Effect of phytohormones on changes in biochemical activity of maize hybrid COH(M) 8 under drought stress

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Abstract

Maize is an important cereal crop cultivated worldwide. However, the temperature extremes are threatening the yield sustainability of maize. Maize plants are sensitive to drought stress and there is a strong decline in grain yield as plants face drought stress above the threshold level for a prolonged duration. The various biochemical and physiological parameters gets altered due to the drought stress. Higher activities of antioxidant enzymes under abiotic stress condition might be useful for the seedlings to cope up with oxidative damage by heat stress. The exogenous application of phytohormones enhanced the activities of antioxidant enzymes such as catalase, peroxidase and superoxide dismutase. The present study was conducted at the control condition (ambient) and in the drought stress condition (exposing drought at 45 DAS) to evaluate the influence of phytohormones on the enzyme activities such as catalase, peroxidase and superoxide dismutase. These studies were carried out in the maize seeds COH(M) 8 for identifying the suitable phytohormone for the drought stress mitigation. The foliar spray treatments were carried out in various phytohormones such as salicylic acid @ 75 ppm, brassinolides @ 0.5 ppm and sodium nitroprusside @ 50 µM along with control. The results of the study revealed that the plants foliar sprayed with sodium nitroprusside 50 µM enhanced the activity of antioxidant enzymes viz., catalase, peroxidase and SOD etc., under both ambient condition and at the drought stress when compared to control and other treatments.

Key Words: Drought stress, antioxidant activity, Phytohormones, maize, foliar spray

Introduction

Maize is one of the most important crops being cultivated as a cereal grain. Maize is also called as the 'Queen of Cereals' since it has higher genetic yield potential. Among cereals, maize has highest growth rate with respect to area and productivity. Maize productivity is found to be the highest among food crops which has been increasing over 50 kg/ha/yr since 2010 (IIMR, 2015). During the year 2020-2021, maize production in India is around 30 million tonnes in an area of 9.9 million hectares (agricoop.nic.in).

Climate change can decrease the global maize productivity and grain quality. High temperature induced drought stress affects the growth and development of the plants by a series of morphological, physiological and biochemical changes. Maize plants are sensitive to drought stress and there is a strong decline in grain yield as plants face stress above this threshold for a prolonged duration. Maize crop requires an optimal temperature for better harvest productivity. A suboptimal temperature at any critical stage for a prolonged duration can negatively affect the growth and yield formation processes and cause severe drought condition. The reproductive stage is the most sensitive to drought stress. A swing from the normal condition to severe drought stress decreases the growth rate and grain yield through a decrease in seed setting ratio and disturbance of several physiological processes. It limits pollen viability and silk receptivity, leading to a significant reduction in seed setting and grain yield. Likewise, alterations in the antioxidant enzyme activities under high temperature collectively limit maize productivity.

Phytohormones play an important role under temperature induced drought stress to confer drought tolerance. The enzyme activity is modulated by phytohormones like brassinosteroids, salicylic acid and sodium nitroprusside. The exogenous application of these hormones at optimum concentration can help plants to manage drought condition. Enzymes such as catalase, peroxidase, superoxide dismutase are effective scavengers of reactive oxygen species. These enzyme levels were increased by the application of phytohormones in maize subjected to water deficit stress. With this context, the current study was formulated to evaluate the influence of various phytohormones *viz.*, brassinosteroids, salicylic acid and sodium nitroprusside on enzyme activity of maize under drought stress.

Materials and Methods

The freshly harvested hybrid maize seeds COH (M) 8 obtained from the Department of Millets, Tamil Nadu Agricultural University, Coimbatore has formed the base materials for the present study. The field experiments were carried out in the Department of seed sicence

and technology, Tamil Nadu Agricultural University, Coimbatore. Drought stress was simulated by withholding irrigation at 45 DAS and control plants were raised with full irrigation thorough the period.

