**Original Research Article**

**Mitigating High Temperature Stress in Rice (*Oryza sativa* L.) with Osmoprotectant Foliar Sprays at Various Phenophases**

**ABSTRACT**

*Pot experiment was conducted to assess the effects of high-temperature stress on rice (Oryza sativa L.) and to identify effective mitigation strategies in kharif season (2023) in the Department of Agricultural Meteorology, Kerala Agricultural University in a factorial CRD layout. The rice variety Jyothi was exposed to high-temperature (HT) in a temperature monitored polyhouse to impart stress and ambient conditions for comparison with the stressed condition. The pots were exposed to high temperature stress in polyhouse at 3 stages: active tillering, heading and milking respectively. Four different treatments were administered as foliar sprays at three stages. Variations were observed in physiological, biochemical, and yield components across the different conditions and treatments. Plants exposed to the high temperature showed lower values of the physiological parameters and also in yield. Temperatures above 29°C during the milking stage reduced the yield and number of filled grains drastically. The external application of osmoprotectants proved effective in mitigating the adverse effects of high temperature. The treatment salicylic acid (400ppm) recorded the highest chlorophyll content and chlorophyll stability index. With regards to the yield, highest yield was seen in the salicylic acid (400ppm) treated set of plants. Temperatures above 29°C during the milking stage reduced the yield and number of filled grains drastically.*

**Key words**: **High temperature stress, Salicylic acid, chlorophyll content, yield, chlorophyll stability index**

**Introduction**

Rice (*Oryza sativa* L.) ranks second among the cereal crops in terms of annual global production. It is the primary staple food for over three billion people worldwide, making it more widely consumed than any other crop [1]. Rice is grown in a wider variety of ecological conditions than any other food crop, including irrigated lowlands, rainfed areas, uplands, deep water environments, and regions below mean sea level. Rice productivity is constrained by various abiotic stresses such as salinity, drought, flooding, and cold temperatures [2]. Abiotic stress can affect a variety of physiological and biochemical processes in plants. The generation of reactive oxygen species (ROS) is harmful at high quantities and causes degradation of RNA, DNA, and plant cell membrane [3]. Autocatalytic peroxidation of membrane lipids and pigments by ROS leads to loss of membrane semi-permeability. The hydroxyl radical (OH-) can damage chlorophyll, protein, DNA, lipids, and other important macromolecules, thus fatally affecting plant metabolism and limiting growth and yield [4]. Rice plants are more vulnerable to heat stress injury during the reproductive stage compared to other growth stages [5]. High temperatures during anthesis decrease anther dehiscence, pollen shedding, stigma receptivity, pollen germination, and pollen tube penetration, resulting in lower pollen viability and spikelet fertility percentage [6,7]. This ultimately leads to reduction in the yield of the crop. It was reported that the yield of dry season rice crop decreased by 15% for each 10̊ C temperature increase in the growing season mean temperature [8].

Common osmoprotectants include salicylic acid, ascorbic acid and citric acid. They can also stabilise membrane bilayers, perhaps serving as an adaptive mechanism to cope with heat stress [9]. Several studies indicate that these compounds mitigate the ill effects of high temperature stress in plants through various mechanisms, like preventing the degradation of chlorophyll, reducing electrolytic leakage and maintaining or sometimes increasing antioxidant enzyme activities, and consequently the yield of the crop. Salicylic acid, a common phenolic molecule, works as a plant growth regulator and enhances photosynthesis under heat stress by regulating different physiological processes and biochemical reactions [10]. Ascorbic acid is a small, water-soluble anti-oxidant molecule which acts as a primary substrate in the cyclic pathway of enzymatic detoxification of hydrogen peroxide [11]. With the increasing population, the need to fulfil the demand of food security has also increased. Keeping in mind the above problem relating to rice production under high temperature stress, the present investigation was undertaken to study the efficiency of some osmoprotectants in improving crop physiological parameters and seed yield of rice. The main focus was to reduce the impact of heat stress in rice during vegetative and reproductive stage of rice. Hence the present study is to find the best treatment which can help mitigate the heat stress in rice at different stages.

**MATERIALS AND METHODS**

The study was conducted at Instructional farm, College of Agriculture, Vellanikkara, Thrissur, Kerala Agricultural University during the *kharif* season of 2023. The rice variety used for this study was *Jyothi*, Kerala’s leading short duration variety. Seedlings were raised in pot trays and transplanted to mud pots 18 days after sowing. The experiment was laid out in factorial CRD with two factors. Factor 1 was the different conditions of exposure to high temperature (8 sets) and factor 2 had 4 treatments (3 sprays and 1 unsprayed control). All the sets had two replications.

