PHYSIOLOGICAL INTERVENTION FOR MITIGATING TEMPERATURE STRESS IN RICE

FEMINA K

(2018-11-094)

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

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By

FEMINA K

(2018-11-094)

THESIS

Submitted in partial fulfilment of the Requirement for the degree of

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DEPARTMENT OF PLANT PHYSIOLOGY

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2020

DECLARATION

I hereby declare that the thesis entitled "**Physiological intervention for mitigating temperature stress in rice**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other university or society.

Vellanikkara

Date: 29-12-2020

FEMINA K

(2018-11-094)

CERTIFICATE

Certified that the thesis entitled **"Physiological intervention for mitigating temperature stress in rice"** is a record of research work done independently by **Ms. Femina K (2018-11-094)** 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 her.

Vellanikkara

Date: 29-12-2020

Dr. T. Girija

(Major Advisor) Professor and Head

Department of Plant Physiology

College of Horticulture, Vellanikkara

CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Femina. K (2018-11-094)**, a candidate for the degree of **Master of Science in Agriculture**, with major field in **Plant Physiology**, agree that the thesis entitled **"Physiological intervention for mitigating temperature stress in rice"** may be submitted by **Ms. Femina. K** in partial fulfillment of the requirement for the degree.

29/12/2020

Dr. T. Girija
Professor and Head
Department of Plant Physiology
College of Horticulture, Vellanikkara.

Dr. P. S. Abida
Professor and Head and ADR
Centre for Plant Biotechnology & Molecular Biology
College of Horticulture, Vellanikkara.

Dr. P. Prameela
Professor and Head
Department of Agronomy
College of Horticulture, Vellanikkara.

Dr. C. Laly John
Professor and Head
Department of Agricultural Statistics
College of Horticulture, Vellanikkara.

knowledgement

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Femina. K

DEDICATED TO

MY FAMILY

MY ADVISOR

TEACHERS

FRIENDS

FARMERS

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LIST OF ABBREVIATIONS

^{0}C	Degree Celsius
per cent	Per cent
m^{-2}	Per square meter
CD	Critical difference
Cm	Centimeter
et al.	Co-authors/Co-workers
Fig.	Figure
mM	Milli Molar
M	Molar
g	Gram
h	Hour
Fr. wt	Fresh weight
i.e.	That is
µ	Micro
CRD	Completely randomized design
RBD	Randomized block design
OD	Optical Density
A_{663}	Absorbance at 663 nm
A_{645}	Absorbance at 645 nm
IAA	Indole-acetic acid

Introduction

1. INTRODUCTION

Global mean surface air temperature has raised by 0.5° C in the 20th century and is predicted to increase further by 1.5 to 4.5°C in this century (IPCC, 2007). High temperature stress is estimated to happen more frequently in coming years because of global warming due to climate change. High temperature condition adversely affects plant growth and development in numerous ways which can even lead to death of plants.

Rice (*Oryza sativa* L.) is a major cereal crop most widely consumed as staple food by millions of people across the globe. Rice is an important cereal which provides more than one-fifth of the calories consumed worldwide by humans and has the third-highest production after sugarcane and maize. With the probable increase of world's population toward 10 billion by 2050 (United Nations), the demand for rice will be much higher than the other crops. Rice requires a fairly high temperature for growth and development ranging from 20ºC to 35ºC (Yoshida, 1981), being a tropical plant. Exposure of rice plants to temperatures higher than this limit leads to heat injuries, reduction in metabolism and finally cause a huge yield loss.

High temperature stress during growth period can lead to major changes in morphological, phenological and physiological characteristics of rice. During early growth stages, high temperature stress leads to alterations in metabolism such as decrease in stomatal conductance, net photosynthetic rate, total chlorophyll content and amount of soluble proteins High temperature stress causes a rapid increase in production of ROS molecules which alter the membrane lipid composition and leads to membrane leakage. Rice plant is reported to be highly vulnerable to high temperature at flowering stage. High temperature stress during this stage causes impaired pollen germination, loss of pollen viability and spikelet sterility which results in production of more chalky grains. Grain yield in rice is predicted to decline by 10% for each 1°C raise in minimum temperature (Peng *et al*., 2004).

High temperature stress is a common occurrence in the third crop season where temperatures can go up to 40 ^{0}C in the reproductive stage leading to yield loss. Plants have evolved complex defence mechanisms to withstand metabolic imbalances due to high

temperature stress. Plants counteracts adverse temperature stress conditions by adjusting molecular mechanisms including proteins, antioxidants, metabolites, regulatory factors and membrane lipids. Application of additional inputs which can boost inherent tolerant mechanisms can be an alternate way to assist plants to cope up with temperature stress. During the last decade, exogenous application of some plant growth regulators, nutrient solutions and antioxidant compounds has been recognized as means to combat the adverse effects of high temperature.

Present study has been proposed with an objective of addressing productivity decline in rice due to high temperature stress by identifying suitable plant protectants that can be applied exogenously to mitigate stress and to enhance productivity.

Review of literature

2. REVIEW OF LITERATURE

Effect of elevated temperature on morphological, physiological, biochemical and yield attributes of rice plant and influence of exogenously applied ameliorants on mitigation of high temperature stress has been reviewed under this chapter

2.1 Effect of elevated temperature and exogenously applied chemical ameliorants on physiological parameters of rice

2.1.1 Photosynthetic rate

Photosynthesis is highly sensitive to fluctuations in atmospheric temperature. Photosynthetic rate is reported to be reduced to half when the air temperature surpass 35^0 C (Taniyama *et al.,* 1988). Zhang *et al.* (2007) reported that, net photosynthetic rate and pollen viability of rice flag leaves get reduced with increasing temperature (beyond 35°). High temperature stress during vegetative stage, grain filling stage and panicle initiation stage caused a reduction in photosynthetic rate in rice (Yun-ying *et al*., 2009). Under high temperature conditions, net photosynthesis of rice cultivars reduced distinctly as compared to control plants grown under normal temperature conditions (Xie *et al*., 2011). Studies conducted in rice genotypes showed a 35 percent reduction in net photosynthesis when they were subjected to temperatures beyond 40 ^{0}C at panicle initiation stage (Jumaitun *et al.,* 2016).

Exogenous application of manganese reduced the impact of temperature stress indirectly by improving photosynthesis and nitrogen metabolism in plants (Waraich *et al*., 2012). Study conducted by Ding *et al*. (2016) in cucumber plant showed that foliar spray of glutathione improved net photosynthesis as compare to control. In another study conducted in grape seedlings, Zhong (2020) revealed that net photosynthesis increased in all treatments supplied with melatonin compared to control plants under high temperature stress.

2.1.2 Stomatal conductance

Taiz and Zeiger (2002) reported that, under high temperature conditions the stomatal conductance increased which allow diffusion of more CO_2 into leaves and thereby increasing the photosynthetic rate. According to Rane *et al*. (2003) stomatal conductance is more important in determining productivity. Munjal and Dhanda (2004) reported reduction in stomatal conductance in rice under high temperature and they stated that it is due to closing of stomata to save water and maintain its functional integrity. A decreasing trend for stomatal conductance was found in four rice genotypes under higher temperature stress compared to normal temperature (Ramesh *et al*., 2017).

Foliar spray of 10^{-5} M salicylic acid was found to increase net photosynthetic rate, internal CO_2 concentration and stomatal conductance in *Brassica juncea* plants grown under high temperature condition (Fariduddin *et al*., 2003). Khan et al. (2003) also recorded increased stomatal conductance in response to exogenous application of 0.5 mM salicylic acid in corn and soybean. Shah *et al.* (2011) reported a hike in stomatal conductance in rice plants grown under high temperature conditions by exogenous application of ascorbic acid. Stomatal conductance of the grape seedling was increased when they were treated with 150 µM melatonin (Zhong, 2020).

2.1.3 Total chlorophyll

Total chlorophyll content of flag leaves is one of the important parameter which determines photosynthetic capability of rice plant. Chlorophyll content in rice was reported to decrease under high temperature stress (Xie *et al*., 2011). Sailaja *et al.* (2014) reported a reduction in chlorophyll content in two rice cultivars N22 and Vandana grown under high temperature condition for a long time.

Hayat *et al*. (2005) recorded an increase in pigment content especially total chlorophyll of wheat seedlings treated with 10^{-3} M concentration of salicylic acid grown under high temperature condition. Mengutay *et al*. (2013) observed an increase in total chlorophyll content in wheat plants supplied with adequate magnesium. Foliar application of 0.32 M boron solution enhanced total chlorophyll contents of rice grown under high temperature (Rehman *et al*., 2014). In *Viciafaba*, exogenous application of magnesium

solution resulted in enhanced total chlorophyll content (Siddiqui *et al*., 2016). Glutathione spray treatment under high temperature stress was reported to increase total chlorophyll and soluble protein content of plants as compare to control in cucumber (Ding *et al*., 2016). Chlorophyll *a*, chlorophyll *b* and total chlorophyll content in grape seedlings were significantly increased by exogenous application of melatonin, compared to control (Zhong *et al*., 2020).

2.1.4 Total soluble protein

Under high temperature stress, physiological changes like enzyme inactivation, inhibition of protein formation, protein degradation can happen in rice (Howarth, 2005). Soluble sugar, soluble protein and free proline content in flag leaf of rice plant decreased under high temperature stress (Zhang *et al*., 2007)

Exogenous application of 1 mM concentration of salicylic acid in three different cotton varieties lead to increase in total protein content under high temperature stress as compared to un- treated control (Galani *et al*., 2016). High temperature + glutathione treatment enhanced chlorophyll and soluble protein content compared to the high temperature treatment in cucumber (Ding *et al*., 2016). Soluble protein content of grape seedling leaves were reported to increase in all the melatonin treatments (50, 100, 150, and 200 µmol / L) as compared to control (Zhong *et al*., 2020). Xue *et al*. (2016) reported that cellular protein content can be enhanced by exogenous application of melatonin.

2.1.5 Proline

Rice plants exposed to high temperature stress conditions had higher content of proline in leaves than those grown under normal temperature condition (Zhang *et al*., 2007). Tang *et al.* (2008) reported that even a small period of exposure to higher temperature resulted in high proline accumulation in rice.

Exogenous application of magnesium enhanced proline content in *Vicia faba* plants grown under heat stress conditions (Siddiqui *et al*., 2016). In tomato plants, exogenously

applied ascorbic acid (0.5 mM) increased the proline content during temperature stress (Abdullah and Alayafi, 2019). Studies revealed that in cucumber plants grown under high temperature, exogenously applied glutathione enhanced proline accumulation (Ding *et al*., 2016).

2.1.6 Nitrate reductase enzyme activity

Nitrate reductase enzyme activity depends on concentration of NO_3 (Solomonson and barber, 1990) and it is reported to decreases in response to increasing temperature. Under 41 to 43 ^{0}C temperatures, nitrate reductase activity was observed to decrease in barley seedlings (*Hordeum vulgare* L.) (Deane *et al.,* 1980). Reduction in nitrate reductase enzyme activity were reported in many crops such as mustard (Hayat *et al*., 2009), soybean (Joseph *et al*., 1976), maize (Jain and Srivastava. 1981) and wheat (Khan *et al*., 2013).

Exogenous application of 0.1 mM salicylic acid has been reported to enhance nitrate reductase enzyme activity (Srivastava, 1980).

2.1.7 IAA content

Kabir *et al*. (2017) reported an increase in IAA under high temperature during the early grain filling period and then it showed a sudden decline in IAA content up to 35 days after anthesis in rice.

In *Brassica juncea,* exogenously applied melatonin increased IAA content by 1.4 times (Chen *et al*., 2009). Exogenous application of salicylic acid increased accumulation of IAA (Shakivora, 2007) in plants.

2.1.8 Reactive oxygen species

Under high temperature condition, there will be a hike in production of reactive oxygen species viz, singlet oxygen, superoxide radicals and hydrogen peroxide (H_2O_2) at cellular level (Almeselmani *et al*., 2006). High temperature stress interferes with the

photosynthetic machinery and enhances production of damaging reactive oxygen species (ROS), including superoxide anions (O^{-}), hydroxyl radicals ($^{\cdot}OH$) and hydrogen peroxide and these ROS affect proteins, DNA and lipids (Suzuki et al., 2012). High temperature treatment exhibited an increased malondialdehyde (MDA) concentration and $O2^{\bullet -}$ synthesis rate in cucumber plants (Ding *et al*., 2016).

Adverse effect of high temperature in mung bean seedlings was negated by exogenous application of glutathione as the chemical was effective in reducing the superoxide and H_2O_2 levels in seedlings (Hasanuzzaman *et al*., 2015). Cucumber plants grown under high temperature condition showed significant increase in reactive oxygen species production, which was decreased by exogenous application of glutathione (Ding *et al*., 2016). Foliar application of magnesium solution was reported to decrease DNA damage in *Vicia faba* by reducing the concentration of H_2O_2 (Siddiqui et al., 2016). Xue et al. (2016) reported a reduction of ROS production in cucumber seedlings of variety 'Jinchun 4' sprayed with melatonin grown under high temperature conditions.

2.1.9 Chalkiness

Chalkiness is an important parameter which determines quality of rice grain. Grain quality is affected by both day and night temperature and more chalky grains are observed when there is a large difference between these temperatures (Yoshida and Hara, 1977). Yoshida (1981) reported that high temperature stress at the ripening stage decreased grain weight and grain filling resulting in white chalky grains in rice. According to Mitsui *et al.* (2016) high temperature stress at grain- filling stage decreased starch synthesizing enzymes and increased starch degrading enzymes interfering in starch assimilation, leading to chalkiness.

Exogenous application of micro-nutrient mixtures (40% zinc sulfate, 6% manganese sulfate, 1% ferric chloride) in two rice decreased amylose content, chalkiness degree and chalky rice percentage (Wang *et al.*, 2017).

2.1.10 Pollen viability

An average daily temperature higher than 35^0 C during the reproductive growth period of rice for several days can lead to pollen sterility and cause yield loss (Matsui *et al*., 2001). Exposure of rice to high temperature stress for even a short period of time can significantly lessen pollen viability which lead to decrease in grain yield (Song *et al*., 2001, Wassmann and Dobermann, 2007). Tao *et al*. (2008) also observed the same result that high temperature at flowering stage causes a drastic reduction in grain yield due to pollen sterility and poor seed setting. Extend of decrease in pollen viability is reported to depend on duration of exposure of plants to high temperature (Tang *et al*., 2008). Rice plants subjected to high temperature stress during reproductive stage exhibited spikelet sterility (Shahid *et al*., 2018). Plants grown under high temperature condition showed minimum pollen viability compared to control plants (Amjath, 2018).

Exogenous application of boron solution in three rice cultivars (Annapurna, Naveen and Shatabdi) showed a significantly higher number of viable pollen as compare to control and different varieties exhibited variation in number of viable pollens (Shahid *et al*., 2018). Rice plants supplied with salicylic acid (0.1 and 1.0 mM concentration) recorded higher pollen viability than control plants under high temperature stress conditions (Feng *et al*., 2018).

2.1.11 Membrane stability

Under high temperature stress ROS production surpasses normal levels in plants, which interferes with cellular integrity (Kreiner *et al*., 2002) and as a result membrane thermal stability (MTS) will be lost. According to Howarth (2005) reduction of membrane integrity is due to slow heat exposure. Plants exposed to high temperature showed increase in electrolytic leakage as compared to plants grown under normal temperature condition (Shahid *et al*., 2018).

Studies conducted in mung bean showed that exogenous application of ascorbic acid resulted in significant decrease of electrolyte leakage (Kumar *et al*., 2011). Improvement in cell membrane integrity was also recorded in cotton seedlings treated with salicylic acid as compared to non-treated plants (Galani *et al*., 2016). Exogenous spray of boron solution (0.2 % foliar spray) reduced electrolytic leakage of rice plants grown under high temperature conditions by 13.9 % compared to control plants (Shahid *et al*., 2018).

2.2 Effect of elevated temperature and exogenously applied chemical ameliorants on morphological parameters of rice

2.2.1 Plant height

Height of rice plant is reported to increase with increase in temperature up to 35ºC (Osada *et al*., 1973 and Yoshida, 1981). A distinct variation in plant height was observed by Oh-e *et al.* (2007) in rice cultivars grown under different temperature gradient chambers. Under high temperature stress there was decrease in number of tillers, plant height and biological yield in rice (Mitra and Bhatia, 2008).

Salicylic acid foliar treatment was found to enhance seedling growth in wheat grown under high temperature condition (Shakirova, 2007). Galani *et al*. (2016) reported that exogenous application of different concentration of salicylic acid under high temperature condition lead to increased shoot length. Cucumber plants grown under high temperature condition sprayed with glutathione showed an increased shoot length in comparison with plants grown without glutathione spray (Ding *et al*., 2016). Exogenous application of salicylic acid resulted in increased shoot length in rice plants grown under high temperature stress condition in growth chambers (Feng *et al*., 2018).

2.2.2 Number of tillers

Tillering in rice plant was reported to be maximum when day temperature and night temperature are 25^0 C and 20^0 C respectively (Sato, 1972). According to Yoshida (1973) up to 31º C number of rice tillers will increase with increase in temperature. Oh-e *et al.* (2007) observed that higher temperatures reduced the number of tillers in rice. Reduced

number of tillers was recorded in rice plants grown under temperature higher than normal range (Mitra and Bhatia. 2008). Wassmann *et al*. (2009) also reported reduction in tiller number due to higher temperature.

Shahid *et al*. (2018) noted that exogenous application of boron solution in three rice varieties Annapurna, Naveen and Shatabdi leads to an increase in number of tillers. Goldbach *et al*. (2001) also observed increase in tiller number by exogenous application of boron solution.

2.3 Effect of elevated temperature and exogenously applied chemical ameliorants on yield of rice

Response of rice to high temperature was found to be influenced by the growth stage of the crop. Highest sensitivity was recorded at reproductive stage. Panicle differentiation needs temperatures ranging from 18 to 30^0 C.

Temperature less than 20 ^{0} C during early tillering stage is reported to increase number of panicles (Yamamoto *et al*., 1985), but at late growth stages including maturity high temperatures decreases the number of panicles. Plants exposed to higher temperature during seed set, resulted in spikelet sterility and yield loss (Nakagawa *et al*., 2003).

High temperature stress during flowering affected cellular structures of vegetative and reproductive organs, thus, decreasing grain yield in rice (Morita *et al*., 2005). From a study conducted in two varieties of rice, Tang *et al*. (2008) found that the percentage of fertility decreased drastically in both varieties under 39^0 C at flowering stage. Jagadish *et al*. (2010) reported that yield loss under high temperature stress is due to decreased pollen viability, abnormal anther dehiscence and impaired pollination which ultimately lead to floret sterility.

Satake and Yoshida (1978) reported that exposure of spikelets to a temperature beyond 35^0 C for more than four days during flowering period caused formation of sterile spikelet and lead to low seed set. Amjath (2018) reported increased chaff percentage (up to 50 percentage) in plants grown under high temperature conditions in rice variety Uma.

From a study conducted in wheat, Arif *et al.* (2006) revealed that foliar application of micro nutrients (at the rate of 0.8 g L^{-1}) showed significant increase in number of spikes m^{-2}, number of grains per spikelet, thousand grain weight and grain yield.

Study conducted in rice cultivar Changyou 1 (heat-sensitive) at experimental farm of China National Rice Research Institute revealed that foliar spray of salicylic acid (1 mmol L^{-1} and 10 mmol L^{-1} concentration) resulted in higher grain yield, spikelet number per panicle and setting rate as compare to control under heat stress conditions (zhang, 2017) .

Exogenous application of micro-nutrient mixture (40% zinc sulfate, 6% manganese sulfate, 1% ferric chloride) enhanced yield by improvement in grain numbers per panicle, filled grain (%) and 1000 grain weight (Wang *et al.*, 2017).

Exogenously applied boron reported to increase number of panicles per plant (Rashid *et al*., 2007), Spikelets per panicle and 1000 seed weight (Rehman *et al*., 2014) and lead to overall increase in grain yield (Dunn *et al*., 2005) in different varieties of rice.

Higher straw yield was reported by exogenous application of salicylic acid in creeping bentgrass (Larkindale and Huang, 2005) and kentucky blue grass (He *et al*., 2005)

Materials and methods

3. MATERIALS AND METHODS

Investigation on mitigation of high temperature stress in rice (*Oryza sativa* L.) by use of chemical ameliorants was carried out as a pot culture study followed by a field trial at College of Horticulture, Vellanikkara. Materials used and methods adopted for the study is detailed in this chapter.

3.1 Experiment 1 – Pot culture study

3.1.1 Location

Pot culture experiment was conducted in glass house at College of Horticulture Vellanikkara. The geographical co-ordinates of the location are 10°32 N and 76°16 E.

3.1.2 Season

The experiment was conducted in the Puncha season (January – April, 2019).

3.1.3 Variety

Rice varieties Uma (MO-16), developed by Rice Research Station, Mancompu and Manuratna developed by Agricultural Research Station, Mannuthy were used in this study. Uma is a red kernelled, long, bold, non-lodging medium duration variety (115-120 days). Manuratna is red kernelled, non-lodging, short duration variety (95-99 days) with medium sized grains.

3.1.4 Details of experiment

The experiment was carried out in mud pots of nine inch height.Soil was collected from paddy field of. Seeds of varieties Uma and Manuratna were germinated in plastic trays and 10 day old seedlings were planted in pots (two seedlings per pot) supplied with basal dose of fertilizers and kept in the glasshouse. Experiment was laid out in completely randomized design (CRD) with 3 factors and 3 replications.

Factor 1 – Variety

V1 – Uma

V2 – Manuratna

Factor 2 – Chemical ameliorants (Table 1.)

Sl. NO	Ameliorants used	Concentration
1	T_1 - Ascorbic acid	10 µM
2	T_2 - Glutathione	1 mM
3	T_3 - Melatonin	200 µM
4	T_4 - Salicylic acid	1 mM
5	T_5 - Hoagland solution	¼ Strength
6	T_6 – Water spray	Water spray
7	T_7 - Control	No spray

Factor 3 – Time of spray

- **S_1** - At active growth stage
- **S_2** - Booting stage
- **S_3** - At both active growth and booting stages

Layout of experiment

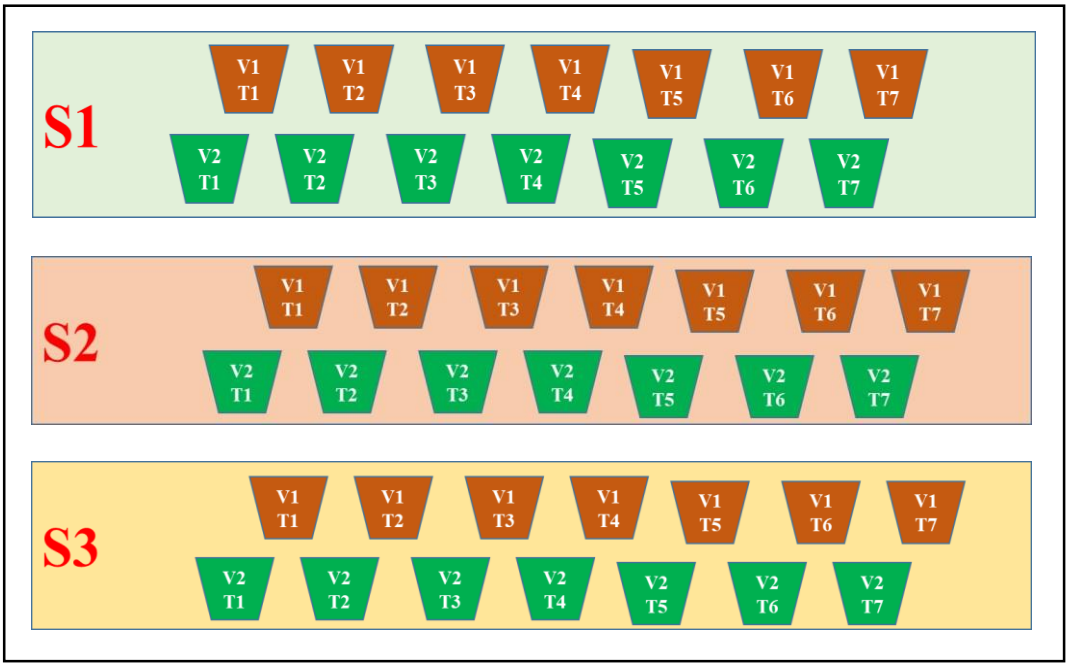

Plate 1. Layout of experiment 1 – Glass house study

3.1.1 Observations recorded

Samples were taken one week after foliar application of ameliorants for estimation of physiological and biochemical parameters. Pollen viability and chalkiness of grain were observed after flowering and harvest respectively.

