

Superoxide dismutase Assay

The activity of Superoxide Dismutase (SOD) was determined according to method described by [12]. About 200 mg fresh tissue (root/shoot) were homogenized in 5ml of 100 mM K-phosphate buffer at pH 7.8 containing 0.1 mM EDTA. 0.1% (v/v) Triton X-100 and 2% (w/v) polyvinyl pyrrolidone (PVP). The extract was filtered and centrifuged at 22 000 x g for 10mins at 4 ^{0}C. The supernatant was dialyzed in cellophane membrane tubings against the cold extraction buffer for 4hrs with 3-4 changes of the buffer and later used for the assay. The assay mixture in a total volume of 3ml contained 50 mM sodium carbonate-bicarbonate buffer (pH 9.8), 0.1 mM EDTA, 0.6mM epinephrine and enzyme while epinephrine was added last. The adrenochrome formation after 5mins was recorded at 475nm in a UV-Vis spectrophotometer.

Catalase Assay

The activity of catalase was examined according to [13]. 200 mg Fresh tissue (roots/shoot) were homogenized in 5ml of 50 mM EDTA. 2% (w/v) PVP and 0.5% (v/v) Triton X -100. The homogenate was centrifuged at 22 000 x g for 10mins at 4^0C and after which the supernatant was used for the enzyme assay. The assay mixture in total volume of 1.5ml contained 1000µl of 100µl enzyme at 240 nm.

Glutathione reductase Assay

Glutathione reductase was assayed according to [14]. 200mg Fresh tissue (root/shoot) were homogenized using chilled mortar and pestle in 5ml of 50 mM Tris-HCl buffer (pH 7.6). The homogenate was centrifuged at 22 000 x g for 30mins at 4 ^{0}C and the supernatant was used for the enzyme. The reaction mixture in a total volume of 1ml contained 50 mM Tris-HCl buffer (pH 7.6), 0.15 mM NADPH, 1 mM GSSG, 3 mM $MgCl_2$ and 200µl enzyme extract. The activity of the enzyme was monitored with absorbance at 340nm.

Results

Rice Pots	lead levels in root (mg/kg)	lead levels shoot (mg/kg)
A	15.2	7.25
B	20.5	12.5
C	16.7	10.8
D	12.4	8.5

Table 1: Uptake of lead by growing rice seedlings (Sand) in Toga area for 40-days.

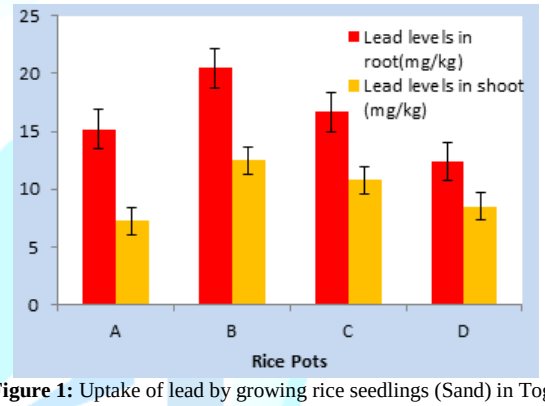

Figure 1: Uptake of lead by growing rice seedlings (Sand) in Toga area for 40-days.

Rice Pots	lead levels in root (mg/kg)	lead levels shoot (mg/kg)
A	6.82	5.12
B	15.5	8.65
C	11.59	6.88
D	7.85	5.52

Table 2: Uptake of lead by growing rice seedlings (clay) in Owode area for 40-days.

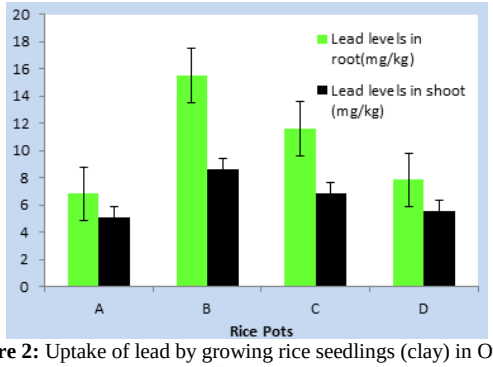

Figure 2: Uptake of lead by growing rice seedlings (clay) in Owode area for 40-days.

