IMPACT OF FOLIAR APPLICATION OF PLANT GROWTH REGULATORS AND NUTRIENTS ON HIGH TEMPERATURE STRESS MITIGATION IN RICE (*Oryza sativa* L.)

by

RAGHUNATH MP

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Impact of foliar application of plant growth regulators and nutrients on high temperature stress mitigation in rice (*Oryza sativa* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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Certified that this thesis entitled **"Impact of foliar application of plant growth regulators and nutrients on high temperature stress mitigation in rice (*Oryza sativa* L.)"** is a record of research work done independently by Mr. Raghunath MP under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Raghunath MP

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LIST OF ABBREVIATIONS

IPCC	Intergovernmental Panel on Climate Change
U. S.	United States
ROS	Reactive oxygen species
HSP	Heat shock proteins
PGRs	Plant growth regulators
CMT	Cell membrane thermo-stability
IAA	Indole-3-acetic acid
CO_2	Carbon dioxide
BADH	Betaine-aldehyde dehydrogenase
RuBP	Ribulose 1,5-Biphosphate
BR	Brassinosteroid
B	Boron
$CaCl_2$	Calcium chloride
SA	Salicylic acid
GB	Glycine betaine
PPFM	Pink-pigmented facultative methylotrophs
1-MCP	1-Methylcyclopropene
GA_3	Gibberellic acid
MeJA	Methyl jasmonate
CMSI	Cell membrane stability index
CSI	Chlorophyll stability index
POD	Peroxidase
MDA	Malondialdehyde
SOD	Superoxide dismutase
EDTA	Ethylenediaminetetraacetic acid
PS II	Photosystem II
Fv	Variable fluorescence

Fm	Maximum fluorescence
F_0	Minimum fluorescence
DAS	Days After Sowing
DAT	Days After Transplanting
SE(m)	Standard Error (Mean)
%	per cent
^{0}C	Degree Celsius
cm	Centimetre
g	Gram
ml	Milliliter
µl	Microliter
µg	Microgram
mg	Milligram
mM	Millimolar
nm	Nanometer
rpm	rotations per minute
et al.	and other co-workers
viz.	namely
i.e.	that is
FYM	Farm Yard Manure
kg	kilo grams

Introduction

1. INTRODUCTION

Rice (*Oryza sativa* L.) occupies extremely desirable prime position among the food crops grown around the world 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).

India has the largest rice-growing area among all nations and ranks second in rice production. In India, rice occupies an area of 43.79 million hectares with total production of 112.91 million tonnes in the year of 2017-2018 (https://eands.dacnet.nic.in).

Abiotic stresses like high temperature, drought, and salinity widen the gap of genetic potential for the production of grain. Among the abiotic stresses, elevated temperature stress is one of the major environmental factors that limit crop growth and productivity (Berry and Bjorkman, 1980; Lobell and Asner, 2003). Plant growth and yield are maximum when grown as close as to its optimum temperature (Wahid *et al.,* 2007) and a marginal rise in the above optimum temperature has a major impact on growth rate (Howarth, 1998).

By the end of the 21st century, the earth's climate is forecasted to rise in temperature by an estimate of 1.8^{0}C to 4.0^{0}C (IPCC, 2007) due to both human activity and environmental factors (Eitzinger *et al.,* 2010). While some nations in the temperate region may get advantage from climate change, many nations in the tropical and subtropical areas seem more prone to the potential effects of global warming (Rosenweig and Parry, 1994). Maximum and minimum temperatures

have rised an average of 0.35^{0}C to 1.13^{0}C respectively in the period of 1979-2003 at International Rice Research Institute (IRRI), Philippines (Peng *et al.,* 2004).

Moreover, as the world population expands exponentially, crop production and productivity need to be enhanced by extending productive regions to warmer environments and this results in the exposure of crops to high temperature stress. This rise in temperature has subjected most of the world's crops to high temperature stress during some phases of their life cycle. The yield of rice crop has been expected to decline by 41 per cent by the end of the 21st century due to the exposure to high temperature stress (Ceccarelli *et al.,* 2010).

Rice grows optimum in the temperature between 20^{0}C to 35^{0}C and rise in ambient temperature of more than 10^{0}C to 15^{0}C compared to the optimum rise in temperature may lead to heat stress (Wahid *et al.*, 2007). Heat injury occurs at vegetative and reproductive phases, when rice is subjected to more than 35^{0}C and this increase in temperature results in high sterility of spikelets (Yoshida, 1981). Even just one hour of exposure to heat stress at anthesis stage could cause sterility and reduced grain yield (Jagadish *et al.*, 2007). Decreased in the anther dehiscence, less production of pollen, and reduces the number of germinating pollen grains on the stigma ultimately results in the sterility of spikelets (Matsui *et al.,* 2001; Prasad *et al.,* 2006).

In rice, it was reported that heat stress immediately before anthesis or during anthesis induced a significant reduction in the measured growth parameters (Wollenweber *et al.*, 2003) and grain yield (Russel & Wilson, 1994). Discrete reports are also available to show beneficial effects of some compounds like brassinosteroid (Cao and Zhao, 2008; Thussagunpanit *et al.,* 2012), boron (Rasheed *et al.,* 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),

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1-methyl cyclopropane (Djanaguiraman *et al.*, 2011) and methyl jasmonate (Zeng *et al.*, 1999; Fahad *et al.*, 2016) 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. Hence, it is of vital importance to study 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.

Hence, keeping in view of all the above facts, this study was conducted to test the effectiveness of selected plant growth regulators and nutrients in improving grain yield by enhancing the tolerance capacity of the rice crop against high temperature stress.

Hence the current study entitled "Impact of foliar application of plant growth regulators and nutrients on high temperature stress mitigation in rice (*Oryza sativa* L.)." was focused on minimizing the effect of heat stress during reproductive stages of rice so as to suggest a farmer friendly strategy to improve heat stress tolerance in rice growing under high temperature condition. The experiment was planned and carried out at College of Agriculture, Vellayani during 2017-19 under high temperature condition (5-6°C more than ambient condition) in a temperature controlled polyhouse with the objectives.

To study the effect of foliar application of plant growth regulators and nutrients on high temperature stress mitigation and to advance the flowering time by using methyl jasmonate (MeJA) in rice (*Oryza sativa* L.).

Review of Literature

2. REVIEW OF LITERATURE

Rice (*Oryza sativa* L.) is an important cereal crop and around half of the world's population is dependent on rice, increase in production of rice by 0.6 - 0.9 per cent annually is essential to meet the rising demand (Carriger and Vallee, 2007). It is also estimated that by 2050, the world population will reach around 900 crore (International Data Base, U. S., 2016). So in order to feed burgeoning population, farmers will need to produce around 50% more food grains by the year 2020 (Kumar and Gautham, 2014). Even though, the production of rice is increasing over years the rate of increase is not enough for the present demand. So we need to produce 116 million tonnes of additional rice by the year 2035 (Kumar and Gautham, 2014). Farmers are adopting better cultural and management practices for rice production, but the productivity of rice is not increasing as expected. It is because of the deleterious effects of external environment, which include both biotic and abiotic factors.

Climate change is mainly expected to have a negative effect on rice production and productivity. Among abiotic factors, high temperature drastically reduces the rice production. It is also estimated that, for every 1°C elevation in temperature, there will be about 10 per cent reduction in the rice grain yield (Peng *et al.,* 2004). Rice is majorly grown in irrigated condition allowing production under warmer and summer months with high radiation post monsoon. Rice production also got intensified in upland and rainfed-lowland cropping systems, many of which were prone to high temperature and drought condition (Jagadish *et al.,* 2007). In these environmental conditions, day temperature periodically exceeded critical temperature of about 33^0C for seed set which resulted in reduction in the fertility of spikelets and lead to reduced in yield (Nakagawa *et al.,* 2003). By the end of this century, the projected global mean surface temperature will elevation of 2.0 to 4.5^0C (IPCC, 2007). So, in future, rice has to be grown under much warmer environmental condition and it is more vulnerable to crops (Battisti and Naylor, 2009).

2.1. HEAT STRESS THRESHOLD TEMPERATURE

A threshold temperature is referred as a value of daily mean temperature at which detectable reduction of growth begins. Lower and upper developmental threshold temperature had been determined for many species of plants in a controlled laboratory experiments. An upper developmental threshold is one above which growth and development cease. Similarly, a base temperature or a lower developmental threshold is the temperature below which plant growth and development stop (Wahid *et al.,* 2007).

2.2. RICE PRODUCTION vs HIGH TEMPERATURE STRESS

The number of hot days and warm nights, and the minimum and maximum daily temperatures in a year, are estimated to elevate over most areas of land (IPCC, 2013). The normal rice development for the optimum temperature is in between 27 to 32°C (Yin *et al.,* 1996). Elevated temperature affects almost all the rice growth stages from emergence to the ripening and harvesting stage. The developmental stage at which crop is directly exposed to high temperature stress determines the extremity of the possible injury to the crop (Wahid *et al.,* 2007). Although, flowering (anthesis and fertilization) and to the lesser extent the foregoing stage booting (microsporogenesis) are mainly considered to be the developmental stages which are highly susceptible to the high temperature in rice (Satake and Yoshida, 1978).

A maximum temperature exceeding 35.8°C at heading stage of rice induced about 15% spikelet sterility (Hasegawa *et al.,* 2009) whereas, 38°C caused about 8–63% spikelet sterility over diverse genotypes of rice (Jagadish *et al.,* 2014). Increase in temperature for 4 days or more during the pre stage of anther development induced pollen grains abortion (Sakata *et al.,* 2000).

Maximum temperature of majorly rice growing regions are touching the maximum limit and rice is the major staple food for most of the asia's population and increasing the reproductive stage stress tolerance is essential for the food security (Wassmann *et al.*, 2009). Rice cultivars showed genotypic variance on heat tolerance; although, the regulation of hormones and their mechanisms are rarely studied under high temperature stress (Cao *et al.,* 2009).

2.3. THE IMPACTS OF HEAT STRESS

The main impacts of heat stress are denaturation of protein, instabilities in cytoskeletal structure and nucleic acids, enhancement of the membrane fluidity, inactivation of the synthesis of proteins, and reduction in membrane integrity (Howarth, 2005; Wahid *et al.,* 2007). Acute cellular injuries occur at moderately high temperature condition, after long-term exposure or severely short-term exposure to very high temperature condition (Wahid *et al.,* 2007). It may decrease the ion flux which leads to ROS production and other toxic compounds which extremely affects growth of the plants (Howarth, 2005). Expression of HSP and also other types of protective proteins is an effective adaptive strategy under high temperature conditions interlinked with stress tolerance (Wahid *et al.,* 2007), water use efficiency and photosynthesis (Camejo *et al.,* 2005), cellular hydration maintenance (Wahid and Close, 2007) and membrane stability (Ahn and Zimmerman, 2006).

2.4. PLANT GROWTH REGULATORS

"Organic compounds other than nutrients, which in small amounts, promote, inhibit or modify any physiological process in plants" (Van overbeek *et al.,* 1954).

Plant growth regulators have been shown to perform an important function in the tolerance of abiotic and biotic stress by the regulation of signaling networks

and the developmental processes in plants (Khan *et al.,* 2012). They are either synthetic compounds or natural hormones that are applied directly to the plants which alters their life processes in some beneficial way to increase in yield and quality and to facilitate withstanding under stress condition.

The application of plant growth regulators in agriculture was started in 1930 in United States. Synthetic substances that act like naturally occurring plant hormones were also produced and hence the plant growth regulators use has been increasing significantly and it become one of the major component in modern agriculture (Krishnan *et al.,* 2011).

2.5. IMPACT OF HIGH TEMPERATURE STRESS ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS.

2.5.1. Cell membrane stability index

Blum and Ebercon, (1981) suggested that maintaining proper functioning of cellular membranes, it is necessary for the processes alike photosynthesis and also respiration under stress condition. High temperature stress increased kinetic energy and the movement of several molecules through the membranes thereby loosening the chemical bonds within the biological membranes molecules. Hence, by proteins denaturation or enhancing the unsaturated fatty acids, the lipid bilayer becomes more fluid (Savchenko *et al.*, 2002). This will increase in the leakage of solutes and this is the indication of reduced cell membrane thermo-stability (CMT) and it has been used as an indirect measure of high temperature tolerance.

Prasad *et al.* (2006) reported that increase in relative injury due to high temperature stress by the leakage of electrolytes from flag leaf of various cultivars of rice ranged between 44 to 56 per cent.

Nysanth (2018) has revealed that pink-pigmented facultative methylotrophs (PPFMs) increase the cell membrane stability and chlorophyll content with increase in the IAA and proline content in rice.

Wahid and Shabbir, (2005) worked with the different levels of glycine betaine (GB) and found that 20 mM concentration was highly effective in increasing seed germination rate, shoot dry and fresh weight and water content of shoot under high temperature stress in barley seedlings. GB was absorbed by seeds and after translocation to the seedlings stage which increased their capacity to maintain higher water content and enhanced the seedling vigour by means of increase in net photosynthesis rate, reduction in the relative membrane permeability as well as reduction in ions leakage under high temperature stress. Also the results revealed that seedlings developed from the 20 mM GB treated seeds had higher net photosynthetic rate, shoot dry weight and water potential of leaf. But the relative membrane permeability was reduced compared to untreated plants under high temperature stress.

2.5.2. Chlorophyll stability index

The high chlorophyll stability index (CSI) value helps the plants to overcome the stress by increasing the chlorophyll availability in the plants leads to enhanced net photosynthetic rate, high dry matter production and increase in productivity. The high values of CSI showed that the plants are have more ability to produce proline by conversion of glutamate into proline and this proline is having various roles under abiotic stresses. (Kaloyereas, 1958)

Verma, (1999) reported that high chlorophyll and proline accumulation in plants indicates that the plant can withstand under abiotic stress environment. Mahla *et al.* (2011) reported that there was a reduction in chlorophyll stability (%) in terms of lesser chlorophyll content in all cultivars of wheat under heat stress

conditions and decrease in chlorophyll stability index in susceptible cultivar and the highest value in tolerant cultivar.

Cao and Zhao, (2008) reported that brassinolide (BR) application greatly improved chlorophyll and protein content, enhanced POD and SOD activities, and decreased MDA content and electrolyte leakage under high temperature stress in rice seedlings. This suggested that BR having protective role of rice seedlings from high temperature stress by increasing the expression level or activities of protective enzymes within the leaves.

2.5.3. Leaf temperature

Leaf temperature is a plant sensitive indicator under stress level and it is associated with the stomatal conductance. Leaf temperature based scheduling of irrigation for rice helps to minimize the heat injury. (Wanjura and Upchurch, 1997)

Batts *et al.* (1998) suggested that use of nitrogen fertilizers in a heat-tolerant variety was more effective by means of reducing the heat injury through minimising the leaf temperature and by increasing evapotranspiration.

2.5.4. Photosynthetic rate

Photosynthesis is the important metabolic process which is directly affected by the heat stress. Scafaro *et al.* (2009) reported the effect of high temperature on *Oryza sativa* and *Oryza meridionalis* on net photosynthetic rate and they observed that net photosynthetic rate for *Oryza meridionalis* 22.4 µmol CO_2 m^{-2} s^{-1} decreased to 13 µmol CO_2 m^{-2} s^{-1} at 45°C. *Oryza sativa* was 26.6 µmol CO_2 m^{-2} s^{-1} at 27°C and it decreased to 12.6 µmol CO_2 m^{-2} s^{-1} at high temperature condition.

Wang *et al.* (2010) reported that depending upon the plant species the effect of drought, high temperature stress and their combination on photosynthesis varies. Simultaneous occurrence of both drought and high temperature stress has shown adverse effect on the net photosynthetic rate of wheat.

Khan *et al.* (2013) suggested that the salicylic acid (SA) treatment eased the heat stress by enhancing proline production through the rise in γ- glutamylkinase and reduction in proline oxidase activity, leading to increase of osmotic potential and water potential, which is necessary for balancing the photosynthetic activity. Along with this, SA treatment reduced the formation of ethylene in high temperature stressed plants by inhibiting the activity of 1- aminocyclopropanecarboxylic acid (ACC) synthase (ACS). This resulted in improved proline metabolism, photosynthesis and nitrogen assimilation. The results indicate that SA interacts with proline metabolism and avoid formation of ethylene to alleviate the adverse effects of high temperature stress on photosynthesis in wheat.

Sivakumar *et al.* (2017) reported that pink-pigmented facultative methylotrophs are effectively enhancing the photosynthetic rate, relative water content and proline content. Yang *et al.* (2005) reported that under heat stress in tobacco, glycine betaine (GB) maintained higher Rubisco activation by preventing the Rubisco activase sequestration to thylakoid membrane from the soluble stroma fractions and hence it increased the tolerance of CO_2 assimilation to heat stress. Results were revealed that engineering of GB biosynthesis by transformation with BADH gene which might be an effective method for increasing heat tolerance of plants.

2.5.5. Stomatal conductance

Stomatal conductance and net photosynthesis were affected by high temperature stress in several species of plants due to suppression in the activation state of rubisco (Crafts-Brander and Salvucci, 2002; Morales *et al.*, 2003).

Stomatal conductance and rate of transpiration is closely interlinked with the photosynthesis in rice (Kuroda and Kumura, 1990; Miah *et al.*, 1997; Ohsumi *et al.*, 2007). Brassinosteroid is reported to plays a major role in improving seed size and weight which showed that brassinosteroid regulates the initial carboxylation activity of Rubisco, and thus having impact on photosynthetic carbon-dioxide assimilation process.

2.5.6. Transpiration rate

Plants maintaining the water status is one of the most important functions under high temperature condition. Disastrously, heat stress generally coincides with scarcity of water under field condition mainly in tropical and sub tropical regions (Simoes-Araujo *et al.,* 2003).

Rapid reduction in water contents in leaf tissue was shown in sugarcane on exposure to heat stress even with sufficient amount of water present in the soil (Wahid and Close, 2007). It reveals that high temperature stress could also have ill effect on root conductance. Similarly, decrease in water content in tissues and root conductance were reported in tomato under high temperature stress condition (Morales *et al.,* 2003).

Generally, loss of water under high temperature stress is high during day time mainly due to increase in transpiration rate which ultimately leads to impairing the important plant physiological processes. High temperature stress decreases the number, mass and growth of the roots and finally it limits the water and nutrients supply to the aerial parts of plants (Wahid *et al.,* 2007).

Beena *et al.* (2014) identified that high temperature stress (33°C for 5 days) leads to increase in the transpiration rate in rice.

2.5.7. Chlorophyll a/b ratio

Exposure to heat stress normally results in reduction in the biosynthesis of chlorophyll in plants. Decrease in the chlorophyll accumulation in the plants may be due to reduced chlorophyll biosynthesis or due to enhanced chlorophyll degradation or combined effect of both under heat stress. The inhibition of the biosynthesis of chlorophyll is actually due to various enzymes deactivation under high temperature stress (Dutta *et al.,* 2009).

For example, the activity of 5-aminolevulinate dehydratase is a main enzyme in the biosynthesis pathway of pyrrole which reduced remarkably in wheat under high temperature stress (Mohanty *et al.,* 2006). Biosynthesis of chlorophyll in cucumber was decreased by 60% at 42°C was majorly due to the inhibition of the biosynthesis of 5-aminolevulinate under heat stress condition and also biosynthesis of the protochlorophyllide was reduced by 70 per cent under elevated temperature condition was observed (Tewari and Tripathy, 1998). Karim *et al.* (1999) reported that high temperature stress which caused high accelerated chlorophyll a and b degradation in developed leaves. These impacts on photosynthetic apparatus and the chlorophyll pigments are mainly associated with oxidative damage (Guo *et al.,* 2006).

An enhanced chlorophyll a/b ratio was observed in heat tolerant varieties of sugarcane and tomato plants compared to heat susceptible varieties (Camejo *et al.,* 2005). It reveals that change in the chlorophyll a/b ratio plays a key role in the tolerance against heat shock.

Meenakshi and Savalgi, (2009) reported that foliar spray of Methylobacterium enhanced the chlorophyll content in soyabean plants.

2.5.8. Carbohydrate content

Photosynthesis in rice plants during the grain-filling period contributes to 60 - 100% of the final grain carbon content (Yoshida, 1981). The remainder is

made up from remobilized storage carbohydrate in leaf sheaths and culms laid down before anthesis (Watanabe *et al.*, 1997).

Non-structural carbohydrate (NSC) stored in the culms and leaf sheaths until heading or anthesis contribute to a part of grain yield through translocation to panicle during grain filling (Ntanos and Koutroubas, 2002).

The rate and duration of grain filling are dependent on the rate and duration of carbohydrate production. If carbohydrates are produced at a higher rate than they are translocated into the grains, excess carbohydrates will be stored in the stem or leaves. When the assimilation rate is decreasing below the carbohydrate utilisation rate and previously stored carbohydrates can be used to maintain a constant grain filling rate. Evans *et al.* (1975) reported that the duration of the grain filling is dependent on the green leaf area duration. A decrease in the rate of assimilates production leads to a slower grain-filling rate and if it is not compensated for by translocation of previously stored carbohydrates. The rate and the duration of grain filling depend on the rate and the duration of assimilate production.

Slow grain filling and low grain weight of inferior spikelets have often been attributed to a limitation in carbohydrate supply (Murty and Murty, 1982). Grain-filling rate in cereals is closely associated with sink strength (Liang *et al.*, 2001). Cao *et al.* (2000) reported that during the grain filling period, rice grains are strong carbohydrate sinks. The sink strength can be described as the product of sink size and sink activity (Venkateswarlu and Visperas, 1987).

The process of the grain filling in cereals is controlled by four major enzymes *viz.,* sucrose synthase, starch synthase, starch branching enzyme, and adenosine diphosphate glucose pyrophosphorylase (Taiz and Zeiger, 2002). A decreased activity of these enzymes has been reported under stress conditions which have a negative impact on the yield of major cereals (Ahmadi and Baker,

2001). Synthesis of starch and sucrose is highly affected due to the reduction in the important enzymes activities such as sucrose phosphate synthase, adenosine diphosphate-glucose pyrophosphorylase and invertase under high temperature stress (Fahad *et al.,* 2017).

Nawaz *et al.* (2017) found that brassinosteroid helps to increase the total carbohydrate accumulation compare to control. The key role of boron in plants are transport of sugar, production of flower, pollen tube elongation and germination, carbohydrates and sugar translocation to reproductive organs, which in turn enhance the number of spikelet and fertility of spikelet that regulates yield and productivity (Rasheed *et al.,* 2009).

Pandey and Gupta, (2013) observed that foliar application of Boron at 0.1% at three different phases of reproductive development, *i.e.* earlier to flowering, initiation of bud formation and after bud formation enhanced yield attributes such as number of pods, pod size and number of seeds produced per crop and also enhanced the seed quality and seed yield (in aspects of seed protein and carbohydrates) in black gram.

2.5.9. Malondialdehyde content

With regards to ROS, enhanced malondialdehyde content has been reported which is a pure sign of oxidative damage (Moller *et al.,* 2007). Hence, maintaining greater rates of the anti-oxidants are useful strategy by the plants to counter the adverse impacts of ROS (Sharma and Dubey, 2005). Plant hormones are also known as natural protection molecules in crops that maintain greater concentrations of antioxidants under stress. They help the crops to acclimatize under different environmental condition by facilitating growth, development, source or sink movements and nutrient distribution (Fahad *et al.,* 2015).

Under high temperature stress, the application of macronutrients such as Ca, K, and micronutrients such as B, Mn and Se are known to alter the function of stomata, which help to stimulate physiological and metabolic processes that contribute to the preserving elevated tissue water potential and thus improving high temperature stress tolerance (Waraich *et al.,* 2012). The foliar application of nutrients such as N, K, Ca, and Mg has also been revealed to decrease toxicity to ROS by raising the antioxidant enzymes concentration in plant cells (Waraich *et al.,* 2012).

Leibler *et al.* (1986) suggested that lesser membrane stability or higher injury reflects the extent of MDA content and which in turn shows the increasing the susceptibility to the oxidative stress due to several biotic and abiotic stresses.

Wang *et al.* (2006) reported that loosely bound calcium is mainly presented on the cell walls under normal conditions and on the otherhand when exposed to heat condition it moves into the cytoplasm. Also the instantaneous assay on the antioxidant system changes was reported. Oxidative injury, as measured by production of ROS, MDA content, elevated significantly throughout heat stress and calcium pre-treatment attenuated the oxidative injury. The assay on the antioxidant enzymes activities were reduced when exposed to heat stress; however calcium pre-treatment effectively reduced the inhibition and concluded that homeostasis of calcium plays an important role in *Lonicera Japonica* when exposed to heat and calcium pre-treatment might boost up its thermo tolerance.

2.5.10. Anti-oxidants

Changes in the environmental condition and variation in developmental processes in plants result in oxidative stress and it mainly accumulates ROS. This will result in disruption of enzymes, membrane proteins and homeostasis of cellular components and finally it enhances the fluidity of the membrane. So in order to detoxify the ROS in the plant cells it is produces both non-enzymatic and

enzymatic compounds of low molecular weight. While catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), peroxidase (POX), mono de-hydro ascorbate reductase (MDHAR), glutathione-s-transferase (GST), and de-hydro ascorbate reductase (DHAR) forms the enzymatic antioxidants. Ascorbate, carotenoids, phenolic compounds and tocopherols mainly contribute to the non-enzymatic antioxidants (Asthir, 2015).