3.1.5.1 Physiological observations

a) Leaf gas exchange parameters

Photosynthetic rate (μ mol CO_2 m^{-2} s^{-1}) and stomatal conductance (mol H_2O m^{-2} s^{-1}) were recorded using portable photosynthesis system (PPS- Model –LI-6400 of Licor Inc. Lincoln, Nebraska, USA). Readings were made on physiologically active leaf (3rd leaf from top) and a total of three plants were selected from each treatment for measurement. Observations were recorded between 9.00 am to 10.30 am.

b) Membrane stability

Membrane stability was determined by measuring electrolytic leakage (Compact conductivity meter, LAQUAtwin-EC-33) from leaf tissue after exposure to high temperature (Sullivan, 1972). Data was interpreted assuming that, if membrane injury index in control tissues was 100, any value higher than 100 indicates extend of membrane stability in respective treatments.

$$\textbf{Membrane injury index} = \frac{\textit{Electric conductivity of control}}{\textit{Electric conductivity of treatment}} \boldsymbol{X\ 100}$$

c) Pollen viability

Anther was taken from different plants of all treatments and viability was determined by staining with methylene blue. Pollen grains stained uniformly were considered as viable. Pollen viability was estimated as ratio of number of stained pollen to total number of pollen grains visible in the microscopic field and expressed in percentage. An average of five field was observed for each treatment.

d) Chalkiness of grain

Chalkiness of grain was determined qualitatively by taking photographs. Grains were dehusked and placed on purity work board. The chalky portions of grains appeared as translucent portions.

3.1.5.2 Biochemical characters

a) Chlorophyll content

Chlorophyll a, chlorophyll b and total chlorophyll were measured by the method adopted by Hiscox and Israelstam (1979). Chlorophyll content was estimated in spectrophotometer (SAKS QUQNTUM VIS 50) at three wavelengths 663 nm, 645nm and 652 nm. It was expressed as milligram per gram fresh weight of plant tissue. Calculation was done by using the following formula.

$$\textbf{Chlorophyll a} = [(12.7 \times A663) - (2.69 \times A645)] \times V/1000 \times W$$

$$\textbf{Chlorophyll b} = [(22.9 \times A645) - (4.68 \times A663)] \times V/1000 \times W$$

$$\textbf{Total chlorophyll} = [(20.2 \times A663) + (8.02 \times A645)] \times V/1000 \times W$$

Where,

A = Absorption at given wavelength

V = Total volume of sample in extraction medium

W = Weight of sample

b) Chlorophyll stability index

Chlorophyll stability index was calculated by method described by Murthy and Majumdhar (1962).

$$\textbf{Chlorophyll stability index} = \frac{\textbf{Total chlorophyll content (Treatment)}}{\textbf{Total chlorophyll content (Control)}} \textbf{ X 100}$$

c) Total soluble protein

The total soluble protein was estimated using the method suggested by Lowry *et al*. (1951) and expressed as mg g^{-1} of fresh weight.

d) Proline content

Proline content was estimated by the method of Bates *et al*. (1973) and expressed as mg g^{-1} of fresh weight. Absorbance was taken at 520 nm, using spectrophotometer.

e) Nitrate reductase activity

Nitrate reductase enzyme activity in leaf was estimated by the method given by Hageman and Flesher (1960). Nitrite formed was estimated by the method described by Nicholas *et al*. (1976), by measuring absorbance of pink colour at 540 nm using spectrophotometer and expressed in µM of NO^{2-} formed per g fresh weight per hour.

f) IAA Content

Indole Acetic Acid (IAA) was estimated by the method proposed by Parthasarathy *et al*. (1970) with little modification using Garden weber reagent. IAA content was expressed as mg of unoxidised auxin g^{-1} fresh weight.

g) Histochemical detection of super oxide

Histochemical detection of super oxide was done by using nitrotetrazolium blue chloride (NBT). NBT reacts with superoxide and forms dark blue colour (Kumar *et al*, 2014). Changes in colour gradient was scored visually.

h) Histochemical detection of hydrogen peroxide

Histochemical detection of hydrogen peroxide was done by using 3, 3-Diaminobenzidine (DAB). DAB is oxidized by H_2O_2 and produces reddish brown colour (Kumar *et al*, 2014). Changes in colour gradient was scored visually.

3.1.5.3 Weather parameters

Maximum and minimum temperature and relative humidity were recorded using hygrometer (HTC, 288-CTH) from glass house at fortnightly intervals.

Interval	Temperature (^{0} C)	Relative Humidity (%)
Jan 30 – Feb 11	36.5	58
Feb 12 – Feb 25	38.05	56
Feb 26 – Mar 11	37.1	67
Mar 12 – mar 25	39.25	58
Mar 26 – Apr 8	39.14	66
Apr 9 – Apr 22	39.05	69
Apr 23- May 6	37.47	72
May 7 - May 20	37.24	74
May 21- June 3	38.15	73

Table 2. Variation in temperature (^{0}C) during the growth period inside glasshouse

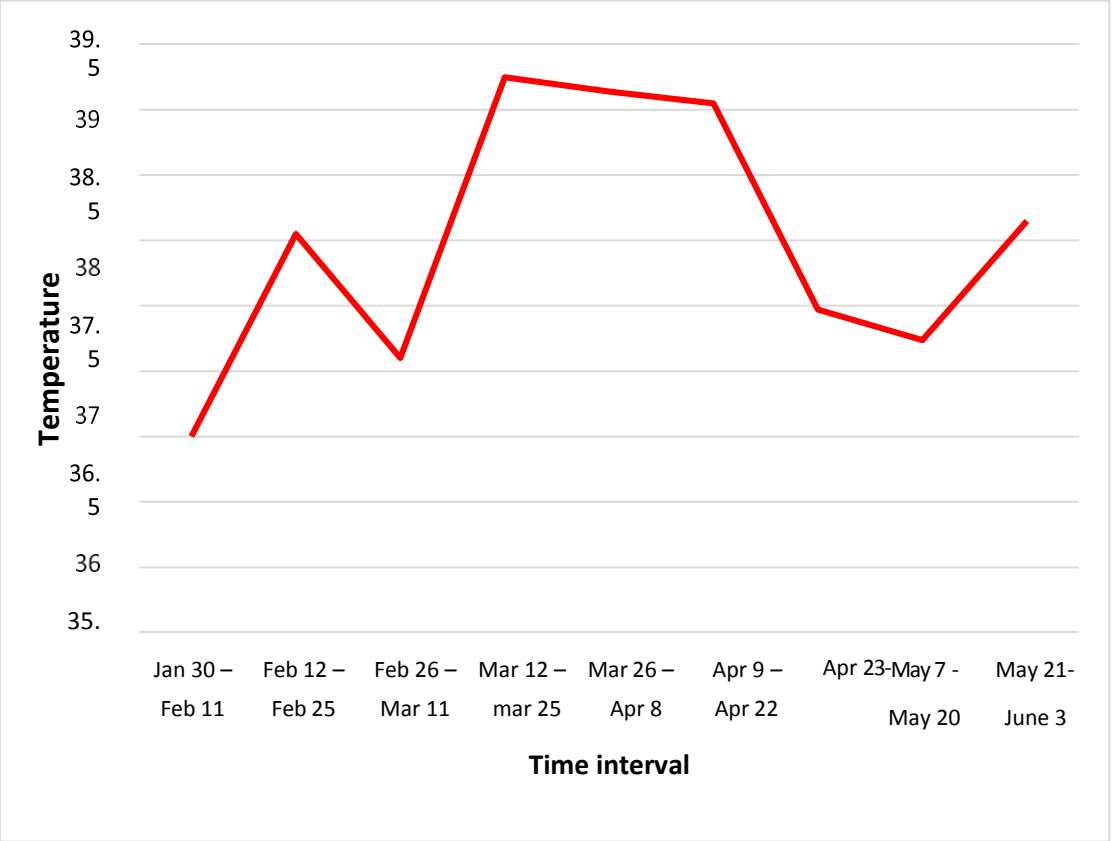

Fig. 1 Variation in temperature (^{0}C) during the growth period inside glasshouse

Plate 2. Inside view of glass house

Plate 3. Tillering stage

Plate 4. Booting stage

Plate 5. Flowering stage

3.2 Experiment 2 - Field study

3.2.1 Location

Field experiment was conducted at Agronomy farm, College of Horticulture, Vellanikkara. The geographical co-ordinates of the location are 10°33 N and 76°16 E.

3.2.2 Season and variety

The experiment was conducted in Puncha season (February – May, 2020). The same varieties Uma and Manuratna were used for field trial.

3.2.3 Details of experiment

Rice seeds were grown in mud pots and transplanting was done on the 19th day. Field trial was laid out in randomized block design (RBD) with 2 factors and 2 replications.

Factor 1 – Variety

Factor 2 – Ameliorants Plot size - 2 × 2 m (4 m^2)

Spacing – 15 x 20 cm^2

Treatments were applied as foliar spray at booting stage. 5 plants were tagged from each plot and biometric observations were recorded at 20 days interval. Yield attributes were measured after harvest.

Layout of experiment 2

Plate 6. Layout of field

Plate 7. Field view

3.2.4 Chemical ameliorants (Table 3)

Sl. NO	Ameliorants used	Concentration
1	T_1 - Ascorbic acid	10 µM
2	T_2 - Glutathione	1 mM
3	T_3 - Melatonin	200 µM
4	T_4 - Salicylic acid	1 mM
5	T_5 - Hoagland solution	¼ Strength
6	T_6 – Water spray	Water spray
7	T_7 - Control	No spray

3.2.5 Observations recorded

3.2.5.1 Morphological observations

a) Plant height

From each plot five plants were tagged. Throughout the experiment, morphological parameters of these plants were recorded. Before panicle initiation, plant height was measured from base to the longest leaf and after panicle initiation, measurement was taken from base to the longest panicle. It was expressed in cm.

b) Tiller decline percentage

Tiller decline percentage =

$$\frac{\boldsymbol{Number\ of\ tillers\ at\ active\ tillering\ stage - productive\ tiller}}{\boldsymbol{Number\ of\ tillers\ at\ active\ tillering\ stage}}\ \boldsymbol{X\ 100}$$

3.2.5.2 Growth indices

Growth indices were computed at 20 days interval up to harvest stage. Sampling unit contained 6 plants per treatment (three from each replication). Plant samples were uprooted and dried and growth indices were calculated as per the procedures given below.

a) Relative growth rate

Relative growth rate is the rate of increase in the dry weight per unit time. It was estimated using the formula suggested by Blackman (1919) and expressed as mg g^{-1} d^{-1}.

$$\boldsymbol{RGR} = (\log \boldsymbol{W2} - \log \boldsymbol{W1})/\boldsymbol{T2} - \boldsymbol{T1}$$

W1 and W2 = Dry weight of plants at time intervals T1and T2 respectively.

b) Crop growth rate

Crop growth rate (CGR) is the rate of dry matter production per unit ground area per unit time. It was calculated using the formula of Watson (1952) and expressed as mg cm^{-2} d^{-1}.

$$CGR = \frac{W2-W1}{\boldsymbol{T2}-T1} \text{ X } \frac{1}{A}$$

W1 and W2 = Dry weight of plants at time intervals T1 and T2 respectively

A = Unit land area occupied by the plant (cm^2)

3.2.5.3 Yield and Yield attributes

a) Number of productive tillers per m^2

Number of productive tillers were counted by selecting 5 hills from each replication during harvest and expressed as productive tiller per m^2.

b) Number of spikelets per panicle

Five panicle were selected randomly from each treatment for counting the number of spikelet. Mean value was expressed as number of spikelets per panicle.

c) Chaff percentage

Samples were collected from randomly selected ten plants. Chaff percentage was calculated and expressed in percentage.

d) 1000 grain weight

1000 grains were randomly selected from each replication and weighed using electronic balance. It was recorded as 1000 grain weight in gram.

e) Grain yield

Manual harvest was done in all plots. Grain yield was taken using electronic balance and recorded as grain yield per plot in grams.

f) Straw yield

Straw weight was taken after removing grains using electronic balance and recorded as straw yield per plot in grams.

3.3 Statistical analysis

Statistical analysis was done in R software for both the experiment.

Result

4. RESULTS

4.1 Effect of ameliorants sprayed during different growth stages on physiological and biochemical characters (Pot culture study – Experiment 1)

A pot culture experiment was laid out in the glass house of COH, Vellanikkara during the third crop season (February to May, 2019). Effect of high temperature on physiological parameters were studied to identify the best stage for application of ameliorants to get the maximum response from the plants.

4.1.1 Chlorophyll a content

Table 4. Effect of ameliorants and time of spray on Chlorophyll a (mg g^{-1} fr.wt) content in Uma and Manuratna

		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
S_1	V_1	0.447	0.663	0.950	0.540	0.740	0.323	0.247	0.619 ^{C}
	V_2	0.550	0.833	1.087	0.647	0.970	0.433	0.240	
S_2	V_1	0.570	0.757	1.077	0.640	0.867	0.470	0.450	1.017 ^{b}
	V_2	1.273	1.530	1.967	1.327	1.773	0.950	0.600	
S_3	V_1	1.320	1.457	2.050	1.647	1.867	0.830	0.727	1.488 ^{a}
	V_2	1.370	1.863	2.190	1.537	2.060	1.083	0.840	
	Mean	0.92 ^{e}	1.18 ^{c}	1.55 ^{a}	1.05 ^{d}	1.37 ^{b}	0.68 ^{f}	0.51 ^{g}	
Varietal mean		Uma – 0.887 ^{b}				Manuratna – 1.19 ^{a}			

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	**0.007**	**0.015**
Between ameliorants	**0.014**	**0.028**
Between time of sprays	**0.009**	**0.018**
Variety-Treatment-Time of Spray	**0.035**	**0.070**

T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control

V1 – Uma V2 – Manuratna

S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage

The effect of the varieties Uma (V_1) and Manuratna (V_2) on chlorophyll a content was similar (Table 4). Among the varieties Manuratna (1.19 mg g^{-1}fr.wt) had significantly higher chlorophyll a content than Uma (0.887 mg g^{-1}fr.wt).

Compared to control (T_7- 0.51) all the ameliorants significantly improved the chlorophyll a content. Among treatments plants sprayed with melatonin (T_3- 1.55 mg g^{-1}fr.wt) showed significantly higher chlorophyll a content followed by Hoagland solution (T_5- 1.37 mg g^{-1}fr.wt) and glutathione (T_2- 1.18 mg g^{-1}fr.wt). Low chlorophyll a content was recorded in plants sprayed with water (T_6- 0.681 mg g^{-1}fr.wt) followed by ascorbic acid (T_1- 0.921 mg g^{-1}fr.wt) and salicylic acid (T_4– 1.05 mg g^{-1}fr.wt).

Spraying of ameliorants at both tillering stage and booting stage (S_3– 1.48 mg g^{-1}fr.wt) has given a better result which is followed by spraying at booting stage (S_2– 1.01 mg g^{-1}fr.wt) alone which was significantly superior to spraying at active tillering (S_1– 0.619 mg g^{-1}fr.wt) stage alone.

Generally the variety Manuratna showed significantly higher response to all the ameliorant treatments at all three stages of application as compared to Uma this was also true for untreated control. The interaction effect was also significant.

4.1.2 Chlorophyll b content

Table 5. Effect of ameliorants and time of spray on Chlorophyll b content (mg g^{-1} fr.wt) in Uma and Manuratna

		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
S_1	V_1	0.270	0.410	0.510	0.364	0.470	0.170	0.093	0.34 [c]
	V_2	0.290	0.453	0.550	0.426	0.490	0.190	0.078	
S_2	V_1	0.304	0.433	0.523	0.383	0.480	0.206	0.110	0.36 [b]
	V_2	0.313	0.470	0.566	0.436	0.513	0.216	0.123	
S_3	V_1	0.336	0.449	0.546	0.407	0.506	0.240	0.128	0.38 [a]
	V_2	0.350	0.490	0.590	0.456	0.533	0.250	0.146	
	Mean	0.310 e	0.451 c	0.547 a	0.412 d	0.498 b	0.212 f	0.113 g	
Varietal mean		Uma – 0.349 [b]				Manuratna – 0.377 [a]			

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	0.003	0.007
Between ameliorants	0.006	0.013
Between time of sprays	0.004	0.008
Variety-Treatment-Time of Spray	0.016	0.033

T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control

V1 – Uma V2 – Manuratna

S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage

The effect of the varieties Uma (V_1) and Manuratna (V_2) on chlorophyll b content was similar. Among the varieties Manuratna (0.377 mg g^{-1}fr.wt) showed higher chlorophyll b content than Uma (0.349 mg g^{-1} fr.wt) (Table 5).

Compared to control (T_7 -0.113 mg g^{-1} fr.wt) all the ameliorants improved the chlorophyll b content. Among treatments plants sprayed with melatonin (T_3 – 0.547 mg g-^{1} fr.wt) showed a higher chlorophyll b content followed by Hoagland solution (T_5 – 0.498 mg g^{-1} fr.wt) and glutathione (T_2 – 0.451 mg g^{-1} fr.wt). Low chlorophyll b content was recorded in plants sprayed with water (T_6 – 0.212 mg g^{-1} fr.wt) followed by ascorbic acid (T_1 – 0.310 mg g^{-1} fr.wt) and salicylic acid (T_4 – 0.412 mg g^{-1} fr.wt).

Spraying of ameliorants at both tillering stage and booting stage (S3– 0.387 mg g^{-1} fr.wt) has given a better result when compare to giving spray at only one stage (Active tillering (S1 – 0.340 mg g^{-1} fr.wt) or booting stage (S2 – 0.3621 mg g^{-1} fr.wt). The interaction effect was also significant.

Application of melatonin in Manuratna at both active tillering and booting stage gave the best result followed by application of melatonin at only booting stage in Manuratna.

4.1.3 Total chlorophyll content

Table 6. Effect of ameliorants and time of spray on total chlorophyll content (mg g^{-1} fr.wt) of Uma and Manuratna

		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
S_1	V_1	0.717	1.073	1.460	0.904	1.210	0.493	0.340	0.95 ^{c}
	V_2	0.840	1.287	1.637	1.073	1.460	0.623	0.318	
S_2	V_1	0.874	1.190	1.600	1.024	1.347	0.677	0.557	1.38 ^{b}
	V_2	1.587	2.00	2.533	1.763	2.287	1.166	0.723	
S_3	V_1	1.65	1.906	2.596	2.054	2.373	1.070	0.855	1.87 ^{a}
	V_2	1.720	2.353	2.780	1.993	2.593	1.333	0.987	
Mean		1.23 e	1.63 c	2.10 a	1.46 d	1.87 b	0.893 f	0.630 g	
Varietal mean		Uma – 1.23 ^{b}				Manuratna – 1.57 ^{a}			

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	**0.009**	**0.018**
Between ameliorants	**0.016**	**0.033**
Between time of sprays	**0.011**	**0.022**
Variety-Treatment-Time of Spray	**0.041**	**0.082**

T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control

V1 – Uma V2 – Manuratna

S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage

The effect of the varieties Uma (V_1) and Manuratna (V_2) on total chlorophyll content was similar. Among the varieties Manuratna (01.57 mg g^{-1}fr.wt) showed significantly higher total chlorophyll content than Uma (1.23 mg g^{-1}fr.wt) (Table 6).

Compared to control all the ameliorants improved the total chlorophyll content. Among treatments plants sprayed with melatonin (T_3– 2.10 mg g^{-1}fr.wt) showed a higher total chlorophyll content followed by Hoagland solution (T_5 – 1.87 mg g^{-1}fr.wt) and glutathione (T_2 – 0.1.63 mg g^{-1}fr.wt). Low total chlorophyll content was recorded in plants sprayed with water (T_6 – 0.893 mg g^{-1}fr.wt) followed by ascorbic acid (T_1 – 1.23 mg g^{-1}fr.wt) and salicylic acid (T_4 – 1.46 mg g^{-1}fr.wt).

Spraying of ameliorants at both tillering stage and booting stage (S_3 –1.87 mg g^{-1}fr.wt) has given a better result in both varieties when compare to giving spray at only one stage, active tillering (S_1 – 0.959 mg g^{-1}fr.wt) or booting stage (S_2 – 1.38 mg g^{-1}fr.wt).

Highest total chlorophyll content was noted in Manuratna sprayed with melatonin at both active tillering and booting stage. Exogenous application of melatonin at only booting stage also gave a better result in Manuratna.

4.1.4 Chlorophyll stability index

Table 7. Effect of ameliorants and time of spray on chlorophyll stability index of Uma and Manuratna

		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
S_1	V_1	211	316	429	266	356	145	100	291 [a]
	V_2	262	402	511	335	456	195	100	
S_2	V_1	159	216	291	186	245	123	101	213 [b]
	V_2	220	278	352	245	316	162	100	
S_3	V_1	195	224	305	242	279	126	101	205 [c]
	V_2	176	240	284	203	265	136	101	
Mean		203 e	279 c	362 a	246 d	319 b	147 f	100 g	
Varietal mean		Uma – 219 [b]				Manuratna – 254 [a]			

Critical difference for comparison

	SE ± Mean	CD at 5 %
Between varieties	1.67	3.32
Between ameliorants	3.12	6.21
Between time of sprays	2.04	4.06
Variety-Treatment-Time of Spray	7.69	15.22

T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control

V1 – Uma V2 – Manuratna

S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage

The effect of the varieties Uma (V_1) and Manuratna (V_2) on chlorophyll stability index was similar. Among the varieties Manuratna (254) showed significantly higher chlorophyll stability index value than Uma (219) (Table 7).

Compared to control all the ameliorants improved the chlorophyll stability index. Among treatments plants sprayed with melatonin (T_3– 362) showed a higher chlorophyll stability index value followed by Hoagland solution (T_5 – 319) and glutathione (T_2 – 279). Low chlorophyll stability index was recorded in plants sprayed with water (T_6 – 147) followed by ascorbic acid (T_1 – 203) and salicylic acid (T_4 – 246).

4.1.5 Total soluble protein

Table 8. Effect of ameliorants and time of spray on total soluble protein content (mg g^{-1} fr.wt) in Uma and Manuratna

		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
S_1	V_1	5.99	6.83	8.62	5.56	5.16	4.60	4.44	5.44 b
	V_2	5.31	6.42	8.24	3.60	5.06	3.35	3.06	
S_2	V_1	4.78	5.15	6.42	4.46	3.80	2.81	2.73	3.44 c
	V_2	3.92	4.26	4.59	3.62	3.28	2.33	2.17	
S_3	V_1	6.70	7.60	8.88	5.81	5.40	4.85	4.77	5.98 a
	V_2	6.50	7.40	8.72	4.83	5.31	3.61	3.40	
	Mean	5.53 c	6.27 b	7.57 a	4.64 d	4.66 d	3.59 e	3.42 f	
Varietal mean		Uma – 5.49 a				Manuratna – 4.71 b			

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	**0.042**	**0.084**
Between ameliorants	**0.079**	**0.158**
Between time of sprays	**0.052**	**0.103**
Variety-Treatment-Time of Spray	**0.194**	**0.387**

T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control
V1 – Uma V2 – Manuratna
S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage

The effect of the varieties Uma (V_1) and Manuratna (V_2) on total soluble protein content was similar. Among the varieties Uma (5.49 mg g^{-1} fr.wt) had significantly higher total soluble protein content than Manuratna (4.71 mg g^{-1} fr.wt) (Table 8).

Compared to control (T_7 -3.42 mg g^{-1} fr.wt) all the ameliorants significantly improved the soluble protein content. Among treatments, plants sprayed with melatonin (T_3- 7.57 mg g^{-1} fr.wt) showed higher soluble protein content followed by glutathione (T_2 – 6.27 mg g^{-1} fr.wt) and ascorbic acid (T_1 – 5.53 mg g^{-1} fr.wt). Low soluble protein content was recorded in plants sprayed with water spray (T_6 -3.59 mg g^{-1} fr.wt) followed by salicylic acid (T_4 - 4.64 mg g^{-1} fr.wt) and Hoagland solution (T_5 - 4.66 mg g^{-1} fr.wt). Effect of Hoagland solution and salicylic acid were on par.

