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Influence of Water Stress on Antioxidative Enzymes and Yield of Banana Cultivars and Hybrids

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Abstract: The main aim for this field experimental study is to screening of various banana cultivars and hybrids for water deficit tolerance through antioxidative enzymes and yield. The field experiment was conducted at National Research Centre for Banana to screen the banana cultivars and hybrids for water deficit tolerance and to elucidate information on antioxidative enzymes mechanism correlated with yield of banana cultivars and hybrids. The methodology of this experimental were analyzed by split plot design and has two treatments considered as main plot (M) viz., control plot taken as M₁ and water deficit plot taken as M₂ and also the cultivars and hybrids were considered as sup plots (S). The stress was imposed according to the Available Soil Moisture (ASM) and this ASM was measured by using pressure plate membrane apparatus. The experimental data were significantly varied between the treatments and also cultivars and hybrids. The antioxidative enzymes of catalase, super oxide dismutase and ascorbate peroxidase were significantly enhanced during water deficit conditions. Among the twelve cultivars and hybrids, Karpuravalli, Karpuravalli x Pisang Jajee, Saba and Sannachenkathali was identified as tolerant to water stress with highly accelerated by water stress treatment in the range of 23 to 32% over Control in Catalase (CAT), Super Oxide Dismutase (SOD) and Ascorbate Peroxidase (APX) leads to reduced the cellular membrane damaged by reactive oxygen species and get higher yield; whereas, Matti, Pisang Jajee x Matti, Matti x Anaikomban and Anaikomban x Pisang Jajee were notified as sensitive cultivars and hybrids with lesser increase in antioxidative enzyme activity of 15% than control which is leads to get very low yield.

Key words: Water deficit, banana, CAT, APX, SOD, yield

INTRODUCTION

The occurrence of unfavorable environmental factors such as moisture deficit/excess, high radiation, low and high temperature, salinity of water and soil, nutrient deficiency or toxicity and pollution of atmosphere, soil and water are likely to affect the crop growth in terms of morphology (plant size, architecture, malformation of plant organs, growth (height, volume, weight), physiological and metabolic processes and yield of crop plants (Munns, 2005). The limited water resources in the area and the cost of pumping irrigation water are the most important factors that force many farmers to reduce irrigation in many arid and semi-arid regions. Banana is an important tropical fruits. In production viz., Tamil Nadu rank first in banana. Among the all fruit crops banana is the fourth largest fruit crop in the world. It is one of the most important fruit crops that can tolerate short periods of water deficit (Turner, 1998). The various environmental stresses affects source and sink strengths by its effects on photosynthesis, growth and general metabolism. The

plants are exposed to water deficit situation leads to higher production of ROS (Reactive Oxygen Species) like singlet oxygen, superoxide radical, hydrogen peroxide, hydroxyl ion and free hydroxyl radical and it will damage the plant cell membranes leads to efflux of ions from the cytoplasm (Larson, 1988; Burke and Mahan, 1991). Some of the plants can tolerate during water deficit conditions through the detoxifying the ROS with higher synthesis of Antioxidative enzymes such as Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX) and Catalase (CAT) (Smimoff, 1998). The level of damages may be limited by enzymatic and nonenzymatic scavengers of free radicals (Foyer and Noctor, 1999). To screen the banana cultivars and hybrids to water deficit is a base idea for physiological responses to environmental stresses. Based on the above background, the field experiments were performed to determine the antioxidative enzymes responses of twelve banana cultivars and hybrids to water deficit and to evaluate the response of antioxidant enzyme contents, APX, CAT and SOD, associated to high yield potential under water deficit conditions.

MATERIALS AND METHODS

The experimental design was a split plot design with three replications. The main plots are, M₁ (control) with the soil pressure maintained from -0.69 to -6.00 bar, M₂ (water deficit) with the Soil pressure maintained from -0.69 to -14.00 bar. Soil pressure of -14.00 bar was reached at 30 days and measured by using soil moisture release curve and measured the soil moisture by using the pressure plate membrane apparatus. The sub plots considered as twelve banana cultivars and hybrids (S₁: Karpuravalli (ABB), S₂: Karpuravalli x Pisang Jajee, S₃: Saba (ABB), S₄: Sanna Chenkathali (AA), S₅: Poovan (AAB), S₆: Ney poovan (AB), S₇: Anaikomban (AA), S₈: Matti x Cultivar Rose, S₉: Matti (AA), S₁₀: Pisang Jajee x Matti, S₁₁: Matti x Anaikomban and S₁₂: Anaikomban x Pisang Jajee.) were randomly distributed within the sub-plots in each of the drought stress treatments (main plots). The antioxidative enzymes were estimated during 3rd, 5th, 7th, 9th month after planting and at harvest stages of the crop.

