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Exogenous Application of Phytohormones on Growth, Flowering Phenology, Enzyme Activity and Seed Yield of Maize Under Heat Stress

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ABSTRACT

Nowadays, the air temperature has risen as a consequence of climatic variability. The extremely high temperature causes heat stress and serious injuries to plant growth and development. Maize plants can operate most of its physiological process normally in the range of 0-400C. Heat stress reduces the uptake of water and nutrients which results in the reduction of plant growth, the source-sink relationships gets altered and it reduces the hormonal content which is being produced in roots and it affects the nutrient concentration of the plants which in-turn affect the productivity by causing injury to the plants.

To mitigate the heat stress, a field experiment was carried out in the high-temperature zone at Agricultural Research Station, Bhavanisagar, TNAU, India to reduce the heat stress-induced squandering to maize (Zea mays L.) plants with exogenous application of phytohormones during two consecutive years 2021 and 2022. The foliar spray treatments with phytohormones include salicylic acid 50 and 75 ppm, brassinolides 0.2 and 0.5 ppm, and sodium nitroprusside 50 and 75 µM along with control. The experiment was laid out in a Randomized Block Design (RBD) with four replicates. The result revealed that plants foliar sprayed with sodium nitroprusside 50 µM and salicylic acid 75 ppm at 40 and 47 days after sowing recorded the maximum plant height, early flowering, pollen viability, chlorophyll content and enzyme activities viz., catalase, peroxidase, and superoxide dismutase when compared to control. The yield parameters such as cob length, cob weight, and seed yield were significantly influenced by the foliar spraying treatments. The plants foliar sprayed with sodium nitroprusside 50 µM outperformed the other treatments during 2021 and 2022 by recording the highest seed yield by modulating the heat stress-induced losses to maize plants whereas the minimum yield was recorded in control.

Keywords: *Phytohormones, Heat stress, Flowering phenology, Enzyme activity, Seed yield, Exogenous application, Maize, Crop growth*

INTRODUCTION

Maize is one of the most important crops cultivated as a cereal grain. Maize is also called as the 'Queen of Cereals' since it has higher genetic yield potential. It has been domesticated about 10,000 years ago by the native people of southern Mexico. It is now almost grown in all countries throughout the year under a more varied range of climate. Maize has also occupied an important position in the industries since 12% of maize is used for industrial purposes and 14% is used in the starch industries,7% is used as a processed food and 6% is used for the export and other purposes.

In India, 83% of the maize area is grown under Kharif season and 17% is grown under Rabi season. Owing to the occurrence of various biotic and abiotic stresses, around 70% of Kharif maize area is grown under rainfed conditions. The susceptible ecosystem to stress contributes to lower productivity. Heat stress is a major concern for crop production. Approaches for sustaining higher yields under heat stress are the main agricultural goals. Plant responses vary with degree as well as the duration of heat stress and also the plant type. The growth and productivity of maize also gets decreased due to temperature fluctuations[1]. An increase in temperature causes serious damage to the plants.

External application of various phytohormones were found to be effective in heat stress mitigation. Plant hormones like salicylic acid, brassinolides and sodium nitroprusside play an important role in alleviating heat stress by acting as messenger molecules and also plays a serious role in the growth and development of plants [2]. Plant hormones play a vital role in imparting thermo tolerance. They are vigorously involved in the response of plants to high-temperature stress and protect the plants from high temperature stress. The hormonal cross talk coordinates the defense responses in plants to heat stress.

Foliar application of brassinolides protects the plants through amplification responses to heat stress and saving the expression of plant growth promoters. Foliar application of sodium nitroprusside reduced heat stress by decreasing ion leakage and increasing the activity of antioxidant enzymes [3].

The effectiveness of exogenously applied phytohormones at optimum concentration for protecting the crop against high temperatures needs to be studied. So that crop production can be sustained under stressful environmental conditions.

