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EXOGENOUS APPLICATION OF PLANT GROWTH PROMOTERS AND THERMOTOLERANCE-INDUCED SEEDS IMPROVES THE GROWTH, YIELD AND YIELD COMPONENTS OF SUMMER MAIZE

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ABSTRACT

Heat stress is a major challenge for maize production; it influences the yield and quality of the crop. Therefore, there is an urgent need to develop new strategies and approaches to improve the heat tolerance of maize under global warming scenarios. The present study investigated the different physiological methods for mitigating heat stress in maize under natural heat stress conditions during *summer*. The experiment was laid out in the split-plot design. The main treatments consisted of two maize hybrids, namely RCRMH-2 and 900MG, and the sub-treatments consisted of different mitigating measures such as foliar application of salicylic acid (200ppm), silicon (200ppm), calcium chloride (2000ppm) and cytokinin (20ppm) and thermotolerance-induced seeds. The results showed that the hybrid RCRMH-2 performed better for most of the physiological parameters and recorded a significantly higher yield by 10.58% than 900MG. Mitigating measures had varied effects on different physiological parameters and hybrids. Cytokinin treatment enhanced photosynthesis and cob size, while silicon application increased transpiration and cob weight and salicylic acid treatment boosted the number of grains per cob. The foliar application of cytokinin (20 ppm) was the most effective and increased the yield by 29.40%, followed by silicon treatment by 25.19% over control plantsnatural under heat stress compared to control plants. The study revealed new methods to enhance the heat tolerance and yield of maize under global warming conditions.

Key words : Heat stress, Maize, Thermotolerance induced seeds, Mitigation, Climate change.

Introduction

The survival of life on Earth is in jeopardy due to the persistent problem of global warming. The National Oceanic and Atmospheric Administration (NOAA) temperature records show that the planet has been heating up by 0.08°C every ten years since 1880 and the pace has doubled to 0.18°C since 1981. The global mean temperature is increasing by 0.3°C every ten years and could reach about 3°C higher than the current level by 2100 (IPCC, 2023). This increase is largely caused by human activities, especially the emissions of greenhouse gases such as CO_2, CH_4 and N_2O.

Maize (*Zea mays* L.) is a diverse cereal crop that grows in various climates and has different types and uses. Maize is an essential crop for billions of people for food, feed and industry. The world produces about 1147.7 million metric tonnes of maize from 193.7 million ha with an average yield of 5.75 t ha^{-1}. Maize is a major industrial crop used for feed, starch and bio-fuel industries in 83% of its production (FAOSTAT, 2021). Global warming severely affects maize and its yield decreases by an average of 7.4 ± 4.5% for every degree Celsius increase in global mean temperature (Zhao *et al.*, 2017). Heat stress impacts maize growth and development and is most harmful during the reproductive phase, when it causes dryness of silks, pollen sterility, poor seed setting and grain abortion. Heat stress also lowers the photosynthesis, biomass accumulation and grain filling of maize plants (El-Sappah *et al.*, 2017). Physiological and biochemical interventions for heat tolerance involve applying plant growth regulators, antioxidants, osmoprotectants and other substances that enhance the heat tolerance of maize plants by modulating their stress responses (Waqas *et al.*, 2021). Otherwise, for every 1°C increase in temperature, maize yields would decline by 7.4% (Zhao *et al.*, 2017).

Salicylic acid (SA) is a phenolic compound that acts as a plant signalling molecule to induce heat tolerance. SA activates the expression of heat shock proteins, which are molecular chaperones that protect cellular proteins from denaturation and aggregation under high temperatures. SA also boosts the activity of antioxidant enzymes, which eliminate the ROS produced by heat stress and prevent oxidative damage to the cell membrane and organelles (Khanna *et al.*, 2016). SA also raises the proline content, a compatible solute stabilising the cell membrane, maintaining the water potential and acting as an osmoprotectant under heat stress. SA also regulates the stomatal conductance and transpiration rate, affecting the leaf temperature and water status of maize plants under heat stress (Iqbal *et al.*, 2020a).

Calcium chloride ($CaCl_2$) is a salt that protects plants from heat-induced oxidative damage by modulating calcium and calmodulin signalling (Shen *et al.*, 2022). Calcium and calmodulin are involved in the expression of heat shock proteins, which help the prevention of protein denaturation and aggregation under high temperatures. Calcium chloride also reduces other abiotic stress in maize by enhancing germination and seedling growth (Lin *et al.*, 2019).

Silicon (Si) is a beneficial element that improves the performance of crops under heat stress by triggering physio-biochemical mechanisms. Si raises the photosynthetic pigments, such as chlorophyll and carotenoids, which improves the light harvesting and energy conversion in leaves (Amin *et al.*, 2018). Si also lowers the transpiration rate and stomatal conductance, reducing the leaf temperature and water loss under heat stress. Si also activates the antioxidant enzymes, which eliminate the ROS and prevent oxidative damage to the cell organelles (Ahmed *et al.*, 2023).

Cytokinins (CK) are phytohormones that help plants with growth, development and stress. They work by controlling cytokinin-target gene expression through phosphorylation cascades (Li *et al.*, 2023). The study showed that a synthetic compound that prevents cytokinin breakdown increased heat stress tolerance in maize plants when used with heat acclimation, which increased cytokinins in roots and auxin in all tissues after heat shock treatment. The study also suggested that heat stress activates a CK signalling pathway through proteins and Ca^{2+} ions to regulate osmolytes for heat tolerance in maize (Prerostova *et al.*, 2020).

The temperature induction response (TIR) technique is widely used to screen and identify thermotolerant genotypes in various crop species. It involves exposing seeds or plantlets to a sublethal temperature (induction temperature) followed by a lethal temperature (challenging temperature) and measuring the survival and growth of the seeds or plantlets after a recovery period (Dar *et al.*, 2016). The principle behind this technique is that the induction temperature induces the heat shock proteins (HSPs) and other stress responses that protect the seeds and plantlets from the lethal temperature (Raghavendra *et al.*, 2017). The TIR has been successfully standardised in maize at the seed level by Raviteja *et al.* (2023) and was employed in the current investigation to study its performance under natural heat-stress conditions.