Enzyme extractions and assays: Catalase (CAT) activity was determined by monitoring the disappearance of H₂O₂, measuring a decrease in the absorbance at 240 nm (Aebi, 1984). The reaction was carried in a reaction mixture containing 1.0 mL of the 0.5 M (pH 7.2) phosphate buffer, 3 mM EDTA, 0.1 mL of the enzyme extract and 0.3% H₂O₂ and allowed to run for 3 min. The enzyme activity was calculated using the extinction coefficient 0.036 mM⁻¹ cm⁻¹. One enzyme unit (U) determines the amount of enzyme necessary to decompose 1 μmol of H₂O₂ per mg protein per min at 25°C and expressed as U mg⁻¹ protein.

SOD activity was assayed by measuring the inhibition of photo-reduction of nitroblue tetrazolium (NBT) at 560 nm using UV-Vis spectrophotometer. A unit of SOD is defined as that being present in the volume of extract that caused inhibition of the photoreduction of NBT by 50% and was expressed in enzyme units (mg⁻¹ protein).

Ascorbate Peroxidase (APX) activity of the leaf samples was estimated by the method proposed by Nakano and Asada, 1981 and expressed as units mg of protein⁻¹ min⁻¹. A fresh leaf sample of 0.5 g was macerated with 10 mL of sodium phosphate buffer (0.1 M pH 7.0) using a pestle and mortar. The extract was centrifuged at 10000 rpm at 4°C for 20 min. 0.2 mL of supernatant was taken in a test tube and 1.8 mL of 50 mM sodium phosphate buffer, 0.5 mL of 0.05 M ascorbic acid and 0.5 mL of 50 μmols of H₂O₂ was added. The reaction

was started by the addition of 0.5 mL of ascorbic acid (50 mM) at final concentration and its consumption was measured 30 sec interval for 2 min at 290 nm. The Ascorbate peroxidase (APX) activity was decreased.

Statistical analysis: The experimental research treatments fixed by split plot design with three replications. The data were analyzed by using AGRESS software.

RESULTS

Catalase: The CAT content was affected by water deficit and Hybrid as well as the interaction of M at S and S at M were significant (Table 1). Water deficit increased CAT content in banana cultivars and hybrids. Among the twelve cultivars and hybrids, Karpuravalli, Karpuravalli x Pisang Jajee, Saba and Sannachenkathali had significant differences in catalase content under the main plot treatments. The highest CAT content was observed in Karpuravalli (6.50) under the water deficit at 7th MAP. The lowest CAT content was observed in Matti, Pisang Jajee x Matti, Matti x Anaikomban and Anaikomban x Pisang Jajee cultivars and hybrids (2.94, 2.92, 2.88 and 2.39) under the water deficit at 7th MAP, respectively. There was a high and positive correlation (R² = 0.618) between CAT level and yield water deficit conditions (Fig. 1).

Table 1: Effect of water stress on catalase activity (μ mol H₂O₂ s⁻¹ mg⁻¹ of protein) at different growth stages of banana cultivars and hybrids

Treatments	3rd MAP	5th MAP	7th MAP	9th MAP	Harvest	Mean
Main plot						
M ₁	2.530	2.680	3.460	2.970	2.280	2.78
M ₂	3.450	3.540	4.380	3.830	3.100	3.66
Mean	2.990	3.110	3.920	3.400	2.690	3.22
SEd	0.050	0.051	0.055	0.054	0.049	
CD (p = 0.05)	0.219	0.223	0.239	0.232	0.210	
Sub plot						
S ₁	6.280	6.400	6.500	6.230	5.550	6.19
S ₂	5.290	5.410	6.110	5.750	5.050	5.52
S ₃	4.620	4.740	5.440	5.080	4.380	4.85
S ₄	3.540	3.660	4.360	4.000	3.300	3.77
S ₅	2.850	2.870	3.670	3.210	2.460	3.01
S ₆	2.420	2.490	3.330	2.830	2.080	2.63
S ₇	2.290	2.360	3.400	2.700	1.950	2.54
S ₈	2.010	2.080	3.100	2.420	1.670	2.26
S ₉	1.820	1.890	2.940	2.230	1.530	2.08
S ₁₀	1.790	1.870	2.920	2.210	1.500	2.06
S ₁₁	1.780	1.850	2.880	2.190	1.490	2.04
S ₁₂	1.190	1.670	2.390	1.970	1.320	1.71
Mean	2.990	3.110	3.920	3.400	2.690	3.22
SEd	0.052	0.053	0.059	0.056	0.050	
CD (p = 0.05)	0.106	0.108	0.120	0.114	0.102	
Interaction SEd						
M at S	0.087	0.089	0.098	0.093	0.084	
S at M	0.074	0.076	0.084	0.080	0.072	
CD (p = 0.05)						
M at S	0.244	0.249	0.270	0.260	0.235	
S at M	0.150	0.153	0.170	0.161	0.145	