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Considering the above facts, the present study was conducted in 2021 and 2022 with the objective to assess the performance of exogenous application of phytohormones in mitigating heat stress on growth and yield of maize hybrid COH (M) 8 under field conditions.

MATERIALS AND METHODS

The field experiment was laid out in the high temperature zone at Agricultural Research Station, Bhavanisagar, Erode District, TNAU, India to reduce the heat stress-induced squandering to maize (Zea mays L.) plants with exogenous application of phytohormones during two consecutive years 2021 and 2022. The foliar spray treatments with phytohormones include salicylic acid 50 and 75 ppm, brassinolides 0.2 and 0.5 ppm and sodium nitroprusside 50 and 75 µM along with control. The experiment was laid out in a Randomized Block Design (RBD) with four replicates on 12th March 2021 and 2022. The crop was foliar sprayed with above-mentioned phytohormones using a knapsack sprayer at two growth stages viz.,, boot leaf initiation stage (40 DAS) and second spray (47 DAS) was given a week after the first spray to find out the effects of phytohormone on mitigating the temperature stress. The unsprayed plot served as control.

The maize crop was grown with recommended package of practices in addition to foliar spray treatments with phytohormones. The weather statistics from maize sowing to final harvesting was collected from the observatory of Agricultural Research Station, Bhavanisagar during 2021 and 2022 (Table 1). The growth parameters and physiological parameters were observed and the leaf samples were collected 3 days after the second foliar spray and analyzed for enzyme activity.

Data collection:

The growth parameters on plant height (cm) at 60 and 90 days after sowing, days to first flowering, and days to 50% flowering were recorded. Pollen grains from the anthers of randomly selected ten spikelets from each treatment were collected on to measure the pollen viability [4]. Standard protocols were adopted to measure relative water content (%)[5], chlorophyll content [6] and enzyme activities viz., proline content[7], catalase and peroxidase activity [8] superoxide dismutase [9] from the leaves collected three days after the second spray.

The yield and yield attributing parameters on cob length (cm), cob weight (g), number of rows cob-1, number of seeds row-1, number of seeds cob-1 were recorded after harvest, shelled manually to record 1000 seed weight (g), seed yield kg plot-1 and seed yield kg ha-1. The shelling percentage was calculated using the weight of cob and seed. It is the ratio between the seed weight and cob weight and expressed in percentage.

$$\text{Shelling}(\%) = \frac{\text{Weight of seed}}{\text{Weight of cob}} \times 100$$

Statistical analysis

The data obtained from various experiments were analysed for the 'F' test of significance [10]. Wherever necessary, the per cent values were transformed to angular (Arc-sine) values before analysis. The critical difference (CD) was calculated at 5 per cent (P = 0.05) probability level and wherever 'F' value is non-significant it is denoted by 'NS'.

RESULT AND DISCUSSION

Effect of foliar spray with phytohormones on growth characters Plant height (cm)

The plant height was significantly influenced by the foliar spaying with phytohormones during both growing years (Table 1). Salicylic acid 75 ppm recorded the highest plant height of 200.5 cm and 234.5 cm at both 60 and 90 DAS, respectively. The lowest plant height of 175.5 and 209.3 cm on 60 and 90 DAS was observed in control during 2021. A similar trend was recorded in the raised during 2022 also (Table 2).

Days to first flowering

Days taken to first flowering were significantly influenced by the foliar spraying treatments. Plants foliar sprayed with sodium nitroprusside 50 and 75 µM, salicylic acid 75 ppm flowered 3 days earlier (49 days) when compared to control (52 days) (Table 2).

Days to 50% flowering

Statistically significant variation was noticed for days taken to 50% flowering among the foliar application with phytohormones. Days taken for 50% flowering was 51 days in plants foliar sprayed with sodium nitroprusside 50 µM and salicylic acid 75 ppm while control seeds have taken 54 days for 50% flowering during 2021 and 52 days in plants foliar sprayed with sodium nitroprusside 50 µM while control seeds has taken 54 days for 50% flowering during 2022 (Table 2).

