Indian Journal of Traditional Knowledge Vol 19(1), January 2020, pp 92-100

Enhancement of growth and seed yield of rice (*Oryza sativa* L.) through foliar spray of osmoprotectants under high temperature stress

Sumita Das*^{+,+}, Simanta Mohanty, Debadatta Dash & Kumuda Chandra Muduli

Department of Seed Science and Technology,

College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar 751 003, Odisha, India

E-mail: ⁺sumitadas.sst@gmail.com

Received 26 March 2019, revised 06 August 2019

An investigation was undertaken during rabi-summer season of 2016-17 in the Department of Seed Science and Technology, OUAT, Bhubaneswar to study the efficiency of some osmoprotectants in mitigating high temperature stress by improving growth, seed yield and quality of rice cv. Naveen. The field experiment was conducted in Split Plot Design, with three replications. The main plot factor was three dates of sowing $(D_1 - 30^{th} \text{ November}, D_2 - 15^{th} \text{ December} \text{ and } D_3 - 30^{th} \text{ December})$. The sub-plot factor was foliar spray of chemicals at vegetative and seed filling stages: Control (T_0) , 600 ppm Glycine betaine (T_1) , 400 ppm Salicylic acid (T₂), 800 ppm Salicylic acid (T₃), 10 ppm Ascorbic acid + 1.3% Citric acid (T₄), 150 ppm α -Tocopherol (T_5) , 1.0% Potassium chloride (T_6) , 5 ppm Brassinolides (T_7) and 10 ppm Brassinolides (T_8) . Observations on various phenological, seed yield and quality parameters were recorded. With advancement in sowing time, there was significant decrease in the chlorophyll content of leaves. The chlorophyll content of leaves after two days of spraying as slightly higher than that prior to spraying in all the treatments. Maximum chlorophyll content was recorded with spray treatment of 800 ppm salicylic acid at both the stages of observation. Significantly higher pollen viability and seed set were recorded with first date of sowing (D₁), compared to second and third dates of sowing (D₂ and D₃, respectively). Among the treatments, pollen viability was highest with 10 ppm brassinolides and 400 ppm salicylic acid. Seed set was highest with 10 ppm brassinolides and 800 ppm salicylic acid. With advancement in sowing time, there was decrease in membrane stability index of leaves. The MSI of leaves after two days of spraying was slightly higher than that prior to spraying in all treatments. Maximum MSI was recorded with spray treatment of 400 ppm salicylic acid (T_2) during vegetative and seed filling stages. The treatments T_2 , T_4 and T_8 produced highest increase in MSI compared to that before spraying during both stages. Higher seed yield was obtained from D₁, compared to delayed sowing. Mean seed yield from D1 was 53.76 q/ha, as against 48.99 q/ha and 44.51 q/ha from D2 and D3, respectively. Spraying of 800 ppm salicylic acid and 10 ppm brassinolides gave highest seed yields from each date of sowing. Seed yields from D_3T_0 and D_2T_0 (35.33 q/ha and 39.72 q/ha, respectively) were significantly lower than all treatment combinations, including $D_1T_0(51.83 q/ha)$. Increase in seed yields due to various treatments as against the control was more pronounced in D_2 and D_3 . The first date of sowing (D_1) produced seeds with higher 1000-seed weight (22.62 g), compared to D_2 and D_3 (20.27 g and 19.36 g, respectively). Among the treatments, higher 1000-seed weights were recorded with the treatments T_3 and T_8 (21.51 g and 21.43 g, respectively), while lowest 1000-seed weight (18.76 g) was recorded with the Control. Seeds produced from D₁ recorded higher germination and seed vigour. All the treatments recorded improvement in germination and vigour of seeds with three dates of sowing. Treatments T_3 , T_7 and T_8 gave highest improvement in seed germination over control. Under accelerated ageing treatment, T₃ recorded highest germination followed by T₈. Dates of sowing and treatment sprays had no influence on electrical conductivity of seed leachate. Hence, the present study demonstrated that foliar sprays at vegetative and seed filling stages with 800 ppm salicylic acid or 10 ppm ascorbic acid + 1.3% citric acid or 10 ppm brassinolides were quite effective in alleviating adverse effects of high temperature during flowering and seed set, through increased chlorophyll content of leaves, improved membrane stability, higher pollen viability and seed set, thus improving seed yield and quality of rice.

Keywords: High temperature stress, Osmoprotectant, Rice, Seed yield, Seed quality **IPC Code:** Int. Cl.²⁰: A01G 22/22, A01C 1/08

Climate change is harshly affecting cereal production all over the world through increased temperature and CO_2 concentration¹, which is one of the main causes of heat stress². Rice is the second highest produced cereal crop in the world, after maize. Major rice producing countries are in Asia, with the farmers of the subcontinent

accounting for about 92% of the world's total rice production. It is estimated that there will be a need for increasing the food production by about 30-40% in the next 30 years, so as to feed the ever-increasing world population. The changing climate scenario is making it increasingly difficult, if not impossible, to achieve the goal. Though high temperature and other abiotic stresses are limiting factors for future crop production goals,

^{*}Corresponding author

crop productivity many a times also suffers from random environmental fluctuations.

Direct injuries due to high temperature include protein denaturation, aggregation and increased fluidity of membrane lipids. Indirect or slower heat injuries include inactivation of enzymes in chloroplast and mitochondria, inhibition of protein synthesis, protein degradation and loss of membrane integrity³. High temperature during seed germination may slow down or totally inhibit germination, depending on plant species and intensity of the stress. At later stages, high temperature may adversely affect vital physiological processes like photosynthesis, respiration, water relations and membrane stability and also modulate levels of hormones and primary and secondary metabolites⁴. Floral abnormalities induced by heat stress (i.e., stamen hypoplasia and pistil hyperplasia), leading to spikelet sterility, represent significant problems in rice production. High temperature stress ultimately leads to lower yields. The yield of dry season rice crop decreased by 15% for each 1°C increase in mean temperature during the growing season⁵.

