

COMBINED EFFECT OF HIGH TEMPERATURE AND SALINITY ON GROWTH AND PHYSIOLOGY OF RICE (*Oryza sativa* L.)

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ABSTRACT

High temperature and salinity are the major climatic factors affecting the productivity of rice. Combined effect of both the stresses can cause severe yield reduction in rice. Therefore, objective of this study was to assess the combined effect of high temperature and salinity on three rice genotypes (MO-16 - popular high yielding variety; N-22 - tolerant to high temperature and drought and NL-44 - tolerant to high temperature). The experiment was laid out in completely randomized design with three replications. The three genotypes of rice were grown in non-saline conditions at optimum temperature until booting stage. At booting stage, plants were irrigated with 250mM NaCl for three days and exposed to high temperature (38°C) for 10 days. High temperature combined with salinity significantly affected the physiological processes and yield parameters. Tolerant genotype, N-22 showed higher proline content, cell membrane stability index, superoxide dismutase activity, pollen viability, spikelet fertility percentage, yield per plant and less malondialdehyde content and Na⁺/K⁺ ratio compared to other genotypes.

Keywords: High temperature, Interactive effect, Physiological traits, Rice, Salinity

Rice (*Oryza sativa* L.) is a widely consumed food crop, which is grown worldwide (Beena *et al.*, 2021). The global population is expected to be nine billion by 2050, which demands 60–110% more rice production than the present-day (Ray *et al.*, 2013). However, it is very difficult to combat the challenges like change in climatic factors, less availability of land area for cultivation, lack of high yielding stress tolerant varieties. Studies show that average temperature is expected to rise by 2–3°C over the next 30–50 years (Hatfield and Prueger, 2015). Rice growth and development is affected above a threshold temperature of 32°C: the most critical temperature was found to be 33°C during the flowering stage (Jagadish *et al.*, 2007). High temperature is detrimental to most physiological processes including stomatal opening, photosynthesis, growth, and grain yield in rice (Beena *et al.*, 2018).

Salinity is the second most devastating constraint in rice production after drought, affecting approximately 1 billion ha of land globally (Fageria *et al.*, 2012). This is equal to more than 6% of the world's total farming area (Ismail and Horie, 2017) and nearly 20% of the globally irrigated area. Most abiotic stress studies concentrated only a single stress condition, whereas, under field conditions, a combination of stresses may occur. The effects of combined heat and salinity stress have not been studied extensively. Hence, in the present study, a pot culture experiment was designed to understand the

combined effect of high temperature and salinity in rice.

MATERIALS AND METHODS

This experiment was laid out as a pot culture study in the Department of Plant Physiology, College of Agriculture, Vellayani, Kerala Agricultural University during the year 2017-2018. Three varieties *viz.*, MO-16- popular high yielding variety; Nagina-22- an *aus* rice type tolerant to high temperature and drought; and NL-44 - tolerant to high temperature were selected for this study. Seeds were sown in pottrays filled with potting mixture (coir pith compost and vermicompost @ 2:1 ratio) and labelling was done properly. Irrigation was provided regularly. Eighteen days after sowing, seedlings were transplanted to pots with potting mixture made from soil, sand and cow dung on equal volume by volume basis. Six replications were maintained for each variety as control and treatment until booting stage. The experiment was laid out in completely randomized design with two treatment levels *i.e.* control and treatment with three replications each. Five plants per each replication were maintained. During booting stage, a set of three genotypes with three replicates was transferred to stress condition. At booting stage, plants were irrigated with 250mM NaCl for three days and exposed to high temperature (38°C) for 10 days. After three days regular irrigation was given. Physiological observations were taken ten days after stress induction from the fully expanded third leaf and yield data were taken at harvest. Physiological parameters and yield traits were taken as per standard procedures;

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Chlorophyll content (Hiscox and Israelstam, 1979); Cell membrane stability (Blum and Ebercon, 1981); Proline content (Bates et al., 1983); Na⁺/K⁺ ratio (Zasoski and Buraum, 1977); Malondialdehyde content (Wang et al., 2013); Superoxide dismutase activity (Beauchamp and Fridovich, 1971); and pollen viability test (Baker and Baker, 1979) were also recorded.

