EFFECT OF FOLIAR APPLICATION ON PLANT METABOLISM AND GRAIN YIELD UNDER ELEVATED CARBON DIOXIDE IN RICE (*Oryza sativa* L.)

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Abstract. Environmental factors influencing plant metabolic process, the increase in the concentration of atmospheric carbon dioxide with fluctuating temperature are important factors. The environmental factors that cause stress in rice affect the reproductive growth and development of the crop and cause high grain yield loss. The experiment was conducted in India to study the impact of elevated carbon dioxide (CO_2) and the associated temperature stress showed that these factors have a significant negative impact on anther, pollen, yield, and resultant seed quality characteristics. The observations include reduction in anther length and width, pollen viability, pollen length, pollen radius, and pollen surface area, and yield. Extrinsic foliar applications of boric acid (BA), salicylic acid (SA) and Glycine betaine (GB) visually alleviated the effects of stress. A direct effect on mitigating the effects of the stress was observed which was confirmed by using antioxidant enzyme activities. Antioxidant enzymes like catalase, peroxidase, and superoxide dismutase show high activity in the treated plants. The results indicate the beneficial impact of the application of foliar treatment with BA (400 and 500 ppm), SA (600 and 800 ppm) and GB (400 and 600 µM), on the anther, pollen and yield characters by mitigating the metabolic irregularities by the stress induced.

Keywords: *environment, open top chamber, salicylic acid, boric acid, glycine betaine and seed quality*

Introduction

Changing atmospheric condition due to increasing emission of greenhouse gases, especially carbon dioxide CO_2, is a major constrain for the global food production. In comparison to the preindustrial times, 40 per cent elevation of CO_2 was observed which

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raised to the current level of 400 µmol/mol (IPCC, 2013; Tans and Keeling, 2013). The prompt increase in the concentration of CO_2 resulted in the rise of atmospheric temperature leading to climatic uncertainties. As the studies indicated that there will be increase in global temperature by an extend of 3^0C due to the increased level of CO_2 up to 670 µmol/mol (IPCC, 2013). One-third of global population feeds on rice, as a major stable food. In the changing climatic conditions, the productivity should be stable. Rice being cultivated in wide ranges of agro climatic conditions in the world, major risk factor for the cultivation and yield is varying weather conditions like increase in the level of CO_2 and relatively changing temperature (Cho and Oki, 2012).

The effect of temperature at the critical stages, flower development and reproductive phase has high impact on the global food security as it causes reproductive sterility (Sage et al., 2015). The high-temperature stress is affecting both male and female reproductive parts throughout the stages of gametogenesis to the fertilization (Djanaguiraman et al., 2019). The male reproductive parts are more exposed to the environmental condition hence having high impact and are very subtle. The synthesis of reactive oxygen species (ROS) due to the plant stress causes enormous irregularities in plant function by hindering the metabolic pathways. The major detrimental effect of reactive oxygen species (ROS) is by actuating the degeneration of pollen tissues (Djanaguiraman et al., 2019).

The elevated CO_2 have no direct effect on pollination and fertilization, in turn worsening the effect of induced sterility due to high temperature in most of the plants. The increase in panicle temperature is increased by the elevated CO_2, by closing the stomata and stomatal conductance (Li et al., 2020). The elevated CO_2 reduces the pollen deposition on stigma was also suggested, there are some beneficial effects reported like increase in anther size (Kobayasi et al., 2019). To overcome the constraints the pollen characters should be studied at elevated CO_2 and associated temperature stress. Rice is a C_3 plant hence has the capability to augment the photosynthesis in elevated CO_2 conditions resulting in higher yield (Fitzgerald et al., 2016). But the impact of elevated temperature with the change in CO_2 had high impact on the plant physiology. The results imply that combination of stresses have detrimental effect on the grain yield (Jena et al., 2018).

Impacts of elevated CO_2 on C_3 photosynthetic rates are the subject of the numerous CO_2 enrichment studies and are reputed in numerous papers. The most of the studies indicates that there is positive influence of elevated CO_2 during the initial exposure of plant. The increased availability of CO_2 at the chloroplasts and reduction in the photorespiration due to the change in ratio of CO_2 to O_2 have caused the current augmentation in rate of photosynthesis (Urban et al., 2014). The studies reported that there is decrease in rate of photosynthesis (down-regulation) from the initial exposure to raised CO_2 and the high rate of photosynthesis cannot be maintained for prolonged period. In this way, transient estimations of photosynthetic rate may overestimate the potential for carbon assimilation of plants exposed to long-term exposure to raised CO_2 (Jothiramshekar et al., 2018). At the physiological dimension, down-regulation of photosynthesis is frequently identified with decreased sink quality (the part that consumes the photosynthates) and low nutrient accessibility. The photosynthetic down-regulation in elevated CO_2 is prominent in the soil with nutrient limitations especially the nitrogen. The plants grown in the nitrogen-poor soils will have the problem in the production of ample amount of photosynthetic enzymes and this condition worsens in the increased CO_2 conditions (Li et al., 2023).

Plant growth regulators and nutrients are discovered recently which can overcome the biotic and abiotic stresses (Fahad et al., 2016). Majority of substances have positive influence during growth of the plant, substances include boric acid (BA), salicylic acid (SA) and glycine betaine (GB). Micronutrient, BA have major role in reproductive development; whereas SA and GB during unfavorable conditions like heat, cold, salinity etc. regulates the crop yield in various plants. Hence these chemicals can effectively influence the plant in withstanding elevated CO_2 condition and associated high-temperature stress. The can increase the antioxidant enzyme activities regulating the reactive oxygen species, bringing down the oxidative stress (Zandalinas et al., 2018). This study is focused on the deviations in the anther and pollen characters including anther length and width, pollen viability, pollen length, pollen radius and pollen surface area, seed yield and resultant seed quality during the elevated CO_2 conditions. The beneficial effects of BA, SA and GB application on the pollen, anther and yield characters in different conditions along with the variations in the antioxidant enzymes were also studied.

Materials and methods

Field experiment

The field experiments were conducted in Open Top Chamber (OTC) at Wetland Farm, Department of Farm Management, Tamil Nadu Agricultural University, Coimbatore, India during 2022 to 2023 (11°N latitude, 77°E longitude and an elevation of 427 m from the mean sea level), with elevated CO_2 550 ppm (eCO_2) and ambient CO_2 (aCO_2) conditions. To analyze various constraints of anther (width and length), pollen viability percentage, effect of foliar spray under elevated CO_2 conditions and the antioxidant activity. The variations in the temperature during the experiments are given in *Table 1*.

Table 1. *Variations in the temperature during the period of a field experiment*

Conditions	**Ambient CO_2 (400±9 ppm)**				**Elevated CO_2 (550 ppm)**			
Year	**Temperature °C**				**Temperature °C**			
	2022		2023		2022		2023	
Month	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**
June	34	23	34	22	36	24.75	36	23.75
July	34	22	34	22	36	23.75	36	23.75
August	35	22	32	22	37	23.75	34	23.75
September	34	21	36	21	36	22.75	38	22.75

OTC Structure

Open top chambers were constructed with superior quality multilayer polycarbonate sheet polygal, USA (4-6 mm thickness) and Galvanized Iron Jindal (GI) of circular type structure with top side partially open for experimental purpose. Those chambers are equipped with CO_2 monitoring and control by SCADA (Supervisory Control and Data Acquisition) integration technology (Nedunchezhiyan et al., 2020) (*Fig. 1*).