Pollen viability (%)

Pollen viability (%) was highly significant due to the foliar spaying with phytohormones. The maximum viability (85 and 86%) was registered for the plants foliar sprayed with sodium nitroprusside 50 µM and the minimum viability was recorded in control (68 and 70 %) during both the crop growing years (Table 2).

Effect of foliar spray with phytohormones on enzyme activity Catalase activity (µg of H2O2/g/minute)

Statistically significant influence on the catalase activity was observed due to the foliar spray with phytohormones. Plants foliar sprayed with sodium nitroprusside 50 µM registered maximum catalase activity (135.1 and 131.6µg of H2O2/g/minute) while minimum catalase activity was recorded in control (125.4 and 119.5 µg of H2O2/g/minute) during both the crop growing years (Table 3).

Peroxidase activity (g tissue/min)

The differences in the peroxidase activity was significant due to the foliar spray with phytohormones. The peroxidase activity was maximum in sodium nitroprusside 50 µM (140.9 and 137.3 g tissue/min). The minimum peroxidase activity was recorded in control (130.2 and 124.3 g tissue/min) during both the crop growing years (Table 3).

Superoxide dismutase activity (U mg1 protein/ min)

The differences in the superoxide dismutase activity were significant among the foliar spraying treatments. The maximum superoxide dismutase activity was observed in sodium nitroprusside 50 µM (1.44 and 1.42 U mg1 protein/min). The minimum superoxide dismutase activity was observed in control (1.22 and 1.23 U mg1 protein/ min) during both the crop growing years (Table 3).

Proline content(mg/g)

The differences in the proline content was significant due to the foliar spray with phytohormones. The maximum proline content was observed in sodium nitroprusside 50 μM (430 and 428 mg/g) whereas the minimum proline content was observed in control (330 and 322 mg/g) during both the crop growing years (Table 3).

Chlorophyll content (mg/g)

The plant foliar sprayed with salicylic acid 75 ppm recorded the maximum chlorophyll-a of 0.445mg/g,chlorophyll'b'of 0.612mg/g and total chlorophyll of 0.956 mg/g which was on par with sodium nitroprusside 50μM (0.921mg/g) (Table3) whereas the content of chlorophyll 'a' of 0.370 mg/g, chlorophyll 'b' of 0.528 mg/g and total chlorophyll of 0.852 mg/g content was minimum in control during 2021 and the similar trend was registered in crop raised during 2022 also (Table 3).

Effect of foliar spray with phytohormones on yield attributing characters and yield in Cob length (cm)

Results showed that cob length was significantly influenced by the foliar spraying treatments during both the crop growing years. During both the crop growing years, the cob length was longest for the plants foliar sprayed with salicylic acid 75ppm (20.7 and 23.8cm) and sodium nitroprusside 50 μM (20.5 and 23.9 cm) whereas the shortest cob length was recorded by the control seeds (17.8 and 20.1 cm) (Table 4).

Cob weight (g)

The difference in cob weight was statistically significant among the foliar spraying treatments. Among the foliar application, foliar spraying with sodium nitroprusside 50 μM recorded the highest cob weight (171.50 and 174.57 g) followed by salicylic acid 75 ppm (169.41 and 170.25g) when compared to control (158.8 and 161.80 g) in both the crop growing years (Table 4).

No. of seeds /row

The difference in the number of seeds per row was statistically significant among the foliar spraying treatments. Among the foliar application, sodium nitroprusside 50 μM (39 and 48) recorded more seeds per row which was on par with salicylic acid 75ppm (38 and 46) when compared to the control (32 and 35) (Table 4).

No. of seeds/cob

The difference in the number of seeds/cob was statistically significant among the foliar spraying treatments during both the crop growing period. More number of seeds/cob was recorded for the plants foliar sprayed with sodium nitroprusside 50 μM (546 and 545) whereas minimum no. of seeds/cob was recorded in control, brassinolides 0.2 and 0.5 ppm (448 and 453 seeds) (Table 4).