Cell membrane stability index was estimated as per the procedure described by Blum and Ebercon (1981). Samples collected from both control and stress imposed plants were washed three times in deionised water to remove electrolytes adhered on the surface. Samples were kept in a capped vial (20ml) containing 10ml of deionised water and incubated in the dark for 24 hours at room temperature. The conductance was measured with a conductivity meter. After the first measurement, the vials were autoclaved for 15 minutes to kill the leaf tissue and release the electrolytes. After cooling, the second conductivity reading was taken. These two measurements were carried out individually for both control and stress treated plants. Cell membrane stability index was calculated by using following formula and expressed as per cent. $CMS (\%) = [1 - (T_1/T_2) / 1 - (C_1/C_2)] \times 100 / 36$ Where, T and C refer to the stress and control samples, respectively. Where, T and C refer to the stress and control samples, respectively. The subscripts 1 and 2 refer to the initial and final conductance readings, respectively.

Statistical analysis

The overall effects of treatment and variety and their interaction were analyzed by means of two-way ANOVA with combined effect of heat treatment with salinity and genotypes taken as fixed factors. Genotypes were treated as fixed factors because we were interested in the response of the specific genotypes used in this experiment. The statistical analysis was done using OPSTAT software (CRD with two factors).

RESULTS AND DISCUSSION

The results of the combined effect of high temperature and salinity with control are presented in Table 1.

The combined stress of high temperature and salinity ($T_h \times S_h$) caused a significant reduction in mean plant yield (5.66 g/plant) compared to control conditions (14.83 g/plant). Among the genotypes, the highest yield per plant was expressed by N-22 (6.75 g/plant) under the stress conditions, while MO-16 showed the highest yield (21.5 g/plant) followed by N-22 (12.5 g/plant) under the control conditions. In this study, grain yield per plant was significantly affected in all genotypes and higher reduction was reported in high yielding variety (MO-16). Studies reported that grain yield and harvest index were

reduced under high temperature stress in rice (Beena et al., 2018; Pravallika et al., 2020). Heat stress hastens the rate of grain filling, but reduces the duration of grain filling, as reported in wheat (Farooq et al., 2011), rice (Beena et al., 2012) which may be due to direct effects of heat stress on the source-sink relationship that reduce photoassimilate supply to developing seeds (Calderini et al., 2006).

The pollen viability of plants grown under the combined stress conditions ($T_h \times S_h$) was significantly reduced with a mean of 73.2%, whereas the control grown plants showed a higher pollen viability percentage of 93.01%. Among the genotypes, NL-44 exhibited the highest pollen viability percentage (79.12%) under the combined stress treatment followed by N-22 (74.52%). Pollen viability and germination are generally adversely affected under saline stress conditions. Pollen viability was reduced in all varieties following treatments compared to their corresponding controls. This may be due to the tapetum, the innermost cell layer of the anther wall which plays a crucial role in supplying nutrients to these microspores and in regulating their release. Temperature and salinity stress during tapetal development (such as early degeneration, hypertrophy, or mutations in the archesporial cell) leads to aborted micro gametogenesis and male sterility (Chaudhury et al., 1993).

Plants grown under the combined stress of highest tolerated level of temperature and salinity ($T_h \times S_h$) exhibited spikelet fertility percentage of 51.1% which was significantly lower than the control grown plants which had a higher mean of 82.3%. Among the genotypes, N-22 had the highest spikelet fertility percentage (62.82%) among the combined stress treatment ($T_h \times S_h$) whereas under the control conditions, the spikelet fertility percentage was highest for MO-16 (86.57%). In this study, spikelet fertility percentage was significantly reduced under combined stress of high temperature and salinity. This result agrees with other studies, which concluded that there was a reduction of grain number per plant under high temperature stress (Pradhan et al., 2012; Pravalika et al., 2020), under salinity condition (Sairam et al., 2002), under combined salinity and high temperature stress (Anjum et al., 2008)). Another study showed that the actual number of spikelet is determined by the length of the reproductive phase (Rahman et al., 1978). Stress induction during the critical reproductive stage may have affected the spikelet formation in rice.