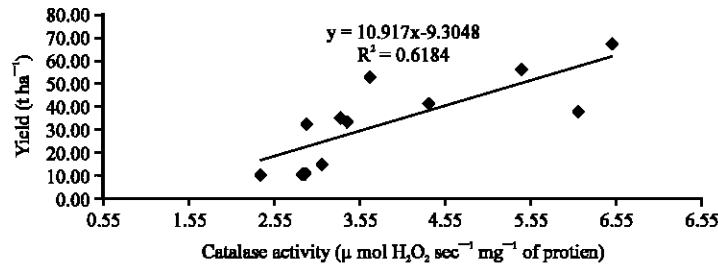


Fig. 1: Correlation of catalase activity ($\mu\text{mol H}_2\text{O}_2 \text{ sec}^{-1} \text{ mg}^{-1}$ of protein) with yield

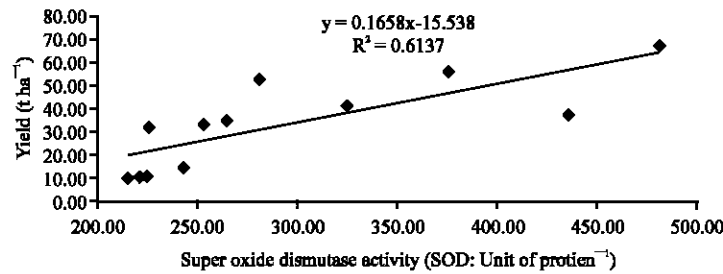


Fig. 2: Correlation of super oxide dismutase activity (SOD: unit mg of protein⁻¹) with yield

Superoxide dismutase: The result on SOD content was affected by water deficit and Hybrid as well as the interaction of M at S and S at M were significant (Table 2). Water deficit increased SOD content in banana cultivars and hybrids. Among the twelve cultivars and hybrids, Karpuravalli, Karpuravalli x Pisang Jajee, Saba and Sannachenkathali had significant differences in SOD content under the main plot treatments. The highest SOD content was observed in Karpuravalli (481.8) under the water deficit. The lowest SOD content was observed in Matti, Pisang Jajee x Matti, Matti x Anaikomban and Anaikomban x Pisang Jajee (226.2, 225.2, 221.2 and 215.2) cultivars and hybrids under the water deficit at 7th MAP, respectively. The positive correlation ($R^2 = 0.613$) were showed between SOD level and yield under the water deficit conditions (Fig. 2).

Ascorbate peroxidase: The data on APX content was affected by water deficit and Hybrid as well as the interaction of M at S and S at M were significant (Table 3). Water deficit increased APX content in banana cultivars and hybrids. Among the twelve cultivars and hybrids, Karpuravalli, Karpuravalli x Pisang Jajee, Saba and Sannachenkathali had significant differences in catalase content under the main plot treatments. The highest APX content was observed in Karpuravalli (64.32) under the water deficit at 7th MAP. The lowest APX content was observed in Matti, Pisang Jajee x Matti, Matti x Anaikomban and Anaikomban x Pisang Jajee (58.44, 58.38,

Table 2: Effect of water stress on Super Oxide Dismutase activity (SOD: unit mg of protein⁻¹) at different growth stages of banana cultivars and hybrids