The sets were kept under high temperature condition in a polyhouse and at ambient temperature from seedling to maturity stage for different intervals. The pots were divided into 8 sets (C1, C2, C3, C4, C5, C6, C7 & C8) based on their exposure to open and high temperature exposure at different phenophases as shown in the Table 1.

Treatments [Salicylic acid (SA)-400ppm (S1), citric acid (CA)-1.3%+Ascorbic acid (AA)-10ppm (S2), water spray(S3) and control (no spray) (S4)] were sprayed at active tillering, heading and milking stage as shown in the Figure 1. Pots exposed to their specific condition 7 days after the spraying.

**Chlorophyll content**

The chlorophyll content of leaves was estimated using the method of Arnon (1949)[12]. Leaf bits of 0.100g were taken in a test tube with 8mL of dimethyl sulphoxide (DMSO). The tubes were kept in dark for about 12 hours and volume was made up to 10mL. The total chlorophyll content was then calculated using the following formula**.** The intensity of colour was read using Spectronic 20 spectrophotometer at 663 nm and 645 nm. The formula for calculating chlorophyll content is given below:

Total Chlorophyll (mg/g) = (18.02 x OD at 663) + (20.2 x OD at 645) x V/ (1000 x W)

Where, OD – Optical Density

V – Final volume of extractant

W – Weight of leaf sample (g)

**Chlorophyll stability index**

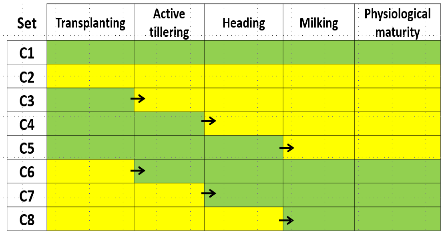
The chlorophyll stability index was measured using a green seeker. The Green seeker readings are NDVI values that give a measure of the stress on plants.

**Yield attributes**

The no. of tillers, panicles, no. of filled grains and chaff per panicle was recorded during harvest.

**Table 1. Conditions of different factors**

|  |  |
| --- | --- |
| Sets | Conditions |
| C1 | Ambient(open) condition from T- Harvest |
| C2 | High temperature (HT) from T- harvest |
| C3 | Open from T- AT/ HT from AT-Harvest |
| C4 | Open from T- H / HT from H- Harvest |
| C5 | Open from T- M/ HT from M- Harvest |
| C6 | HT from T- AT/ open from AT- Harvest |
| C7 | HT from T-H /open from H- Harvest |
| C8 | Heat stress from T-M / open from M- Harvest |
| T- Transplanting | |
| AT- Active tillering | |
| H- Heading | |



**Figure 1: Sets (factors) exposed to different conditions and stage of spraying**

**Arrows represents the stages of shifting between conditions**

**RESULTS AND DISCUSSIONS**

**Maximum temperature**

The temperature in the polyhouse and the ambient condition was recorded by a campbell scientific instrument. The temperature at different phenophases in the factors can be seen in Table 2.

**Phenophases and their duration**

Under heat stress condition, the growth stages of factors C2, C3 and C8 got arrested at the heading stage without proceeding further as a result, no milking stage was observed. The factor C7, which was under stress till heading stage, proceeded to the milking stage after it was shifted to the ambient condition.

In addition to that, heat stress caused a delay in the arrival of the phenophases. Active tillering under the stress condition was achieved with a delay of 3 days as compared to the ambient condition. Same was seen for the heading stage, where the stressed plants took around 8-13 days more than that taken in the ambient condition which can be seen in the Figure 2.

During the reproductive stages, high temperature of about 46.1̊ C in the factors C2, C3, and C8 restricted the flowering stage. Hence, there was no grain filling (milking) and no yield was obtained (Figure 3).

**Physiological parameters**

The data on the physiological parameters of variety *Jyothi* was taken 7 days after spraying of the osmoprotectants from both the conditions at active tillering, heading and milking stage. The yield data was taken after the harvest.

**Chlorophyll content**

When the spraying was done at the vegetative stage i.e. active tillering stage, out of the different treatments, the highest chlorophyll content was seen in the plants sprayed with salicylic acid (400ppm) followed by ascorbic acid (10ppm) + citric acid (1.3%) spray (Table 3). The least chlorophyll content was found to be in the water spray and the unsprayed plant set. The same results were obtained in heading as well as the milking stage (Table 4& 5). When the total chlorophyll content in the different factors were compared, it was found that, the C1 set (completely under ambient condition) showed the highest content, the least value was shown by C2 (completely in polyhouse) and C6(under high temperature till active tillering) with no significant difference between them (Figure 4, 5 & 6). The plants in the stress condition did not reach the milking stage, so no observations of heat stress conditions were taken. Chlorophyll content provides insight into plant physiological status [13]. Heat stress can limit chlorophyll accumulation in plants by decreased production, increased breakdown, or a combination of both. Heat stress can degrade chlorophyll, resulting in loss of photosynthetic activity [14] It is believed that a number of enzymes become inactive under high temperature stress, inhibiting the formation of chlorophyll [15].