There is no significant difference between treatments for plant height. Mean height for both the treatments was 111cm. Among the genotypes, N-22 recorded the highest plant height in both the treatments. Since the stress was imposed during the booting stage, there was no significant difference in plant height.

These differences may be due to the genetic control of the trait. On contrary, reduction in plant height under temperature stress is due to decreased cell growth especially cell elongation (Vijayakumar *et al.*, 2021). Zinn *et al.* (2010) reported that high temperature stress mainly affect the reproductive stage.

The highest number of productive tillers under control conditions were found in MO-16 (15.6) whereas, N-22 plants had the highest productive tiller number (5.5) under combined stress treatments. The difference in the productive tiller number between the genotypes under the control conditions was greater as compared to the plants grown under combined stress treatments. Salinity adversely affects a number of yield elements including the panicles, tillers and spikelets per plant, floret sterility, individual size of grain, and even delayed heading (Grattan *et al.*, 2002). Increasing temperatures from 24 to 32°C resulted in reductions in tiller number in rice (Harsant *et al.*, 2013).

Under combined stress condition, highest proline content was recorded by N-22 (27.33 µg/g), followed by NL-44 (26.12 µg/g) and MO-16 (24.76 µg/g). Under control condition, highest proline content was recorded by N-22 (24.90 µg/g), followed by NL-44 (23.48 µg/g) and MO-16 (19.97 µg/g). Average proline content under control condition was 22.79 µg/g and under stress condition was 26.07 µg/g. Salinity and high temperature induced an accumulation of proline in rice leaves. In fact, the accumulation of amino acids in plants has been widely reported as a response to salinity and temperature (Dadkhah and Rassam, 2016). The increase in proline content may be due to osmotic regulation. Some plants are able to stand salinity by reducing the cellular osmotic potential as a result of a net increase in inorganic and solute accumulation. In the present study, an increase of proline was seen in all genotypes tested. The present result was in agreement with other results on wheat seedling treated with high temperature and salinity, which also found that high temperature and salinity treatments resulted in a significant increase in proline in some genotypes (Hamada and Khulaef, 1995). Proline has various roles, such as stabilizing proteins, membranes and sub cellular structures, and protecting cellular functions by scavenging reactive oxygen species under abiotic stress conditions (Kishor *et al.*, 2005). Proline acts as an osmoprotectant and helps plants to maintain cellular homeostasis under saline stress condition (Huang *et al.*, 2013).

N-22 plants expressed the highest level of cell membrane stability index (69.17%) under the combined stress treatment ($T_h \times S_n$). The mean cell membrane stability index was 59.62% with MO-16 expressing the least value (49.52%) followed by NL-44 (60.19%). Cell

Table 1. Variation in physio-morphological and yield traits under combined stress of highest tolerated level of high temperature (T_h) and salinity (S_n) levels