Spraying of ameliorants at both tillering and booting stages (S_3 –5.983 mg g^{-1} fr.wt) has given significantly better result when compared to giving spray at only one stage, which was followed by spraying at active tillering stage (S_1 – 5.446 mg g^{-1} fr.wt) and at booting stage (S_2 – 3.879 mg g^{-1} fr.wt). Melatonin spray at both tillering and booting stage in Uma recorded highest soluble protein content (V_1 T_3 S_3 - 8.88 mg g^{-1} fr.wt). Melatonin application at tillering stage in Uma also gave a better result.

4.1.1 Proline content

Table 9. Effect of ameliorants and time of spray on proline content (µmol g^{-1} fr. wt) in Uma and Manuratna

		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
S_1	V_1	234	293	258	190	214	168	152	223.27 c
	V_2	248	308	275	212	227	184	164	
S_2	V_1	253	328	274	186	231	185	172	245.47 b
	V_2	273	344	288	236	254	222	190	
S_3	V_1	272	358	294	238	259	219	195	269.92 a
	V_2	287	376	302	252	268	240	218	
	Mean	261.22 c	334.31 a	282.00 b	218.94 e	242.16 d	203.11 f	181.83 g	
Varietal mean		Uma – 255 a				Manuratna – 236 b			

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	1.88	3.73
Between ameliorants	3.51	6.98
Between time of sprays	2.3	4.57
Variety-Treatment-Time of Spray	NS	NS

T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control

V1 – Uma V2 – Manuratna

S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage

The effect of the varieties Uma (V_1) and Manuratna (V_2) on proline content was similar. Among the varieties Manuratna (255.6 µmol g^{-1} fr.wt) had significantly higher proline content than Uma (236.8 µmol g^{-1} fr.wt). There was no significant difference between effects of ameliorants on Uma and Manuratna (Table 9).

Compared to control all the ameliorants improved the proline content. Among treatments, plants sprayed with glutathione (T_2 – 334.31 µmol g^{-1}fr.wt) showed higher proline content followed by melatonin (T_3– 282.00 µmol g^{-1} fr.wt) and ascorbic acid (T_1– 261.22 µmol g^{-1} fr.wt). Low proline content was recorded in plants sprayed with water (T_6– 203.11 µmol g^{-1} fr.wt) followed by salicylic acid (T_4 – 218.94 µmol g^{-1} fr.wt) and Hoagland solution (T_5 – 242.16 µmol g^{-1} fr.wt).

Spraying of ameliorants at both tillering and booting stages (S_3 – 269.92 µmol g^{-1} fr.wt) has given significantly higher result when compared to a single spray at only one stage (Active tillering or booting stage). Spraying at booting stage (S_2– 245.47 µmol g^{-1} fr.wt) gave significantly better result as compare to active tillering stage (S_1 – 223.27 µmol g^{-1} fr.wt).

Highest proline content was observed in Manuratna sprayed with glutathione at both tillering and booting stage. A single spray of glutathione at active tillering stage also gave better result in Manuratna.

4.1.2 Nitrate reductase enzyme content

Table 10. Effect of ameliorants and time of spray on Nitrate reductase enzyme content (µ mol of NO^{2-} formed g^{-1} FW h^{-1}) in Uma and Manuratna

		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
S_1	V_1	333	350	356	403	401	325	316	351.61 c
	V_2	328	340	347	398	385	320	312	
S_2	V_1	434	445	448	460	450	415	375	429.14 b
	V_2	422	440	442	451	449	408	363	
S_3	V_1	475	488	496	514	501	466	449	482.41 a
	V_2	469	484	492	510	498	462	445	
	Mean	410.59 e	425.12 d	430.65 c	456.27 a	447.7 b	399.85 f	377.08 g	
Varietal mean		Uma – 424.24 [a]				Manuratna – 417.86 [b]			

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	0.356	0.708
Between ameliorants	0.666	1.32
Between time of sprays	0.436	0.867
Variety-Treatment-Time of Spray	1.63	3.24

T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control

V1 – Uma V2 – Manuratna

S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage

The effect of the varieties Uma (V_1) and Manuratna (V_2) on nitrate reductase enzyme content was similar. Among the varieties Uma showed significantly higher nitrate reductase enzyme activity than Manuratna (Table 10).

Compare to control all the ameliorants significantly improved the nitrate reductase enzyme activity. Among treatments plants sprayed with salicylic acid (T_4 – 456.27 µ mol of NO^{2-} formed g^{-1} FW h^{-1}) showed a higher nitrate reductase enzyme activity followed by Hoagland solution (T_5 – 447.79 µ mol of NO^{2-} formed g^{-1} FW h^{-1}) and melatonin (T_3 – 430.65 µ mol of NO^{2-} formed g^{-1} FW h^{-1}).

Low nitrate reductase enzyme activity was recorded in plants sprayed with water (T_6 – 399.85 µ mol of NO^{2-} formed g^{-1} FW h^{-1}) followed by ascorbic acid (T_1 – 410.59 µ mol of NO^{2-} formed g^{-1} FW h^{-1}) and glutathione (T_2 – 425 µ mol of NO^{2-} formed g^{-1} FW h^{-1}).

Spraying of ameliorants at both tillering stage and booting stage (S_3 – 482 µ mol of NO^{2-} formed g^{-1} FW h^{-1}) has given significantly higher nitrate reductase enzyme content when compare to giving spray at only one stage. Spraying at booting stage (S_2 – 429.14 µ mol of NO^{2-} formed g^{-1} FW h^{-1}) resulted in significantly higher nitrate reductase enzyme content than spraying at active tillering stage (S_1 – 351.6 µ mol of NO^{2-} formed g^{-1} FW h^{-1}).

Highest nitrate reductase enzyme content was observed in Uma sprayed with salicylic acid at both tillering and booting stage. Salicylic acid spray at booting stage alone also gave a good result in Uma. Response of different varieties to chemicals on nitrate reductase enzyme content was not significant.

4.1.3 IAA content

Table 11. Effect of ameliorants and time of spray on IAA content (mg of unoxidised auxin g^{-1} h^{-1}) in Uma and Manuratna

		T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
S_1	V_1	2.53	3.55	4.16	3.23	5.31	2.68	2.39	3.47 b
	V_2	2.65	2.93	5.49	3.00	5.37	2.81	2.61	
S_2	V_1	2.49	3.43	3.69	2.81	5.09	2.50	2.31	3.31 c
	V_2	2.41	2.87	5.40	2.89	5.21	2.76	2.50	
S_3	V_1	3.07	6.50	6.68	6.89	6.91	3.80	2.68	4.68 a
	V_2	2.85	3.09	5.69	6.03	5.85	2.89	2.67	
	Mean	2.66 f	3.72 d	5.18 b	4.14 c	5.62 a	2.90 e	2.52 g	
Varietal mean		Uma – 3.93 a				Manuratna – 3.71 b			

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	0.006	0.013
Between ameliorants	0.012	0.024
Between time of sprays	0.008	0.016
Variety-Treatment-Time of Spray	0.030	0.060

T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control

V1 – Uma V2 – Manuratna

S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage

The effect of the varieties Uma (V_1) and Manuratna (V_2) on IAA content was similar. Among the varieties Uma showed significantly higher IAA content (3.93 mg of unoxidised auxin g^{-1} h^{-1}) than Manuratna (3.71 mg of unoxidised auxin g^{-1} h^{-1}) (Table 11).

Compare to control all the ameliorants significantly improved IAA content. Plants sprayed with Hoagland solution (T_5 – 5.62 mg of unoxidised auxin g^{-1} h^{-1}) showed significantly higher IAA content followed by melatonin (T_3 - 5.18 mg of unoxidised auxin g^{-1} h^{-1}) and salicylic acid (T_4 - 4.14 mg of unoxidised auxin g^{-1} h^{-1}). Low IAA content was recorded in plants sprayed with ascorbic acid (T_1 – 2.66 mg of unoxidised auxin g^{-1} h^{-1}) followed by water (T_6 - 2.90 mgof unoxidised auxin g^{-1} h^{-1}) and glutathione (T_2 – 3.72 mg of unoxidised auxin g^{-1} h^{-1}).

Spraying of ameliorants at both tillering stage and booting stage (S_3 – 4.68 mgof unoxidised auxin g^{-1} h-1) showed significantly higher IAA content compare to giving spray at only one stage (Active tillering – 3.47 mg of unoxidised auxin g^{-1} h^{-1}or booting stage – 3.31 mg of unoxidised auxin g^{-1} h^{-1}).

4.1.4 Photosynthetic rate

Table 12. Effect of ameliorants and time of spray on photosynthetic rate (µmol CO_2 m^{-2} $s^{-1)}$ in Uma and Manuratna

		T1	T2	T3	T4	T5	T6	T7	Mean
S1	V1	14.26	16.95	17.74	14.39	19.45	14.07	13.70	16.65 c
	V2	15.86	18.55	19.59	16.85	21.12	15.96	14.61	
S2	V1	14.35	18.33	18.53	14.47	20.13	14.28	14.13	17.22 b
	V2	16.02	19.35	20.73	17.03	21.89	16.82	15.13	
S3	V1	14.78	18.45	18.74	15.23	21.14	14.37	14.25	17.96 a
	V2	17.83	20.15	21.24	19.34	23.73	16.97	15.35	
	Mean	15.51 e	18.63 c	19.42 b	16.21 d	21.24 a	15.41 f	14.52 g	
Varietal mean		Uma – 16.27				Manuratna – 18.29			

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	**0.015**	**0.031**
Between ameliorants	**0.029**	**0.059**
Between time of sprays	**0.019**	**0.038**
Variety-Ameliorants-Time of Spray	**0.073**	**0.145**

T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control

V1 – Uma V2 – Manuratna

S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage

The effect of the varieties Uma (V1) and Manuratna (V2) on photosynthetic rate was similar. Among the varieties Manuratna (18.29 µM CO_2 m^{-2} s^{-1}) showed higher photosynthetic rate than Uma (16.14 µM CO_2 m^{-2} s^{-1}) (Table 2).

All the ameliorants significantly improved photosynthetic rate as compared to control (T7-4.07 µM CO_2 m^{-2} s^{-1}). Among treatments, plants sprayed with Hoagland solution (T_5 - 21.24 µM CO_2 m^{-2} s^{-1}) showed highest photosynthetic rate followed by melatonin (T_3 - 19.42 µM CO_2 m^{-2} s^{-1}) and glutathione (T_2 - 18.63 µM CO_2 m^{-2} s^{-1}).Low photosynthetic rate was recorded in plants sprayed with water (T_6 - 15.41 µM CO_2 m^{-2} s^{-1}) followed by ascorbic acid(T_1 – 15.51 µM CO_2 m^{-2} s^{-1}) and salicylic acid (T_4 - 16.21 µM CO_2 m^{-2} s^{-1}).

Spraying of ameliorants at both tillering stage and booting stage (S_3 –17.91 µM CO_2 m^{-2} s^{-1}) has given a significantly higher values when compared to giving spray at only one stage either active tillering (S_1 – 16.56 µM CO_2 m^{-2} s^{-1}) or booting stage (S_2 – 17.17 µM CO_2 m^{-2} s^{-1}).However between the two stages the plant response was higher for booting stage than active tillering stage.

The highest photosynthetic rate was recorded in variety Uma sprayed with Hoagland solution at both tillering and booting stage followed by spraying Hoagland solution at booting stage only.

4.1.5 Stomatal conductance

Table 13. Effect of ameliorants and time of spray on stomatal conductance (mol H_2O m^{-2} s^{-1}) in Uma and Manuratna

		T1	T2	T3	T4	T5	T6	T7	Mean
S1	V1	0.149	0.175	0.189	0.164	0.200	0.098	0.091	0.155 c
	V2	0.154	0.178	0.201	0.169	0.211	0.107	0.095	
S2	V1	0.158	0.190	0.203	0.176	0.214	0.111	0.096	0.168 b
	V2	0.161	0.204	0.212	0.184	0.227	0.125	0.097	
S3	V1	0.170	0.209	0.222	0.180	0.248	0.123	0.098	0.184 a
	V2	0.180	0.221	0.235	0.203	0.252	0.135	0.101	
	Mean	0.162 e	0.196 c	0.210 b	0.179 d	0.225 a	0.116 f	0.096 g	
Varietal mean		Uma – 0.165				Manuratna – 0.174			

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	0.0005	0.001
Between ameliorants	0.001	0.002
Between time of sprays	0.0006	0.0011
Variety-Treatment-Time of Spray	0.002	0.0043

T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control

V1 – Uma V2 – Manuratna

S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage

The effect of the varieties V_1 (Uma) and V_2 (Manuratna) on stomatal conductance was similar. Among the varieties Manuratna (0.174 mol H_2O m^{-2} s^{-1}) showed significantly higher stomatal conductance than Uma (0.165 mol H_2O m^{-2} s^{-1}) (Table 13).

All the ameliorants significantly improved the stomatal conductance as compared to control (T_7- 0.096 mol H_2O m^{-2} s^{-1}). Among the treatments, plants sprayed with Hoagland solution (T_5- 0.225 mol H_2O m^{-2} s^{-1}) showed significantly higher stomatal conductance followed by melatonin (T_3 - 0.210 mol H_2O m^{-2} s^{-1}), glutathione (T_2 - 0.196 mol H_2O m^{-2} s^{-1}) and salicylic acid (T_4 - 0.179 mol H_2O m^{-2} s^{-1}). Low stomatal conductance was recorded in plants sprayed with water (T_6 - 0.115 mol H_2O m^{-2} s^{-1}) followed by ascorbic acid (T_1 -0.096 mol H_2O m^{-2} s^{-1}).

Spraying of ameliorantxs at both tillering and booting stages (S_3 –0.184 mol H_2O m^{-2} s^{-1}) gave significantly higher results followed by a single spray at booting stage (S_2 – 0. mol H_2O m^{-2} s^{-1}), which was significantly higher than a single spray at Active tillering (S_1 – 0.155 mol H_2O m^{-2} s^{-1}).

Highest value for stomatal conductance was observed in Manuratna sprayed with Hoagland solution at both tillering and booting stage. Application of Hoagland solution at booting stage in Manuratna also gave good result.

4.1.6 Histochemical detection of superoxide dismutase

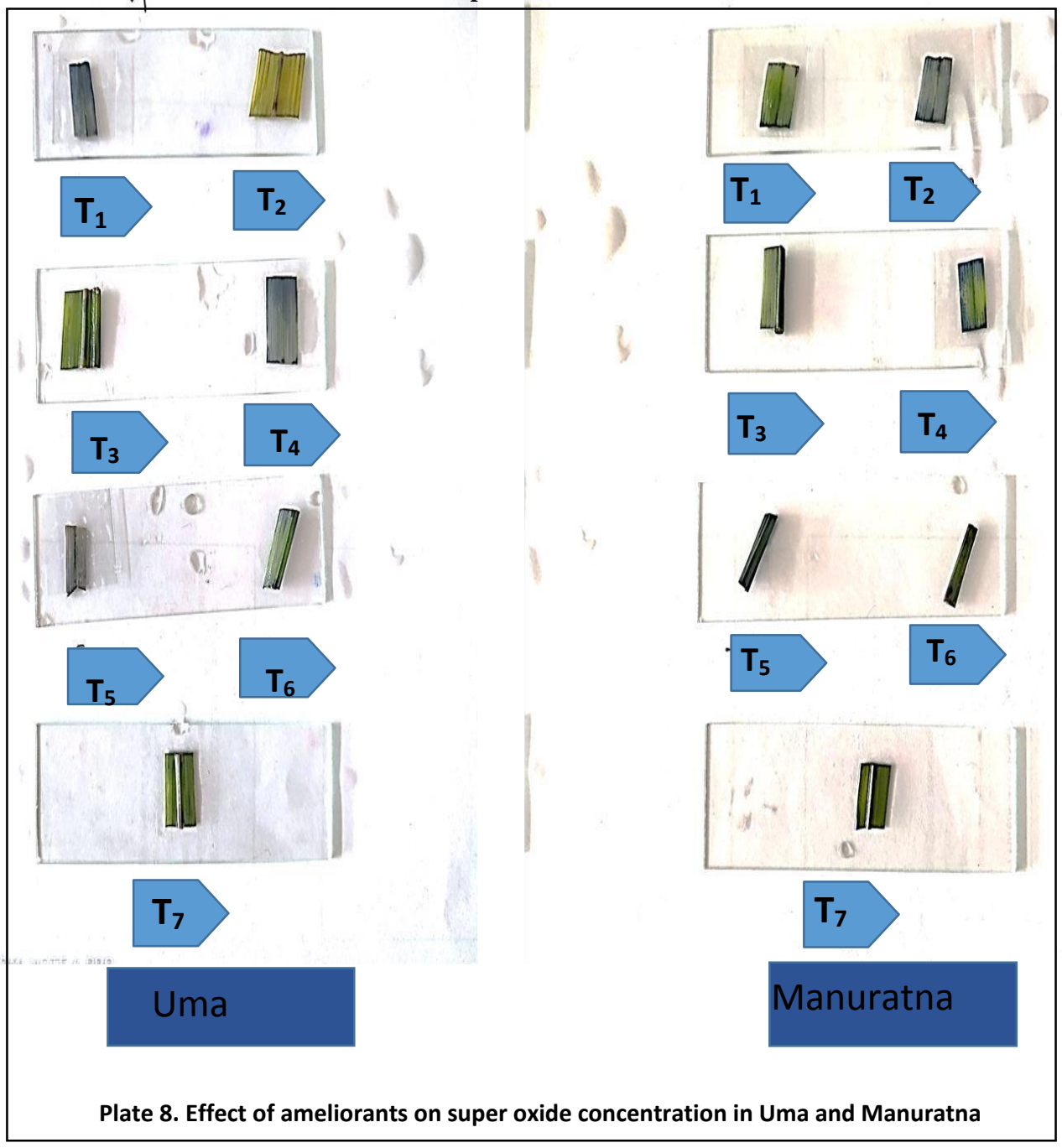

Plate 8. Effect of ameliorants on super oxide concentration in Uma and Manuratna

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control

Histochemical analysis of superoxide showed a lesser concentration in plants sprayed with glutathione and melatonin in Uma and ascorbic acid and melatonin in Manuratna (Plate 8).

4.1.12 Histochemical detection of hydrogen peroxide

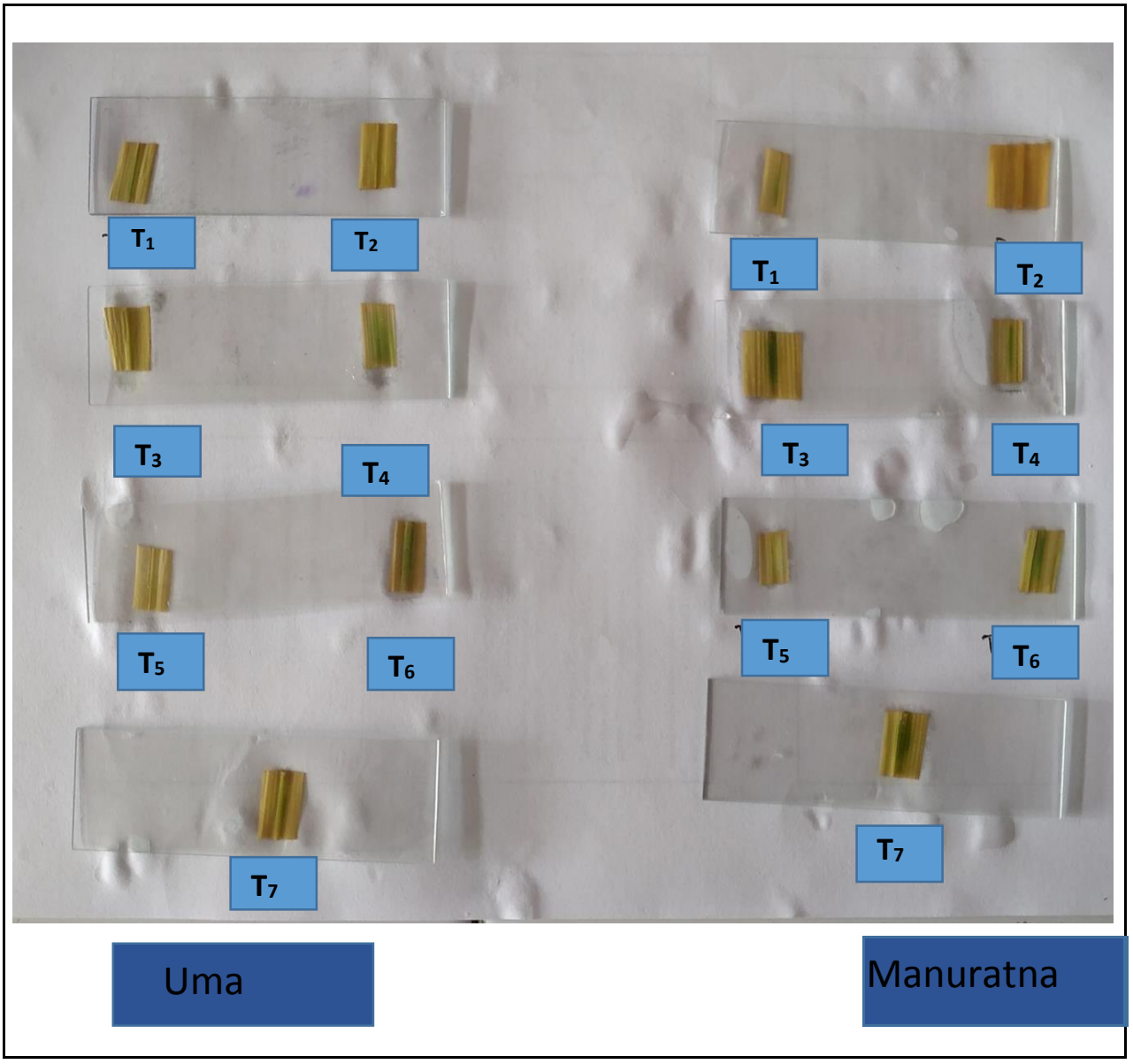

Plate 9. Effect of ameliorants on hydrogen peroxide concentration in Uma and Manuratna

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control

Histochemical detection of hydrogen peroxide showed variations between different ameliorants. Salicylic acid treated plant leaves showed less concentration of hydrogen peroxide in Uma. Melatonin and Hoagland solution sprayed plants showed comparatively low H_2O_2 concentration in Manuratna (Plate 9).

4.1.13 Chalkiness of grains

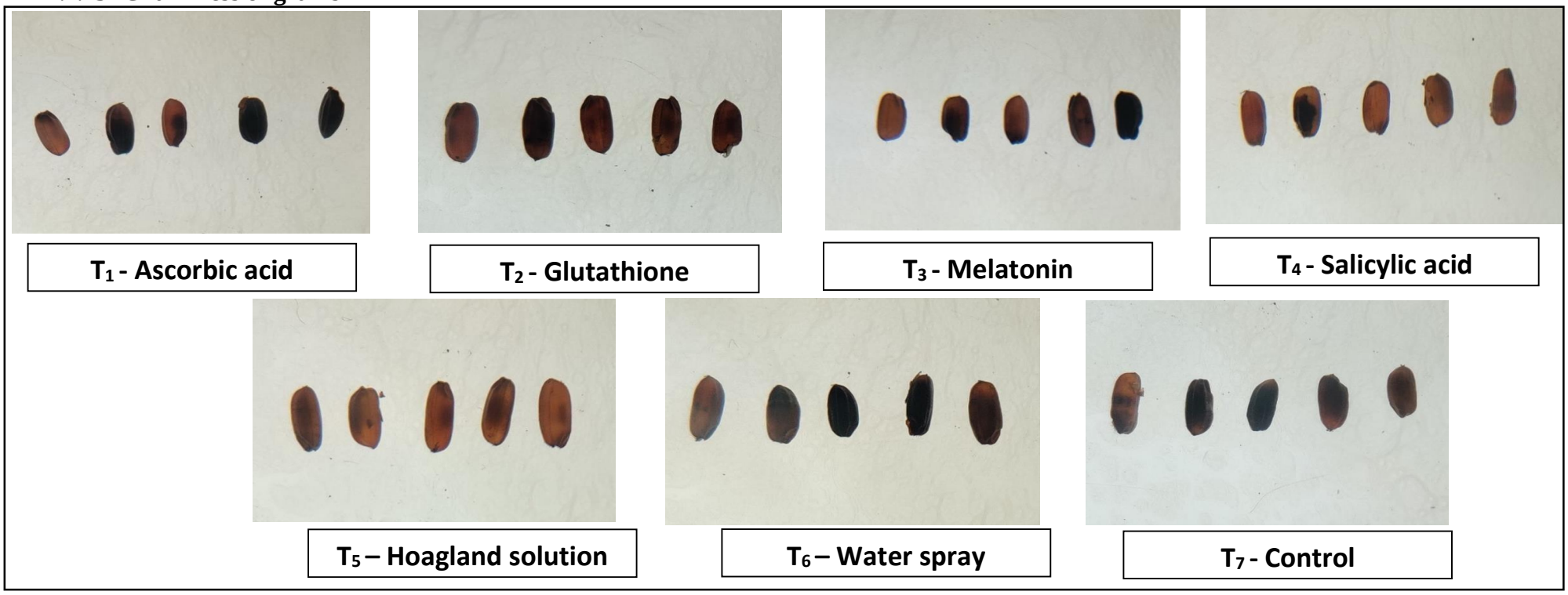

Plate 10. Effect of ameliorants on chalkiness of grains in Uma

Hoagland solution and salicylic acid spray plants showed grains with less chalkiness. Water sprayed and control plants produced grains with more chalkiness (Plate 10).

