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Analysis of Anti-oxidant Enzyme Activity in Sugarcane Varieties under Moisture Stress

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Authors' contributions

This work was carried out in collaboration between all authors. Author TVM carried out the whole experiment. Authors GSMB and MVK helped in experiments, literature and statistical analysis. Author CSN gave the guidance. All authors read and approved the final manuscript.

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ABSTRACT

The bio-chemical activity in eight sugarcane varieties was studied in a poly bag experiment at Agricultural Research Station, Basanthpur, Medak, India. The anti-oxidant enzyme activity in terms of superoxide dismutase and peroxidase activity in sugarcane genotypes increased upon exposure to moisture stress for 20 days at formative stage (120 DAP) and the maximum was found in the varieties Co 95020 and Co 87025 ranging from 109 to 122 and 20 to 21 eu/100 ml/gram fresh weight, respectively. The relative water content ranged from 10.92 to 37.72% among the varieties due to water stress with maximum reduction in Co 8014 (37.72%). Moisture stress at formative stage has also reduced the cane yield by 19.62 per cent as compared to irrigated treatment. The least reduction in cane yield was noticed in Co 87025 (11.76%) and Co 95020 (11.21%). The increase in anti-oxidant enzyme activity during stress was also found significant and positively correlated with relative water content and cane yield up to 78 and 63%, respectively.

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Keywords: Superoxide dismutase; peroxidase; relative water content; cane yield; moisture stress.

1. INTRODUCTION

In the present scenario of climate change, plants are exposed to adverse environmental conditions and drought has become a major abiotic factor that can damage their growth and development. As for any crop, water is essentially required during vegetative growth to harness maximum yield and drought events at this stage can significantly affect productivity of the crop. Sugarcane is a C_4 crop which produce higher amount of biomass per unit cultivated area and its water requirement varies with the development stages, thus the highest water requirement being observed at maximum tillering and culm development stages than during the maturation stage, when this need would be diminished. This indicates that formative stage is the most important one for growth and cane yield.

When plants are subjected to various abiotic stresses, some reactive oxygen species (ROS) such as the superoxide radical, hydrogen peroxide and hydroxyl radical can cause lipid peroxidation and consequently membrane injury which leads to leakage of cellular content, protein degradation, enzyme inactivation, pigment bleaching and disruption of DNA strands and thus cell death [1]. They initiate destructive oxidative processes. To protect against the harmful effects of ROS produced during oxidative stress, plants have developed a complex defense mechanism consisting of antioxidant enzymes and metabolites. The antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) are sensitive enough to abiotic stress conditions to serve as indicators of stress [2]. Studies on sugarcane have used the responses of antioxidant enzymes to measure its ability to defend against oxidative stress, such as that induced by water deficit [3,4,5] salinity [6] increased temperature [7]. When the plant is exposed to drought conditions, other components of the photosynthetic process are also affected as result of decrease in the relative water content in the plant. Therefore, this study was aimed for detailed understanding of anti-oxidant enzyme activity during stress at formative stage (120 DAP) of sugarcane in relation to relative water content of the plant and cane yield which otherwise may provide information about strategies that could be used in genetic programs for selecting drought-tolerant cultivars.

2. MATERIALS AND METHODS

A poly bag experiment was carried out during *rabi,* 2013-14 at Agricultural Research Station, Basanthpur, Medak district of Telangana state situated at 17° 47' East longitude and 77° 32' North latitude reflecting peninsular India situated at an altitude of 645 m above mean sea level. The experiment was conducted in strip plot design in three replications with stress and non-stress (control) in main plots and varieties in the sub plots. Eight Sugarcane varieties (Co 86032, Co 87025, Co 8014, 2003 V 46, 97 R 129, Co 94012, 97 R 401 and Co 95020) were raised in six sets of poly bags as per the recommended practices to quantify the growth and yield data. Each poly bag was of size 4' x 4'. Bud chip seedlings of the test varieties raised in nursery were planted in poly bags @ 12 seedlings per bag. The seedlings in control/non stress sets of bags (3 sets) were raised under normal conditions. Whereas, the seedlings in the remaining three sets of poly bags were exposed to moisture stress by withholding irrigation for 20 days during formative stage i.e., at 120 DAP (Days after planting). After relieving of stress, leaf samples from stress and non stress plants were taken to analyze antioxidant enzyme activity in terms of superoxide dismutase (SOD) and peroxidase activity (POD) as affected by stress at formative stage. The activity of SOD and POX in the plants was measured as per the standard procedures given by Misra and Fridovich [8] and Chance and Meahley [9] respectively and was expressed in eu/100 ml/ gram fresh weight of leaf sample of one gram. The antioxidant enzyme activity under moisture stress conditions was later related to leaf tissue water content studied as relative water content (RWC) and cane yield (t ha^{-1}). The relative water content was estimated by the method.