Therefore, we hypothesised that the exogenous application of the different plant growth regulators, thermotolerance-induced seeds and silicon application possibly improves the grain yield and yield components by influencing the physiological processes of maize under high temperatures. Thus, the present study was designed to evaluate the potentials of foliar application of salicylic acid (SA), calcium chloride ($CaCl_2$) Silicon (Si), cytokinin (6-Benzyl adenine) and thermotolerance-induced seeds on different physiological traits, yield and yield components of maize under natural heat stress conditions.

Materials and Methods

Plant materials

The RCRMH-2 and 900M Gold (900MG) were used in the present study. The RCRMH-2 is a high-yielding, medium-duration maize hybrid released from the University of Agricultural Sciences, Raichur, Karnataka, India. The 900MG is a high-yielding hybrid from Monsanto Pvt. Ltd.

Location of the experiment

The experiment was conducted at the Agricultural Research Station, Bheemarayanagudi, University of

Agricultural Sciences, Raichur, during the summer of 2022 and 2023, which is situated in the North-Eastern dry zone of Karnataka (Region II, Zone II) located between 160 43' N and 760 51' E longitude at an elevation of 411.75 meters above MSL characterised by dry climate with an average annual rainfall of 774.1 mm.

Weather parameters

The experiment was conducted under natural high-temperature conditions from March to July of 2022 and 2023 that matched the maize plants' flowering and grain-filling in filling stages. The weather data, such as maximum and minimum temperature, maximum and minimum relative humidity and rainfall, were collected during the crop growth period in 2022 and 2023. The data showed a rise in temperature and RH. The highest weekly average temperature was 40°C in the 8th week of 2022 and 42.14°C in the 11^{th} week of 2023. The lowest weekly average temperatures were 20.57°C in the first week of 2022 and 19.3°C in the first week of 2023. The supplementary material (Supplimentary Table 1) contains the daily changes in the weather data. Hence, it is clear that the maize plants in this study faced heat stress.

Details of the experiment

The experiment was laid out in a split-plot design with two main treatments and six sub-treatments replicated thrice during the *summer* of 2022 and 2023. The main plot size was 25.2 m × 24 m, and the subplot size was 4.2m × 4m. Following the standard package of practices for maize. The sowing was taken on March 26, 2022 and March 19, 2023. The plants were irrigated by drip irrigation and optimal soil moisture was maintained during the period of the experiment.

Treatment details

The main treatments (M) were maize hybrids, namely RCRMH-2 (M_1) and 900MG (M_2). The sub-treatments (S) were heat stress mitigating measures *viz*., S_1: Control (No foliar spray), S_2: Foliar spray of salicylic acid (SA) at the rate of 200 ppm, S_3: Foliar spray of $CaCl_2$ at the rate of 2000 ppm, S_4: Foliar spray of silicon (Si) (Orthosilicic acid) at the rate of 200 ppm, S_5: Thermotolerance-induced seeds (TIR-treated seeds), S_6: Foliar spray of cytokinin (CK) (Benzyl adenine) at the rate of 20 ppm. The TIR seeds are the seeds that are exposed to a graded temperature of 32-50°C for 4.5 hours, followed by exposure to a lethal temperature of 56°C for 3 hours. A recovery period of 24 hours under room conditions was maintained and then used for sowing in the field (Raviteja *et al*., 2023).

Harvesting and collection of experimental data

Five plants per treatment were randomly selected and tagged for observation. Flag leaf was used for the analysis of different physiological parameters. The experiment was harvested on July and June 27 of 2022 and 2023, respectively, when the husk covers had turned brown and the silks had dried entirely. Yield and yield components were assessed at harvest on the tagged plants. The mean value of each treatment was computed based on the observations from the five plants in both seasons.

Physiological parameters

Gas exchange parameters

The photosynthetic measurements were made using an infrared gas analyser portable photosynthesis system (CI-340-Handheld photosynthesis system, CID Bio-Science, Inc 1554 NE 3rd Ave, Camas, WA 98607). All measurements were made on the flag leaf between 8 a.m. and 12:00 noon. Measurements were recorded within a leaf area of 2 cm^2 at photosynthetic photon flux density (PPFD) of 1200 µmol m^{-2} s^{-1} and block temperature of 28°C. The relative humidity (RH) was maintained at 60%. The photosynthetic rate (Pn), transpiration rate (E) and stomatal conductance (gs) were averaged over three log events for individual replications and treatments. Photosynthesis, transpiration and stomatal conductance were expressed in µ mol CO_2 m^2 s^1, m mol H_2O m^2 s^1 and mol m^2 s^1, respectively.

Estimation of chlorophyll and carotenoid concentration in leaf tissue

The non-maceration method of Hiscox and Israelstam (1979) was used to determine the chlorophyll content. The concentrations of chlorophyll a, b and total chlorophyll were calculated according to the formulae of Lichtenthaler and Wellburn (1983) and expressed as mg g^{-1} F.W. The carotenoid concentration was also calculated by measuring the absorbance at 470 nm and expressed as mg g^{-1} F.W.

$$\text{Chlorophyll a} = \frac{(12.7\times OD663)-(2.69\times OD645)}{1000\times W\times 1}\times V$$

$$\text{Chlorophyll b} = \frac{(22.9\times OD645)-(4.68\times OD663)}{1000\times W\times 1}\times V$$

$$\text{Total chlorophyll} = \frac{(20.2\times OD645)+(8.02\times OD663)}{1000\times W\times 1}\times V$$

$$\text{Total carotenoid} = \frac{(1000\times OD470)-(3.29\times Ca)-(104\times Cb)}{198}$$

Where,

OD663 = Absorbance values at 663 nm, OD645 = Absorbance values at 645 nm, OD470 = Absorbance values at 470 nm, W = Weight of the sample in mg, V = Volume of the solvent used (ml), 1 = Path length of light (cm), Ca = (12.21 × OD663) – (2.81 × OD645), Cb = (20.13 × OD645) – (5.03 × OD663)

Pollen viability

Pollen viability is determined by using the 2% acetocarmine staining method (Marutani *et al*., 1993) and expressed in per cent.

Anthesis-silking interval

Anthesis-silking interval (ASI) is the difference, in days, between the emergence of female and male flowering.