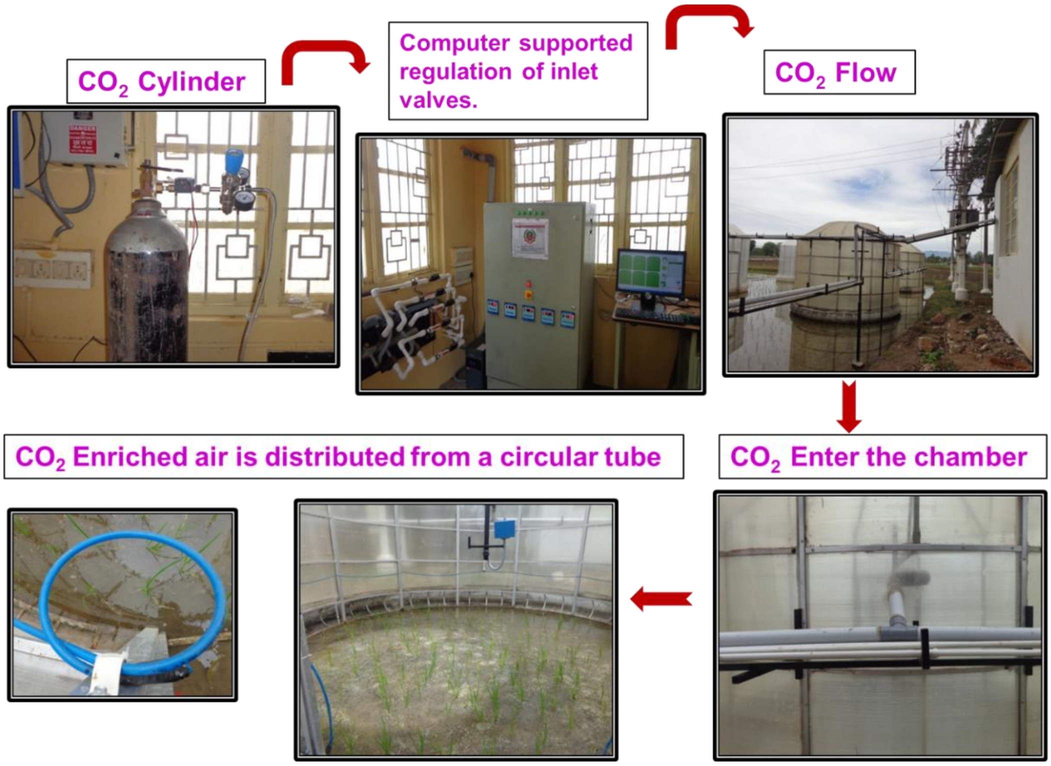

Figure 1. *Structure of automated open top chamber (OTC) with elevated CO_2 under field condition*

CO_2 gas distribution

One time capacity of a CO_2 gas cylinder is 47 kg. To each OTC through the manifold independent solenoid valve and PU tubing, constant concentration of 550 ppm of CO_2 gas is released (Nedunchezhiyan et al., 2020).

Field experimental details

Rice Seeds of CO 51 variety was sown in a nursery and after 21 days, the seedlings were transplanted in the E_1 - Ambient CO_2 (aCO_2 400 ppm ± 9 ppm) and E_2 – OTC elevated CO_2 (eCO_2 550 ppm) throughout the growing period in 2022 and 2023 (from transplanting up to harvesting). The plants grown outside the OTC were served as control (atmospheric CO_2 condition). The plant was maintained in a plot size of 20 m^2 to provide adequate space for its growth and development. The crop was managed in accordance with the 2022 and 2023 Crop Production Guide released by Directorate of Agriculture and Tamil Nadu Agricultural University. The following chemicals Salicylic acid, Boric acid and Glycine betaine were used for the foliar spray to mitigate the effect temperature on anther (length and width), pollen viability %, chlorophyll content, antioxidant enzymes activity, tiller production, yield attributes, physiological and biochemical seed quality attributes of resultant seeds under elevated CO_2 conditions in four replications of factorial randomized block design under field condition. Foliar spray of the above-mentioned chemicals was given at the time of boot leaf initiation stage and the following observations were recorded replication (4) wise on ten random plants.

Treatment details

S.no.	Treatments and concentration
1	F1 – Control (water spray)
2	F2 - Salicylic acid (600 ppm)
3	F3 - Salicylic acid (800 ppm)
4	F4 - Boric acid (400 ppm)
5	F5 - Boric acid (500 ppm)
6	F6 - Glycine betaine (400µM)
7	F7 - Glycine betaine (600µM)

Anther length and width (µm)

At the time of 50 % floweing, a randomly selected three florets (18 anthers plant^{-1}) in ten plants were used for measurement of anther length and width according to Standard Evaluation System (SES, 2002). The length and width of anther were measured with a micrometre under a binocular microscope (Software: ScopeImage 9.0.exe) and expressed in µm.

Pollen viability

The pollen grains from anthers of randomly selected spikelets were collected and taken on cavity slides and stained with Iodine-potassium iodide solution (0.44 g Iodine + 20.08 g potassium iodide in 500 ml of 70 % alcohol). The viable pollen stained immediately dark blue and the non-viable ones remained as light yellow. The number of viable and non-viable ones was counted using a microscope (Software: ScopeImage 9.0.exe). The viability percentage was calculated from the mean of three microscopic field counts (Jensen, 1962) at the time of 50% floweing.

$$\text{Viability (\%)} = \frac{\text{Number of viable pollen grains}}{\text{Total number of pollen grains}} \times 100 \qquad \text{(Eq.1)}$$

Pollen length and radius (µm), pollen surface area (µm^2)

At the time of 50% floweing, a randomly selected 5 pollen grains in each field (20 pollen grains plant^{-1}) in ten plants were used for measurement of pollen length and radius (µm) and pollen surface area (µm^2) according to Standard Evaluation System (SES, 2002). The above parameters were measured under a binocular microscope (Software: ScopeImage 9.0.exe).

Catalase activity (µmol H_2O_2 min^{-1} g $^{-1}$ protein)

Luck method (1974) was used to determine the catalase activity in the leaf sample after spraying at the time of boot leaf initiation stage in four replications. Calculated the catalase activity with the following formula.

$$\frac{17}{13.9} = 1.22\ \textit{units in the assay mixture (or)}$$
$$\frac{1.22\ \times 10}{0.01} = 1220\ \textit{units/ml extract i.e.,}\ 2.44\ \times\ 10^4 \textit{units/g tissue} \qquad \text{(Eq.2)}$$

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Peroxidase activity (U mg^{-1}protein min^{-1})

Malik and Singh (1980) method was used to assay peroxidase activity of leaf samples after spraying at the time of boot leaf initiation stage in four replications.

$$\mathit{Peroxidase\ activity} = \frac{\mathit{Difference\ in\ OD\ value}}{10} \times \frac{1000}{500} \times 60 \qquad \text{(Eq.3)}$$

Superoxide dismutase (SOD) (unit×10^{2} mg^{-1}FW min^{-1})

Using the protocol developed by Dhindsa et al. (1981), superoxide dismutase activity was measured in four replications after spraying at the time of boot leaf initiation stage.

$$Enzyme\ Unit\ (EU) = \frac{{Enzyme^{(-)}}_{light} - \left({Enzyme^{(+)}}_{light} - {Enzyme^{(+)}}_{dark}\right)}{{Enzyme^{(-)}}_{light}/2} \qquad \text{(Eq.4)}$$

Chlorophyll a, b and total content

The non-maceration method by Hiscox and Israelstam (1979), with reagent dimethyl sulphoxide was followed for chlorophyll extraction after spraying at the time of boot leaf initiation stage.

The formula suggested by Arnon (1949) was used for the calculation of chlorophyll content (mg g^{-1} leaf fresh weight).

$$\text{Chlorophyll ‘a’} = \frac{(12.7 \times \text{O.D at 663 nm}) - (2.69 \times \text{O.D at 645 nm})}{1000 \times \text{W}} \times \text{V}(\text{mg g}^{-1})$$

$$\text{Chlorophyll ‘b’} = \frac{(22.9 \text{ x O.D at 645 nm}) - (4.68 \text{ x O.D at 663 nm})}{1000 \times \text{W}} \times \text{V}(\text{mg g}^{-1}) \qquad \text{(Eq.5)}$$

$$\text{Total Chlorophyll} = \frac{(\text{O.D at 652 nm})}{1000 \times \text{W}} \times \text{V}(\text{mg g}^{-1})$$

Total number of tillers plant^{-1}

At maximum tillering stage the total number of tillers per plant was counted and recorded.

Number of reproductive tillers plant^{-1}

The number of reproductive tillers per hill at maturity was counted and recorded.

1000 seed weight (g)

A thousand seeds were counted in eight replicates and the weight was recorded and expressed in gram.

Seed yield plant^{-1} (g)

Seeds harvested at physiological maturity, and are processed for each plant separately, the safe moisture content of 12% is maintained, seeds are weighed and expressed in g per plant.

Seed yield plot^{-1} (kg)

Seeds harvested at physiological maturity, and are processed for each plot separately, the safe moisture content of 12 % is maintained, seeds are weighed and expressed in kg per plot.