Ten discs from the leaves of three hills were collected randomly in each treatment and weighed accurately up to fourth decimal on an electrically operated single pan analytical balance. This was considered as the fresh weight. The weighed leaf discs were allowed to float on distilled water in a petri dish and allowed to absorb water for four hours. After four hours the leaf discs were taken out and their surface was blotted gently and weighed. This was referred to as turgid weight after drying these leaf discs in an oven at 70℃ for 48 hours, the dry weight was recorded and designated as dry weight. The RWC was calculated by the following formula.

$$\text{RWC (\%)} = \frac{\text{Fresh weight — Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Anti-oxidase Enzyme Activity

Stress imposed during formative stage in sugarcane varieties showed significant difference in SOD activity which was high as compared to that under control conditions (Table 1). The Superoxide dismutase activity of different Sugarcane varieties up on exposure to moisture stress for 20 days at 120 DAP ranged from 50.04 to 122.04 eu/100 ml/ gram fresh weight. Significantly highest SOD activity was observed in the variety, Co 95020 (122.04 eu/100 ml/ gram fresh weight) followed by Co 87025 (109.12 eu/100 ml/ gram fresh weight). Conversely, lowest SOD value of 50.04 eu/100 ml/ gram fresh weight was registered by Co 8014. Significant interaction of varieties with moisture also indicated the Co 95020 under stress conditions has highest SOD activity followed by Co 87025 under stress conditions. The increase in SOD activity in the varieties Co 95020 and Co 87025 by combating oxidative stress and by decreasing the deleterious effect of H_2O_2 under drought stress [10] might had made them capable to tolerate drought as compared to other varieties. The results are in agreement with Halliwel and Gutteridge [11].

Similar to SOD values, the peroxidase activity in Sugarcane varieties also showed significant variations when exposed to moisture stress for 20 days during the formative stage. The POD activity was high when the crop was exposed to stress as compared to control conditions. The peroxidase activity was significantly highest in Co 95020 (21.24 eu/100 ml/ gram fresh weight) and Co 87025 (20.60 eu/100 ml/ gram fresh weight) compared to other genotypes. The varieties viz., Co 8014, 97 R 401 and 2003 V 46, and being on par registered low POD values ranging from 13.21 to 14.10 eu/100 ml/ gram fresh weight. The interaction of varieties with moisture however, was not found significant.

Moisture stress leads to oxidative stress through an increase in ROS such as superoxide ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{-\bullet}$) which attack the most sensitive biological macromolecules like lipids, proteins and nucleic acids, cause lipid peroxidation mainly at membranes to impair their function. Plants have an enzymatic and non-enzymatic system that protects them against the damage of ROS. The anti oxidative enzymes include superoxide dismutase, peroxidase, glutathione reductase and catalase. Peroxidase and catalase detoxify the hydrogen peroxide, which accumulates in the plants under water stress. Thus the increase in peroxidase activity in the varieties Co 95020 and Co 87025 might be due to formation of large amount of H_2O_2 during water stress.