Exogenous foliar spray of osmoprotectants like Glycinebetaine, Salicylic acid, Ascorbic acid, Citric acid, a-Tocopherol, Potassium chloride, Brassinolides, etc. has been reported to be effective in reducing the loss of yield by improving tolerance to heat stress under drier and warmer climate or due to late sowing of rice. 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 electrolyte leakage and thus, help to maintain or sometime increase pollen viability, seed setting and yield of crops.

The present investigation was undertaken to study the efficiency of some osmoprotectants in improving growth, seed yield and quality of rice by enhancing tolerance to high temperature stress. The main focus was to reduce the impact of heat stress in rice during vegetative and seed filling stages.

Methodology

The field experiment was conducted during rabi-summer season of 2016-17 in the Department of Seed Science and Technology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar. The field experiment was laid out in Split Plot Design, with three replications, the main plot factor being three dates of sowing $(D_1 - 30^{th}$ November, $D_2 15^{\text{th}}$ December and $D_3 - 30^{\text{th}}$ December) and the sub-plot factor being foliar spray of chemicals at vegetative and seed filling stage (9 treatments). Rice variety Naveen (120 days duration) was chosen for the experiment. The foliar sprays included: Control (T₀), 600 ppm (5.121 mM) Glycine betaine (T_1) , 400 ppm (2.896 mM) Salicylic acid (T₂), 800 ppm (5.792 mM) Salicylic acid (T₃), 10 ppm (0.056 mM) Ascorbic acid + 1.3% Citric acid (T₄), 150 ppm (0.348 mM) α -Tocopherol (T₅), 1.0% Potassium chloride (T_6), 5 ppm (0.0104 mM) Brassinolides (T₇) and 10 ppm (0.0208 mM) Brassinolides (T_8) . The mean weekly maximum and minimum temperatures during the period of study are given in Table 1. Observations on total chlorophyll

| Table 1 — Mean weekly maximum and minimum temperatures during the period of study corresponding to stages of crop growth | | | | | | | | |
|--|--|------------------------------|----------------|-----------------------------|------------------|-----------------------------|----------------|--|
| Days after Stage of crop growth | | Mean weekly temperature (°C) | | | | | | |
| sowing | | | D ₁ | | D_2 | | D ₃ | |
| | | (30 th Nov 2016) | | (15 th Dec 2016) | | (30 th Dec 2016) | | |
| | | T _{max} | T_{min} | T _{max} | T _{min} | T _{max} | T_{min} | |
| 4-10 | Seedling emergence | 30.6 | 16.2 | 29.8 | 12.6 | 29.2 | 16.0 | |
| 11-17 | Seedling stage | 30.5 | 15.8 | 29.5 | 14.6 | 29.5 | 14.6 | |
| 18-24 | Seedling stage | 29.8 | 12.6 | 29.2 | 16.0 | 29.2 | 12.7 | |
| 25-31 | Transplanting | 29.5 | 14.6 | 29.5 | 14.6 | 30.7 | 14.1 | |
| 32-38 | Seedling establishment | 29.2 | 16.0 | 29.2 | 12.7 | 31.3 | 17.6 | |
| 39-45 | Vegetative growth | 29.5 | 14.6 | 30.7 | 14.1 | 33.3 | 17.2 | |
| 46-52 | Tillering | 29.2 | 12.7 | 31.3 | 17.6 | 33.6 | 17.4 | |
| 53-59 | Maximum tillering | 30.7 | 14.1 | 33.3 | 17.2 | 35.1 | 22.4 | |
| 60-66 | Panicle initiation, primary and secondary branch primordial | 31.3 | 17.6 | 33.6 | 17.4 | 35.7 | 20.6 | |
| 67-73 | Panicle development (stamen and pistil primordia, pollen mother cells) | 33.3 | 17.2 | 35.1 | 22.4 | 33.2 | 22.6 | |
| 74-80 | Panicle development (meiotic division) | 33.6 | 17.4 | 35.7 | 20.6 | 33.1 | 21.6 | |
| 81-87 | Panicle development (mature pollen) | 35.1 | 22.4 | 33.2 | 22.6 | 35.9 | 22.7 | |
| 88-94 | Completed spikelets and anthesis | 35.7 | 20.6 | 33.1 | 21.6 | 35.6 | 25.3 | |
| 95-101 | Seed development | 33.2 | 22.6 | 35.9 | 22.7 | 35.6 | 26.0 | |
| 102-108 | Seed filling | 33.1 | 21.6 | 35.6 | 25.3 | 37.8 | 26.0 | |
| 109-115 | Seed filling | 35.9 | 22.7 | 35.6 | 26.0 | 36.0 | 25.9 | |
| 116-122 | Seed maturity | 35.6 | 25.3 | 37.8 | 26.0 | 38.3 | 27.1 | |

content of leaves $(mg/g FW)^6$ and membrane stability $(\%)^7$ were recorded before foliar spray and 2 days after spray of chemicals. Pollen viability (%) was recorded by collecting the spikelet sample early in the morning just before anthesis (07:00-08:00 AM). After harvest, seed set (%), seed yield (q/ha), 1000-seed weight (g), seed germination $(\%)^8$, seedling length (cm), seedling dry weight (mg), seed vigour indices I and II⁹, EC of seed leachate $(dS/m)^{10}$ and germination (%) after accelerated ageing were also recorded.

Results and discussion

The present investigation was aimed at studying the role of osmoprotectants in rice cv. Naveen under high temperature stress. Foliar application of such osmoprotectants showed positive effects in mitigating high temperature stress. The possible mechanisms of positive effects of osmoprotectants are discussed here under. With delay in sowing time, there was significant decrease in the chlorophyll content of leaves (Table 2). The chlorophyll content of leaves after two days of spraying was slightly higher than that prior to spraying in all the treatments. Maximum chlorophyll content was recorded with spray treatment of 800 ppm salicylic acid (T₃) at both the stages of observation. Salicylic acid is a common phenolic compound that promotes photosynthesis under heat stress by influencing various physiological and biochemical process¹¹. The treatments T_3 , T_4 and T₈ recorded the highest percent increase in chlorophyll content in comparison to the chlorophyll content before spray both during vegetative and seed filling stages (Fig. 1 & Fig. 2). Application of Brassinolides under high temperature stress conditions was reported to significantly increase the chlorophyll content in rice seedlings¹².