4.1.14 Pollen viability

Table 14. Effect of ameliorants sprayed at booting stage on pollen viability (%) in Uma and Manuratna

	T1	T2	T3	T4	T5	T6	T7	Mean
V1	58.0	59.3	67.4	72.5	72.6	50.2	43.4	60.48 [a]
V2	56.0	52.5	64.4	66.5	69.0	44.9	40.4	56.24 [b]
Mean	56.99 [d]	55.87 [e]	65.88 [c]	69.49 [b]	70.81 [a]	47.57 [f]	41.92 [g]	

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	0.136	0.292
Between ameliorants	0.254	0.546
Variety – Ameliorant	0.360	0.773

T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 – Hoagland solution T6 - Water spray T7 – Control
V1 – Uma V2 – Manuratna
S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage

Estimation of pollen viability at booting stage for the varieties Uma and Manuratna showed that the response of both the verities to ameliorants was similar (Table 14). Among the varieties Uma (60.48 %) showed significantly higher percentage of viable pollens than Manuratna (56.24 %).

Compare to control all the ameliorants significantly improved pollen viability in both rice varieties. Plants sprayed with Hoagland solution (T_5 - 70.81 %) showed significantly higher pollen viability followed by salicylic acid (T_4 - 69.49 %),

melatonin (T_3 - 65.88 %), ascorbic acid (T_1 - 56.99 %) and glutathione (T2 - 55.87 %). Low pollen viability was recorded in plants sprayed with and water (T_6 - 47.57 %).

4.1.13 Membrane thermo stability

Table 15. Effect of ameliorants sprayed at booting stage on membrane thermo stability in Uma and Manuratna

	T1	T2	T3	T4	T5	T6	T7	Mean
V1	133	145	151	137	118	112	100	128.1 a
V2	121	138	145	129	116	111	100	122.9 b
Mean	127 d	141 b	148 a	133 c	117 e	111 f	100 g	

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	1.12	2.41
Between ameliorants	2.10	4.52
Variety – Ameliorant	2.98	6.39

T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control

V1 – Uma V2 – Manuratna

S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage

Estimation of membrane thermo stability at booting stage for the varieties Uma and Manuratna indicated that the response of both the verities to ameliorants was similar (Table 15). Among the varieties Uma showed significantly higher percent increase in membrane thermal stability than Manuratna.

Compare to control all the ameliorants significantly improved the membrane thermal stability in both rice varieties. Plants sprayed with melatonin showed a higher percent increase in membrane thermal stability followed by glutathione, salicylic acid and ascorbic acid. Low membrane thermal stability was recorded in plants sprayed with Hoagland solution and water. Interaction effect of ameliorants and varieties were not significant.

4.2 Effect of ameliorants sprayed during booting stage on morphological and yield parameters (Field trial – Experiment 2)

The results from pot culture study indicated that best response for the ameliorants was observed when chemicals were sprayed at both active tillering and booting stage (S3) of crop and the next best spray was at booting stage (S2). Due to corona virus disease 2020 lockdown, the second best spray was adopted for field trial.

4.2.1 Plant height

Table 16. Effect of ameliorant sprayed at booting stage on plant height in Uma

Treatment	60 DAS	80 DAS	100 DAS	120 DAS
T_1	36.66 abc	55.66 c	71.03 d	80.03 d
T_2	36.16 abc	56.66 c	76.66 cd	85.66 cd
T_3	37.16 a	64.33 ab	86.00 ab	95.00 ab
T_4	37.00 ab	62.50 b	80.66 bc	89.66 bc
T_5	37.00 ab	68.33 a	88.50 a	97.50 a
T_6	35.00 bc	57.00 c	77.66 cd	86.66 cd
T_7	34.66 c	46.33 d	63.83 e	72.83 e
Mean	36.23	58.69	77.76	86.76
CD at 5%	2.07	5.41	7.02	7.03

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control

Ameliorants were sprayed after 60 days. Compared to control all the ameliorants increased plant height in Uma. Among the ameliorants plants sprayed with Hoagland solution significantly increased height in all intervals followed by melatonin and salicylic acid (Table 16).

Table 17. Effect of ameliorant sprayed at booting stage on plant height in Manuratna

Treatment	40DAS	60 DAS	80 DAS	100 DAS
T_1	25.33 ab	39.00 bc	59.33 cd	68.00 e
T_2	27.33 ab	41.30 abc	64.66 b	76.00 c
T_3	27.50 ab	42.50 ab	71.66 a	81.66 b
T_4	24.83 b	37.83 c	69.00 a	79.00 bc
T_5	25.83 ab	42.00 abc	72.33 a	85.66 a
T_6	30.00 a	44.00 a	61.66 bc	71.66 d
T_7	28.33 ab	41.33 abc	58.00 d	67.33 e
Mean	27.01	41.13	65.23	75.61
CD at 5%	4.75	4.38	3.48	3.26

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control

Ameliorants were sprayed after 60 days. Compared to control all the ameliorants increased plant height in Manuratna. Among the ameliorants plants sprayed with Hoagland solution significantly increased height in all intervals followed by melatonin and salicylic acid (Table 17).

4.2.2 Relative growth rate

Table 18. Effect of ameliorant sprayed at booting stage on relative growth rate (mg g^{-1} d^{-1}) in Uma

Treatment	40-60 DAS	60-80 DAS	80-100 DAS
T_1	62.55	32.04	8.25
T_2	60.43	36.73	8.24
T_3	61.59	38.98	9.44
T_4	63.30	38.15	9.14
T_5	60.28	40.56	9.52
T_6	62.43	31.73	5.15
T_7	60.88	30.43	5.03
CD at 5%	0.554	0.153	0.076

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control

There was not much difference between relative growth rates of plants up to 60 DAS in Uma. After ameliorant application (In 60 to 100 days) Hoagland solution (T_5) sprayed plants showed higher relative growth rate compared to control followed by melatonin (T_3) and salicylic acid (T_4). Less growth rate was observed in plots sprayed with water spray (T_6) and glutathione (T_2) (Table 18).

Table 19. Effect of ameliorant sprayed at booting stage on relative growth rate (mg g^{-1} d^{-1}) in Manuratna

Treatment	40-60 DAS	60-80 DAS	80-100 DAS
T_1	59.75	29.69	2.67
T_2	61.34	31.73	2.75
T_3	62.27	34.95	3.08
T_4	62.82	34.45	3.01
T_5	61.67	36.85	3.40
T_6	60.39	29.27	2.66
T_7	58.85	28.98	2.42
CD at 5%	0.393	0.270	0.068

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control

There was not much difference between relative growth rates of plants up to 60 DAS in Manuratna. After ameliorant application (In 60 to 100 days) Hoagland solution (T_5) sprayed plants showed higher relative growth rate compared to control followed by melatonin (T_3) and salicylic acid (T_4). Less growth rate was observed in plots sprayed with water spray (T_6) and glutathione (T_2) (Table 19).

4.2.3 Crop growth rate

Table 20. Effect of ameliorant sprayed at booting stage on crop growth rate (mg cm^{-2} d^{-1}) in Uma

Treatment	40-60 DAS	60-80 DAS	80-100 DAS
T_1	4.10	4.02	0.65
T_2	3.56	4.22	0.63
T_3	4.03	4.34	0.86
T_4	4.69	4.41	0.81
T_5	3.50	4.53	0.93
T_6	3.99	3.86	0.62
T_7	3.60	3.73	0.60
CD at 5%	0.042	0.046	0.033

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solutionT_6 - Water spray T_7 – Control

There was not much difference between crop growth rates of plants up to 60 DAS in Uma. After ameliorant application (In 60 to 100 days) Hoagland solution (T_5) sprayed plants showed higher relative growth rate compared to control followed by melatonin (T_3) and salicylic acid (T_4). Less growth rate was observed in plots sprayed with water spray (T_6) and glutathione (T_2) (Table 20).

Table 21. Effect of ameliorant sprayed at booting stage on crop growth rate (mg cm^{-2} d^{-1}) in Manuratna

Treatment	40-60 DAS	60-80 DAS	80-100 DAS
T_1	3.19	3.47	0.51
T_2	3.21	4.02	0.56
T_3	3.28	4.18	0.61
T_4	3.27	4.09	0.63
T_5	3.13	4.40	0.69
T_6	3.25	3.48	0.53
T_7	3.30	3.42	0.47
CD at 5%	0..49	0.033	0.029

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control

There was not much difference between crop growth rates of plants up to 60 DAS in Manuratna (Table 21). After ameliorant application (In 60 to 100 days) Hoagland solution (T_5) sprayed plants showed higher relative growth rate compared to control followed by melatonin (T_3) and salicylic acid (T_4). Less growth rate was observed in plots sprayed with water spray (T_6) and glutathione (T_2).

4.2.4 Number of productive tillers

Table 22. Effect of ameliorants sprayed at booting stage on productive tillers per meter square in Uma and Manuratna

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
V_1	240	255	285	315	360	240	225	274.28 a
V_2	240	255	270	300	345	240	225	267.85 a
Mean	240 de	255 cd	277.5 c	307.5 b	352.5 a	240 de	225 e	

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control V_1- Uma V_2- Manuratna

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	NS	NS
Between ameliorants	10.60	22.74
Variety – Ameliorant	NS	NS

Among the varieties Uma (274.28) had higher number of spikelets per panicle than Manuratna (267.85) (Table 22).

Compare to control all the ameliorants except ascorbic acid (T_1 – 240) and water spray (T_6 - 240) significantly improved productive tillers per meter square. Plants sprayed with Hoagland solution (T_5 - 352) showed significantly higher productive tillers per meter square followed by salicylic acid (T_4 - 307.5), melatonin (T_3 - 14.17) and glutathione (T_2 - 12.71).

There was no significant difference on productive tillers per meter square in Uma and Manuratna in response to ameliorants.

4.2.5 Tiller decline

Table 23. Effect of ameliorant sprayed at booting stage on tiller decline percentage in Uma

Ameliorant	Tiller decline(percentage)
T_1	43.85 ^{ab}
T_2	41.69 ^{ab}
T_3	35.57 ^{bc}
T_4	29.58 ^{cd}
T_5	21.65 ^{d}
T_6	44.44 ^{ab}
T_7	47.95 ^{a}
CD at 5%	9.52

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control

Compared to control all the ameliorants sprayed at booting stage decreased tiller decline percentage in Uma (Table 23). Among the ameliorants plants sprayed with Hoagland solution maintained significantly higher number of tillers (T_5 - 21 %) followed by salicylic acid (T_4 - 29%) and melatonin (T_3 - 35%). Plants sprayed with water showed higher decline in tiller number (T_6 -44%) followed by ascorbic acid (T_1- 43%) and glutathione (T_2- 41 %).

Table 24. Effect of ameliorant sprayed at booting stage on tiller decline percentage in Manuratna

Ameliorant	Tiller decline(percentage)
T_1	44.50 ab
T_2	41.69 abc
T_3	39.66 bc
T_4	34.42 c
T_5	24.11 d
T_6	45.26 ab
T_7	49.27 a
CD at 5%	7.85

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control

Compared to control all the ameliorants sprayed at booting stage decreased tiller decline percentage in Manuratna (Table 24). Among the ameliorants plants sprayed with Hoagland solution maintained significantly higher number of tillers (T_5 - 24 %) followed by salicylic acid (T_4 - 34%) and melatonin (T_3 - 39%). Plants sprayed with water showed higher decline in tiller number (T_6 - 45%) followed by ascorbic acid (T_1 - 44%) and glutathione (T_2 -41%).

4.2.6 Yield and yield parameters

4.2.6.1 Number of spikelets

Table 25. Effect of ameliorants sprayed at booting stage on number of spikelets per paniclein Uma and Manuratna

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
V_1	111	107	119	120	118	109	107	113.14 a
V_2	109	107	118	120	116	104	102	111.00 b
Mean	110.0 c	107.0 d	118.7 ab	120.0 a	117.5 b	106.5 de	104.7 e	

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	0.439	0.941
Between ameliorants	0.821	1.761
Variety – Ameliorant	NS	NS

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control V_1- Uma V_2- Manuratna

Among the varieties Uma (113.14) had significantly higher number of spikelets per panicle than Manuratna (111) (Table 25).

Compare to control all the ameliorants significantly improved number of spikelets per panicle. Plants sprayed with salicylic acid (T_4 -120) had significantly higher number of spikelets per panicle followed by melatonin (T_3 -118.7), Hoagland solution (T_5 -117.5) and ascorbic acid (T_1 -110). Lower improved number of spikelets per panicle was observed in plants sprayed with glutathione (T_2 -107) and water spray (T_6 -106.5).

Varietal difference in response to ameliorants on number of spikelets per panicle was not significant (Table 25).

4.2.6.2 Filled grains per panile

Table 26. Effect of ameliorants sprayed at booting stage on filled grains per panicle in Uma and Manuratna

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
V_1	15	16	21	22.5	29	8.5	7	17.00 a
V_2	10	11.5	15.5	17	17.5	6.5	4.5	11.78 b
Mean	12.50 e	13.75 d	18.25 c	19.75 b	23.25 a	7.50 f	5.75 g	

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	0.276	0.593
Between ameliorants	0.517	1.109
Variety – Ameliorant	0.732	1.569

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control V_1- Uma V_2- Manuratna

Among the varieties Uma (17.00) had significantly higher number of filled grains per panicle than Manuratna (11.78) (Table 26).

Compare to control all the ameliorants significantly improved number of filled grains per panicle. Plants sprayed with Hoagland solution (T_5-23.25) showed significantly higher number of filled grains per panicle followed by salicylic acid (T_4- 19.75), melatonin (T_3-18.25) and glutathione (T_2-13.75). Lower number of filled grains per panicle was observed in plants sprayed with water (T_6-7.50) and ascorbic acid (T_1- 12.50).

The best result was noted in Uma sprayed with Hoagland solution. Hoagland solution gave better result in Manuratna also (Table 26).

4.2.6.3 Chaff percentage

Table 27. Effect of ameliorants sprayed at booting stage on chaff percentage in Uma and Manuratna

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
V_1	86.5	85	82.4	81.3	75.5	92.2	93.5	85.20 a
V_2	90.8	89.2	86.9	85.8	85.4	93.8	95.6	89.64 a
Mean	88.65 c	87.14 d	84.6 e	83.55 f	80.46 g	92.97 b	94.53 a	

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	0.211	0.454
Between ameliorants	0.396	0.849
Variety – Ameliorant	0.560	1.201

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control V_1- Uma V_2- Manuratna

Among the varieties Manuratna (89.64 %) showed significantly higher chaff percentage than Uma (85.20) (Table 27). Compare to control all the ameliorants significantly reduced chaff percentage. Plants sprayed with Hoagland solution (T_5- 80.46 %) significantly reduced chaff percentage followed by salicylic acid (T_4- 83.55 %), melatonin (T_3- 84.6 %) and glutathione (T_2- 87.14 %). Higher chaff percentage was recorded in plants sprayed with ascorbic acid (T_1- 88.65 %) and water (T_6- 92.97 %) (Table 27).

4.2.6.4 Thousand grain weight

Table 28. Effect of ameliorants sprayed at booting stage on thousand grain weight (g) in Uma and Manuratna

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
V_1	16.9	16.6	17.3	17.6	22.2	16.3	16.1	17.55 a
V_2	16.7	16.3	17.1	17.2	20.4	16.0	16.0	17.09 b
Mean	16.78 d	16.42 e	17.18 c	17.40 b	21.28 a	16.17 f	16.03 g	

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	0.030	0.066
Between ameliorants	0.057	0.124
Variety – Ameliorant	0.081	0.175

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control V_1- Uma V_2- Manuratna

The response of the varieties uma and manuratna to ameliorants was similar. Among the varieties Uma (15.55 g) showed significantly higher 1000 seed weight than Manuratna (17.09 g) (Table 28).

Compare to control all the ameliorants significantly improved thousand grain weight. Plants sprayed with Hoagland solution (T_5-21.28 g) showed a high 1000 seed weight followed by salicylic acid (T_4-17.40 g), melatonin (T_3-17.18 g) and ascorbic acid (T_1- 16.78 g). Lower 1000 seed weight was recorded in plants sprayed with water (T_6-16.17 **g)** and glutathione (T_2- 16.42 g) (Table 28).

4.2.6.5 Grain yield

Table 29. Effect of ameliorants sprayed at booting stage on grain yield (g/plot) in Uma and Manuratna

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
V_1	243	271	414	499	926	133	101	396.6 a
V_2	160	191	286	351	493	100	64	235.2 b
Mean	201.5 d	231.0 d	350.35 c	425.14 b	709.6 a	116.6 e	82.85 e	

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	11.26	24.15
Between ameliorants	21.07	45.18
Variety – Ameliorant	29.79	63.88

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control V_1- Uma V_2- Manuratna

Among the varieties Uma had significantly higher grain yield (396.6 g/plot) than Manuratna (235.2 g/plot) (Table 29).

Compare to control all the ameliorants except water spray (T_6- 116.6 g) significantly improved grain yield. Plants sprayed with Hoagland solution (T_5- 709.6 g) showed significantly higher number of filled grains per panicle followed by salicylic acid (T_4- 425.2 g) and melatonin (T_3-350.3 g). Effect of glutathione (T_2- 231 g) and ascorbic acid (T_1- 201.5 g) were on par (Table 29).

4.2.6.6 Straw yield

Table 30. Effect of ameliorants sprayed at booting stage on straw yield (Kg/plot) in Uma and Manuratna

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean
V_1	4.90	6.35	6.48	8.55	7.12	4.53	4.20	6.01 a
V_2	2.83	3.82	4.86	6.77	6.09	2.82	2.31	4.25 b
Mean	3.86 d	5.08 c	5.67 bc	7.66 a	6.60 ab	3.67 d	3.25 d	

Critical difference for comparison

	SE ± Mean	CD at 5%
Between varieties	0.281	0.603
Between ameliorants	0.526	1.13
Variety – Ameliorant	NS	NS

T_1 -Ascorbic acid T_2 –Glutathione T_3 –Melatonin T_4 - Salicylic acid T_5 –Hoagland solution T_6 - Water spray T_7 – Control V_1- Uma V_2- Manuratna

Among the varieties Uma (6.01 Kg/plot) had significantly higher straw yield than Manuratna (4.25 Kg/plot) (Table 30).

Compare to control all the ameliorants except ascorbic acid (T_1 – 3.86 g) and water spray (T_6 – 3.67 g) significantly improved straw yield. Plants sprayed with salicylic acid (T_4 -7.66 g) showed significantly higher straw yield followed by Hoagland solution (T_5 -6.60 g), melatonin (T_3 – 5.67 g) and glutathione (T_2 – 5.08 g).

Varietal difference in response to different ameliorants on straw yield was not significant (Table 30).

Discussion

5. DISCUSSION

Atmospheric temperature is reported to be more than 40 ^{0}C during third crop season which accounts for low productivity of rice grown during the period. Effect of different chemical ameliorants on the physiology of rice under high temperature conditions and its impact on productivity of the crop is discussed in this chapter.

5.1 Effect of chemical ameliorants on physiological parameters

The present study conducted in the glass house during the third crop season of 2019 showed that ameliorant sprays can improve the photosynthetic rate and stomatal conductance in rice plants. Lowest photosynthetic rate was observed in control plants (Table 12) compared to ameliorant applied plants. This might be due to degradation of chlorophyll and closure of stomata to reduce transpiration loss under high temperature condition.

Plants sprayed with Hoagland solution showed higher photosynthetic rate in both the varieties. The result is substantiated by higher total chlorophyll content (Table 6) and stomatal conductance (table 13). This result is in conformity with Waraich *et al.* (2012) who found that exogenous application of manganese could improve photosynthesis and nitrogen metabolism in plants. Exogenously applied melatonin also improved photosynthetic rate in both Uma and Manuratna. This is supported by higher total chlorophyll content and stomatal conductance as has been reported in a recent study on melatonin treatment to ameliorate heat stress in rice (Barman *et al.*, 2019). Similar result was reported in grape seedlings, where exogenously applied melatonin reduced the degradation of chlorophyll and increased photosynthesis (Zhong *et al*., 2020).

Stomatal conductance is an important factor which can control rate of photosynthesis and transpiration. Under high temperature condition, stomatal conductance reduces as stomata closes to prevent transpirational loss of water from plants (Munjal and Dhanda. 2004). Our study showed lower stomatal conductance in control plants under high temperature compared to plants sprayed with ameliorants. Highest stomatal conductance was noted in Hoagland solution sprayed plants (Table 13). It might be due to role of boron in controlling K^+ efflux of guard cells in epidermal strips which enhances stomatal opening (Bejerano and Itai. 1981).