Treatments	3rd MAP	5th MAP	7th MAP	9th MAP	Harvest	Mean
Main plot						
M ₁	168.30	219.80	249.30	245.30	235.30	223.6
M ₂	196.30	249.30	342.00	330.90	317.20	287.2
Mean	182.30	234.60	295.70	288.10	276.30	255.4
SEd	0.23	0.22	1.16	1.17	1.18	
CD (p = 0.05)	1.01	0.96	5.00	5.06	5.11	
Sub plot						
S ₁	308.00	346.50	481.80	477.80	467.80	416.4
S ₂	292.00	345.50	435.80	431.80	421.80	385.4
S ₃	222.00	275.50	375.80	371.80	361.80	321.4
S ₄	186.00	239.50	324.80	320.80	310.80	276.4
S ₅	168.00	221.50	281.20	267.20	252.20	238.0
S ₆	162.00	215.50	265.20	256.20	241.20	228.0
S ₇	150.00	203.50	253.20	244.20	229.20	216.0
S ₈	145.00	198.50	243.20	234.20	219.20	208.0
S ₉	143.00	196.50	226.20	217.20	207.20	198.0
S ₁₀	142.00	195.50	225.20	217.20	206.20	197.2
S ₁₁	138.00	191.50	221.20	212.20	202.20	193.0
S ₁₂	132.00	185.50	215.20	207.20	196.20	187.2
Mean	182.30	234.60	295.70	288.10	276.30	255.4
SEd	6.43	7.96	10.40	10.23	9.88	
CD (p = 0.05)	12.96	16.05	21.04	20.61	19.91	
Interaction SEd						
M at S	8.70	10.7	14.1	13.90	13.40	
S at M	9.00	11.2	14.7	14.40	13.90	
CD (p = 0.05)						
M at S	17.50	21.7	28.8	28.20	27.30	
S at M	18.30	22.7	29.7	29.10	28.10	

58.32 and 58.26) cultivars and hybrids under the water deficit during 7th MAP, respectively. The positive correlation ($R^2 = 0.615$) were showed between SOD level and yield under the water deficit conditions (Fig. 3).

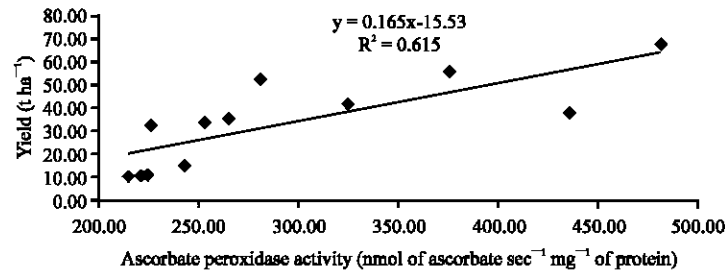


Fig. 3: Correlation of ascorbate peroxidase activity (nmole of ascorbate sec⁻¹ mg⁻¹ of protein) with yield

Table 3: Effect of water stress on ascorbate peroxidase activity (nmole of ascorbate sec⁻¹ mg⁻¹ of protein) at different growth stages of banana cultivars and hybrids

Treatments	3rd MAP	5th MAP	7th MAP	9th MAP	Harvest	Mean
Main plot						
M ₁	46.370	54.750	59.560	57.400	57.200	55.06
M ₂	50.240	58.610	60.490	58.260	58.020	57.13
Mean	48.310	56.680	60.030	57.830	57.610	56.09
SEd	0.145	0.193	0.394	0.367	0.208	
CD (p = 0.05)	0.626	0.831	1.697	1.583	0.896	
Sub plot						
S ₁	52.000	60.370	64.320	62.160	61.960	60.16
S ₂	50.960	59.330	62.980	60.820	60.620	58.94
S ₃	50.210	58.580	62.330	60.170	59.970	58.25
S ₄	48.960	57.330	60.930	58.770	58.570	56.91
S ₅	47.750	56.120	59.460	57.200	56.950	55.50
S ₆	47.510	55.880	59.120	56.910	56.660	55.22
S ₇	47.360	55.730	58.970	56.760	56.510	55.07
S ₈	47.260	55.630	58.820	56.610	56.360	54.94
S ₉	47.030	55.400	58.440	56.230	56.030	54.63
S ₁₀	46.970	55.340	58.380	56.180	55.970	54.57
S ₁₁	46.910	55.280	58.320	56.110	55.910	54.51
S ₁₂	46.850	55.220	58.260	56.060	55.850	54.45
Mean	48.310	56.680	60.030	57.830	57.610	56.09
SEd	0.071	0.157	0.209	0.185	0.183	
CD (p = 0.05)	0.143	0.316	0.422	0.374	0.369	
Interaction SEd						
M at S	0.174	0.287	0.486	0.445	0.324	
S at M	0.100	0.222	0.296	0.262	0.259	
CD (p = 0.05)						
M at S	0.629	0.876	1.712	1.593	0.959	
S at M	0.202	0.447	0.597	0.529	0.522	