Age (days)	Treatment	Root (nmol/mg)	Shoot (nmol/mg)
10	Rice + distilled water	1.51±0.17	0.87±0.23
	Rice + 500mg/kg lead acetate	2.19±0.97	1.98±0.07
20	Rice + distilled water	1.58±0.12	0.73±0.43
	Rice + 500mg/kg lead acetate	6.20±0.41	2.94±0.56
30	Rice + distilled water	1.58±012	0.73±0.43
	Rice + 500mg/kg lead aceatate	10.15±0.44	5.85±0.73
40	Rice + distilled water	1.55±0.13	0.73±0.53
	Rice + 500mg/kg lead acetate	17.99±088	7.73±0.53

Table 3: Levels of total lipid peroxides in root and shoot of rice seedlings in Toga area.

Age (days)	Treatment	Root (nmol/mg)	Shoot (nmol/mg)
10	Rice + distilled water	0.10±0.08	0.02±0.01
	Rice + 500mg/kg lead acetate	1.98±0.07	1.06±0.19
20	Rice + distilled water	0.02±0.01	0.02±0.01
	Rice + 500mg/kg lead acetate	3.97±0.44	2.94±0.56
30	Rice + distilled water	0.06±0.04	0.05±0.09
	Rice + 500mg/kg lead acetate	7.42±0.79	5.85±0.73
40	Rice + distilled water	0.04±0.02	0.10±0.08
	Rice + 500mg/kg lead acetate	10.74±1.17	7.42±0.79

Table 4: Levels of total lipid peroxides in root and shoot of rice seedlings in Owode area.

Extract (mg/ml)	Absorbance	% Inhibition	IC50 (mg/ml)
Basal	0.08	-	-
Control	0.4 8	-	-
10	0.33	30.9	1.2
20	0.32	32.4	1.34
40	0.21	55.9	3.41
80	0.16	66.6	4.36
160	0.14	70.4	4.69

Table 5: Inhibitory potential of garlic aqueous extract on lead acetate induced oxidative stress in root of rice plant.

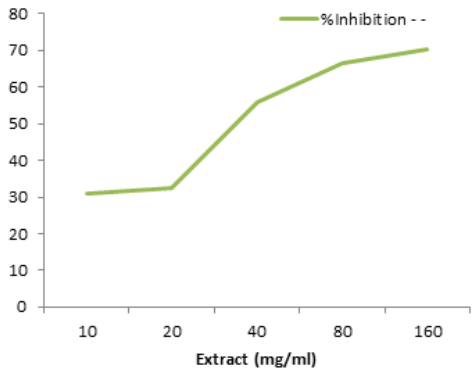

Figure 3: Inhibitory potential of garlic aqueous extract on lead acetate induced oxidative stress in root of rice plant.

Extract (mg/kg)	Absorbance	% Inhibition	IC50 (mg/kg)
Basal	0.09	-	-
Control	0.48	-	-
10	0.24	49.3	0.55
20	0.13	72.6	3.49
40	0.12	74.72	3.86
80	0.11	75.65	4.01
160	0.1	76.6	4.19

Table 6: Inhibitory potential of garlic aqueous extract on lead acetate induced oxidative stress in shoot of rice plant.

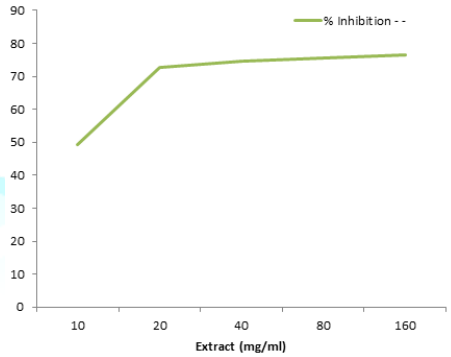

Figure 4: Inhibitory potential of garlic aqueous extract on lead acetate induced oxidative stress in shoot of rice plant.