Seed yield ha^{-1} (kg)

Computed seed yield was calculated from plot yield, which was expressed as kg per hectare.

Germination (%)

Based on the standard procedure outlined in (ISTA, 2012) using paper (between papers) medium germination test was conducted. Four replications of seeds germinated in the germination room (25 ± 2^{o}C temperature and 95 ± 3% RH), containing 100 seeds each.

Seedling length (cm)

From each replication, the root length of 10 random selected normal seedlings at the time of final count were measured.

Vigour index

Following formula of Vigour index was used for computing Vigour index and the mean values were expressed as whole number (AbdulBaki and Anderson, 1973).

$$\text{Vigour index} = \text{Germination (\%)} \times \text{ Seedling length (cm)} \qquad \text{(Eq.6)}$$

Biochemical resultant seed quality parameters

Protein content (%), Starch content (mg g^{-1}) and α- amylase activity (mg maltose min^{-1}) was estimated by the method suggested by AliKhan and Youngs (1973); Hodge and Hofreiter (1962) and Paul (1970).

Statistical analysis

The analysis of variance was carried out and comparison was done by least significant difference (LSD). The mean difference is significant at the P-values < 0.05. Statistical analysis was performed using the SPSS 16.0 software (SPSS Inc., Chicago, USA).

Results

Anther length and width

Significant differences were observed for length and width due to environmental conditions and increased temperature (2ºC) under artificially induced CO_2 condition in 2022 and 2023. Among the environments, the anther length and width were significantly higher in the aCO_2 condition in both years and the lowest anther length and width was recorded in eCO_2. The anther length and width differed significantly with treatments. The salicylic acid @ 800 ppm and boric acid @ 500 ppm treatments exhibited lengthier anther (202.4 and 201.4 µm, respectively in 2022 and 205.2, 203.5 µm, respectively in 2023) and wider anther (48.1, 47.5 µm, respectively in 2022 and 48.9, 48.1 µm, respectively in 2023). Under eCO$_2$ condition salicylic acid 800 ppm resulted in the significantly higher length of anther during both the years (195.2, 197.4 µm) and width of anther was 44.5, 45.8 µm, which was on par with boric acid 500 ppm. Control plants showed shorter anther length (188.5, 190.0 µm) and width (37.8, 38.1 µm). Increased length and width of anther was observed during 2022 compared to 2023 in both environmental conditions. A significant interaction between environment and treatments was observed only in another width during 2022 (*Table 2*, *Fig. 2*).

Table 2. *Effect of elevated CO$_2$ and foliar spray on anther characters and pollen viability of rice*

Treatments		**Anther (µm)**				**Pollen viability (%)**	
		2022		2023			
EC	**FS**	**Length**	**Width**	**Length**	**Width**	2022	2023
E_1	F_1	195.8 ± 1.3 ^{ab}	46.1 ± 0.5 ^{ac}	197.2 ± 2.7 ^{ac}	46.4 ± 0.5 ^{acd}	95 ± 1.1 ^{ab}	96 ± 0.7 ^{ab}
	F_2	199.6 ± 2.0 ^{ab}	47.3 ± 0.6 ^{ab}	201.6 ±1.5 ^{abc}	47.9 ± 0.5 ^{ab}	95 ± 0.9 ^{aa}	96 ± 0.8 ^{aa}
	F_3	202.4 ± 2.5 ^{aa}	48.1 ± 0.3 ^{aa}	205.2 ± 1.8 ^{aa}	48.9 ± 0.6 ^{aa}	96 ± 1.0 ^{aa}	97 ± 1.0 ^{aa}
	F_4	199.5 ± 1.6 ^{ab}	47.1 ± 0.6 ^{ab}	201.3 ± 2.8 ^{abc}	47.7 ± 0.8 ^{abc}	96 ± 1.6 ^{aa}	97 ± 0.9 ^{aa}
	F_5	201.4 ± 1.8 ^{aab}	47.5 ± 0.4 ^{ab}	203.5 ± 1.3 ^{aab}	48.1 ± 0.5 ^{ab}	96 ± 0.6 ^{aa}	97 ± 0.7 ^{aa}
	F_6	196.1 ± 1.9 ^{ab}	46.5 ± 0.7 ^{abc}	197.7 ± 2.4 ^{abc}	47.0 ± 0.8 ^{ac}	95 ± 0.7 ^{aab}	96 ± 0.8 ^{aab}
	F_7	196.8 ± 1.9 ^{ab}	46.8 ± 0.4 ^{ab}	198.6 ± 2.2 ^{abc}	47.3 ± 0.8 ^{abc}	95 ± 1.5 ^{aab}	96 ± 0.7 ^{aab}
E_2	F_1	188.5 ± 2.3 ^{bb}	37.8 ± 1.1^{bc}	190.0 ± 2.3 ^{bc}	38.1 ± 0.5 ^{bcd}	85 ± 0.9 ^{bb}	86 ± 1.0 ^{bb}
	F_2	190.1 ± 2.5 ^{bb}	41.3 ± 1.4 ^{bb}	192.0 ± 1.8 ^{bbc}	42.1 ± 0.8 ^{bb}	89 ± 1.4 ^{ba}	92 ± 0.9 ^{ba}
	F_3	195.2 ± 1.3 ^{ba}	44.5 ± 1.2 ^{ba}	197.4 ± 1.0 ^{ba}	45.8 ± 0.7 ^{ba}	90 ± 1.1 ^{ba}	92 ± 0.8 ^{ba}
	F_4	189.7 ± 1.4 ^{bb}	41.0 ± 1.2 ^{bb}	191.5 ± 1.2 ^{bbc}	41.8 ± 0.7 ^{bbc}	90 ± 1.1 ^{ba}	93 ± 1.1 ^{ba}
	F_5	190.4 ± 1.4 ^{bab}	41.5 ± 1.0 ^{bb}	192.4 ± 1.3 ^{bbc}	42.3 ± 0.9 ^{bb}	91 ± 1.1 ^{ba}	93 ± 1.1 ^{ba}
	F_6	189.0 ± 1.9 ^{bb}	39.8 ± 0.7 ^{bbc}	190.4 ± 1.6 ^{bbc}	40.4 ± 0.6 ^{bc}	88 ±1.1 ^{bab}	91 ± 1.1 ^{bab}
	F_7	189.4 ± 1.7 ^{bb}	41.0 ± 0.9 ^{bb}	191.0 ± 1.3 ^{bbc}	41.7 ± 0.6 ^{bc}	88 ± 1.0 ^{bab}	91 ± 1.0 ^{bab}

Data presented are means from four replicates with standard errors. Within each treatment, different letters at each column indicate significant differences by Duncan's multiple range test at $P < 0.05$. Environmental condition; E_1 - Ambient condition and E_2 - Elevated co$_2$ (550 ppm); Foliar spray; F_1 - Control, F_2 - Salicylic acid (600 ppm), F_3 - Salicylic acid (800 ppm), F_4 - Boric acid (400 ppm), F_5 - Boric acid (500 ppm), F_6 - Glycine betaine (400µM), F_7 - Glycine betaine (600µM)

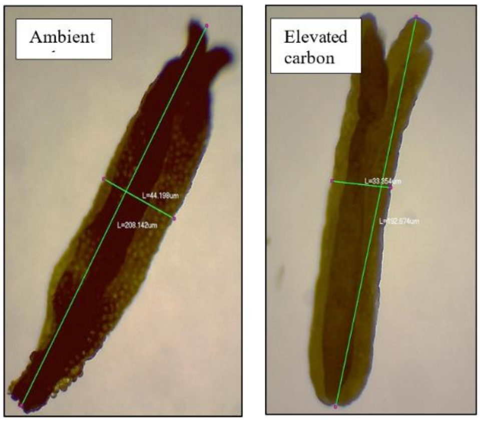

Figure 2. *Effect of elevated CO$_2$ and foliar spray on anther size of rice*

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Pollen viability

Both years maximum pollen viability % was recorded in aCO_2, while minimum under eCO_2 condition. In eCO_2 condition, foliar application of boric acid @ 500 ppm recorded significantly higher pollen viability than control plants. Foliar application significantly increased the pollen viability by 1% under aCO_2, while in eCO_2 condition, there was an increase of 7.1 %, 7.6 %, 8.2 %, 4.7% in pooled data on spraying with boric acid (500 ppm) boric acid (400 ppm), salicylic acid (800 ppm) and glycine betaine (400 and 600 µM) respectively over other treatments and control (*Table 2*, *Fig. 3*).