3.2 Relative Water Content

The relative water content of sugarcane was also significantly influenced by moisture regimes and varieties (Table 2). The effect of moisture stress was evident as the relative water content of all the varieties under the study was highest under

Table 1. Anti-oxidant enzyme activity during moisture non stress and stress periods at formative stage in different sugarcane genotypes

S. no.	Variety	Super oxide dismutase (eu/100 ml/ gram fresh weight)		Peroxidase (eu/100 ml/ gram fresh weight)	
		Stress	**Non Stress**	**Stress**	**Non Stress**
1	Co 86032	50.45	99.76	13.22	15.46
2	Co 87025	65.09	109.12	16.57	20.60
3	Co 8014	30.15	50.04	10.78	13.21
4	2003 V 46	36.54	68.60	11.00	14.10
5	97 R 129	46.74	75.81	14.15	15.60
6	Co 94012	43.24	73.30	11.03	14.99
7	97 R 401	31.56	57.90	10.99	13.23
8	Co 95020	68.04	122.04	18.10	21.24
		SeM	**CD (0.05)**	**SeM**	**CD (0.05)**
Moisture		1.253	5.390	0.214	0.921
Variety		2.586	5.546	0.488	1.046
Moisture x variety		2.873	6.162	0.896	NS

non stress conditions as compared to stressed conditions. The decrease in relative water content due to water stress however, varied among the varieties and ranged from 10.92 to 37.72%. The reduction being maximum in Co 8014 (37.72%).

The genotypes Co 87025 (86.54 and 97.72%), Co 95020 (85.16 and 96.17%) and Co 86032 (82.96 and 97.72%) being on par maintained higher tissue water contents at the formative stage under both stress and non-stress conditions while the sensitive genotype Co 8014 (52.84 and 84.87%) recorded the lowest relative water content under both the conditions. Similar observation was reported as they observed marginal decrease in relative water content per unit decrease in leaf water potential of a drought resistant species than in drought susceptible species.

The interaction of moisture stress and varieties on relative water content on the other side was not significant.

3.3 Cane Yield

The data on cane yield recorded after harvest indicated significant variations due to moisture stress and varieties, but their interaction was not significant (Table 2). Moisture stress reduced the cane yield by 19.62 per cent as compared to irrigated treatment. Among the varieties, reduction in the cane yield due to moisture was maximum in Co 8014 (32.35%). The least reduction in cane yield was noticed in Co 87025 (11.76%) and Co 95020 (11.21%). Both under stress and non stress conditions, highest yield was recorded with the variety Co 95020 (125.23 and 148.54 t/ha, respectively) followed by Co 86032 (119.65 and 140.26 t/ha, respectively). Yield in sugarcane is reported to be as sensitive to soil water stress as it is for total growth. Yield data recorded during both the seasons have shown that soil water deficit resulted in decreased cane yield. The water stress reduced cane yield more in the susceptible genotypes, Co 8014 followed by 97 R 401. The reduction in cane yield in drought sensitive varieties may be due to marked decline in the number of millable canes at formative phase. [12] observed more reduction in cane yield at 25 per cent available soil moisture regime in certain varieties under sub-tropical Indian conditions. Singh and Reddy (1980) also found that the cane yield was affected most adversely when available soil moisture decreased from 60 to 20 per cent under tropical Indian conditions.

3.4 Inter-relation of Anti-oxidant Enzyme Activity with Relative Water Content and Cane Yield

The assessed correlation of anti-oxidant enzyme activity during stress with relative water content and cane yield was linear and positively significant (Fig. 1). However, the correlation of superoxide dismutase activity with relative water content and cane yield was found significantly higher and dependable up to 78 and 63%, respectively than that of peroxidase activity in

Table 2. Relative water content and cane yield during moisture non stress and stress periods at formative stage in different sugarcane genotypes

S. no.	Variety	Relative water content (%)			Cane yield (t/ha)		
		Non stress	**Stress**	**% Decrease over non stress treatment**	**Non stress**	**Stress**	**% Decrease over non stress treatment**
1	Co 86032	97.72	82.96	15.10	140.26	119.65	14.69
2	Co 87025	97.15	86.54	10.92	136.42	115.37	15.43
3	Co 8014	84.87	52.85	37.72	111.3	98.3	11.68
4	2003 V 46	91.41	68.04	25.56	119.55	100.89	15.61
5	97 R 129	94.05	75.32	19.91	126.36	105.56	16.46
6	Co 94012	93.42	73.35	21.48	108.56	94.49	12.96
7	97 R 401	88.73	62.40	29.68	130.33	111.71	14.29
8	Co 95020	96.17	85.16	11.45	148.54	125.23	15.69
		SeM	**CD (0.05)**		**SeM**	**CD (0.05)**	
Moisture		1.65	7.11		2.4	10.33	
Variety		1.50	3.22		3.31	7.09	
Moisture x variety		1.99	NS		5.56	NS	

