

Effect of Water Deficit on Bunch Yield and Antioxidants Content in Ratoon Banana Cultivars and Hybrids

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Abstract The field experiment was conducted at national research centre for banana to screen the ratoon banana cultivars and hybrids for water deficit tolerance and to elucidate information on growth attribute mechanism of banana cultivars and hybrids. Stress was imposed at different critical stages viz., 3rd, 5th, 7th and 9th month after planting. The stress was given by scheduling irrigation at the 50 per cent available soil moisture (ASM) characteristic during critical stages. The soil moisture content was analyzed by using pressure plate membrane apparatus. In control plots, the irrigation was given at the ASM of 80% with the soil water potential of around -6 bars and in the case of stressed plots; the irrigation was given when an ASM reached 50% with the soil water potential of -14 bars. In stressed plots, 50% ASM was reached around 30 days. In this present study conducted with twelve cultivars and hybrids with three replications. The data were analyzed by using split plot design. The antioxidative enzymes of catalase, super oxide dismutase and ascorbate peroxidase were significantly enhanced during water deficit conditions. Among the twelve ratoon cultivars and hybrids, Karpuravalli, Karpuravalli×Pisang Jajee, Saba, and Sannachenkathali was identified as tolerant to water stress with highly accelerated by water stress treatment in the range of 20% to 28% 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×Matti, Matti×Anaikomban and Anaikomban×Pisang Jajee were notified as sensitive ratoon cultivars and hybrids with lesser increase in antioxidative enzyme activity of 13 per cent than control which is leads to get very low yield.

Keywords Water deficit; Ratoon banana; CAT; APX; SOD; Yield

Abbreviations: CAT: Catalase; APX: Ascorbate peroxidase; ROS: Reactive oxygen species; SOD: Superoxide dismutase; **: Highly significant; *: Significant

Background

Plants are frequently exposed to diverse stress conditions, including salt, drought, heat, low temperature, heavy metals, and oxidative stress (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 the 'queen of tropical fruits' and is one of the oldest fruits known to mankind from pre-historic times. Today, it is the leading tropical fruit in the world market with a highly organized and developed industry. It is the fourth largest fruit crop in the world after grapes, citrus fruits and apples. 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. In plants under water deficit conditions, an oxidative brush system may be observed (Larson, 1988). Such a response is due to disturbances in the balance between production of reactive oxygen species (ROS) such as singlet oxygen, superoxide radical, hydrogen peroxide, hydroxyl ion and free hydroxyl radical ($\cdot\text{O}^-2$, H_2O_2 , OH^- and $\cdot\text{OH}$) and their scavenging by the antioxidant system (Del Río et al., 2002). The antioxidant system, including antioxidant enzymes such as Superoxide dismutase (SOD), Ascorbate Peroxidase (APX), and Catalase (CAT) play a key role in controlling ROS levels (Smirnoff, 1998). The level of damages may be limited by enzymatic and nonenzymatic scavengers of free radicals (Foyer and Noctor, 1999). The biochemical and physiological responses of plants are a base of agronomic response to environmental stress. Characterization of the

agronomic response of banana cultivars and hybrids to water stress could help to stabilize production at present levels, and to identify appropriate stress tolerance mechanisms for use in future breeding efforts. The field experiments were performed to determine the agronomic and physiological 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.

1 Result

1.1 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; Figure 1). Water deficit increased CAT content in banana cultivars and hybrids. Among the twelve cultivars and hybrids, Karpuravalli, Karpuravalli×Pisang Jajee, Saba, and Sannachenkathali had significant differences in catalase content under the main plot treatments. The highest CAT content was observed in Karpuravalli under the water deficit. The lowest CAT content was observed in Matti, Pisang Jajee×Matti, Matti×Anaikomban and Anaikomban×Pisang Jajee cultivars and hybrids under the water deficit, respectively. There was a high and positive correlation between CAT level and yield water deficit conditions.