Significantly higher pollen viability (%) and seed set (%) were recorded with first date of sowing, as compared to second and third dates of sowing (Table 2). With delay in sowing time, there was gradual decrease in both the parameters. High temperatures $\geq 30^{\circ}$ C have been reported to cause pollen sterility in rice¹³. Among the treatments, pollen viability was highest in T₈ (10 ppm brassinolides), followed by T₂ (400 ppm salicylic acid), though both the treatments were statistically at par. However, seed set was highest in T₈, followed by T₃ (800 ppm salicylic acid), though both the treatments were statistically at par. The treatments T₈ and T₂ recorded highest percent increase in pollen viability over Control (Fig. 3), whereas the treatments T₈ and T₃ recorded highest percent increase in seed set over Control (Fig. 4). Salicylic acid is a phenolic compound that naturally exists in plants at a very low concentration. Salicylic acid can reduce pollen sterility when encountering high temperature stress at the pollen mother cell meiosis stage. Under heat stress, rice plants sprayed with Salicylic acid at 0.1-10 mM is reported to have attained a significantly higher seed-setting rate and pollen viability compared to those of the non-Salicylic acid treatment¹⁴. The most effective Salicylic acid concentration was found to be 10 mM under heat stress and no inhibition was found on the rice plants when they were sprayed with 50 mM Salicylic acid. Similarly, exogenous application of Vitamin E, Glycine betaine and Salicylic acid has been reported to significantly improve pollen germination and spikelet fertility in rice¹⁵. Two rice cultivars, IR-64 and Huanghuazhan, subjected to high day temperature (HDT), high night temperature (HNT) and control temperature (CT) in controlled growth chambers showed severely reduced the pollen fertility, anther dehiscence, pollen retention, germination and metabolites synthesis in pollens of both rice cultivars¹⁶. Exogenous application of four different combinations of growth regulators (Ascorbic acid, a-Tocopherol, Brassinosteroides, Methyl jasmonates and Traiazoles) could mitigate high temperature stress by increased pollen fertility, anther dehiscence, pollen retention, germination rate of both rice cultivars.

As the sowing time was delayed, there was significant decrease in the membrane stability index of leaves (Table 3). The membrane stability index of leaves after two days of spraying was slightly higher than that prior to spraying in all the treatments. Maximum membrane stability index was recorded with spray treatment of 400 ppm salicylic acid (T₂) at both the stages of observation. The treatments T₂, T₄ and T₈ recorded the highest percent increase in membrane stability index in comparison to that before spray both during vegetative and seed filling stages (Fig. 5 & Fig. 6).

Higher seed yield was obtained with first date of sowing (Table 3), owing mainly to exposure of the crop to relatively lower temperatures during vegetative and seed filling stages, thus recording higher pollen viability and higher seed set percentage. The mean seed yield from first date of sowing was 53.76 q/ha, as against 48.99 q/ha and 44.51 q/ha from the second and third dates of sowing, respectively. Among the treatments, T_3 (800 ppm salicylic acid)

and T_8 (10 ppm brassinolides) gave the highest seed yields from each date of sowing, which were significantly higher than rest of the treatments. With

delay in sowing, there was drastic reduction in the seed yield. The seed yields from D_3T_0 and D_2T_0 (35.33 q/ha and 39.72 q/ha, respectively) were significantly