Relative water content

Relative water content (RWC) was determined by the method suggested by Barrs and Weatherley (1962). The top-most fully expanded leaves were sampled and three replications were taken from a single treatment. The calculated RWC was expressed in per cent.

RWC (%) = [(Fresh weight – Dry weight) / (Turgid Weight – Dry Weight)] × 100

Membrane stability index

The leaf membrane stability index (MSI) was determined by the method suggested by Premchandra *et al*. (1990). The formula below was used to calculate the MSI and expressed in per cent.

$$M(\%) = 1 - \frac{C_1}{C_2} \times 100$$

Where, C_1 = Electrical conductivity at 40^0C and C_2 = Electrical conductivity at 100^0C.

Yield and yield parameters

Yield and yield parameters were determined using the measuring scale, counting kernels manually and using an electronic weighing balance. The yield parameters determined are cob length (cm), cob diameter (cm), cob weight (g), number of grains per cob, 100-seed weight (g), grain weight per plant (g) and total grain yield (kg ha^{-1}).

Statistical analysis

The experimental data was averaged over the two seasons. Online statistical tool OPSTAT was used for the statistical analysis, to perform ANOVA and to determine LSD, significance level at 5% probability ($P < 0.05$), CD and CV (%). Microsoft Excel was used to create graphs and tables. The hybrids were compared to each other, mitigating measures were compared to control and interactive effects due to hybrids and mitigating measures were also discussed.

Results

The experiment aimed to study the different mitigating approaches to combat heat stress in maize under natural heat stress conditions. The heat stress tolerance was measured in terms of different physiological parameters. The pooled data from the two seasons are discussed below.

Anthesis-Silking Interval

The hybrids showed significant differences (Table 1). The RCRMH-2 recorded the lowest ASI with a mean value of 1.47 days, while 900MG recorded an ASI of 1.61 days. Among the mitigating measures, a significant difference was observed. A reduction in ASI with a mean value of 1.42 days was observed in all the mitigating measures except for thermotolerance-induced seed treatment, which recorded slightly higher ASI (1.58 days). Control plants recorded the highest ASI of 2 days. The interactive effect due to hybrids and sub-treatments was found to be non-significant.

Pollen viability

Hybrids did not differ significantly, but treatments showed statistical differences on pollen viability (Table 1). Thermotolerance-induced seed treatment showed the highest pollen viability (80.96%), followed by salicylic acid (80.7%). Control plants recorded the lowest (69.17%). The hybrid-mitigation treatment interaction was also found significant. Thermotolerance-induced RCRMH-2 showed the highest pollen viability (81.04%), while control 900MG had the lowest (69.17%).

Physiological parameters

Gas exchange parameters

The gas exchange parameters like photosynthesis, transpiration and stomatal conductance were measured at 40, 60 and 80 DAS are discussed below.

Photosynthesis

The effects of hybrids and mitigating measures on stomatal conductance were studied at three stages of growth: 40, 60 and 80 DAS. Table 2 shows the measurements taken at 40, 60 and 80 days after sowing (DAS). Between the hybrids, RCRMH-2 recorded a significantly higher photosynthetic rate than 900MG at both 40 and 60 DAS, with mean values of 30.30 and 38.17 μ mol CO_2 m^2 s^{-1}, respectively. While at the 80 DAS, the 900MG recorded a significantly higher

Table 1 : Effect of mitigating measures on anthesis-silking-interval and pollen viability inmaize hybrids.

Treatment	Anthesis-Silking Interval (Days)			Pollen Viability (%)		
M / S	RCRMH-2	900MG	Mean	RCRMH-2	900MG	Mean
Control	2[a]	2[a]	2[a]	71.17[c]	69.17[c]	70.17[c]
Salicylic acid	1.33[bc]	1.5[bc]	1.42[b]	80.89[a]	80.54[a]	80.71[a]
$CaCl_2$	1.33[bc]	1.5[bc]	1.42[b]	73.58[b]	73.73[b]	73.66[b]
Silicon	1.17[c]	1.67[ab]	1.42[b]	80.04[a]	79.87[a]	79.96[a]
TIR	1.5[bc]	1.67[ab]	1.58[ab]	81.04[a]	80.88[a]	80.96[a]
Cytokinin	1.5[bc]	1.33[bc]	1.42[b]	79.93[a]	80.33[a]	80.13[a]
Mean	1.47	1.61		**77.77**	**77.42**	
SOV	M	S	M x S	M	S	M x S
S. Em ±	0.020	0.124	0.176	0.131	0.228	0.323
CD at 5 %	0.120	0.367	NS	NS	0.673	0.952

M: Main treatments: Maize hybrids. S: Sub-treatment: Different mitigating measures. Same letters are not significantly different at $\alpha = 0.05$

Table 2 : Effect of mitigating measures on photosynthetic rate in maize hybrids at different growth stage.

Photosynthesis (μ mol CO_2 m^2 s^1)									
Treatment	40 DAS			60 DAS			80 DAS		
M / S	RCRMH-2	900MG	Mean	RCRMH-2	900MG	Mean	RCRMH-2	900MG	Mean
Control	26.3[d]	25.17[d]	25.74[c]	32.66[g]	32.77[g]	32.71[d]	19.13[e]	19.03[e]	19.08[c]
Salicylic acid	31.09[ab]	30.23[bc]	30.66[ab]	38.39[cd]	37.94[de]	38.16[b]	21.4[cd]	23.5[ab]	22.45[b]
$CaCl_2$	29.59[c]	28.98[c]	29.28[b]	35.96[f]	36.73[ef]	36.35[c]	20.63[d]	21.77[cd]	21.2[b]
Silicon	31.25[ab]	31.26[ab]	31.24[a]	39.59[bc]	38.69[cd]	39.14[ab]	22.43[bc]	22.67[bc]	22.55[b]
TIR	31.27[ab]	31.28[ab]	31.27[a]	40.65[ab]	39.67[bc]	40.16[a]	21.7[cd]	21.71[cd]	21.7[b]
Cytokinin	32.3[a]	32.19[a]	32.25[a]	41.75[a]	39.59[bc]	40.67[a]	24.57[a]	24.33[a]	24.45[a]
Mean	30.3	29.85		38.17	37.56		21.64	22.17	
SOV	M	S	M x S	M	S	M x S	M	S	M x S
S. Em ±	0.058	0.086	0.121	0.029	0.085	0.12	0.184	0.336	0.475
CD at 5 %	0.354	0.253	0.357	0.175	0.251	0.355	NS	0.99	NS