Recent studies have confirmed that production of ROS in programmed cell death is interlinked with the pollen sterility of some cytoplasmic male sterile lines in rice (Li *et al.*, 2004) and wheat (Wan *et al.*, 2007).

Djanaguiraman *et al.* (2011) reported that plants under heat stress had increased ethylene production rate and reactive oxygen species (ROS) production by lowered the antioxidant activity, which lead to higher membrane damage. The beneficial outcome of 1-methyl cyclopropane were eminent under heat stress compared to optimum condition, which regarding reduced in ethylene and ROS production, enhanced in antioxidant protection and also reduced in flower abscission.

An early reaction to heat stress was observed to cause Ca^{2+} influx and cytoskeletal reorganization, which triggers the upregulation of mitogen-activated protein kinases and calcium-dependent protein kinase signaling cascades (Wahid *et al.,* 2007; Ashraf and Harris, 2013). This signaling cascade results in the antioxidants production, compatible osmolytes (for osmotic adjustment) and heat shock proteins expression.

2.5.10.1. Superoxide dismutase activity

Exposure of plants to heat stress which leads to reduction in the activity of SOD and CAT and this SOD reduction was closely interlinked with the severity of high temperature stress in rice (Karuppanapandian *et al.*, 2011). Zhao *et al.*

(2017) conducted an experiment to access the activity of SOD in different rice cultivars under high temperature condition. They observed that under heat stress condition SOD activity of HT tolerant cultivar Qianjiang 3 was reduced by 29.8 per cent as compared to the control condition and the SOD activity in susceptible cultivar Xieqingzao reduced by 35.9 per cent.

Tan *et al.* (2011) suggested that the application of $CaCl_2$ improved the carboxylation efficiency, net photosynthetic rate, apparent quantum yield and also maximum photochemical efficiency of photochemistry (Fv/Fm) under heat stress. High temperature stress reduced the activities of SOD, ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD) and glutathione reductase (GR), whereas the activities of those enzymes enhanced in plants pre-treated with $CaCl_2$. There was an accumulation of O_2 and H_2O_2 under heat stress, however application of $CaCl_2$ minimize the level of O_2 and H_2O_2 under high temperature conditions and also it enhanced the level of heat shock protein 70 (HSP70).

El-Gawad *et al.* (2015) examined the impact of pink-pigmented facultative methylotrophs (PPFMs) bacteria on antioxidant enzymes, growth and yield of snap beans. Results indicated that the application of PPFMs to the crops altered the amount of antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD), polyphenol oxidase (PPO), ascorbate peroxidase (APX) and catalase (CAT). This research demonstrated the beneficial impact of PPFMs on the growth and yield of snap bean crops.

2.5.11. PS II photochemistry (Fv/Fm ratio)

Light-dependent chemical reactions occurring in the thylakoid and the carbon metabolism occurring in the stroma are the major places of injury taking place as a consequence of elevated temperature stress. Elevated leaf and photon flux density temperature affects the PSII thermo-tolerance adjustment (Crafts-Brandner and Salvucci, 2002). The PSII is highly heat sensitive and its function is highly affected and even partly terminated under elevated heat stress (Camejo *et*

al., 2005). Oxygen evolving complex is also exposed to severe harm at elevated temperatures that may lead in imbalanced stream of electrons to the PSII acceptor site (De Ronde *et al.,* 2004). The proteins D1 and D2 are subject to denaturation at high temperature (De Las Rivas and Barber, 1997).

Different PSII elements were injured under high temperature stress in barley and wheat (Sharkova, 2001; Toth *et al.,* 2005). Likewise, photosynthesis was limited due to damage of the electron transport chain and reduced RuBP regeneration capacity in cotton crop (Wise *et al.,* 2004). In a latest research, Fahad *et al.* (2016) noted that high day and night temperatures considerably decreased the photosynthetic functions of two rice cultivars (IR64 and Huanghuazhan). The decrease in photosynthesis was attributed to damage to chlorophyll pigments, decrease in leaf nitrogen content, blockage of PSII reaction center and electron transport, reduced quantum efficacy (Fv/Fm) and down-regulation of PSII photochemistry.

Xu *et al.* (2013) reported that treatment of $CaCl_2$ on zoysiagrass leaves under water stress condition showed that chlorophyll fluorescence Fv/Fm, was higher in treated leaves and also it enhanced the antioxidant enzyme activities. Meanwhile, it lowered the proline and malondialdehyde (MDA) contents. As the result shows, the drought tolerance of zoysiagrass increased to some extent by the application of $CaCl_2$.

Wang *et al.* (2014) reported that under heat stress condition, foliar application of SA maintain or increase the activities of antioxidative enzymes such as SOD, catalase and ascorbate peroxidase which are known to increase the protection against oxidative stress for wheat crops. They also suggested that foliar application of SA protect the PSII complex from photo-damage through increased transcription of psbA gene (encoding D1 protein) and also mitigating photo-oxidation by a increase in activities of antioxidative enzymes, that permits faster functional recovery of PSII from heat stress.

2.6. IMPACT OF HIGH TEMEPERATURE ON MORPHOLOGICAL AND YIELD PARAMETERS

2.6.1. Plant height

Heat stress can trigger serious protein damage, disrupt their synthesis bring inactivation of significant enzymes, and also cause membrane damage. It also has significant impacts on the process of division of cell (Smertenko *et al.,* 1997). These kind of damages can severely restrict plant growth and also influence on oxidative damage.

Severe heat stress reduced the growth of stem resulting in reduction in the height of the plant (Prasad *et al.*, 2006a). In wheat and mung bean increased the height of the plant which helps the plants from heat stress through enhanced in transpiration cooling effect (Hasanuzzaman *et al.*, 2013). Under high temperature stress, plant height was more in tolerant rice variety N22 (4.59%) and mutant NH219 (12.82%) (Poli *et al.*, 2013).

2.6.2. Days to 50% flowering

The adverse impacts of elevated temperatures on cereals differ with the timing, duration and severity of high temperature stress (Fahad *et al.,* 2016). Sailaja *et al.* (2015) stated that in all rice genotypes, the total number of days to 50% flowering was reduced. High temperature tolerant variety Nagina-22 showed more number of days to 50% flowering (67 days) under control condition. But under high temperature condition the number of days to 50% flowering (63 days) was reduced.

2.6.3. Productive tillers per plant

Djanaguiraman *et al.* (2010) observed that under high temperature (30/25 °C, day/night) productive tillers per plant were drastically reduced. Mitra and Bhatia, (2008) reported that under high temperature condition the number of tillers, plant height and total biomass declined in rice genotypes.

On exposure of wheat plants to heat stress, reduction in the number of tillers was observed (Kumar *et al.*, 2011). Park *et al.* (1999) observed there was a reduction in plant height, leaf area, panicle numbers per hill, number of tiller, and number of spikelets per panicle under moisture stress in the cultivars of Japonica and Dongjinbyeo.

2.6.4. Pollen viability

Hedhly (2011) reported that in flowering plants the reproductive processes which involved viability of pollen and stigma, pollination, anthesis, growth of the pollen tube, and early development of embryo were mainly susceptible to high temperature stress (Giorno *et al.,* 2013). Moreover, male reproductive tissues were found more sensitive to high temperature stress at all stages of development than female reproductive tissues.

Matsui and Omasa, (2002) reported that heat stress given a day before to flowering affected the normal function of pollen sac, anther dehiscence and viability of pollen in rice.

Prasad *et al.* (2006) reported that heat stress reduced the production of pollen by 51 per cent and the pollen grains number on stigma was reduced by 43 per cent. Also it was reported that heat stress reduced the viability of pollen from 91 to 75 per cent, when averaged across all the cultivars of rice.

Bahuguna *et al.* (2015) concluded from his study that the effect of heat stress on viability of pollen and found that HT-tolerant rice varieties *i.e.* NL-44 and N22 both showed about 87% fertility under extremely hot field conditions.

Guru *et al.* (2016) conducted an experiment to examine the effects of boron (B) on reproductive growth and yield qualities of seven rice genotypes with the application of boron at different concentrations (control, 0.2 ppm, 0.4 ppm and 0.8 ppm) during flowering stage. The results revealed that IET 20979 and IET 21519 responded highly positive to application of boron at 0.4 ppm and this enhancement in the grain yield was mainly due to increase in pollen viability and stigma receptivity of rice.

Thussagunpanit *et al.* (2012) reported that 24-epibrassinolide (EBR) application reduced the harmful effects of high temperature stress on crop growth by enhancing the photosynthetic efficiency and pollen germination which allowed rice to withstand heat stress condition.

2.6.5. Time of anthesis

Flowering occurrence during early in the morning is a beneficial characteristic for tolerance to stress condition (mainly high temperature stress). In most of the rice cultivars the peak anthesis mainly occurs in between 10.00 am to 12.00 pm (Sheehy *et al.*, 2005). Satake and Yoshida, (1978) reported that heat stress during or after anthesis (1 to 3 hour after anthesis in rice) induces the sterility of spikelet. Early morning flowering character can be effectively used for the escape from the heat stress which induce the sterility of the spikelet during anthesis by shedding of the viable pollen in the cooler hours in the morning on to the receptive stigma.

Ishimaru *et al.* (2009) transferred a promising EMF allele or trait from wild rice (*Oryza officinalis*) to reduce the heat injury during anthesis. Kumar and Sarlach, (2015) suggested that foliar application of bio-regulators like salicylic

acid, potassium nitrate and calcium chloride increase thermo-tolerance and flowering in plants under high temperature stress conditions in using two varieties of forage cowpea.

Zeng *et al.* (1999) reported that methyl jasmonate (MeJA) remarkably induced the opening of rice florets within 30 minutes, with the most rapid induction of the opening of rice florets occurring just 6 minutes after treatment. Induced numbers of opening florets are corresponding with the concentrations of MeJA. Higher concentration of MeJA induced more florets.

2.6.6. Panicle length

Fahad *et al.* (2016) reported that exogenous application of combination treatment of plant growth regulators such as alpha-tocopherol (Ve), ascorbic acid (Vc), brassinosteroids (Br), triazoles (Tr) and methyl jasmonates (MeJA) helped to increase the length of the panicle in rice.

Under elevated temperature stress IR64, Mahamaya and Danteshwari varieties of rice showed maximum panicle length, while N-22 and R-RF-76 showed the minimum panicle length (Shrivastava *et al.,* 2012).

Panicle length, spikelets per panicle and height of the plant were mainly affected by heat stress and it varied with the tolerance of rice cultivars (Kovi *et al.,* 2011).

2.6.7. Grain yield per plant

Ferris *et al.* (1998) observed a notable decrease in total number of grains and grain weight in wheat under high temperature.

Heat stress decreased the yield of rice by decreasing the efficiency of growth and yield characters. Fahad *et al.* (2016) suggested that tillering stage was very susceptible to high night temperature in rice.

High temperature stress induced significant yield reduction in peanut (*Arachis hypogea* L.) and common beans (*Phaseolus vulgaris* L.) (Parasad *et al.,* 1999; Rainey and Griffiths, 2005).

A significant impact of high temperature stress was observed in tomatoes (*Lycopersicum esculentum*) due to its effect on meiosis, fertilization, and growth of fertilized embryos and eventually leading to a significant decrease in yield (Camejo *et al.,* 2005). Sailaja *et al.* (2015) reported that there was a great reduction in grain yield per hill (29.4%) under heat stress and the maximum reduction was reported in the variety BPT5204 (72%) whereas minimum value for Nagina-22. Radhika *et al.* (2008) reported that plants sprayed with pink-pigmented facultative methylotrophs produced highest maize cob yield compared to untreated plants.

2.6.8. Spikelet fertility percentage

Heat stress decreased the number of spikes and the number of florets per rice plant and also in the case of sorghum, seed set was found negatively impacted under similar condition (Prasad *et al.,* 2006; Fahad *et al.,* 2016).

Anthers and pollens were more sensitive to elevated temperature than ovules. Under heat stress, floret sterility was reported to be associated with decreased anther dehiscence, poor pollen shedding, poor pollen grains germination, reduced pollen tube elongation and decrease in the germination of invivo pollen (Fahad *et al.,* 2015, 2016).

Prasad *et al.* (2006) reported that there is a significant reduction in the fertility of spikelet and grain yield at high temperature above 5°C compared with the ambient temperature in various rice genotypes like Gainesville and Florida.

Ekanayake *et al.* (1989) mentioned that availability of water plays a key role in the incidence of spikelet sterility in rice. Moriya and Nara, (1971) reported that in rice, sterility percentage increased and at the same time, there was partially filling of grains when the daily mean temperature of 31.5°C (daily maximum temperature was 36°C and daily minimum temperature was 27°C) especially durimg flowering stage.

Prasad *et al.* (2006) reported that in rice highly positive correlation between the fertility of spikelet, production of pollen and pollen receptivity. Spikelet fertility for HT-tolerant variety Nagina-22 at ambient temperature was recorded 89.4% while under high temperature it was 81.1%.

Zhang *et al.* (2017) reported that salicylic acid mitigated the damage to rice plants induced by high temperature stress, but its function in preventing the degeneration of spikelet under high temperature stress has not been documented.

Fahad *et al.* (2016) reported that exogenous application of PGRs was helpful in alleviating the adverse effects of high temperature in rice. The combination treatment of plant growth regulators such as alpha-tocopherol (Ve), ascorbic acid (Vc), brassinosteroids (Br), triazoles (Tr) and methyl jasmonates (MeJA) was shown as the most effective treatment under heat stress. The highest grain production by Ve+Vc+Br+Tr+MeJA treated plants was due to increased in the photosynthesis, fertility of spikelet and grain filling, which helped to overcome the ill effects of high temperature stress and helpful in mitigating the adverse effects of high temperature.

2.6.9. 1000 grain weight

Prasad *et al.* (2017) reported that in cereals there was a severe reduction in number of seeds, individual weight of seed, yield of grains and also biomass production under heat stress condition which is directly reflected in the harvest index.

In a stress-free environment, grain weights for rice cultivars are almost constant (Mohammed and Tarpley, 2010). But, under high night temperature condition a significant reduction in the individual grain weight as well as grain production of rice per unit area (Fahad *et al.*, 2016).

Ishimaru *et al.* (2009) stated that one of the main reasons for chalky grains formation was heat stress during grain ripening stage. Sailaja *et al.* (2015) observed that there was a great decline in 1000 grain weight in several cultivars of rice under high temperature condition and there was a 5 per cent mean reduction observed in 1000 grain weight. Maximum reduction was reported in the variety BPT5204 whereas minimum value for N22.

Anjum *et al.* (2016) reported that methyl jasmonate spray enhanced the dry biomass, number of grains per spikelet, and grain weight which resulted in higher grain and biological yield in wheat under drought condition.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled "Impact of foliar application of plant growth regulators and nutrients on high temperature stress mitigation in rice (*Oryza sativa* L.)" was conducted in the polyhouse maintained by Instructional farm, College of Agriculture, Vellayani, Kerala Agricultural University during the years from 2017-2019. The main objective of the programme was to study the effect of foliar application of plant growth regulators and nutrients on high temperature mitigation and to advance the flowering time by using methyl jasmonate (MeJA) in rice. The details of the materials used and methods adopted for this experiment as well as the procedures followed for laboratory analysis during the course of experimentation are described in this chapter.

3.1 TO STUDY THE EFFECT OF FOLIAR APPLICATION OF PLANT GROWTH REGULATORS AND NUTRIENTS ON HIGH TEMPERATURE MITIGATION IN RICE.

3.1.1. Plant materials

Rice variety used for this study was Uma (Mo 16) collected from Integrated Farming System Research Station, Karamana (plate1).

3.1.2. Location

The study was conducted in the temperature controlled polyhouse maintained by Instructional farm, College of Agriculture, Vellayani during 2018.

3.1.3. Preparation of potting mixture and transplanting

Earthen pots were filled with potting mixture of soil, sand and FYM in the ratio of 3:2:1. Seedlings were raised in plastic pro-trays filled with soil and coir pith in the ratio of 2:1 (plate 4). Foliar spray of 19:19:19 mixture was given on seedlings in pro-trays and on 15th DAT. Eighteen days old seedlings were transplanted to the pots at the rate of three seedlings per pot. Thinning and gap filling was done on 6th DAT and one healthy seedling was maintained in each pot.

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Crop was applied with recommended dose of fertilizer as per package of practices of Kerala Agricultural University, Thrissur. The cultural operations including weeding and plant protection measures were carried out as per the Package of Practices recommendations of Kerala Agricultural University, Thrissur.

3.1.4. Methodology

In this study, seedlings were raised in pot trays and transplanted to mud pots on 18th DAS (plate 5). The pots were kept under high temperature condition (5-6°C more than ambient condition) in a temperature controlled polyhouse from seedling to maturity stage (plate 2). Maximum and minimum temperature was measured daily using a thermo-hygrometer (plate 3). Foliar spray of plant growth regulators and nutrients were given at panicle initiation (plate 6), heading (plate 7) and flowering stage (plate 8). Physiological observations were taken at 50% flowering stage and yield parameters were taken at harvest stage (plate 9, 10).

Table 1. Particulars of experiment 1.

1.Crop	Rice: Uma (Mo 16) variety
2.Design	Completely Randomized Design (CRD)
3.Number of treatments	Ten T1: Brassinosteroid (50 ppm) T2: Boron (100 ppm) T3: Calcium chloride (0.6%) T4: Salicylic acid (50 ppm) T5: Glycine betaine (20 ppm) T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%) T7: 1-methyl cyclopropane (50ppm) T8: Gibberllic acid (50ppm) T9: Water spray T10: Control
4.Replication	Three

Plate 1. Uma (Mo 16) variety seeds

Plate 2. General view of temperature controlled polyhouse

Plate 3. Maximum and minimum temperature was measured daily using Thermo-hygrometer

Plate 4. Seedlings were raised in plastic pro-trays

Plate 5. General view of transplanted seedlings to mud pots

Plate 6. General view of panicle initiation stage

Plate 7. General view of heading stage

Plate 8. General view of flowering stage

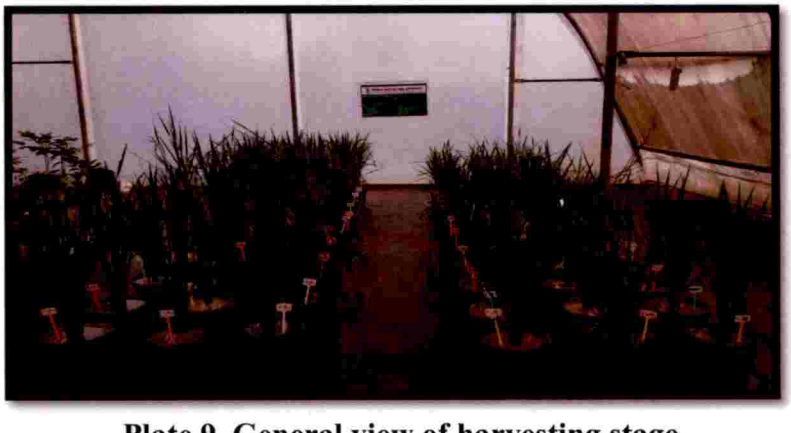


Plate 9. General view of harvesting stage

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 10. General view of treated plants compared with control of exp. 1

3.1.5. Observations

3.1.5.1. Physiological and biochemical parameters

3.1.5.1.1. Cell membrane stability index (%)

Cell membrane stability index was estimated as per the procedure outlined by Blum and Ebercon (1981) during 50% flowering stage. Samples were collected from all treatments and wiped thrice in deionized water to remove all adhered electrolytes on the surface. Samples were kept in a capped vial (20ml) which containing 10ml of deionized water and incubated at room temperature for 24 hours in a dark condition. The conductance was recorded by using conductivity meter. Then these vials were autoclaved for 15 minutes to kill the leaf tissues and release the electrolytes. The second conductivity measurements were done after cooling. For all treatments, these two readings were taken separately. Cell membrane stability index was determined by using the following formula and expressed as percentage.

$$CMS\ (\%) = [1-(T_1/T_2)/1-(C_1/C_2)] \times 100$$

Where, T and C refer to the treatment and control samples respectively. The subscripts 1 and 2 refer to conductance before and after autoclaving, respectively.

3.1.5.1.2. Chlorophyll stability index (%)

Chlorophyll content of leaf samples was calculated as per the procedure by Arnon (1949) during 50% flowering stage. 100 mg of the leaf sample was taken from the fully expanded third leaf and cut into tiny bits. Then 5 ml of DMSO (Dimethyl sulfoxide): 80% of Acetone (1:1) mixture was added to the samples and incubated it for overnight. The supernatant was collected and absorbance was measured at 645 and 663 nm. Total chlorophyll content and chlorophyll stability index were calculated by using the formula given below and it was expressed in mg g^{-1} of fresh leaf weight.

Total chlorophyll = {[20.2(OD at 645) + 8.01(OD at 663)] x V} / (Wx1000)

Where V = volume of the solution made up and W= fresh leaf weight.

$$\text{Chlorophyll stability index (\%)} = \frac{\text{Total chlorophyll in treated sample}}{\text{Total chlorophyll in control sample}} \times 100$$

3.1.5.1.3. Leaf temperature (°C)

Leaf temperature was measured at morning time between 8:30 am and 11 am using a portable photosynthetic system (CIRAS-3, PP systems U.S.A) and was expressed in ^{o}C.

3.1.5.1.4. Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})

Photosynthetic rate was measured using Portable Photosynthetic System (CIRAS-3, PP systems U.S.A) at morning time between 8 am and 10 am and was expressed in µmol CO_2 m^{-2} s^{-1}.

3.1.5.1.5. Stomatal conductance (mmol H_2O m^{-2} s^{-1})

Stomatal conductance was measured at morning time between 8 am and 10 am using Portable Photosynthetic System (CIRAS-3, PP systems U.S.A) and was expressed in mmol H_2O m^{-2} s^{-1}.

3.1.5.1.6. Transpiration rate (mmol H_2O m^{-2} s^{-1})

Transpiration rate was measured using Portable Photosynthetic System (CIRAS-3, PP systems U.S.A) at morning time between 8 am and 10 am and were expressed in m moles H_2O m-2 s-1.

3.1.5.1.7. Chlorophyll a/b ratio

Chlorophyll content of leaf samples was calculated as per the procedure by Arnon (1949) during 50% flowering stage. 100 mg of the leaf sample was taken

from the fully expanded third leaf and cut into tiny bits. Then 5 ml of DMSO (Dimethyl sulfoxide): 80% of Acetone (1:1) mixture was added to the samples and incubated it for overnight. The supernatant was collected and absorbance was measured at 645 and 663 nm. Chlorophyll a and chlorophyll b were calculated by using the formula given below.

Chlorophyll a = {[12.7(OD at 663) – 2.65 (OD at 645)] x V} / (Wx1000)

Chlorophyll b = {[22.9(OD at 645) – 4.68(OD at 663)] x V} / (Wx1000)

Where V = volume of the solution made up and W= fresh leaf weight.

$$\text{Chlorophyll a/b ratio} = \frac{\text{Chlorophyll a}}{\text{Chlorophyll b}}$$

3.1.5.1.8. Carbohydrate content (mg g^{-1})

The total carbohydrate content was measured by using anthrone method (Hedge and Hofreiter, 1962). 100 mg leaf sample were measured from all the treated and control plants individually. It was hydrolysed with 5 ml of 2.5N hydrochloric acid (HCI) in a boiling water bath. The hydrolyzate was neutralized with solid sodium carbonate until the effervescence stopped. The volume was made up to 100 ml and it was centrifuged at 5000 rpm for 15 minutes. From the supernatant 0.5 ml aliquot was collected and it was made up to 1 ml by adding distilled water. To this 4 ml of anthrone reagent was added and then heated for 8 minutes in a boiling water bath. This was cooled suddenly and by using spectrophotometer the absorbance was taken at 630 nm. Carbohydrate content was estimated from the standard graph prepared by using glucose and it was expressed in terms of milligrams of carbohydrate per gram of fresh weight leaf tissue.

3.1.5.1.9. Malondialdehyde content (mmol g^{-1})

The malondialdehyde content was measured by the method described by Dionisio-Sese and Tobita (1998) using leaf samples used for measuring ROS enzyme activities and ascorbic acid (AsA). Grind 0.5g of fresh leaf tissue was ground to a fine powder in liquid nitrogen. The ground powder was homogenized in 5 ml of ice-cold 50 mM potassium phosphate buffer (pH 7.0). Then the extract was centrifuged at 4 ^{0}C for 30 min at 20,000 g and one ml aliquot of the extract was then mixed with the same volume of a 0.5% (w/v) thiobarbituric acid solution containing 20% (w/v) of trichloroacetic acid. The mixture was heated at 95 ^{0}C for 30 min and the reaction was stopped quickly by placing the sample in an ice bath and this cooled mixture was centrifuged at 10000 g for 10 min. Then the absorbance of the supernatant was measured at 532 and 600 nm by using spectrophotometer. After subtracting the non-specific absorbance at 600 nm, the malondialdehyde concentration was determined using the extinction coefficient of 155 mM $^{-1}$cm^{-1}.