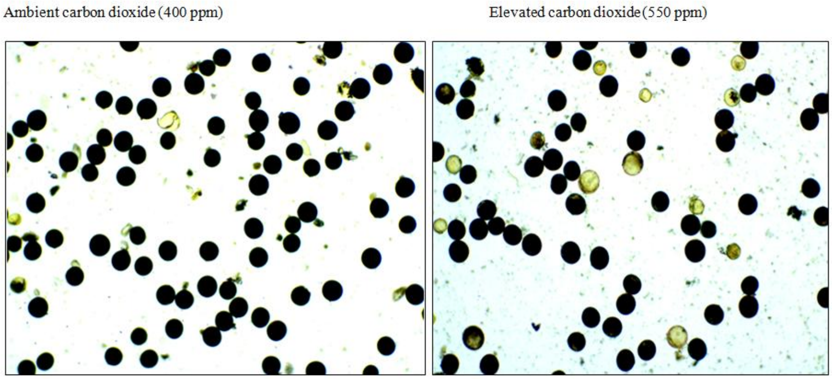


Figure 3. *Effect of elevated CO_2 and foliar spray on pollen viability (%) of rice*

Pollen length, pollen radius and pollen surface area

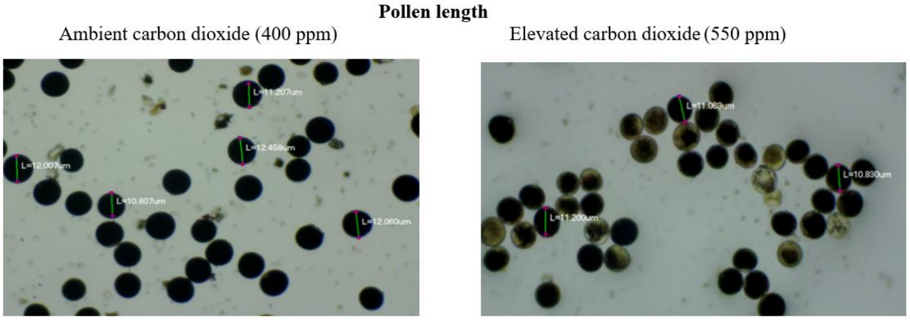
There was a significant difference between the environments in all the pollen characters in both years. There was no effect of the treatments on pollen length in 2022. But in all other parameters, there were significant influences of the treatments in both the conditions and years. In pooled data, a reduction of pollen length by 3.6%, pollen radius by 6.2% and pollen surface area by 8.2 was observed under eCO_2 condition. The foliar application had significant effect on all the pollen parameters. Maximum increase in pollen length (4.1% and 3.6%) and the pollen radius (4.1% and 2.5%) was observed at Foliar application with boric acid @ 500 ppm and boric acid @ 400 ppm. All the foliar treatments showed an increase in pollen surface area from 6.2% to 8.4% with a maximum in an application with boric acid @ 500 ppm in pooled data under eCO_2 condition (*Table 3, Fig. 4*).

Table 3. *Effect of elevated CO_2 and foliar spray on pollen characters of rice*

Treatments		**Pollen Length (µm)**		**Pollen Radius (µm)**		**Pollen Surface area (μm^2)**	
EC	**FS**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**
E_1	F_1	11.5 ± 0.04^{a}	11.5 ± 0.05^{ab}	6.1 ± 0.04^{ac}	6.1 ± 0.01^{ad}	120 ± 0.8^{ac}	120 ± 1.4^{ad}
	F_2	11.5 ± 0.03^{a}	11.6 ± 0.13^{aab}	6.3 ± 0.02^{ab}	6.3 ± 0.01^{ac}	123 ± 0.1^{ab}	123 ± 1.7^{abc}
	F_3	11.6 ± 0.12^{a}	11.7 ± 0.01^{aa}	6.4 ± 0.10^{ab}	6.4 ± 0.04^{aac}	123 ± 1.3^{ab}	124 ± 1.0^{abc}
	F_4	11.6 ± 0.07^{a}	11.7 ± 0.18^{aa}	6.3 ± 0.06^{ab}	6.4 ± 0.05^{aa}	125 ± 0.6^{ab}	126 ± 0.8^{ab}
	F_5	11.6 ± 0.14^{a}	11.7 ± 0.01^{aa}	6.5 ± 0.10^{aa}	6.7 ± 0.09^{aa}	127 ± 1.1^{aa}	129 ± 1.0^{aa}
	F_6	11.5 ± 0.07^{a}	11.6 ± 0.13^{aab}	6.3 ± 0.10^{ab}	6.3 ± 0.08^{ac}	122 ± 2.0^{ab}	122 ± 1.8^{ac}
	F_7	11.5 ± 0.12^{a}	11.6 ± 0.04^{aab}	6.3 ± 0.05^{ab}	6.3 ± 0.03^{ac}	123 ± 1.0^{ab}	123 ± 1.3^{abc}

E_2	F_1	11.1 ± 0.06^{b}	11.1 ± 0.06^{bb}	5.9 ± 0.03^{bc}	5.9 ± 0.02^{bd}	113 ± 1.2^{bc}	113 ± 1.3^{bd}
	F_2	11.4 ± 0.04^{b}	11.4 ± 0.08^{bab}	6.1 ± 0.09^{bb}	6.2 ± 0.10^{bc}	120 ± 0.5^{bb}	120 ± 0.8^{bbc}
	F_3	11.5 ± 0.10^{b}	11.6 ± 0.19^{ba}	6.1 ± 0.05^{bb}	6.2 ± 0.05^{bac}	121 ± 0.8^{bb}	122 ± 1.1^{bbc}
	F_4	11.5 ± 0.17^{b}	11.5 ± 0.09^{ba}	6.2 ± 0.03^{bb}	6.3 ± 0.02^{ba}	120 ± 0.8^{bb}	121 ± 1.7^{bb}
	F_5	11.5 ± 0.11^{b}	11.6 ± 0.10^{ba}	6.3 ± 0.09^{ba}	6.4 ± 0.11^{ba}	122 ± 0.8^{ba}	123 ± 1.3^{ba}
	F_6	11.3 ± 0.03^{b}	11.3 ± 0.03^{bab}	6.1 ± 0.07^{bb}	6.1 ± 0.04^{bc}	120 ± 1.4^{bb}	120 ± 0.7^{bc}
	F_7	11.3 ± 0.13^{b}	11.3 ± 0.18^{bab}	6.1 ± 0.03^{bb}	6.1 ± 0.04^{bc}	120 ± 0.7^{bb}	120 ± 1.6^{bbc}

Data presented are means from four replicates with standard errors. Within each treatment, different letters at each column indicate significant differences by Duncan's multiple range test at $P < 0.05$. Environmental condition; E_1 - Ambient condition and E_2 - Elevated co_2 (550 ppm); Foliar spray; F_1 - Control, F_2 - Salicylic acid (600 ppm), F_3 - Salicylic acid (800 ppm), F_4 - Boric acid (400 ppm), F_5 - Boric acid (500 ppm), F_6 - Glycine betaine (400µM), F_7 - Glycine betaine (600µM)



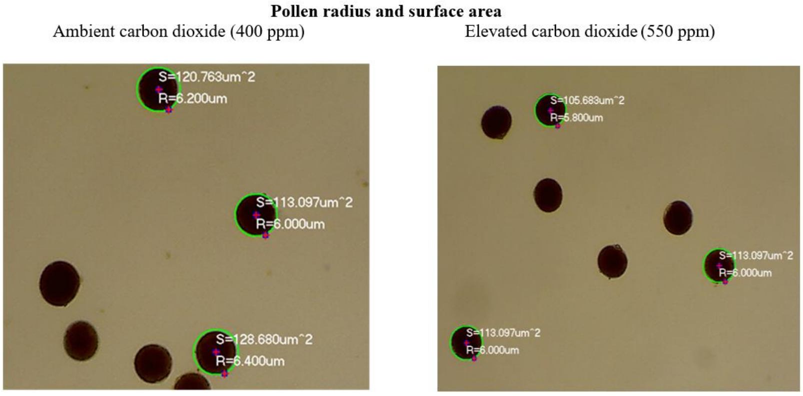


Figure 4. *Effect of elevated CO_2 and foliar spray on pollen length, surface area and radius of pollen in rice*

Antioxidant enzyme activity

The maximum antioxidant activity was recorded under eCO_2 condition, while minimum under aCO_2 condition in both the years. In, eCO_2 condition, foliar application salicylic acid (600 ppm) recorded significantly higher catalase, peroxidase and superoxide dismutase activity than untreated plants. Foliar application with salicylic acid (600 ppm) was recorded higher catalase (17.6, 18.7 µmol H_2O_2 min^{-1} g^{-1} protein), peroxidase (19.8, 21.3U mg^{-1}protein min^{-1}) and superoxide dismutase (24.5, 26.4 U mg^{1}protein min^{-1}) activities followed by glycine betaine (600µM) compared to control plants in both the years. All the foliar treatments showed an increase in catalase, peroxidase and superoxide dismutase from 5.9% to 26.5%, 10.4% to 30.1% and 3.2% to 24.4% with a maximum in an application with salicylic acid (600 ppm) in pooled data under eCO_2 condition (*Fig. 5*).