The crop was foliar sprayed with phytohormones as detailed below *viz.*, salicylic acid 50 and 75 ppm, brassinolides 0.2 and 0.5 ppm, sodium nitroprusside 50 and 75 µM using knapsack sprayer at two growth stages *viz*, boot leaf initiation stage (40 DAS) and second spray (47 DAS) was given a week after first spray as detailed below to find out the effects of phytohormone on mitigating the drought stress. The growth parameters and physiological parameters were observed and the leaf samples were collected before spray and 3 days after second foliar spray and analysed for the enzyme activity. The experiment was conducted with four replications in Completely Randomized Block Design (CRD).

Treatment details

Foliar spray treatments		**Drought stress**	**Stage of spray**
Control		Simulating drought stress by withholding irrigation @ 45 DAS	First spray - 40 DAS Second spray - 45 DAS
Salicylic acid	50 ppm		
	75 ppm		
Brassinolides	0.2 ppm		
	0.5 ppm		
Sodium nitroprusside	50 µM		
	75 µM		

Catalase (µg of H_2O_2/g/minute)

In the enzymatic estimation of catalase, sodium perborate acts as a hydrogen donor. Higher catalase activity results in lower hydrogen peroxide accumulation. It indicates higher stress tolerance. Catalase requires two hydrogen molecules for conversion of H_2O_2 to hydrogen and oxygen. It has higher turnover reaction.

Procedure

500 mg of leaf sample was weighed and macerated with 10 ml of phosphate buffer. The contents were centrifuged at 3000 rpm for 10 minutes. 1 ml of each supernatant was taken in 5 different beakers. 5 ml of 1.5% sodium perborate and 1.5 ml of phosphate buffer was added. 10 ml of 2 N sulphuric acid was later added at the time interval of 1 min, 2 mins, 3 mins, 4 mins after the addition of enzyme extracts in first four beakers respectively. Simultaneously, blank was prepared by adding 10 ml of sulphuric acid before addition of enzyme extract. The contents were titrated against 0.05 N $KMnO_4$. Pink colour development

persists for 30 seconds which is the end point. The volume of $KMnO_4$ consumed was noted. The enzyme activity was expressed as µg of H_2O_2.

$$\text{Catalase activity} = \frac{x}{1 \times 0.5} \times 10 \times 1 \times 0.85 \text{ µg of } H_2O_2\text{/g/minute}$$

Peroxidase activity (g tissue/min)

500 mg of leaf sample was weighed and macerated with 10 ml of phosphate buffer. The contents were centrifuged at 5000 rpm for 15 minutes. 1 ml of supernatant was taken in a test tube and 3 ml of pyrogallol was added. The contents were transferred to cuvette and it was read as a blank in a spectrophotometer. 0.5 ml of H_2O_2 was added as substrate and change in OD value was recorded at 430 nm for 2 minutes with 30 seconds interval. The difference in OD was calculated and the average of the differences were computed.

$$\text{Peroxidase activity} = \frac{x \times 60 \times 10 \times 1000}{1 \times 30 \times 500}$$

Superoxide dismutase (U mg^1 protein min^{-1})

The 2 ml assay reaction mixture contained 50 mM phosphate buffer (pH 7.8), 2 mM EDTA, 9.9 mM L-methionine and 55 µM NBT,40 µl diluted sample and 20 µl of 1 mM riboflavin. The reaction was started by lighting the materials for 10 minutes using a 15W fluorescent lamp. The test tubes were placed in an aluminium foil-lined box. The test tubes were placed in a box that was slowly oscillating at a distance of about 12 cm from the light source. The tubes with the same reaction mixture were kept in the dark and used as blanks. After the process was stopped, the absorbance of the samples was measured at a wavelength of 560 nm (Beyer Jr and Fridovich, 1987).

Proline content (mg/g)

Proteins were precipitated as a complex during the selective extraction with aqueous sulphosalicylic acid. The extracted proline was made to react with ninhydrin in acid medium conditions to form the chromophore and read at 520 nm.

Preparation of acid ninhydrin

2.5 g of acid ninhydrin was prepared and 600 ml of glacial acetic acid, 40 ml of 6 M orthophosphoric acid was added and stirred well until the contents gets dissolved.