| Table 2 - | — Chlorophyll con | | iability and seed s application of osn | | influenced by date of so | wing and foliar | |
|----------------------------------|---|-------------------------|--|--|--------------------------|-------------------|--|
| Treatments | Chlorophyll content (mg/g FW) of leaves during vegetative stage | | | ontent (mg/g FW) of g seed filling stage | Pollen viability* (%) | Seed set** (%) | |
| | Before spray | 2 days after spray | Before spray | 2 days after spray | | | |
| D_1 | 2.326 | 2.398 | 2.313 | 2.381 | 78.06 (62.10) | 90.16 (9.49) | |
| \mathbf{D}_2 | 2.233 | 2.397 | 2.220 | 2.379 | 76.68 (61.17) | 86.42 (9.29) | |
| D_3 | 2.231 | 2.398 | 2.224 | 2.386 | 69.65 (56.59) | 85.10 (9.22) | |
| S.E.m (±) | 0.0005 | 0.0003 | 0.0006 | 0.0006 | 0.082 | 0.008 | |
| CD _{0.05} | 0.0021 | NS | 0.0024 | 0.0022 | 0.323 | 0.033 | |
| T_0 | 1.894 | 1.895 | 1.876 | 1.882 | 67.77 (55.45) | 81.22 (9.01) | |
| T_0 T_1 | 2.258 | 2.385 | 2.246 | 2.372 | 75.18 (60.19) | 86.08 (9.28) | |
| T_1 T_2 | 2.274 | 2.427 | 2.265 | 2.410 | 76.98 (61.40) | 88.54 (9.41) | |
| T_2 T_3 | 2.388 | 2.571 | 2.378 | 2.544 | 75.72 (60.53) | 90.07 (9.49) | |
| T_4 | 2.354 | 2.529 | 2.357 | 2.513 | 75.15 (60.13) | 88.82 (9.42) | |
| T_{5} | 2.231 | 2.328 | 2.217 | 2.332 | 74.02 (59.41) | 85.01 (9.22) | |
| T_6 | 2.308 | 2.439 | 2.298 | 2.447 | 75.25 (60.22) | 87.44 (9.35) | |
| T ₆ T ₇ | 2.299 | 2.454 | 2.298 | 2.432 | 75.95 (60.71) | 87.53 (9.35) | |
| T_7 T_8 | 2.362 | 2.549 | 2.347 | 2.509 | 77.14 (61.51) | 90.34 (9.50) | |
| $^{1_{8}}$ S.E.m (±) | 0.0010 | 0.0006 | 0.0010 | 0.0010 | 0.122 | 0.020 | |
| $CD_{0.05}$ | 0.0028 | 0.0018 | 0.0028 | 0.0028 | 0.346 | 0.020 | |
| | 2.010 | 2.013 | 1.992 | 1.996 | 72.18 (58.17) | 86.50 (9.30) | |
| D_1T_0 D T | 2.316 | 2.367 | 2.302 | 2.359 | | | |
| D_1T_1 | | | | | 78.98 (62.71) | 90.89 (9.53) | |
| D_1T_2 | 2.331 | 2.415 | 2.329 | 2.405 | 79.85 (63.33) | 90.63 (9.52) | |
| D_1T_3 | 2.444 2.406 | 2.558 | 2.438 | 2.533 | 78.34 (62.26) | 92.27 (9.61) | |
| D_1T_4 | | 2.512 | 2.384 | 2.468 2.321 | 77.88 (61.95) | 90.72 (9.52) | |
| D_1T_5 | 2.288 | 2.315 | 2.276 | | 77.09 (61.40) | 87.77 (9.37) | |
| D_1T_6 | 2.362 | 2.423 | 2.357 | 2.438 | 78.64 (62.47) | 89.81 (9.48) | |
| D_1T_7 | 2.354 | 2.441 | 2.340 | 2.418 | 79.04 (62.76) | 90.88 (9.53) | |
| D_1T_8 | 2.419 | 2.535 | 2.402 | 2.493 | 80.52 (63.81) | 91.96 (9.59) | |
| D_2T_0 | 1.887 | 1.889 | 1.873 | 1.879 | 68.36 (55.77) | 79.76 (8.93) | |
| D_2T_1 | 2.226 | 2.388 | 2.213 | 2.372 | 77.16 (61.45) | 83.38 (9.13) | |
| D_2T_2 | 2.241 | 2.427 | 2.233 | 2.411 | 79.36 (62.98) | 88.09 (9.39) | |
| D_2T_3 | 2.355 | 2.571 | 2.339 | 2.539 | 77.86 (61.93) | 89.91 (9.48) | |
| D_2T_4 | 2.322 | 2.531 | 2.296 | 2.482 | 76.18 (60.79) | 88.78 (9.42) | |
| D_2T_5 | 2.195 | 2.324 | 2.182 | 2.329 | 76.15 (60.77) | 83.68 (9.15) | |
| D_2T_6 | 2.277 | 2.442 | 2.267 | 2.449 | 77.06 (61.38) | 86.95 (9.32) | |
| D_2T_7 | 2.268 | 2.457 | 2.252 | 2.432 | 78.86 (62.63) | 86.33 (9.29) | |
| D_2T_8 | 2.324 | 2.542 | 2.322 | 2.519 | 79.17 (62.85) | 90.93 (9.54) | |
| D_3T_0 | 1.785 | 1.784 | 1.762 | 1.771 | 62.78 (52.40) | 77.39 (8.80) | |
| D_3T_1 | 2.231 | 2.399 | 2.223 | 2.384 | 69.40 (56.42) | 83.97 (9.16) | |
| D_3T_2 | 2.251 | 2.438 | 2.234 | 2.413 | 71.73 (57.88) | 86.89 (9.32) | |
| D_3T_3 | 2.365 | 2.583 | 2.356 | 2.561 | 70.97 (57.40) | 88.02 (9.38) | |
| D_3T_4 | 2.333 | 2.544 | 2.391 | 2.588 | 71.40 (57.67) | 86.95 (9.32) | |
| D_3T_5 | 2.211 | 2.344 | 2.194 | 2.345 | 68.81 (56.05) | 83.58 (9.14) | |
| D_3T_6 | 2.285 | 2.453 | 2.270 | 2.454 | 70.04 (56.82) | 85.56 (9.25) | |
| D_3T_7 | 2.274 | 2.465 | 2.264 | 2.446 | 69.95 (56.76) | 85.38 (9.24) | |
| D_3T_8 | 2.344 | 2.569 | 2.318 | 2.514 | 71.73 (57.88) | 88.12 (9.39) | |
| S.E.m (±) | 0.0017 | 0.0011 | 0.0017 | 0.0017 | 0.211 | 0.034 | |
| CD _{0.05} | 0.0049 | 0.0031 | 0.0048 | 0.0048 | 0.599 | 0.097 | |
| N.B.: Figures | in the parenthesis | are arc sine (*) / squa | re root (**) transf | ormed values. | | | |

lower than all the other treatments, as well as D_1T_0 (51.83 q/ha). All the treatments had a positive influence in minimising the adverse effect of high temperature on the seed yield of rice. The increase in seed yields due to various treatments as against the control (T_0) was more pronounced in the 2nd and 3rd dates of sowing (Fig. 7). Correlation studies revealed highly significant correlation of the seed yield with chlorophyll content in seed filling stage after the

spray treatments, pollen viability, seed set and membrane stability in seed filling stage after the spray

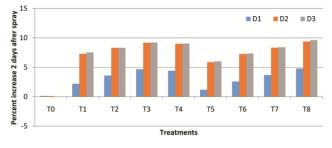


Fig. 1 — Percent increase in leaf chlorophyll content 2 days after spray during vegetative stage of rice cv. Naveen due to foliar spray of osmoprotectants under high temperature stress

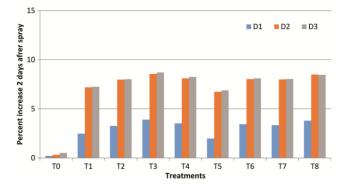


Fig. 2 — Percent increase in leaf chlorophyll content 2 days after spray during seed filling stage of rice cv. Naveen due to foliar spray of osmoprotectants under high temperature stress

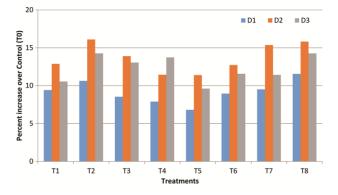


Fig. 3 — Percent increase in pollen viability of rice cv. Naveen over Control as influenced by foliar spray of osmoprotectants under high temperature stress

treatments (0.559**, 0.912**, 0.884**, 0.497**, respectively). Foliar spray of the osmoprotectants had

