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High temperature stress mitigation in rice (*Oryza sativa* L.): Foliar application of plant growth regulators and nutrients

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ABSTRACT

A two years (years?) experiment was conducted to make certain the effects of exogenously applied plant growth regulators (PGR), nutrients and pink-pigmented facultative methylotrophs on rice growth and yield attributes under high day temperature (HDT). Rice variety Uma (MO-16) was subjected to HDT in controlled polyhouse and ambient temperature with ten different treatments were applied as foliar spray at three stages viz., panicle initiation, heading and flowering stage. Physiological observations were taken at 50% flowering stage and yield parameters were taken at harvest stage. There was significant variation for physiological, biochemical, morphological and yield components among the treatments. Exogenous application of PGRs was helpful in alleviating the adverse effects of high temperature. Brassinosteroid treatment recorded high pollen viability, spikelet fertility and grain yield plant^{-1} by improving the physiological traits such as cell membrane stability index (CMSI), photosynthetic rate (Pn), stomatal conductance (Gs), Fv/Fm ratio, chlorophyll stability index (CSI). These results will be of importance for further understanding the adaptation and survival mechanisms of rice to high temperature.

Keywords: High temperature stress, plant growth regulators, physiological traits, nutrients

Rice (*Oryza sativa* L.) occupies the extremely desirable prime position among the food crops grown around the world and remains as the most significant food crop in Asia. Rice is consumed by around three billion people and is the main staple food for multitude of people on earth more than any other crop (Krishnan *et al.,* 2011). The term "Rice is life" rightly explains the significance of rice in food and nutritional safety, especially in Asian countries. More than two billion people in Asia receive 60-70 per cent of their food energy from rice (Changchui, 2003). Rice is cultivated under more diverse ecologies than any other food crop, ranging from irrigated (lowland), rainfed, upland, to deepwater conditions and below mean sea level. Still the productivity of rice is limited by many abiotic stresses including salinity, drought, submersion and cold (Wani and Sah, 2014). Among various abiotic stresses, high temperature is one of the important stress which affects rice productivity (Berry and Bjorkman, 1980; Lobell and Asner, 2003; Beena, 2013). The vulnerability of rice cultivation will be further exacerbated by the predicted global climate change. Among the future global climate change events, an increase of 1.4–5.8 °C in the global average surface temperature by 2100 and the possibility of increased variability about this mean (IPCC, 2007) will be very significant to global rice production. Temperatures above 35°C at flowering induce floret sterility in rice (Satake and Yoshida, 1978; Matsui *et al*., 2001; Jagadish *et al*., 2010; Beena *et al*., 2018a). One of the effective way to mitigate the adverse effects of global warming is to adopt high temperature-tolerant cultivars (Nakagawa *et al*., 2003). However, the extent to which we can improve the high-temperature tolerance of rice is unclear. Although the genetic and physiological mechanisms of high temperature-induced floret sterility are unclear, the tolerance to high temperatures varies by more than 3°C in rice (Satake and Yoshida, 1978). It is estimated that the increasing temperature would reduce rice production by 41% at the end of the 21st century (Khan *et al*., 2019).

For many decades, concerted efforts have been made to overcome harmful effects of environmental stresses such as alternate wetting and drying, aerobic rice cultivation, use of tolerant cultivars and divergent cultural practices, temperature induction response technique (Neumann, 2008; Beena *et al*., 2013; Beena *et al*., 2018b). Other approaches adopted involve plant breeding which create useful genetic variations to withstand a specific environmental stress (Mickelbart *et al*., 2015). However, the process of breeding tolerant rice cultivars is very slow owing to numerous concerns including a paucity of knowledge of the mechanisms underlying tolerance, intricacies of the traits associated with the stress, inadequate selection criteria and absence of rigorous, consistent and reproducible screening

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methodologies (Gregorio *et al*., 2002). Moreover, attempts to improve stress tolerance through conventional plant breeding methods are time consuming, laborious and dependent on existing genetic variability (Prince *et al*., 2015; Beena *et al*., 2018c).

The sufficient utilization of the genotypic alterations in rice (Krishnan *et al*., 2007) and the use of different plant growth regulators and chemical applications could improve the rice performance against escalating temperatures. However, due to the scarce literature on these two mechanisms, studies concerning the potential effects of these strategies on both quantitative and qualitative traits of rice are needed. In this context, the essential role of phytohormones and nutrients in stress regulatory processes of plant is being explored in various laboratories across the world. Although, phytohormones are usually synthesized at low concentration in plants, however, they control surfeit of developmental events in a plant under normal conditions. Phytohormones control plant's functions by regulating their own biosynthesis, modulating their available pool for a particular action, or influencing various signaling cascades. Discrete reports are also available to show beneficial effects of some compounds like brassinosteroid (Cao and Zhao, 2008; Thussagunpanit *et al.,* 2012), boron (Rasheed *,* 2009; Pandey and Gupta, 2013; Guru *et al*., 2016), calcium chloride (Wang *et al.* 2006; Tan *et al*. 2011; Kumar and Sarlach, 2015), salicylic acid (Khan *et al.,* 2013; Zhang *et al*., 2017), glycine betaine (Rhodes and Hanson, 1993; Wahid and Shabbir, 2005; Yang and Lu, 2005; Ashraf and Fooland, 2007) and 1-methyl cyclopropane (Djanaguiraman *et al*., 2011) in many crops including rice when applied exogenously under abiotic stresses like high temperature. The main principal roles of these compounds were involved in reactive oxygen species detoxification by enhancing antioxidants, protection of chlorophyll, maintenance of water balance and photosynthates in plants under high temperature stress condition. Plant functions are regulated by the biosynthesis of phytohormones and influence various signaling cascades. Exogenous application of PGRs to heat-stressed plants have established their role in imparting tolerance or resilient to heat stress in terms of less damage to membranes, improved photosynthesis and leaf water status, and carbon allocation than the untreated control plants, suggesting the strong potential of these molecules in improving the performance of food crops grown under high temperature (Sharma *et al*., 2020). Among these compounds, brassinosteroid plays an important role in mitigating high temperature stress in rice. Hence, it is of vital importance to study the responses of rice crop to high temperature with special emphasis on the morphological, physiological and biochemical traits in order to assess the tolerance of rice crop.