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

Superoxide dismutase activity was measured according to the method of Beauchamp and Fridovich (1971). Grind 1g of fresh leaf tissue in 10 ml ice cold 50 mM potassium phosphate buffer (pH 7.8) in a pre-chilled pestle and mortar. Then centrifuge the homogenate at 10000 rpm for 10 min at 4°C and the supernatant was used for estimation. Mix a 3 ml of reaction mixture containing 50 mM potassium phosphate buffer, 13mM methionine, 2 µM riboflavin, 0.1 mM EDTA, 75 µM NBT and 50 µL of crude enzyme extract, in duplicate. Make up the volume equal by adding double distilled water. Set a blank without enzyme and NBT to calibrate the spectrophotometer. Set another control having NBT but no enzyme as reference control. Expose all the tubes to 400 W bulb (4 x 100 W bulbs) for 15 min. Read the absorbance immediately at 560 nm by using spectrophotometer and then calculate the percentage inhibition. The 50% inhibition of the reaction between riboflavin and NBT in the presence of methionine was taken as 1 unit of SOD activity.

3.1.5.1.11. PS II photochemistry (Fv/Fm ratio)

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

3.1.5.1.12. Minimum and maximum temperature (°C)

Minimum and maximum temperature was measured daily by using a thermo-hygrometer.

3.1.5.2. Morphological and yield parameters:

3.1.5.2.1. Plant height (cm)

Plant height was measured at the time of maturity from the base of the plant to the tip of the primary panicle and it was expressed in centimeter.

3.1.5.2.2. Days to 50% flowering

The number of days was taken from sowing to the exertion of 50% of panicles in each replication was taken as days to 50% flowering

3.1.5.2.3. Productive tillers per plant

In each replication, the number of panicle bearing tillers at the time of harvest was counted and recorded.

3.1.5.2.4. Pollen viability (%)

Pollen viability was measured by using 1% iodine- 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 were denoting the fertile pollen and unstained, shriveled, empty grains which indicate sterile grains. Fertile pollen grains were visually

counted under compound microscope, Leica. The pollen viability was calculated by using the formula given below and expressed as percentage.

$$\text{Pollen viability} = \frac{\text{Number of pollen grains stained}}{\text{Total number of pollen grains}} \times 100$$

3.1.5.2.5. Time of anthesis

Time of anthesis was observed from 09.00 am to 12.00 pm. Visual observation was taken directly from the location.

3.1.5.2.6. Panicle length (cm)

Ten panicles were selected randomly from the each treatment. The panicle length was measured from the neck to the tip and the mean value was calculated and it was expressed in centimeters.

3.1.5.2.7. Grain yield per plant (g)

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

3.1.5.2.8. 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

$$\text{Spikelet fertility (\%)} = \frac{\text{Number of fertile spikelets}}{\text{Total number of spikelets}} \times 100$$

3.1.5.2.9. 1000 grain weight (g)

One thousand seeds were taken randomly from each replication, weighed and expressed in grams.

3.2 TO STUDY THE EFFECT OF FOLIAR APPLICATION OF METHYL JASMONATE ON EARLY MORNING FLOWERING (EMF) TRAIT ON HIGH TEMPERATURE MITIGATION IN RICE.

3.2.1. Plant materials

Rice variety used for this study was Uma (Mo 16) collected from Integrated Farming System Research Station, Karamana.

3.2.2. Location

The study was conducted in the temperature controlled polyhouse maintained by Instructional farm, College of Agriculture, Vellayani during 2018.

3.2.3. Methodology

In this study, seedlings were raised in pot trays and transplanted to mud pots on 18th DAS. The pots were kept under high temperature condition (5-6°C more than ambient condition) in a temperature controlled polyhouse from seedling to maturity stage. Maximum and minimum temperatures were measured daily using a thermo-hygrometer. Foliar spray of methyl jasmonate was sprayed to the spikelet in varying concentration with different time. Physiological observations were taken at 50% flowering stage and yield parameters were taken at harvest stage (plate 11).

3.2.4. Preparation of potting mixture and transplanting

Seedlings were raised in pot trays and maintained in mud pots as in the case of Experiment I.

Table 2. Particulars of experiment 2.

1.Crop	Rice: Uma (Mo 16) variety
2.Design	Completely Randomized Design (CRD)
3.Number of treatments	Ten T1: 2mM L^{-1} Methyl jasmonate at 7 am T2: 2mM L^{-1} Methyl jasmonate at 8 am T3: 2mM L^{-1} Methyl jasmonate at 9 am T4: 4mM L^{-1} Methyl jasmonate at 7 am T5: 4mM L^{-1} Methyl jasmonate at 8 am T6: 4mM L^{-1} Methyl jasmonate at 9 am T7: Water spray at 7 am T8: Water spray at 8 am T9: Water spray at 9 am T10: Control
4.Replication	Three

3.2.5. Observations

3.2.5.1. Anthesis time

Time of anthesis was observed from 07.00 am to 12.00 pm. Visual observation were taken directly from the location.

3.2.5.2. Pollen viability (%)

Pollen viability was measured by using 1% iodine- 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 were denoting the fertile pollen and unstained, shriveled, empty grains which indicate sterile grains. Fertile pollen grains were visually

counted under compound microscope, Leica. The pollen viability was calculated by using the formula given below and expressed as percentage.

$$\text{Pollen viability} = \frac{\text{Number of pollen grains stained}}{\text{Total number of pollen grains}} \times 100$$

3.2.5.3. 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

$$\text{Spikelet fertility (\%)} = \frac{\text{Number of fertile spikelets}}{\text{Total number of spikelets}} \times 100$$

3.2.5.4. Yield per plant (g)

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

3.2.5.5. 1000 grain weight (g)

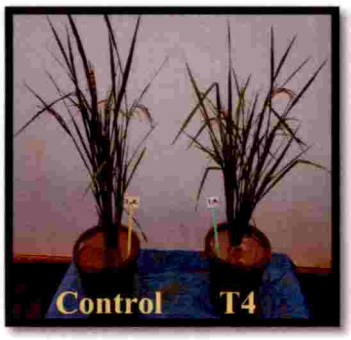
One thousand seeds were taken randomly from each replication, weighed and expressed in grams.

Results

T1: 2mM L^{-1} MeJA at 7 am

T2: 2mM L^{-1} MeJA at 8 am

T3: 2mM L^{-1} MeJA at 9 am

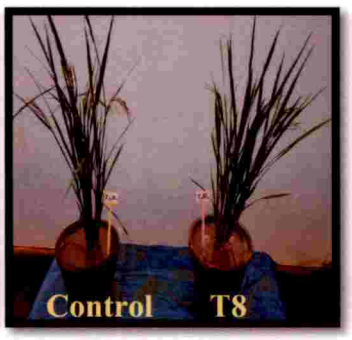


T4: 4mM L^{-1} MeJA at 7 am

T5: 4mM L^{-1} MeJA at 8 am

T6: 4mM L^{-1} MeJA at 9 am

T7: Water spray at 7 am



T8: Water spray at 8 am

T9: Water spray at 9 am

Plate 11. General view of treated plants compared with control of exp. 2

4. RESULTS

The present study "Impact of foliar application of plant growth regulators and nutrients on high temperature stress mitigation in rice (*Oryza sativa* L.)" was implemented in two experiments in the Department of Plant Physiology, College of Agriculture, Vellayani. The objective of the first experiment was to assess the effect of foliar application of plant growth regulators and nutrients on high temperature stress mitigation in rice. Second experiment was conducted to study the effect of foliar application of methyl jasmonate on early morning flowering (EMF) trait on high temperature stress mitigation in rice. The data obtained during the course of investigation were statistically analysed and the results are presented in this chapter with suitable tables.

4.1 EXPERIMENT 1 - TO ASSESS THE EFFECT OF FOLIAR APPLICATION OF PLANT GROWTH REGULATORS AND NUTRIENTS ON HIGH TEMPERATURE STRESS MITIGATION IN RICE

4.1.1. Physiological and biochemical parameters

Cell membrane stability index, chlorophyll stability index, leaf temperature, photosynthetic rate, stomatal conductance, transpiration rate, chlorophyll a/b ratio, carbohydrate content, malondialdehyde content, superoxide dismutase activity, PS II photochemistry (Fv/Fm ratio), minimum and maximum temperature were recorded during the experiment.

4.1.1.1. Cell membrane stability index (%)

Cell membrane stability of rice was studied at 50% flowering stage and the data is presented in table 3. It was observed that among the treatments brassinosteroid-50 ppm (141.57%) and glycine betaine-20 ppm (134.31%) showed the highest cell membrane stability and the least membrane stability was recorded in 1-methyl cyclopropane-50 ppm (109.95 %), gibberllic acid-50 ppm (108.18%) and water spray (103.65%).

4.1.1.2. Chlorophyll stability index (%)

Data on chlorophyll stability index of rice was taken at 50% flowering stage under different treatments are depicted in table 4. In all the treatments chlorophyll stability was increased in under high temperature stress. Among the treatments, brassinosteroid-50 ppm (109.32%) recorded the highest chlorophyll stability. Gibberllic acid-50 ppm (102.75%), 1-methyl cyclopropane-50 ppm (102.26%) and water spray (101.09%) recorded the lowest value.

4.1.1.3. Leaf temperature (°C)

Data recorded for leaf temperature of rice at 50% flowering stage under different treatments is presented in table 5. Observed mean value was minimum for calcium chloride-0.6% (30.32°C), glycine betaine-20 ppm (30.40°C), pink-pigmented facultative methylotrophs-1% (30.42°C), brassinosteroid–50 ppm (30.45°C) and boron-100 ppm (30.65°C) whereas, control (31.20°C) recorded the highest value.

4.1.1.4. Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})

Data recorded for photosynthetic rate of rice at 50% flowering stage under different treatments is presented in table 6. Observed mean value was maximum for brassinosteroid–50 ppm (17.50 µmol CO_2 m^{-2} s^{-1}), pink-pigmented facultative methylotrophs-1% (16.90 µmol CO_2 m^{-2} s^{-1}) and minimum was recorded in water spray (10.10 µmol CO_2 m^{-2} s^{-1}) and control (9.50 µmol CO_2 m^{-2} s^{-1}).

4.1.1.5. Stomatal conductance (mmol H_2O m^{-2} s^{-1})

Data pertaining to stomatal conductance of rice at 50% flowering stage under different treatments is presented in table 7. Mean stomatal conductance under different treatments was maximum in brassinosteroid–50 ppm (583.7 mmol H_2O m^{-2} s^{-1}) and minimum for water spray (341.3 mmol H_2O m^{-2} s^{-1}) and control (318.1 mmol H_2O m^{-2} s^{-1}).

Table 3. Effect of foliar application of plant growth regulators and nutrients on cell membrane stability index, %

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	141.57
2	T2: Boron (100 ppm)	117.18
3	T3: Calcium chloride (0.6%)	124.98
4	T4: Salicylic acid (50 ppm)	112.52
5	T5: Glycine betaine (20 ppm)	134.31
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	126.75
7	T7: 1-methyl cyclopropane (50ppm)	109.95
8	T8: Gibberllic acid (50ppm)	108.18
9	T9: Water spray	103.65
	SE m ±	3.55
	CD (0.05)	10.64

Table 4. Effect of foliar application of plant growth regulators and nutrients on chlorophyll stability index, %

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	109.32
2	T2: Boron (100 ppm)	106.31
3	T3: Calcium chloride (0.6%)	104.41
4	T4: Salicylic acid (50 ppm)	106.38
5	T5: Glycine betaine (20 ppm)	103.39
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	105.60
7	T7: 1-methyl cyclopropane (50ppm)	102.26
8	T8: Gibberllic acid (50ppm)	102.75
9	T9: Water spray	101.09
	SE m ±	0.77
	CD (0.05)	2.33

Table 5. Effect of foliar application of plant growth regulators and nutrients on leaf temperature, °C

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	30.45
2	T2: Boron (100 ppm)	30.65
3	T3: Calcium chloride (0.6%)	30.32
4	T4: Salicylic acid (50 ppm)	30.75
5	T5: Glycine betaine (20 ppm)	30.40
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	30.42
7	T7: 1-methyl cyclopropane (50ppm)	30.70
8	T8: Gibberllic acid (50ppm)	30.62
9	T9: Water spray	30.82
10	T10: Control	31.20
	SE m ±	0.12
	CD (0.05)	0.35

Table 6. Effect of foliar application of plant growth regulators and nutrients on photosynthetic rate, µmol CO_2 m^{-2} s^{-1}

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	17.50
2	T2: Boron (100 ppm)	13.10
3	T3: Calcium chloride (0.6%)	13.40
4	T4: Salicylic acid (50 ppm)	14.20
5	T5: Glycine betaine (20 ppm)	12.40
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	16.90
7	T7: 1-methyl cyclopropane (50ppm)	14.90
8	T8: Gibberllic acid (50ppm)	12.80
9	T9: Water spray	10.10
10	T10: Control	9.50
	SE m ±	0.27
	CD (0.05)	0.78

4.1.1.6. Transpiration rate (mmol H_2O m^{-2} s^{-1})

Data recorded for transpiration rate of rice at 50% flowering stage under different treatments is presented in table 8. Observed mean value was minimum for glycine betaine-20 ppm (3.51 mmol H_2O m^{-2} s^{-1}) and maximum for control (4.95 mmol H_2O m^{-2} s^{-1}) recorded the highest value.

4.1.1.7. Chlorophyll a/b ratio

Data on chlorophyll a/b ratio of rice was taken at 50% flowering stage under different treatments are depicted in table 9. In all the treatments chlorophyll a/b ratio was increased in under high temperature stress. Among the treatments, brassinosteroid–50 ppm (1.28), pink-pigmented facultative methylotrophs-1% (1.24), boron-100 ppm (1.22), salicylic acid-50 ppm (1.22), glycine betaine-20 ppm (1.21), calcium chloride-0.6% (1.20) and 1-methyl cyclopropane-50 ppm (1.20) recorded the highest chlorophyll stability whereas, water spray (1.11) and control (1.06) recorded the lowest value.

4.1.1.8. Carbohydrate content (mg g^{-1})

Carbohydrate content of rice was studied at 50% flowering stage and the data is presented in table 10. It was observed that among the treatments boron-100 ppm (39.58 mg g^{-1}), pink-pigmented facultative methylotrophs-1% (37.16 mg g^{-1}), glycine betaine-20 ppm (36.78 mg g^{-1}), salicylic acid-50 ppm (35.29 mg g^{-1}) and brassinosteroid-50 ppm (33.61 mg g^{-1}) showed the highest carbohydrate content and the least carbohydrate content was recorded in gibberllic acid-50 ppm (29.90 mg g^{-1}), water spray (27.70 mg g^{-1}) and control(26.24 mg g^{-1}).

4.1.1.9. Malondialdehyde content (µg g^{-1})

Data on malondialdehyde content of rice was taken at 50% flowering stage under different treatments are depicted in table 11. In all the treatments malondialdehyde content was decreased under high temperature stress. Among

the treatments, calcium chloride-0.6% (1.03 μg g^{-1}), glycine betaine-20 ppm (1.14 μg g^{-1}) and brassinosteroid–50 ppm (1.15 μg g^{-1}) recorded the lowest malondialdehyde content and control (2.36 μg g^{-1}) recorded the maximum value.

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

Data recorded for superoxide dismutase activity of rice was taken at 50% flowering stage under different treatments is presented in table 12. Among the treatments, brassinosteroid–50 ppm (0.33 g^{-1} min^{-1}), 1-methyl cyclopropane-50 ppm (0.31 g^{-1} min^{-1}), salicylic acid-50 ppm (0.30 g^{-1} min^{-1}) and pink-pigmented facultative methylotrophs-1% (0.30 g^{-1} min^{-1}) recorded the highest superoxide dismutase activity and the minimum activity was shown by water spray (0.16 g^{-1} min^{-1}) and control (0.15 $g^{-1}min^{-1}$).

4.1.1.11. PS II photochemistry (Fv/Fm ratio)

PS II photochemistry (Fv/Fm ratio) of rice was studied at 50% flowering stage and the data is presented in table 13. It was observed that among the treatments brassinosteroid-50ppm (0.74) showed the highest Fv/Fm ratio and the least Fv/Fm ratio was recorded in control (0.67).

4.1.1.12. Minimum and maximum temperature (°C)

Minimum and maximum temperature recorded inside the polyhouse and ambient condition from 18^{th} DAS to maturity stage and the data is presented in table 14. 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.

Table 7. Effect of foliar application of plant growth regulators and nutrients on stomatal conductance, mmol H_2O m^{-2} s^{-1}

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	583.7
2	T2: Boron (100 ppm)	426.6
3	T3: Calcium chloride (0.6%)	456.8
4	T4: Salicylic acid (50 ppm)	477.1
5	T5: Glycine betaine (20 ppm)	415.8
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	541.8
7	T7: 1-methyl cyclopropane (50ppm)	484.4
8	T8: Gibberllic acid (50ppm)	415.9
9	T9: Water spray	341.3
10	T10: Control	318.1
	SE m ±	11.18
	CD (0.05)	32.47

Table 8. Effect of foliar application of plant growth regulators and nutrients on transpiration rate, mmol H_2O m^{-2} s^{-1}

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	4.26
2	T2: Boron (100 ppm)	4.33
3	T3: Calcium chloride (0.6%)	3.84
4	T4: Salicylic acid (50 ppm)	4.54
5	T5: Glycine betaine (20 ppm)	3.51
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	4.01
7	T7: 1-methyl cyclopropane (50ppm)	3.94
8	T8: Gibberllic acid (50ppm)	4.31
9	T9: Water spray	4.51
10	T10: Control	4.95
	SE m ±	0.08
	CD (0.05)	0.25

Table 9. Effect of foliar application of plant growth regulators and nutrients on chlorophyll a/b ratio

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	1.28
2	T2: Boron (100 ppm)	1.22
3	T3: Calcium chloride (0.6%)	1.20
4	T4: Salicylic acid (50 ppm)	1.22
5	T5: Glycine betaine (20 ppm)	1.21
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	1.24
7	T7: 1-methyl cyclopropane (50ppm)	1.20
8	T8: Gibberllic acid (50ppm)	1.19
9	T9: Water spray	1.11
10	T10: Control	1.06
	SE m ±	0.02
	CD (0.05)	0.08

Table 10. Effect of foliar application of plant growth regulators and nutrients on carbohydrate content, mg g^{-1}

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	33.61
2	T2: Boron (100 ppm)	39.58
3	T3: Calcium chloride (0.6%)	31.40
4	T4: Salicylic acid (50 ppm)	35.29
5	T5: Glycine betaine (20 ppm)	36.78
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	37.16
7	T7: 1-methyl cyclopropane (50ppm)	33.41
8	T8: Gibberllic acid (50ppm)	29.90
9	T9: Water spray	27.70
10	T10: Control	26.24
	SE m ±	2.06
	CD (0.05)	6.12

Table 11. Effect of foliar application of plant growth regulators and nutrients on malondialdehyde content, μg g^{-1}

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	1.15
2	T2: Boron (100 ppm)	1.40
3	T3: Calcium chloride (0.6%)	1.03
4	T4: Salicylic acid (50 ppm)	1.68
5	T5: Glycine betaine (20 ppm)	1.14
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	1.37
7	T7: 1-methyl cyclopropane (50ppm)	1.91
8	T8: Gibberllic acid (50ppm)	1.49
9	T9: Water spray	2.11
10	T10: Control	2.36
	SE m ±	0.07
	CD (0.05)	0.21

Table 12. Effect of foliar application of plant growth regulators and nutrients on superoxide dismutase activity, activity g^{-1} min^{-1}

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	0.33
2	T2: Boron (100 ppm)	0.25
3	T3: Calcium chloride (0.6%)	0.22
4	T4: Salicylic acid (50 ppm)	0.30
5	T5: Glycine betaine (20 ppm)	0.24
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	0.30
7	T7: 1-methyl cyclopropane (50ppm)	0.31
8	T8: Gibberllic acid (50ppm)	0.23
9	T9: Water spray	0.16
10	T10: Control	0.15
	SE m ±	0.01
	CD (0.05)	0.03

Table 13. Effect of foliar application of plant growth regulators and nutrients on PS II photochemistry, Fv/Fm ratio

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	0.74
2	T2: Boron (100 ppm)	0.71
3	T3: Calcium chloride (0.6%)	0.70
4	T4: Salicylic acid (50 ppm)	0.71
5	T5: Glycine betaine (20 ppm)	0.71
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	0.72
7	T7: 1-methyl cyclopropane (50ppm)	0.70
8	T8: Gibberllic acid (50ppm)	0.71
9	T9: Water spray	0.69
10	T10: Control	0.67
	SE m ±	0.005
	CD (0.05)	0.013

Table 14. Minimum and maximum temperature (°C) recorded inside the polyhouse and ambient condition

Sl.No.	DAS	Minimum temperature (°C)		Maximum temperature (°C)	
		Inside the polyhouse condition	Ambient condition	Inside the polyhouse condition	Ambient condition
1	18	25.2	23.2	39.4	30.6
2	19	24.9	23.8	39.9	30.4
3	20	24.6	23.8	40.8	30.6
4	21	24.7	22.3	41.0	29.8
5	22	24.7	22.8	41.4	29.6
6	23	24.9	23.2	40.3	30.4
7	24	24.3	24.2	39.9	30.6
8	25	25.3	23.4	40.4	30.6
9	26	25.4	23.8	40.1	30.6
10	27	25.1	22.0	37.8	29.0
11	28	25.1	21.2	39.2	29.2
12	29	25.2	22.8	39.7	28.0
13	30	25.1	22.0	39.0	28.0
14	31	24.8	22.8	39.3	28.2

15	32	24.8	23.2	39.8	29.8
16	33	24.6	24.0	40.7	30.6
17	34	24.9	24.2	41.5	30.4
18	35	25.4	24.2	42.3	30.6
19	36	25.6	24.4	41.0	31.0
20	37	25.4	24.0	40.4	32.0
21	38	25.4	24.0	41.8	32.3
22	39	25.5	24.8	40.6	31.8
23	40	24.7	24.0	43.3	32.4
24	41	24.9	24.0	44.1	31.8
25	42	24.6	24.8	38.8	32.0
26	43	25.0	24.8	39.3	31.8
27	44	24.3	24.4	39.6	32.0
28	45	25.1	24.4	40.7	32.0
29	46	25.4	24.6	41.4	32.0
30	47	25.0	24.0	39.8	32.0
31	48	24.8	24.0	41.0	32.3
32	49	24.9	24.0	40.2	32.4
33	50	25.0	24.0	41.3	32.0
34	51	24.8	23.8	42.1	32.3
35	52	23.9	24.0	43.0	32.2
36	53	24.6	24.2	42.8	33.2
37	54	24.0	24.0	38.9	33.1
38	55	24.2	24.0	39.3	32.9
39	56	25.1	24.0	40.2	32.9
40	57	24.6	24.0	40.0	32.9
41	58	23.8	24.1	41.3	33.0
42	59	26.1	24.2	41.1	33.0
43	60	25.8	24.4	41.6	32.2
44	61	25.7	24.8	40.9	32.0
45	62	25.3	24.0	42.3	31.8
46	63	24.9	24.0	40.4	31.8
47	64	24.7	24.0	41.7	31.6
48	65	25.0	24.2	43.2	32.4
49	66	24.9	24.2	41.9	32.2
50	67	25.4	24.0	42.0	32.2
51	68	25.6	24.8	39.8	32.8
52	69	26.1	25.0	40.3	32.6
53	70	26.3	24.8	43.2	32.8
54	71	25.8	24.8	41.9	32.8
55	72	24.9	24.0	41.7	32.5
56	73	26.2	24.8	41.4	32.1
57	74	24.7	25.2	42.7	32.4
58	75	25.0	24.8	43.3	31.3

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59	76	25.8	24.2	43.1	31.5
60	77	25.7	24.5	40.9	30.7
61	78	26	24.6	41.8	31.8
62	79	24.4	24.8	39.3	31.4
63	80	23.8	25.0	39.4	31.1
64	81	23.6	25.1	41.4	31.2
65	82	24.7	23.8	40.0	28.2
66	83	25.2	23.8	39.8	30.3
67	84	24.8	24.0	39.9	30.4
68	85	24.8	24.3	41.6	31.2
69	86	25	24.2	41.0	31.8
70	87	25.4	24.8	41.9	31.6
71	88	26.1	24.8	41.4	31.2
72	89	24.9	23.8	42.1	32.2
73	90	25	24.0	42.1	32.2
74	91	25.1	24.2	40.0	32.2
75	92	26.4	24.8	41.5	32.1
76	93	26.9	24.8	40.3	32.2
77	94	26.4	24.8	41.6	32.1
78	95	26.3	23.8	41.2	31.2
79	96	25.6	24.8	40.4	32.2
80	97	26	23.8	41.3	31.5
81	98	26.7	23.8	39.8	31.6
82	99	25.9	24.8	44.0	29.7
83	100	24.7	24.5	44.1	31.8
84	101	27.2	24.2	42.3	31.8
85	102	25.8	24.8	41.1	31.8
86	103	23.7	24.2	40.6	31.8
87	104	23.7	24.3	40.0	31.6
88	105	25.1	24.0	40.4	32.4
89	106	25.4	24.0	40.6	31.8
90	107	26.2	23.8	42.1	31.2
91	108	26.1	24.8	42.0	31.8
92	109	26.6	24.8	41.3	31.2
93	110	23.8	24.8	40.6	32.1
94	111	23.5	24.0	33.4	30.8
95	112	24.7	23.8	37.2	30.2
96	113	23.4	24.3	39.5	30.2
97	114	25.7	24.2	40.4	31.1
98	115	25.1	24.0	39.7	31.8
99	116	25.4	24.0	40.1	31.2
100	117	23.2	23.8	38.4	32.1
	Mean	25.1	24.1	40.8	31.5

4.1.2. Morphological and yield parameters:

Plant height, days to 50% flowering, productive tillers per plant, pollen viability, time of anthesis, panicle length, grain yield per plant, spikelet fertility percentage and 1000 grain weight were recorded during the experiment.