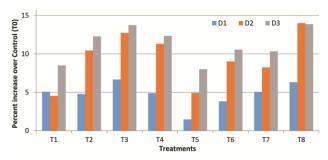


Fig. 4 — Percent increase in seed set of rice cv. Naveen over Control as influenced by foliar spray of osmoprotectants under high temperature stress

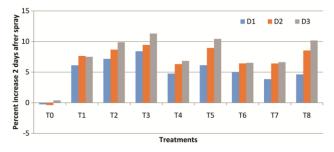


Fig. 5 — Percent increase in membrane stability index 2 days after spray during vegetative stage of rice cv. Naveen due to foliar spray of osmoprotectants under high temperature stress

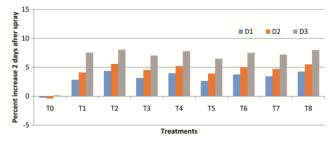


Fig. 6 — Percent increase in membrane stability index 2 days after spray during seed filling stage of rice cv. Naveen due to foliar spray of osmoprotectants under high temperature stress

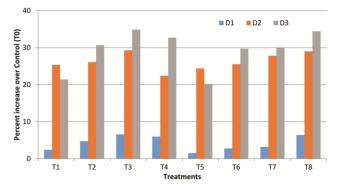


Fig. 7 — Percent increase in seed yield of rice cv. Naveen over Control as influenced by foliar spray of osmoprotectants under high temperature stress

a positive influence on the seed yield attributing characters of rice, which corroborates the findings of previous workers. Glycine betaine is a low molecular weight organic compound, which has been successfully applied to induce high temperature tolerance in plants. Capacity to synthesize glycine betaine under stress conditions differs from species to species¹⁷. In assessing the functional significance of accumulation of compatible solutes, it is suggested that proline or glycine betaine synthesis may buffer

Table 3 — Membrane stability index, seed yield and 1000-seed weight of rice cv. Naveen as influenced by date of sowing and foliar application of osmoprotectants

| Treatments | Membrane stability index (%) during vegetative stage | | | ability index (%) d filling stage | Seed yield (q/ha) | 1000-seed weight (g) |
|--------------------|---|--------------------|--------------|--------------------------------------|----------------------|-------------------------|
| | Before spray | 2 days after spray | Before spray | 2 days after spray | | |
| D_1 | 77.61 | 85.59 | 87.67 | 89.08 | 53.76 | 22.62 |
| D_2 | 78.48 | 85.86 | 87.79 | 89.28 | 48.99 | 20.27 |
| $\overline{D_3}$ | 78.72 | 86.13 | 88.07 | 89.58 | 44.51 | 19.36 |
| S.E.m (±) | 0.436 | 0.377 | 0.116 | 0.178 | 0.120 | 0.131 |
| CD _{0.05} | NS | NS | NS | NS | 0.471 | 0.513 |
| T ₀ | 78.83 | 77.80 | 76.50 | 74.93 | 42.29 | 18.76 |
| T_1 | 78.63 | 85.10 | 87.70 | 89.70 | 48.58 | 20.80 |
| T_2 | 78.67 | 88.90 | 91.23 | 92.90 | 50.18 | 21.23 |
| T_3 | 78.97 | 85.67 | 88.53 | 90.30 | 51.41 | 21.51 |
| T_4 | 77.13 | 88.13 | 90.20 | 91.90 | 50.14 | 20.61 |
| T ₅ | 78.73 | 83.80 | 86.77 | 89.13 | 48.16 | 20.60 |
| T ₆ | 77.73 | 87.60 | 89.67 | 91.47 | 49.65 | 20.93 |
| T ₇ | 77.80 | 87.17 | 89.20 | 90.93 | 50.06 | 20.89 |
| T_8 | 77.93 | 88.57 | 90.77 | 92.53 | 51.29 | 21.43 |
| S.E.m (±) | 0.605 | 0.604 | 0.610 | 0.444 | 0.378 | 0.171 |
| CD _{0.05} | NS | 1.717 | 1.735 | 1.262 | 1.075 | 0.487 |
| D_1T_0 | 79.40 | 78.40 | 77.60 | 75.70 | 51.83 | 20.97 |
| D_1T_1 | 77.50 | 84.80 | 87.20 | 89.30 | 53.07 | 22.67 |
| D_1T_2 | 78.60 | 88.60 | 90.80 | 92.60 | 54.28 | 22.93 |
| D_1T_3 | 78.70 | 85.40 | 88.30 | 89.90 | 55.24 | 23.27 |
| D_1T_4 | 74.50 | 87.60 | 89.80 | 91.60 | 54.92 | 22.07 |
| D_1T_5 | 76.30 | 83.50 | 86.60 | 88.70 | 52.61 | 22.40 |
| D_1T_6 | 77.20 | 87.10 | 89.40 | 91.10 | 53.26 | 22.73 |
| D_1T_7 | 78.40 | 86.70 | 88.80 | 90.50 | 53.48 | 23.20 |
| D_1T_8 | 77.90 | 88.20 | 90.50 | 92.30 | 55.15 | 23.30 |
| D_2T_0 | 80.20 | 77.70 | 76.10 | 74.80 | 39.72 | 18.07 |
| D_2T_1 | 79.80 | 85.10 | 87.70 | 89.70 | 49.79 | 20.40 |
| D_2T_2 | 79.50 | 88.90 | 91.30 | 92.90 | 50.09 | 20.90 |
| D_2T_3 | 78.40 | 85.70 | 88.50 | 90.30 | 51.35 | 21.10 |
| D_2T_4 | 77.60 | 88.10 | 90.10 | 91.80 | 48.63 | 20.30 |
| D_2T_5 | 79.30 | 83.80 | 86.80 | 89.10 | 49.41 | 20.20 |
| D_2T_6 | 77.80 | 87.70 | 89.70 | 91.50 | 49.86 | 20.48 |
| D_2T_7 | 76.50 | 87.10 | 89.20 | 90.90 | 50.78 | 20.18 |
| D_2T_8 | 77.20 | 88.60 | 90.70 | 92.50 | 51.24 | 20.80 |
| D_3T_0 | 76.90 | 77.30 | 75.80 | 74.30 | 35.33 | 17.23 |
| D_3T_1 | 78.60 | 85.40 | 88.20 | 90.10 | 42.89 | 19.33 |
| D_3T_2 | 77.90 | 89.20 | 91.60 | 93.20 | 46.17 | 19.87 |
| D_3T_3 | 79.80 | 85.90 | 88.80 | 90.70 | 47.65 | 20.15 |
| D_3T_4 | 79.30 | 88.70 | 90.70 | 92.30 | 46.88 | 19.46 |
| D_3T_5 | 80.60 | 84.10 | 86.90 | 89.60 | 42.47 | 19.19 |
| D_3T_6 | 78.20 | 88.00 | 89.90 | 91.80 | 45.83 | 19.58 |
| D_3T_7 | 78.50 | 87.70 | 89.60 | 91.40 | 45.91 | 19.28 |
| D_3T_8 | 78.70 | 88.90 | 91.10 | 92.80 | 47.49 | 20.19 |
| S.E.m (±) | 1.048 | 1.046 | 1.057 | 0.769 | 0.655 | 0.296 |
| CD _{0.05} | NS | NS | NS | NS | 1.862 | NS |