**Chlorophyll stability index**

The high chlorophyll stability index (CSI) value gives an indication of stress tolerance. At the active tillering stage, the highest CSI was observed in the C1 set followed by C3. The least values were observed in case of C2 and C6 which were under high temperature (41̊ C) during the active tillering stage (Figure 7 & Table 6). The foliar spray S1 showed the highest value in all the factors.

When spraying was done in heading stage, the sets kept in ambient condition i.e C1 and C4 showed the highest chlorophyll stability index as compared to the one kept at stress i.e. C2 and C7. The foliar spray S2 (Ascorbic acid 10ppm + Citric acid 1.3%) showed highest chlorophyll stability in the factor C7 (under high temperature condition till heading stage) (Figure 8 & Table 7). Similar results were observed in wheat, where reduced chlorophyll content and chlorophyll stability index under elevated heat stress was observed by [16]

**Yield attributes**

**Number of tillers and panicles**

The number of tillers and panicles were highest in the plants kept under ambient condition as compared to all other sets. Among all the spray treatments, the salicylic acid treated plants resulted in higher number of tillers and panicles. The set C6 that was exposed to high temperature of around 48.8°C (Fig 10.a) from transplanting to active tillering stage resulted in lower number of tillers.

The same trend was seen in the case of number of panicles. The highest number of panicles was seen in the set C1 where the maximum temperature from active tillering to heading stage was 31.51°C (Fig 10.b). as the temperature starting increasing more than that, there was a decrease in the panicle numbers.

High temperatures during the active-tillering period reduce the number of panicles, particularly at maturity [17]. It was reported that stress conditions reduced number of panicles in all the genotypes and the reduction was more under combined heat and drought stress and genotype Ptb-7 produced more number of panicles[18].

**Number of filled grains and chaff**

The number of filled grains were also higher in the case of C1 set with salicylic acid spray followed by ascorbic acid and citric acid spray. The lowest number of filled grains was seen in the set C4 and C5. These two sets i.e C4 & C5 were exposed to high temperature conditions at the heading and milking stage repectively. the highest number of filled grains were seen when the maximum temperature at heading to milking stage was 29.5°C (Fig 10.c). When the temperature increased more than this, the number of filled grains drastically reduced in C4 where maximum temperature was 49.77°C. Number of filled grains per panicle decreased by 26 per cent when plants were exposed to high air temperature compared to normal air temperature [19] (Liu *et al*., 2013).

The number of chaff was highest in the set C4 & C5 which were exposed to high temperature at heading and milking stage respectively. The lowest number of chaffs were seen in case of the set C1 with salicylic acid treatment. The same trend was seen in the case of all the sets. The lowest number of chaff was seen when maximum temperature was 26°C (Fig 10.d). When the maximum temperature at milking was more than 26°C, the number of chaffs also increased drastically as in set C4 (maximum temperature- 53°C) and C5 (maximum temperature- 52°C).

**Total grain weight**

The sets C2, C3 and C8 were under high temperature at the reproductive phase (Table 2) and their growth got restricted at heading. Further growth was not noticed and thus they did not have any yield.

So, the sets which had their reproductive stages in the ambient condition (C1, C4, C5 and C6) gave yield.

The total grain weight was found to be highest in factor C1(which was kept completely outside and foliar spraying was done in all stages) with the spray S1 (salicylic acid 400ppm) which was followed by S2 (Ascorbic acid+ citric acid) spray.

The highest yield was obtained when the maximum temperature at milking to maturity stage was 26°C (Fig 10.e). When the temperature increased more than this, there was a decrease in the yield.

High temperature may cause reduced tillering at the vegetative stage [20].

Temperatures above 30̊ C reduces grain filling duration, photosynthesis, and impede starch formation in the endosperm. The rise in temperature affects the ratio of different proteins and carbohydrates [21]. The grain weight as well as grain number is reduced due to the high temperature during the pre-anthesis stage[22]. High temperatures at this stage greatly reduce spikelet sterility in rice[23]. This spikelet sterility reduces yield while also affecting development and physiological responses during the reproductive growth stage [24].