4.1.2.1. Plant height (cm)

Plant height of rice was studied at harvest stage and the data is presented in table 15. It was observed that among the treatments gibberllic acid-50 ppm (142.75 cm) showed the maximum plant height and the minimum plant height was in water spray (94.75 cm) and control (91.00 cm) under high temperature stress condition.

4.1.2.2. Days to 50% flowering

Data regarding days to 50% flowering of rice under different treatments is recorded in table 16. In general among the treatments, early days to 50% flowering was recorded in plants under high temperature condition without treatment compared to treated plants. Among the treatments, early days to 50% flowering was showed by control (81.75) and water spray (82.50). Delay in days to 50% flowering was observed in pink-pigmented facultative methylotrophs-1% (86.00), brassinosteroid-50 ppm (85.75) and boron-100 ppm (85.25) under high temperature stress condition.

4.1.2.3. Productive tillers per plant

Data pertaining to productive tillers per plant was studied at harvest stage of rice under different treatments is recorded in table 17. Among the treatments, brassinosteroid-50 ppm produced more number of productive tillers per plant (10.25) whereas, gibberllic acid-50 ppm (8.00), water spray (7.75) and control (7.25) produced least number of productive tillers per plant under high temperature stress condition.

4.1.2.4. Pollen viability (%)

Data related to pollen viability of rice under different treatments is recorded in table 18. Brassinosteroid-50 ppm (80.23%), pink-pigmented facultative methylotrophs-1% (78.79%), glycine betaine-20 ppm (77.73%) and boron-100 ppm (77.67%) showed higher percent of pollen viability whereas, Control (37.52%) recorded the minimum pollen viability (Plate 12).

4.1.2.5. Time of anthesis (am)

Flower opening time of rice plants under different treatments shown in table 19. Among the ten treatments, plants sprayed with boron-100 ppm flowered at 11:13 am. Flowers of control plants opened at 11:54 am with respect to other treatments.

4.1.2.6. Panicle length (cm)

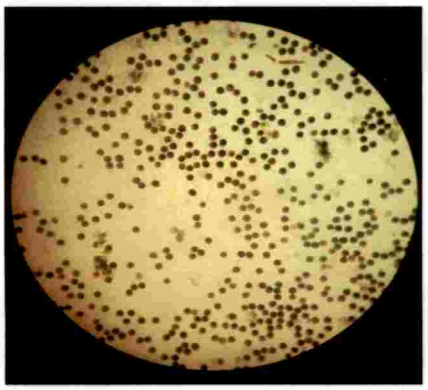
Panicle length of rice was studied at harvest stage and the data is presented in table 20. It was observed that among the treatments gibberllic acid-50 ppm (21.33 cm), brassinosteroid-50 ppm (20.90 cm) and boron-100 ppm (20.83 cm) treatment showed the maximum panicle length and the minimum panicle length was in water spray (19.03 cm) and control (18.98 cm).

4.1.2.7. Grain yield per plant (g)

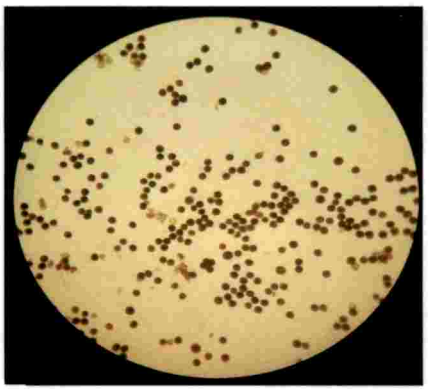
Grain yield per plant recorded under different treatments is presented in table 21. The results showed that significant reduction was observed in grain yield per plant under high temperature stress conditions. The mean grain yield per plant of treatments was maximum for brassinosteroid-50 ppm (15.87 g), whereas the minimum value recorded in control plants (4.16 g) under high temperature stress.

4.1.2.8. Spikelet fertility percentage (%)

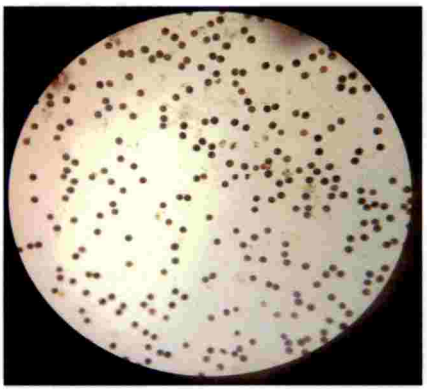
Data recorded for spikelet fertility of rice under different treatments is recorded in table 22. Highest spikelet fertility was recorded in brassinosteroid-50 ppm (75.40 %) and pink-pigmented facultative methylotrophs-1% (73.46 %) whereas, control recorded the minimum spikelet fertility (31.96 %).



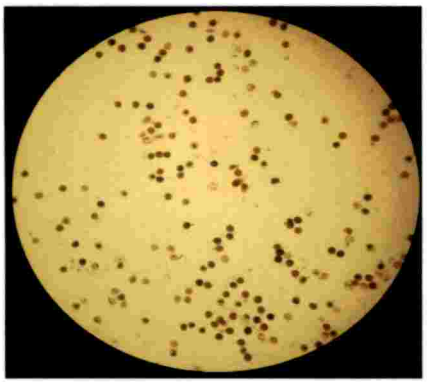
T1: Brassinosteroid (50 ppm)



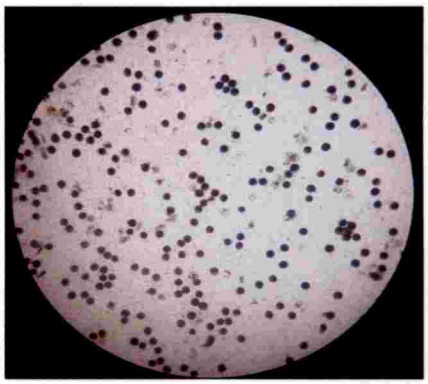
T2: Boron (100 ppm)



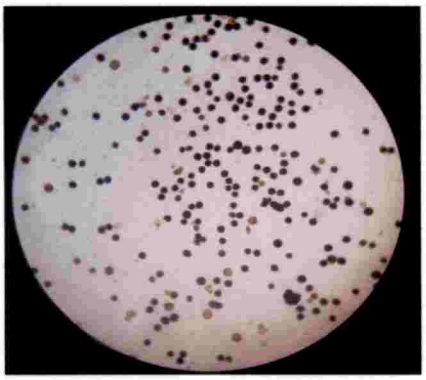
T3: Calcium chloride (0.6%)



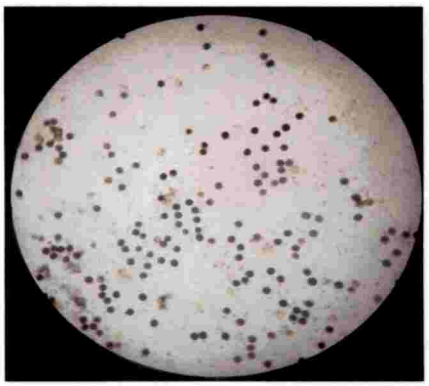
T4: Salicylic acid (50 ppm)



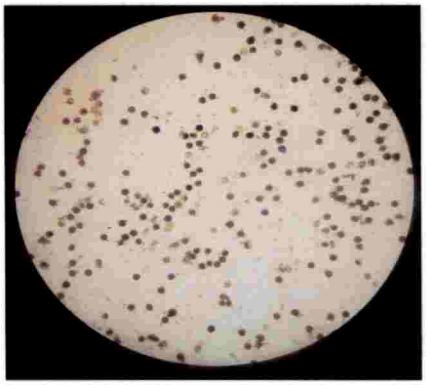
T5: Glycine betaine (20 ppm)



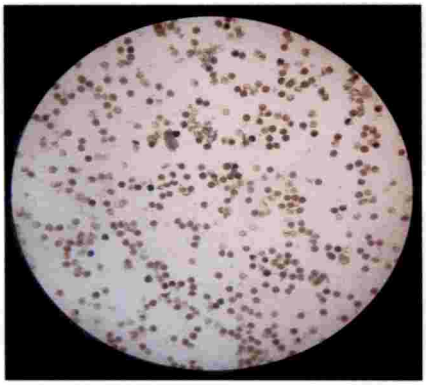
T6: PPFM (1%)



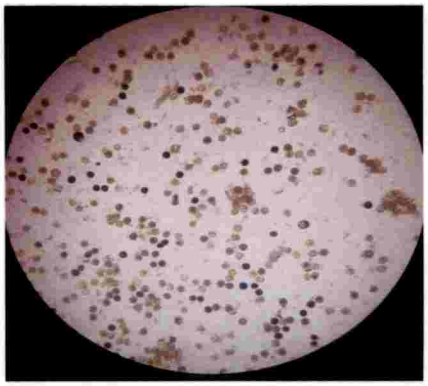
T7:1-methyl cyclopropane (50ppm)



T8: Gibberllic acid (50ppm)



T9: Water spray



T10: Control

Plate 12. Effect of foliar application of plant growth regulators and nutrients on pollen viability, %

Table15. Effect of foliar application of plant growth regulators and nutrients on plant height, cm

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	99.50
2	T2: Boron (100 ppm)	103.00
3	T3: Calcium chloride (0.6%)	105.00
4	T4: Salicylic acid (50 ppm)	109.00
5	T5: Glycine betaine (20 ppm)	105.75
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	99.50
7	T7: 1-methyl cyclopropane (50ppm)	108.25
8	T8: Gibberllic acid (50ppm)	142.75
9	T9: Water spray	94.75
10	T10: Control	91.00
	SE m ±	1.56
	CD (0.05)	4.53

Table 16. Effect of foliar application of plant growth regulators and nutrients on days to 50% flowering

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	85.75
2	T2: Boron (100 ppm)	85.25
3	T3: Calcium chloride (0.6%)	84.00
4	T4: Salicylic acid (50 ppm)	83.75
5	T5: Glycine betaine (20 ppm)	83.75
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	86.00
7	T7: 1-methyl cyclopropane (50ppm)	83.25
8	T8: Gibberllic acid (50ppm)	84.75
9	T9: Water spray	82.50
10	T10: Control	81.75
	SE m ±	0.32
	CD (0.05)	0.92

Table 17. Effect of foliar application of plant growth regulators and nutrients on productive tillers per plant

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	10.25
2	T2: Boron (100 ppm)	8.75
3	T3: Calcium chloride (0.6%)	8.75
4	T4: Salicylic acid (50 ppm)	8.50
5	T5: Glycine betaine (20 ppm)	8.50
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	9.25
7	T7: 1-methyl cyclopropane (50ppm)	8.25
8	T8: Gibberllic acid (50ppm)	8.00
9	T9: Water spray	7.75
10	T10: Control	7.25
	SE m ±	0.30
	CD (0.05)	0.89

Table 18. Effect of foliar application of plant growth regulators and nutrients on pollen viability, %

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	80.23
2	T2: Boron (100 ppm)	77.67
3	T3: Calcium chloride (0.6%)	73.56
4	T4: Salicylic acid (50 ppm)	61.63
5	T5: Glycine betaine (20 ppm)	77.73
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	78.79
7	T7: 1-methyl cyclopropane (50ppm)	68.95
8	T8: Gibberllic acid (50ppm)	72.81
9	T9: Water spray	51.46
10	T10: Control	37.52
	SE m ±	1.30
	CD (0.05)	3.87

Table 19. Effect of foliar application of plant growth regulators and nutrients on time of anthesis, am

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	11:25
2	T2: Boron (100 ppm)	11:13
3	T3: Calcium chloride (0.6%)	11:33
4	T4: Salicylic acid (50 ppm)	11:26
5	T5: Glycine betaine (20 ppm)	11:42
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	11:22
7	T7: 1-methyl cyclopropane (50ppm)	11:46
8	T8: Gibberllic acid (50ppm)	11:35
9	T9: Water spray	11:44
10	T10: Control	11:54

Table 20. Effect of foliar application of plant growth regulators and nutrients on panicle length, cm

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	20.90
2	T2: Boron (100 ppm)	20.83
3	T3: Calcium chloride (0.6%)	20.05
4	T4: Salicylic acid (50 ppm)	20.33
5	T5: Glycine betaine (20 ppm)	20.70
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	20.38
7	T7: 1-methyl cyclopropane (50ppm)	19.83
8	T8: Gibberllic acid (50ppm)	21.33
9	T9: Water spray	19.03
10	T10: Control	18.98
	SE m ±	0.17
	CD (0.05)	0.50

Table 21. Effect of foliar application of plant growth regulators and nutrients on grain yield per plant, g

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	15.87
2	T2: Boron (100 ppm)	13.84
3	T3: Calcium chloride (0.6%)	12.33
4	T4: Salicylic acid (50 ppm)	9.55
5	T5: Glycine betaine (20 ppm)	12.54
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	13.82
7	T7: 1-methyl cyclopropane (50ppm)	10.08
8	T8: Gibberllic acid (50ppm)	11.21
9	T9: Water spray	6.31
10	T10: Control	4.16
	SE m ±	0.34
	CD (0.05)	0.99

Table 22. Effect of foliar application of plant growth regulators and nutrients on spikelet fertility percentage, %

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	75.40
2	T2: Boron (100 ppm)	70.77
3	T3: Calcium chloride (0.6%)	67.17
4	T4: Salicylic acid (50 ppm)	54.56
5	T5: Glycine betaine (20 ppm)	70.06
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	73.46
7	T7: 1-methyl cyclopropane (50ppm)	56.29
8	T8: Gibberllic acid (50ppm)	63.47
9	T9: Water spray	42.55
10	T10: Control	31.96
	SE m ±	1.04
	CD (0.05)	3.02

4.1.2.9. 1000 grain weight (g)

1000 grain weight recorded in rice under different treatments is presented in table 23. The results showed that significant reduction was observed in 1000 grain weight under high temperature stress condition. The mean 1000 grain weight of treatments was maximum for pink-pigmented facultative methylotrophs-1% (23.34 g), brassinosteroid-50 ppm (23.21 g), glycine betaine-20 ppm (23.04 g) and boron-100 ppm (22.85 g) whereas the minimum value recorded in control plants (17.85 g) under high temperature condition.

Table 23. Effect of foliar application of plant growth regulators and nutrients on 1000 grain weight, g

Sl.No.	Treatments	Mean
1	T1: Brassinosteroid (50 ppm)	23.21
2	T2: Boron (100 ppm)	22.85
3	T3: Calcium chloride (0.6%)	22.31
4	T4: Salicylic acid (50 ppm)	21.44
5	T5: Glycine betaine (20 ppm)	23.04
6	T6: Pink-Pigmented Facultative Methylotrophs (PPFM) (1%)	23.34
7	T7: 1-methyl cyclopropane (50ppm)	21.09
8	T8: Gibberllic acid (50ppm)	21.73
9	T9: Water spray	19.39
10	T10: Control	17.85
	SE m ±	0.20
	CD (0.05)	0.60

4.2 EXPERIMENT 2 - TO STUDY THE EFFECT OF FOLIAR APPLICATION OF METHYL JASMONATE ON EARLY MORNING FLOWERING (EMF) TRAIT ON HIGH TEMPERATURE STRESS MITIGATION IN RICE.

4.2.1. Morphological and yield parameters:

Anthesis time, pollen viability, spikelet fertility percentage, yield per plant and 1000 grain weight were recorded during the experiment.

4.2.1.1. Anthesis time (am)

Flower opening time of rice plants under different treatments shown in table 24. Among the ten treatments, 4mM L^{-1} methyl jasmonate at 7 am (08:11 am) showed early flowering. Flowers of control opened in late hours (11:54 am) with respect to other treatments.

4.2.1.2. Pollen viability (%)

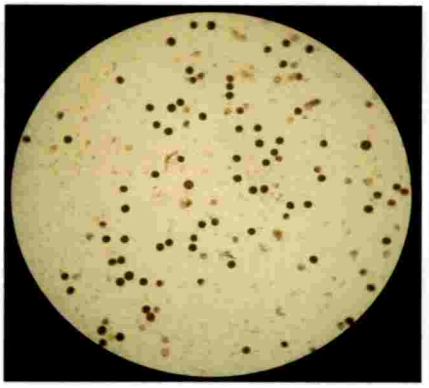
Data related to pollen viability of rice under different treatments is recorded in table 25. 4mM L^{-1} methyl jasmonate at 7 am (61.93 %) and 2mM L^{-1} methyl jasmonate at 7 am (58.64 %) showed higher percent of pollen viability whereas, control (37.52 %) recorded the minimum pollen viability (Plate 13).

4.2.1.3. Spikelet fertility percentage (%)

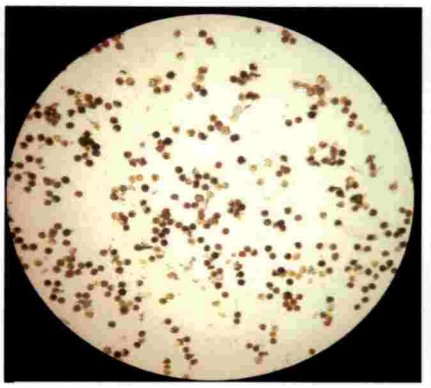
Data recorded for spikelet fertility of rice under different treatments is recorded in table 26. Highest spikelet fertility was recorded in 4mM L^{-1} methyl jasmonate at 7 am (56.07%) whereas, control (33.09%) recorded the minimum spikelet fertility.

4.2.1.4. Yield per plant (g)

Grain yield per plant recorded under different treatments is presented in table 27. The results showed that significant reduction was observed in grain yield per plant under high temperature stress conditions. The mean grain yield per plant



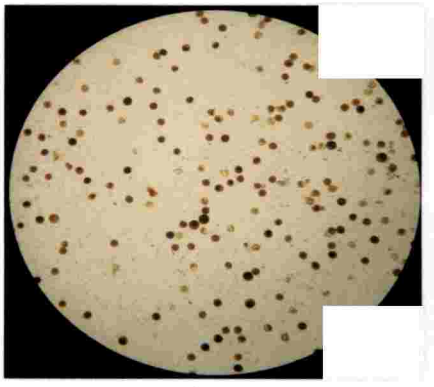
T1: 2mM L^{-1} MeJA at 7 am



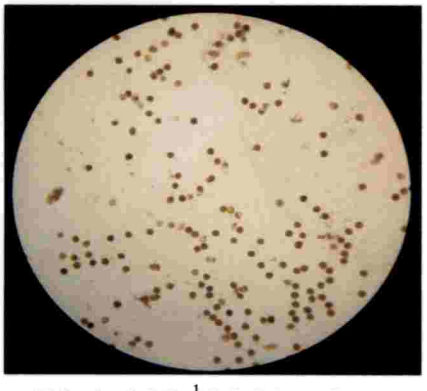
T2: 2mM L^{-1} MeJA at 8 am

T3: 2mM L^{-1} MeJA at 9 am

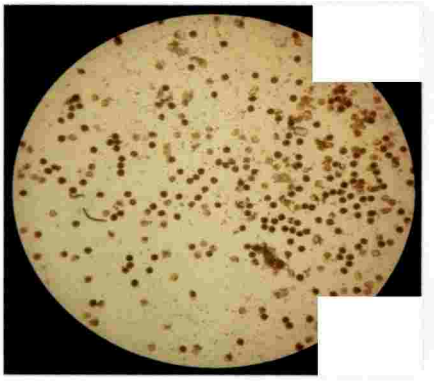
T4: 4mM L^{-1} MeJA at 7 am



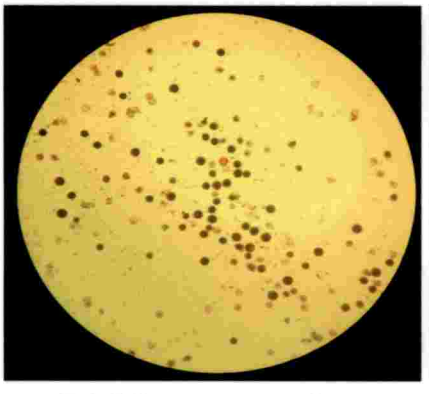
T5: 4mM L^{-1} MeJA at 8 am



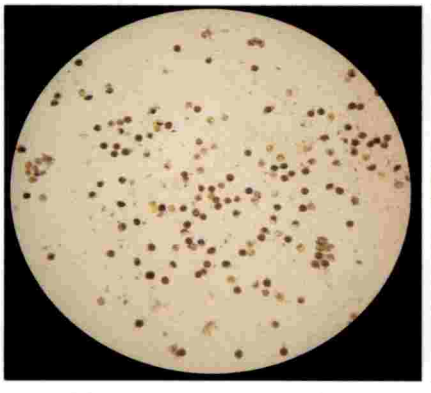
T6: 4mM L^{-1} MeJA at 9 am



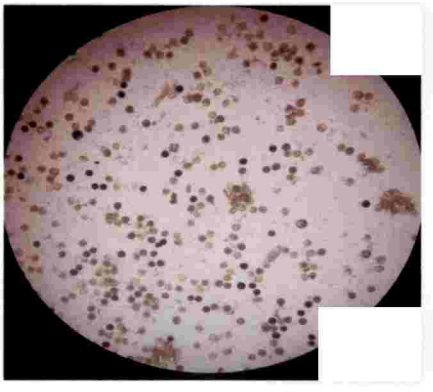
T7: Water spray at 7 am



T8: Water spray at 8 am



T9: Water spray at 9 am



T10: Control

Plate 13. Effect of foliar application of MeJA and water spray on pollen viability, %

of treatments was maximum for 4mM L^{-1} methyl jasmonate at 7 am (8.55 g), 2mM L^{-1} methyl jasmonate at 7 am (8.06 g) and 4mM L^{-1} methyl jasmonate at 8 am (7.89 g) whereas, the minimum value recorded in control plants (4.22 g) under high temperature stress.

4.2.1.5. 1000 grain weight (g)

1000 grain weight recorded in rice under different treatments is presented in table 28. The results showed that significant reduction was observed in 1000 grain weight under high temperature stress condition. The mean 1000 grain weight of treatments was maximum for 4mM L^{-1} methyl jasmonate at 7 am (21.33 g), 2mM L^{-1} methyl jasmonate at 7 am (21.03 g), 2mM L^{-1} methyl jasmonate at 8 am (20.86 g) and 4mM L^{-1} methyl jasmonate at 8 am (20.83 g) whereas, the minimum value recorded in water spray at 7 am (18.26 g), water spray at 8 am (18.21 g), water spray at 9 am (18.19 g) and control plants (17.85 g) under high temperature condition.

Table 24. Effect of foliar application of methyl jasmonate and water spray on time of anthesis, am

Sl.No.	Treatments	Mean
1	T1: 2mM L^{-1} Methyl jasmonate at 7 am	08:26
2	T2: 2mM L^{-1} Methyl jasmonate at 8 am	09:18
3	T3: 2mM L^{-1} Methyl jasmonate at 9 am	10:30
4	T4: 4mM L^{-1} Methyl jasmonate at 7 am	08:11
5	T5: 4mM L^{-1} Methyl jasmonate at 8 am	09:04
6	T6: 4mM L^{-1} Methyl jasmonate at 9 am	10:18
7	T7: Water spray at 7 am	11:29
8	T8: Water spray at 8 am	11:30
9	T9: Water spray at 9 am	11:40
10	T10: Control	11:54

Table 25. Effect of foliar application of methyl jasmonate and water spray on pollen viability, %

Sl.No.	Treatments	Mean
1	T1: 2mM L^{-1} Methyl jasmonate at 7 am	58.64
2	T2: 2mM L^{-1} Methyl jasmonate at 8 am	55.05
3	T3: 2mM L^{-1} Methyl jasmonate at 9 am	51.55
4	T4: 4mM L^{-1} Methyl jasmonate at 7 am	61.93
5	T5: 4mM L^{-1} Methyl jasmonate at 8 am	58.33
6	T6: 4mM L^{-1} Methyl jasmonate at 9 am	52.87
7	T7: Water spray at 7 am	45.89
8	T8: Water spray at 8 am	45.38
9	T9: Water spray at 9 am	42.10
10	T10: Control	37.52
	SE m ±	1.02
	CD (0.05)	3.05

Table 26. Effect of foliar application of methyl jasmonate and water spray on spikelet fertility percentage, %

Sl.No.	Treatments	Mean
1	T1: 2mM L^{-1} Methyl jasmonate at 7 am	52.10
2	T2: 2mM L^{-1} Methyl jasmonate at 8 am	49.78
3	T3: 2mM L^{-1} Methyl jasmonate at 9 am	47.60
4	T4: 4mM L^{-1} Methyl jasmonate at 7 am	56.07
5	T5: 4mM L^{-1} Methyl jasmonate at 8 am	49.38
6	T6: 4mM L^{-1} Methyl jasmonate at 9 am	48.66
7	T7: Water spray at 7 am	44.01
8	T8: Water spray at 8 am	39.78
9	T9: Water spray at 9 am	36.88
10	T10: Control	33.09
	SE m ±	1.31
	CD (0.05)	3.89

Table 27. Effect of foliar application of methyl jasmonate and water spray on grain yield per plant, g

Sl.No.	Treatments	Mean
1	T1: 2mM L^{-1} Methyl jasmonate at 7 am	8.06
2	T2: 2mM L^{-1} Methyl jasmonate at 8 am	7.57
3	T3: 2mM L^{-1} Methyl jasmonate at 9 am	7.16
4	T4: 4mM L^{-1} Methyl jasmonate at 7 am	8.55
5	T5: 4mM L^{-1} Methyl jasmonate at 8 am	7.89
6	T6: 4mM L^{-1} Methyl jasmonate at 9 am	7.57
7	T7: Water spray at 7 am	6.01
8	T8: Water spray at 8 am	5.42
9	T9: Water spray at 9 am	5.01
10	T10: Control	4.22
	SE m ±	0.27
	CD (0.05)	0.82

Table 28. Effect of foliar application of methyl jasmonate and water spray on 1000 grain weight, g

Sl.No.	Treatments	Mean
1	T1: 2mM L^{-1} Methyl jasmonate at 7 am	21.03
2	T2: 2mM L^{-1} Methyl jasmonate at 8 am	20.86
3	T3: 2mM L^{-1} Methyl jasmonate at 9 am	20.50
4	T4: 4mM L^{-1} Methyl jasmonate at 7 am	21.33
5	T5: 4mM L^{-1} Methyl jasmonate at 8 am	20.83
6	T6: 4mM L^{-1} Methyl jasmonate at 9 am	20.50
7	T7: Water spray at 7 am	18.26
8	T8: Water spray at 8 am	18.21
9	T9: Water spray at 9 am	18.19
10	T10: Control	17.85
	SE m ±	0.17
	CD (0.05)	0.52

Discussion

5. DISCUSSION

Rice is consumed around three billion people and is the main staple food for multitude of people on earth (Krishnan *et al.,* 2011). More than two billion peoples in Asia receive 60-70 per cent of their food energy from rice (Changchui, 2003). Climate change is mainly expected to have a negative effect on rice production and productivity. Among abiotic factors, high temperature drastically reduces the rice production. It is also estimated that, for every 1°C elevated in temperature, there will be about 10% reduction in the rice grain yield (Peng *et al.,* 2004).