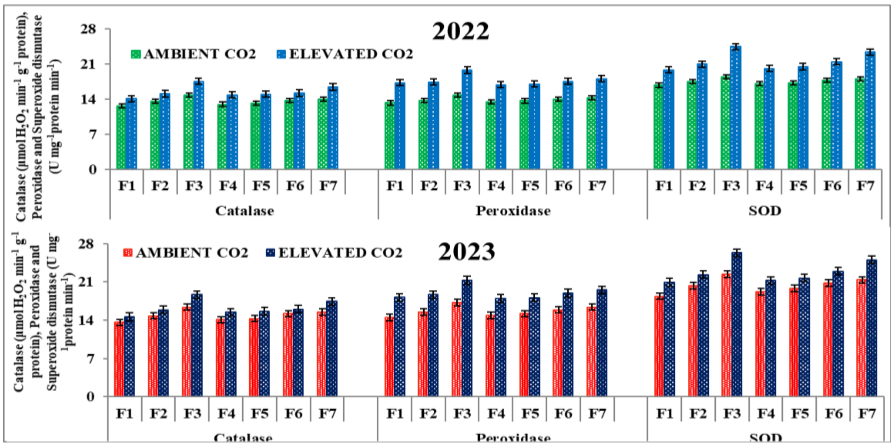


Figure 5. *Antioxidant enzymes activity in rice as influenced by foliar spray under elevated* CO_2 *condition. Data presented are means from four replicates with standard errors (Mean ± SE, n = 4) of different foliar spray (F) (*F_1 *– Control,* F_2 *- Salicylic acid 600 ppm,* F_3 *- Salicylic acid 800 ppm,* F_4 *- Boric acid 400 ppm,* F_5 *- Boric acid 500 ppm,* F_6 *- Glycine betaine 400µM,* F_7 *- Glycine betaine 600µM) under two environmental conditions (*E_1 *– Ambient* CO_2*,* E_2 *– Elevated* CO_2*). The analysis of variance was carried out and comparison was done by Duncan's multiple range test (DMRT) at 5% level of significance*

Chlorophyll a, b and total content

Chlorophyll a, b and total chlorophyll showed a significant difference due to environmental conditions, foliar spray and their interaction were significant in both *kharif* 2022 -2023. The maximum chlorophyll 'a' (0.665 and 0.693), chlorophyll 'b' (0.583 and 0.599) and total chlorophyll was registered in salicylic acid 800 ppm (1.25 and 1.29) under ambient condition when compared to OTC+ elevated CO_2. While lowest was recorded in control under ambient condition and OTC+ elevated CO_2 irrespective of the seasons (*Fig 6*).

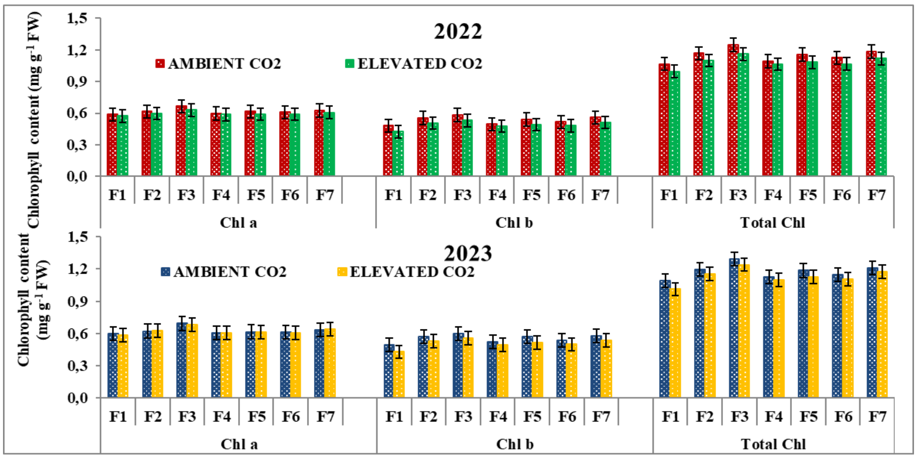


Figure 6. *Effect of elevated* CO_2 *and foliar spray on chlorophyll content of rice*

Tiller production plant^{-1}, 1000 seed weight (g) and seed yield plant^{-1} (g)

The effect of different environmental conditions had a significant influence on a total number of tillers and reproductive plant^{-1}. Total number of tillers plant^{-1} was maximum at ambient condition (24 to 27) compared to OTC+ elevated CO_2 (14 to 16) irrespective of the seasons. The maximum reproductive tillers plant^{-1} (20 to 24) recorded under ambient condition when compared to OTC+ elevated CO_2 (14 to 16) irrespective of the seasons. The increased 1000 seed weight was registered in salicylic acid 800 ppm (19.0 and 19.3) under OTC+ elevated CO_2 when compared to ambient condition (17.3 and 17.7), while lowest was recorded in control under OTC+ elevated CO_2 (17.2 and 17.3) and ambient condition (15.8 and 16.0) irrespective of the seasons. Seed yield plant^{-1} showed a significant difference due to environmental conditions, foliar spray and their interaction was significant in both *kharif* 2022 -23. The maximum seed yield plant^{-1} (28.7 and 32.1) registered in salicylic acid 800 ppm under ambient condition when compared to OTC+ elevated CO_2 (21.4 and 22.7), while the minimum was registered in control under ambient condition (25.2 and 27.3) and OTC+ elevated CO_2 (17.3 and 18.4) irrespective of the seasons (*Fig. 7*).

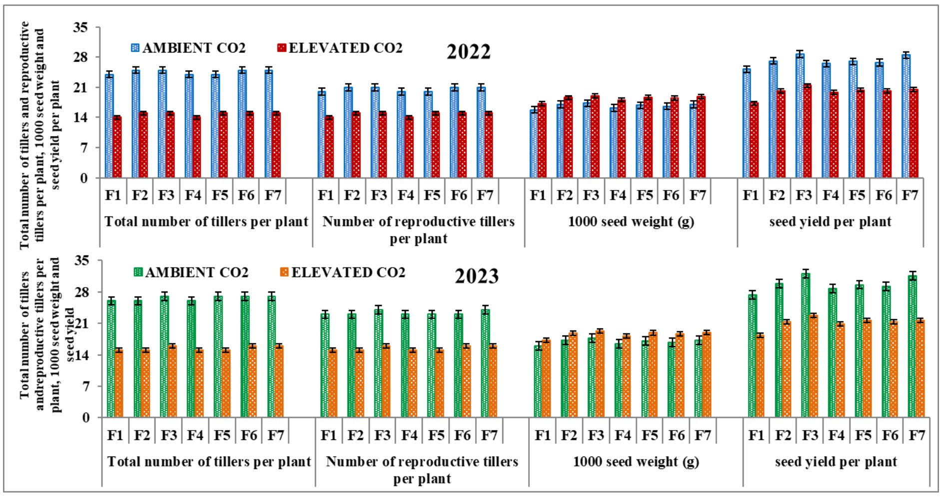


Figure 7. *Impact of elevated CO_2 and foliar spray on tiller production and yield attributes of rice. Data presented are means from four replicates with standard errors (Mean ± SE, n = 4) of different foliar spray (F). (F_1 – Control, F_2 - Salicylic acid 600 ppm, F_3 - Salicylic acid 800 ppm, F_4 - Boric acid 400 ppm, F_5 - Boric acid 500 ppm, F_6 - Glycine betaine 400µM, F_7 - Glycine betaine 600µM) under two environmental conditions (E_1 – Ambient CO_2, E_2 – Elevated CO_2). The analysis of variance was carried out and comparison was done by Duncan's multiple range test (DMRT) at 5% level of significance*

Seed yield plot-1 (kg) and seed yield ha^{-1} (kg)

Seed yield plot^{-1} and ha^{-1} showed a significant difference due to environmental conditions; foliar spray and their interaction was significant in both kharif 2022 -23. The maximum seed yield plot^{-1} (11.5 and 11.8) and seed yield ha^{-1} (7188 and 7375) registered in salicylic acid 800 ppm under ambient condition when compared to OTC+ elevated CO_2 (8.6 and 9.1) and (5350 and 5675) while the minimum was registered in control under ambient condition (9.1 and 10.0) and (6269 and 6275) and OTC+ elevated CO_2 (6.9 and 7.4) (4325 and 4600) irrespective of the seasons (*Table 4*).