Shelling (%)

Statistically significant influence was observed on the shelling (%) due to the foliar spraying with phytohormones. Among the foliar application, shelling (%) was maximum for the plants foliar sprayed with sodium nitroprusside 50 μM (78%) while the minimum shelling per cent was observed for control (71 and 72 %) during both the crop growing period (Table 4).

1000 seed weight (g)

Foliar spraying with phytohormones had no significant influence on the seed weight (Table 4).

Seed yield/plot (kg)

The seed yield plot-1 differed significantly among the foliar spraying treatments during both the crop growing period of 2021 and 2022. The seed yield recorded from the plant's foliar sprayed with sodium nitroprusside 50μM was higher (8.6 and 9.3 kg) when compared to control (7.5 and 8.1kg) (Table 4).

Seed yield/ha (kg)

The seed yield per ha showed statistically significant variation due to the foliar spraying treatments. Among the foliar spraying treatments, plants foliar sprayed with sodium nitroprusside 50 μM (7166 and 7217 kg) whereas minimum seed yield was recorded in control (6250 and 6247 kg) during both the crop growing period (Table 4).

DISCUSSION

Heat stress causes a lot of physiological changes in plants especially in the disruption of the enzymes which are capable of solarization and crystallization because of high temperature. This in turn affects the metabolic process such as photosynthesis and respiration. The application of phytohormones alleviated different stresses in plants caused by different temperatures. The recent changes in the environment might be due to various reasons especially global warming is posing as ever threat to plants due to temperature fluctuations. In recent times, the exogenous application of phytohormones viz., salicylic acid, brassinolides and sodium nitroprusside have been found effective in mitigating high temperature stress induced damage in plants [11].

The increase in temperature beyond the optimum level is one of the most important environmental factors influencing metabolic processes of the plants. It mainly affects the reproductive growth and development leading to more yield losses in maize. Hence, the present experiment was carried out to study the impact of temperature stress on plant growth and development. The results revealed a significant negative impact on crop growth and pollen. The exogenous application of foliar treatments included salicylic acid, brassinolides and sodium nitroprusside imparted a visible effect in mitigating the effects of heat stress which was confirmed by the activities of antioxidant enzymes. The activity of catalase, peroxidase and superoxide dismutase were significantly higher in the treated plants. The present study revealed that the application of foliar treatments with sodium nitroprusside 50 μM and salicylic acid 75 ppm at 40 and 47 DAS had a positive impact on the growth and yield of maize by alleviating the stress-induced metabolic irregularities compared to control under temperature stress.

The major threat to global food security is the increased temperature during the flower development and reproductive phase which leads to reproductive sterility [12]. Heat stress influences the crop yield by influencing reproductive components during development that contribute to a reduction in the harvest index and these responses vary with the severity and stress duration [13]. Heat stress reduces the flowering number, branches, and thus the number of flowers per plant. Heat stress disturbs male and female gametophytes which results in poor pollen viability, poor pollen germination, and poor seed set [14].

Heat stress is accompanied by oxidative stress as noticed by the burst of hydrogen peroxide in cells after high temperature exposure [15] Reactive oxygen species production is triggered when plants are subjected to oxidative stresses. Among the cellular and molecular structures, damage to chloroplasts and the photosynthetic pigments decrease the photosynthetic capacity of the plant thus reducing yield [16]. To combat the generation of reactive oxygen species, plants produce the antioxidant enzymes such as superoxide dismutase, catalase and peroxidase [17].

The exogenous application of phytohormones such as sodium nitroprusside @ 50 µM, salicylic acid @ 75 ppm, and brassinolides @ 0.5 ppm when compared to control either through seed soaking and foliar spray was found effective in mitigating heat stress in maize which is proved by the field study conducted at high-temperature zone at Agricultural Research Station, Bhavanisagar, Erode District, Tamil Nadu, India since it helps in adapting the plant to heat stress and significantly reduces the effect of high temperature.