Plant growth regulators have been shown to perform an important function in the tolerance of abiotic and biotic stress by the regulation of signaling networks and the developmental processes in plants (Khan *et al.,* 2012). In practical purpose, they are also called as either natural or synthetic compounds or hormones that are applied directly to the plants which alters its life processes in some beneficial way like increase in yield, improve its quality and facilitate to withstand under stress condition. The application of plant growth regulators and nutrients in agriculture helps to overcome the limitations in productivity and environmental constraints.

The main goal of the present study is to focus on the impact of plant growth regulators and nutrients through physiological mechanisms to improve the productivity of rice under high temperature condition. The results obtained are discussed in this chapter to evaluate the factors that contribute for higher productivity in rice as influenced by the exogenous application of plant growth regulators and nutrients under high temperature condition.

5.1. EFFECT OF FOLIAR APPLICATION OF PLANT GROWTH REGULATORS AND NUTRIENTS ON HIGH TEMPERATURE STRESS MITIGATION IN RICE ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS

Scenarios of high temperature stress will negatively impact on physiological functions during sensitive periods. Biological membrane integrity and functions are highly vulnerable to high temperature, as tertiary and quaternary membrane protein structures are highly susceptible to changes in the environment, especially in high temperature stress. In the current research, the cell membrane stability of rice under elevated temperature stress, control was reduced and improved significantly by exogenous application of plant growth regulators and nutrients as shown in the figure 1. Brassinosteroid-50 ppm recorded the highest cell membrane stability index (41.57%) and on par with glycine betaine-20 ppm (34.31%). These results are in line with findings of Cao and Zhao, (2008). They reported that brassinosteroid treated plants showed increase in stability as compared to the plants which were not treated at seedling stage. 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. Falcone *et al.* (2004) mentioned that since PSI and PSII are integral thylakoid membrane protein complexes, any changes in membrane fluidity will adversely affect PSI and PSII ultimately Fv/Fm complexes and it is associated with the membrane integrity.

High temperature affects chloroplast stroma and thylakoid membrane (Wang *et al.*, 2010). In the present study variation in exogenous application of plant growth regulators and nutrients on chlorophyll stability index of rice under high temperature stress conditions were presented in figure 2. Stability of

chlorophyll membrane was reduced significantly under high temperature stress and the highest reduction observed under without treatment (control) condition. Stability of treated plants were higher than control plants. Highest increased in chlorophyll stability index recorded in brassinosteroid-50 ppm was 9.32% compare to control. Brassinosteroid increases the chlorophyll content by reducing the activity of chlorophyllase and hence brassinosteroid treated plants increase the chlorophyll stability index of rice under high temperature stress. 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 in chlorophyll content and chlorophyll stability index in wheat under elevated heat stress (Sairam *et al.,* 1997). Chlorophyll content was decreases due to enhanced activity of chlorophyllase under elevated temperatures (Todorov *et al.*, 2003).

Leaf temperature considerably increased under elevated temperature stress and subsequently increased chlorophyll breakdown leading to reduced photosynthetic rate (Nainanayake and Bandara, 1998). In the present study, minimum leaf temperature recorded in calcium chloride-0.6% (30.32°C) compare to control (31.20°C) were showed in figure 3. Calcium acts as a secondary messenger and helps in reduced leaf temperature for metabolic homeostasis. Similar results were obtained by Attia *et al.* (2014) they reported that calcium treated showed reduced in leaf temperature as compared to the plants which were not treated under stress condition in *Vitis vinifera.*

The reduction in photosynthetic rate was mainly due to various factors under stress conditions. Lea and Leegood, (1999) revealed that under elevated temperature stress, oxygen solubility is reduced to a lesser extent than CO_2, leading in enhanced photorespiration and reduced photosynthesis. High temperature is able to decrease the photosynthetic rate at mid-ripening by 40-60 per cent, leading to rapid flag leaf senescence (Oh-e *et al.,* 2009). In the present

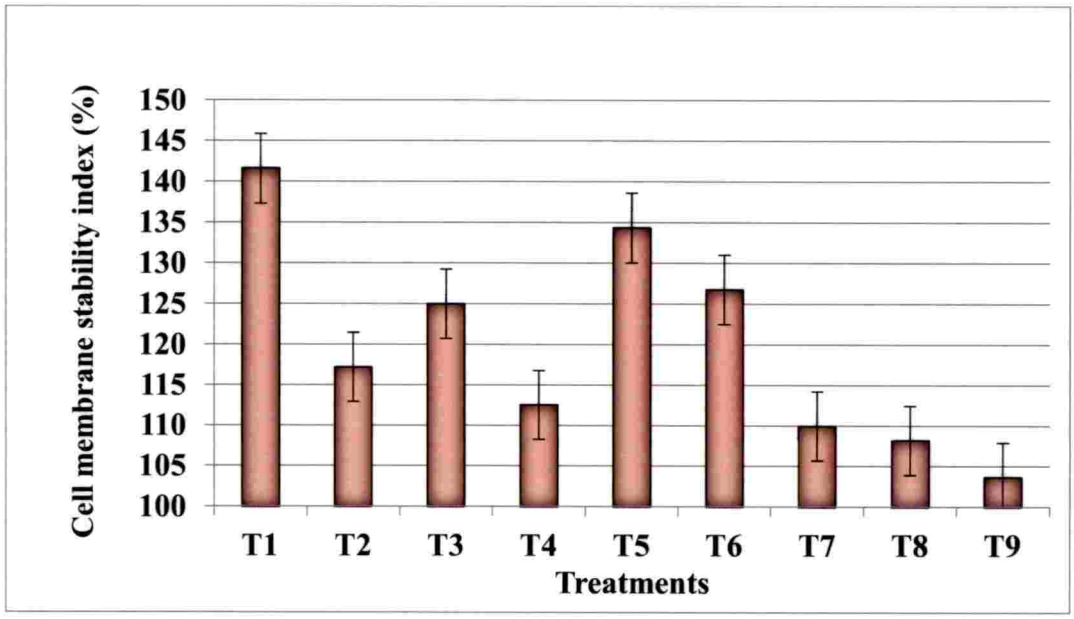


Figure 1. Variation in cell membrane stability index (%) of different treatments under high temperature stress conditions.

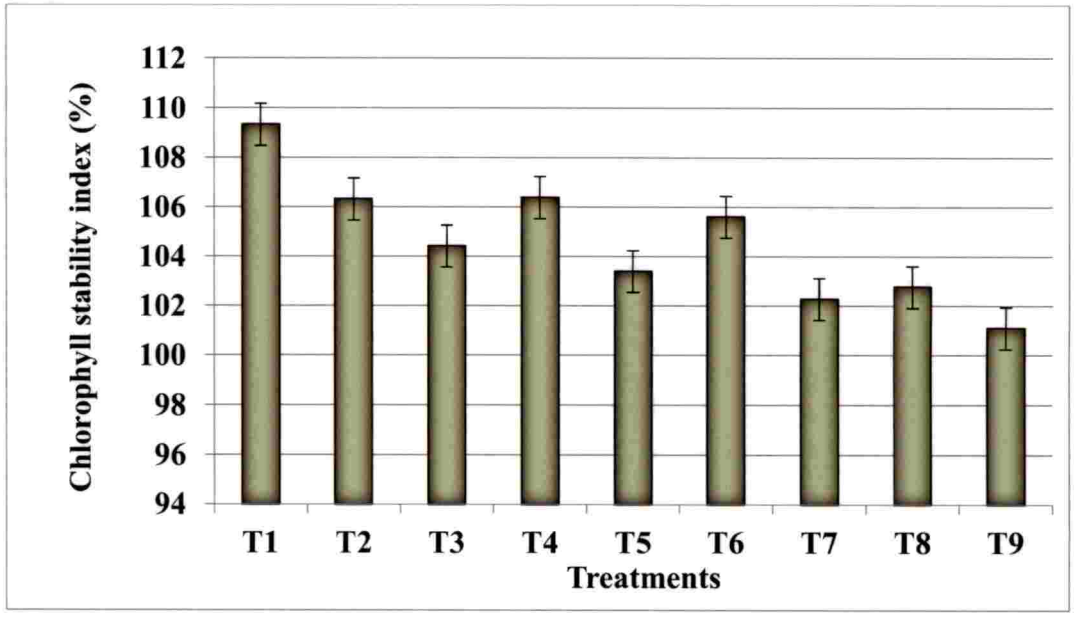


Figure 2. Variation in chlorophyll stability index (%) of different treatments under high temperature stress conditions.

Fig 1 and 2. T1: Brassinosteroid (50 ppm); **T2:** Boron (100 ppm); **T3:** Calcium chloride (0.6%); **T4:** Salicylic acid (50 ppm); **T5:** Glycine betaine (20 ppm); **T6:** Pink-Pigmented Facultative Methylotrophs (PPFM) (1%); **T7:**1-methyl cyclopropane (50 ppm); **T8:**Gibberllic acid (50 ppm); **T9:** Water spray.

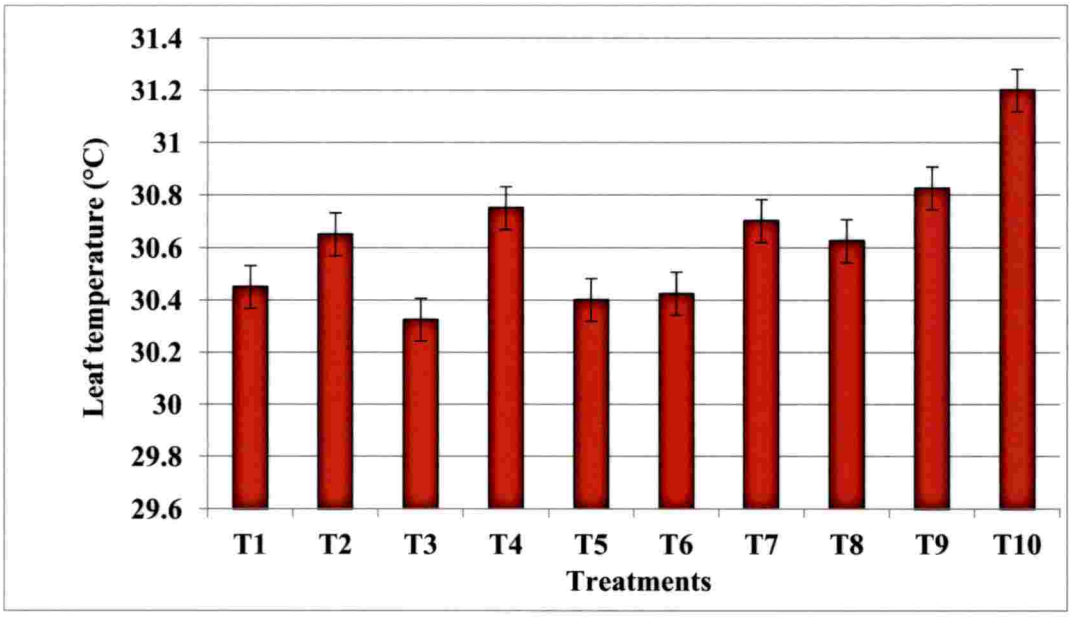


Figure 3. Variation in leaf temperature (°C) of different treatments under high temperature stress conditions.

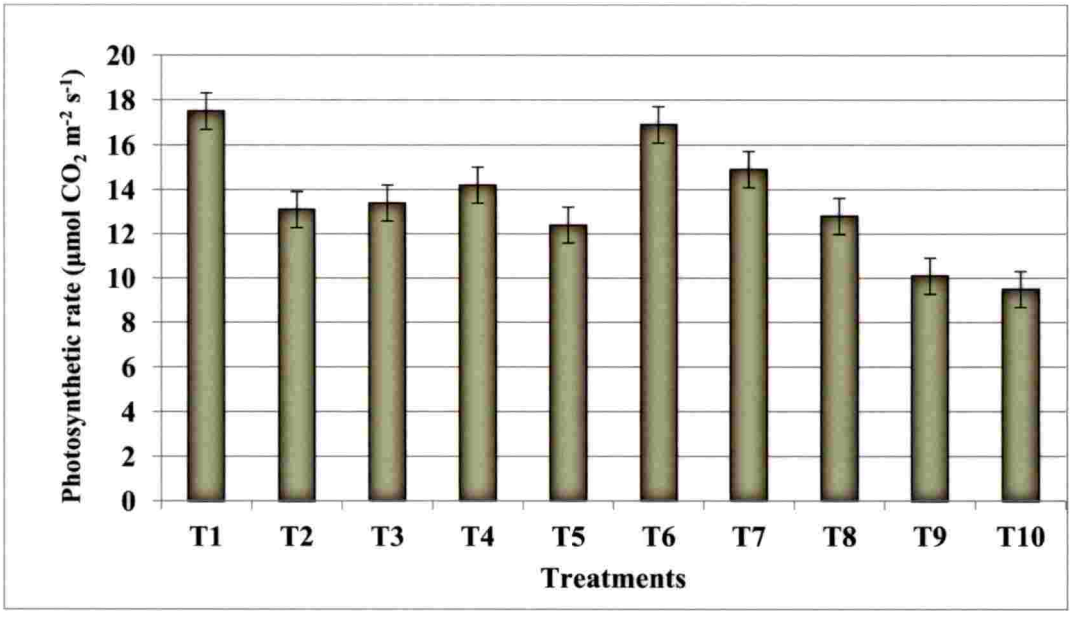


Figure 4. Variation in photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) of different treatments under high temperature stress conditions.

Fig 3 and 4. T1: Brassinosteroid (50 ppm); **T2:** Boron (100 ppm); **T3:** Calcium chloride (0.6%); **T4:** Salicylic acid (50 ppm); **T5:** Glycine betaine (20 ppm); **T6:** Pink-Pigmented Facultative Methylotrophs (PPFM) (1%); **T7:**1-methyl cyclopropane (50 ppm); **T8:**Gibberllic acid (50 ppm); **T9:** Water spray; **T10:** Control.

study, highest increased in photosynthetic rate recorded in brassinosteroid-50 ppm (17.50 μmol CO_2 m^{-2} s^{-1}) and pink-pigmented facultative methylotrophs-1% (16.90 μmol CO_2 m^{-2} s^{-1}) compare to water spray (10.10 μmol CO_2 m^{-2} s^{-1}) and control (9.50 μmol CO_2 m^{-2} s^{-1}) were showed in figure 4. These findings are in line up with the findings of Thussagunpanit *et al.* (2012) they reported that brassinosteroid treated plants showed increase in photosynthetic rate 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 stomatal conductance is the initial cause of reduced in photosynthesis. In the present study, highest increased in stomatal conductance recorded in brassinosteroid-50 ppm (583.7 mmol H_2O m^{-2} s^{-1}) compare to control (318.1 mmol H_2O m^{-2} s^{-1}) were showed in figure 5. These findings are in line up with the findings of Thussagunpanit *et al.* (2012) they 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.

Glycine betaine application helps to mitigate the harmful effects of stress on grain biomass. Glycine betaine's repairing impact can be attributed to the fact that it decreases the transpiration rate from leaves (Aldesuquy *et al.,* 2012), which could be potentially lead to the excess water accumulation, leading in an increase in fresh grain mass. Plant retains water status under elevated temperature conditions is one of the most significant functions. Plants mainly try to balance

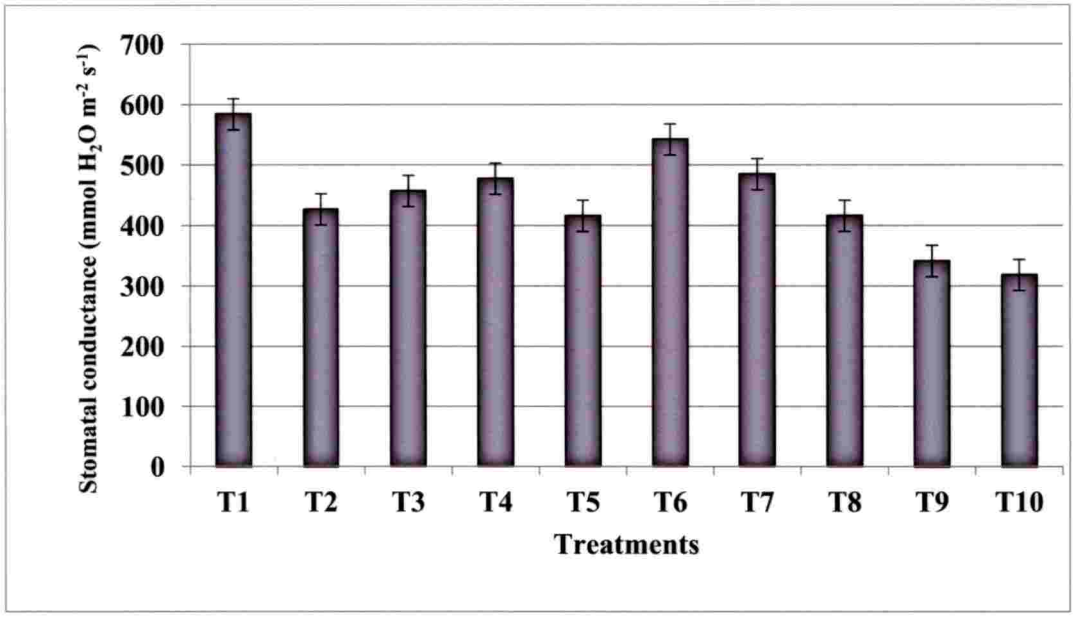


Figure 5. Variation in stomatal conductance (mmol H_2O m^{-2} s^{-1}) of different treatments under high temperature stress conditions.

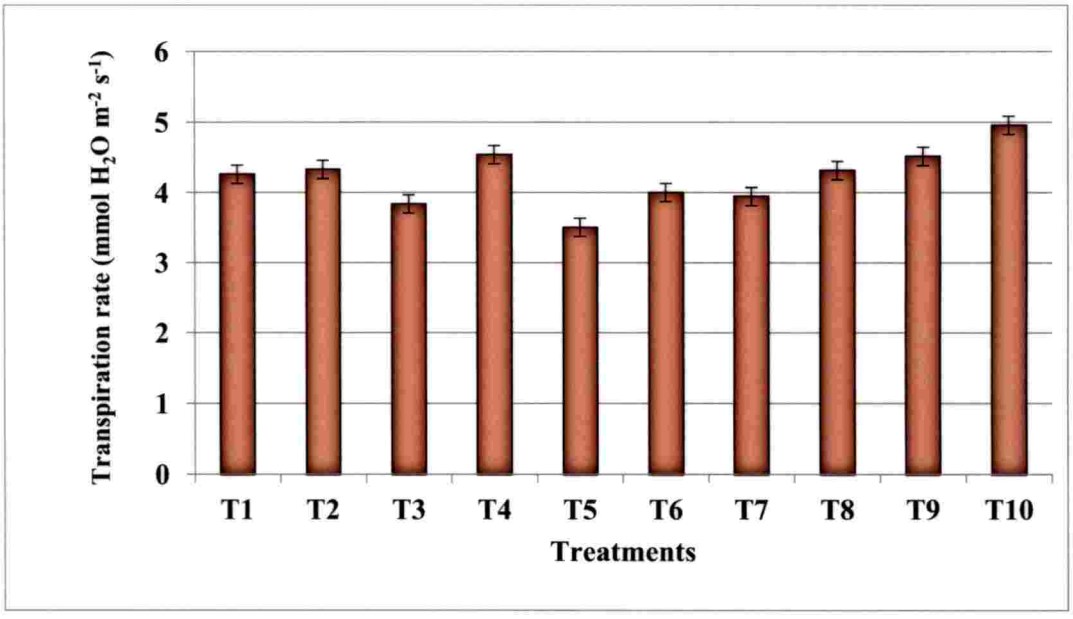


Figure 6. Variation in transpiration rate (mmol H_2O m^{-2} s^{-1}) of different treatments under high temperature stress conditions.

Fig 5 and 6. T1: Brassinosteroid (50 ppm); **T2:** Boron (100 ppm); **T3:** Calcium chloride (0.6%); **T4:** Salicylic acid (50 ppm); **T5:** Glycine betaine (20 ppm); **T6:** Pink-Pigmented Facultative Methylotrophs (PPFM) (1%); **T7:**1-methyl cyclopropane (50 ppm); **T8:**Gibberllic acid (50 ppm); **T9:** Water spray; **T10:** Control.

their water status of tissue under different temperature regimes (Abo-Hamed *et al.,* 1990). In the present study, reduced in transpiration rate recorded in glycine betaine-20 ppm (3.51 mmol H_2O m^{-2} s^{-1}) compare to control (4.95 mmol H_2O m^{-2} s^{-1}) were showed in figure 6. Similar results were obtained by Aldesuquy *et al.* (2012) they reported that glycine betaine helps to maintain tissue water level by reduced the transpiration rate. Osmoprotectants accumulation is a major adaptive mechanism in plants subjected to high temperature, as primary metabolites directly participate in the osmotic adjustment (Sakamoto and Murata, 2000). For instance, accumulation of glycine betaine, proline and soluble sugars is necessary to maintain osmotic activities and it protects the cellular structures from increased temperatures by regulating the water balance of cell, membrane stability, and buffering the redox potential of cell (Farooq *et al.,* 2008). Glycine betaine plays a major role as compatible solute in plants subjected to high temperature conditions (Sakamoto and Murata, 2002). Production of glycine betaine in chloroplasts which maintains the Rubisco activation by sequestering Rubisco activase near thylakoids and thereby preventing its thermal injury (Allakhverdiev *et al.,* 2008).

When leaves were subjected to high temperature, chlorophyll a degraded faster than chlorophyll b. Chlorophyll b is found only in the pigment antenna, but chlorophyll a is present both in the pigment antenna and photosystems I and II reaction center. The chlorophyll a/b ratio is the most important finding for light adaptation of the photosynthetic apparatus and the functional pigment equipment (Lichtenthaler *et al.,* 1981). When reduces in chlorophyll a/b ratio, it may be described as an enlargement of the antenna system of PS II (Yuzbasioglu *et al.,* 2017). In the present study, highest increased in chlorophyll a/b ratio recorded in brassinosteroid-50 ppm (1.28) and on par with pink-pigmented facultative methylotrophs-1% (1.24) compare to control (1.06) were shown in figure 7. These findings are in line up with the findings of Wu *et al.* (2014) and Niu *et al.* (2016) they reported that brassinosteroid treated plants showed increase in chlorophyll

a/b ratio as compared to the control plants under high temperature stress condition by reducing the chlorophyll a degradation in the pigment antenna and the photosystems I and II reaction centers in eggplant and *Leymus chinensis* respectively.