MATERIALS AND METHODS

The study was conducted at Instructional farm, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala Agricultural University during the years of 2018-2019 and 2019-2020. The experiment was laid out in CRD with 10 treatments replicated thrice at two conditions of temperature: high temperature condition in a temperature controlled polyhouse facility and at ambient condition. Treatments [Brassinosteroid (BR)-5 ppm, Boron (B)-100 ppm, Calcium chloride ($CaCl_2$)-0.6 per cent, Salicylic acid (SA)-50 ppm, Glycine betaine (GB)-20 ppm, Pink-Pigmented Facultative Methylotrophs (PPFM)-1 per cent, 1-methyl cyclopropane (1-MCP)-50 ppm, Gibberllic acid (GA_3)-50 ppm, water spray and control (no spray)] were sprayed at panicle initiation, heading and flowering stage. Seedlings were raised in pot trays and transplanted to mud pots on 18th days after sowing. The pots were kept under high temperature condition in a temperature controlled polyhouse from seedling to maturity stage and at ambient temperature. Maximum and minimum temperature was measured daily using a thermo-hygrometer. Foliar spray of plant growth regulators and nutrients were given at panicle initiation, heading and flowering stage. Physiological observations were taken at 50 % flowering stage and yield parameters were taken at harvest stage.

The variety used for this study was Uma (MO-16). This is a high yielding variety with an average yield of 6t/ha, but susceptible to high temperature condition.

Cell membrane stability index (%)

Leaf MSI was estimated according to Sairam *et al*. (1997). For MSI estimation, 100 mg of leaf material, in two sets, was taken in test tubes containing 10 ml of double distilled water. One set was heated at 40°C for 30 min in a water bath, and the electrical conductivity of the solution was recorded on a conductivity bridge (C1) (*Elico, CM183EC-TDS analyzer*, India). Another sample was boiled at 100°C on a boiling water bath for 10 min, then its conductivity was measured on a conductivity bridge (C2). MSI was calculated as: MSI = [1 – (C1/C2)] ×100.

Chlorophyll stability index (%)

Estimation of CSI was carried out based on the protocol of Koleyoras (1958). Two clean test tubes (Control and treatment) were taken. Two 250 mg of leaf samples were weighed and cut into 8 to 10 leaf bits and

transferred to test tubes. 20 ml of distilled water to control tube and 20 ml of hot water (55°C) to treatment test tube were added. The treatment tube was kept in a hot water bath for exactly 30 minutes control tube in the lab condition. After the completion of the reaction time, the leaf bits were taken out from the test tube and macerated with 10 ml of 80% acetone. The contents were centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and made up the volume to 25 ml by using 80% acetone. OD was measured at 652 nm in a spectrophotometer and total chlorophyll content of control and treated samples were calculated. CSI expressed in terms of per cent by using following formula. Chlorophyll stability index (CSI) = Total chlorophyll content (Treated)/Total chlorophyll content (Control) X 100.

Photosynthetic rate (ìmol CO_2 m^{-2} s^{-1}), stomatal conductance (mmol H_2O m^{-2} s^{-1}) and PS II photochemistry (Fv/Fm ratio)

Pn, Gs and Fv/Fm ratio were measured using Portable Photosynthetic System (CIRAS-3, PP systems U.S.A) at morning time between 8 am and 10 am.

Superoxide dismutase activity (activity g^{-1} min^{-1})

SOD was measured according to the method of Beauchamp and Fridovich (1971).

Pollen viability (%)

Pollen viability was measured by using 1% iodo-potassium iodide (IKI) solution which was prepared by dissolving 2.5 g of KI with 250 mg of iodine and made up to 125 ml. Just before anthesis spikelets were collected from each treatment. Then it was crushed and stained by using IKI solution in a glass slides. Fully stained grains denoted the fertile pollen and unstained, shriveled, empty grains indicated sterile grains. Fertile pollen grains were visually counted under compound microscope, Leica. The pollen viability was calculated by using the formula pollen viability (%) = (Number of pollen grains stained) / (Total number of pollen grains) X 100.

Spikelet fertility percentage (%):

The total numbers of filled and unfilled spikelets of three randomly selected primary tillers of the target plants in each treatment were counted. Then, spikelet fertility (%) was calculated by using the formula spikelet fertility (%) = (Number of fertile spikelets)/ (Total number of spikelets) X 100.

Grain yield per plant (g)

The each treatment plant was harvested separately, cleaned, dried and weighed and it was expressed in grams.

RESULTS AND DISCUSSION

Minimum and maximum temperature (°C)

Minimum and maximum temperature recorded inside the polyhouse and ambient condition by using thermo-hygrometer from 18th DAS to maturity stage (Fig. 1). It was observed that the mean minimum temperature (°C) recorded inside the polyhouse condition was 25.1°C and ambient condition was 24.1°C. Difference of mean minimum temperature was 1.0°C. The mean maximum temperature (°C) recorded inside the polyhouse condition was 40.8°C and ambient condition was 31.5°C. Difference of mean maximum temperature was 9.3°C. Plate 1 shows the change in relative growth of plants under different treatments under high temperature condition.

Physiological traits

Data on various physiological traits taken at 50 % flowering stage and yield determining traits of rice variety Uma (MO-16) at ambient temperature and high temperature conditions are depicted in Table 1.

Cell membrane stability index

There was significant difference among treatments for cell membrane stability index both under high temperature and ambient conditions. Under high temperature and ambient conditions, brassinosteroid - 5ppm recorded the highest cell membrane stability index of 82.26 and 84.49%, respectively. Under both conditions, absolute control recorded the lowest cell membrane stability index. These results are in line with findings of Cao and Zhao, (2008), was reported that brassinosteroid treated rice seedlings showed an increase in cell membrane stability as compared to the plants which were not treated. Electrolyte leakage was enhanced in leaves of rice seedlings under elevated temperature stress. The electrolyte leakage in the brassinosteroid-treated rice seedling was lower than those in control. Electrolyte leakage was reduced after brassinosteroid treatment and higher electrolyte leakage occurred in control plants that were consistent with the malondialdehyde (MDA) content. External application of brassinosteroid leads to the modification of cell-wall architecture and modification of the membrane system thus providing a first line of defense against environmental stresses to plants (Clouse, 1996). Dhaubhadel *et al*. (1999) observed that application of 24-epiBL resulted in enhanced basic thermo-tolerance of tomato seedlings which might have been due to the protection of the translational machinery as well as heat-shock protein synthesis by BR-application.