Table 4. *Effect of elevated CO$_2$ and foliar spray on seed yield of rice*

Treatments		**Seed yield plot^{-1} (kg)**		**Seed yield ha^{-1} (kg)**	
EC	**FS**	**2022**	**2023**	**2022**	**2023**
E$_1$	F$_1$	10.0 ± 0.13^{ae}	10.0 ± 0.06^{ad}	6269 ± 18.5^{afe}	6275 ± 20.3^{aff}
	F$_2$	10.8 ± 0.12^{abc}	11.0 ± 0.07^{ab}	6775 ± 18.4^{acc}	6875 ± 19.2^{add}
	F$_3$	11.5 ± 0.09^{aa}	11.8 ± 0.05^{aa}	7188 ± 21.7^{aaa}	7375 ± 18.8^{aaa}
	F$_4$	10.6 ± 0.13^{ad}	10.8 ± 0.14^{ac}	6625 ± 20.3^{aed}	6750 ± 22.3^{aee}
	F$_5$	10.8 ± 0.09^{abc}	11.1 ± 0.09^{ab}	6750 ± 17.4^{acc}	6938 ± 17.7^{acc}
	F$_6$	10.7 ± 0.13^{abcd}	11.0 ± 0.07^{ab}	6675 ± 21.0^{add}	6875 ± 15.5^{add}
	F$_7$	11.0 ± 0.08^{ab}	11.2 ± 0.05^{ab}	6875 ± 19.3^{abb}	7000 ± 15.4^{abb}
E$_2$	F$_1$	6.9 ± 0.05^{be}	7.4 ± 0.06^{bd}	4325 ± 20.1^{bfj}	4600 ± 20.3^{bfk}
	F$_2$	8.1 ± 0.04^{bbc}	8.6 ± 0.04^{bb}	5050 ± 20.7^{bch}	5350 ± 16.4^{bdi}
	F$_3$	8.6 ± 0.04^{ba}	9.1 ± 0.04^{ba}	5350 ± 17.8^{baf}	5675 ± 22.5^{bag}
	F$_4$	7.9 ± 0.07^{bd}	8.4 ± 0.06^{bc}	4950 ± 19.4^{bei}	5225 ± 20.2^{bej}
	F$_5$	8.2 ± 0.03^{bbc}	8.6 ± 0.08^{bb}	5100 ± 20.5^{bcgh}	5400 ± 22.5^{bchi}
	F$_6$	8.1 ± 0.07^{bbcd}	8.6 ± 0.07^{bb}	5050 ± 17.7^{bdh}	5350 ± 17.8^{bdi}
	F$_7$	8.2 ± 0.05^{bb}	8.7 ± 0.06^{bb}	5125 ± 17.4^{bbg}	5425 ± 19.1^{bbh}

Data presented are means from four replicates with standard errors. Within each treatment, different letters at each column indicate significant differences by Duncan's multiple range test at $P < 0.05$. Environmental condition; E$_1$ - Ambient condition and E$_2$ - Elevated co$_2$ (550 ppm); Foliar spray; F$_1$ - Control, F$_2$ - Salicylic acid (600 ppm), F$_3$ - Salicylic acid (800 ppm), F$_4$ - Boric acid (400 ppm), F$_5$ - Boric acid (500 ppm), F$_6$ - Glycine betaine (400µM), F$_7$ - Glycine betaine (600µM)

Resultant seed quality parameters

Germination per cent was not significantly influenced due to a different environmental conditions and foliar spray during first season. Significant difference was observed due to different environmental conditions in second season. Statistically, significant difference was observed for seedling length between the environmental conditions and foliar spray and their interaction was significant in both *kharif* 2022 -23. Different environmental conditions had a significant influence on vigour index and biochemical seed quality parameters. Significant differences were observed between the environmental conditions, foliar spray for the vigour index and their interaction was significant in both *kharif* 2022 -23.

Vigour indices depend upon the germination, dry matter production and seedling length. Elevated CO$_2$ registered high germination, seedling length, vigour index, starch content and α- amylase enzyme activity but there was a reduction in protein content also observed. Among the foliar sprays, salicylic acid @ 800 ppm recorded maximum seedling length, vigour index, starch content, α- amylase enzyme activity and protein content under both ambient and elevated CO$_2$ conditions compared to other treatment and control (*Tables 5, 6*).

Table 5. *Effect of elevated CO_2 and foliar spray on physiological seed quality attributes of resultant seeds of rice*

Treatments		**Germination (%)**		**Seedling length (cm)**		**Vigour index**	
EC	**FS**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**
E_1	F_1	$92(73.6) \pm 1.0^{ns}$	$93(74.7) \pm 1.0^{b}$	26.3 ± 0.6^{bd}	26.8 ± 0.8^{bd}	2420 ± 12^{bg}	2492 ± 12^{bg}
	F_2	$93(74.7) \pm 1.0^{ns}$	$93(74.7) \pm 0.9^{b}$	28.4 ± 0.6^{babc}	29.1 ± 0.8^{babc}	2641 ± 13^{bc}	2706 ± 11^{bc}
	F_3	$93(74.7) \pm 0.9^{ns}$	$94(75.8) \pm 0.7^{b}$	29.7 ± 0.8^{ba}	30.6 ± 0.5^{ba}	2762 ± 13^{ba}	2876 ± 12^{ba}
	F_4	$92 (73.6) \pm 0.8^{ns}$	$93(74.7) \pm 0.6^{b}$	27.1 ± 0.9^{bcd}	27.6 ± 0.8^{bcd}	2493 ± 12^{bf}	2567 ± 12^{bf}
	F_5	$93(74.7) \pm 1.3^{ns}$	$93(74.7) \pm 0.8^{b}$	28.0 ± 0.6^{bbc}	28.7 ± 0.8^{bbc}	2604 ± 11^{bd}	2669 ± 11^{bd}
	F_6	$92(73.6) \pm 1.4^{ns}$	$93(74.7) \pm 0.6^{b}$	27.4 ± 0.8^{bbc}	27.9 ± 0.9^{bbcd}	2521 ± 9^{be}	2595 ± 10^{be}
	F_7	$93 (74.7) \pm 0.7^{ns}$	$94(75.8) \pm 1.0^{b}$	28.8 ± 0.9^{bab}	29.5 ± 0.8^{babc}	2678 ± 8^{bb}	2773 ± 11^{bb}
E_2	F_1	$93 (74.7) \pm 0.8^{ns}$	$94(75.8) \pm 1.2^{a}$	27.9 ± 0.8^{ad}	28.6 ± 0.6^{ad}	2595 ± 8^{ag}	2688 ± 12^{ag}
	F_2	$94(75.8) \pm 0.9^{ns}$	$95(77.1) \pm 0.9^{a}$	30.2 ± 0.7^{aabc}	31.1 ± 0.9^{aabc}	2839 ± 9^{ac}	2955 ± 10^{ac}
	F_3	$95(77.1) \pm 1.1^{ns}$	$95(77.1) \pm 0.8^{a}$	31.3 ± 0.9^{aa}	32.6 ± 0.8^{aa}	2974 ± 11^{aa}	3097 ± 11^{aa}
	F_4	$93(74.7) \pm 1.2^{ns}$	$94(75.8) \pm 0.8^{a}$	28.8 ± 0.8^{acd}	29.5 ± 0.8^{acd}	2678 ± 10^{af}	2773 ± 11^{af}
	F_5	$94(75.8) \pm 1.0^{ns}$	$95(77.1) \pm 1.6^{a}$	30.1 ± 0.8^{abc}	30.9 ± 1.0^{abc}	2829 ± 10^{ad}	2936 ± 9^{ad}
	F_6	$93(74.7) \pm 0.9^{ns}$	$94(75.8) \pm 1.3^{a}$	29.8 ± 1.0^{abc}	30.5 ± 0.9^{abcd}	2771 ± 10^{ae}	2867 ± 12^{ae}
	F_7	$94(75.8) \pm 0.7^{ns}$	$95(77.1) \pm 0.8^{a}$	30.5 ± 0.7^{aab}	31.4 ± 0.6^{aabc}	2867 ± 7^{ab}	2983 ± 11^{ab}