Heat stress decreases the accumulation of carbohydrate by altering assimilates partitioning and changing the balance between apoplastic and symplastic loading of phloem (Taiz and Zeiger, 2002). High temperature stress reduces the synthase of sucrose and several cell wall and vacuolar invertases; as a result, the turnover of sucrose and starch is interrupted and therefore soluble carbohydrates accumulate at low concentrations (Sato *et al.,* 2006). In tomatoes, reducing sink and source strength even at moderately high temperature results in decreased in carbohydrates available at critical phases of plant development, resulting in decreased fruit setting and other yield-related parameters (Sato *et al.,* 2006). In the present study, highest increased in carbohydrate content recorded in boron-100 ppm (39.58 mg g^{-1}), pink-pigmented facultative methylotrophs-1% (37.16 mg g^{-1}), glycine betaine-20 ppm (36.78 mg g^{-1}), salicylic acid-50 ppm (35.29 mg g^{-1}) and brassinosteroid-50 ppm (33.61 mg g^{-1}) compare to control was (26.24 mg g^{-1}) were shown in figure 8. These findings are in line up with the findings of Sisler *et al.* (1956) they reported that boron helps in the translocation of carbohydrate and it enhances the carbohydrate content. Boron is vital for many plant activities such as keeping a equilibrium between sugar and starch, sugar and carbohydrate translocation, pollination and seed reproduction, cell division, nitrogen metabolism and protein formation, and the formation of cell walls. It plays significant role in the normal functioning of cell membranes and the transport of potassium ion to guard cells for the internal water balance control system (Blaser-Grill *et al.,* 1989, Blevins and Lukaszewski 1998, Camacho-Cristobal *et al.,* 2005, Camacho-Cristobal and Gonzalez-Fontes, 2007, Goldbach *et al.*, 2001, Pilbeam and Kirkby 1983 and Yu *et al.,* 2003). Studies suggest that high carbohydrate availability (e.g., glucose and sucrose) during high temperature

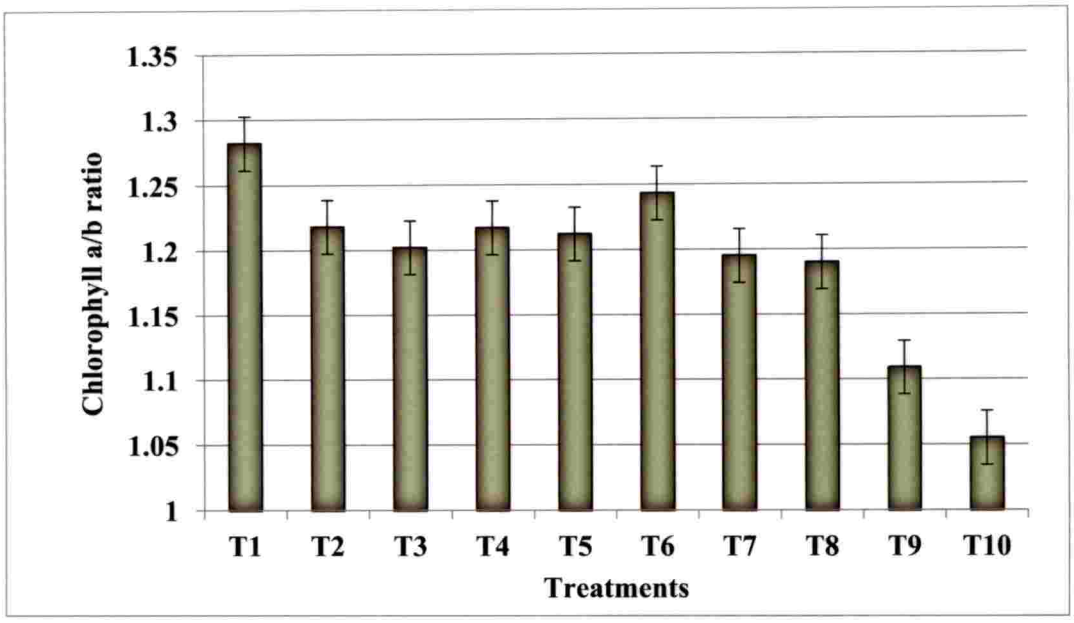


Figure 7. Variation in chlorophyll a/b ratio of different treatments under high temperature stress conditions.

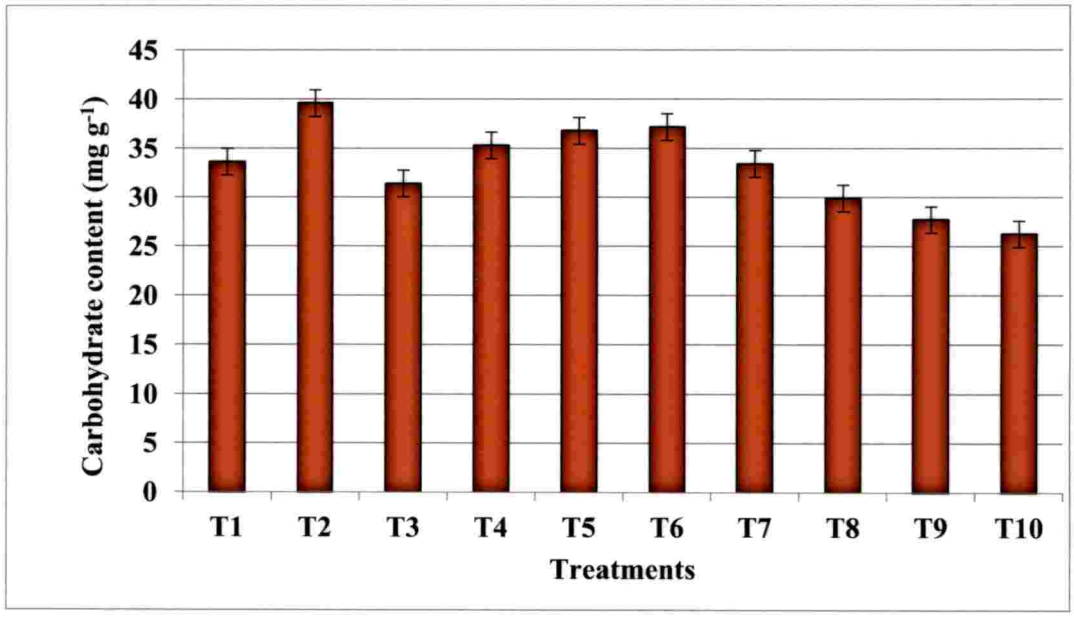


Figure 8. Variation in carbohydrate content (mg g^{-1}) of different treatments under high temperature stress conditions.

Fig 7 and 8. T1: Brassinosteroid (50 ppm); **T2:** Boron (100 ppm); **T3:** Calcium chloride (0.6%); **T4:** Salicylic acid (50 ppm); **T5:** Glycine betaine (20 ppm); **T6:** Pink-Pigmented Facultative Methylotrophs (PPFM) (1%); **T7:**1-methyl cyclopropane (50 ppm); **T8:**Gibberllic acid (50 ppm); **T9:** Water spray; **T10:** Control.

stress represents an important physiological character associated with temperature stress tolerance (Liu and Huang, 2000). Sucrose is the main photosynthesis end product that translocates through the phloem from source leaves to sink organs. Sucrose and its cleavage products control the growth and response of plants to stresses by allocating carbon and sugar signaling (Roitsch and Gonzalez, 2004). Likewise, the carbohydrate content of developing and mature pollen grains can be an important factor in determining the quality of pollen, as heat-tolerant tomato varieties appear to have a mechanism for maintaining the appropriate carbohydrate content under high temperature stress (Firon *et al.,* 2006). Furthermore, sugars have been shown to also act as antioxidants in plants (Lang-Mladek *et al.,* 2010). At low concentrations of sucrose which acts as signaling molecule while it has becomes a ROS scavenger in high concentrations (Sugio *et al.,* 2009).

Lipid peroxidation levels were evaluated by MDA content, the final product of lipid peroxidation. H_2O_2 is generated as a by-product of fatty acid oxidation during lipid catabolism. MDA and H_2O_2 play a major role in the metabolism of plant tissue, but any excess production of MDA and H_2O_2 is harmful to the membranes. H_2O_2 and MDA enhanced significantly, combined with activities of antioxidants enzymes indicating that effectiveness of scavenging O_2^- and H_2O_2 reduced along with reduction in the activities of antioxidants enzymes, which leads to damage in cellular membrane during heat stress (Shen *et al.,* 2016). In the present study, highest reduced in MDA recorded in calcium chloride-0.6% (1.03 $\mu g\ g^{-1}$), glycine betaine-20 ppm (1.14 $\mu g\ g^{-1}$) and brassinosteroid-50 ppm (1.15 $\mu g\ g^{-1}$) compare to control (2.36 $\mu g\ g^{-1}$) were showed in figure 9. These findings are in line up with the findings of Shen *et al.* (2016) they reported that calcium chloride treated plants showed reduced in MDA content as compared to the control plants under high temperature stress condition in rhododendron. Antioxidant enzymes activities were decreased when exposed to high temperature stress; moreover calcium pre-treatment significantly reduced the

inhibition and hence concluded that calcium homeostasis plays an key role in *Lonicera japonica* when subjected to high temperature, and calcium pre-treatment can boost up its thermo-tolerance by decreased the MDA content (Wang *et al.,* 2006).

Changes in the environmental condition and varies in developmental process in plants which results in oxidative stress and it mainly accumulates ROS. This will resulted in disruption of enzymes, membrane proteins and homeostasis of cellular components, finally it enhance the fluidity of the membrane. So in order to detoxify the ROS in the plant cells are done by both non-enzymatic and enzymatic antioxidants. Zhao *et al.* (2017) and Karuppanapandian *et al.* (2011) also reported reduction in SOD activity under high temperature. Cao *et al.* (2009) explained that increased in antioxidant activity in plants could be one of the mechanisms for stress tolerance in rice. In the present study, SOD was increased in brassinosteroid–50 ppm (0.33 g^{-1} min^{-1}), 1-methyl cyclopropane-50 ppm (0.31 g^{-1} min^{-1}), salicylic acid-50 ppm was (0.30 g^{-1} min^{-1}) and pink-pigmented facultative methylotrophs-1% (0.30 g^{-1} min^{-1}) and the considerable decrease was observed under water spray (0.16 g^{-1} min^{-1}) and control (0.15 $g^{-1}min^{-1}$) under high temperature stress were showed in figure 10. These findings are in line up with the findings of Cao and Zhao, (2008) 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 ROS.

Photosynthesis which converts light energy into chemical energy for plant 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). In *Populus euphratica*, high temperature stress causes a reduced

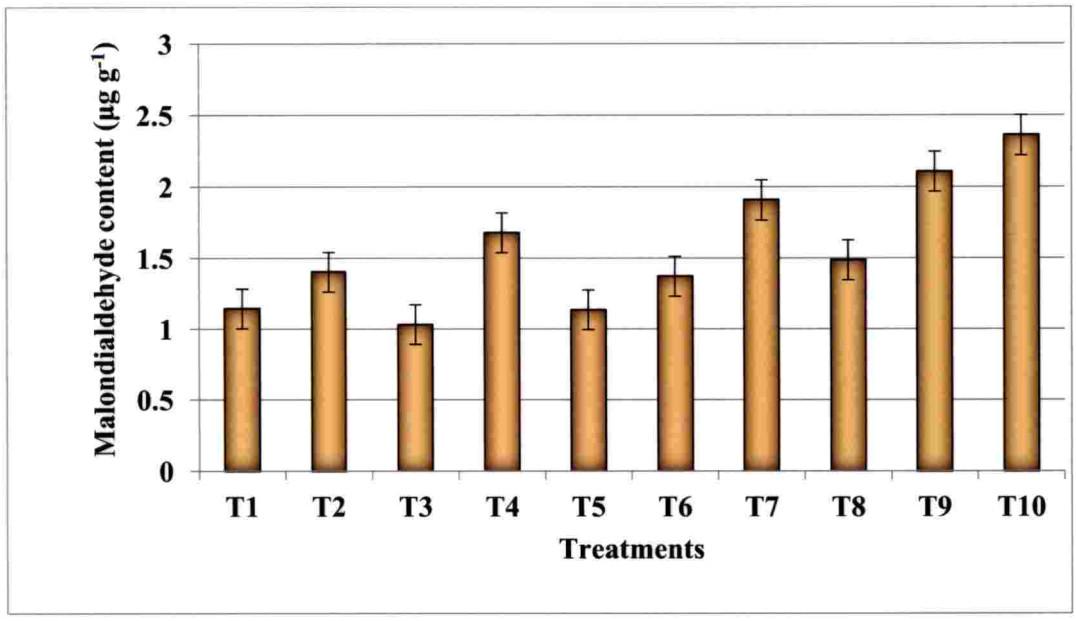


Figure 9. Variation in malondialdehyde content (µg g^{-1}) of different treatments under high temperature stress conditions.

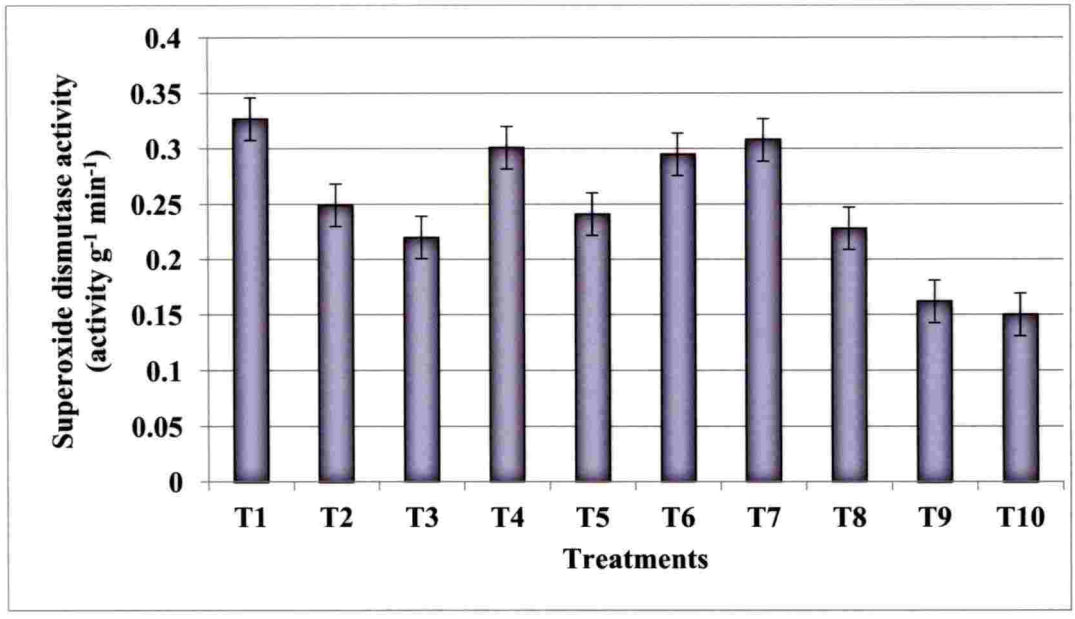


Figure 10. Variation in superoxide dismutase activity (activity g^{-1} min^{-1}) of different treatments under high temperature stress conditions.

Fig 9 and 10. T1: Brassinosteroid (50 ppm); **T2:** Boron (100 ppm); **T3:** Calcium chloride (0.6%); **T4:** Salicylic acid (50 ppm); **T5:** Glycine betaine (20 ppm); **T6:** Pink-Pigmented Facultative Methylotrophs (PPFM) (1%); **T7:**1-methyl cyclopropane (50 ppm); **T8:**Gibberllic acid (50 ppm); **T9:** Water spray; **T10:** Control.

in Photosystem II abundance and an increase of Photosystem I; it also enhance photosynthetic linear electron flow (Ferreira *et al.,* 2006). Sharkey, (2005) reported that mild heat stress also reduces the Rubisco activities. The side reaction of Rubisco oxygenase promotes H_2O_2, which can be toxic to plant cells. Photosystem II photochemistry (Fv / Fm ratio) study can identify environmental stress impacts and provide insight into a plant ability to tolerate environmental stress (Maxwell and Johnson, 2000). 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 temperature, which suggesting that the photosystem might be inhibited by high temperature stress. In the present study, photochemistry (Fv/Fm ratio) was increased in brassinosteroid-50ppm (0.74) and the considerable decrease was observed in control (0.67) were showed in figure 11. These findings are in line up with the findings of Wu *et al.* (2014) 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.

5.2. EFFECT OF FOLIAR APPLICATION OF PLANT GROWTH REGULATORS AND NUTRIENTS ON HIGH TEMPERATURE STRESS MITIGATION IN RICE ON MORPHOLOGICAL AND YIELD PARAMETERS

Heat stress directly affects the stem growth and plant height. Mitra and Bhatia, (2008) observed that plant height, tillers number and total biomass were decreased in rice cultivar when subjected to high temperature stress. In the present study, an overall plant height reduction observed, water spray recorded the plant height of 94.75 cm, control was 91.00 cm and gibberllic acid-50 ppm treated plants showed significant increase in plant height of 142.75 cm (figure 12). Similar results were reported by Lo *et al.* (2008) in rice, where plant height was found to increase in gibberllic acid as compared to control. High temperature stress has significant impacts on process of cell division. These kinds of injury can significantly limit the plant growth and also it impacts on oxidative damage (Prasad *et al.*, 2006). Gibberllic acid treated plants showed an increase in plant height is mainly due to the vegetative growth promotion through active division of cell, enlargement of cell and elongation of cell.

Flowering time and duration has a key role in determining crop duration and grain yield under high temperature. Delay in days to 50% flowering which helps to accumulation of photosynthates for further reproductive phases under high temperature condition (Ritchie and Nesmith, 1991). In the present study, days to 50% flowering was advanced in plants exposed to high temperature condition (figure 13). Days to 50% flowering was increased in pink-pigmented facultative methylotrophs-1% (86 days), brassinosteroid–50 ppm (85.75 days) and boron-100 ppm (85.25 days) and the considerable decrease was observed in control (81.75 days) and water spray (82.50 days) under high temperature stress. These findings are in line up with the findings of Madhaiyan *et al.* (2004) where day to 50% flowering was increased in pink-pigmented facultative methylotrophs might be due

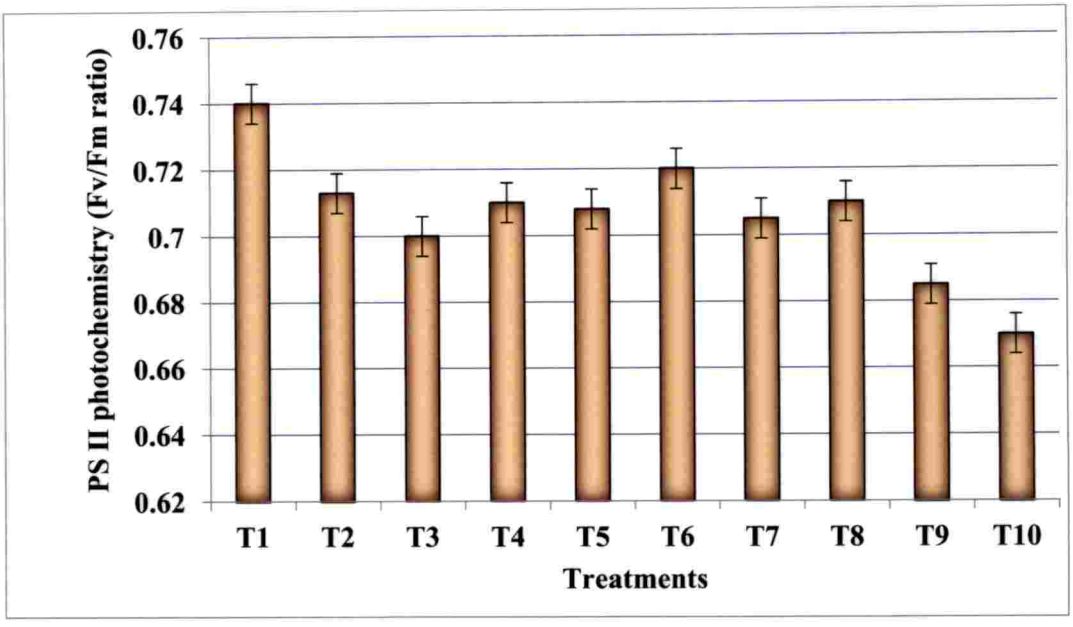


Figure 11. Variation in PS II photochemistry (Fv/Fm ratio) of different treatments under high temperature stress conditions.

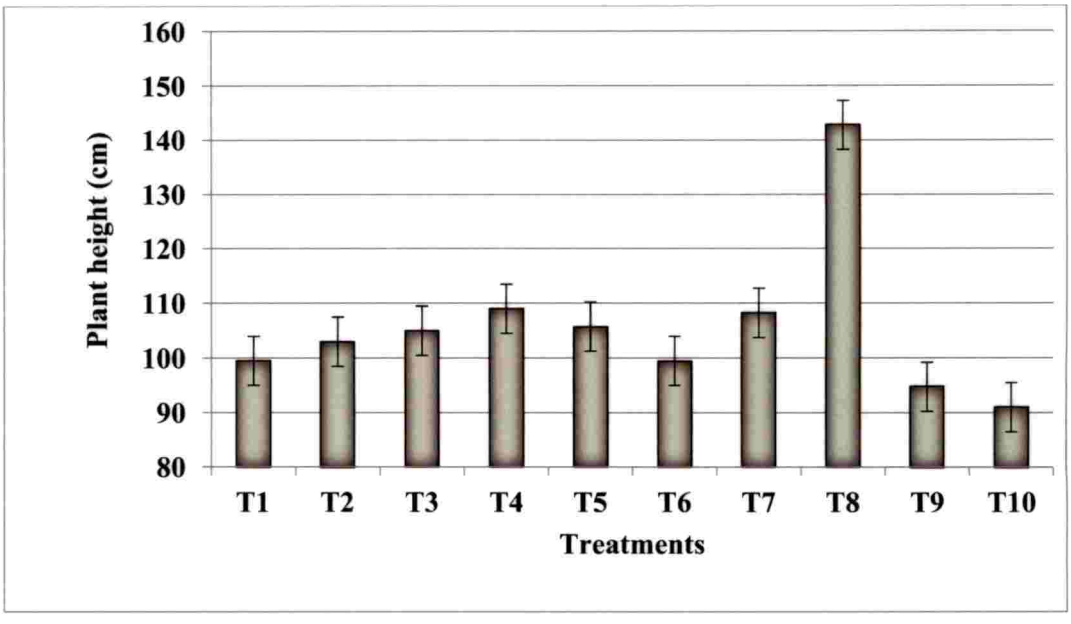


Figure 12. Variation in plant height (cm) of different treatments under high temperature stress conditions.

Fig 11 and 12. T1: Brassinosteroid(50 ppm); **T2:** Boron(100 ppm); **T3:** Calcium chloride (0.6%); **T4:** Salicylic acid (50 ppm); **T5:** Glycine betaine (20 ppm); **T6:** Pink-Pigmented Facultative Methylotrophs (PPFM) (1%); **T7:**1-methyl cyclopropane (50 ppm); **T8:**Gibberllic acid (50 ppm); **T9:** Water spray; **T10:** Control.

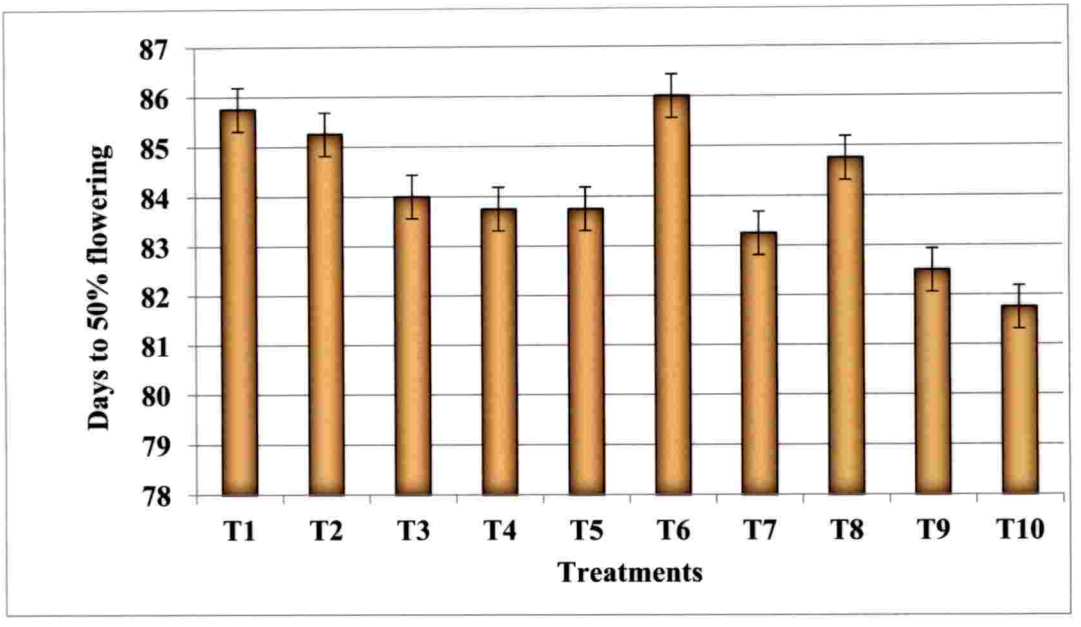


Figure 13. Variation in days to 50% flowering of different treatments under high temperature stress conditions.