Chlorophyll stability index (CSI)

Plants grown under ambient condition recorded higher chlorophyll stability index than high temperature condition. Under high temperature and ambient conditions, brassinosteroid -5ppm recorded the highest chlorophyll stability index of 76.29 and 86.28 % respectively. The difference between the treatments, conditions as well as the interaction effects were found to be statistically significant. Similar results were obtained by Viswan (2013) observed that plants treated with brassinosteroids showed an improvement in stability compared to plants not treated in groundnut under stress condition. Reduced chlorophyll content and chlorophyll stability index in wheat under elevated heat stress was observed by Sairam *et al.,*(1997). Chlorophyll content was decreased due to enhanced activity of chlorophyllase under elevated temperatures (Todorov *et al*., 2003). But brassinosteroids increase chlorophyll content and reduce the activity of chlorophyllase responsible for catabolism of chlorophyll pigment under abiotic stress (Sharma *et al*., 2015)

Photosynthetic rate

Brassinosteroid (5 ppm) showed the highest photosynthetic rate of 18.1 ìmol CO_2 $m^{-2}s^{-1}$ under high temperature conditions which was significantly higher compared to other treatments while the absolute control (without any spray) showed the least photosynthetic rate of 10.53 ìmol CO_2 $m^{-2}s^{-1}$ (Fig 2). Under ambient conditions, the highest photosynthetic rate was observed with brassinosteroid- 5 ppm (25.7 ìmol CO_2 $m^{-2}s^{-1}$) compared to the absolute control (without any spray) which recorded the least photosynthetic rate of 20.22 ìmol CO_2 $m^{-2}s^{-1}$. Statistical significance was recorded within the treatments and the conditions. The interaction effect between the treatments and the conditions was also found to be significant at 0.05 probability level. These findings are in line up with the findings of Thussagunpanit *et al*. (2012) who reported that brassinosteroid treated plants showed increase in Pn as compared to the plants which were not treated under high temperature stress condition in rice. Hayat *et al*. (2012) reported that brassinosteroids can enhance the net photosynthetic rate, intercellular CO_2 concentration, transpiration rate and stomatal conductance under stress. Sonjaroon *et al*.(2017) reported that brassinosteroids were effective in enhancing the heat tolerance in rice by increasing the net photosynthesis, transpiration rate, and stomatal conductance.

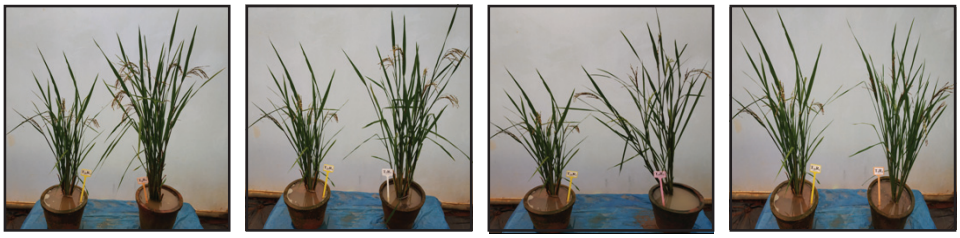
Stomatal conductance

Pink Pigmented Facultative Methylotrophs (PPFM) recorded the highest stomatal conductance of 533.39 mmol H_2O m^{-2} s^{-1} and 499.03 mmol H_2O m^{-2} s^{-1} under high temperature and ambient conditions, respectively. The difference within the treatments was statistically significant at 0.01 probability level (Fig. 3). The difference between the conditions as well as the interaction effects was recorded to be statistically non-significant. These findings are in line up with the findings of Thussagunpanit *et al*. (2012) who reported that brassinosteroid treated plants showed increase in stomatal conductance as compared to the plants which were not treated under high temperature stress condition in rice. Photosynthesis reduction can be affected either by regulating the pathway through stomatal closure and reducing CO_2 flow into mesophyll tissue (Flexas *et al.,* 2004) or directly impairing metabolic activity (Farquhar *et al.,* 1989). Reduced in regeneration of ribulose bisphosphate (RuBP) and protein content of ribulose 1,5-bisphosphate carboxylase/ oxygenase (Rubisco) (Bota *et al*., 2004) was reported and hence decreased Rubisco activity (Parry *et al*., 2002). Cornic (2000) reported that decline in Gs is the initial cause of reduced in photosynthesis. Exogenous application of brassinosteroids regulates signal transduction pathways by enhancing the biosynthesis of endogenous hormones, such as brassinosteroids, zeatin riboside, indole-3-acetic acid, jasmonic acid, and gibberellic acid, and stimulates stress tolerance (Anwar *et al*., 2018). Previous studies also indicated that, under high-temperature stress, application of brassinosteroids induced thermal tolerance by increasing the synthesis of HSP, and also by increasing expression of genes for protective enzymes (Dhaubhadel *et al*., 2002; Sonjaroon *et al*., 2017).

Chlorophyll fluorescence-PS II photochemistry-(Fv/Fm ratio)