DISCUSSION

Reactive Oxygen Species (ROS), such as the superoxide radical (O₂⁻), H₂O₂ and the hydroxyl radical (OH_·) are generated as byproducts of normal metabolism in different subcellular compartments including the chloroplasts, mitochondria, peroxisomes and plasma membrane-linked electron transport system (Elstner, 1991; Del Rio *et al.*, 1998; Asada, 1999). These ROS can damage DNA, proteins, chlorophyll and membrane functions. Furthermore, the imposition of biotic or abiotic stress might give rise to an excessive concentration of ROS, resulting in oxidative damage at a cellular level. To mitigate and repair damage initiated by ROS, plants have developed a complex antioxidant system (Del Rio *et al.*, 2002). Water deficit induced accumulation of ROS is

detrimental to cells as they caused oxidative damage to membrane lipids, proteins and nucleic acids (Fridovich, 1997). In banana plants, some detoxifying enzymes such as catalase, ascorbate peroxidase and superoxide dismutase would scavenge the free radicals from the metabolic sites thereby imparting tolerance to water deficits situations (Smirnov, 1998).

The enzyme catalase is generally regarded as H₂O₂ scavenger (Fridovich, 1976) and H₂O₂ reported to be involved in the enhancement of damage of cell oxidation function (Reddy and Raghavendra, 2006). Higher accumulation of H₂O₂ coupled with low rate of enzyme activity indicates the susceptible nature of the crop to water stress. The twelve cultivars employed in the present study showed differential responses to the water deficit treatments. The cultivars like Karpuravalli, Karpuravalli x Pisang jajee, Saba and Sannachenkathali maintained higher activity than cultivars of Matti, Matti x Anaikomban, Matti x cultivar rose and Pisang jajee x Matti. Similar results were made by McKersie and Leshem, 1994, who reported that catalase was responsible for the better protection against oxidative injury in banana.

Environmental stresses exert their effects directly or indirectly through production of ROS. Such effects have been reported for water deficit in banana (Alscher *et al.*, 2002).

In order to prevent oxidative damage to cellular components from occurring, a number of protective enzymes have been evolved. The Superoxide Dismutases (SODs) remove superoxide anions (O₂⁻) by catalysing their conversion into hydrogen peroxide which in turn is broken down by catalase to yield oxygen and water. The SOD is the first line of defense against injury caused by ROS. The importance of antioxidant enzymes in stress defense has been demonstrated in transgenic plants over expressing superoxide dismutase and ascorbate peroxidases which showed enhanced oxidative stress tolerance (McKersie *et al.*, 1996; Van Breusegem *et al.*, 1999; Allen *et al.*, 1997). In the present study, cultivars like Karpuravalli, Karpuravalli x Pisang jajee, Saba and Sannachenkathali had higher per cent increase of about

65 in SOD activity at 7th MAP over control. Similar results was also observed by McKersie and Leshem (1994) stating that higher SOD activity was therefore associated with better protection against water stress induced oxidative injury in banana cultivars; The cultivars of Matti, Matti x Anaikomban, Matti x cultivar rose and Pisang jajee x Matti registered about 2% increase in SOD activity over control at 7th MAP. It was also established that lower SOD activity was detected in water stressed jute plants, showing an association of lower SOD activity with greater degree of oxidative damage (Chowdhury and Choudhuri, 1985).

The enzyme Ascorbate Peroxidase (APX) is a hydrogen peroxide scavenging enzyme (McKersie and Leshem, 1994) and H₂O₂ has been observed to be involved in the damage of cell oxidation function (Reddy and Raghavendra, 2006). Higher accumulation of H₂O₂ coupled with low rate of enzyme activity indicate the sensitive nature of the crop to a water deficit. In the present study, twelve cultivars were evolved and showed various responses to the water deficit treatment for APX activity. Among these ratoon cultivars and hybrids, Karpuravalli, Karpuravalli x Pisang jajee, Saba and Sannachenkathali maintained higher enzyme activity under water deficit conditions over control. Similar results were made by Chai *et al.* (2005), who stated that ascorbate peroxidase activity might be a more crucial antioxidant defense than CAT in water stressed banana plants.

CONCLUSION

Plants respond to drought stress through alteration in physiological and biochemical processes. Our results showed that the activities of antioxidant enzymes increased under the water deficit condition. However, the water deficit reduced seed yield and some yield components. The ratoon banana cultivars and hybrids of Karpuravalli, Karpuravalli x Pisang jajee, Saba and Sannachenkathali with highest antioxidative enzymes were produced more bunch yield when the plants endured water deficit. The findings of this research also showed that the APX and CAT content can be used as a drought tolerance index to selection tolerant genotypes under water deficit conditions in ratoon banana cultivars and hybrids.

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