Rice Pot	SOD Activity in Root (nmol/mg)	SOD Activity in Shoot (nmol/mg)
A	2.19±0.97	2.94±0.56
B	17.99±0.88	20.57±0.57
C	26.51±0.48	39.91±0.68
D	10.74±1.17	15.95±0.96

Table 7: Effect of lead uptake on superoxide dismutase activity in rice seedlings.

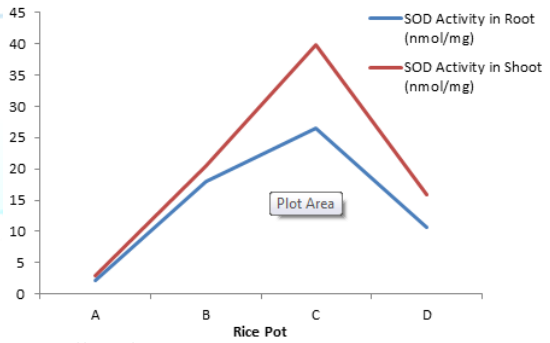

Figure 5: Effect of lead uptake on superoxide dismutase activity in rice seedlings.

Rice Pot	CAT Activity in Root (nmol/mg)	CAT Activity in Shoot (nmol/mg)
A	0.21±0.03	0.28±0.02
B	0.15±0.04	0.22±0.01
C	1.56±0.06	1.61±0.30
D	1.69±0.47	0.71±0.19

Table 8: Effect of lead uptake on catalase activity in rice seedlings.

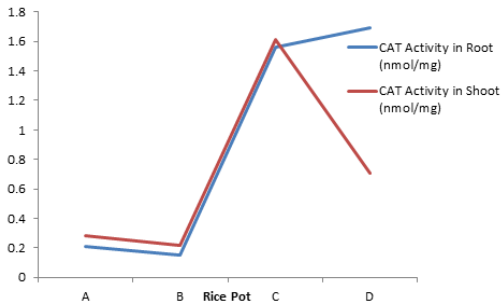

Figure 6: Effect of lead uptake on catalase activity in rice seedlings.

Rice Pot	GR Activity in Root (nmol/mg)	GR Activity in Shoot (nmol/mg)
A	0.67±0.05	1.16±0.02
B	1.56±0.06	1.21±0.03
C	0.91±0.12	0.82±0.10
D	1.07±0.10	1.11±0.11

Table 9: Effect of lead uptake on glutathione reductase activity in rice seedlings.

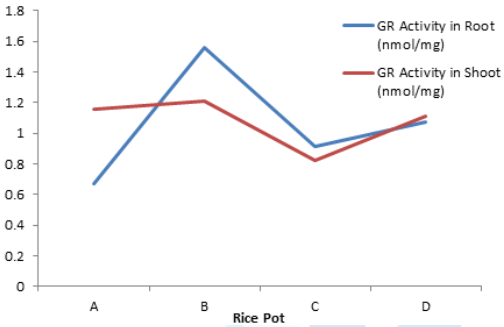

Figure 7: Effect of lead uptake on glutathione reductase activity in rice seedlings.

Discussion

Lead uptake highest level (20.50mg/kg) was observed in root treated with 500mg/kg lead acetate in pot B. lowest lead level was obtained in pot D in root treated with garlic extract only in Toga area for 40-days. However, highest lead level obtained in shoot (12.50mg/kg) in pot B treated with lead acetate only was much more reduced compared to root, while lowest lead level (8.50mg/kg) was obtained in D. This was reasonably possible as plant roots normally absorb more lead than shoot. In addition at Owode area, (15.50mg/kg) and (8.65) were the highest and lowest lead levels obtained in rice root and shoot respectively. These values were comparatively lower to the values of lead concentrations obtained for both root and shoot of rice plant in Toga area. This could be attributed to enhanced lead absorption aided by wet or flooded soils in Toga area. However, due to antioxidant property of garlic, lead absorption was restricted in pot D where remarkable lowest lead level was obtained. Besides, the production of lipid peroxides in both root and shoot of rice seedling showed increased lead concentration relative to seedling age or period of growth. Seedlings grown under 10-days had (1.5nmol/mg and 0.87nmol/mg) lowest concentration of lipid peroxides in rice root and shoot respectively. Those grown under 40-days had (17.99nmol/mg and 7.73nmol/mg) highest concentration of lipid peroxides in rice root and shoot respectively in Toga swampy area. However, same trend was observed in Owode area where highest lipid peroxidation levels (10.74nmol/mg and 7.42nmol/mg) in root and shoot respectively were obtained under 40-days while lowest lipid peroxidation levels (0.10nmol/mg and 0.02nmol/mg) in root and shoot were respectively obtained under 10-days.