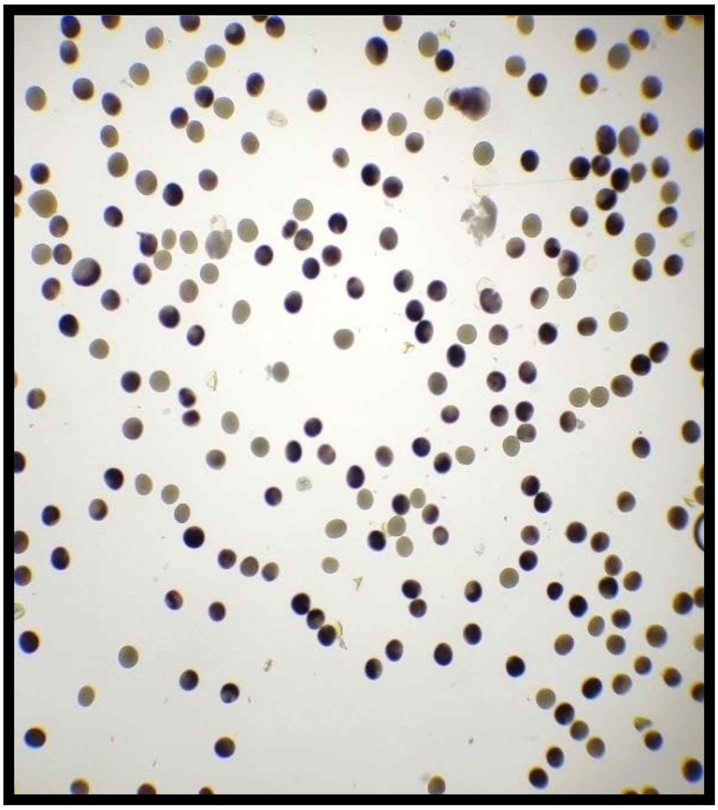
Effect of phytohormones on growth parameters of maize

To know the influence of phytohormones under heat stress the plants foliar sprayed with salicylic acid 75 ppm at 40 & 47 DAS recorded the maximum plant height of 234.5cm. The minimum plant height was observed in control. This is due to the fact that exogenous application of salicylic acid in seedlings causes stimulation in cell division in apical meristem [18]. In Cicer arietinum, foliar application of salicylic acid 0.1 µM increased the plant growth, shoot and root biomass, nodule number, and leghemoglobin content [19]. The exogenous application of salicylic acid helps in improving plant growth and development and protects the plant membranes under heavy metal stress [20]. In Rosmarinus officinallis, salicylic acid application of 100-300 ppm induced the plant growth specifically branching and biomass [21].