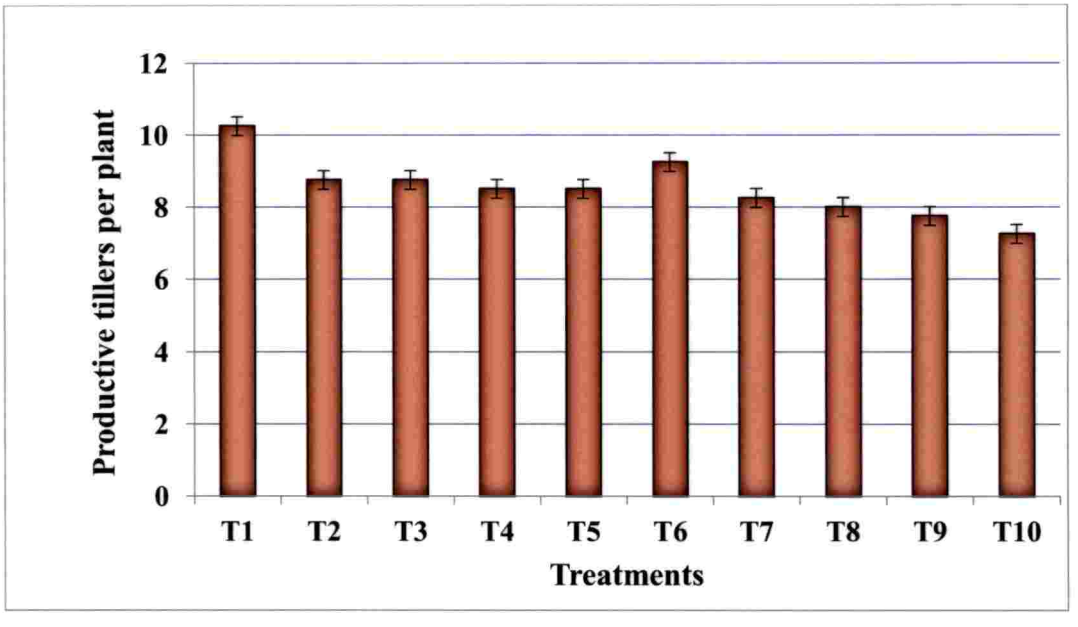


Figure 14. Variation in productive tillers per plant of different treatments under high temperature stress conditions.

Fig 13 and 14. T1: Brassinosteroid(50 ppm); **T2:** Boron(100 ppm); **T3:** Calcium chloride (0.6%); **T4:** Salicylic acid (50 ppm); **T5:** Glycine betaine (20 ppm); **T6:** Pink-Pigmented Facultative Methylotrophs (PPFM) (1%); **T7:**1-methyl cyclopropane (50 ppm); **T8:**Gibberllic acid (50 ppm); **T9:** Water spray; **T10:** Control.

to supply of cytokinin. Cytokinin helps in the promotion of cell division, delay of senescence, translocation of assimilates and thereby delay in days to 50% flowering which helps to accumulation of photosynthates for further reproductive phases.

The increase in yield was mainly due to increase in number of productive tillers per plant. Productive tillers number was decreased under high temperature stress and it was also reported in the study conducted by Mitra and Bhatia (2008); Kumar *et al.* (2011); Djanaguiraman *et al.* (2010). In the present study, productive tiller per plant was increased in brassinosteroid-50 ppm (10.25) and the considerable decrease was observed in gibberllic acid-50 ppm (8.00), water spray (7.75) and control (7.25) showed in figure 14. These findings are in line up with the findings of Wu *et al.* (2008) where brassinosteroid treated plants showed increase in productive tillers per plant as compared to the control plants under high temperature stress condition. Brassinosteroid treated plants showed increase in productive tillers per plant might be due to synergistic action of brassinosteroid with indigenous auxin in cell elongation and cell proliferation of meristematic tissue (Sairam, 1994).

Plants subjected to high temperature stress which drops in the soluble sugars concentration in the anther walls, developing pollen grains and in the locular fluid which result in reduced concentration of sugar in the mature pollen grains and leads to declined in pollen viability (Ismail and Hall, 1999). In sorghum, high temperature stress reduces the carbohydrate accumulation in pollen grains and ATP in the stigmatic tissue (Jain *et al.,* 2007). 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 which undergo programmed cell death, thus results in pollen sterility (Oshino *et al.,* 2007; Sakata and Higashitani, 2008). For example, under heat stress, the structural

abnormalities was observed in developing snap bean anthers microspores have been associated with degeneration of tapetal due to malformations in endoplasmic reticulum (Suzuki *et al.,* 2001). Reduced in tomato anthers dehiscence under high temperature stress is also associated by the closure of locules and thus reduced in pollen dispersal in several plants (Peet *et al.,* 2002). In the present study, pollen viability was increased in brassinosteroid-50 ppm (80.23%), pink-pigmented facultative methylotrophs-1% (78.79%), glycine betaine-20 ppm (77.73%) and boron-100 ppm (77.67%) and the considerable decrease was observed in control (37.52%) were showed in figure 15. These findings are in line up with the findings of Thussagunpanit *et al.* (2012) they 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.

Flowering occurrence during early in the morning was a beneficial characteristic for tolerance to stress condition mainly high temperature stress. In most of the rice cultivars the peak anthesis mainly occurs in between 10.00 am to 12.00 pm (Sheehy *et al.*, 2005). In the present study, anthesis time for boron-100 ppm was 11:13 am and the control was 11:54 am (figure 16). These findings are in line up with the findings of Pandey and Gupta, (2013) they reported that boron helps to early blooming of flowers in black gram and it also improving pollen viability and stigma receptivity (Guru *et al.,* 2016).

Panicle length, spikelets per panicle and height of the plant were mainly affected with high temperature stress and it varies with the tolerance of rice cultivars (Kovi *et al.,* 2011). In the present study, panicle length was increased in gibberllic acid-50 ppm (21.33 cm), brassinosteroid-50 ppm (20.90 cm), boron-100 ppm (20.83 cm) and the considerable decrease was observed in water spray (19.03 cm) and control (18.98 cm) were showed in figure 17. These findings are in line up with the findings of Milach *et al.* (2002) they reported that gibberllic acid increase the panicle length in oat dwarf lines.

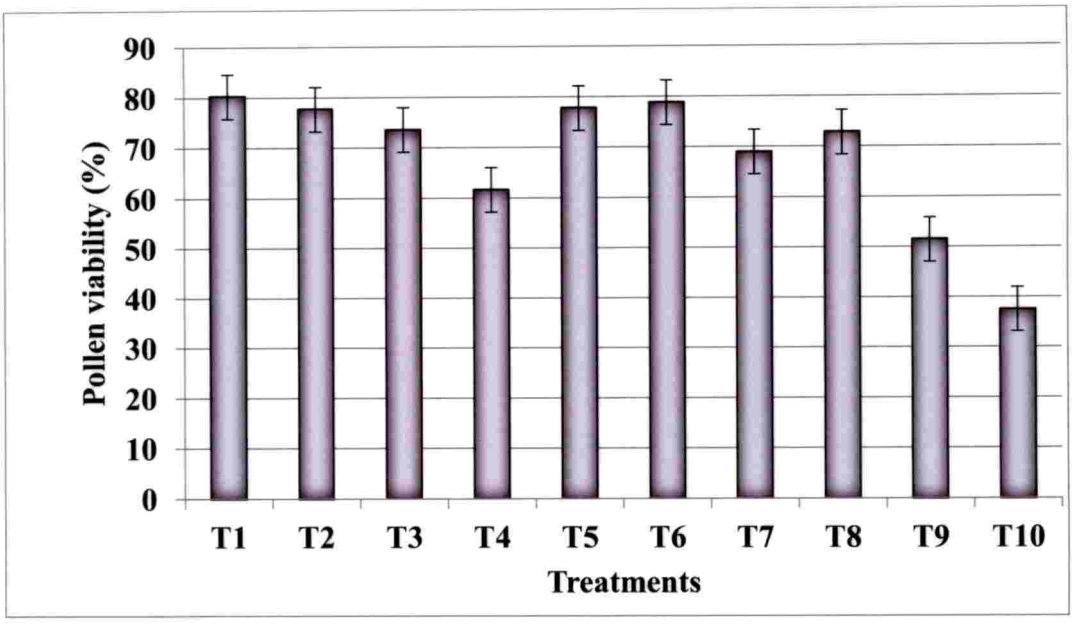


Figure 15. Variation in pollen viability (%) of different treatments under high temperature stress conditions.

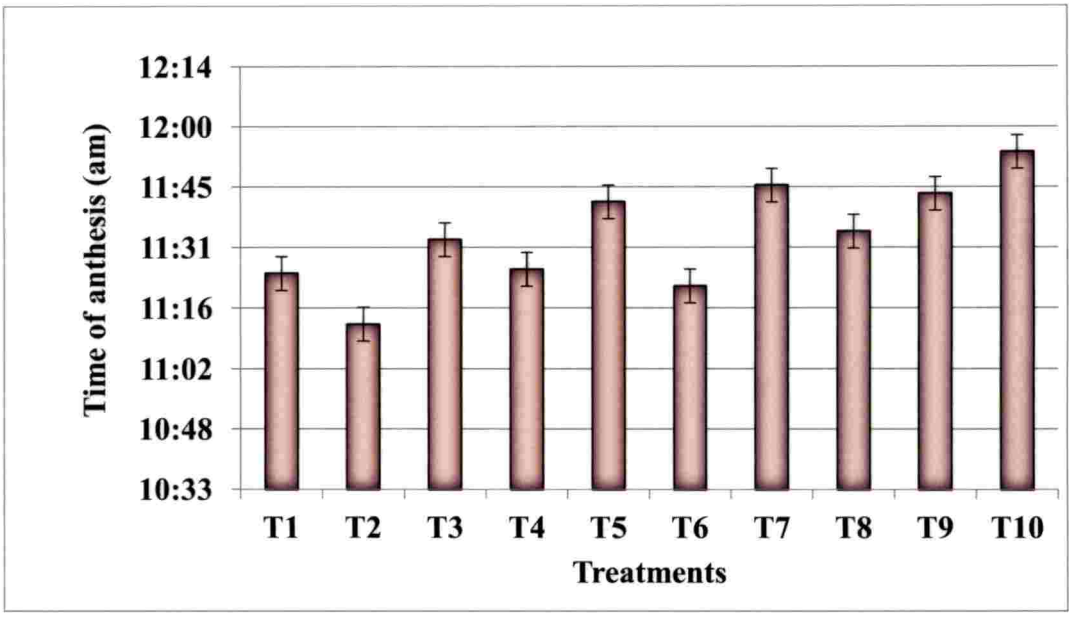


Figure 16. Variation in time of anthesis (am) of different treatments under high temperature stress conditions.

Fig 15 and 16. T1: Brassinosteroid(50 ppm); **T2:** Boron(100 ppm); **T3:** Calcium chloride (0.6%); **T4:** Salicylic acid (50 ppm); **T5:** Glycine betaine (20 ppm); **T6:** Pink-Pigmented Facultative Methylotrophs (PPFM) (1%); **T7:**1-methyl cyclopropane (50 ppm); **T8:**Gibberllic acid (50 ppm); **T9:** Water spray; **T10:** Control.

A significant impact of high temperature stress is affects meiosis, fertilization, and growth of fertilized embryos eventually leading to a significant decrease in yield (Camejo *et al.,* 2005). In the present study, reduction was observed in grain yield per plant under high temperature stress conditions and there is increase in treated plants. Maximum grain yield per plant observed in brassinosteroid-50 ppm was 15.87 g, whereas the minimum grain yield per plant recorded in control plants was 4.16 g (figure 18). These findings are in line up with the findings of Thussagunpanit *et al.* (2012) they reported that brassinosteroid treated plants showed increase in grain yield per plant as compared to the plants which were not treated under high temperature stress condition in rice.

High temperature stress reduces the floret fertility was associated with decreased anther dehiscence, poor pollen shedding, poor pollen grains germination, reduced in pollen tubes elongation and decreased the germination of invivo pollen (Fahad *et al.,* 2015, 2016). In the present study, reduction was observed in spikelet fertility under high temperature stress conditions and increasing trend for treated plants. Maximum increase in spikelet fertility percentage observed in brassinosteroid-50 ppm (75.40%) and pink-pigmented facultative methylotrophs-1% (73.46%) whereas, the minimum spikelet fertility percentage recorded in control plants (31.96%) was showed in the figure 19. These findings are in line up with the findings of Thussagunpanit *et al.* (2012) they 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.

In a stress-free environment, grain weights for a rice cultivar are almost constant (Mohammed and Tarpley, 2010). Although, under high temperature,

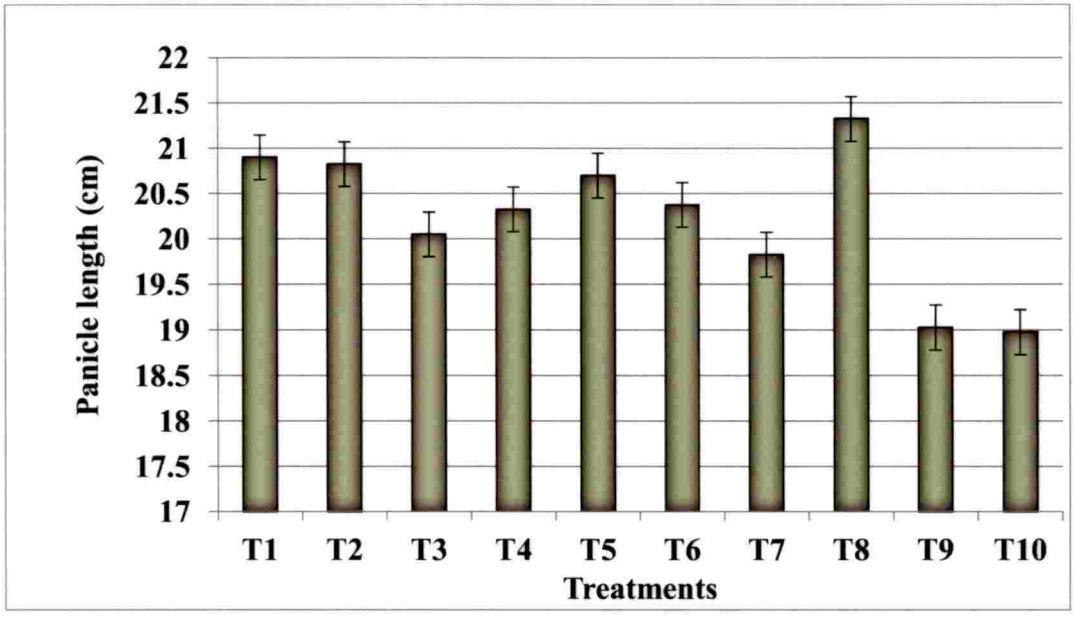


Figure 17. Variation in panicle length (cm) of different treatments under high temperature stress conditions.

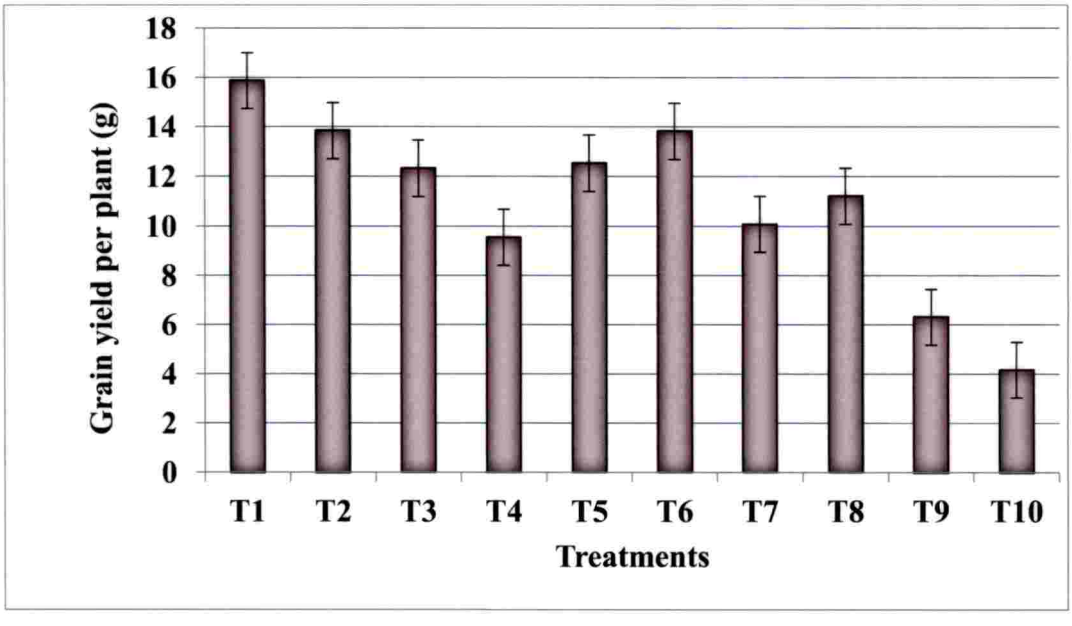


Figure 18. Variation in grain yield per plant (g) of different treatments under high temperature stress conditions.

Fig 17 and 18. T1: Brassinosteroid(50 ppm); **T2:** Boron(100 ppm); **T3:** Calcium chloride (0.6%); **T4:** Salicylic acid (50 ppm); **T5:** Glycine betaine (20 ppm); **T6:** Pink-Pigmented Facultative Methylotrophs (PPFM) (1%); **T7:**1-methyl cyclopropane (50 ppm); **T8:**Gibberllic acid (50 ppm); **T9:** Water spray; **T10:** Control.

reduction in the individual grain weight which showed in significant reduction in grain production of rice per unit area (Fahad *et al.,* 2016). In the present study, reduction was observed in 1000 grain weight under high temperature stress conditions and increase in treated plants. Maximum increase in 1000 grain weight observed in pink-pigmented facultative methylotrophs-1% (23.34 g), brassinosteroid-50 ppm (23.21 g), glycine betaine-20 ppm (23.04 g) and boron-100 ppm (22.85 g) whereas, the minimum 1000 grain weight recorded in control plants (17.85 g) were shown in figure 20. These findings are in line up with the findings of Nysanth, (2018) reported that pink-pigmented facultative methylotrophs treatments increase the 1000 grain weight in rice compare to control plants.

5.3. EFFECT OF FOLIAR APPLICATION OF METHYL JASMONATE AND WATER SPRAY ON HIGH TEMPERATURE STRESS MITIGATION IN RICE ON PHYSIOLOGICAL AND YIELD PARAMETERS

The susceptibility to high temperature which induced sterility of floret is more at the flowering stage, followed by booting stage (Sato *et al.,* 1973; Satake and Yoshida, 1978). The temperature at which sterility occurs is varies and depends on the cultivars. Sterility which occurs at a high temperature was due to poor anther dehiscence, reduce in the pollen grain number on the stigma, and poor pollen germination on the stigma (Satake and Yoshida, 1978; Imaki, 1983; Matsui *et al.,* 1997, 2001). Imaki *et al.* (1982) reported that the time of flowering in rice differed among various cultivars and some cultivars flowered early in the morning. Such cultivars might be helpful to avoid the damage by high temperatures during flowering time (Imaki, 1983). High temperature stress during or after anthesis (1 to 3 hour after anthesis in rice) which induce the sterility of spikelet (Satake and Yoshida, 1978). Ishimaru *et al.* (2009) transferred a promising early morning flowering allele or trait from wild rice (*Oryza officinalis*) to reduce the heat injury during anthesis. In the present study, anthesis time was

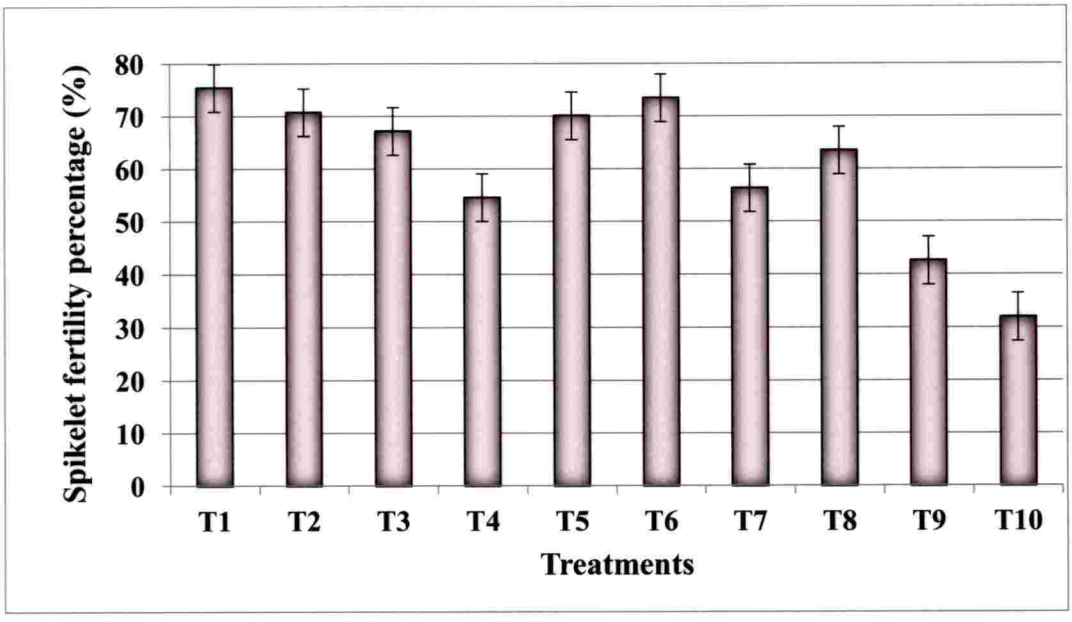


Figure 19. Variation in spikelet fertility percentage (%) of different treatments under high temperature stress conditions.

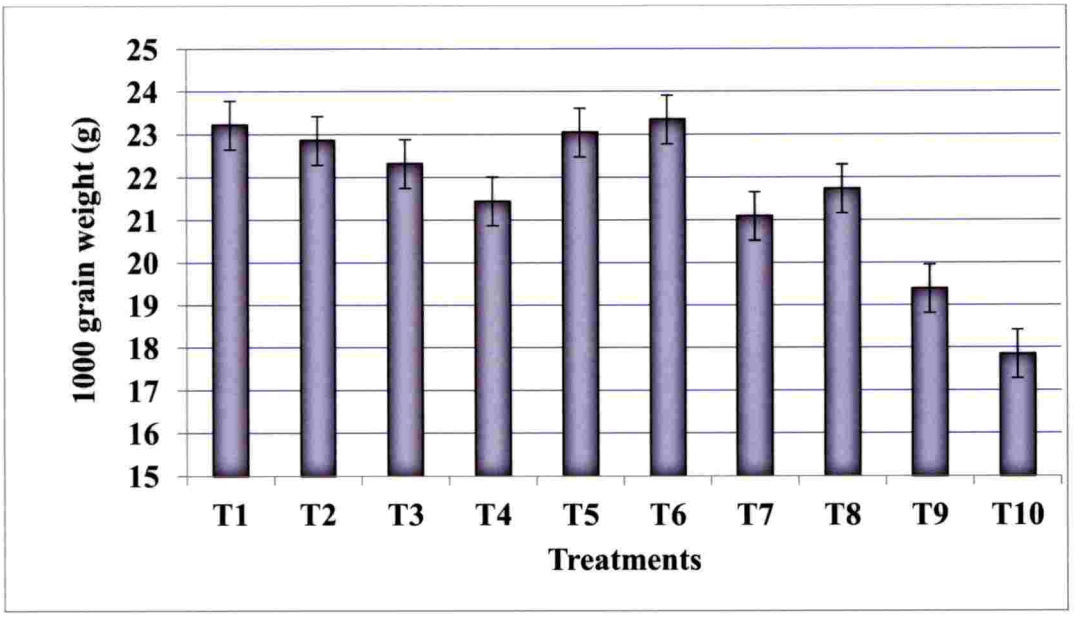


Figure 20. Variation in 1000 grain weight (g) of different treatments under high temperature stress conditions.

Fig 19 and 20. T1: Brassinosteroid(50 ppm); **T2:** Boron(100 ppm); **T3:** Calcium chloride (0.6%); **T4:** Salicylic acid (50 ppm); **T5:** Glycine betaine (20 ppm); **T6:** Pink-Pigmented Facultative Methylotrophs (PPFM) (1%); **T7:**1-methyl cyclopropane (50 ppm); **T8:**Gibberllic acid (50 ppm); **T9:** Water spray; **T10:** Control.

showed early in plants sprayed with 4mM L^{-1} methyl jasmonate at 7 am was 08:11 am whereas for control, anthesis time was 11:54 am (figure 21). These findings are in line up with the findings of Zeng *et al.* (1999) they reported that methyl jasmonate induced rice florets opening within about 30 minutes of different rice cultivars.

High temperature stress given a day before to flowering which affected the normal function of pollen sac, anther dehiscence and pollen viability in rice (Matsui and Omasa, 2002). Early morning flowering character can be effectively used for the escape from the heat stress which induced the sterility of the spikelet during anthesis by shedding of the viable pollen while in the cooler hours in the morning on to the receptive stigma (Satake and Yoshida, 1978). In the present study, pollen viability was drastically reduced in control plants (37.52%) whereas, the considerable increase in plants sprayed with 4mM L^{-1} methyl jasmonate at 7 am (61.93 %) and 2mM L^{-1} methyl jasmonate at 7 am (58.64 %) were showed in figure 22. These findings are in line up with the findings of Fahad *et al.* (2016) reported that methyl jasmonate improves the pollen viability compared to the plants which were not treated under high temperature stress condition in rice.

