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Assessment of Antioxidant Defense System in Transplanted Rice Varieties (in Catalase and Nitrate Reductase)

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Abstract: ***Water loss in rice (Oryza sativa L.) can induce production of highly reactive molecules such as hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH), singlet oxygen which all are named reactive oxygen species (ROS). ROS are regarded as main source of damage to cell of abiotic stress, which particularly synthesized in Chloroplast and Mitochondria salt tolerant varieties (Usar Dhan-3 and CSR-23 and salt susceptible varieties IR-42 and CSR-28) showed good result in stress condition increased or decreased under sodic condition in all salt tolerant varieties.***

Keywords: Antioxidants, *Oryza sativa* L. , reactive oxygen species (ROS)

1. Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop that provides 43% of calories requirement for more than 70% India as well as world population that is why the rice production always holds a key in the overall food situation of the whole world . In India, rice production was 217.28 mt from 44% mha cultivated area (Anonymous, 2008). In Uttar Pradesh, rice occupies an area of about 5-69 mha with production and productivity of 11.73 mt tones and 20.62 q/ha resp. (Directorate of statistics, U.P. 2008).

The production always holds a key role in the over all food situation of the world, because it is the most important cereal crop that provides 43% of calories requirement for more than 70% India as well as world population. Water scarcity resulting from global climate change is accompanied by more frequent and more severe summer drought in many regions (Seneviratne fail., 2006; Kolves and Alexandra, 2008; Jaeger and Seneviratne, 2011). This cause drought stress in plants and limits crop yield world wide (Handy *et al.*, 2003) The agricultural practice for drought tolerant crops turns into tropical demand (Cazanes *et al.*, 2010). Water loss can induce production of highly reactive molecules such as hydrogen peroxide, superoxide anion and hydroxyl radicals, singlet oxygen which all are named reactive oxygen species (ROS). Plant response to drought is after acompained by oxidative damage (chaves *et al.*, 2003; Noctor *et al.,* 2002; Reddy *et al*., 2004; Slesak *et al.,* 2007 and Foyer and Noctar, 2009). Antioxidant protection in plant cells is a complex and highly compartmentized phenomenon and includes both enzymatic and non-enzymatic components (Mittler, 2002).

ROS detoxifying enzymes are induced during different kinds of biotic and abiotic stresses for maintenance of normal growth. The key role of antioxidant enzymes is to reduce or scavenge ROS which are normally produced in different cell organelles and the cytosol. Their activities increase considerably under stress conditions Catalase and Nitrate reductase are the important ROS scavenging enzymes. They participate in removal of superoxide radical and hydrogen peroxide (H_2O_2), produced directly or indirectly by Mehler reaction on and photorespiration in plants, preventing the formation of the highly toxic hydroxyl radical via Haber-weiss or Fenton reactions (Mittler, 2002). Hydrogen peroxide was considered also as a signaling molecule involved in plant response to wide range of biotic and abiotic stresses. (Laloi *et al*., 2004). It was demonstrated that elevated levels of reactive oxygen species, such as hydroxyl radicals, under drought are capable to induced oxidative stress, causing lipid peroxidation and consequently membrane injury (Mittler, 2002). Plants with high levels of constitutive or induced antioxidants have been reported to have higher resistance to oxidative damage (Chaparzadeh *et al*., 2004; Feroj Lopez *et al.,* 2009, 2010). The basic objective of the present work was, therefore, to investigate in details that how the biochemical enzymatic activities such as catalase, peroxidase and nitrate reductase are associated with sodicity tolerance in transplanted rice from transplanting to mature stage.

2. Materials and Methods

The present experiments were conducted for two *Kharif* seasons during 2010-2011 and 2011-2012. The investigations were carried out in either pot culture with four varieties/genotypes of rice (*Oryza sativa* L.) salt tolerant (Usar Dhan-3 and CSR-23) and salt sensitive IR-42 and CSR-28) at the experimental site of the Department of Botany, M.L.K. P.G. College, Balrampur in collaboration with Narendra Dev Uni. of agri. & Tech, crop physiology Department, Kumarganj Faizabad under the supervision of Dr. S.P. Singh (Assis. Prof.). The experiments were laid out at four stages and two types of soil all in three replicates. The catalase activity was assayed with colorimeter according to the method described in Analytical Biochemistry (Sinha, 1972).

200 mg. of fresh leaves were homogenized in 10ml of 0.1 M phosphate buffer (P^H 7.0) and centrifuged at 10,000 rpm

for 30 minutes at 4^0c. The enzyme extract was stored at low temperature until assay.

The activity of enzyme was assayed by taking 1.25 ml H_20_2, 0.5 ml enzyme extract, 3.25 ml 0.1 M phosphate buffer (P^H7.0) . The reaction mixture was taken in Erlenmeyer flask and mixed rapidly at 37^0C. at 3 minutes interval, 2.0 ml of reaction mixture was withdrawn and poured into 4 ml potassium dichromate acetic acid solution and kept in boiling water bath for 10 min. The mixture was cooled and the colour intensity was measured at 570 nm on spectrophotometer against a reagent blank prepared similarly expect for the substitution of the enzyme extract with phosphate buffer.