Table 1 Effect of water stress on catalase activity ($\mu \text{ mol H}_2\text{O}_2 \text{ s}^{-1} \text{ mg}^{-1}$ of protein) at different growth stages of banana cultivars and hybrids

Treatments	3 rd MAP	5 th MAP	7 th MAP	9 th MAP	Harvest	Mean
Main plot						
M ₁	2.05	2.21	2.98	2.50	1.81	2.31
M ₂	2.98	3.06	3.91	3.36	2.63	3.19
Mean	2.52	2.63	3.45	2.93	2.22	2.75
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	5.80	5.92	6.02	5.75	5.07	5.72
S ₂	4.81	4.93	5.63	5.27	4.57	5.05
S ₃	4.14	4.26	4.96	4.60	3.90	4.38
S ₄	3.06	3.18	3.88	3.52	2.82	3.30
S ₅	2.38	2.40	3.20	2.74	1.99	2.54
S ₆	1.95	2.02	2.86	2.36	1.61	2.16
S ₇	1.82	1.89	2.93	2.23	1.48	2.07
S ₈	1.54	1.61	2.63	1.95	1.20	1.78
S ₉	1.35	1.42	2.47	1.76	1.06	1.61
S ₁₀	1.32	1.40	2.45	1.74	1.03	1.59
S ₁₁	1.31	1.38	2.41	1.72	1.02	1.57
S ₁₂	0.72	1.20	1.92	1.50	0.85	1.24
Mean	2.52	2.63	3.45	2.93	2.22	2.75
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	*	**	**	**	**	
S at M	**	*	**	**	*	
CD (P=0.05)						
M at S	**	**	*	**	**	
S at M	**	**	**	**	**	

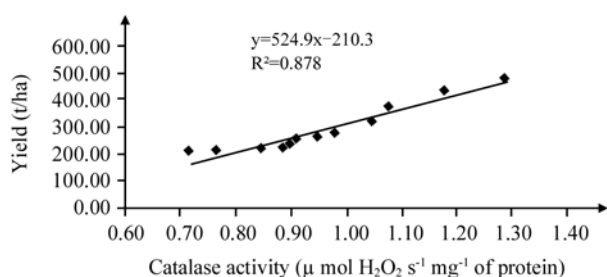


Figure 1 Correlation of catalase activity ($\mu \text{ mol H}_2\text{O}_2 \text{ s}^{-1} \text{ mg}^{-1}$ of protein) with yield in banana cultivars and hybrids

1.2 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; Figure 2). Water deficit increased SOD content in banana cultivars and hybrids. Among the twelve cultivars and hybrids, Karpuravalli, Karpuravalli×Pisang Jajee, Saba, and Sannachenkathali had significant differences in SOD content under the main plot treatments. The highest SOD content was observed in Karpuravalli under the water deficit. The lowest SOD content was observed in Matti, Pisang Jajee×Matti, Matti×Anaikomban and Anaikomban×Pisang Jajee cultivars and hybrids under the water deficit, respectively. The positive correlation were showed between SOD level and yield under the water deficit conditions.

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	3 rd MAP	5 th MAP	7 th MAP	9 th MAP	Harvest	Mean
Main plot						
M ₁	298.6	350.1	379.6	375.6	365.6	353.9
M ₂	326.6	379.6	472.3	461.1	447.5	417.4
Mean	312.6	364.8	425.9	418.4	406.5	300.6
SEd	0.24	0.21	1.15	1.15	1.16	
CD (P=0.05)	1.00	0.95	4.98	5.04	5.09	
Sub plot						
S ₁	438.3	476.8	612.0	608.0	598.0	546.6
S ₂	422.3	475.8	566.0	562.0	552.0	515.6
S ₃	352.3	405.8	506.0	502.0	492.0	451.6
S ₄	316.3	369.8	455.0	451.0	441.0	406.6
S ₅	298.3	351.8	411.4	397.4	382.4	368.2
S ₆	292.3	345.8	395.4	386.4	371.4	358.2
S ₇	280.3	333.8	383.4	374.4	359.4	346.2
S ₈	275.3	328.8	373.4	364.4	349.4	338.2
S ₉	273.3	326.8	356.4	347.4	337.4	328.2
S ₁₀	272.3	325.8	355.4	347.4	336.4	327.4
S ₁₁	268.3	321.8	351.4	342.4	332.4	323.2
S ₁₂	262.3	315.8	345.4	337.4	326.4	317.4
Mean	312.6	364.8	425.9	418.4	406.5	385.6
SEd	6.42	7.95	10.38	10.20	9.86	
CD (P=0.05)	12.94	16.02	21.02	20.57	19.88	
Interaction SEd						
M at S	**	**	**	**	**	
S at M	**	*	**	**	**	
CD (P=0.05)						
M at S	**	**	**	**	**	
S at M	*	**	*	**	**	

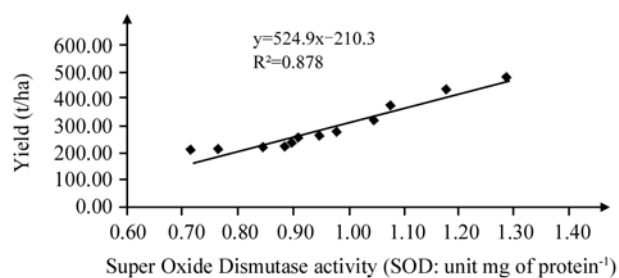


Figure 2 Correlation of Super Oxide Dismutase activity (SOD: unit mg of protein⁻¹) with yield in banana cultivars and hybrids