In rice the spikelet fertility, pollen viability, pollen production and pollen receptivity are highly positive correlated (Prasad *et al.*, 2006a). MeJA was a crucial cellular regulators involved in several plants developmental process (Ueda and Saniewski, 2006; Norastehnia *et al.,* 2007). Foliar application of MeJA which brings alteration in various physiological processes and hence stimulates plant defense responses against a abiotic and biotic stresses (Walia *et al.,* 2007). Clarke *et al.* (2009) reported that exogenous application of MeJA in *Arabidopsis thaliana* improves basal thermo-tolerance and protected from the heat shock damage. In the present study, the spikelet fertility percentage was increased in 4mM L^{-1} methyl jasmonate at 7 am (56.07%) whereas, there is considerable reduction in spikelet fertility percentage in control (33.09%) were showed in figure 23. These

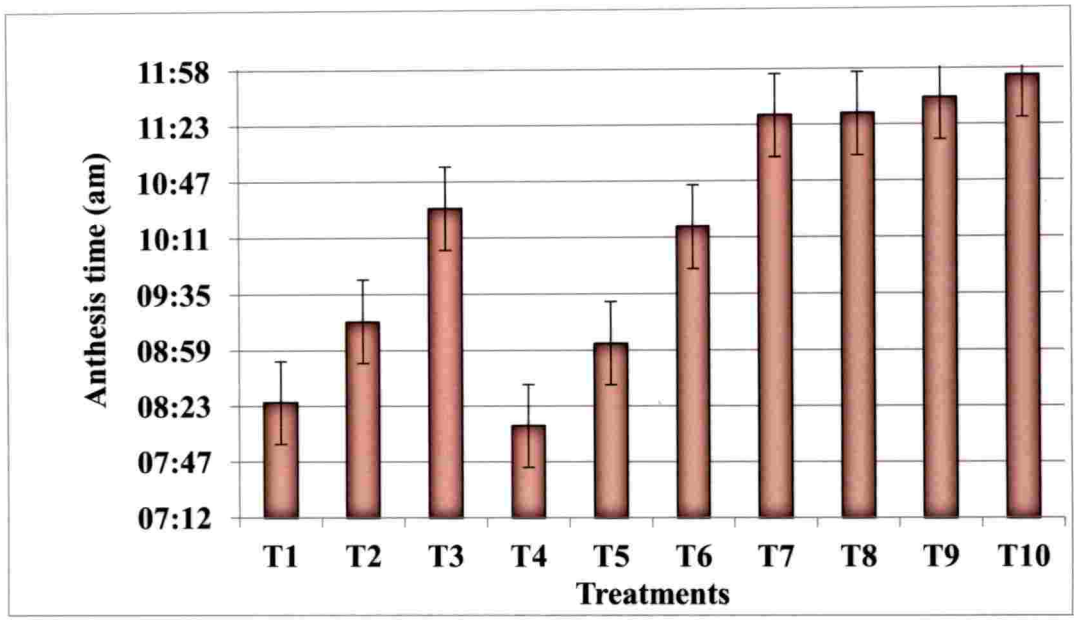


Figure 21. Variation in anthesis time (am) of different treatments under high temperature stress conditions.

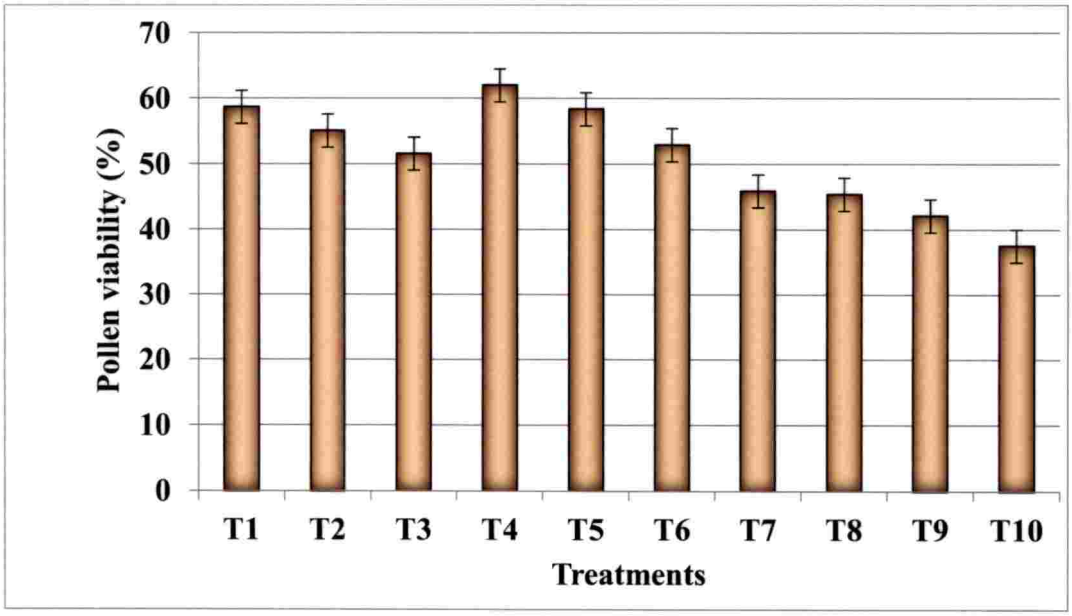


Figure 22. Variation in pollen viability (%) of different treatments under high temperature stress conditions.

Fig 21 and 22. T1: 2mM L^{-1} Methyl jasmonate at 7 am; **T2:** 2mM L^{-1} Methyl jasmonate at 8 am; **T3:** 2mM L^{-1} Methyl jasmonate at 9 am; **T4:** 4mM L^{-1} Methyl jasmonate at 7 am; **T5:** 4mM L^{-1} Methyl jasmonate at 8 am; **T6:** 4mM L^{-1} Methyl jasmonate at 9 am; **T7:** Water spray at 7 am; **T8:** Water spray at 8 am; **T9:** Water spray at 9 am; **T10:** Control.

findings are in line up with the findings of Fahad *et al.* (2016) they reported that methyl jasmonate improves the spikelet fertility compared to the plants which were not treated under high temperature stress condition in rice.

Rice grain yield is dependent on panicles number, spikelets number per panicle, grain filling rate and total grain weight (Kim *et al.,* 2009). Heat stress decreased the yield of rice by decreasing the efficiency of rice growth and yield characters (Fahad *et al.,* 2016). A notable decrease in total number of grains and grain weight under high temperature (Ferris *et al.,* 1998). High temperature stress induced significant yield reductions in peanut (*Arachis hypogea* L.) and common beans (*Phaseolus vulgaris* L.) (Prasad *et al.,* 1999; Rainey and Griffiths, 2005). A significant impact of high temperature stress is mainly observed in tomatoes (*Lycopersicum esculentum*) as it affects meiosis, fertilization, and growth of fertilized embryos eventually leading to a significant decrease in yield (Camejo *et al.,* 2005). Early morning flowering trait can be effectively used for the escape from the high temperature stress which helps to reduced the sterility of the spikelet during anthesis by shedding of the viable pollen while in the cooler hours in the morning on to the receptive stigma and which leads to significant increase in grain yield (Satake and Yoshida, 1978). In the present study, grain yield per plant in 4mM L^{-1} methyl jasmonate at 7 am was 8.55 g, 2mM L^{-1} methyl jasmonate at 7 am was 8.06 g and 4mM L^{-1} methyl jasmonate at 8 am was 7.89 g whereas, the considerable reduce in grain yield per plant in control was 4.22 g (figure 24). These findings are in line up with the findings of Fahad *et al.* (2016) reported that methyl jasmonate improves the grain yield per plant compared to the plants which were not treated under high temperature stress condition in rice.

Severe reduction in number of seeds, individual weight of seed, yield of grains and also biomass production under high temperature stress condition which is directly impacted in the harvest index (Prasad *et al.,* 2017). Early morning flowering trait which leads to significant increase in grain yield and grain weight

(Satake and Yoshida, 1978). In the present study, 1000 grain weight in 4mM L^{-1} methyl jasmonate at 7 am was 21.33 g, 2mM L^{-1} methyl jasmonate at 7 am was 21.03 g, 2mM L^{-1} methyl jasmonate at 8 am was 20.86 g and 4mM L^{-1} methyl jasmonate at 8 am was 20.83 g whereas, the considerable reduction in 1000 grain weight in water spray at 7 am was 18.26 g, water spray at 8 am was 18.21 g, water spray at 9 am was 18.19 g and control plants was 17.85 g (figure 25). These findings are in line up with the findings of Anjum *et al.* (2016) they reported that methyl jasmonate induce the 1000 grain weight compare to control.

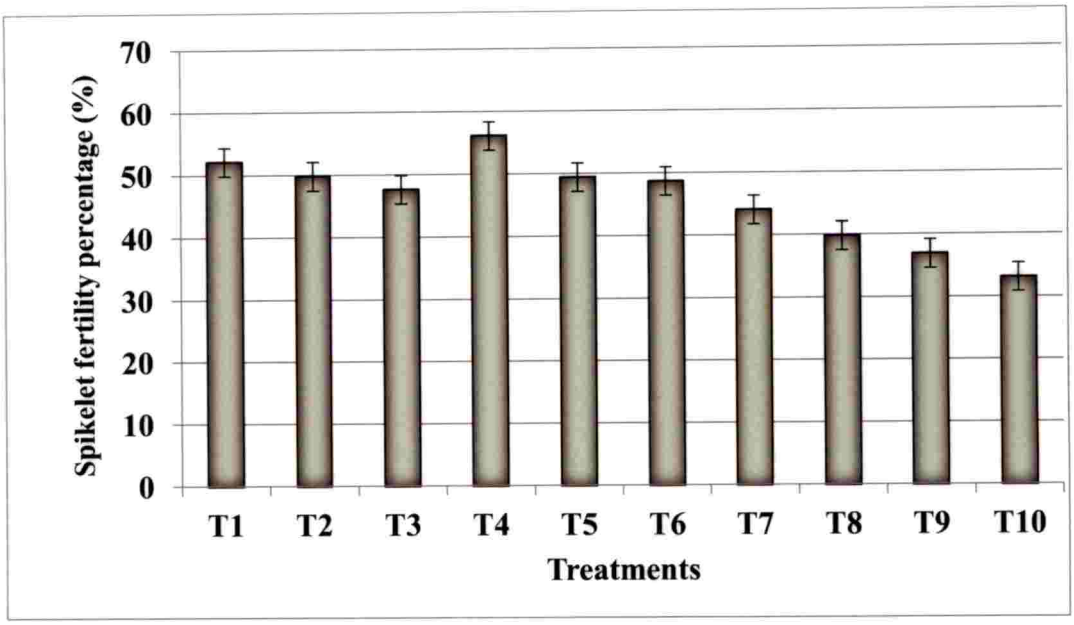


Figure 23. Variation in spikelet fertility percentage (%) of different treatments under high temperature stress conditions.

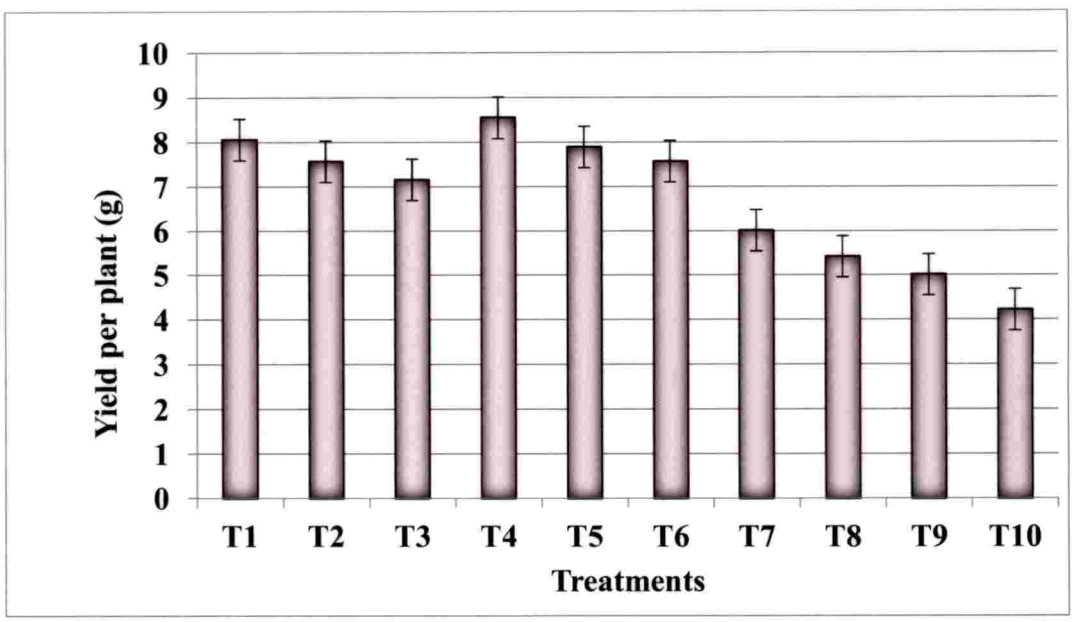


Figure 24. Variation in yield per plant (g) of different treatments under high temperature stress conditions.

Fig 23 and 24. T1: 2mM L^{-1} Methyl jasmonate at 7 am; **T2:** 2mM L^{-1} Methyl jasmonate at 8 am; **T3:** 2mM L^{-1} Methyl jasmonate at 9 am; **T4:** 4mM L^{-1} Methyl jasmonate at 7 am; **T5:** 4mM L^{-1} Methyl jasmonate at 8 am; **T6:** 4mM L^{-1} Methyl jasmonate at 9 am; **T7:** Water spray at 7 am; **T8:** Water spray at 8 am; **T9:** Water spray at 9 am; **T10:** Control.

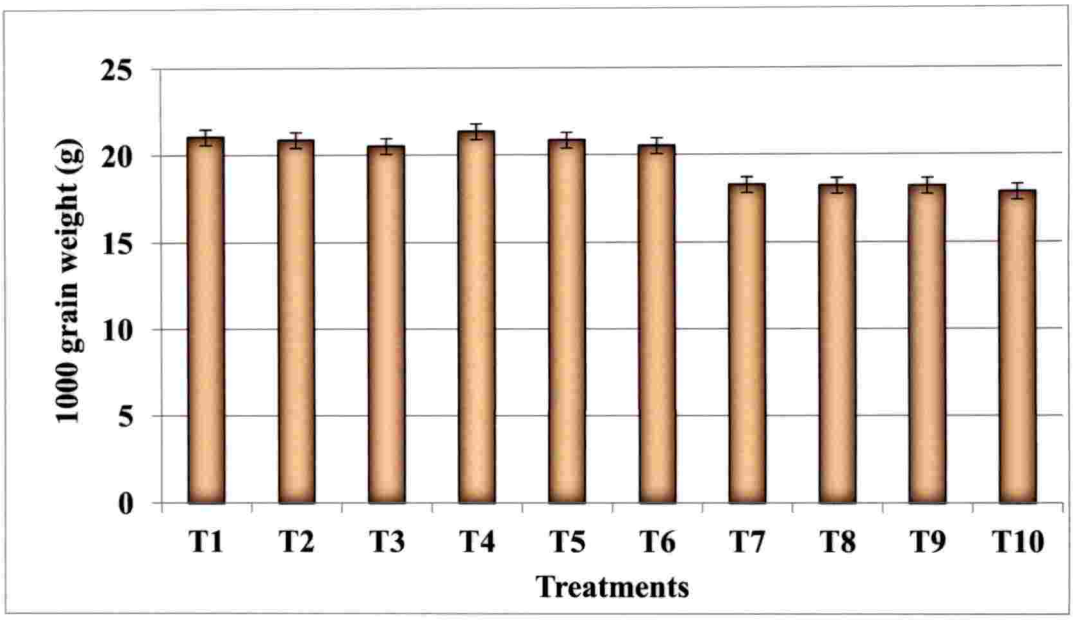


Figure 25. Variation in 1000 grain weight (g) of different treatments under high temperature stress conditions.

Fig 25. T1: 2mM L^{-1} Methyl jasmonate at 7 am; **T2:** 2mM L^{-1} Methyl jasmonate at 8 am; **T3:** 2mM L^{-1} Methyl jasmonate at 9 am; **T4:** 4mM L^{-1} Methyl jasmonate at 7 am; **T5:** 4mM L^{-1} Methyl jasmonate at 8 am; **T6:** 4mM L^{-1} Methyl jasmonate at 9 am; **T7:** Water spray at 7 am; **T8:** Water spray at 8 am; **T9:** Water spray at 9 am; **T10:** Control.

Summary

6. SUMMARY

The present programme was conducted to study the impact of foliar application of plant growth regulators and nutrients on high temperature stress mitigation in rice. It was conducted in two experiments and the salient findings are given below.

In the first experiment the objective of the study was to assess the effect of foliar application of plant growth regulators and nutrients on high temperature stress mitigation in rice. In this study, seedlings were raised in pot trays and transplanted to mud pots on 18th DAS. The pots were kept under high temperature condition (5-6°C more than ambient condition) in a temperature controlled polyhouse from seedling to maturity stage. Maximum and minimum temperatures were measured daily using a thermo-hygrometer. Foliar spray of plant growth regulators and nutrients was given at panicle initiation, heading and flowering stage. Physiological observations were taken at 50% flowering stage and yield parameters were taken at harvest stage.

Physiological, biochemical, morphological and yield parameters were studied in all the treatments under high temperature stress. Physiological and biochemical parameters such as cell membrane stability index, chlorophyll stability index, photosynthetic rate, stomatal conductance, chlorophyll a/b ratio, carbohydrate content, superoxide dismutase activity and PS II photochemistry (Fv/Fm ratio) were decreased in control as compared to treatments whereas, leaf temperature, transpiration rate and malondialdehyde content were increased in control as compared to treatments under high temperature stress condition. Brassinosteroid treatment showed significant increase in physiological and biochemical parameters such as cell membrane stability index, photosynthetic rate, stomatal conductance, Fv/Fm ratio, chlorophyll stability index, chlorophyll a/b ratio and superoxide dismutase activity compare to all other treatments.

All the morphological and yield parameters were reduced in control whereas, increased with the treatments. Gibberllic acid treated plants exhibited increase in plant height and panicle length compared to all other treatments. Days to 50% flowering and 1000 grain weight were increased in pink pigmented facultative methylotrophs compared to control. Time of anthesis of boron treated plants observed at 11:13 am. Flowers of control opened at 11:54 am with respect to other treatments. Spikelet fertility and pollen viability were significantly reduced under high temperature stress. Treated plants were showed higher pollen viability and hence increased in spikelet fertility. Brassinosteroid treatment showed significant increase in pollen viability, spikelet fertility, productive tillers per plant and grain yield per plant compared to all other treatments. All the morphological and yield parameters were significantly affected control plants compared to treated plants. As a consequence of improvement in physiological, biochemical, morphological and yield parameters, brassinosteroid treated plants exhibited improved in stress tolerance, which led to better performance under high temperature stress.

In the second experiment to study the effect of foliar application of methyl jasmonate on early morning flowering trait on high temperature stress mitigation in rice. Seedlings were raised in pot trays and maintained in mud pots as in the case of first experiment. Methyl jasmonate in varying concentration and water spray were given on spikelet at different time. Physiological observations were taken at 50% flowering stage and yield parameters were taken at harvest stage.

Physiological and yield parameters were studied in all the treatments under high temperature stress. Physiological and yield parameters such as anthesis time, pollen viability, spikelet fertility percentage, yield per plant and 1000 grain weight were decreased in control whereas, increased with the treatments. Time of anthesis of 4mM L^{-1} methyl jasmonate at 7 am treated plants observed at 08:11 am. Flowers of control opened at 11:54 am with respect to other treatments. 4mM L^{-1} methyl jasmonate at 7 am treated plants exhibited increase in pollen

viability, spikelet fertility percentage, yield per plant and 1000 grain weight compare to control.

It is therefore, concluded that, in the first experiment there was significant variation in physiological, biochemical, morphological and yield components among treatments. Brassinosteroid treatment recorded high pollen viability, spikelet fertility percentage, and grain yield per plant by improving the physiological and biochemical traits like cell membrane stability index, photosynthetic rate, stomatal conductance, Fv/Fm ratio, chlorophyll stability index, chlorophyll a/b ratio and superoxide dismutase activity. Hence, concluded that BR treatment can mitigate the ill effects of high temperature stress in rice. In the second experiment there was significant variation for physiological and yield components among treatments. 4mM L^{-1} methyl jasmonate at 7 am showed better performance for all the parameters such as anthesis time, pollen viability, spikelet fertility percentage, yield per plant and 1000 grain weight compared to all the treatments under high temperature stress condition. Hence, it is found that methyl jasmonate can advance anthesis time and thereby plants can escape from the severity of temperature experiencing at the normal flowering time.

Future line of work

These results will be worth for further understanding the adaptation and survival mechanisms of rice to high temperature and also further studies are needed to validate these results from controlled-environment studies to field conditions.

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Abstract

IMPACT OF FOLIAR APPLICATION OF PLANT GROWTH REGULATORS AND NUTRIENTS ON HIGH TEMPERATURE STRESS MITIGATION IN RICE (*Oryza sativa* L.)

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ABSTRACT

The study entitled "Impact of foliar application of plant growth regulators and nutrients on high temperature stress mitigation in rice (*Oryza sativa* L.)" was conducted in the Department of Plant Physiology, College of Agriculture, Vellayani during August to December of 2018 with the objectives to study the effect of foliar application of plant growth regulators and nutrients on high temperature mitigation and to advance the flowering time by using methyl jasmonate (MeJA) in rice.

The investigation comprised two experiments. The extent of variation for various physiological, biochemical, morphological and yield parameters were assessed as an indicator of high temperature stress mitigation by using plant growth regulators and nutrients. The rice variety (Uma) utilized in the experiment was collected from IFSRS, Karamana. Plants were maintained under high temperature condition in a temperature controlled polyhouse from seedling to maturity stage with three replications. Maximum and minimum temperatures were measured daily using a thermo-hygrometer.

The first experiment was laid out in CRD with 10 treatments [Brassinosteroid (BR)-50 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. Physiological observations and yield parameters were recorded at 50 per cent flowering and harvesting stage respectively.

The study revealed that physiological and biochemical parameters such as cell membrane stability index (%), photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), stomatal conductance (mmol H_2O m^{-2} s^{-1}) and Fv/Fm ratio were found to increase

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significantly in most of the treatments under high temperature stress condition, whereas leaf temperature(°C) and transpiration rate (mmol H_2O m^{-2} s^{-1}) decreased. Among the treatments, BR spray significantly increased in the cell membrane stability index (141.57%), photosynthetic rate (17.50 µmol CO_2 m^{-2} s^{-1}), stomatal conductance (583.70 mmol H_2O m^{-2} s^{-1}), Fv/Fm ratio (0.74), chlorophyll stability index (109.32%), chlorophyll a/b ratio (1.28) and superoxide dismutase activity (0.33 activity g^{-1} min^{-1}).

Treatments had significant effect on morphological and yield parameters over control plants. Among the treatments, GA_3 spray resulted in significant increase in plant height (142.75 cm) and panicle length (21.33 cm). BR spray significantly increased the productive tillers per plant (10.25), pollen viability (80.23%), spikelet fertility percentage (75.4%) and grain yield per plant (15.87 g).

The second experiment was laid out in CRD with 10 treatments. Foliar spray of MeJA in varying concentrations was given on spikelet at different time [2mM L^{-1} MeJA at 7 am, 2mM L^{-1} MeJA at 8 am, 2mM L^{-1} MeJA at 9 am, 4mM L^{-1} MeJA at 7 am, 4mM L^{-1} MeJA at 8 am, 4mM L^{-1} MeJA at 9 am, water spray at 7 am, water spray at 8 am, water spray at 9 am and control (no spray)]. Physiological observations were taken at 50% flowering stage and yield parameters were taken at harvesting stage.

The study revealed that among the treatments, 4mM L^{-1} MeJA at 7 am treatment showed early anthesis (08:11 am) and also significant increase in pollen viability (61.93%), spikelet fertility (56.07%), yield per plant (8.55 g) and 1000 grain weight (21.33 g).

In the first experiment there was significant variation for physiological, biochemical, morphological and yield components among treatments. BR treatment recorded high pollen viability, spikelet fertility and grain yield per plant by improving the physiological and biochemical traits such as cell membrane

stability index, photosynthetic rate, stomatal conductance, Fv/Fm ratio, chlorophyll stability index, chlorophyll a/b ratio and superoxide dismutase activity. Hence, BR treatment can mitigate the ill effects of high temperature stress in rice.

In the second experiment there was significant variation for physiological and yield components among treatments. 4mM L^{-1} MeJA at 7 am showed better performance for all the parameters such as anthesis time, pollen viability, spikelet fertility, yield per plant and 1000 grain weight. Hence, MeJA can advance anthesis time thereby enabling plants to escape from the severe temperature experienced at normal flowering time.

APPENDICES

APPENDIX-I

Anthrone reagent

Anthrone reagent made by dissolving 200 mg of Anthrone in 100 ml ice cold 95 per cent concentrated sulphuric acid.

APPENDIX-II

Buffer for biochemical analysis

1. Potassium phosphate buffer (50 mM) - pH. 7.8.

A: 50 mM solution of K_2HPO_4 - 4.35 g in 500 ml.

B: 50 mM solution of KH_2PO_4 - 3.40 g in 500 ml.

Solution A and solution B were added with constant stirring until pH 7.8 reached.

APPENDIX-III

Iodine- potassium iodide (IKI) solution

1 per cent iodine- potassium iodide solution which was prepared by dissolving 2.5 g of potassium iodide with 250 mg of iodine and made up to 125 ml.