Procedure

500 mg of leaf sample was weighed and macerated with 10 ml of 3% sulphosalicylic acid and the contents were centrifuged at 3000 rpm for 10 minutes. The supernatant solution of 10 ml was taken in a test tube and the acid ninhydrin of 2 ml, glacial acetic acid of 2 ml, 6 M orthophosphoric acid of 2 ml was added. The test tube with the contents were kept in hot water bath for one hour and after that cooled under tap water. The solutions were transferred to a separating funnel and 4 ml of toluene was added. The separating funnel was uniformly shaken for 30 seconds and the formation of two different layers was noted. The colourless bottom layer was discarded and the upper pink colour solution was collected and the OD value was measured at 520 nm.

$$\text{Amount of proline} = \frac{X}{2} \times \frac{10}{500} \times 1000$$

Relative Water Content (%)

Physiologically functional leaf from the top portion was selected for relative water content estimation and 50 uniform leaf discs were made. Fresh weight of the leaf samples were recorded. The leaf discs were made to float in the water for one hour to attain full turgidity. After that, leaf discs were taken out and the excess droplets sticking on the leaf surface were wiped out by using the filter paper. After an hour of floating, turgid weight was recorded immediately. The leaf discs were transferred to a butter paper cover and kept in hot air oven at 80℃ for two days. Then, dry weight was recorded.

Relative water content was calculated using the formula given below,

$$RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Results

Catalase activity

The differences in the catalase activity was found non-significant before spray at both ambient condition and in the drought condition. Statistically significant influence on the

catalase activity was observed due to the foliar spraying with phytohormones for the plants kept at the ambient condition and in the drought stress after spray.

The maximum catalase activity was observed in the leaves foliar sprayed with sodium nitroprusside 50 µM (128.4 µg of H_2O_2/g/minute). The minimum catalase activity was observed in the control (120.0 µg of H_2O_2/g/minute) at the ambient condition. However, the catalase activity was increased in the plants at drought stress. The maximum catalase activity was observed in the leaves foliar sprayed with sodium nitroprusside 50 µM (131.4 µg of H_2O_2/g/minute). The minimum catalase activity was observed in the control (123.4 µg of H_2O_2/g/minute) (Table 1).

Peroxidase activity

The differences in the peroxidase activity was recorded non-significant before spray at both ambient condition and in drought stress condition.

Statistically significant influence on the peroxidase activity was observed due to the foliar spraying with phytohormones for the plants kept under moisture deficit stress after spray. The peroxidase activity was maximum for the plants foliar sprayed with sodium nitroprusside 50 µM (132.1 g tissue/min) whereas the minimum peroxidase activity was observed in the control seeds (128.3 g tissue/min) (Table 2).

Superoxide dismutase

The differences in the superoxide dismutase activity were non-significant before spray at both ambient condition and in the drought stress condition.

Statistically significant influence was observed due to the foliar spraying with phytohormones for the plants kept at the ambient condition and in drought stress condition. The maximum superoxide dismutase activity was observed in the plants foliar sprayed with sodium nitroprusside 50 µM (1.47) whereas the minimum superoxide dismutase activity was observed in the control (1.12) at the ambient condition. However, the catalase activity was increased in the plants kept at the drought stress condition. Among the foliar spraying treatments, the plants foliar sprayed with sodium nitroprusside 50 µM registered maximum superoxide dismutase activity (1.60) followed by salicylic acid 75 ppm (1.40). The minimum superoxide dismutase activity was observed in control (1.10) (Figure. 1).

Proline content

The differences in the proline content was non significant at both ambient condition and under drought stress before spray.

Statistically significant variation was observed due to the foliar spraying with phytohormones for the plants kept at the control condition and in drought condition after spray. The plants foliar sprayed with salicylic acid 75 ppm and sodium nitroprusside 50 µM registered maximum proline content (359 mg/g) when compared to control 322 mg/g at the ambient condition. Similar trend was observed for the plants kept at the drought stress. However, the proline content was increased when compared to the plants kept at the ambient condition. The plants foliar sprayed with sodium nitroprusside 50 µM registered maximum proline content (420) followed by salicylic acid 75 ppm (410). The minimum proline content was observed in control (340) (Table: 3).