It was revealed that grain yield reduced when heat stress was imposed from booting and flowering stage and the heat stress during ripening stage did not affect yield. [25]

**Figure 2: Temperature inside polyhouse and ambient condition throughout the growth stages**

**Table 2: Temperature of the sets at different phenophases**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sets** | **Active tillering** | **heading** | **milking** | **milking to harvest** |
| **C1** | 29.4 | 31.4 | 30.1 | 30.9 |
| **C2** | 41.4 | 46.1 | - | - |
| **C3** | 29.4 | 46.1 | 45.9 | - |
| **C4** | 29.4 | 31.4 | 46.0 | 45.8 |
| **C5** | 29.4 | 31.4 | 30.1 | 48.1 |
| **C6** | 41.4 | 31.3 | 30.1 | 30.9 |
| **C7** | 41.4 | 39.2 | 32.2 | - |
| **C8** | 41.4 | 46.1 | - | - |

**Figure 3: Phenophase duration of all the factors and treatments**

**Table 3: Chlorophyll content at active tillering stage in open and stress condition**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Sets exposed to stress | | Sets exposed to ambient condition | | | |  |
|  | Stages | T-AT | T-Harvest | T-AT | | T-Harvest | |  |
|  |  | C6 | C2 | C3 | | C1 | | mean |
| Treatment  (sprays) | S1 | 0.34 | 0.24 | 0.34 | | 0.46 | | 0.35a |
| S2 | 0.26 | 0.31 | 0.28 | | 0.34 | | 0.30ab |
| S3 | 0.24 | 0.19 | 0.27 | | 0.23 | | 0.25b |
| S4 | 0.16 | 0.28 | 0.28 | | 0.31 | | 0.26b |
|  | mean | 0.25b | 0.26b | 0.30ab | | 0.35a | |  |
| CD (0.05) | Sets | | | 0.09 |  | |
| Treatments | | | 0.09 |  | |
| Set × Treatments | | | 0.10 |  | |

**Figure 4: The variations in chlorophyll content in different treatments at active tillering**

**Table 4: Chlorophyll content at heading stage in open and stress condition**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | Sets exposed to stress | | Sets exposed to ambient condition | |  |
|  | Stages | T-H | T-Harvest | T-H | T-Harvest | mean |
|  |  | C7 | C2 | C4 | C1 |  |
| Treatment  (sprays) | S1 | 0.10 | 0.16 | 0.30 | 0.32 | 0.22a |
| S2 | 0.09 | 0.10 | 0.19 | 0.30 | 0.17b |
| S3 | 0.07 | 0.07 | 0.12 | 0.22 | 0.12c |
| S4 | 0.15 | 0.08 | 0.08 | 0.20 | 0.13c |
|  | mean | 0.11c | 0.11c | 0.17b | 0.26a |  |
| CD (0.05) | Sets | | | 0.12 |  |
| Treatments | | | 0.12 |  |
| Set × Treatments | | | 0.24 |  |

**Figure 5: Variations in chlorophyll content at heading stage in open and stress condition**

**Table 5: Chlorophyll content at milking stage in open and stress condition**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | Sets exposed to ambient condition | |  |
|  | Stages | T-M | T-Harvest |  |
|  |  | C5 | C1 | mean |
| Treatment  (sprays) | S1 | 0.19 | 0.19 | 0.20a |
| S2 | 0.19 | 0.19 | 0.20a |
| S3 | 0.05 | 0.04 | 0.05b |
| S4 | 0.08 | 0.08 | 0.09b |
|  | mean | 0.13a | 0.13a |  |
| CD (0.05) | Sets | | 0.05 |  |
| Treatments | | 0.08 |  |
| Set × Treatments | | 0.12 |  |

**Figure 6: The variations in chlorophyll content in different treatments at milking stage**

**Table 6: Chlorophyll stability index at active tillering in open and stress condition**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | Sets exposed to stress | | Sets exposed to ambient condition | |  |
|  | Stages | T-AT | T-Harvest | T-AT | T-Harvest |  |
|  |  | C6 | C2 | C3 | C1 | mean |
| Treatment  (sprays) | S1 | 0.60 | 0.59 | 0.74 | 0.76 | 0.67a |
| S2 | 0.57 | 0.56 | 0.58 | 0.73 | 0.61a |
| S3 | 0.45 | 0.50 | 0.52 | 0.63 | 0.53b |
| S4 | 0.59 | 0.44 | 0.49 | 0.64 | 0.54b |
|  | mean | 0.55b | 0.52b | 0.58b | 0.69a |  |
| CD (0.05) | Sets | | | 0.10 |  |
| Treatments | | | 0.10 |  |
| Set × Treatments | | | 0.20 |  |