cellular redox potential under heat and other environmental stresses¹⁸. Ascorbic acid (Vitamin C) acts as a modulator of plant development through hormone signalling and as coenzyme in reactions by which carbohydrates and proteins are metabolized. They catch the free radicals or the reactive oxygen species produced during altered photosynthesis and respiration process under heat stress. They also regulate photosynthesis flowering and senescence¹⁹ under elevated temperature. High night temperature was reported to increase the malondialdehyde (MDA) content in two varieties of rice, while exogenous application of ascorbic acid reduced it almost to the level of control night temperature²⁰. Tocopherol (Vitamin E) is a lipophylic antioxidant which establishes membranes, scavenges various ROS²¹ and preserves PS-II photo inactivation and membrane lipids from photo oxidation. Brassinolide is a relatively new phytohormone with pleiotropic effect²² and influences varied physiological processes like germination, growth, flowering and senescence and confers resistance to plants against various abiotic stresses, through its induced effects on membrane stability and osmo-regulation²³. Citric acid increases high temperature stress tolerance during different growth stages of plants. The main functions of these sprayed compounds are protection of chlorophyll, detoxification of reactive oxygen species, maintenance of favourable balance of water and photosynthesis in plants under stressful condition. Lower spikelet fertility at elevated temperature has been reported to result in fewer filled grains, lower grain weight per panicle, and decreased harvest index in rice¹³.

The first date of sowing (D₁) produced seeds with significantly higher 1000-seed weight (22.62 g), compared to the second and third dates of sowing (20.27 g and 19.36 g). Among the treatments, significantly higher 1000-seed weights were recorded in T₃ and T₈ (21.51 g and 21.43 g, respectively), while the untreated control produced seeds with the least 1000-seed weight (18.76 g). The other treatments were also able to significantly improve the seed weight (Fig. 8). Seeds produced from the first date of sowing recorded higher germination and seed vigour (Table 4). All the treatments were able to improve germination and vigour of seeds from three dates of sowing. The treatments T₃, T₇ and T₈ gave the highest improvement in seed germination over control (T₀) (Fig. 9).

For seeds subjected to accelerated ageing treatment, T_3 (800 ppm salicylic acid) recorded

highest germination followed by T_8 (10 ppm brassinolides) (Table 3). Moreover, the two treatments produced highest increase in germination after accelerated ageing, as compared to the Control (T_0) (Fig. 10). Electrical conductivity (EC) of seed leachate was neither influenced by the date of sowing nor by any of the treatment sprays (Table 4).

Delayed sowing of rice in rabi-summer season adversely affects the crop due to exposure to high temperatures during flowering and seed filling stages.

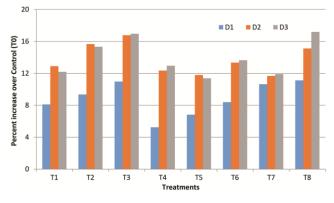


Fig. 8 — Percent increase in 1000-seed weight of rice cv. Naveen over Control as influenced by foliar spray of osmoprotectants under high temperature stress

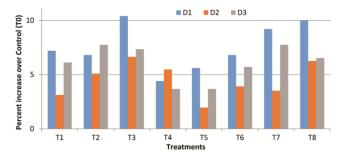


Fig. 9 — Percent increase in seed germination of rice cv. Naveen over Control as influenced by foliar spray of osmoprotectants under high temperature stress

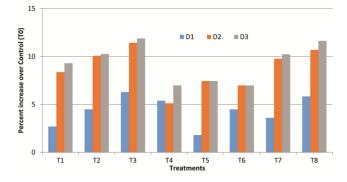


Fig. 10 — Percent increase in seed germination after accelerated ageing of rice cv. Naveen over Control as influenced by foliar spray of osmoprotectants under high temperature stress

The effects of high temperature is manifested through lower chlorophyll content of leaves, reduced membrane stability, decreased pollen viability and seed set, and ultimately lower seed yield. Exposure to high temperature also lowers 1000-seed weight in the crop. In case a long duration rice cultivar was grown in kharif season and the sowing of rabi rice crop is delayed, farmers may choose heat-tolerant varieties to minimize the yield losses. The present study demonstrated the role of a few osmoprotectants in