Relative water content

Statistically significant variation was observed due to the foliar spraying with phytohormones for the plants kept at the ambient condition and in the drought stress condition. The relative water content (%) was maximum in the plants foliar sprayed with sodium nitropursside 50 µM which recorded 20 in control condition and at control condition and salicylic acid 75 ppm (15 percent) moisture deficit condition respectively. The relative water content was minimum in control 16 and 11 per cent at control/ambient condition and moisture stress condition respectively (Table: 4).

Table 1. Effect of foliar spray with phytohormones on catalase (µg of H_2O_2/g/minute) activity in maize COH (M) 8 under drought stress (45 DAS)

Foliar spray treatments		Ambient condition		Drought stress (45 DAS)	
		Before spray	After spray	Before spray	After spray
Control		119.2.	120.0	115.6	124.5
Salicylic acid	50 ppm	118.5	122.9	115.5	123.4
	75 ppm	117.6	126.4	117.8	125.6
Brassinolides	0.2 ppm	118.1	123.7	116.2	126.9
	0.5 ppm	119.2	123.14	117.9	128.3
Sodium nitroprusside	50 µM	122.7	128.4	116.8	131.4
	75 µM	123.5	125.6	117.8	129.3
Mean		**121.247**	**125.1**	**116.0**	**128.1**
SEd		1.431	1.591	1.831	1.610
CD (P=0.05)		NS	3.308	NS	3.348

Table 2. Effect of foliar spray with phytohormones on peroxidase (g tissue/min) activity in maize COH (M) 8 under drought stress (45 DAS)

Foliar spray treatments		Ambient condition		Drought stress (45 DAS)	
		Before spray	After spray	Before spray	After spray
Control		127.3	128.3	126.5	123.4
Salicylic acid	50 ppm	129.1	130.2	128.4	125.2
	75 ppm	128.3	131.5	127.1	145.7
Brassinolides	0.2 ppm	125.4	133.4	125.5	137.3
	0.5 ppm	129.0	129.6	127.3	134.5
Sodium nitroprusside	50 µM	124.1	132.1	129.9	147.3
	75 µM	123.4	131.6	128.5	145.4
Mean		**127.28**	**130.1**	**129.16**	**139.51**
SEd		1.512	1.702	1.824	1.274
CD (P=0.05)		NS	S	NS	2.650

Figure 1. Effect of foliar spraying with phytohormones on superoxide dismutase activity (U mg^{1} protein min^{-1}) in maize COH (M) 8 under drought stress (45 DAS)