1.3 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; Figure 3). Water deficit increased APX content in banana cultivars and hybrids. Among the twelve cultivars and hybrids, Karpuravalli, Karpuravalli×Pisang Jajee, Saba, and Sannachenkathali had significant differences in catalase content under the main plot treatments. The highest APX content was observed in Karpuravalli under the water deficit. The lowest APX content was observed in Matti, Pisang Jajee×Matti, Matti×Anaikomban and Anaikomban×Pisang Jajee cultivars and hybrids under the water deficit, respectively.

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

Treatments	3 rd MAP	5 th MAP	7 th MAP	9 th MAP	Harvest	Mean
Main plot						
M ₁	54.7	63.1	67.9	65.7	65.5	63.4
M ₂	58.5	66.9	68.8	66.6	66.3	65.4
Mean	56.6	65.0	67.3	66.1	65.9	63.8
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	60.3	68.7	72.6	70.5	70.3	68.5
S ₂	59.3	67.6	71.3	69.1	68.9	67.2
S ₃	58.5	66.9	70.6	68.5	68.3	66.6
S ₄	57.3	65.6	69.2	67.1	66.9	65.2
S ₅	56.0	64.4	67.8	65.5	65.3	63.8
S ₆	55.8	64.2	67.4	65.2	65.0	63.5
S ₇	55.7	64.0	67.3	65.1	64.8	63.4
S ₈	55.6	63.9	67.1	64.9	64.7	63.2
S ₉	55.3	63.7	66.7	64.5	64.3	62.9
S ₁₀	55.3	63.6	66.7	64.5	64.3	62.9
S ₁₁	55.2	63.6	66.6	64.4	64.2	62.8
S ₁₂	55.1	63.5	66.5	64.4	64.2	62.8
Mean	56.6	65.0	68.3	66.1	65.9	64.4
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	*	**	**	**	**	
S at M	**	**	*	**	**	
CD (P= 0.05)						
M at S	*	**	**	**	**	
S at M	**	**	**	*	**	

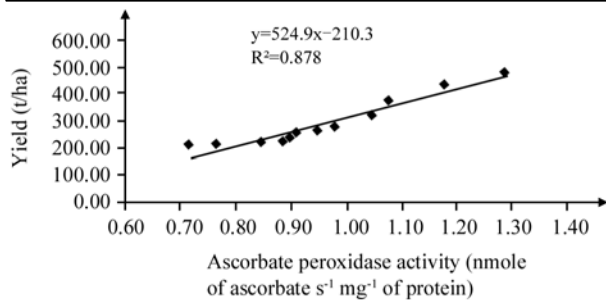


Figure 3 Correlation of ascorbate peroxidase activity (nmole of ascorbate s⁻¹ mg⁻¹ of protein) with yield in banana cultivars and hybrids

2 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 Río 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 Río 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. 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, 1997) and H₂O₂ reported to be involved in the enhancement of damage of cell oxidation function (Madhava et al., 2004). 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×Pisang jaje, Saba and Sannachenkathali maintained higher

activity than cultivars of Matti, Matti×Anaikomban, Matti x cultivar rose and Pisang jaje×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., 1997).

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 peroxidase, which showed enhanced oxidative stress tolerance (McKersie et al., 1996; Van Breusegem et al., 1999; Allen, 1997). In the present study, cultivars like Karpuravalli, Karpuravalli×Pisang jaje, 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×Anaikomban, Matti×cultivar rose and Pisang jaje×Matti registered about 22% 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 (Madhava et al., 2004). 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×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.

3 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×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.

4 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×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×Matti, S₁₁: Matti×Anaikomban and S₁₂: Anaikomban×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.

4.1 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 mmol/L 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 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 & mortar. The extract was centrifuged at 10000 rpm at 4 °C for 20 minutes. 0.2 mL of supernatant was taken in a test tube and 1.8 mL of 50 mmol/L sodium phosphate buffer, 0.5 mL of 0.05M ascorbic acid and 0.5 ml of 50 μmol of H₂O₂ was added. The reaction was started by the addition of 0.5 mL of ascorbic acid (50 mmol/L) at final concentration and its consumption was measured 30 seconds interval for 2 minutes at 290 nm. The Ascorbate peroxidase (APX) activity was decreased.

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