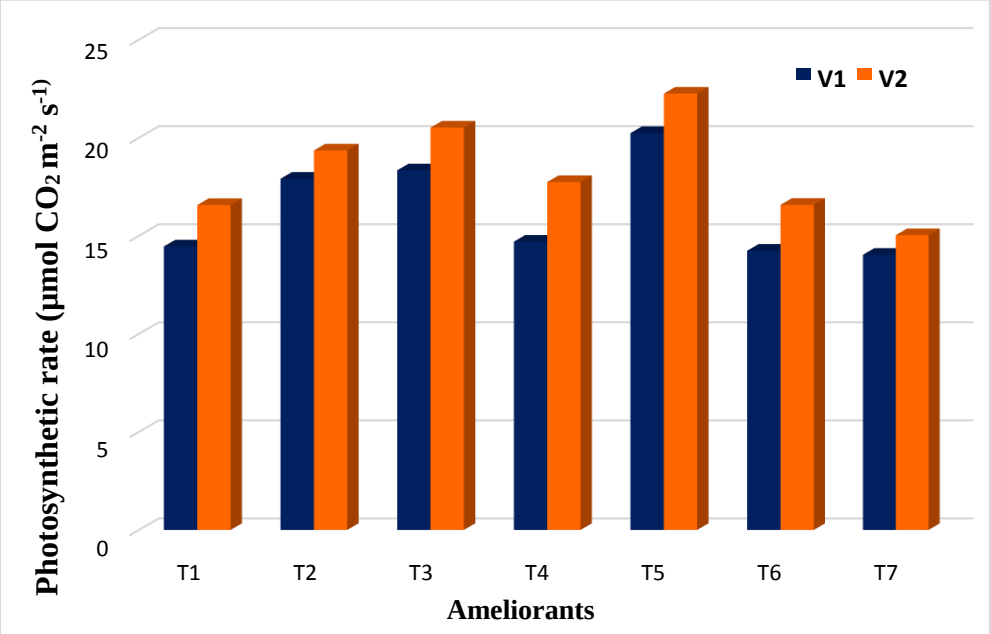

Fig. 2 Effect of ameliorants on photosynthetic rate (µmol CO_2 m^{-2} $s^{-1)}$ in Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna**]**

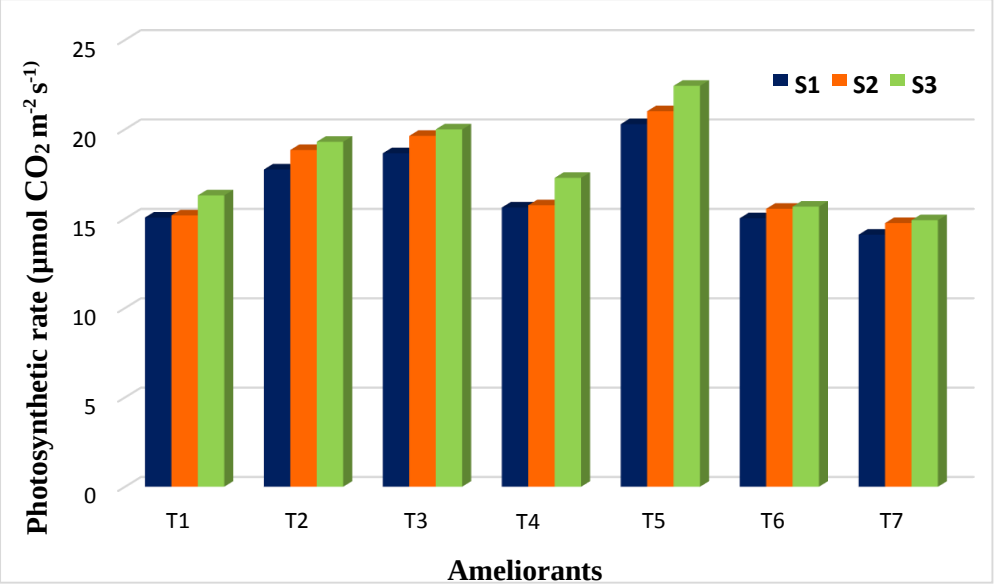

Fig. 3 Effect of ameliorants and time of spray on photosynthetic rate (µmol CO_2 m^{-2} s^{-1})

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage]

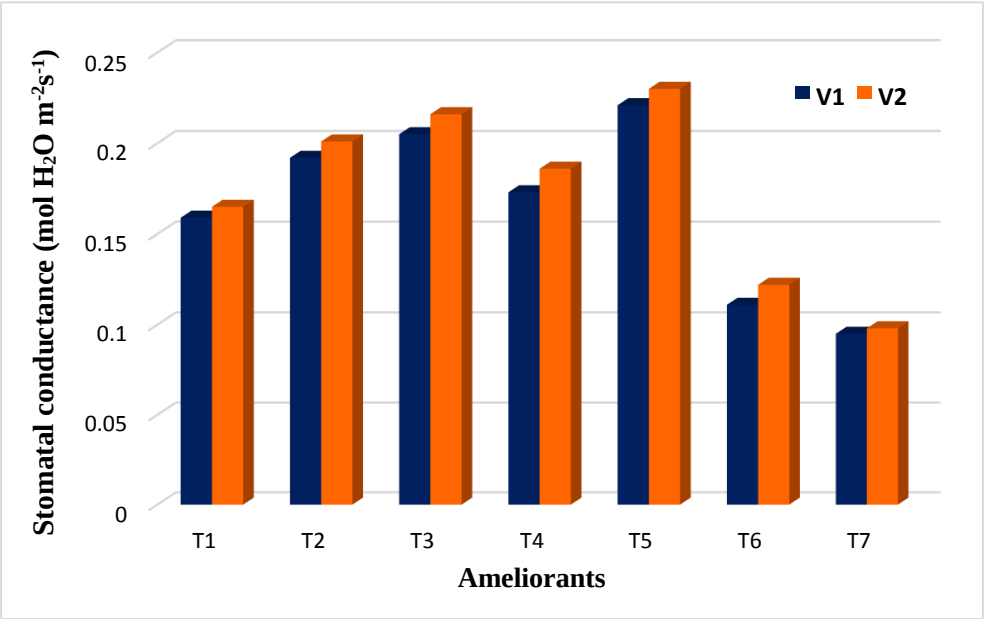

Fig. 4 Effect of ameliorants on stomatal conductance (mol H2O m^{-2} s^{-1}) in Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna**]**

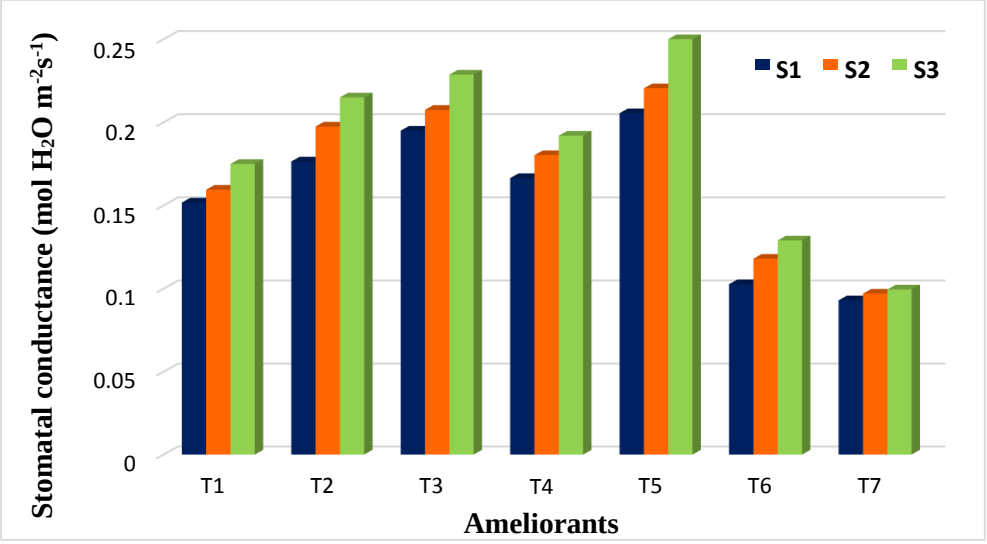

Fig. 5 Effect of ameliorants and time of spray on stomatal conductance (mol H2O m -2 s -1)

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage]

5.2 Effect of ameliorants on biochemical parameters

Chlorophyll is an important plant pigment which determines the photosynthetic capacity of a plant. High temperature degrades chlorophyll and cause peroxidative damage of chloroplasts which leads to reduction in photosynthesis (Mengutay *et al*., 2013). In the current study, exogenously applied melatonin increased total chlorophyll content in plants grown under high temperature conditions (Table 6). The same result was noted by Zhong *et al*. (2020) in grape seedlings and this might be due to reduction in chlorophyll degradation by melatonin through down regulation of chlorophyll degrading enzymes (Xue *et al*., 2011). Hoagland solution also improved chlorophyll a (Table 4), chlorophyll b (Table 5) and total chlorophyll content (Table 6). This may be ascribed to the combined effect of boron on increased production of chloroplasts (Sharma, 1990) and magnesium on increased chlorophyll biosynthesis (Waraich *et al.*, 2012).

Soluble proteins are highly sensitive to increasing temperature. Lower protein content was recorded in control plants compared to plants sprayed with ameliorants (Table 8). It might be due to reduction in protein biosynthesis and loss of structure of proteins as they undergo denaturation and degradation under high temperature conditions. Study revealed the effect of ameliorants in maintaining stability of soluble protein as all the ameliorants improved protein content compared to control. Among the ameliorants, melatonin and glutathione sprayed plants showed higher total soluble protein content. This is supported by the higher membrane thermo stability in plants sprayed with melatonin and glutathione (Table 15). Study conducted by Xue *et al*. (2016) reported an increased protein content in tomato plants sprayed with melatonin as melatonin can reduce ubiquitinated protein build up in plants and enhance the expression of heat shock proteins under heat stress. Plants sprayed with glutathione also had high soluble protein content. This result was in accordance with high membrane thermo stability value in glutathione applied plants. Effect of glutathione on soluble protein content was also observed by Hasanuzzaman *et al*. (2017) in mung bean. He observed that glutathione protects oxidative degradation of proteins by protecting thiol group of proteins from denaturation.

Plants respond to abiotic stress by accumulating special metabolites which help plants to withstand unfavorable conditions. Proline is one of them which acts as an osmoprotectant, and stabilizes cellular structure and enzymes during stress conditions. Proline scavenges reactive oxygen species (ROS) and maintains membrane stability. Plants accumulates more proline under high temperature than normal temperature conditions (Zhang *et al*., 2007). From the study it was evident that plants accumulated more proline when they were treated with glutathione and melatonin (Table 9). This is in agreement with high membrane stability index in glutathione and melatonin treated plants (Table 15). Glutamate is an amino acid component of glutathione, the concentration of which increases by exogenous application of glutathione. The increase in proline precursors glutamate and ornithine respectively might have contributed to increased biosynthesis of proline (Meena *et al*., 2019). Exogenous melatonin also enhances proline accumulation by up regulating the function of enzymes like pyroline 5-carboxylate synthetase (P5CS) and ornithine aminotransferase (OAT) which catalyzes proline biosynthesis and inhibits proline dehydrogenase (PDH) gene expression (Aghdam *et al*., 2019).

Nitrate reductase enzyme activity depends on the availability and concentration of its substrate NO_3^- (Solomonson and Barber, 1990) and it decreases in response to increasing temperature. This decrease might be to reduce assimilation of nitrate to prevent energy loss during increasing temperature conditions (Hayat *et al*., 2009). Current study reveals that exogenous application of salicylic acid is effective in enhancing nitrate reductase enzyme activity (Table 10). Similar results were reported in mustard (Hayat *et al*., 2009), soybean (Joseph *et al*., 1976), maize (Jain and Srivastava, 1981) and wheat (Khan *et al*., 2013). Salicylic acid has been reported to down regulate NR specific inhibitor (Srivastava, 1980). Moreover, being a phenolic compound, it has chelating property. Salicylic acid chelates with ions that impart cell membrane stability leading to higher membrane permeability, which in turn enhances the free movement of metabolites involved in nitrate reductase enzyme synthesis (Jain and Srivastava, 1981).

According to Kabir *et al*. (2017) indole acetic acid (IAA) content increases at early growth stages of rice in response to increasing temperature and then shows a decreasing trend. The same result was obtained in the present study (Table 11). Exogenous application

of Hoagland solution and melatonin were effective in improving IAA content in plants grown under high temperature. Similar findings were reported in *Brassica juncea*, where exogenous application of melatonin increased IAA content by 1.4 times (Chen *et al*., 2009). This might be because both melatonin and IAA have a common precursor, tryptophan (Zhong *et al*., 2020).

Pollen viability is one of the important physiological characters which determines the grain yield in cereals. High temperature stress lead to drastic reduction in pollen viability in rice (Matsui *et al*., 2001, Song *et al*., 2001, Wassmann and Dobermann, 2007). These reports substantiate decreased pollen viability in control plants in the present study. This decrease might be due to loss of carbohydrate mobilization capacity of floral buds due to high temperature (Dinar and Rudich, 1985). Exogenous application of Hoagland solution showed an improvement in pollen viability (Table 14) and the same findings were reported in three rice varieties by Shahid *et al*. (2018). Boron is reported to enhance mobilisation of sugars, hence increased metabolism and mobilization of photosynthates to reproductive parts can be attributed as the reason for improvement in pollen viability (Shahid *et al*., 2018).

Metabolic changes occurring in plants grown under high temperature include over production of reactive oxygen species (ROS). These highly reactive molecules interfere with cell integrity and lead to loss of membrane stability and electrolyte leakage. From the current study it was clear that exogenous ameliorants could improve membrane stability compared to control plants. Higher membrane thermo stability (MTS) value was observed in plants sprayed with melatonin (Table 15). This is supported by lower ROS content observed in histochemical detection analysis (Fig. 18 (a) and (b)). Similar observations were made in tomato by Xue *et al*. (2016). Melatonin maintains membrane stability by directly scavenging free radicals and also by activating the production of antioxidant enzymes such as superoxide dismutase and catalase (Zhong *et al*., 2020). Exogenously applied glutathione also increased membrane stability in plants. The same result was reported in mung bean (Hasanuzzaman *et al*., 2015) where exogenous spray of glutathione decreased ROS content. This might be due to the ability of glutathione to regenerate antioxidants via AsA-GSH cycle. It also acts as a substrate of antioxidant enzymes like glutathione S-transferases (GST) and glutathione peroxidase (GPx) (Szalai *et al*., 2009)

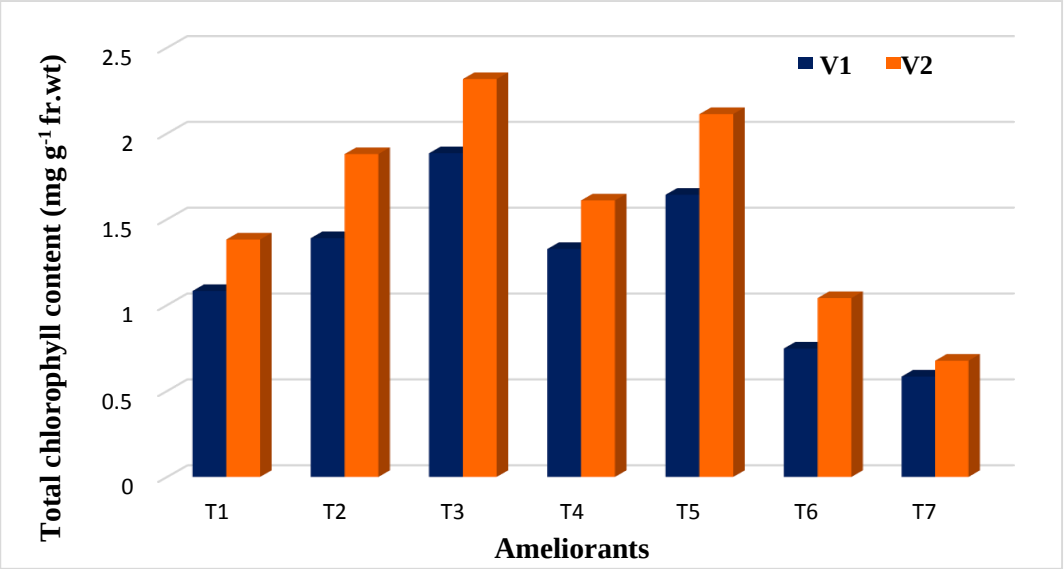

Fig. 6 Effect of ameliorants on total chlorophyll content (mg g^{-1} fr.wt) of Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna**]**

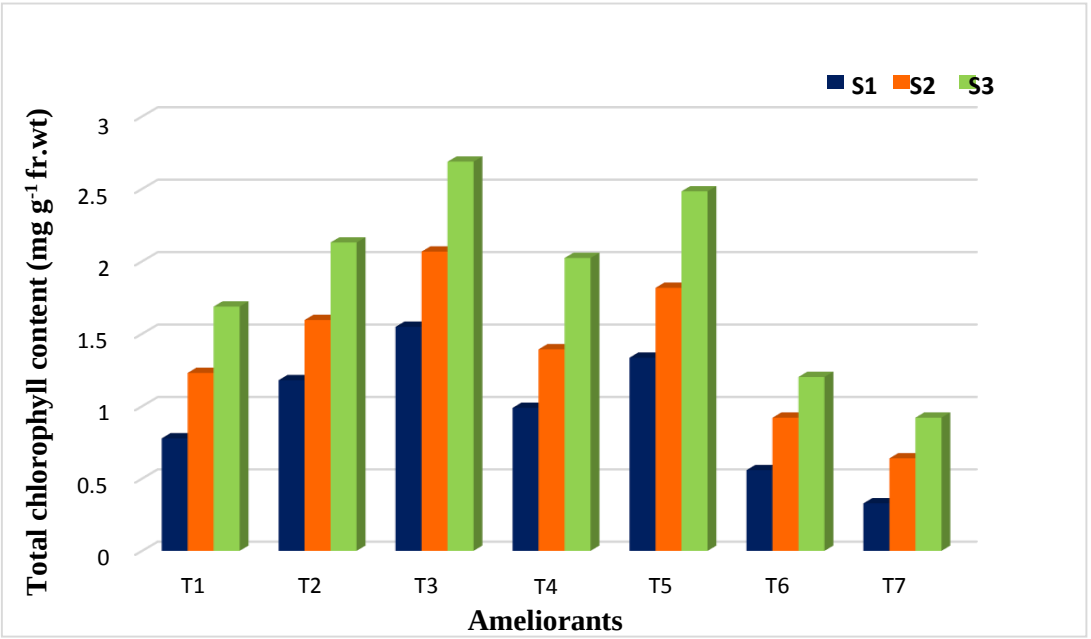

Fig. 7 Effect of ameliorants and time of spray on total chlorophyll content (mg g^{-1} fr.wt)

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage]

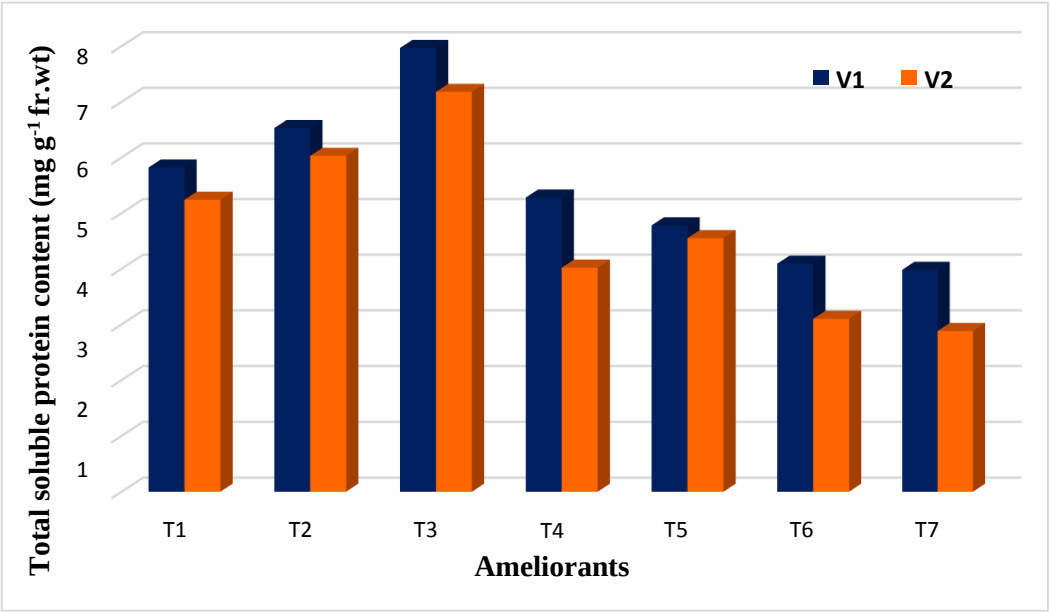

Fig. 8 Effect of ameliorants on total soluble protein content (mg g-1 fr.wt)) in Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna**]**

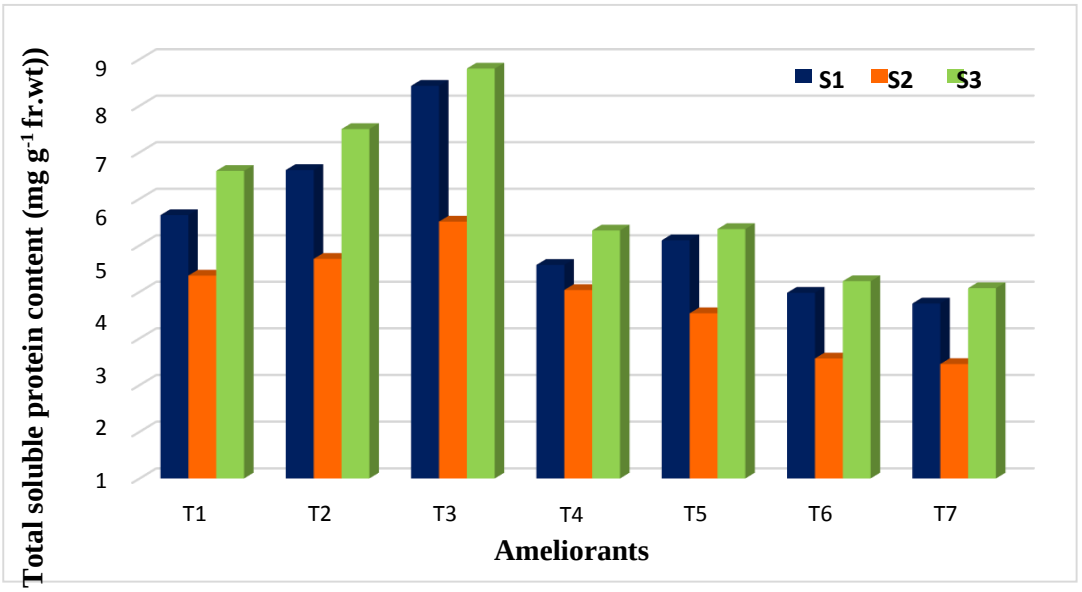

Fig. 9 Effect of ameliorants and time of spray on total soluble protein content (mg g-1 fr.wt))

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage]

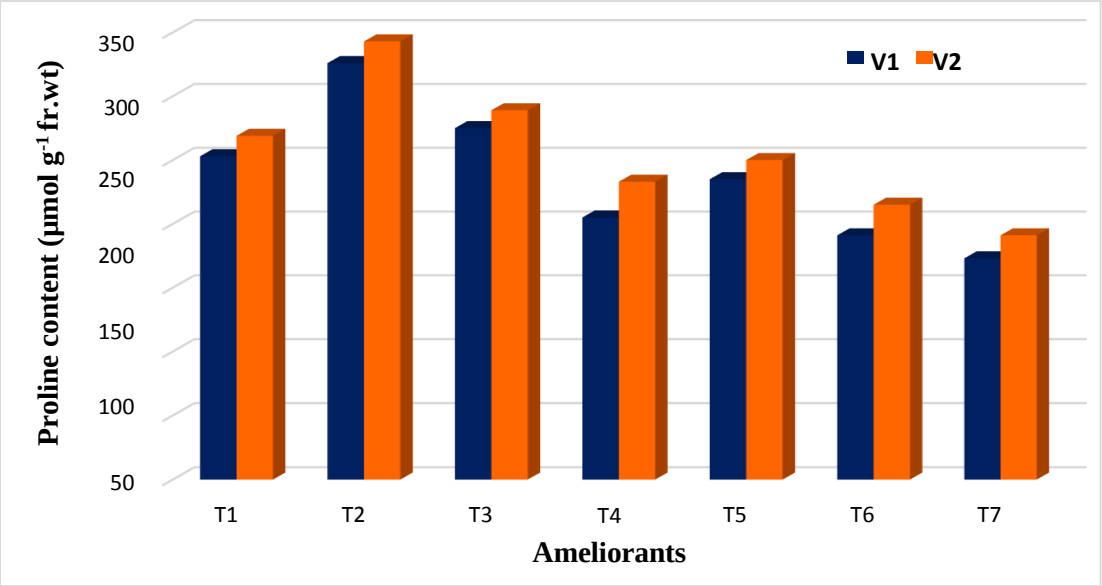

Fig. 10 Effect of ameliorants on proline content (µmol g-1 fr.wt)) in Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna]

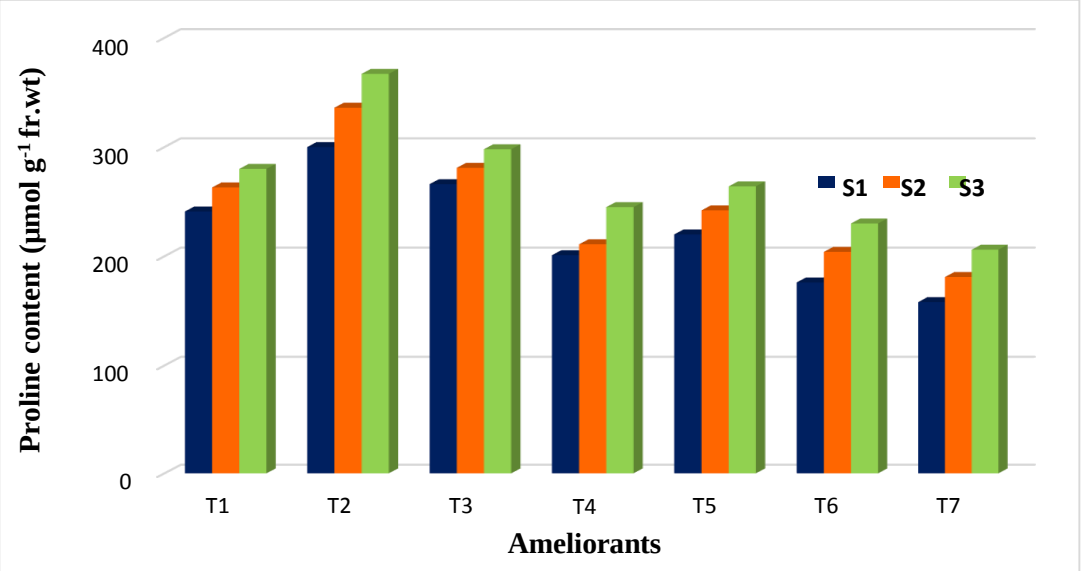

Fig. 11 Effect of ameliorants and time of spray on proline content (µmol g-1 fr.wt)