M: Main treatments: Maize hybrids. S: Sub-treatment: Different mitigating measures. Same letters are not significantly different at $\alpha = 0.05$

photosynthetic rate (22.17 μ mol CO_2 m^2 s^{-1}). The mitigating measures had a significant effect on the photosynthetic rate. The plants treated with cytokinin showed a substantial increase in photosynthesis at all stages of growth, with mean values of 32.25, 40.67 and 24.45 μ mol CO_2 m^2 s^{-1} at 40, 60 and 80 DAS, respectively. The control plants had the lowest photosynthetic rate at all stages, with mean values of 25.74, 32.71 and 19.08 μ mol CO_2 m^2 s^{-1} at 40, 60 and 80 DAS, respectively. The hybrids and the mitigating measures also had a significant interaction effect. The cytokinin-treated RCRMH-2 plants recorded the highest photosynthetic rate among all the treatments at all the measured stages, with mean values of 32.30, 41.75 and 24.57 μ mol CO_2 m^2 s^{-1} at 40, 60 and 80 DAS, respectively. The control 900MG recorded the lowest photosynthetic rate at 40 and 80 DAS, with mean values of 25.17 and 19.03 μ mol CO_2 m^2 s^{-1}, respectively. However, at 60 DAS, the control RCRMH-2 recorded the lowest photosynthetic rate (32.66 μ mol CO_2 m^2 s^{-1}.)

Transpiration

The effects of hybrids and mitigating measures on stomatal conductance were studied at three stages of growth: 40, 60 and 80 DAS. The hybrids recorded significant differences in transpiration rate (Table 3). The RCRMH-2 showed a significantly lower transpiration rate than 900MG with mean values of 4.03, 4.76 and 2.23 m mol H_2O m^2 s^1 at 40, 60 and 80 DAS, respectively. The mitigating measures had a significant effect on the transpiration rate. The plants treated with salicylic acid, $CaCl_2$ and silicon had significantly higher transpiration rates than the control plants, with mean values of 4.39, 5.82 and 2.82 m mol H_2O m^2 s^1 at 40, 60 and 80 DAS, respectively. The control plants had the lowest

Table 3 : Effect of mitigating measures on transpiration rate in maize hybrids at different growth stages.

Transpiration (m mol H_2O m^2 s^1)									
Treatment	**40 DAS**			**60 DAS**			**80 DAS**		
M / S	**RCRMH-2**	**900MG**	**Mean**	**RCRMH-2**	**900MG**	**Mean**	**RCRMH-2**	**900MG**	**Mean**
Control	3.51^{bc}	3.27^{c}	3.39^{b}	5.01^{c}	5.22^{c}	5.12^{b}	5.12^{d}	1.56^{g}	1.58^{b}
Salicylic acid	4.54^{a}	4.24^{a}	4.39^{a}	5.33^{bc}	5.35^{abc}	5.34^{ab}	5.34^{c}	2.43^{f}	2.45^{a}
$CaCl_2$	4.26^{a}	4.01^{ab}	4.13^{a}	5.82^{a}	5.81^{a}	5.82^{a}	5.82^{a}	2.67^{e}	2.71^{a}
Silicon	3.48^{bc}	3.49^{bc}	3.48^{b}	5.39^{abc}	5.7^{ab}	5.54^{ab}	5.54^{b}	2.82^{e}	2.82^{a}
TIR	4.19^{a}	4.05^{ab}	4.12^{a}	5.68^{ab}	5.68^{ab}	5.68^{a}	5.68^{ab}	2.24^{f}	2.4^{a}
Cytokinin	4.24^{a}	4.13^{a}	4.19^{a}	5.77^{ab}	5.76^{ab}	5.76^{a}	5.76^{a}	2.33^{f}	2.47^{a}
Mean	4.03	3.87		5.5	5.59		2.34	2.47	
SOV	**M**	**S**	**M x S**	**M**	**S**	**M x S**	**M**	**S**	**M x S**
S. Em ±	0.007	0.029	0.041	0.007	0.009	0.013	0.01	0.016	0.022
CD at 5%	0.04	0.085	0.12	0.042	0.027	0.038	0.061	0.047	0.066

M: Main treatments: Maize hybrids. S: Sub-treatment: Different mitigating measures. Same letters are not significantly different at $\alpha = 0.05$

Table 4 : Effect of mitigating measures on stomatal conductance in maize hybrids at different growth stages.

Stomatal conductance to water vapor (mol m^2 s^1)									
Treatment	**40 DAS**			**60 DAS**			**80 DAS**		
M / S	**RCRMH-2**	**900MG**	**Mean**	**RCRMH-2**	**900MG**	**Mean**	**RCRMH-2**	**900MG**	**Mean**
Control	0.146^{a}	0.117^{a}	0.132^{a}	0.217^{a}	0.208^{a}	0.212^{a}	0.125^{a}	0.126^{a}	0.125^{a}
Salicylic acid	0.185^{a}	0.17^{a}	0.178^{a}	0.256^{a}	0.277^{a}	0.267^{a}	0.217^{a}	0.204^{a}	0.21^{a}
$CaCl_2$	0.16^{a}	0.152^{a}	0.156^{a}	0.253^{a}	0.267^{a}	0.26^{a}	0.224^{a}	0.217^{a}	0.22^{a}
Silicon	0.197^{a}	0.187^{a}	0.192^{a}	0.277^{a}	0.268^{a}	0.273^{a}	0.227^{a}	0.232^{a}	0.23^{a}
TIR	0.179^{a}	0.183^{a}	0.181^{a}	0.28^{a}	0.254^{a}	0.267^{a}	0.217^{a}	0.217^{a}	0.217^{a}
Cytokinin	0.185^{a}	0.181^{a}	0.183^{a}	0.278^{a}	0.271^{a}	0.274^{a}	0.222^{a}	0.212^{a}	0.216^{a}
Mean	0.175	0.165		0.26	0.258		0.205	0.201	
SOV	**M**	**S**	**M x S**	**M**	**S**	**M x S**	**M**	**S**	**M x S**
S. Em ±	0.058	0.086	0.121	0.029	0.085	0.12	0.184	0.336	0.475
CD at 5 %	0.354	0.253	0.357	0.175	0.251	0.355	NS	0.99	NS