**Figure 7: The variations in chlorophyll stability index at active tillering**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | Sets exposed to stress | | Sets exposed to ambient condition | |  |
|  | Stages | T-H | T-Harvest | T-H | T-Harvest | mean |
|  |  | C7 | C2 | C4 | C1 |  |
| Treatment  (sprays) | S1 | 0.54 | 0.57 | 0.72 | 0.75 | 0.64a |
| S2 | 0.67 | 0.57 | 0.65 | 0.72 | 0.65a |
| S3 | 0.46 | 0.56 | 0.63 | 0.70 | 0.58ab |
| S4 | 0.53 | 0.56 | 0.65 | 0.71 | 0.61b |
|  | mean | 0.55c | 0.56c | 0.66b | 0.72a |  |
| CD (0.05) | Sets | | | 0.08 |  |
| Treatments | | | 0.08 |  |
| Set × Treatments | | | 0.15 |  |

**Table 7: Chlorophyll stability index at heading in open and stress condition**

**Figure 8: The variations in chlorophyll stability index at heading**

**Table 8: Chlorophyll stability index at milking stage in open and stress condition**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | Sets exposed to ambient condition | |  |
|  | Stages | T-Milking | T-Harvest |  |
|  |  | C5 | C1 | mean |
| Treatment  (sprays) | S1 | 0.60 | 0.77 | 0.69a |
| S2 | 0.60 | 0.75 | 0.68a |
| S3 | 0.59 | 0.59 | 0.59b |
| S4 | 0.56 | 0.61 | 0.59b |
|  | mean | 0.59b | 0.68a |  |
| CD (0.05) | Sets | | 0.07 |  |
| Treatments | | 0.10 |  |
| Set × Treatments | | 0.14 |  |

**Figure 9: The variations in chlorophyll stability index at milking**

**Table 9: Yield attributes and the total grain yield from each factor and treatment**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sets | Spray | tillers | panicles | filled grains | chaff | Grain yield (in grams) |
| C1 | S1 | 23.13 | 19.13 | 67.75 | 23.63 | 180 |
| C1 | S2 | 18.38 | 14.50 | 50.75 | 22.50 | 158.6 |
| C1 | S3 | 19.13 | 14.75 | 39.00 | 24.38 | 134.6 |
| C1 | S4 | 20.50 | 15.88 | 53.88 | 25.25 | 106.8 |
| C4 | S1 | 22.88 | 19.13 | 28.75 | 28.25 | 140 |
| C4 | S2 | 22.38 | 9.63 | 43.50 | 33.00 | 125.5 |
| C4 | S3 | 21.63 | 15.50 | 33.25 | 29.75 | 123.8 |
| C4 | S4 | 20.63 | 17.13 | 30.63 | 33.13 | 106.9 |
| C5 | S1 | 21.60 | 21.00 | 27.60 | 45.60 | 122 |
| C5 | S2 | 20.20 | 18.40 | 24.60 | 45.80 | 110 |
| C5 | S3 | 19.13 | 15.25 | 16.75 | 35.00 | 117 |
| C5 | S4 | 22.75 | 19.25 | 15.25 | 34.75 | 121 |
| C6 | S1 | 15.43 | 13.71 | 43.86 | 24.29 | 153 |
| C6 | S2 | 15.33 | 13.17 | 41.83 | 18.33 | 103 |
| C6 | S3 | 12.17 | 9.67 | 48.00 | 20.17 | 103.5 |
| C6 | S4 | 14.25 | 11.75 | 47.25 | 11.25 | 100 |

**(b)**

**(a)**

**Fig 10. Effect of maximum temperature (Tmax) at different stages on (a) tillers (b) panicles (c) filled grains (d) chaff (e) yield**

**Conclusion**

From the present investigation, it can be concluded that, under high temperature, rice plant is adversely affected due to heat stress leading to changes in many physiological characters. Very high temperatures retard the growth of the plants and restricts their development into the next phenophases. Especially during reproductive phase, the flowering stage is highly affected by heat stress leading to spikelet sterility and flower drop which results in reduced or negligible yield. Heat stress during the heading stage, leads to reduced yield. The same during the milking stage results in improper grain filling and chalkiness. Spray of Salicylic acid (400 ppm) showed higher values of chlorophyll content and chlorophyll stability index as compared to the other sprays. Ascorbic acid (10 ppm) + Citric acid (1.3%) spray is also be effective in mitigating the effects of heat stress on crop physiological parameters up to a considerable extent. Overall, salicylic acid (400ppm) and Ascorbic acid (10ppm) +Citric acid (1.3%) can be used as foliar sprays to improve the plant characteristics and result in high yield.

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