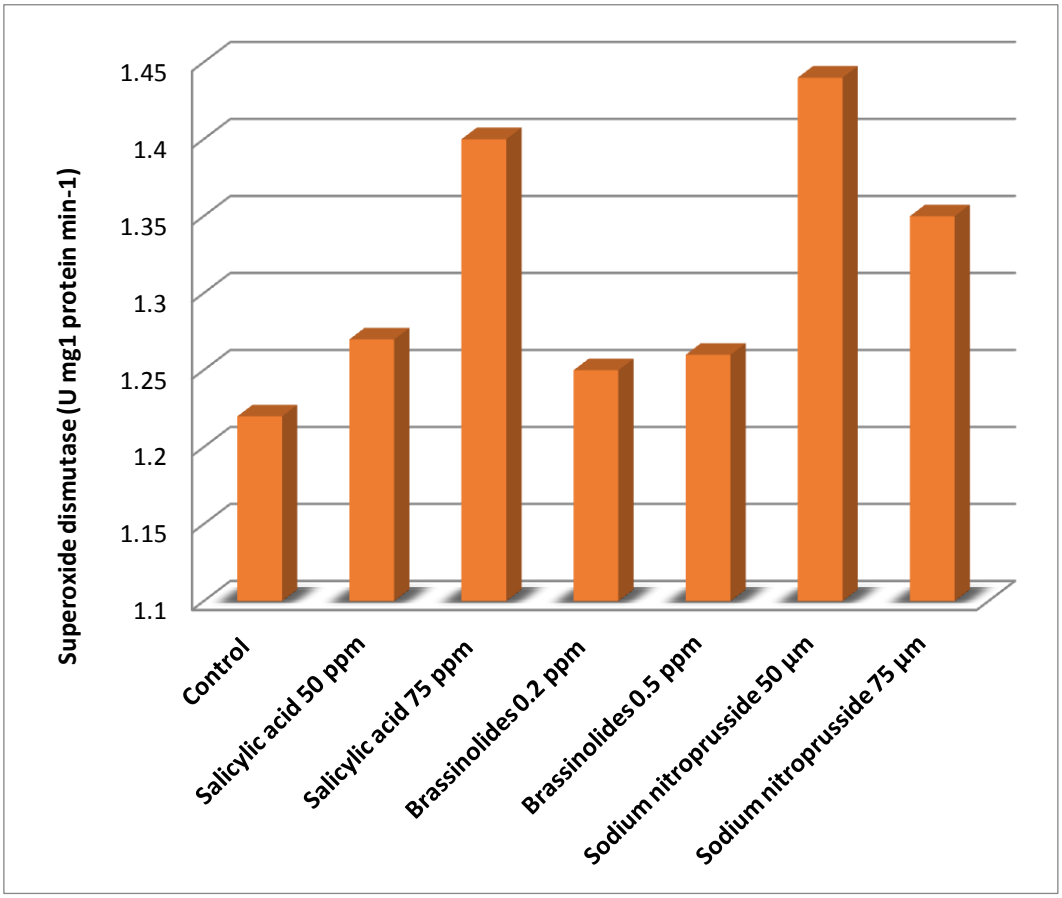

Table 3: Effect of foliar spray with phytohormones on proline (mg g^{-1} DW) content in maize COH (M) 8 under drought stress (45 DAS)

Foliar spray treatments		Ambient condition		Drought stress (45 DAS)	
		Before spray	**After spray**	**Before spray**	**After spray**
Control		334	322	341	340
Salicylic acid	50 ppm	335	343	338	350
	75 ppm	322	359	339	410
Brassinolides	0.2 ppm	329	337	341	360
	0.5 ppm	319	348	342	370
Sodium nitroprusside	50 µM	321	359	329	420
	75 µM	322	362	328	390
Mean		**325.16**	**345.21**	**336.82**	**378.21**
SEd		5.318	6.654	5.551	4.739
CD (P=0.05)		NS	13.839	NS	9.857

Table 4. Effect of foliar spray with phytohormones on Relative Water Content (%) in maize COH (M) 8 under drought stress (45 DAS)

Foliar spray treatments		Ambient condition	Drought stress (45 DAS)
Control		17 (23.57)	12 (19.37)
Salicylic acid	50 ppm	19 (25.10)	14 (21.13)
	75 ppm	17 (25.10)	15 (21.97)
Brassinolides	0.2 ppm	18 (24.33)	14 (21.13)
	0.5 ppm	19 (24.35)	14 (21.13)
Sodium nitroprusside	50 µM	20 (24.35)	15 (21.97)
	75 µM	21 (24.35)	14 (21.13)
Mean		**18.143**	**14.00**
SEd		0.334	0.163
CD (P=0.05)		0.694	0.339