M: Main treatments: Maize hybrids. S: Sub-treatment: Different mitigating measures. Same letters are not significantly different at $\alpha = 0.05$

transpiration rates at all stages. There was also a significant interaction effect due to the hybrids and the mitigating measures. The salicylic acid-treated RCRMH-2 had the highest transpiration rate among all the treatments at 40 DAS (4.54 m mol H_2O m^2 s^1). The $CaCl_2$-treated RCRMH-2 plants recorded the highest transpiration rate at 60 DAS (5.82 m mol H_2O m^2 s^1). The silicon-treated RCRMH-2 recorded the highest transpiration rate at 80 DAS (2.82 m mol m mol H_2O m^2 s^1). The control 900MG plants showed the lowest transpiration rate at 40 and 80 DAS, with mean values of 3.27 and 1.56 m mol m mol H_2O m^2 s^1, respectively. However, at 60 DAS, the control RCRMH-2 plants had the lowest transpiration rate (5.01 m mol H_2O m^2 s^1).

Stomatal Conductance

The effects of hybrids and mitigating measures on stomatal conductance were studied at three stages of growth: 40, 60 and 80 DAS (Table 4). A significant increase in stomatal conductance was observed in the RCRMH-2 at 40 and 60 DAS, with mean values of 0.175 and 0.260 mol m^2 s^1, respectively, compared to 900MG. However, at 80 DAS, both hybrids recorded similar stomatal conductance values. Among the treatments, silicon application increased stomatal conductance significantly at 40 and 80 DAS, with mean values of 0.192 and 0.230 mol m^2 s^1, respectively, while cytokinin application had the highest effect at 60 DAS, with a mean value of 0.274 mol m^2 s^1. The control plants had the lowest stomatal conductance values at all stages of growth. The interaction between hybrids and treatments was significant at 40 and 60 DAS, with the highest values recorded by silicon-treated RCRMH-2 and

Table 5 : Effect of mitigating measureschlorophyll a, b and total chlorophyll in maize hybrids.

Treatment	Chlorophyll 'a' (mg g^{-1} F.W.)			Chlorophyll 'b' (mg g^{-1} F.W.)			Total Chlorophyll (mg g^{-1} F.W.)		
M / S	**RCRMH-2**	**900MG**	**Mean**	**RCRMH-2**	**900MG**	**Mean**	**RCRMH-2**	**900MG**	**Mean**
Control	3.16^{b}	3.02^{b}	3.09^{b}	1.37^{b}	1.27^{b}	1.32^{b}	4.07^{b}	3.9^{b}	3.99^{b}
Salicylic acid	3.98^{a}	4.07^{a}	4.03^{a}	2.18^{a}	1.91^{ab}	2.05^{a}	5.22^{a}	5.05^{a}	5.13^{a}
$CaCl_2$	4.04^{a}	3.91^{a}	3.98^{a}	2.47^{a}	2.04^{a}	2.26^{a}	5.5^{a}	5.07^{a}	5.28^{a}
Silicon	4.07^{a}	3.97^{a}	4.02^{a}	2.51^{a}	2.32^{a}	2.41^{a}	5.55^{a}	5.34^{a}	5.44^{a}
TIR	3.79^{a}	3.94^{a}	3.86^{a}	1.89^{ab}	2.06^{a}	1.98^{a}	4.86^{a}	5.1^{a}	4.98^{a}
Cytokinin	4.06^{a}	4.08^{a}	4.06^{a}	2.33^{a}	2.46^{a}	2.39^{a}	5.39^{a}	5.51^{a}	5.45^{a}
Mean	3.850	3.830		2.130	2.010		5.100	4.990	
SOV	**M**	**S**	**M x S**	**M**	**S**	**M x S**	**M**	**S**	**M x S**
S. Em ±	0.015	0.016	0.022	0.024	0.034	0.048	0.028	0.033	0.047
CD at 5%	NS	0.047	0.066	NS	0.101	0.142	NS	0.097	0.137

M: Main treatments: Maize hybrids. S: Sub-treatment: Different mitigating measures. Same letters are not significantly different at $\alpha = 0.05$

Table 6 : Effect of mitigating measures onRWC, MSI and carotenoid content in maize hybrids.

Treatment	Relative Water Content (%)			Membrane Stability Index (%)			Carotenoid (mg g^{-1} F.W.)		
M / S	**RCRMH-2**	**900MG**	**Mean**	**RCRMH-2**	**900MG**	**Mean**	**RCRMH-2**	**900MG**	**Mean**
Control	70.41^{g}	70.61^{g}	70.51^{c}	64.02^{d}	60.41^{e}	62.21^{c}	4.18^{c}	4.41^{c}	4.29^{b}
Salicylic acid	80.35^{b}	77.75^{def}	79.05^{b}	73.52^{a}	68.81^{c}	71.16^{a}	5.26^{ab}	5.45^{a}	5.36^{a}
$CaCl_2$	79.27^{bcd}	76.64^{f}	77.96^{b}	68.61^{c}	67.78^{c}	68.2^{b}	4.66^{bc}	5.33^{a}	4.99^{ab}
Silicon	78.42^{cde}	79.91^{bc}	79.16^{b}	70.79^{b}	71.09^{b}	70.94^{a}	4.84^{abc}	5.41^{a}	5.12^{a}
TIR	79.35^{bcd}	77.12^{ef}	78.24^{b}	71.47^{b}	72.54^{ab}	72.01^{a}	5.34^{a}	5.39^{a}	5.36^{a}
Cytokinin	84.06^{a}	79.31^{bcd}	81.68^{a}	73.64^{a}	71.78^{ab}	72.71^{a}	5.2^{ab}	5.16^{ab}	5.18^{a}
Mean	78.64	76.89		70.34	68.73		**4.91**	**5.19**	
SOV	**M**	**S**	**M × S**	**M**	**S**	**M × S**	**M**	**S**	**M × S**
S. Em ±	0.755	0.59	0.835	0.036	0.084	0.118	0.035	0.094	0.133
CD at 5%	NS	1.741	2.463	0.219	0.247	0.349	0.212	0.277	NS

M: Main treatments: Maize hybrids. S: Sub-treatment: Different mitigating measures. Same letters are not significantly different at $\alpha = 0.05$

thermotolerance-induced seeds with mean values of 0.197 and 0.280 mol $m^2 s^1$ and the lowest values by control 900MG. At 80 DAS, the interaction effect was found to be not significant.