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage]

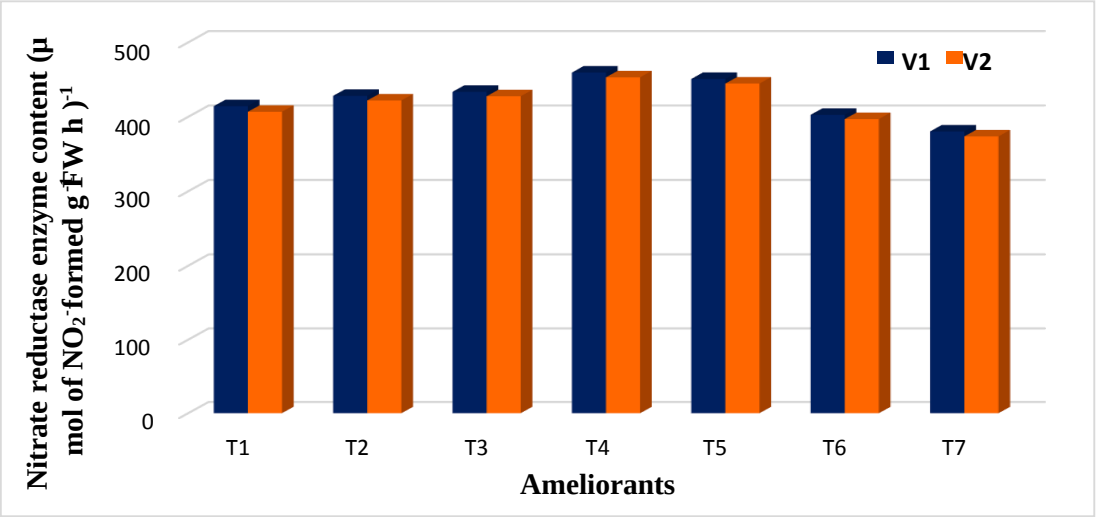

Fig. 12 Effect of ameliorants on Nitrate reductase enzyme content (µmol of NO^{2-} formed g^{-1} FW h^{-1}) in Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna]

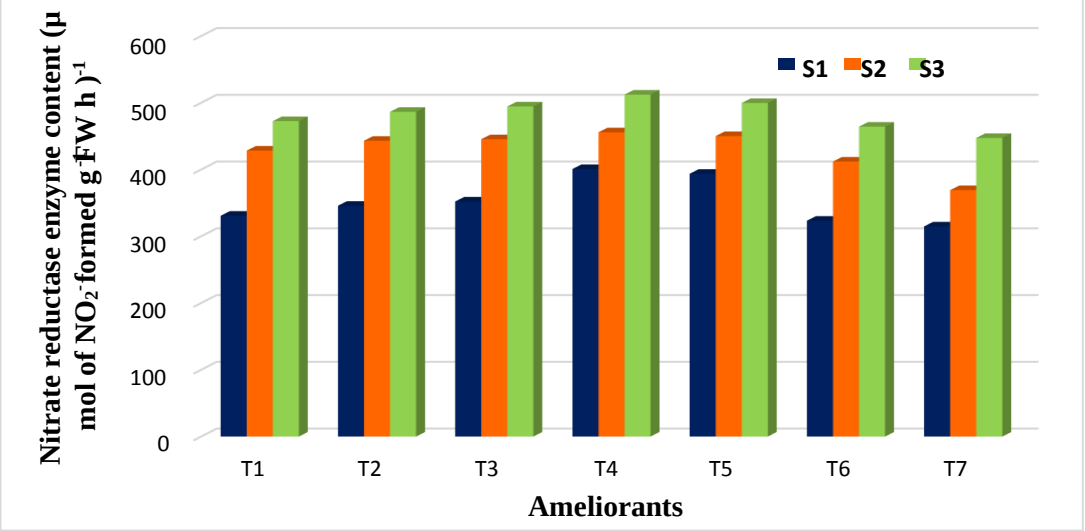

Fig. 13 Effect of ameliorants and time of spray on Nitrate reductase enzyme content (µmol of NO_2^- formed g^{-1} FW h^{-1})

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage]

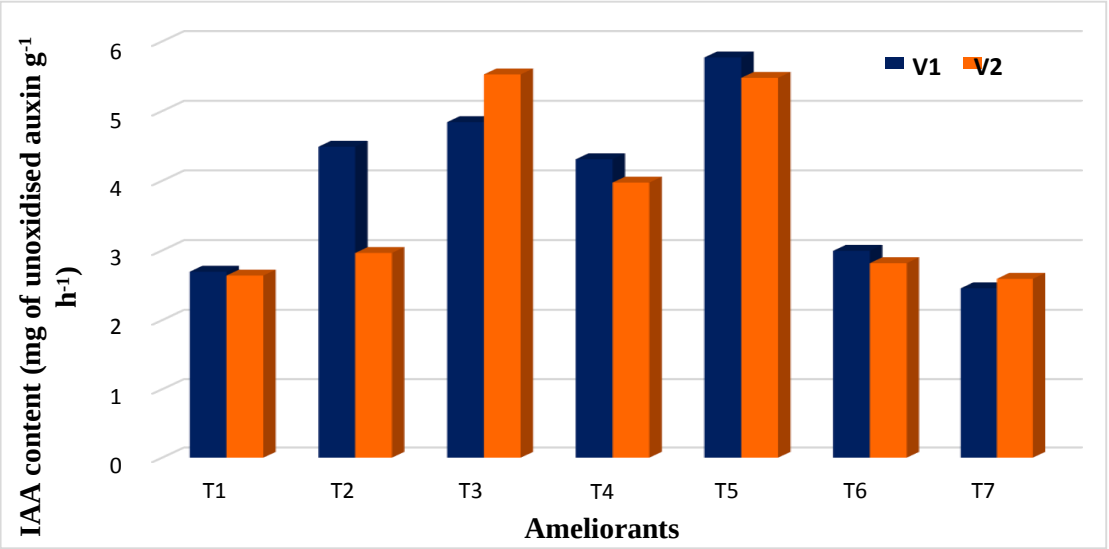

Fig. 14 Effect of ameliorants on IAA content (mg of unoxidised auxin g^{-1} h^{-1}) in Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna]

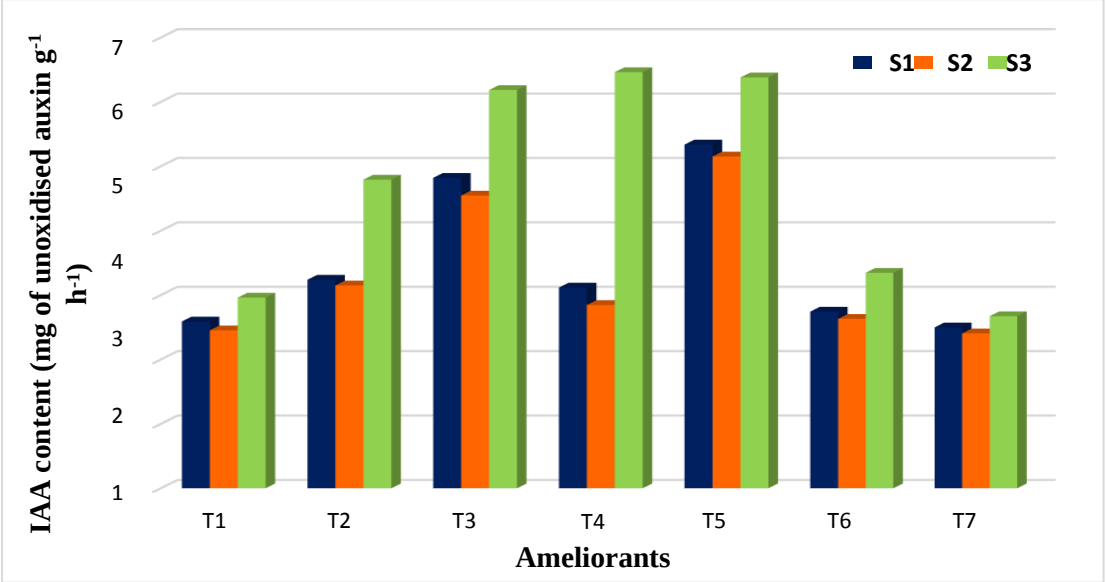

Fig. 15 Effect of ameliorants and time of spray on IAA content (mg of unoxidised auxin g^{-1} h^{-1})

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control S1 – Spray at active tillering stage S2 – Spray at booting stage S3 – Spray at both tillering and booting stage]

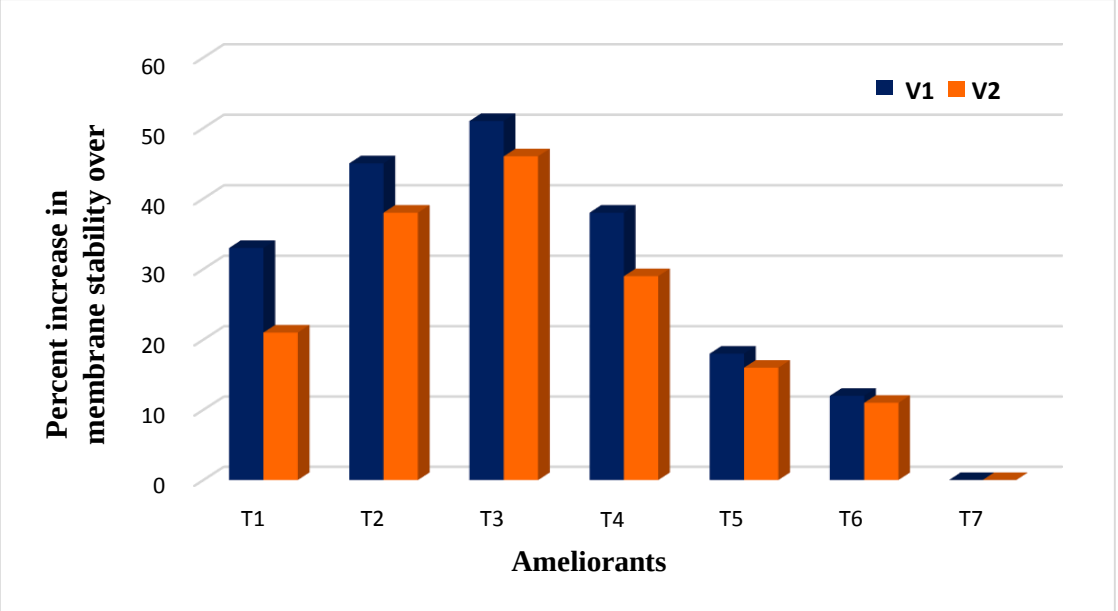

Fig. 16 Effect of ameliorants sprayed at booting stage on membrane thermo stability in Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna]

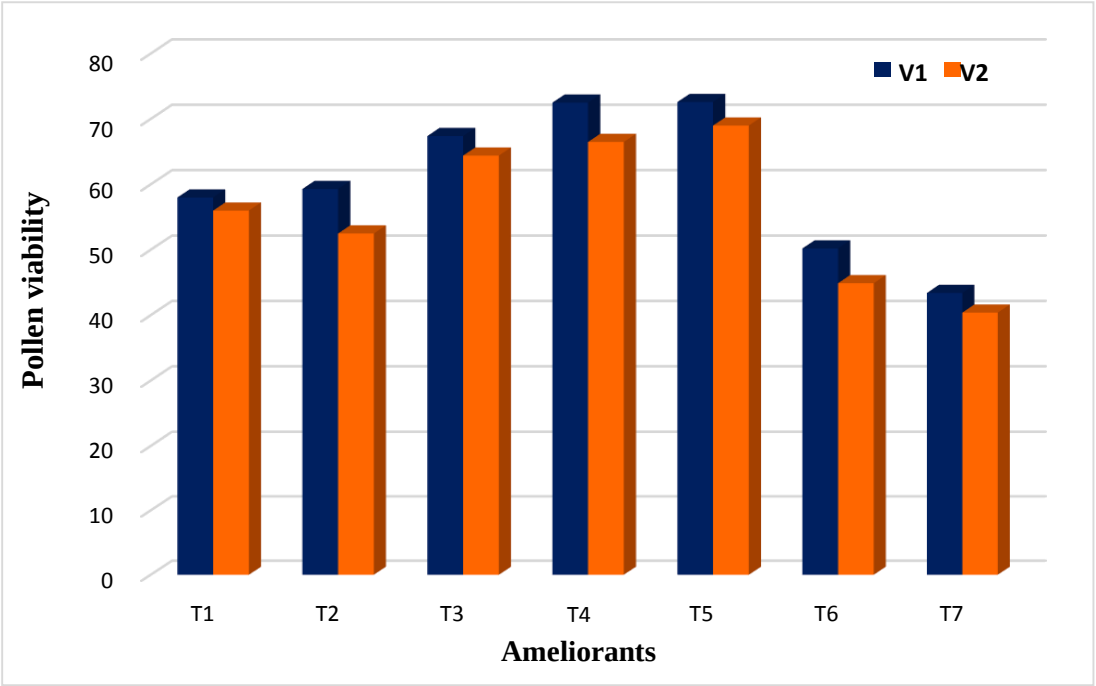

Fig. 17 Effect of ameliorants sprayed at booting stage on pollen viability in Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna]

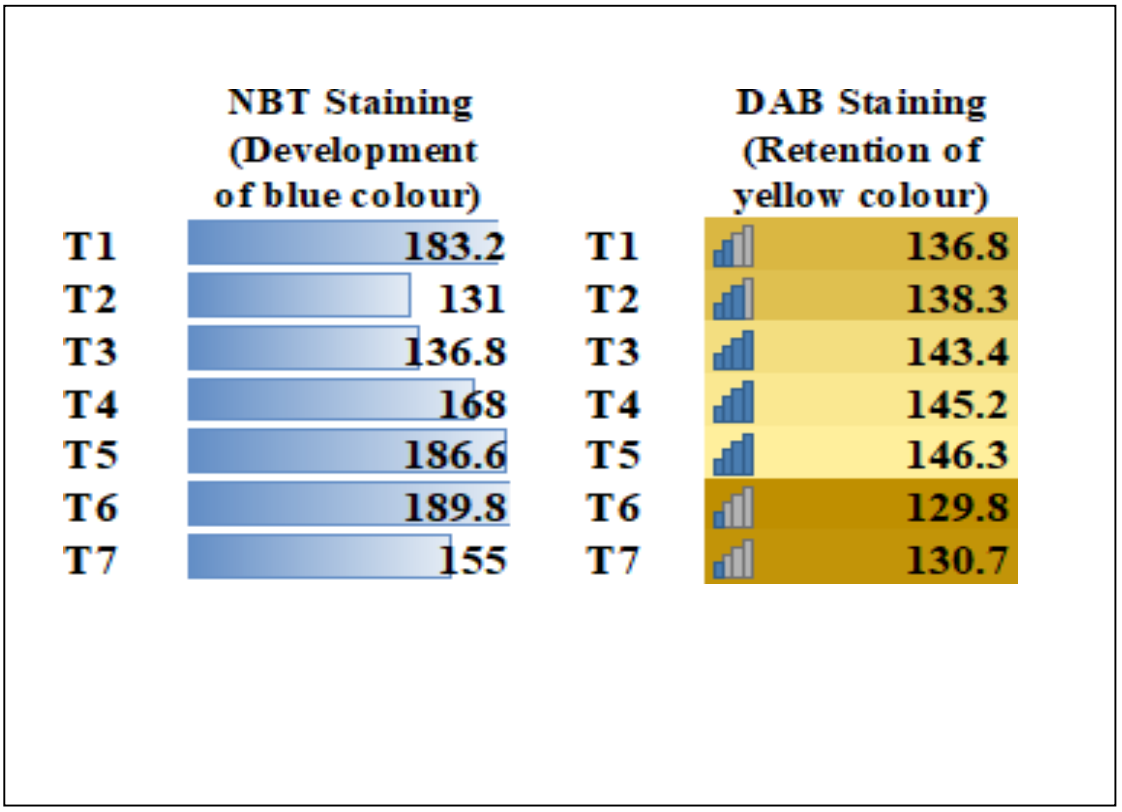

Fig. 18 Histochemical detection of superoxide (a) and Hydrogen peroxide (b)

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control]

5.2 Effect of time of application of ameliorants on physiological parameters

Response of rice plant to increasing temperature depends on its growth stage**.** From current study it was evident that exogenous application of ameliorants at both tillering and booting stages gave better results for physiological characters compared to giving spray at only one stage. The antioxidant properties of the ameliorative sprays during the critical growth stages might help the plant to combat high temperature stress and reduce the usage of its own resources which can be diverted for growth and development activities. The next best treatment was spraying ameliorants at booting stage. As far as productivity of a crop is concerned this is the most sensitive stage hence ameliorative sprays at this stage contributes to improvement in yield parameters and higher pollen viability.

Two rice varieties, Uma and Manuratna were used in the current study. Uma showed higher soluble protein content, proline accumulation, nitrate reductase enzyme activity, IAA content, pollen viability and membrane stability index compared to Manuratna. Manuratna showed improvement only in total chlorophyll content, photosynthetic rate and stomatal conductance.

5.3 Effect of ameliorant spray at booting stage on morphological parameters

Increasing temperature has been reported to alter morphological characters in plants. Plant height, tiller number, days to flowering and growth rates are observed to be affected by increasing temperature. Present study recognized a notable decrease in plant height (Table 16 & 17) growth rates viz. RGR (Table 18 & 19), CGR (Table 20 & 21) and increase in tiller decline (Table 23 & 24). Decreases in plant height and tiller number under high temperature have been substantiated by Mitra and Bhatia (2008). This might be due to drastic decrease in photosynthetic rate and dry matter production (Galani *et al*., 2016). Ameliorants sprays at booting stage improved morphological parameters even under increasing temperature. Hoagland solution spray improved plant height and growth rates which might due to the role of micronutients in enhancing growth parameters (Farooq *et al.*, 2009, Bohnsack and Albert, 1977). Increase in tiller number might be also due to activation of meristems by boron (Goldbach *et al*., 2001). Salicylic acid spray also enhanced plant height and tiller number in rice. This might be due to activation of metabolism and cell division at meristmatic regions by salicylic acid (Feng *et al*., 2018).

Plate 11. Effect of different ameliorants on plant height in Uma

Plate 12. Effect of different ameliorants on plant height in Manuratna

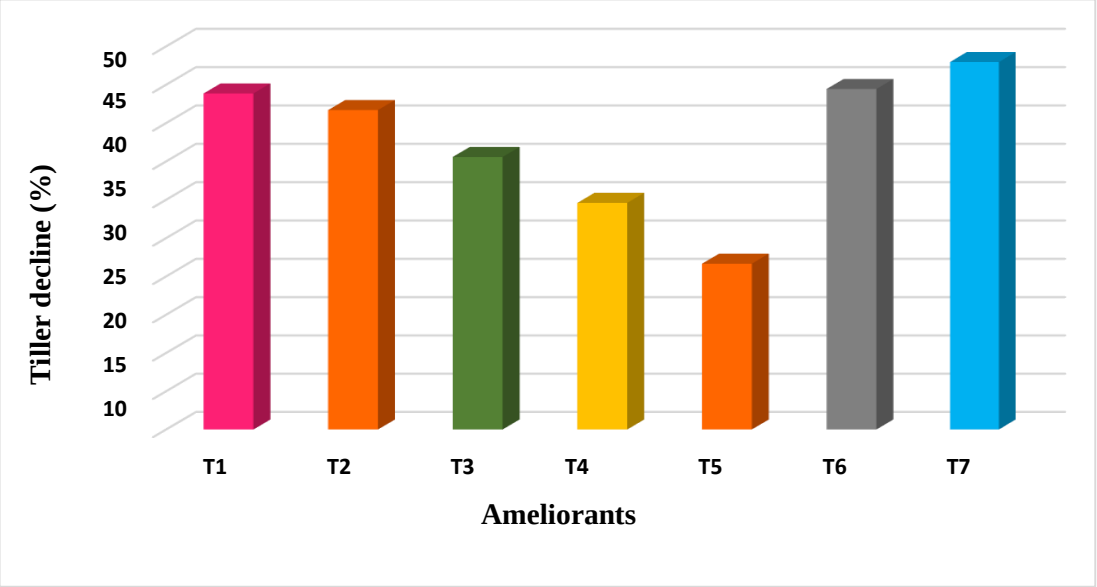

Fig. 19 Effect of ameliorant sprayed at booting stage on tiller decline percentage in Uma

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control]

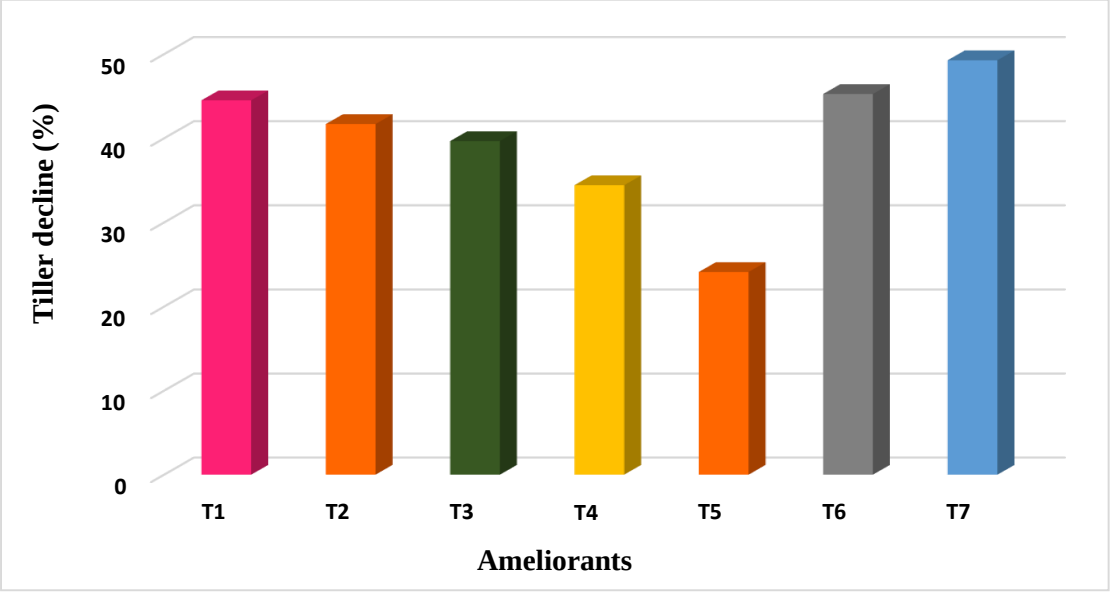

Fig. 20 Effect of ameliorant sprayed at booting stage on tiller decline percentage in Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control]

5.4 Effect of ameliorants on yield characters

The present study conducted at Agronomy farm during third crop season of 2020 with two rice varieties Uma and Manuratna revealed that temperature stress impairs yield and grain quality (Table 22 to table 30). Decrease in tiller number, kernel quality (Rashid *et al*., 2004), increased chaff percentage (Yoshida, 1977), spikelet sterility (Nakagawa *et al*., 2003) and yield loss (Morita *et al.*, 2005) were already reported in previous studies all over the world in different rice varieties grown under high temperature stress. It might be due to reduction in capacity of floral buds to mobilize photosynthates under high temperature conditions (Dinar and Rudich, 1985).