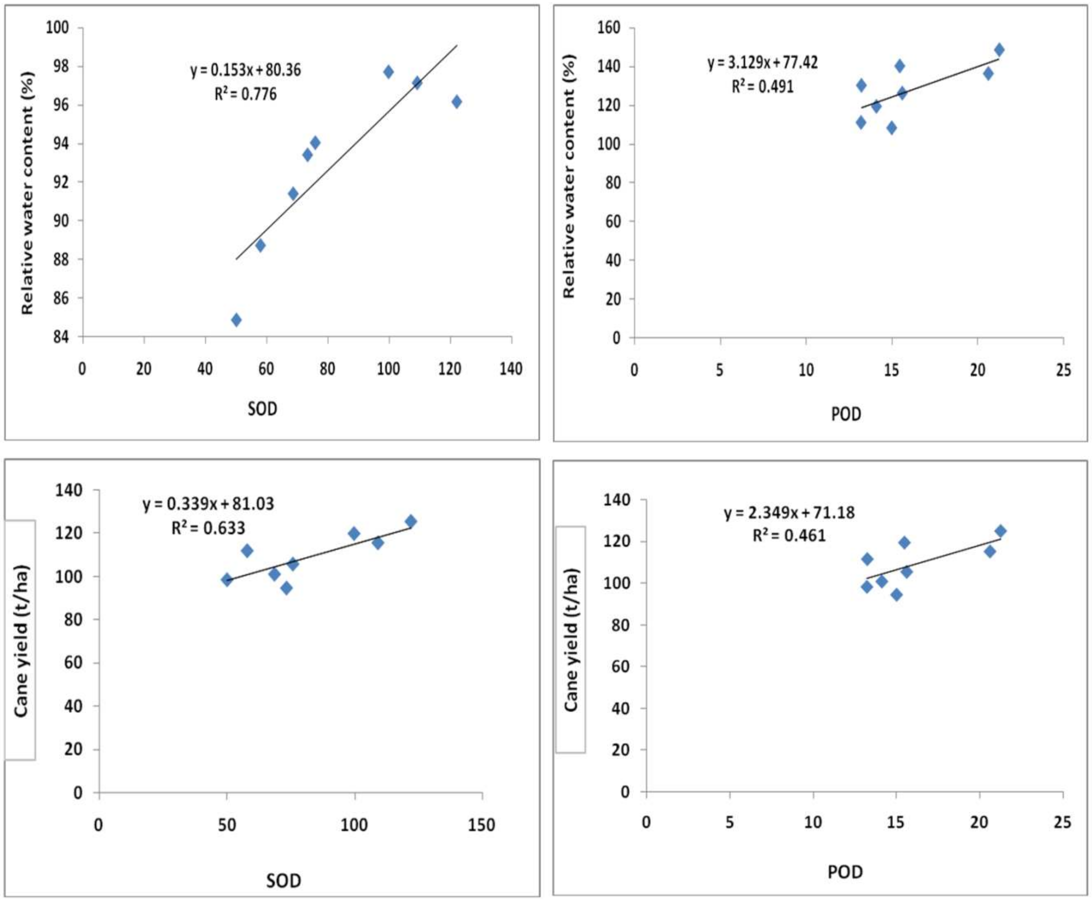

Fig. 1. Interrelation of SOD and POD activity during stress with relative water content and cane yield of sugarcane

relation to relative water content (r^2=0.49) and cane yield (r^2=0.46). The results are similar to those noted by Claudiana et al. [13], who observed that an increase in enzymes SOD, CAT, APX exhibited a positive correlation with Fv/Fm (maximum photochemical efficiency of photosystem II), gs (stomatal conductance), Ψ_w (leaf water potential) and RWC (relative water content) and a negative correlation with LT (leaf temperature).

4. CONCLUSION

Increase in anti-oxidant enzyme activity viz., superoxide dismutase and peroxidase in the varieties Co 95020 and Co 87025 is an indication that these varieties can perform well under moisture stress by tolerating oxidative stress as compared to the other varieties. Also, the variable RWC was found to be a reliable physiological indicator for selecting sugarcane cultivars tolerant to water deficit.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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