Chlorophyll a, b and total chlorophyll

The chlorophyll a, b and total chlorophyll were measured at 60 DAS (Table 5). Hybridsdisplayed only numerical changes in chlorophyll a, b and total chlorophyll but a significant difference was observed among mitigating measures and interaction effects.

Chlorophyll-a

Hybrids displayed only numerical changes in chlorophyll-a. In the sub-treatments, the cytokinin treatment showed a substantial increase in chlorophyll-a with a mean of 4.06 mg g^{-1} F.W. Besides, the least chlorophyll 'a' was noticed in control plants (3.09 mg g^{-1} F.W.). The interaction effect due to hybrids and sub-treatment showed a significant variation. The cytokinin-treated 900MG showed a considerable increase in chlorophyll-a content with mean value of 4.07mg g^{-1} F.W. In contrast, the lowest chlorophyll 'a' was noticed in control 900MG with mean values of 3.02 mg g^{-1} F.W. The present study noted that the cytokinin showed a 31.39% increase inchlorophyll-a content compared to control plants.

Chlorophyll-b

Hybrids displayed only numerical changes in chlorophyll-a The sub-treatments showed a statistically considerable increase in chlorophyll-b. The cytokinin treatment showed a significant increase in chlorophyll-b with mean values of 2.39 mg g^{-1} F.W. However, the leastchlorophyll-b was recorded in control plants, 1.32 mg g^{-1} F.W. The interaction effect due to hybrids and sub-treatment where the cytokinin-treated 900MG showed a considerable increase in Chlorophyll-b with mean values of 2.46 mg g^{-1} F.W. In contrast, the lowest Chlorophyll-b was noticed in control 900MG with mean values of 1.27 mg g^{-1} F.W. Among sub-treatments, cytokinin alone showed an 81% increase in chlorophyll 'b' content compared to control plants.

Total chlorophyll

The total chlorophyll was measured at 60 DAS. The hybrids showed no significant difference in chlorophyll content. However, the mitigating measures showed a significant effect on the total chlorophyll content, with cytokinin treatment having the highest mean value of 5.45 mg g^{-1} F.W. The control plants had the lowest total chlorophyll content (3.99 mg g^{-1} F.W.). The interaction due to hybrids and treatments was significant as well, with the highest total chlorophyll content noticed in cytokinin-treated 900MG, with a mean value of 5.51 mg g^{-1} F.W. and the lowest was seen in control 900MG (3.90 mg g^{-1} F.W.).

Relative water content (RWC)

The RWC was measured at 60 DAS (Table 6). RWC showed no significant difference between the hybrids. The mitigating measures had a significant effect on the RWC with cytokinin treatment, resulting in the highest mean value of 81.68%. The control plants showed the lowest RWC (70.51%). The interaction effect due to hybrids and treatments was significant as well, with the highest RWC noticed in cytokinin-treated RCRMH-2 (86.06%). The lowest RWC was seen in control RCRMH-2 (70.41%).

Membrane stability index (MSI)

The MSI was determined at 60 DAS and the results are shown in Table 6. The results revealed that the hybrids showed significant differences with RCRMH-2 having a higher MSI of 70.34% than 900MG, with a lower MSI of 68.73%. The mitigating measures also significantly affected the MSI with thermotolerance-induced seed treatment and cytokinin resulted in the highest mean value of 72.71%. The control plants showed the lowest MSI (62.21%). The interaction effect between hybrids and treatments was significant as well, with the highest MSI noticed in cytokinin-treated RCRMH-2 (73.64%), while control RCRMH-2 showed the lowest MSI (64.02%).

Carotenoid content

Carotenoids are called antioxidant pigments that protect plants from oxidative stress and are measured at 60 DAS (Table 6). A significant difference was noticed between the hybrid, the 900MG showed a substantial increase in carotenoid content (5.19 mg g^{-1} F.W.). In contrast, the RCRMH-2 showed a lower carotenoid (4.91 mg g^{-1} F.W.). The mitigating treatments showed significant differences, where the silicon and salicylic application considerably increased the carotenoid content, with a mean value of 5.36 mg g^{-1} F.W. Furthermore, the lowest carotenoid content was seen in control plants with a mean value of (4.29 mg g^{-1} F.W). The interaction effect due to hybrids and sub-treatment was found non-significant. The 900MG showed a 5.66% increase in carotenoid content compared to RCRMH-2. Silicon and salicylic acid treatment showed a 24.94% increase in carotenoid content compared to control plants

Yield parameters and grain yield

The results of different yield parameters like cob length, cob diameter, cob weight, kernels per cob, 100 seeds weight, grain weight and total yield recorded are discussed below.

Cob length

The cob length of different hybrids and mitigating measures were presented in Fig. 1a. The cob length showed significant differences among different mitigating measures but showed no difference between Hybrids. Cytokinin treatment resulted in a significant increase in the cob length (16.86 cm), followed by silicon treatment (16.54 cm). Control plants had the lowest (13.92 cm). The hybrid-mitigating measures interaction effect was also found significant. Silicon-treated RCRMH-2 recorded a significantly higher cob length (17.25 cm), while control RCRMH-2 recorded the lowest cob length (13.42 cm).