| Table 4 – | - Seed germination and vigour of rice cv. Naveen as influenced by date of sowing and foliar application of osmoprotectants | | | | | | |
|------------------------------------|--|-------------------------|------------------------|----------------------------|------------------------|-------------------------------------|----------------------------|
| Treatments | Seed germination (%) | Seedling length (cm) | Seed Vigour Index-I | Seedling dry weight (g) | Seed Vigou Index-II | ur Seed germination (%) after AA | EC of seed leachate (dS/m) |
| D_1 | 88.93 (9.43) | 31.50 | 2801.8 | 0.077 | 6.85 | 77.19 (8.78) | 0.155 |
| D_2 | 88.74 (9.42) | 31.04 | 2755.7 | 0.077 | 6.86 | 77.23 (8.79) | 0.156 |
| $\tilde{D_3}$ | 86.08 (9.28) | 30.52 | 2628.4 | 0.079 | 6.81 | 77.62 (8.81) | 0.159 |
| S.E.m (±) | 0.013 | 0.068 | 13.04 | 0.0004 | 0.027 | 0.028 | 0.0012 |
| CD _{0.05} | 0.049 | 0.268 | 51.19 | 0.0015 | NS | NS | NS |
| T_0 | 83.44 (9.13) | 29.45 | 2458.3 | 0.074 | 6.18 | 72.56 (8.52) | 0.152 |
| \mathbf{T}_{1}° | 88.00 (9.38) | 30.88 | 2717.7 | 0.078 | 6.84 | 77.44 (8.80) | 0.157 |
| T_2 | 88.89 (9.43) | 31.35 | 2786.7 | 0.078 | 6.93 | 78.53 (8.86) | 0.156 |
| T_3^2 | 90.22 (9.50) | 31.54 | 2846.5 | 0.080 | 7.22 | 79.68 (8.92) | 0.161 |
| T_4 | 87.22 (9.34) | 31.17 | 2719.3 | 0.078 | 6.77 | 76.78 (8.76) | 0.156 |
| T_5 | 86.56 (9.30) | 30.62 | 2650.6 | 0.077 | 6.69 | 76.56 (8.75) | 0.158 |
| T ₆ | 88.00 (9.38) | 30.65 | 2697.2 | 0.078 | 6.86 | 77.00 (8.77) | 0.156 |
| T_7 | 89.11 (9.44) | 31.61 | 2817.0 | 0.078 | 6.98 | 78.22 (8.84) | 0.158 |
| T_8 | 89.78 (9.47) | 31.90 | 2864.7 | 0.079 | 7.09 | 79.33 (8.91) | 0.155 |
| s.E.m (±) | 0.025 | 0.183 | 21.81 | 0.0007 | 0.085 | 0.060 | 0.0027 |
| CD _{0.05} | 0.071 | 0.522 | 62.01 | 0.0019 | 0.241 | 0.170 | NS |
| $D_1 T_0$ | 83.33 (9.13) | 30.49 | 2540.7 | 0.072 | 6.00 | 74.33 (8.62) | 0.152 |
| $\mathbf{D}_{1}\mathbf{T}_{1}^{0}$ | 89.33 (9.45) | 31.24 | 2790.5 | 0.078 | 6.97 | 76.33 (8.74) | 0.154 |
| D_1T_2 | 89.00 (9.43) | 31.69 | 2820.4 | 0.079 | 7.03 | 77.67 (8.81) | 0.155 |
| D_1T_3 | 92.00 (9.59) | 31.89 | 2934.4 | 0.078 | 7.18 | 79.00 (8.89) | 0.156 |
| D_1T_4 | 87.00 (9.33) | 31.92 | 2776.9 | 0.076 | 6.61 | 78.33 (8.85) | 0.157 |
| $D_1 T_5$ | 88.00 (9.38) | 30.97 | 2725.5 | 0.077 | 6.78 | 75.67 (8.70) | 0.153 |
| D_1T_6 | 89.00 (9.43) | 31.66 | 2817.7 | 0.077 | 6.85 | 77.67 (8.81) | 0.157 |
| D_1T_7 | 91.00 (9.54) | 31.53 | 2869.4 | 0.079 | 7.19 | 77.00 (8.77) | 0.156 |
| $D_1^{'}T_8^{'}$ | 91.67 (9.57) | 32.08 | 2940.9 | 0.077 | 7.06 | 78.67 (8.87) | 0.157 |
| D_2T_0 | 85.33 (9.24) | 29.36 | 2505.2 | 0.075 | 6.40 | 71.67 (8.47) | 0.153 |
| D_2T_1 | 88.00 (9.38) | 30.81 | 2711.3 | 0.077 | 6.78 | 77.67 (8.81) | 0.158 |
| D_2T_2 | 89.67 (9.47) | 31.55 | 2828.8 | 0.077 | 6.91 | 78.89 (8.88) | 0.159 |
| $D_2 T_3^2$ | 91.00 (9.54) | 31.71 | 2885.5 | 0.079 | 7.19 | 79.85 (8.92) | 0.158 |
| D_2T_4 | 90.00 (9.49) | 30.89 | 2780.3 | 0.078 | 7.02 | 75.33 (8.68) | 0.153 |
| D_2T_5 | 87.00 (9.33) | 30.65 | 2666.8 | 0.076 | 6.61 | 77.00 (8.77) | 0.156 |
| D_2T_6 | 88.67 (9.42) | 30.01 | 2660.9 | 0.078 | 6.92 | 76.67 (8.76) | 0.154 |
| D_2T_0 D_2T_7 | 88.33 (9.40) | 32.18 | 2842.4 | 0.077 | 6.80 | 78.67 (8.87) | 0.157 |
| D_2T_8 | 90.67 (9.52) | 32.21 | 2920.3 | 0.078 | 7.07 | 79.33 (8.91) | 0.153 |
| D_3T_0 | 81.67 (9.04) | 28.51 | 2328.9 | 0.075 | 6.13 | 71.67 (8.47) | 0.150 |
| D_3T_1 | 86.67 (9.31) | 30.59 | 2651.2 | 0.078 | 6.76 | 78.33 (8.85) | 0.160 |
| D_3T_2 | 88.00 (9.38) | 30.81 | 2711.0 | 0.078 | 6.86 | 79.02 (8.89) | 0.153 |
| D_3T_3 | 87.67 (9.36) | 31.02 | 2719.6 | 0.083 | 7.28 | 80.18 (8.95) | 0.168 |
| D_3T_4 | 84.67 (9.20) | 30.71 | 2600.6 | 0.079 | 6.69 | 76.67 (8.76) | 0.159 |
| D_3T_5 | 84.67 (9.20) | 30.23 | 2559.6 | 0.079 | 6.69 | 77.00 (8.77) | 0.164 |
| D_3T_6 | 86.33 (9.29) | 30.23 | 2612.8 | 0.079 | 6.82 | 76.67 (8.76) | 0.158 |
| D_3T_6 D_3T_7 | 88.00 (9.38) | 31.13 | 2739.4 | 0.079 | 6.95 | 79.00 (8.89) | 0.160 |
| D_3T_8 | 87.00 (9.33) | 31.41 | 2732.7 | 0.082 | 7.14 | 80.00 (8.94) | 0.156 |
| $S.E.m(\pm)$ | 0.043 | 0.318 | 37.77 | 0.0012 | 0.147 | 0.104 | 0.0047 |
| CD _{0.05} | NS | NS | NS | NS | NS | NS | NS |
| | s in the parenthesis are squa | | | - 10 | | | - 10 |