Discussion

The present experimental study are in line with Karpets *et al.* (2011) and Hasanuzzaman *et al.* (2012) who reported that under drought stress, exogenous application of 0.5 mM sodium nitroprusside enhanced the antioxidant enzymes such as catalase, superoxide dismutase and peroxidase, thereby reducing peroxidation of lipids and electrolyte leakage in *Triticum aestivum*. It was positively correlated with enhanced tolerance to heat shock. Peroxidation of lipids and membrane leakage was reduced up to 48% when compared with the controls in mung bean leaf discs treated with sodium nitroprusside (Yang *et al.*, 2006). It also resulted in increased activity of enzymes such as RuBisCo, carbonic anhydrase, nitrate reductase, as well as enhanced osmolytes such as proline, glycine betaine in*Solanumlycopersicum* (Siddiqui *et al.*, 2017). Upregulation of enzymes such as sucrose phosphate synthase, small heat shock protein 26 and delta-1-pyrroline-5-carboxylate synthase gene transcription were found in *Oryza sativa* seedlings under drought stress (Uchida *et al.*, 2002). Sodium nitroprusside stimulated the activities of L-cysteine disulfhydrase, thereby endorsing the accumulation of H_2S and resulting in high heat survival percentage in maize seedlings (LI *et al.*, 2013). Under drought stress, sodium nitroprusside presoaked *Phaseolus radiatus* leaf discs reduced H_2O_2 production (Yang *et al.*, 2006; Hasanuzzaman *et al.*, 2013). Pre-treatment of rice plants with 1μM sodium nitroprusside ameliorated moisture stress while application of more than 100 mM sodium nitroprusside had a negative impact on growth (Uchida *et al.*, 2002).

In the field conditions, the plants foliar sprayed with salicylic acid 75 ppm recorded the catalase, peroxidase and superoxide dismutase enzyme activity of 133.4 µg of H_2O_2/g/minute, 140.9 g tissue/min and 1.44 U mg^1 protein min^{-1} respectively which is higher than control. The exogenous application of phytohormones such as salicylic acid reduces the damaging effects of abiotic stress in plants (Hasanuzzaman *et al.*, 2014). Salicylic acid improved plant tolerance to drought stress (Larkindale *et al.*, 2005; Wang *et al.*, 2010; Khan *et al.*, 2013a,b). Similarly, Khan *et al.* (2013b) observed that treatment of 0.5 mM salicylic acid can mitigate heat stress in *Triticum aestivum* by restriction of the stress ethylene formation under drought stress. Salicylic acid can control various aspects of plant responses under both stress conditions and optimum environments through cross-talk signaling with other phytohormones (Horváth *et al.*, 2007; Asensi-Fabado and Munné-Bosch, 2011; Khan *et al.*, 2012a,b, 2013b, 2014). In the field conditions, the plants foliar sprayed with brassinolides 0.5 ppm recorded the catalase, peroxidase and superoxide dismutase enzyme activity of 130.4 µg of H_2O_2/g/minute, 133.2 g tissue/min and 1.26 U mg^1 protein min^{-1} respectively which is

higher than control. Brassinolides were reported to play a positive role in mitigating the drought stress (Confraria *et al.,* 2007). Exogenous application of brassinolides increases the production of many antioxidant enzymes in many plant species. Increased production of superoxide dismutase and peroxidases were seen in drought stressed rice seedlings with the exogenous application of brassinolides (Cao *et al.,* 2008). Therefore, scavenging of reactive oxygen species gets enhanced by brassinolide application which provide increased tolerance to oxidative stress induced by drought. Brassinolides are known to mitigate various stresses including temperature stress. Hayat *et al*. (2012) observed that treatment of 28-homoBL to *Vigna radiata* plants mitigated the stress generated by insufficiency in water by improved membrane stability index, leaf water potential, increased activities of antioxidative enzymes as well as proline levels.

Conclusion

The heat stress exposure during the reproductive stages brought severe negative effects on the plant metabolism of maize which ultimately results in poor growth and reduced yield. The exogenous application of phytohormones at optimum concentrations can reduce the productivity loss under the stress conditions. The present study revealed that among various phytohormones used, the foliar spray with sodium nitroprusside 50 µM performed well by enhancing the enzyme activities under both ambient condition (34±2℃) and at the elevated temperature of 42℃ in the Open Top Chamber. The exogenous application of sodium nitroprusside @ 50 µM can be recommended for the heat stress mitigation by enhancing the antioxidant enzyme activities.

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