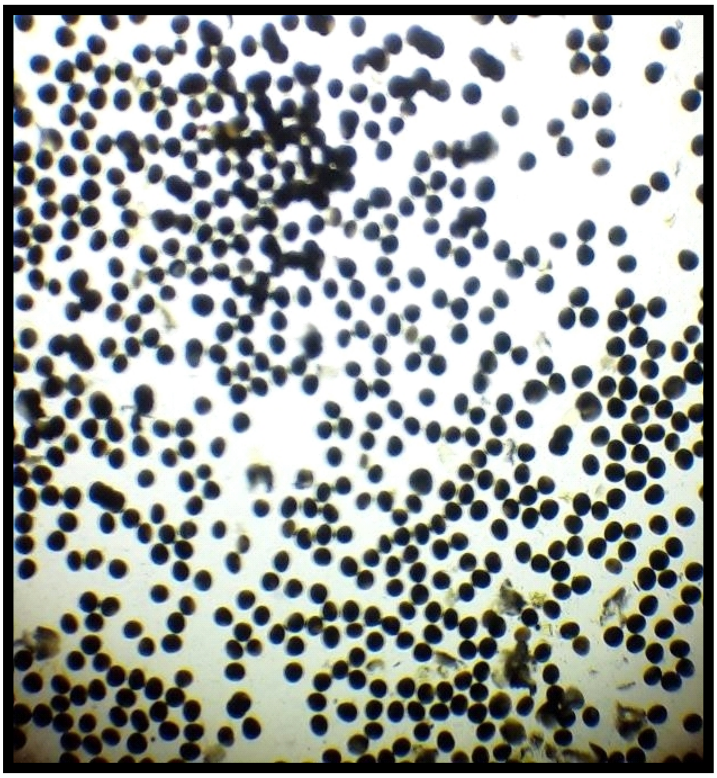
Plants foliar sprayed with sodium nitroprusside 50 µM flowered early by 3 days when compared to control. Foliar spray of sodium nitroprusside 150 and 300 µM concentrations enhanced flowering in chickpea under chilling stress. Sodium nitroprusside 150 and 300 µM resulted in a significant increase in all the morphological characters viz., plant height, number of leaves, leaf area and leaf area index over the control at all the stages. Salicylic acid treatment enhanced flowering and alleviated stress [22] in addition to increased flowering and reduced senescence of flowers [23].

The pollen viability was observed in plants foliar sprayed with phytohormones revealed that plants foliar sprayed with sodium nitroprusside 50 µM recorded the maximum pollen viability of 85% whereas the minimum pollen viability was observed in control (68%). Pollen abortion caused by different stresses is always a result of the abnormal development of tapetum [24], which directly contacts the male gametophyte and plays a serious role in the development and maturation of microspores [25]. However, this tissue is susceptible to abiotic stress, especially during the meiosis stage [26]. Sodium nitroprusside is a signaling molecule capable of improving the growth and physiological function of plants under abiotic and biotic stress conditions [27]. The sodium nitroprusside application of 10 µM had significantly improved the biomass of salt stressed plants [28]. Pollen viability, stigma receptivity and ovule viability was reduced in plants exposed to heat stress but increased with the exogenous application of sodium nitroprusside as a consequence of the improvement in leaf and anther function to significantly increase the pod as well as the seed numbers in heat-stressed lentil plants [29].

Plate.1 Effect of sodium nitroprusside 50 µM on pollen viability (%)



Control



Sodium nitroprusside @ 50µM

Effect of phytohormones on biochemical / enzyme activity in maize

The enzyme activities such as catalase, peroxidase and superoxide dismutase and the osmolyte proline were increased in the plants foliar sprayed with phytohormones such as salicylic acid, sodium nitroprusside and brassinolides when compared to control during the high-temperature stress conditions. The enzyme activities and proline content were more in plants foliar sprayed with sodium nitroprusside 50 µM followed by salicylic acid75 ppm.

In the field conditions, the catalase (135.1µg of H2O2 / g/ minute) and superoxide dismutase activity (1.44 U mg1 protein / min) was higher in the plants foliar sprayed with sodium nitroprusside 50 µM. The peroxidase activity was higher in the plants foliar sprayed with salicylic acid 75 ppm (140.9 g tissue/min). The minimum enzyme activities were observed in the control.

The present experimental study are in line with [30] [31] who reported that under heat stress, at 43°C, 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 [32]. 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 Solanum lycopersicum [33]. 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 heat stress [34]. Sodium nitroprusside stimulated the activities of L-cysteine di sulfhydryls, thereby endorsing the accumulation of H2S and resulting in high heat survival percentage in maize seedlings [35]. Under heat stress, sodium nitroprusside pre soaked Phaseolus radiatus leaf discs reduced H2O2 production [32] [36]. Pre-treatment of rice plants with 1µM sodium nitroprusside ameliorated heat stress while application of more than 100 mM sodium nitroprusside had a negative impact on growth [34].