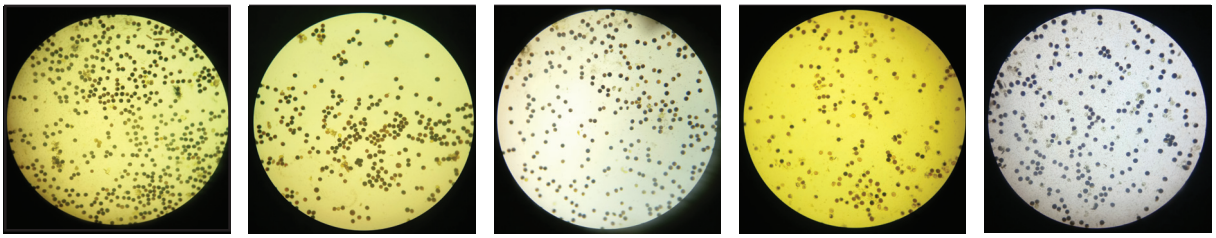
The chlorophyll fluorescence of brassinosteroid- 5 ppm (0.8) was the highest under ambient conditions while the lowest was recorded with Gibberellic acid-50 ppm (0.72). Under high temperature conditions, the Salicylic acid (50 ppm) recorded the highest fluorescence ratio of 0.73 followed closely by Pink Pigmented Facultative Methylotrophs (PPFM) with at-par fluorescence ratio of 0.727 (Fig. 4). The least value was recorded under the absolute control (without any spray) with chlorophyll fluorescence of 0.653 under the stress conditions. The difference between the treatments, conditions as well as the interaction effects was found to be statistically significant at 0.05 probability level. These findings are in line up with the findings of Wu *et al.* (2014) who observed that brassinosteroid treated plants showed increase in Fv/Fm ratio and decreased in F_0 as compared to the control plants under high temperature stress condition in eggplant. Photosynthesis converts light energy into chemical energy for plant

T1: Brassinosteroid **T2: Boron** **T3: Calcium chloride** **T4: Salicylic acid** **T5: Glycine betaine**

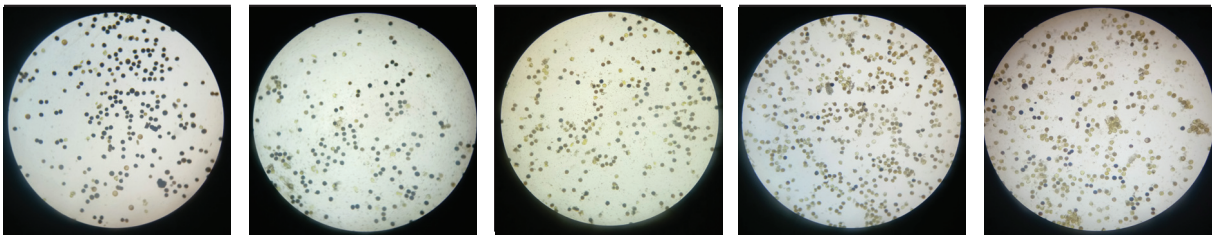


T6: PPFM **T7: 1-MCP** **T8: Gibberllic acid** **T9: Water spray**

Plate 1: General view of treated plants compared with control



T1: Brassinosteroid (5 ppm) T2: Boron (100 ppm) T3: Calcium chloride (0.6%) T4: Salicylic acid (50 ppm) T5: Glycine betaine (20 ppm)



T6: PPFM (1%) T7:1-methyl cyclopropane (50ppm) T8: Gibberllic acid (50ppm) T9: Water spray T10: Control

Plate 2: Effect of foliar application of plant growth regulators and nutrients on pollen viability (%)

growth and development (Pan *et al.,* 2012). Photosynthesis is the most complex physiological process in plants and involves several components, including CO_2 reduction mechanisms, photosynthetic photosystems and the electron transport system (Ashraf and Harris, 2013). Among these, Photosystem II was described as the most heat sensitive element of the photosynthetic apparatus (Berry and Bjorkman, 1980). Fo is the level of fluorescence when all the complexes of antenna pigments associated with the photosystem are presumed to be open (dark adapted). An enhance of Fo denotes the chloroplasts are significantly affected by an environmental stress. Fv/Fm indicates the photosynthetic capability of entire Photosystem II and the maximum quantum efficiency of open Photosystem II centers (Lu and Zhang, 2000). A significant reduction in Fv/Fm suggested an enhance in energy dissipation as heat and photoinhibition to the photosynthetic apparatus (Efeoglu and Terzioglu, 2009). We found that Fo enhanced and Fv/Fm significantly reduced under high

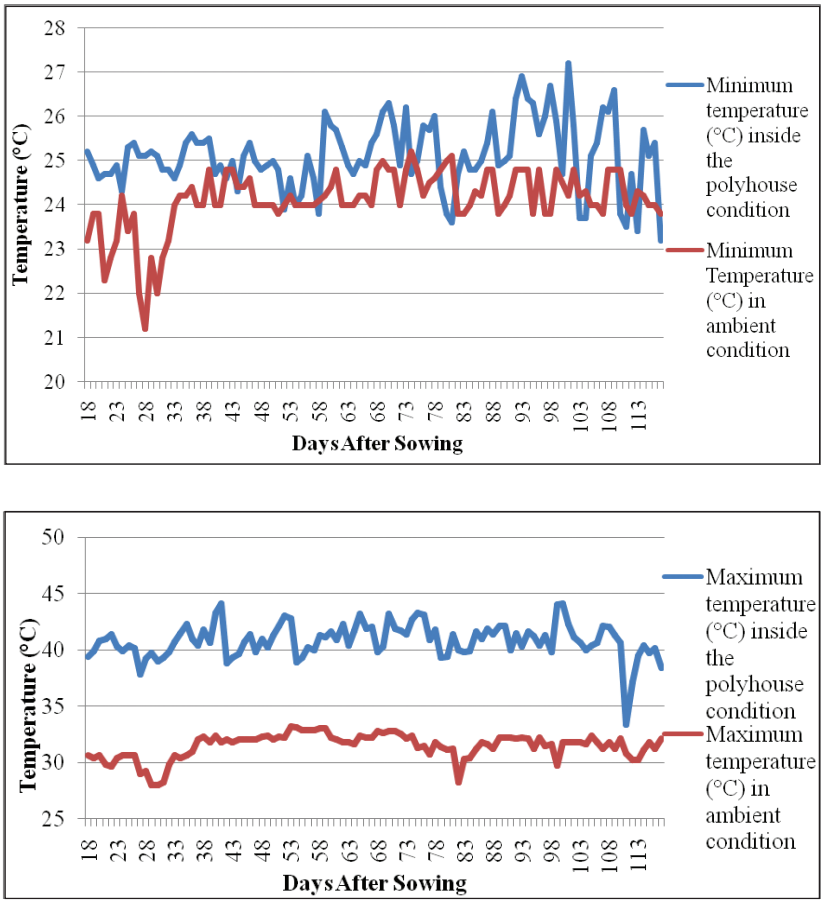


Fig. 1: Minimum and maximum temperature (°C) recorded inside the polyhouse and ambient condition