Cob diameter

The cob diameter of hybrids and mitigating measures are shown in Fig. 1 (b). Hybrids differed significantly, with 900MG having the largest cob diameter (3.05 cm), followed by RCRMH-2 (2.93 cm). Mitigating measures also differed significantly, with cytokinin recorded the largest cob diameter (3.19 cm), followed by silicon (3.11 cm). Control plants showed the smallest cob diameter (2.71 cm). The hybrid-mitigating treatments interaction effect was found to be non-significant. Cytokinin-treated 900MG recorded the largest cob diameter (3.20 cm), while salicylic acid-treated RCRMH-2 recorded the smallest (2.63 cm).

Cob weight

Fig. 1(c) shows the cob weights of hybrids and mitigating measures. The hybrids showed only numerical differences. The mitigating measures resulted in a significant effect on cob weight, with cytokinin treatment

Fig. 1 : Effect of mitigating measures on yield parameters and total grain yield in maize cob length (a), cob diameter (b), cob per grains (c), cob weight (d), total grain weight per plant (e), hundred seed weight (f), total grain yield (g). In bar graphs, means ± standard errors with the same letters are not significantly different at á = 0.05, according to Fisher's least significant difference.

being the most effective treatment, recording the highest cob weight (185.88 g), followed by silicon treatment (169.78 g). The control plants had the smallest cob weight (125.0 g). However, there was no significant interaction effect between hybrids and mitigating measures was seen for cob weight.

Number of grains per cob

The results of the number of grains per cob are shown in Fig. 1 (d). The hybrids varied significantly in grains per cob, with RCRMH-2 having the highest value (348.92), followed by 900MG (303.97). The mitigating measures also affected grains per cob significantly, with cytokinin treatment recorded the highest number of grains per cob (352.53), followed by silicon treatment (351.25). The control plants recorded the lowest number of grains per cob (281.17). The interaction effect between hybrids and mitigating measures showed significant differences. The highest grains per cob were observed in the silicon-treated RCRMH-2 (369.50), while the lowest kernels per cob were recorded in the control 900MG (256.67).

Hundred seed weight

The results of the hundred seed weight are shown in Fig. 1 (e). Hybrids differed significantly in hundred seed weight, with RCRMH-2 recording the highest 100 seed weight (23.67 g), followed by 900MG (19.63 g). The mitigating measures also influenced hundred seed weight significantly, with cytokinin being the most effective treatment (24.22 g), followed by silicon (22.81 g). The control plants had the lowest hundred-seed weight (19.47 g). There was a significant interaction between hybrids and mitigating measures. The significantly highest hundred seed weight was observed in the thermotolerance-induced RCRMH-2 (25.07 g), while the lowest hundred seed weight was recorded in the control 900MG (16.21 g).

Total grain weight per plant

The results of the total grain weight per plant are shown in Fig. 1 (f). The hybrids did not vary significantly. The mitigating measures significantly affected the total grain weight per plant, with cytokinin recorded a considerably higher total grain weight per plant (82.27 g), followed by silicon (75.06 g). The control plants had the lowest total grain weight per plant (57.62g). There was a significant interaction between hybrids and mitigating measures for total grain weight per plant. The highest total grain weight per plant was observed in the calcium chloride-treated 900MG (83.98 g), while the lowest total grain weight per plant was recorded in the control 900MG (57.621 g).

Total grain yield

Fig. 1 (g) shows the total grain yield (kg ha^{-1}) of hybrids and mitigating measures. Hybrids and treatments differed significantly in total grain yield. RCRMH-2 recorded the highest grain yield (3503.47 kg ha^{-1}), followed by 900MG (3135.03 kg ha^{-1}). Cytokinin treatment recorded the significantly highest yield (3601.28 kg ha^{-1}) followed by silicon treatment having 3473.14 kg ha^{-1}. Control plants had the lowest yield (2774.23 kg ha^{-1}). The hybrid-mitigating treatment interaction effect was significant, with cytokinin-treated RCRMH-2 recording the highest yield (4008.5 kg ha^{-1}), followed by silicon-treated RCRMH-2 with a mean value of 3775.32 kg ha^{-1}. Meanwhile, control 900MG recorded the lowest grain yield (2542.40 kg ha^{-1}).

Discussion

The present results show that the mitigating measures significantly increased the growth, development, yield and yield components of the maize under natural heat stress conditions. The prolonged ASI can lead to poor pollination, reduced seed set and decreased grain yield (Yang *et al*., 2017). TIR seed treatment significantly increased the sucrose synthase and sucrose phosphate synthase activities, as well as lower amylolytic activity and increased starch content, providing a continuous energy supply critical for the development of anthers, ovaries and pollen viability; hence, the ASI was reduced significantly, leading to the successful pollination and fertilisation (Abdelrahman *et al*., 2017). Furthermore, the increased gas exchange parameters, pollen viability MSI and RWC contributed to effective pollination and reduced ASI.

The foliar spray of silicon protects pollen grains from heat stress by improving the plant's antioxidant activities, chlorophyll stability, starch, sugar accumulation, germination and pollen tube growth (Hasanuzzaman *et al*., 2017). A study by Huo and Yang (2022) examined the effect of exogenous 6-benzyl adenine on the starch quality of waxy maize under post-silking drought stress. The results indicated that 6-benzyl adenine application enhanced photosynthesis, increased starch and sugar production and provided a steady energy supply to reproductive parts, which is essential for maintaining pollen viability under heat stress (Huo and Yang, 2022). Therefore, cytokinin application plays a potential role in improving pollen viability. Cytokinin treatment induces the stay-green trait in maize and wheat, which maintains the chlorophyll content and biosynthesis in the leaves. Stay green trait delays leaf senescence and enhances photosynthesis even during the grain-filling stages, resulting in higher yield and yield components (Kumar *et al*., 2023). Furthermore, the 6-BAP foliar application increased the total chlorophyll content and photosynthetic rate under combined heat and drought stress, the same was also reported in wheat (Kumari *et al*., 2018). SA foliar spray increases photosynthesis by enhancing stomatal opening, which facilitates gas exchange and transpiration and helps plants to cope with heat stress. SA can also regulate the expression of aquaporins, which are involved in water transport across cells and help plants to maintain their water status, osmotic potential and membrane stability under heat stress (Hasanuzzaman *et al*., 2017).