(Figures in parenthesis indicate arcsine values)

Table 6. *Effect of elevated CO_2 and foliar spray on biochemical seed quality attributes of resultant seeds of rice*

Treatments		**Protein content (%)**		**Starch content (mg g^{-1})**		**α- amylase activity (mg maltose min^{-1})**	
EC	**FS**	**2022**	**2023**	**2022**	**2023**	**2022**	**2023**
E_1	F_1	7.80 ± 0.07^{ad}	7.82 ± 0.06^{a}	9.05 ± 0.09^{b}	9.11 ± 0.07^{be}	1.15 ± 0.01^{be}	1.16 ± 0.01^{be}
	F_2	7.94 ± 0.04^{aabc}	7.97 ± 0.04^{a}	9.21 ± 0.11^{b}	9.34 ± 0.03^{bbc}	1.20 ± 0.02^{bbc}	1.22 ± 0.02^{bbc}
	F_3	7.96 ± 0.06^{aa}	7.99 ± 0.06^{a}	9.28 ± 0.13^{b}	9.43 ± 0.05^{ba}	1.21 ± 0.02^{ba}	1.24 ± 0.02^{ba}
	F_4	7.80 ± 0.06^{acd}	7.82 ± 0.07^{a}	9.17 ± 0.07^{b}	9.26 ± 0.05^{bd}	1.16 ± 0.02^{bde}	1.18 ± 0.02^{bde}
	F_5	7.92 ± 0.06^{aabc}	7.95 ± 0.05^{a}	9.21 ± 0.08^{b}	9.32 ± 0.03^{bbcd}	1.19 ± 0.01^{bbc}	1.21 ± 0.01^{bbcd}
	F_6	7.81 ± 0.05^{abcd}	7.83 ± 0.08^{a}	9.20 ± 0.09^{b}	9.31 ± 0.07^{bcd}	1.16 ± 0.01^{bcd}	1.18 ± 0.01^{bcd}
	F_7	7.94 ± 0.05^{aab}	7.97 ± 0.06^{a}	9.26 ± 0.09^{b}	9.39 ± 0.06^{bab}	1.20 ± 0.01^{bb}	1.22 ± 0.02^{bb}
E_2	F_1	7.13 ± 0.07^{bd}	7.15 ± 0.10^{b}	9.21 ± 0.07^{a}	9.30 ± 0.10^{ae}	1.20 ± 0.01^{ae}	1.22 ± 0.01^{ae}
	F_2	7.37 ± 0.01^{babc}	7.39 ± 0.08^{b}	9.60 ± 0.08^{a}	9.74 ± 0.07^{abc}	1.26 ± 0.02^{abc}	1.30 ± 0.01^{abc}
	F_3	7.51 ± 0.10^{ba}	7.54 ± 0.06^{b}	9.68 ± 0.05^{a}	9.92 ± 0.06^{aa}	1.36 ± 0.02^{aa}	1.40 ± 0.01^{aa}
	F_4	7.31 ± 0.04^{bcd}	7.33 ± 0.06^{b}	9.40 ± 0.05^{a}	9.51 ± 0.07^{ad}	1.22 ± 0.01^{ade}	1.25 ± 0.02^{ade}
	F_5	7.37 ± 0.06^{babc}	7.39 ± 0.09^{b}	9.54 ± 0.04^{a}	9.66 ± 0.06^{abcd}	1.25 ± 0.01^{abc}	1.28 ± 0.02^{bcd}
	F_6	7.35 ± 0.04^{bbcd}	7.37 ± 0.08^{b}	9.45 ± 0.10^{a}	9.56 ± 0.06^{acd}	1.25 ± 0.01^{acd}	1.28 ± 0.02^{acd}
	F_7	7.40 ± 0.09^{bab}	7.42 ± 0.06^{b}	9.61 ± 0.09^{a}	9.78 ± 0.10^{aab}	1.28 ± 0.01^{ab}	1.32 ± 0.02^{ab}

Data presented are means from four replicates with standard errors. Within each treatment, different letters at each column indicate significant differences by Duncan's multiple range test at $P < 0.05$. Environmental condition; E_1 - Ambient condition and E_2 - Elevated co_2 (550 ppm); Foliar spray; F_1 - Control, F_2 - Salicylic acid (600 ppm), F_3 - Salicylic acid (800 ppm), F_4 - Boric acid (400 ppm), F_5 - Boric acid (500 ppm), F_6 - Glycine betaine (400µM), F_7 - Glycine betaine (600µM)

Discussion

Rice is most sensitive to stress during its reproductive period specifically during anthesis stage. Abnormal pollination is caused due to the irregularities in anther and pollen (Fahad et al., 2018). The morphology of the reproductive parts has variations due to the external environment in the experiments conducted there was significant difference in anther length and width at different environmental conditions. The elevated CO_2 had a negative impact on the anther length and width, mainly due to associated high temperature stress. Pollen grain swelling is essential for anther dehiscence have negative impact due to elevated temperature (Matsui et al., 2000). There is negative impact of elevated CO_2 on pollen viability. Many reports suggests that elevated CO_2 have enhanced effect on the heat induced sterility in pollen (Yoshimoto et al., 2005). Even though Kobayasi et al. (2019) reported a positive effect on anther length during elevated CO_2 condition, there was no improvement in pollination and anther dehiscence in those cases.

The pollen radius effect the electrostatic charges in the pollen and the velocity of drift to the stigmatic surface (Bowker and Crenshaw, 2007). The pollen radius also indicates the pollen hydration. Whereas the pollen surface area affects the hydration of pollen grains in turn affecting the viability of pollen (Aylor, 2003). Pollen viability and fertility is determined by the amount of sporopollenin, an important inorganic polymer present in the surface of pollen, which is proportional to the radius and surface area of the pollen grains. There is reduction in pollination due to the reduction in these parameters of the pollen grains during elevated CO_2 and the associated temperature stress conditions. Due to the elevated CO_2 the antioxidant activity was increased in both years, because of the change in antioxidant mechanisms induced by the climate changes (AbdElgawad et al., 2015).

Boron is one of the important micronutrients affecting the metabolic activities in the plant system. There is positive effect of boron on plants during several abiotic stresses (Hanafy et al., 2008). It increases the antioxidant activities during stress condition, creating tolerance to the temperature stress (Waraich et al., 2012). In the experiment conducted, the increase in antioxidant activity was also observed. These mechanisms involved could be the reason for the improvement in anther properties and pollen parameters during elevated CO_2 and associated temperature stress. In addition the external application of boron in the form of boric acid have positive effects on flowering, reproductive development especially on microsporogenesis, anther development, pollination, cell division and cell wall synthesis (Miwa and Fujiwara, 2010). Boron regulates water transport in protoplasm helping in the hydration of pollen grains. The improvement in pollen and anther parameters might be due to the effects of boron in mitigation of stress. This also have influence on the reproductive activities from pollen germination to fertilization.

Heat stress have detrimental effects on the microspore development and can even cause spikelet degeneration (Feng et al., 2018). Another organic compound in alleviation of stresses is salicylic acid. In the combination of stresses, due to elevated CO_2 and high temperature, salicylic acid controls the stress on reproductive parts and reduces the impact. Improved pollen viability and other traits of anther and pollen was attained by the application of salicylic acid. The plant was protected from the oxidative stress during abiotic stress by increasing the antioxidant levels (Mohammed and Tarpley, 2009). During the experiment it was proved that, increased activity of antioxidant enzymes like superoxide dismutase, catalase, peroxidase, ascorbate peroxidase was obtained due to spray of salicylic acid which prevents oxidative damage (Feng et al., 2018). The

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application of salicylic acid induced maximum increase in the total antioxidant production. According to the reports of Feng et al. (2018), the salicylic acid retards the tapetal tissue degradation due to heat stress during high temperature, reversing the pollen abortion. The capability to avoid shrinkage of the cells may help in improving the anther and pollen parameters during the stress.