From the field trial it was evident that exogenously applied ameliorants at booting stage improved grain quality and yield attributes in both the varieties compared to non-treated control plants. Hoagland solution spray significantly increased number of productive tillers, filled grain per panicle, 1000 seed weight and grain yield in Uma and Manuratna. It also reduced the chaff percentage when applied at booting stage. This result was in accordance with previous studies viz. exogenous boron application increased number of panicles per plant (Rashid *et al*., 2007), Spikelets per panicle and 1000 seed weight (Rehman *et al*., 2014) and improved grain yield (Dunn *et al*., 2005) in different varieties of rice.

This might be due to the critical role of boron in translocation of photosynthetic assimilates from source to sink (Marschner, 1995). Boron application is reported to increase the rate of translocation and redistribution of photosynthates to growing points especially fruits which reduces chalkiness (Fig. 28) of grains (Hellal *et al*., 2009). The enhancement in Tiller number might be due to increased growth in meristamatic regions by the activity of boron (Bohnsack and Albert, 1977). Enhancement in Spikelets per panicle and 1000 seed weight plants could be due to role of boron in assimilate partitioning (Woodbridge *et al*., 1971).

Current study revealed that exogenously applied salicylic acid significantly improved straw yield in rice varieties Uma and Manuratna under high temperature conditions. Enhanced forage yield by exogenous application of salicylic acid was reported in creeping bentgrass (Larkindale and Huang, 2005) and kentucky blue grass (He *et al*., 2005).

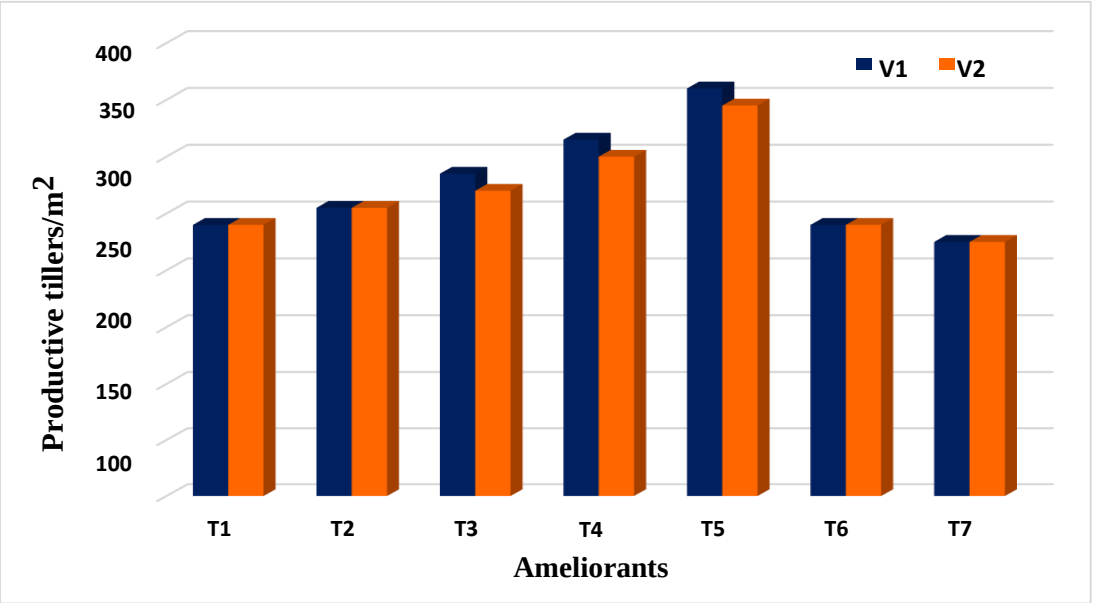

Fig. 21 Effect of ameliorants sprayed at booting stage on productive tillers per meter square in Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna]

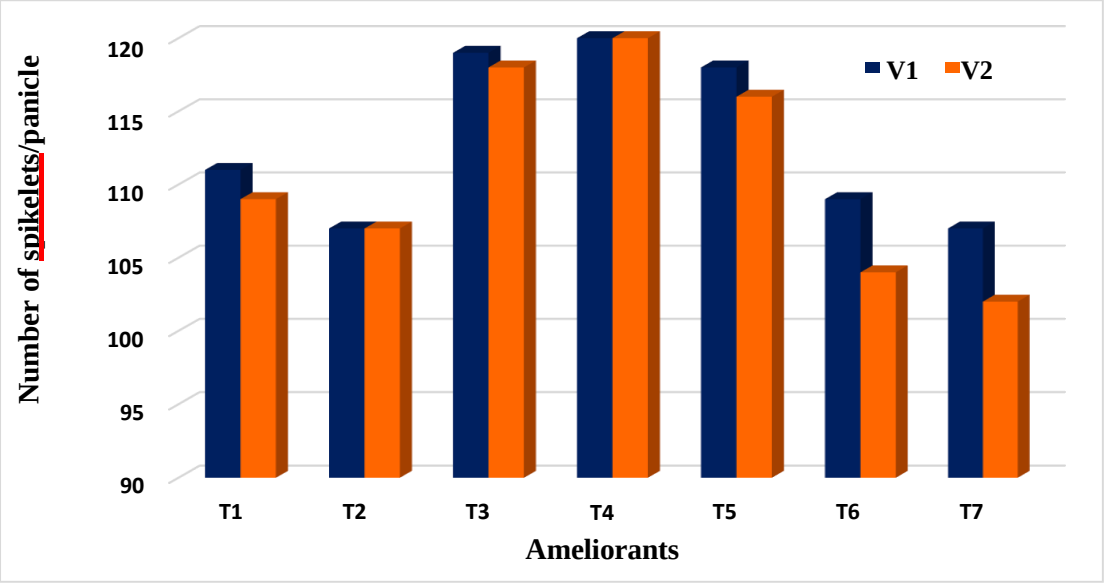

Fig. 22 Effect of ameliorants sprayed at booting stage on number of spikelets per panicle in Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna]

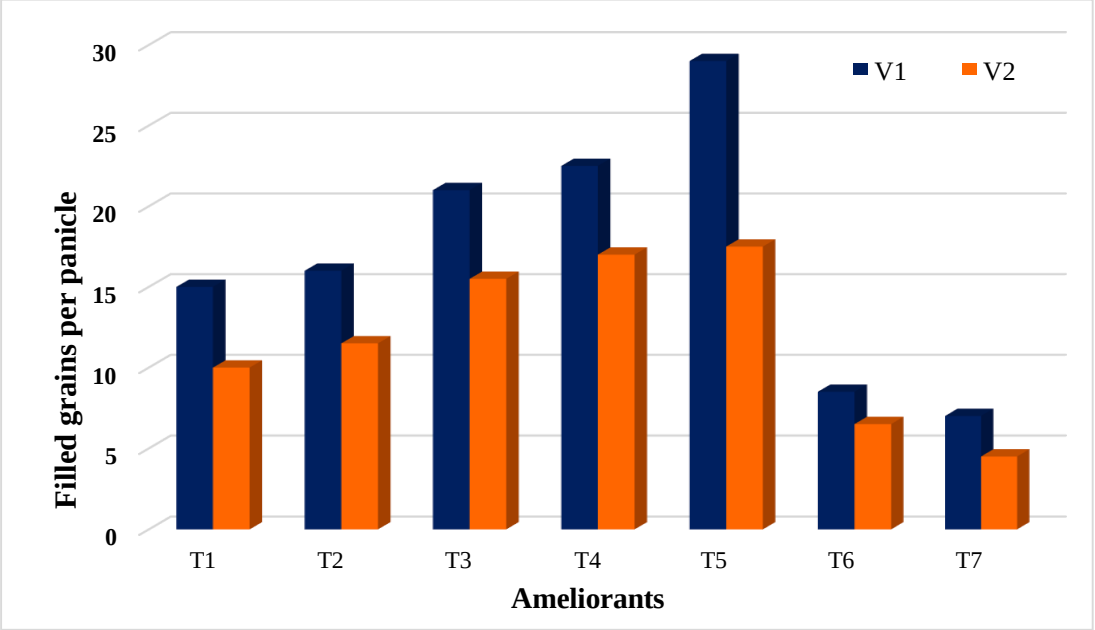

Fig. 23 Effect of ameliorants sprayed at booting stage on filled grains per panicle in Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna]

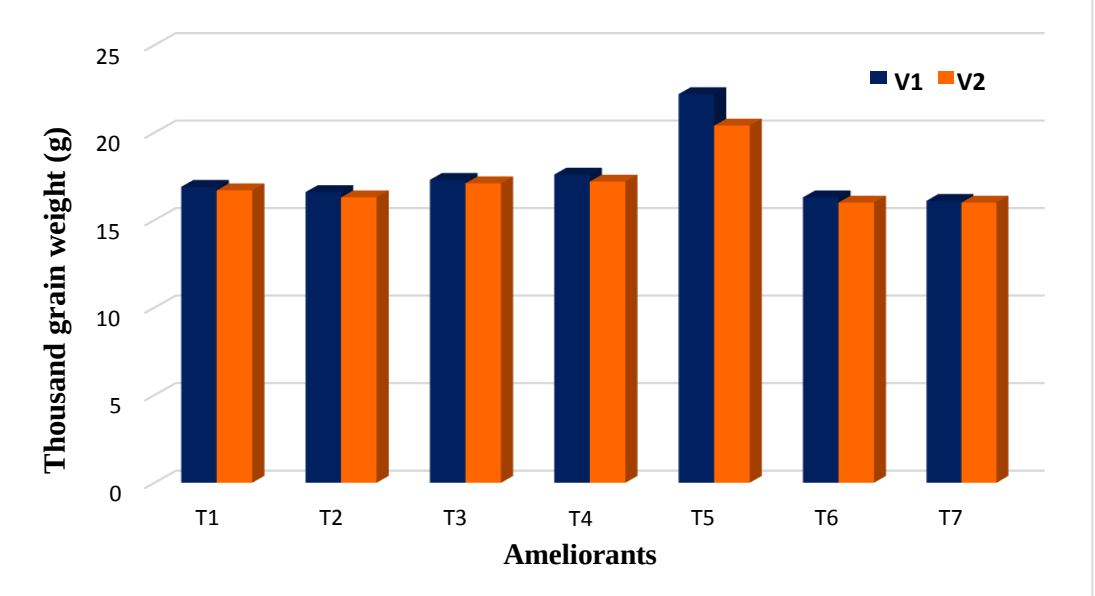

Fig. 24 Effect of ameliorants sprayed at booting stage on thousand grain weight (g) in Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna]

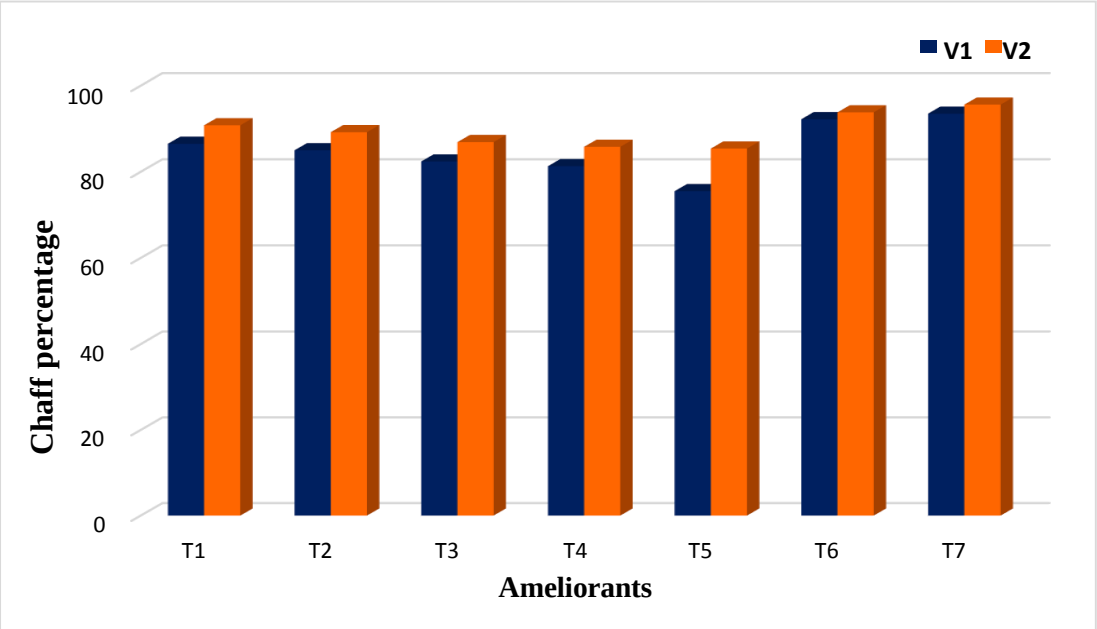

Fig. 25 Effect of ameliorants sprayed at booting stage on chaff percentage in Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna]

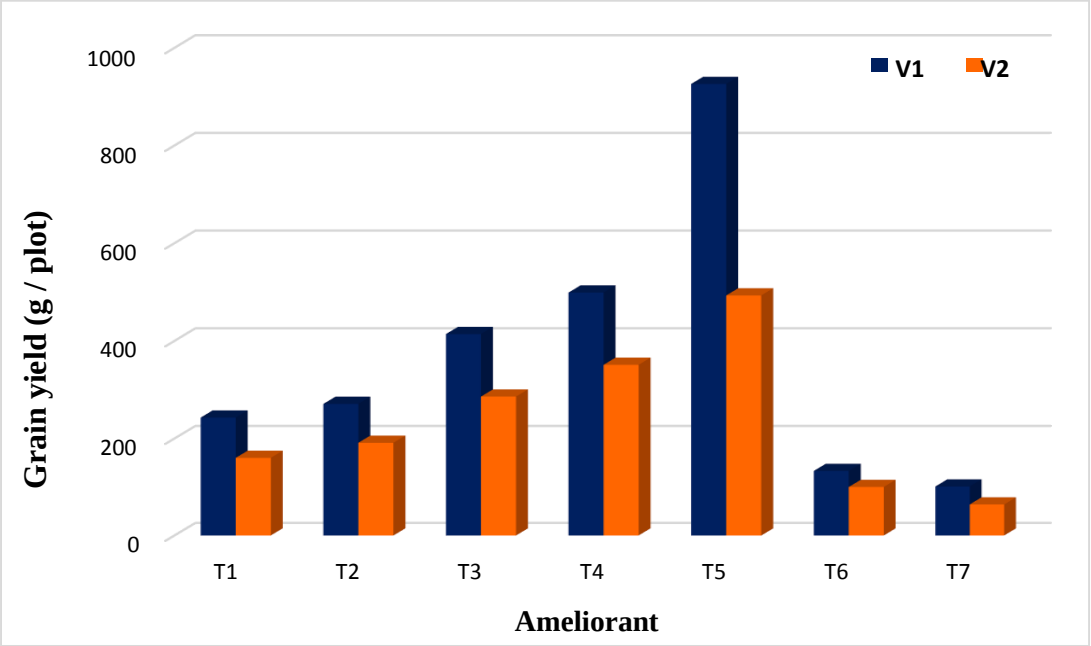

Fig. 26 Effect of ameliorants sprayed at booting stage on grain yield (g / plot) in Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna]

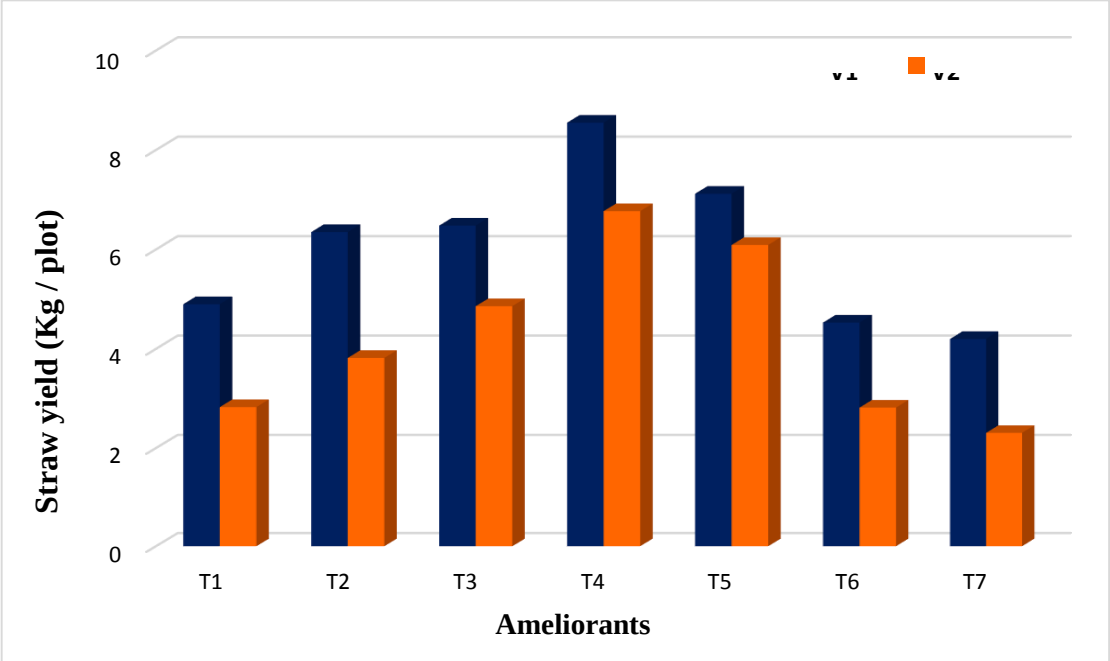

Fig. 27 Effect of ameliorants sprayed at booting stage on straw yield (Kg/plot) in Uma and Manuratna

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna]

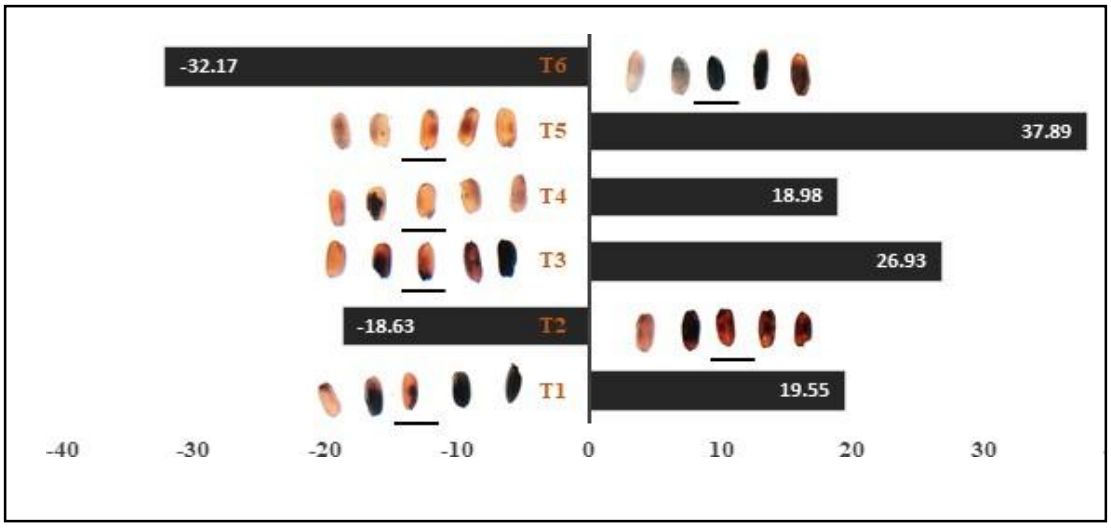

Fig. 28 Effect of ameliorants sprayed at booting stage on percent decrease in grain chalkiness over control in Uma

[T1 -Ascorbic acid T2 –Glutathione T3 –Melatonin T4 - Salicylic acid T5 –Hoagland solution T6 - Water spray T7 – Control V1- Uma V2- Manuratna]

Summary

1. Summary

Rice (*Oryza sativa* L.) is a major cereal crop most widely consumed as staple food by millions of people. Rapid increase in global population demands higher production of rice across the globe. Growth and development of rice depends on changes in environment. Among various abiotic stresses high temperature exerts a high impact on growth and productivity of rice. Third crop season always coincide with high temperature and lead to productivity decline. Chemical ameliorants can be applied to assist plants to mitigate impacts of high temperature stress.

Present study was conducted at COH, Vellanikkara. First experiment was pot culture study at glass house. Effect of ameliorants and time of spray on physiological parameters were studied. Best time of spray was standardized from this study. Second experiment was field trial. Effect of ameliorants on morphological and yield parameters was studied.

The salient findings of the study are as follows

1) Photosynthetic rate, stomatal conductance, IAA content and pollen viability was improved in plants sprayed with Hoagland solution.

2) Exogenous application of melatonin enhanced total soluble protein, chlorophyll a, chlorophyll b, total chlorophyll content and chlorophyll stability index in plants compared to control.

3) Proline accumulation increased in plants sprayed with glutathione. Nitrate reductase enzyme activity was improved by salicylic acid application.

4) Application of ameliorants with antioxidant property (Glutathione, Melatonin and ascorbic acid) scavenged reactive oxygen species and membrane stability was increased.

5) Hoagland solution and salicylic acid sprayed plants had grains with less chalky portions and improved grain quality.

6) Plants sprayed with ameliorants at both tillering and booting stage (S_3) showed better performance than plants sprayed with ameliorants at only one stage.

7) Plant height, RGR and CGR in both the varieties were increased by Hoagland solution and salicylic acid treatments.

8) Reduced tiller decline was observed in Hoagland solution treated plants.

9) Uma showed higher response to ameliorants than Manuratna.

10) Increased grain yield, number of productive tillers, spikelets per panicle, filled grains per panicle and thousand grain weight was observed in Hoagland solution treated plants.

11) Reduction in chaff percentage observed in Hoagland solution applied plants.

12) Salicylic acid improved straw yield in both varieties.

13) Comparing all the yield parameters, Uma performed better than Manuratna.

Conclusion

High temperature affects growth and productivity of rice. Ameliorative chemicals can be used to mitigate the impacts of temperature stress. Exogenous application of Hoagland solution, melatonin and salicylic acid improved physiological, morphological and yield parameters of rice. Spraying of ameliorants at both active tillering and booting stage gave better result. Among the varieties Uma performed better than manuratna.

Future line of work

- Development of commercial formulations of promising ameliorants.
- The mitigative property of different ameliorants have paved way to the identification of key mechanism / traits contributing to high temperature stress tolerance , which can be utilized in crop improvement initiatives

References

REFERENCES

Abdullah, A., and Alayafi, M. 2019. Exogenous ascorbic acid induces systemic heat stress tolerance in tomato seedlings: transcriptional regulation mechanism. *Environ. Sci. Pollut. Res. Int*. 27(16): 19186-19199.

Aghdam, M. S., Jannatizadeh, A., Luo, Z., and Razavi, F. 2019. Impact of exogenous melatonin application on chilling injury in tomato fruits during cold storage. *Food Bioproc. Tech*. 12: 741–750.

Almeselmani, M., Deshmukh, P. S., Sairam, R. K., Kushwaha, S. R., and Singh, T. P. 2006. Protective role of antioxidant enzymes under high temperature stress. *Plant Sci*. 171(3): 382-388.

Amjath, T. 2018. Interactive effect of UV radiation and elevated temperature on rice growth and physiology. M.Sc (Ag.) Thesis. Kerala Agricultural University, Thrissur, 115p.

Arif, M., Chohan, M. A., Ali, S., Gul, R. and Khan, S. 2006. Response of wheat to foliar application of nutrients. *J. Agric. Biol. Sci*. 1(4): 30-34.

Barman, D., Ghimire, O. P., Chinnusamy, V., Kumar, R. R. and Arora, A., 2019. Amelioration of heat stress during reproductive stage in rice by melatonin. *Indian J. Agric. Sci. 89*(7): 91-96.

Bates, L., Waldren, R. P., and Teare, I. D. 1973. Rapid determination of free proline for water- stress studies. *Plant and Soil* 39: 205-207.

Bejerano, N. R., Itai, C. 1981. Effect of boron on stomatal opening in epidermal strips of Commelina communis. *Physiol. plant.* 52(2): 302-304.

Blackman, V. N. 1919. The compound interest law and plant growth. *Annu.Bot.* 33: 353-358.

Bohnsack, C. W., and Albert, L. S. 1977. Early effects of boron deficiency on indole acetic oxidase levels of squash root tips. *Plant Physiol*. 59: 1047-1050.