The total chlorophyll content was maximum in plants foliar sprayed with sodium nitroprusside 50 µM (0.956 mg/g) which is higher than control. This was supported by [37] that application of sodium nitroprusside led to higher levels of chlorophylls a and b due to less degradation of chlorophyll under heat stress. Regular function of biological membranes under stress is critical for important physiological processes such as respiration and photosynthesis. Premature loss of chlorophyll due to heat stress has been reported earlier [38]. Chlorophyll is photo oxidized under high temperatures [39], increase in the chlorophyll content under high temperatures could be a sign of heat stress tolerance [40].

However, decrease in chlorophyll contents was significantly improved by the application of sodium nitroprusside which might be due to the inhibition of reactive oxygen species production or sustaining the stability of photosynthesis [41]. The results of the current study are in line with [42] they indicated that sodium nitroprusside application could improve chlorophyll contents, transpiration rate and photosynthetic efficiency under stress conditions. The sodium nitroprusside application under stress conditions improved photosynthetic pigments by protecting cell membranes containing chlorophyll [43].

Effect of foliar spray with phytohormones in mitigating temperature stress on yield parameters of maize under field conditions

Plant yield is very important in the agricultural point of view to feed food for the growing population. Heat stress is considered as major factor for all important agricultural crops.

The present study clearly examined that the plants foliar sprayed with phytohormones performed well by recording the yield attributing parameters in the field conditions when compared to control under high-temperature stress conditions. The plants foliar sprayed with sodium nitroprusside 50 µM registered the maximum cob length (20.7cm), cob weight (171.5g), the maximum number of seeds per row (39) and a number of seeds per cob (546) followed by salicylic acid 75 ppm and minimum was observed in control (Plate 2). The maximum yield was observed in sodium nitroprusside 50 µM (7166 kg/ha) which is on par with salicylic acid 75 ppm (7083 kg/ha) whereas minimum yield was recorded in control (6250 kg/ha).

Plate.2 Influence of phytohormones on the cob characters of maize COH (M)

T_1 – Control	**T_5 – Brassinolides @ 0.5 ppm**
T_2 - Salicylic acid @ 50ppm	**T_6 – Sodium nitroprusside @ 50**
T_3 - Salicylic acid @ 75 ppm	**µM T_7 – Sodium nitroprusside @**
T_4 – Brassinolides @ 0.2ppm	**75 µM**

Exogenous application of sodium nitroprusside could effectively increase the growth and yield of wheat plants under salt stress. The increase in plant growth and grain yield is positively correlated with the sodium nitroprusside which mitigates salt induced over production of reactive oxygen species possibly by upregulating the antioxidant enzymes. Exogenous application of sodium nitroprusside as seed priming treatment was also found effective in maintaining the plant water relations by upregulating the proline synthesis when sodium nitroprusside of 0.1 mM was applied. Overall, it is recommended that a comparatively low concentration of sodium nitroprusside can be used as a seed priming treatment in wheat for better growth and yield in salt-stressed conditions [44]. Yield components recorded the highest values in plants foliar sprayed with phytohormones; number of grains, spikelets per plant, grain weight per plant except in 1000 grains weight, which showed non-significant difference with all other plants irrigated by different salinity levels. Our results were in harmony with [45] who found that foliar spray with sodium nitroprusside significantly increased yield per plant and number of seeds per plant in wheat and improved grain yield per plant under salt stress.

CONCLUSION

The plants are foliar sprayed with sodium nitroprusside 50 µM at 40 and 47 DAS recorded the maximum growth, enzyme activity and seed yield under heat stress condition.

FUTURE SCOPE OF THE STUDY

External application of phytohormone namely sodium nitroprusside play a vital role in imparting thermo tolerance, alleviating the heat stress by acting as messenger molecules and involved in growth and development of plants. The hormonal cross talk coordinates the defence responses in plants to heat stress. In the present study, foliar application of sodium nitroprusside to maize crop incrased the seed yield under heat stress condition. Similarly, influence of external application of phytohormone to other crops may also be studied.

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