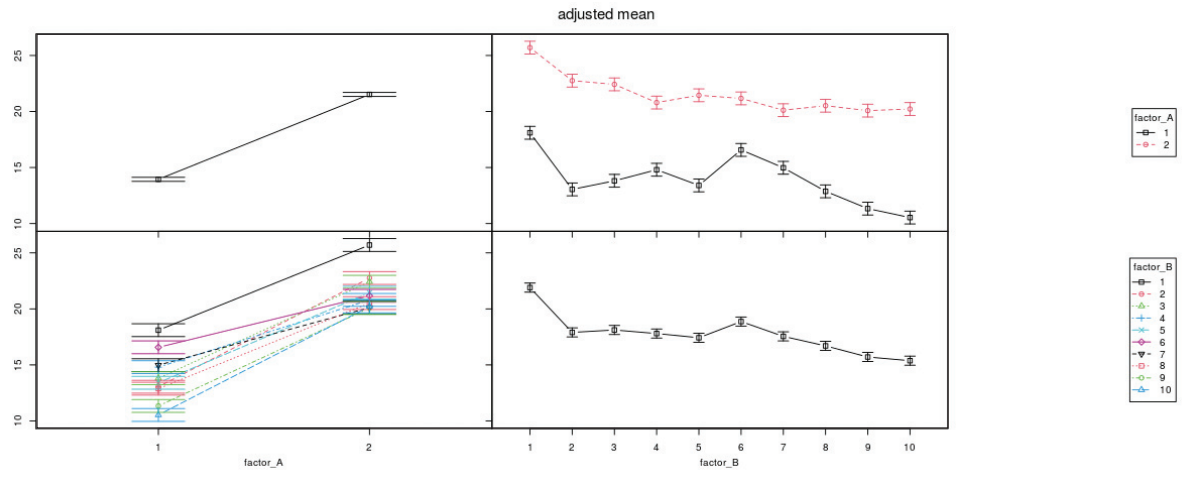


Fig. 2: Changes in photosynthetic rate of Factor A: (1-High temperature; 2-Ambient condition) and Factor B [1. Brassinosteroid (BR)-5 ppm, 2.Boron (B)-100 ppm, 3.Calcium chloride ($CaCl_2$)-0.6 per cent, 4.Salicylic acid (SA)-50 ppm, 5.Glycine betaine (GB)-20 ppm, 6.Pink-Pigmented Facultative Methylotrophs (PPFM)-1 per cent, 7.1-methyl cyclopropane (1-MCP)-50 ppm, 8.Gibberllic acid (GA_3)-50 ppm, 9.water spray and 10.control (no spray)].

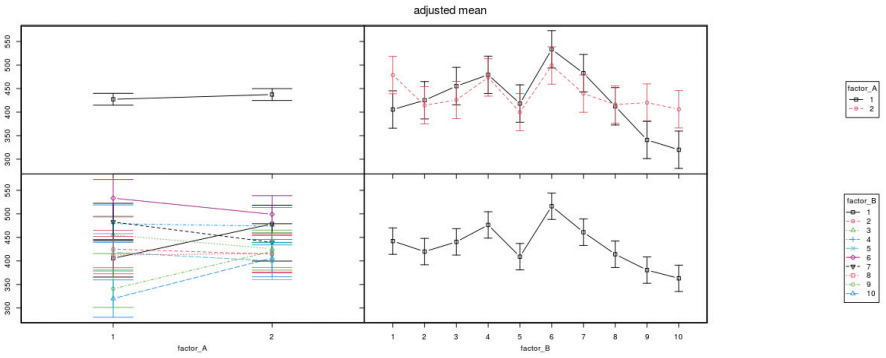


Fig. 3: Changes in stomatal coductance of Factor A: (1-High temperature; 2-Ambient condition) and Factor B [1. Brassinosteroid (BR)-5 ppm, 2.Boron (B)-100 ppm, 3.Calcium chloride ($CaCl_2$)-0.6 per cent, 4.Salicylic acid (SA)-50 ppm, 5.Glycine betaine (GB)-20 ppm, 6.Pink-Pigmented Facultative Methylotrophs (PPFM)-1 per cent, 7.1-methyl cyclopropane (1-MCP)-50 ppm, 8.Gibberllic acid (GA_3)-50 ppm, 9.water spray and 10.control (no spray)].

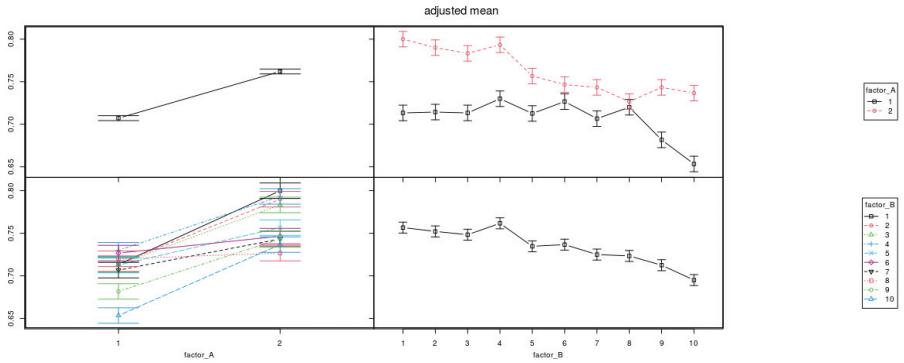


Fig. 4: Changes in Fv/Fm ratio of Factor A: (1-High temperature; 2-Ambient condition) and Factor B [1. Brassinosteroid (BR)-5 ppm, 2.Boron (B)-100 ppm, 3.Calcium chloride ($CaCl_2$)-0.6 per cent, 4.Salicylic acid (SA)-50 ppm, 5.Glycine betaine (GB)-20 ppm, 6.Pink-Pigmented Facultative Methylotrophs (PPFM)-1 per cent, 7.1-methyl cyclopropane (1-MCP)-50 ppm, 8.Gibberllic acid (GA_3)-50 ppm, 9.water spray and 10.control (no spray)].