Chen, Q., Qi, W. B., Reiter, R. J., Wei, W., and Wang, B. M. 2009. Exogenously applied melatonin stimulates root growth and raises endogenous indole acetic acid in roots of etiolated seedling of *Brassica juncea*. *J. Plant Physiol*.166: 324–328.

Deane, C. E., Clarkson, D. T., and Johnson, C. B., 1980. The effect of differential root and shoot temperature on the nitrate reductase activity, assayed in vivo and in vitro in roots of *Hordeum vulgare* (barley). *Planta*. 148(5): 455-461.

Dinar, M., and Rudich, J. 1985. Effect of heat stress on assimilate partitioning in tomato. *Ann*.
Bot. 56: 239–248 [https://doi.org/10.1093/oxfordjournals.aob.a087008].

Ding, X., Jiang, Y., He, L., Zhou, Q., Yu, J., Hui, D., and Huang, D. 2016. Exogenous glutathione improves high root-zone temperature tolerance by modulating photosynthesis, antioxidant and osmolytes systems in cucumber seedlings. *Sci. rep*. 6: 35424p.

Dunn, D., Stevens, G., and Kending, A. 2005. Boron fertilization of rice with soil and foliar applications. *Online Crop Manag*. [https://doi.org/10.1094/CM-2005-0210-01-RS].

Fariduddin, Q., Hayat, S., and Ahmad, A. 2003. Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in *Brassica juncea*. *Photosynthetica* 41: 281–284.

Farooq, M., Basra, S. M. A., Wahid, A., Ahmad, N. and Saleem, B. A., 2009. Improving the drought tolerance in rice (*Oryza sativa* L.) by exogenous application of salicylic acid. *J. Agron. Crop Sci. 195*(4): 237-246.

Feng, B., Zhang, C., Chen, T., Zhang, X., Tao, L., and Fu. G. 2018. Salicylic acid reverses pollen abortion of rice caused by heat stress. *BMC Plant Biol*. 18: 1-16

Galani, S., Hameed, S., and Kazim Ali, M. 2016. Exogenous application of salicylic acid: Inducing thermotolerance in Cotton (*Gossypium hirsutum* L.) seedlings. *Int. J. Agric. Food Res. 5*9-18.

Goldbach, H. E., Yu, Q., Wingender, R., Schulz, M., Wimmer, M., Findeklee, P., and Baluska, F. 2001. Rapid response reactions of roots to boron deprivation. *J. Plant Nutr. Soil Sci. 164*(2): 173-181.

Hageman, R. H., and Flesher, D. 1960. Nitrate reductase activity in corn seedlings as affected by light and nitrate content of nutrient media. *Plant Physiol.* 35(5): p700.

Hasanuzzaman, M., Nahara, K. B., Alama, M., and Fujitaa, M. 2015. Exogenous glutathione confers high temperature stress tolerance in mung bean (*Vigna radiata* L.) by modulating antioxidant defense and methylglyoxal detoxification system. *Environ. Exp. botan*.112: 44-54.

Hasanuzzaman, M., Nahara, K. B., Fujitaa, M., Bhuiyan, T. F., Anee, T.I., Inafuku, M., Oku, H., and Fujita, M. 2017. Salicylic Acid: An All-Rounder in Regulating Abiotic Stress Responses in Plants. *Phytohormones-signaling Mechanisms and Crosstalk in Plant Development and Stress Responses*. pp. 31-75.

Hayat, S., Fariddudin, Q., Ali, B., and Ahmad, A. 2005. Effect of salicylic acid on growth and enzyme activities of wheat seedlings. *Acta. Agron. Hung*. 53: 433-437.

Hayat, S., Masood, A., Yusuf, M., Fariduddin, Q., and Ahmad, A. 2009. Growth of Indian mustard (*Brassica juncea* L.) in response to salicylic acid under high-temperature stress. *Braz. J. Plant Physiol*. 21(3). 187-195.

He, Y. L., Liu, L., Cao, W. X., Huai, M. F., Xu, B. G., and Huang, B. G. 2005. Effects of salicylic acid on heat tolerance associated with antioxidant metabolism in Kentucky bluegrass. *Crop Sci*. 45: 988-995.

Hellal, F. A., Taalab, A. S., and Safaa, A. M. 2009. Influence of nitrogen and boron nutrition on nutrient balance and sugar beet yield grown in calcareous soil. *Ozean J. Appl. Sci*. 2: 95–112.

Hiscox, J. D. and Israelstam, G. F. 1979. A method for extraction of chlorophylls from leaf tissue without maceration. *Can. J. Bot*. 57: 1332-1334.

Howarth, C. J. 2005. Genetic improvements of tolerance to high temperature. In: Ashraf, M., Harris, P. J. C (eds). Abiotic Stresses: Plant Resistance through Breeding and Molecular Approaches. New York, Howarth Press. pp. 277–300.

IPCC (Intergovernmental Panel on Climate Change), 2007. The Physical Science Basis, Summary for Policymakers. [http://www.ipcc.ch/SPM2feb07.pdf].

Jagadish, S. V. K., Muthurajan, R., Oane, R., Wheeler, T. R., Heuer, S., Bennett, J., and Craufurd, P. Q. 2010. Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). *J. Exp. Bot*. 61: 143–156.

Jain, A., and Srivastava, H.S. 1981. Effect of salicylic acid on nitrate reductase activity in maize seedlings. *Physiol. Plant*. 51: 339-342.

Joseph, C., Nicholas, J., Harper, J. E., and Hageman, R. H. 1976. Nitrate Reductase Activity in Soybeans (*Glycine max* [L.J Merr.) I. effects of light and temperature. *Plant Physiol*. 58: 731-735.

Jumiatun, A.J., Lubis, I., Chozin, M.A., and Miyazaki, A. 2016. Morphological, physiological and yield responses of some rice varieties (Oryza sativa L.) as exposed under high temperature in Indonesia. *Am. J. Plant Physiol*. 11: 33-41.

Kabir, M.H., Liu, Q., Su, Y., Huang, Z., and Xiao, L. 2017. Dynamics of phytohormones and their relationship with chalkiness of early indica rice under different post- anthesis temperature regimes. *Bangladesh J. Agric. Res.* 42(1): 53-65.

Khan, W., Prithviraj, B., and Smith, D. L. 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. *J. Plant Physiol*. 160: 485–492.

Khan, M. I. R., Iqbal, N., Masood, A., Per, T. S and Khan, N. A. 2013. Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. *Plant Signal Behav*. 8(11): p.e 26374.

Kreiner, M., Harvey, L. M., and McNeil, B. 2002. Oxidative stress response of a recombinant *Aspergillus niger* to exogenous menadione and H_2O_2 addition. *Enzyme Microb. Technol.* 30: 346– 353.

Kumar, S., Kaur, R., Kaur, N., Bhandhari, K., Kaushal, N., Gupta, K., Bains, T. S. and Nayyar, H. 2011. Heat-stress induced inhibition in growth and chlorosis in mung bean (*Phaseolus aureus* Roxb.) is partly mitigated by ascorbic acid application and is related to reduction in oxidative stress. *Acta Physiol. Plant. 33*(6). p.2091.

Kumar, D., Yusuf, M. A., Singh, P., Sardar, M., Sarin, N. B. and Biosciences, J. M. I., 2014. Histochemical detection of superoxide and H2O2 accumulation in Brassica juncea seedlings. *Bio Protoc*. *4*(8): p.e1108.

Larkindale, J., and Huang, B. 2005. Eff ects of abscisic acid, salicylic acid, ethylene and hydrogen peroxide in thermotolerance and recovery for creeping bent grass. *Plant Growth Regul*. 47: 17–28.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193: 265- 275.

Matsui, T., Omasa, K. and Horie, T., 2001. The difference in sterility due to high temperatures during the flowering period among japonica-rice varieties. *Plant Prod. Sci. 4*(2): 90-93.

Meena, M., Divyanshu, K., Kumar, S., Swapnil, P., Zehra, A., Shukla, V., Yadav, M., and Upadhyay. R. S. 2019. Regulation of L-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. *Heliyon*. 5(12). pp 2952.

Mengutay, M., Ceylan, Y., Kutman, U. B., and Cakmak, I. 2013. Adequate magnesium nutrition mitigates adverse effects of heat stress on maize and wheat. *Plant Soil.* 368: 57–72.

Mitra, R., and Bhatia, C. R. 2008. Bioenergetics cost of heat tolerance in wheat crop. *Curr. Sci*. 94(8):

Mitsui, T., Yamakawa, H., and Kobata, T. 2016. Molecular physiological aspects of chalking mechanism in rice grains under high-temperature stress. *Plant Prod. Sci*. 19: 22–29.

Morita, S., Yonemaru, J. L., and Takanashi, J. 2005. Grain growth and endosperm cell size under high night temperatures in rice *(Oryza sativa* L.). *Ann. Bot*. 95: 695-701.

Munjal, R., Dhanda, S. S. 2004. Physiological and morphological traits associated in bread wheat under heat stress. In: *Proceedings of 91st Indian Science Congress.* (Advance abstracts). 1–3rd January, P.U. Chandigarh, India. pp. 77– 78.

Murthy, K. S. and Majumdar, S. K. 1962. Modification of the technique for determination of chlorophyll stability index in relation to studies of drought resistance in rice. *Curr. Sci.* 32: 470-471.

Nakagawa, H., Horie, T., and Matsui, T. 2003. Effects of climate change on rice production and adaptive technologies. In *International Rice Research Conference*, Beijing, China, 16-19 September 2002. International Rice Research Institute.

Nicholas, J. C., Harper, J., and Hageman, R. H. 1976. Nitrate reductase activity in Soybeans (*Glycine max* L.). *Plant Physiol.* 58: 736.

Oh-e, I., Saitoh, K., and Kuroda, T. 2007. Effects of high temperature on growth, yield and dry-matter production of rice grown in the paddy field. *Plant Prod. Sci*. 10(4): 412–422.

Osada, A., Takahashi, H., Dhammanuvong, S., Sasiprapa, V., and Gunthararom, S. 1973. Seasonal changes in growth pattern of tropical rice: I. Environmental factors affecting plant height, tillering, weight and leaf area. *Jpn. J. Crop Sci*. 42(3): 343- 350.

Parthasarathy, K., Balu, D. R. C., and Rao, P. S. 1970. Studies on sandal spur. VII. Polyphenol oxidase activity and metabolism of sandal (*Santalam album* L.) in healthy and diseased. *Proc. Indian. Acad. Sci.* 72: 277- 284.

Peng, S., Huang, J., Sheehy, J.E., Laza, R.C., Vispera, R.M., Zhong, X., Centeno, G.S., Khush, G.S., and Cassman, K. G. 2004. Rice yields decline with higher night temperature from global warming, *Proceedings of the National Academy of Sciences*, August 2004, 101(27): 9971-9975.

Ramesh, R., Ramesh, T., Rao, P. R., Shankar, V. G., and Bhave, M. H. V. 2017. Evaluation of rice genotypes for high temperature tolerance under field conditions. *J. Pharmacogn. Phytochem.* 6(4): 115-124.

Rane, M. J., Pan, Y., Singh, S., Powell, D. W., Wu, R., Cummins, T., Chen, Q., McLeish, K.

R. and Klein, J. B., 2003. Heat shock protein 27 controls apoptosis by regulating Akt activation. *J. Biol. Chem.* 278(30): 27828-27835.

Rashid, A., Yasin, M., and Ashraf, M. 2004. Boron deficiency in calcareous soil reduces rice yield and impairs grain quality. *Int. Rice Res. Notes*. 29: 58–60.

Rashid, A., Yasin, M., Ali, M. A., Ahmad, Z., and Ullah, R. 2007. An alarming boron deficiency in calcareous rice soils of Pakistan: boron use improves yield and cooking quality. In: Xu F (ed) Advances in plant and animal boron nutrition. *Proceedings of 3rd international symposium on all aspects of plant and animal boron nutrition*, Wuhan, China, 9–13 Sep 2005. Springer, Dordrecht, pp 103–116.

Rehman, A., Farooq, M., Cheema, Z., Nawaz, A., and Wahid, A., 2014. Foliage applied boron improves the panicle fertility, yield and biofortification of fine grain aromatic rice. *J. soil sci. plant nutr. 14*(3): 723-733.

Sailaja, B., Anjum, N., Prasanth, V. V., Sarla, N., Subrahmanyam, D., Voleti, S. R., Viraktamath, B. C., and Mangrauthia, S. K., 2014. Comparative study of susceptible and tolerant genotype reveals efficient recovery and root system contributes to heat stress tolerance in rice. *Plant mol. biol. rep*. 32(6): 1228-1240.

Satake, T. and Yoshida, S., 1978. High temperature-induced sterility in indica rices at flowering. *Japanese J. Crop Sci.* 47(1): 6-17.

Sato, K. 1972. Growth Responses of Rice Plant to Environmental Conditions: I. the effects of air-temperatures on the growth at vegetative stage. *Jpn. J. Crop Sci*. 41(4): 388- 393.

Shah, F., Huang, J., Cui, K., Nie, L., Shah, T., Wu, W., Wang, K., Khan, Z.H., Zhu, L. and Chen, C., 2011. Physiological and biochemical changes in rice associated with high night temperature stress and their amelioration by exogenous application of ascorbic acid (vitamin C). *Aust. J. Crop Sci. 5*(13), p.1810.

Shahid, M., Nayak, A. K., Tripathi, R., Katara, J. L., Bihari, P., Lal, B., and Gautam, P. 2018. Boron application improves yield of rice cultivars under high temperature stress during vegetative and reproductive stages. *Int. j. biometeorol*. *62*(8): 1375- 1387.

Shakirova, F. M. 2007. Role of Hormonal System in the Manisfestation of Growth Promoting and Anti-Stress Action of Salicylic Acid. In: Hayat, S., and Ahmad, A. (Eds.) Salicylic Acid, A Plant Hormone. Springer, Dordrecht, Netherlands.

Sharma, P. N., and T. Ramachandra. 1990. Water relations and photosynthesis in mustard plants subjected to boron deficiency. *Indian J. Plant Physiol.* 33: 150–154.

Siddiqui, M.H., Alamri, S.A., Al-Khaishany, M.Y., Al-Qutami, M.A., Ali, H.M., Al-Whaibi, M.H., Al-Wahibi, M.S. and Alharby, H.F., 2018. Mitigation of adverse effects of heat stress on Vicia faba by exogenous application of magnesium. *Saudi J. Biol. Sci*. 25(7): 1393-1401.

Solomonson, L. P., and Barber, M. J. 1990. Assimilatory nitrate reductase: Functional properties and regulation. *Ann. Rev. Plant. Physiol. Plant. Mol. Biol*. 41: 225-253.

Song, J., Nada, K., and Tachibana, S. 2001. The early increase of S-adenosylmethionine decarboxylase activity is essential for the normal germination and tube growth in tomato (*Lycopersicon esculentum* Mill.) pollen. *Plant Sci*. 161: 507–515.

Srivastava, H. S., 1980. Regulation of nitrate reductase activity in higher plants. *Phytochem.* 19(5): 725-733.

Sullivan, C. Y. 1972. Mechanisms of Heat and Drought Resistance in Grain Sorghum and Methods of Measurement. Oxford and IPH, New Delhi, p. 247– 264.

Suzuki, N., Koussevitzky, S., Mittler, R., and Miller, G. 2012. ROS and redox signalling in the response of plants to abiotic stress. *Plant Cell Environ*. 35: 259–270.

Szalai, G., Kellős, T., Galiba, G., and Kocsy, G.2009. Glutathione as an antioxidant and regulatory molecule in plants under abiotic stress conditions. *Plant Growth Regul*. 28: 66-80

Taiz, L. and Zeiger, E. 2002. Plant Physiology. Sinuaer associates incorporation publishers, Saunderland.

Tang, R.S., Zheng, J.C., Jin, Z.Q., Zhang, D.D., Huang, Y.H., and Chen, L.G. 2008. Possible correlation between high temperature-induced floret sterility and endogenous levels of IAA, GAs and ABA in rice (*Oryza sativa* L.). *Plant Growth Regul*. 54:37–43.

Taniyama, T., Subbaiah, S. V., Rao, M. L. N., and Ikeda, K. 1988. Cultivation and ecophysiology of rice plants in the tropics, 3: photosynthesis of rice cultivars of India, measured by the Tsuno simple method. *Japanese J. Crop Sci.* 57:184–190.

Tao, F., Hayashi, H., Zhang, Z., Sakamoto, T., and Yokozawa, M.2008. Global warming, rice production, and water use in China: developing a probabilistic assessment. *J. Agric. For. Meteorol*.148: 94-110.

Wang, C., Lei, S., Ashraf, U., Mo, Z., Nawaz, M., Ashraf, I., Muzaffar, W., Liu, S. J., and Tang, X. R., 2017. Exogenous application of mixed micro-nutrients improves yield, quality, and 2-acetyl-1-pyrroline. *Appl. Ecol. Environ. Res. 15*(3): 1097-1109.

Waraich, E., Ahmad, R., Halim, A., and Aziz, T. 2012. Alleviation of temperature stress by nutrient management in crop plants: a review. *J. Soil Sci. Plant. Nutr*. 12: 221–244.

Wassmann, R., and Dodermann, A. 2007. Climate change adaptation through rice production in regions with high poverty levels. *J. SAT Agric. Res.* 4.

Zhang, G. L., Chen, L. Y., Zhang, S. T., Liu, G. H., Tang, W. B., He, Z. Z., and Wang, M. 2007. Effects of high temperature on physiological and biochemical characteristics in flag leaf of rice during heading and flowering period. *Scientia Agricultura Sinica*. 40: 1345-1352.

Zhang, C. X., Feng, B. H., Chen, T. T., Zhang, X. F., Tao, L. X. and Fu, G. F., 2017. Sugars, antioxidant enzymes and IAA mediate salicylic acid to prevent rice spikelet degeneration caused by heat stress. *Plant Growth Regul. 83*(2): 313-323.

Zhong, L., Lin, L., Yang, L., Liao, M. A., Wang, X., Wang, J., Deng, H., Liang, D., Xia, H. and Tang, Y. 2020. Exogenous melatonin promotes growth and sucrose metabolism of grape seedlings. *Plos one.* 15(4): p.0232033.

Wassmann, R., Jagadish, S. V. K., Sumfleth, K., Pathak, H., Howell, G., Ismail, A., Serraj, R., Redona, E., Singh, R. K., and Heuer, S., 2009. Regional vulnerability of climate change impacts on Asian rice production and scope for adaptation. *Adv. Agron.* 102: 91-133.

Watson, D. J. 1952. The physiological basis of variation in yield. *Adv. Agron.* 4: 101-145.

Woodbridge, C. G., Venegas. A., and Crandall, P. C. 1971. The boron content of developing pear, apple and cherry flower buds. *J. Amer. Soc. Hort. Sci*. 96: 613–615.

Xie, X.J., Shen, S.H.H., Li, Y.X., Zhao, X.Y., Li, B., and Xu, D.F. 2011. Effect of photosynthetic characteristic and dry matter accumulation of rice under high temperature at heading stage. *Afr. J. Agric. Res.* 6(7): 1931-1940.

Xue, X. D., Sun, Y., Guo, X. Q., Sun, B., and Zhang, J. 2011. Effect of exogenous melatonin on photosynthesis and chlorophyll fluorescence parameters in leaves of cucumber seedlings under high temperature stress. *Acta. Agric. Nucl. Sin*. 25: 179–184.

Xue, W., Cai, S.Y., Zhang, Y., Wang, Y., Ahammed, G.J., Xia, X.J., Shi, K., Zhou, Y.H., Yu, J.Q., Reiter, R.J. and Zhou, J., 2016. Melatonin enhances thermotolerance by promoting cellular protein protection in tomato plants. *J. Pineal Res*. 61(4): 457-469.

Yamamoto, Y., Tamori, T., and Kawaguchi, S. 1985. Relations between weather and growth of rice plant. I. Effects of air-temperature on the growth of rice plant in the first half stage. *Bull. Toyama. Agric. Exp. Stn*.16: 20–26.

Yoshida, S. 1973. Effects of temperature on growth of rice plant (*Oryza sativa* L) in a controlled environment. *Soil Sci. Plant Nutr*.19: 299–310.

Yoshida, S. 1981. Fundamentals of Rice Crop Science. International Rice Research Institute, Los Banos, Laguna, Philippines, 269p.

Yoshida, S. and Hara, T. 1977. Effects of air temperature and light on grain filling of an indica and a japonica rice (*Oryza sativa* L.) under controlled environmental conditions. *Soil Sci. Plant Nutr.* 23(1): 93-107.

Yun-Ying, C., Hua, D., Li-Nian, Y., Zhi-Qing, W., Li-Jun, L., and Jian-Chang, Y. 2009. Effect of high temperature during heading and early filling on grain yield and physiological characteristics in indica rice. *Acta. Agronomica Sinica*. 35(3): 512- 521.

PHYSIOLOGICAL INTERVENTION FOR MITIGATING TEMPERATURE STRESS IN RICE

By

FEMINA K

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ABSTRACT OF THE THESIS

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Faculty of Agriculture

Kerala Agricultural University, Thrissur

Department of Plant Physiology
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA

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KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF HORTICULTURE, VELLANIKKARA

Department of Plant Physiology

Master's Defence Seminar

Physiological intervention for mitigating temperature stress in rice

Abstract

Name : Femina K

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Major Advisor : Dr. T.Girija

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Rice is an important cereal crop which is most widely consumed as staple food by millions of people. Demand for rice is projected to increase across globe with rapid increase in population. Rice being a tropical crop requires a fairly high temperature for optimum growth and development. However, temperatures above 35 ^{0}C cause heat injuries in rice including changes in morphological, physiological and yield characters. Hence, the present study was conducted to identify ameliorative chemicals to mitigate high temperature stress, which is a common occurrence in the third crop season where temperatures can go up to 40 ^{0}C in the reproductive stage leading to yield loss. Popular rice varieties, Uma and Manuratna, which are also suitable for the third crop season were chosen for the study.

The whole study was divided in to two experiments. The first experiment was carried out in glass house at College of Horticulture, Vellanikkara during January to May, 2019. Ten day old rice seedlings of Uma (V_1) and Manuratna (V_2) were planted in pots. Treatments included five ameliorative sprays viz. Ascorbic acid (T_1), Glutathione (T_2), Melatonin (T_3), Salicylic acid (T_4) and Hoagland solution (T_5) along with Water sprayed (T_6) and Unsprayed (T_7) controls. Ameliorants were sprayed at active tillering stage (S_1), booting stage (S_2) and both active tillering and booting stages (S_3). Physiological responses were studied from this experiment.

Pot culture study showed that ameliorants could improve physiological characters of rice under increasing temperature. Hoagland solution spray enhanced photosynthetic rate, stomatal conductance, IAA content and pollen viability in both the varieties. Increased total soluble protein content, chlorophyll a, chlorophyll b and total chlorophyll was noted in plants sprayed with melatonin. Proline accumulation increased in plants sprayed with glutathione while nitrate reductase enzyme activity improved by salicylic acid. Reduced amount of reactive oxygen species as evidenced by histochemical studies was observed in plants sprayed with ameliorants having antioxidant property, like melatonin, glutathione and ascorbic acid. Spraying of ameliorants at both active tillering and booting stages (S_3) was the best followed by spraying at booting stage (S_2) alone.

Second experiment was carried out in Agronomy farm at College of Horticulture, Vellanikkara during February to June, 2020. Twenty day old seedlings of Uma and Manuratna were transplanted to micro plots of 4 m^2. Ameliorants were given as foliar spray at booting stage and one plot of each variety was maintained as control for comparison. Biometric observations and yield characters were studied from this trial.

Hoagland solution and salicylic acid treatments increased plant height, RGR and CGR in both the varieties. Reduced tiller decline was also observed in Hoagland solution treated plants. Uma showed higher response to ameliorants than Manuratna. Yield parameters were analyzed by comparing response of ameliorants with control. Plants sprayed with Hoagland solution showed better performance with higher grain yield. It was due to increased number of productive tillers, spikelets per panicle, filled grains per panicle and thousand grain weight. Chaff percentage decreased by application of ameliorants and among them Hoagland solution spray gave the best result. Salicylic acid applied plants had higher straw yield compared to other treatments. Yield performance was better in Uma compared to Manuratna.