In view of the above, peroxide levels in Toga area was higher than that obtained in Owode area and maximum concentration (17.99nmol/mg) was obtained in rice root. This is because root absorbs lead faster in wet or flooded area and tends to retain absorbed lead which it does not easily transport for prompt uptake by the plant shoot. This results to slow mobility of lead in rice plant where large accumulations are observed in root. The high lipid peroxide levels in root due toaccumulation could as well be attributed to the ability of Pb^{2+} which catalyzes one electron (e-) transfer reaction that generate Reactive Oxygen Species (ROS) such as hydroxyl radicals and hydrogen peroxides via Fenton reaction and thus, generate more lipid peroxides [15]. However, the protective effect demonstrated by garlic in this study could be due to presence of its inherent phenolic and organosulphur components that constitute and enhance its antioxidant activity.

The antioxidant activity has been reported to be concomitant with the development of reducing power [16] and this is due to garlic extract hydrogen donating ability [17]. In this study, garlic antioxidant activity was demonstrated against the reactive oxygen species generated and thus, inhibits lipid peroxidation due to its scavenging potential. Besides, plants generally possess inherent antioxidant defense system used naturally to combat the oxidative damage. In view of this, table 7 shows effect of lead uptake on Superoxide-Dimutase (SOD) activity in rice seedling where a significant ($P<0.05$) increase in SOD activity (26.51 and 17.99) was observed in roots of rice seedlings of pots C and B treated with lead acetate.

Meanwhile similar effect was observed in shoots of rice seedlings of pots C and D treated with lead acetate where significant ($P<0.05$) increase (39.91 and 20.57) in SOD activity respectively. However, SOD activity was observed higher in shoot than root because root absorbs more lead concentrations than shoot. Hence, the excessive lead has the propensity of reducing the defensive potential of SOD in plant root. SOD activity has been reported to increase under water stress [18], heavy metal toxicity [19]. This increase in response to stress could be due to de novo synthesis of the enzyme [20]. The catalase (CAT) activity observed for lead treated rice seedlings in pot B was significantly ($P<0.05$) lower than control for both root (0.15) and shoot (0.22) respectively. However, in pots C and D treated with the sample extract, CAT activity was appreciably higher in the plant tissues when compared to control.

The decline in CAT activity in pot B could be attributed to lead toxicity which could possibly delay the removal of hydrogen peroxide and peroxides mediated by catalase which in turn enhances free radical mediated lipid peroxidation in plant tissues [21]. Besides, a decline in catalase activity has been attributed to the inactivation of enzyme protein due to deleterious activity of reactive oxygen species which either decrease the enzyme synthesis or cause alteration in assembly of enzyme subunits [22]. On the contrary, Glutathione Reductase (GR) activity was higher in both root (1.56) and shoot (1.21) of rice seedlings treated with lead acetate in pot B compared with control. Similarly, GR activity was significantly ($P<0.05$) higher in pot D treated with extract sample than tissues of rice seedlings in pot C. This could be attributed to antioxidant potential of the extract (garlic) which also compliments the GR antioxidant activity [23]. In addition, the increased GR activity suggests possible involvement of GR in regenerating GSH from GSSG under lead toxicity in order to increase GSH/GSSG ratio and thus, increasing total glutathione pool [24]. The study above clearly suggests that lead toxicity induces oxidative stress in rice plants which could be modulated by garlic antioxidant effect, while antioxidant enzymes play a pivotal role in combating oxidative stress in the plants.