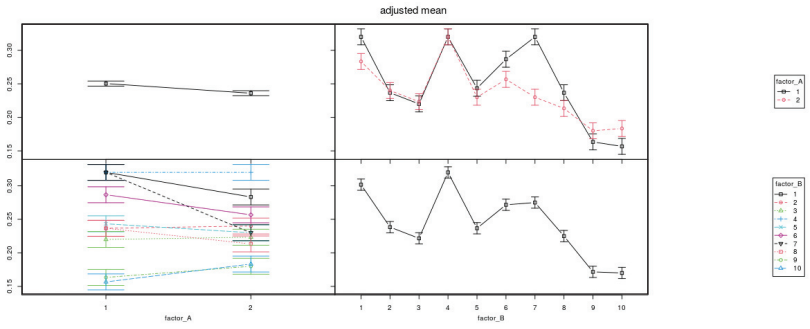


Fig. 5: Changes in super oxide dismutase activity of Factor A: (1-High temperature; 2-Ambient condition) and Factor B [1. Brassinosteroid (BR)-5 ppm, 2.Boron (B)-100 ppm, 3.Calcium chloride ($CaCl_2$)-0.6 per cent, 4.Salicylic acid (SA)-50 ppm, 5.Glycine betaine (GB)-20 ppm, 6.Pink-Pigmented Facultative Methylotrophs (PPFM)-1 per cent, 7.1-methyl cyclopropane (1-MCP)-50 ppm, 8.Gibberllic acid (GA_3)-50 ppm, 9.water spray and 10.control (no spray)].

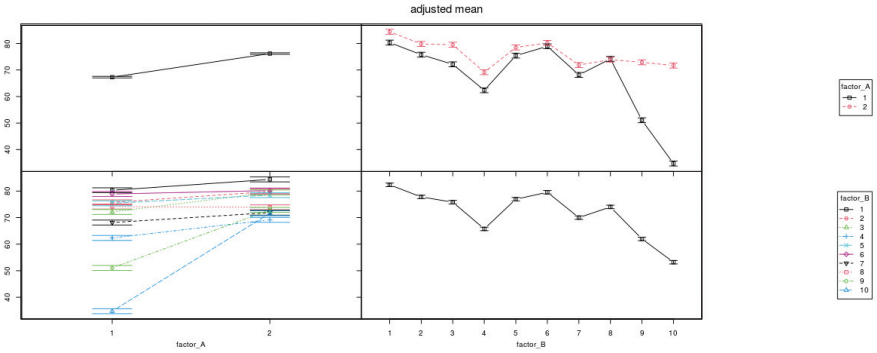


Fig. 6: Changes in pollen viability of Factor A: (1-High temperature; 2-Ambient condition) and Factor B [1. Brassinosteroid (BR)-5 ppm, 2.Boron (B)-100 ppm, 3.Calcium chloride ($CaCl_2$)-0.6 per cent, 4.Salicylic acid (SA)-50 ppm, 5.Glycine betaine (GB)-20 ppm, 6.Pink-Pigmented Facultative Methylotrophs (PPFM)-1 per cent, 7.1-methyl cyclopropane (1-MCP)-50 ppm, 8.Gibberllic acid (GA_3)-50 ppm, 9.water spray and 10.control (no spray)].

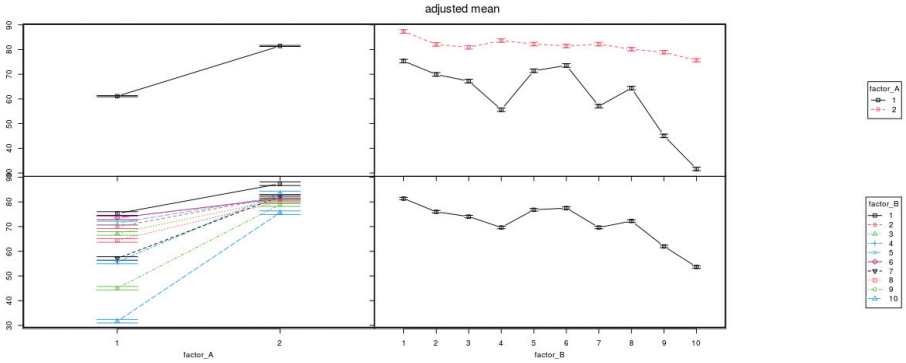


Fig. 7: Changes in spikelet fertility of Factor A: (1-High temperature; 2-Ambient condition) and Factor B [1. Brassinosteroid (BR)-5 ppm, 2.Boron (B)-100 ppm, 3.Calcium chloride ($CaCl_2$)-0.6 per cent, 4.Salicylic acid (SA)-50 ppm, 5.Glycine betaine (GB)-20 ppm, 6.Pink-Pigmented Facultative Methylotrophs (PPFM)-1 per cent, 7.1-methyl cyclopropane (1-MCP)-50 ppm, 8.Gibberllic acid (GA_3)-50 ppm, 9.water spray and 10.control (no spray)].

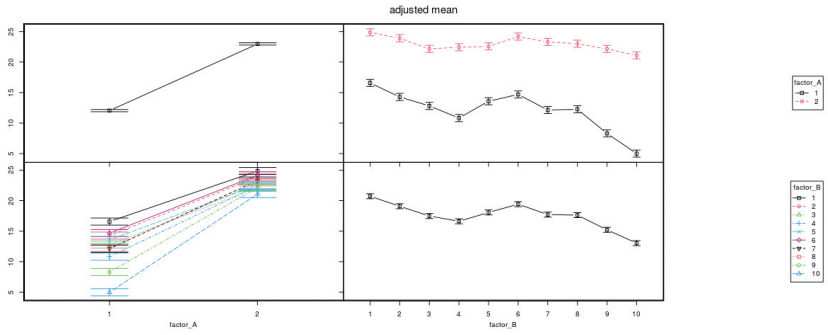


Fig. 8: Changes in grain yield per plant of Factor A: (1-High temperature; 2-Ambient condition) and Factor B [1. Brassinosteroid (BR)-5 ppm, 2.Boron (B)-100 ppm, 3.Calcium chloride ($CaCl_2$)-0.6 per cent, 4.Salicylic acid (SA)-50 ppm, 5.Glycine betaine (GB)-20 ppm, 6.Pink-Pigmented Facultative Methylotrophs (PPFM)-1 per cent, 7.1-methyl cyclopropane (1-MCP)-50 ppm, 8.Gibberllic acid (GA_3)-50 ppm, 9.water spray and 10.control (no spray)].