Nitrate reductase (NR) assayed based on Methods described by Jaworski (1971). 250 Mg of sample was suspended in screen cap vials having 4.5 ml medium containing 0.1 M phosphate buffer (P^H 7.5) 0.02 M. KNO_3.5% propanol. A control was kept on omitting plant samples the vials were capped and kept in tank at 30^0C for desired incubation period, Nitrate released into the medium was determined by treating 0.4 ml aliquot with 0.3 ml each of sulphanilamide and N-1 napthyl- ethylene diamine hydrochloride after 20 minutes the solution was diluted with distilled water to make the volume upto 5 ml and the absorbance was measured at 540 nm using reagent blank standard curve was prepared using graded concentration of $NaN0_2$ solution upto 100 ppm and the amount of $N0_2$ produced by the activity of nitrate reductase in the assay medium was calculated.

RESULTS AND DISCUSSION

In this study (Table-1) shows catalase activity increased under sodic soil in its tolerant varieties, i-e Usar Dhan-3. CSR-23 and in sensitive rice varieties IR-42 and CSR-28 corresponding to the days of 15, 45, 75 and maturity while Usar Dhan-3 shows highest catalase response and CSR-28 show minimum response. Salt tolerance has usually been assessed as the percentage of biomass production in saline versus control conditions over a prolonged period of time Nitrate reductase activity (Table-2) decreased at the age of corresponding to stages at 45 DAT, 75 DAT and at lowest at maturity stages.

In Nitrate reductase, highest enzyme content showed at 40 ESP. Plants possesses a number of antioxidant enzymes like NR and Catalase (CAT) to protect against the damaging effect of reactive oxygen species (ROS) (Asada, 1992 and Pal *et al*., 2004). Antioxidant enzymes play a significant role in rice plants is protect them against the damaging effect of Reactive oxygen species (ROS) generated during salinity stress (Asada, 1992)

Table 1: Catalase

Varieties	15 DAYS					45 DAYS					75 DAYS					AT MATURITY				
	S0	S1	S2	S3	Mean	S0	S1	S2	S3	Mean	S0	S1	S2	S3	Mean	S0	S1	S2	S3	Mean
Usar Dhan-3	330.34	337.22	347.54	361.31	344.10	420.12	428.87	442.00	459.51	437.63	475.49	485.39	500.25	520.07	495.30	365.18	372.79	384.20	399.42	380.40
CSR-23	3233.86	330.60	340.72	354.22	337.35	415.44	424.10	437.08	454.39	432.75	461.57	471.18	485.61	504.84	480.80	347.14	354.37	365.22	379.68	361.60
IR-42	285.10	297.23	309.37	324.53	304.06	357.11	372.30	387.50	406.49	380.85	386.34	402.78	419.22	439.77	412.03	295.02	307.57	320.13	335.82	314.63
CSR-28	281.06	293.02	304.98	319.93	299.75	351.61	366.57	381.53	400.23	374.99	379.95	396.12	412.28	432.49	405.21	288.03	300.28	312.54	327.862 307.187	
Mean	305.09	314.52	325.65	340.00	321.31	386.07	397.96	412.03	430.16	406.55	425.84	438.87	454.34	474.29	448.33	323.84	33.75	345.52	360.69	340.95
SEm+	V= 4.74, S=4.74, VXS= 9.49					V= 5.83, S= 5.83, VXS= 11.67					V= 6.52, S= 6.52, VXS= 13.03					V= 4.92, S= 4.92, VXS=9.83				
CD at 5%	V= 13.40, S= 13.40, VXS= NS					V= 16.48, S= 16.48, VXS= NS					V= 18.41, S= 18.41, VXS= NS					V= 13.89, S= 13.89, VXS= NS				

Table 2: Nitrate reductase

	15 DAT					45 DAT					75 DAT					AT MATURITY				
Varieties	S0	S1	S2	S3	Mean	S0	S1	S2	S3	Mean	S0	S1	S2	S3	Mean	S0	S1	S2	S3	Mean
USAR DHAN - 3	44.12	44.14	44.19	44.18	44.15	136.4	134.4	135.9	133.4	135	135.9	133.7	134.9	132.7	133.8	127.4	126.4	129.4	128.7	127.98
CSR-23	45.49	45.56	45.44	45.67	45.54	140.4	143.9	142.7	141.7	142.2	139.7	142.7	141.7	140.7	141.7	128.19	127.15	130.5	128.5	128.59
IR-42	42.19	42.43	42.61	42.44	42.41	130.4	128.4	132.4	129.4	130.2	129.1	127.1	131.4	128.4	129.0	11.14	115.12	109.12	114.12	112.13
CSR-28	43.14	42.13	43.19	43.45	42.97	132.92	130.4	133.9	130.4	131.9	130.4	130.1	132.7	130.4	131.1	115.1	119.07	110.11	112.12	115.44
Mean	43.735	43.565	43.857	43.935	43.767	135.03	134.275	136.225	133.725	134.825	133.775	133.4	135.175	133.05	133.9	95.462	121.935	121.035	120.86	121.035
SE m^+	V=0.35 , S=0.35 , VxS=0.71					V=0.8 , S=0.8 , VxS=1.61					V=0.75 , S=0.75 , VxS=1.49					V=0.84 , S=0.84 , VxS=1.68				
CD at 5%	V=0.99 , S=0.NS , VxS=NS					V=2.20 , S=NS , VxS=NS					V=2.12 , S=NS , VxS=NS					V=2.38 , S=2.38 , VxS=NS				

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