Similar effects in mitigation of the stresses were shown by glycine betaine. The inert organic solute gets accumlated in plant cells and prevents the osmotic imbalance during abiotic stresses (Masood et al., 2016). It acts on the reactive oxygen produced while the stress and detoxifies (Hasanuzzaman et al., 2019). GB, external application in the form of osmoprotectant is found effective retarding the effect of high temperature on the plant growth and development by increasing the antioxidant activity. GB prevents the oxidative damage to the membrane by increasing the leaf photosynthetic rate and decreasing the internal concentration of CO_2, rate of respiration, scavenging of ROS, protecting enzymes, photosystem II, membrane integrity and antioxidant status of the plant through signal transduction and ion homeostasis which help in reduce pollen sterility (Mohammed and Tarpley, 2009). These protective activities of glycine betaine might have contributed to the improvement of reproductive parameters in the elevated CO_2 and temperature stress.

The change in tertiary and quaternary structures of membrane protein at high temperature determines the biological membrane integrity and functions. The physiological processes including the photosynthesis an respiration to be carried out in the temperature stress, regular functioning of the membrane protein is very crucial (Mittler, 2002). In the present studies at elevated CO_2, there is significant reduction in chlorophyll content. The study also proved that foliar application of salicylic acid @ 800 ppm increase chlorophyll content compared to other treatments. An earlier report says premature loss of chlorophyll due to heat sensitivity (Talukder et al., 2014). Rukmini and Raharjo (2010) are of the opinion that photo-oxidization of chlorophyll occurs under high temperature.

In the young leaves under high temperature there is significant degradation of chlorophyll a and b. the active oxygen species production is associated with the effect on chlorophyll or the photosynthetic apparatus. The reduction in the rate of photosynthesis is due to the structural changes in the thylakoid (Camejo et al., 2006). The effect of high temperature on the photosynthetic membrane in a study revealed to be the loss of grana by stacking or its swelling. The temperature have high influence on the photosynthetic capacity of the C_3 plants than C_4 plants. There is change in carbon metabolism enzymes, specifically rubisco enzyme and alteration of energy distribution. The rate of RuBP regenerated is altered disrupting the electron transport and inactivating the oxygen-evolving enzymes of photosynthetic system II (Salvucci and Crafts-Brandner, 2004).

Under elevated CO_2 associated high temperature stress plants has lower number of tillers plant^{-1} and reproductive tillers plant^{-1} but no difference was observed due to foliar spray. With Ohe et al. (2007) reported increased plant height and earlier tillering with reduced number of tillers under high temperature. Mitra and Bhatia (2008) on the other hand reported that in rice cultivar with the elevated CO_2 and associated high temperature stress there is reduction in number of tillers. Allen et al. (2003) registered that increasing CO_2 boosting the biomass, volume and length of roots, with enhanced biomass allocation to roots and increased root-shoot ratio. There is reduction in growth and yield of plants under higher temperature due to loss of cell water content and cell size. In the study conducted, when under foliar application with salicylic acid @ 800 ppm and boric acid

@ 500 ppm. Enhanced yield attributes of rice is observed by the spray of salicylic acid (Shah et al., 2017). Boron has been postulated to increase the rate of sugars translocation produced during photosynthesis in mature plant leaves to active growing regions and the developing seeds.

The reduction in seed yield per plant was high under elevated CO_2. The foliar application of salicylic acid @ 800 ppm can increase the seed yield significantly. Elevated CO_2 have both beneficial and detrimental impact in rice plant, increase in CO_2 increases the biomass production whereas the overall yield is reduced. For every 75 ppm increase in CO_2 concentration, rice yields increase by 0.5 t ha^{-1}, but for every 1°C increase in temperature the yield decrease by 0.6 t ha^{-1} (IPCC, 2013). In a study, there is reduction in yield from 1°C rise in the daily mean temperature from 5-7 % for the major crops including rice. The yield reduction is associated with the sink, shortening the growth duration and increasing the maintenance respiration (Matthews and Wassman, 2003). Even smaller increase in temperature causes decrease in the yield. Under elevated CO_2 condition both reproductive as well as vegetative biomass growth is increased. Air temperature have great influence on the leaf appearance developmental rate and total growth duration, whereas when the range of CO_2 330 to 660 ppm across the limit (Ashraf and Ali, 2008). Studies shows that the CO_2 increase has significantly increased the biomass and yield at ambient temperature when the associated temperature increase occurs, there is induced spikelet sterility. There is lack of studies on CO_2 along with temperature.

The germination, seedling length and dry matter production is influenced by Vigour indices. Under elevated CO_2, rice showed high germination, seedling length, vigour index, starch content and α- amylase enzyme activity with reduced protein content being observed. Foliar application of salicylic acid @ 800 ppm induced the maximum seedling length, vigour index, starch content, α- amylase enzyme activity and protein content, compared to other foliar applications in both ambient and elevated CO_2 condition compared to other treatments and control. The phenolic compound present in salicylic acid act as an endogenous phytohormone cum regulator playing a vital role in strengthening the plant against the biotic and abiotic stresses with strong defense mechanisms (Szalai, 2002). Rajashekaran et al. (2002) proved that there is improvement in germination percentage by the external application of salicylic acid to seeds. Senaratna et al. (2002) reported that the primary intermediate molecule acting against the environmental stresses is salicylic acid. The biosynthesis of GA may be with the help of salicylic acid and 2-6 dihydroxybenzoic acids and the thermogene inducers which stimulate seed germination are induced (Shah, 2003). A report by Khan et al. (2015) says that when barley seeds are treated with salicylic acid it fastened the seed germination and eliminating the reactive oxygen species with its antioxidant properties.

Photosynthetic acclimation has various explanations reported, one among them is decreased leaf nitrogen (Li et al., 2023). In a study on rice, decline in N allocation into leaf blades, resulted in reduced rubisco and other protein synthesis under elevated CO_2 (Seneweera et al., 2011). Statement by Mishra et al. (2009), higher germination rate and quick seedling growth at initial stage is observed on the varieties with high alpha-amylase activity. They are non-structural carbohydrate which effects the gene transcription by signaling the molecules which is their major role. Photosynthetic acclimation involves sugars containing high amount of carbohydrates lowering the gene transcripts and decreases the photosynthesis by suppressing the protein synthesis under elevated condition (Li et al., 2020). Thus, resulting in photosynthetic acclimation mainly concerned with the products of photosynthesis. El Tayeb and Ahmed (2010) registered that when salicylic acid is used for the treatment of seeds there was noticeable increase in dry weight of seedlings, sugar content, protein and minerals when compared with the untreated seeds.

During elevated CO_2 condition, there is observable increase in stored carbohydrate content in the plants. Increase in the storage carbohydrates is proportional to the conversion of starch to sucrose overnight under the elevated condition (Li et al., 2020). The starch to sucrose conversation is important for the normal growth and development of the plant, but there is accumulation of sucrose during the elevated CO_2 condition. Under optimum conditions plants produces starch and it get utilized during night, in the elevated condition the increased production of sucrose leads to the accumulation of starch in the leaves over time (Aranjuelo et al., 2008).

Conclusion

For mitigating the adverse effects of elevated CO_2 associated with high temperature on seed set of rice, different osmo protectants and secondary metabolites were applied as foliar spray in rice. The chemicals like salicylic acid (600 and 800 ppm), boric acid (400 and 500 ppm) and glycine betaine (400 and 500 μM) were sprayed at the time of booting stage. A significant reduction was observed in anther, pollen characters, chlorophyll content, antioxidant enzyme activity, seed and yield characters due to increased temperature of 2°C under open top chamber with elevated CO_2. The study also proved that foliar spray with salicylic acid @ 800 ppm showed positive effect by recording lengthier and wider anthers and pollen length were increased under elevated CO_2 condition. Among the foliar sprays, salicylic acid @ 800 ppm recorded maximum seed weight, seed yield and resultant seed quality characters in both the environmental conditions.

Conflicts of Interest. The authors declare that they have no conflict of interest.

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