Table 1: Effect of foliar application of plant growth regulators and nutrients on cell membrane stability index (CMSI) (%), chlorophyll stability index (CSI) (%), photosynthetic rate (Pn) (µmol CO_2 m^{-2} S^{-1}); stomatal conductance (Gs) (mmol H_2O m^{-2} S^{-1}) and superoxide dismutase activity (SOD) (activity g^{-1} min^{-1}), pollen viability (PV) (%), spikelet fertility percentage (SF) (%) and grain yield per plant (GY) (g).) (Mean of two years data)

Conditions	Treatments	CMSI	CSI	Pn	Gs	Fv/Fm	SOD	PV	SF	GY
High temperature	T1: Brassinosteroid (5 ppm)	82.26^{a}	76.29^{f}	18.100 ^{e}	405.503^{ns}	0.713 ^{ef}	0.320 ^{a}	80.307 ^{b}	75.320 ^{fg}	16.577 ^{f}
	T2: Boron (100 ppm)	68.21 ^{efg}	73.24^{hi}	13.050 ^{h}	425.233^{ns}	0.714 ^{ef}	0.237 ^{cd}	75.757 ^{c}	69.917 ^{h}	14.277 ^{gh}
	T3: Calcium chloride (0.6%)	71.94^{cd}	73.63 ^{hi}	13.813 ^{gh}	455.27^{ns}	0.713 ^{ef}	0.220 ^{d}	72.123 ^{e}	67.217 ^{i}	12.813 ^{hi}
	T4: Salicylic acid (50 ppm)	64.88 ^{hij}	73.90^{fghi}	14.800 ^{g}	479.253^{ns}	0.730 ^{cdef}	0.320 ^{a}	62.340 ^{h}	55.653 ^{k}	10.827 ^{j}
	T5: Glycine betaine (20 ppm)	77.19 ^{b}	72.15^{ij}	13.403 ^{gh}	418.227^{ns}	0.713 ^{ef}	0.243 ^{cd}	75.403 ^{cd}	71.417 ^{h}	13.580 ^{ghi}
	T6: Pink-Pigmented Facultative Methylotrops (PPFM) (1%)	73.32^{c}	73.70^{ghi}	16.570 ^{ef}	533.393^{ns}	0.727 ^{cdef}	0.287 ^{ab}	78.910 ^{b}	73.550 ^{g}	14.693 ^{g}
	T7: 1-methyl cyclopropane (50ppm)	63.18^{ijk}	71.38^{ijk}	14.977 ^{fg}	482.737^{ns}	0.707 ^{fg}	0.320 ^{a}	68.143 ^{g}	57.080 ^{k}	12.137 ^{ij}
	T8: Gibberllic acid (50ppm)	61.98 ^{jk}	70.65^{jk}	12.877 ^{hi}	412.333^{ns}	0.720 ^{def}	0.237 ^{cd}	74.170 ^{cde}	64.387 ^{j}	12.263 ^{ij}
	T9: Water spray	60.66^{kl}	70.60^{jk}	11.333 ^{ij}	340.903^{ns}	0.682 ^{g}	0.163 ^{f}	51.047 ^{i}	45.040 ^{l}	8.303^{k}
	T10: Absolute control	58.69^{l}	69.54^{k}	10.533 ^{j}	320.043^{ns}	0.653 ^{h}	0.157 ^{f}	34.740 ^{j}	31.667 ^{m}	5.000 ^{l}
Ambient condition	T1: Brassinosteroid (5 ppm)	84.49 ^{a}	86.28^{a}	25.700 ^{a}	478.723^{ns}	0.800 ^{a}	0.283 ^{b}	84.393 ^{a}	87.353 ^{a}	24.867 ^{a}
	T2: Boron (100 ppm)	70.25^{cde}	84.99^{ab}	22.753 ^{b}	414.493^{ns}	0.790 ^{a}	0.240 ^{cd}	79.813 ^{b}	82.080 ^{bcd}	23.903 ^{abc}
	T3: Calcium chloride (0.6%)	70.05 ^{def}	83.98^{abc}	22.420 ^{bc}	425.76^{ns}	0.783 ^{a}	0.223 ^{cd}	79.510 ^{b}	80.897 ^{cde}	22.160 ^{de}
	T4: Salicylic acid (50 ppm)	69.56^{def}	83.24^{bc}	20.797 ^{cd}	473.72^{ns}	0.793 ^{a}	0.320 ^{a}	69.153 ^{fg}	83.627 ^{b}	22.423 ^{cde}
	T5: Glycine betaine (20 ppm)	77.95 ^{b}	76.20^{fg}	21.447 ^{bcd}	399.92^{ns}	0.757 ^{b}	0.230 ^{cd}	78.487 ^{b}	82.203 ^{bc}	22.560 ^{bcde}
	T6: Pink-Pigmented Facultative Methylotrops (PPFM) (1%)	72.01^{cd}	80.17^{de}	21.167 ^{bcd}	499.03^{ns}	0.747 ^{bc}	0.257 ^{bc}	80.197 ^{b}	81.457 ^{cd}	24.163 ^{ab}
	T7: 1-methyl cyclopropane (50ppm)	65.83^{ghi}	81.64^{cd}	20.117 ^{d}	439.35^{ns}	0.743 ^{bcd}	0.230 ^{cd}	71.863 ^{e}	82.183 ^{bcd}	23.300 ^{abcd}
	T8: Gibberllic acid (50ppm)	67.60^{efgh}	81.44^{cde}	20.517 ^{d}	416.033^{ns}	0.727 ^{cdef}	0.213 ^{de}	73.870 ^{cde}	80.133 ^{de}	22.997 ^{bcd}
	T9: Water spray	66.94^{fgh}	79.05^{e}	20.077 ^{d}	420.25^{ns}	0.743 ^{bcd}	0.180 ^{ef}	72.893 ^{de}	78.917 ^{e}	22.143 ^{de}
	T10: Absolute control	63.58^{ijk}	75.09^{fgh}	20.220 ^{d}	405.997^{ns}	0.737 ^{bcde}	0.183 ^{ef}	71.660 ^{ef}	75.637 ^{f}	21.090 ^{e}
Conditions		**	**	**	ns	**	*	**	**	**
Treatments		**	**	**	*	**	**	**	**	**
Conditions X Treatments		*	**	**	ns	**	**	**	**	**
L.S.D. (CxT)		5.483	3.67	1.637	-	0.026	0.034	2.658	2.07	1.662
C.V.		2.757	2.026	5.593	15.905	2.149	8.461	2.245	1.76	5.753

** and * denote significance at the 0.01 and 0.05 probability level, respectively.

Means not sharing a common letter (a, b, c. . .) are significantly different at 0.05 probability level according to least significant difference (LSD). ns: non-significant, CV: Coefficient of variation.