enhancing seed yield and quality of rice cv. Naveen under high temperature stress. Among various foliar spray treatments done at vegetative and seed filling stages, 800 ppm salicylic acid, 10 ppm ascorbic acid + 1.3% citric acid and 10 ppm brassinolides proved to be quite effective in alleviating the adverse effects of high temperature during flowering and seed set, through increased chlorophyll content of leaves, improved membrane stability, higher pollen viability and seed set. All these parameters had a positive influence on seed yield and quality of rice.

Acknowledgement

The authors wish to acknowledge the financial assistance from the ICAR-funded Seed Technology Research, AICRP-NSP (Crops) and the Department of Seed Science and Technology, College of Agriculture, Odisha University of Agriculture & Technology, Bhubaneswar. The facilities rendered by the University in carrying out the field and laboratory study are also hereby acknowledged.

References

- 1 Qin DH, Ding YH & Wang SW, A study of environment changes and its impacts in western China, *Earth Science Frontiers*, 9(2) (2002) 321-328.
- 2 Farooq M, Bramley H, Palta JA & Siddique KHM, Heat stress in wheat during reproductive and grain-filling phases, *Critical Reviews in Plant Sciences*, 30(6) (2011) 491–507.
- 3 Howarth CJ, Genetic Improvements of Tolerance to High Temperature, In: *Abiotic Stresses Plant Resistance Through Breeding and Molecular Approaches*, Edited by M Ashraf & PJC Harris, Howarth Press Inc., New York, 2005, 277-300.
- 4 Wahid A, Gelani S, Ashraf M & Foolad MR, Heat tolerance in plants: An overview. *Environmental and Experimental Botany*, 61(3), (2007) 199-223.
- 5 Peng S, Huang J, Sheehy JE, Laza RC, Visperas RM, Zhong X, Centeno GS, Khush GS &Cassman KG, Rice yields decline with higher night temperature from global warming. *Proceedings of The National Academy of Sciences of the United States of America*, 101(27) (2004) 9971-9975.
- 6 Arnon DI, Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiology*, 24(1) (1949) 1-15.
- 7 Sairam RK, Deshmukh PS & Shukla DS, Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat, *Journal of Agronomy and Crop Science*, 178 (1997) 171-177.
- 8 ISTA (International Seed Testing Association), International rules for seed testing rules, *Seed Science and Technology*, 13 (1996) 299-513.

- 9 Abdul-Baki AA & Anderson JP, Vigour deterioration in soybean by multiplecriteria, *Crop Science*, 13 (1973) 630-637.
- 10 Presley JT, Relation of protoplast permeability of cotton seed viability and predisposition to seedling diseases, *Plant Disease Report*, 42 (1958) 852.
- 11 Suryavanshi P & Buttar GS, Mitigating terminal heat stress in wheat. International Journal of Bio-resource and Stress Management, 7(1) (2016)142-150.
- 12 Yun-ying C & Hua Z, Protective roles of brassinolides on rice seedlings under high temperature stress. *Rice Science*, 15(1) (2008) 63-68.
- 13 Prasad PVV, Boote KJ, Allen Jr. LH, Sheehy JE & Thomas JMG, Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress, *Field Crops Research*, 95 (2006) 398–411.
- 14 Fu GF, Zhang CX, Yang XQ, Yang YJ, Chen TT, Zhao X, Fu WM, Feng BH, Zhang XF, Tao LX & Jin QY, Action mechanism by which SA alleviates high temperature induced inhibition to spikelet differentiation, *Chinese Journal of Rice Science*, 29 (2015) 637–647.
- 15 Mohammed AR & Tarpley L, High night temperatures affect rice productivity through altered pollen germination and spikelet fertility, *Agricultural and Forest Meteorology*, 149 (2009) 999-1008.
- 16 Fahad S, Hussain S, Saud S, Khan F, Hassan S, Amanullah, Nasim W, Arif M, Wang F & Huang J, Exogenously applied plant growth regulator affects heat stressed rice pollens, *Journal of Agronomy and Crop Science*, 202 (2016) 139-150.
- 17 Ashraf M & Foolad MR, Roles of glycine betaine and proline in improving plant abiotic stress resistance, *Environmental and Experimental Botany*, 59(2) (2007) 206–216.
- 18 Wahid A & Close TJ, Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves, *Biologia Plantarum*, 51(1) (2007) 104–109.
- 19 Barth C, Tullio M & Conklin PL, The role of ascorbic acid in the control of flowering time and the onset of senescence. *Journal of Experimental Botany*, 57(8) (2006) 1657–1666.
- 20 Shah F, Huang J, Cui K, Nie L, Shah T, Wu W, Wang K, Khan Z H, Zhu L & Chen C, Physiological and biochemical changes in rice associated with high night temperature stress and their amelioration by exogenous application of ascorbic acid (vitamin C). *Australian Journal of Crop Science*, 5(13) (2011) 1810-1816.
- 21 Maeda H & Dellapenna D, Tocopherol functions in photosynthetic organisms. *Current Opinion in Plant Biology*, 10(3), (2007) 260–265.
- 22 Sasse JM, Recent progress in brassinosteroid research, *Physiologia Plantarum*, 100(3) (1997) 696-701
- 23 Rao SSR, Vardhini BV, Sujatha E & Anuradha S, Brassinosteroids: A new class of phytohormones, *Current Science*, 82(10), (2002) 1239-1245.