The studies of Khan *et al*. (2020) reported that the silicon application increases the stomatal conductance, water potential, gas exchange capacity and epicuticular wax formation under heat stress. Silicon-induced increase in stomatal conductance was because of improved hydraulic properties of the roots and water uptake capacity of the roots under silicon application (Kleiber *et al*., 2020). Similarly, Mir *et al*. (2022) reported that silicon application elevated the abscisic acid and gibberellin ratio under heat stress, leading to the regulation of stomatal aperture and reduced water loss. These studies proposed that silicon can improve heat tolerance in maize by regulating ABA-mediated stomatal conductance and enhancing photosynthetic capacity and water status. Prior studies have also established the positive effects of cytokinin on stomatal conductance. Cytokinin affects the endogenous abscisic acid, which regulates stomatal opening and closure under heat stress (Farber *et al*., 2016).

The results indicate that silicon can reduce heat stress-induced injuries such as chloroplast and nucleus and protect cell membranes by stabilising membrane proteins and lipids from heat damage (Mir *et al*., 2022). A study by Islam *et al*. (2022) found that cytokinin application increased relative water content and transpiration in drought-stressed maize plants. This was achieved through cytokinin-induced improvements that reduced electrolyte leakage and malondialdehyde and increased proline and soluble sugar levels, thereby maintaining osmoregulation and relative water content. Previous studies have also demonstrated that silicon can decrease electrolyte leakage and increase membrane stability index and cell wall thickness in rice under heat stress, which protects membrane proteins and lipids from thermal damage (Younis *et al*., 2020). Cytokinin also improved the membrane stability index in wheat under combined heat and drought stress by increasing total chlorophyll and reducing lipid peroxidation. Moreover, benzyl adenine can lower MDA and electrolyte leakage levels and prevent membrane damage (Kumari *et al*., 2018). Mustafa *et al*. (2021) reported that the application of silicon resulted in a significant increase in carotenoid content in wheat flag leaves. Another study by Li *et al*. (2008) identified cytokinin as a critical factor in delaying leaf senescence and maintaining chlorophyll levels in the photosynthetic apparatus, essential for carotenoid biosynthesis. The study also found that cytokinin upregulated the expression of phytoene synthase (PSY), a vital enzyme for carotenoid synthesis, in maize leaves, improving overall carotenoid content.

Previous studies revealed the activation of specific genes, such as heat shock proteins and heat shock transcription factors, in TIR-treated seeds. These genes are essential for enabling plants to withstand high temperatures by facilitating physiological and biochemical processes. Heat shock proteins are produced more significantly during stress, which is vital for adapting to extreme stress conditions (Vidya *et al*., 2018). The mitigating measures in the current study significantly increased the yield parameters, such as cob length, cob diameter, cob weight, number of grains, hundred seed weight and total grain weight of the maize under heat stress conditions. It is noteworthy that previous studies reported that the exogenous application of salicylic acid, cytokinin, calcium chloride and silicon treatment increased carbon assimilation, resulting in increased biomass production (Ali *et al*., 2020). It also observed that the mitigating treatments improved the photosynthetic performance and the source-sink balance of the plants, leading to higher biomass production and distribution towards sink organs such as cob length, cob diameter, cob weight and grain weight (Seebauer *et al*., 2009). These findings are consistent with the results of the previous studies on rice by Reshma *et al*. (2021), who reported that TIR treatment increased grain yield and quality by enhancing plants physiological and biochemical characteristics in rice.

The RCRMH-2 recorded a significantly higher yield under heat-stress conditions than 900MG, indicating that the RCRMH-2 responded more efficiently to mitigating treatments. The mitigating measures increased the physiological parameters such as gas exchange parameters, ASI, RWC, MSI, pollen viability and chlorophyll content, which contributed to the grain yield. Therefore, the improved yield and yield parameters under heat stress in maize were due to the tolerance mechanism induced by the mitigating measures. Previous studies reported that the 6-BAP application improved grain filling in rice by reducing *CKX* expression and activity, especially in basal spikelets. It also increased cell cycle regulators expression in basal spikelets, facilitating endosperm cell division and G1/S and G2/M phase transition (Panda *et al*., 2018). Jawahar *et al*. (2019) reported that the application of silicon increased plant height, LAI, dry matter production, cob size, number of grains per cob, test weight and grain and stover yield, which resulted in the highest yield and net returns. The silicon application in rice crop resulted in a denser, thicker and more erect leaves, stronger roots, more tillers and fewer white ear heads than the control plants leading to the higher yield. Naeem *et al*. (2017) found that calcium chloride can boost maize yield and yield parameters by enhancing photosynthetic efficiency, membrane stability, osmotic adjustment, antioxidant defence system and hormonal balance in the presence of heat stress. This suggests that using a foliar spray of calcium chloride can effectively improve maize production under heat-stress conditions (Prerostova *et al.*, 2020).

Conclusion

The study noted that the physiological different parameters, yield, and yield components of maize were improved by the mitigating measures under heat stress. However, the treatments had varied effects on different parameters and hybrids. Cytokinin treatment enhanced photosynthesis and cob size, while silicon application increased transpiration and cob weight, and salicylic acid treatment boosted the number of grains per cob. Compared to 900MG, RCRMH-2 had a higher total grain yield of 10.58%. The silicon was the most effective and significantly increased the grain yield by 29.40% compared to the control plants. Cytokinin also increased the grain yield by 25.61% compared to the control plants. The other treatments, such as thermotolerance-induced seeds, salicylic acid and calcium chloride, were also improved the total grain yield by 25.60%, 24.92% and 15.96%, respectively, compared to the control plants. This indicates that silicon can potentially reduce the adverse impacts of heat stress on maize. The findings suggest new ways to improve the heat tolerance and yield of maize in the face of global warming.

Author contributions

Conceptualization: M.M. Dhanoji; Methodology: Raviteja D.H; Formal analysis and investigation: Raviteja D.H; Writing: original draft preparation: Raviteja D.H; Writing: review and editing: Srikanth Barkeer and Amaregouda A; Funding acquisition: R. P Patil and Srikanth Barkeer; Resources: P.H Kuchanur Supervision: M.M. Dhanoji and P.H Kuchanur

Conflict of interest

The authors state no conflict of interest with respect to the research, authorship and/ or publication of this article.

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