CMSI- cell membrane stability index; CSI –chlorophyll stability index; Pn- photosynthetic rate; Gs- stomatal conductance; Fv/Fm ratio- chlorophyll fluorescence; SOD- superoxide dismutase activity; PV- pollen viability percentage; SF- spikelet fertility percentage, GY- grain yield per plant.

temperature, which suggests that the photosystem might be inhibited by high temperature stress. Ahammed *et al*.(2015) reported that exogenous application of brassinosteroids can assuage photoinhibition by significantly improve the photochemical efficiency of PSII, the quantum efficiency of PSII photochemistry and photochemical quenching coefficient.

Superoxide dismutase activity (activity g^{-1} min^{-1})

The SOD activity of Salicylic acid- 50 ppm (0.32 g^{-1} min^{-1}) was observed to be statistically significant under ambient conditions. Brassinosteroid (5 ppm), Salicylic acid (50 ppm) and 1-methyl cyclopropane (50 ppm) were ~~on~~ at par with SOD activity of 0.32 g^{-1} min^{-1} under high temperature conditions. The lowest SOD activity was recorded by absolute control (without any spray) (0.157 g^{-1} min^{-1}) and water spray (0.180 g^{-1} min^{-1}) under high temperature and ambient conditions respectively (Fig 5). The data was statistically significant within the treatments at 0.01 probability level whereas it was significant at 0.05 probability level for the conditions and the interaction effect of treatments and conditions. These findings are in line up with the findings of Cao and Zhao, (2008) who observed that brassinosteroid treated plants showed increase in SOD as compared to the control plants under high temperature stress condition in rice seedlings in order to detoxify the reactive oxygen species.

Yield determining traits

Pollen viability

Brassinosteriod (5 ppm) recorded significantly higher pollen viability with 84.39% and 80.3% under ambient and high temperature conditions respectively (Fig 6). The least percentage was recorded under ambient (71.6%) and high temperature (34.74%) by absolute control (without any spray) (Plate 2). These findings are in line up with the findings of Thussagunpanit *et al*. (2012) who reported that brassinosteroid treated plants showed increase in pollen viability as compared to the plants which were not treated under high temperature stress condition in rice. High temperature stress also increases early abortion of tapetal cells, which results in the pollen mother cells to rapidly progress toward meiotic prophase and finally undergo programmed cell death, thus results in pollen sterility (Oshino *et al.,* 2007; Sakata and Higashitani, 2008).

Spikelet fertility

Under high temperature conditions, the spikelet fertility percentage was significantly higher for brassinosteroid-5 ppm (75.32%) compared to absolute control (without any spray) (31.66%). Similarly, under ambient conditions, the percentage was significantly higher for brassinosteroid-5 ppm (87.35%) compared to absolute control (without any spray) (75.63%) (Fig 7). There was statistical significance even between the conditions as well as the interaction of treatments and conditions. These findings are in line up with the findings of Thussagunpanit *et al*. (2012) who reported that brassinosteroid treated plants showed increase in spikelet fertility percentage as compared to the plants which were not treated under high temperature stress condition in rice. High temperature stress reduces the floret fertility which was associated with decreased anther dehiscence, poor pollen shedding, poor pollen grains germination, reduced in pollen tubes elongation and decreased the germination of *in vivo* pollen (Fahad *et al.,* 2015, 2016).

Grain yield per plant

The grain yield was significantly higher by brassinosteroid- 5 ppm (24.86 g $plant^{-1}$) compared to absolute control (without any spray) (21.09 g $plant^{-1}$) under ambient conditions. Comparatively, under high temperature conditions, brassinosteroid- 5 ppm recorded significantly higher yield of 16.57 g $plant^{-1}$ while absolute control (without any spray) recorded the lowest yield of 5 g $plant^{-1}$ (Fig 8). There was significant difference for interaction effect between conditions and treatments. These findings are in line up with the findings of Thussagunpanit *et al*. (2012) who reported that brassinosteroid treated plants showed increase in grain yield $plant^{-1}$ as compared to the plants which were not treated under high temperature stress condition in rice. A significant impact of high temperature stress affects meiosis, fertilization, and growth of fertilized embryos eventually leading to a significant decrease in yield (Camejo *et al.,* 2005). Chandrakala *et al*. (2013) investigated the effect of foliar application of 24-epi-brassinolide on the physiology of rice cultivars (Pusa Sugandh 5 and Nerica L 44) those were grown under ambient and high temperature (36°C) environments. They found a strong positive relationship between grain yield and leaf photosynthesis by the pre-treatment of 24-epi-brassinolide.

CONCLUSION

The study revealed that physiological and biochemical parameters such as cell membrane stability index, photosynthetic rate, stomatal conductance and Fv/Fm ratio were found to increase significantly by the foliar application of phytohormones and nutrients under high temperature stress condition, whereas leaf temperature and transpiration rate were decreased. Treatments had significant effect on morphological and yield parameters over control plants. Among the

treatments, BR spray significantly increased in the cell membrane stability index, photosynthetic rate, stomatal conductance, Fv/Fm ratio, chlorophyll stability index, chlorophyll a/b ratio and superoxide dismutase activity, productive tillers plant^{-1}, pollen viability, spikelet fertility percentage and grain yield plant^{-1}. Among the treatments, GA_3 spray resulted in significant increase in plant height and panicle length.

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