Acknowledgement

The authors appreciated the financial aid and facilities provided by tertiary education trust fund (TETFUND) via Federal Government of Nigeria.

References

1. Erik MI, Peak JD, Brady PV, Pesek JD. Kinetics of lead absorption and deposition in soil (1999) Soil Sci 64: 28-39.
2. Godbold DI, Kettner C. Lead influence on root growth and mineral nutrition of Picea abies seedlinds (1991) Journal of Plant Physiol 139: 95-99.
3. Mayer DT, Brown K. Roles of peroxidase in development of water impermeable seeds (2008) Journal of Phys. Biochem 193: 265-275.
4. Kasfori R, Petrovic M. Effect of excess lead, cadmium copper and zinc on water relation in sunflower (1992) Journal of Plant Nutri 15: 2427-2439.
5. Malecka A, Jarmuseklewiez W, Tomaszewska B. Antioxidative defense against lead induced oxidative stress in some cellular components of pea root cells (2001) Acta Biochem 48: 687-698.
6. Becana M, Dalton DA, Moran JF, Matamoros MA. Reactive oxygen species and antioxidants in legume nodules (2000) Plant Physiol 109: 372-381.
7. Halliwell B. Free radicals and antioxidant defense system (1994) J Lab Clin Med 119: 598-620.
8. Nwanjo HU. Free radical scavenging potential of the aqueous extract of Viscum album (mistletoe) leaves (2007) International J of Nutrition and wellness 12: 121-127.
9. Aguawa CN, Mittal GC. Study of the antioxidative potentials of Pyrynacanthia staudtii using various models of experiments (1981) Envr J of Pharmacol 40: 215-220.
10. Heath RI, Packer L. Photoperoxidation in isolated chloroplast; kinetics and stochiometry of fatty acid peroxidation (1968) Arch Biochem Biophys 125: 189-198.
11. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides via thiobarbituric acid reactions (1979) Analytical Biochem 95: 351-358.
12. Misra HP, Fridovich I. The role of superoxide anion in auto-oxidation of epinephrine and simple assay for superoxide dismutase (1972) J Biol Chem 247: 3170-3175.
13. Beers RF, Sizer IW. Colorimetric method for estimation of catalase (1982) J Biol Chem 195: 133-139.
14. Schaedle M, Bassham JA. Chloroplast glutathione-reductase (1997) Plant Physiol 59: 1011-1012.
15. Zago MP, Verteraesten SV, Oteiza PI. Role of zinc in the prevention of Fe2+ initiated lipid and protein oxidation (2000) Biological Research 33: 143-150.
16. Tanaka T, Kato T, Nishioka Y, Kimura J. Lead burden and oxidative stress effect (1985) Biol Chem 263: 11646-11651.
17. Baisak R, Rana DA, Kar M, Acharya PBB. Alteration in the activities of active oxygen scavenging enzymes of wheat leaves subjected to water stress (1994) Plant Cell Physiol 35: 489-495.
18. Zhang J, Kirkham MB. Drought stress induced changes in the activities of superoxide dismutase, catalase and peroxidase in wheat species (1994) Plant Cell Physiol 35: 785-791.
19. Shah K, Kumar RG, Verma S, Dubey RS. Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings (2001) Plant Sci 161: 1135-1144.
20. Lozano R, Azoon R, Palm JM. Superoxide dismutase and drought stress in lactna sativa (1996) New Phytol 136: 329-333.
21. Lin CC, Kao CH. Effect of sodium chloride stress on hydrogen peroxide metabolism in rice leaves (2000) Plant Growth Regul 30: 151-155.
22. Mac Rae EA, Ferguson IB. Changes in catalase activity and hydrogen peroxide concentration in plants in response to low temperature (1985) Plant Physiol 65: 51-56.
23. Scandalios JG. Response of plant antioxidant defense genes to environmental stress. (1990) Advance Genet 28: 11-41.
24. Noctor G, Foyer CH. Ascorbate and glutathione; keeping active oxygen under control (1998) Annual Reviews of Plant Mol Biol 499: 249-279.