Varieties	Plant height(cm)	No. of productive tillers/plant	Proline content (µg/g)	Cell membrane stability index (%)	Malondialdehyde content (mmol g ⁻¹)	Chlorophyll a/b ratio	Na ⁺ - K ⁺ ratio	Superoxide dismutase activity (g ⁻¹ min ⁻¹)	Spikelet fertility percentage (%)	Pollen viability (%)	Yield per plant (g/ plant)
MO-16	98.00 ^c	3.50 ^c	24.76 ^c	49.52 ^b	2.63 ^a	1.23	0.72 ^a	0.37	31.87 ^c	65.97 ^c	4.75 ^b
N-22	125.00 ^a	5.50 ^a	27.33 ^a	69.17 ^a	2.13 ^c	1.07	0.46 ^c	0.42	62.82 ^a	74.52 ^b	6.75 ^a
NL-44	110.00 ^b	4.50 ^b	26.12 ^b	60.19 ^a	2.42 ^b	1.01	0.54 ^b	0.34	58.52 ^b	79.12 ^a	5.50 ^b
Mean	111.00	4.500	26.07	59.62	2.39	1.10	0.57	0.38	51.075	73.205	5.666
MO-16	100.00 ^b	15.60 ^a	19.97 ^c	10.556	0.96	2.07	0.69 ^a	0.23	86.57 ^a	90.91 ^b	21.50 ^a
N-22	122.00 ^a	4.60 ^c	24.90 ^a	10.556	0.94	2.08	0.30 ^b	0.24	82.61 ^b	93.66 ^a	12.50 ^b
NL-44	112.00 ^{ab}	6.60 ^b	23.48 ^b	10.556	1.04	2.01	0.29 ^b	0.29	77.58 ^c	94.48 ^a	10.50 ^c
Mean	111.00	8.933	22.79	10.556	0.98	2.05	0.42	0.26	82.256	93.016	14.833
C.D. (p≤0.05)	G = 16.718 ; T= N/S ; G×T =N/S	G = 0.545; T= 0.668 ; G×T = 0.944	G = 0.970 ; T= 0.792 ; G×T =1.372	G = 0.136; T= 0.111 ; G×T = 0.192	G = N/S ; T= 0.201 ; G×T = N/S	Control condition	G = 0.07 ; T= 0.057 ; G×T = -0.099	G = N/S ; T= 0.036; G×T = -0.063	G = 2.714; T= 3.324; G×T = 4.701	G = 2.939; T= 3.599; G×T = 5.090	G = 0.800; T= 0.979; G×T = 1.385

membrane stability was reduced under high temperature condition in rice (Beena *et al.*, 2018). Heat stress primarily affects the stability of plasma membranes, several proteins, cytoskeleton organization, and the efficiency of cell enzymatic reactions and creating metabolic disparity (Xu *et al.*, 2006). Heat-stress-induced oxidative stress causes peroxidation of membrane lipids, proteins, and nucleic acids. Due to reduced membrane stability, electrolyte leakage increases, which intensifies the membrane injuries (Wahid *et al.*, 2007). However, under temperature and salinity stress conditions antioxidant concentration is decreased leading to cell harm (Kreiner *et al.*, 2002). Increased cell damage due to ROS can reduce the thermal stability of the membrane, thereby disrupting the motion of water, ion and organic-solutes.

Plants grown under the combined stress of highest tolerated level of drought and salinity ($T_h \times S_h$) exhibited a mean malondialdehyde content of 0.98 mmol g⁻¹ which was significantly lower than the control grown plants which had a higher mean of 2.39 mmol g⁻¹. Among the genotypes, MO-16 had the highest malondialdehyde content (2.63 mmol g⁻¹) among the combined stress treatments ($T_h \times S_h$) followed by NL-44 (2.42 mmol g⁻¹). The MDA content is linked with the oxidization of the cell membrane and the content of MDA is often used as an indicator of lipid peroxidation resulting from oxidative stress. MDA has been considered an indicator of salt-induced oxidation in cell membranes and a tool for determining salt tolerance in plants (Ghafiyehsanj *et al.*, 2013; Radi *et al.*, 2013). In this study, MDA content were significantly increased by combined stress condition.

The chlorophyll a/b ratio of the plants under control conditions was higher with a mean of 2.05 while the mean ratio of plants grown under the combined stress of high temperature and salinity ($T_h \times S_h$) was 1.10 which was significantly lower. The chlorophyll a/b ratio among the genotypes as well as the interaction between the genotype and treatments was found to be non-significant. As the most significant photosynthetic pigment, chlorophyll plays an important role in controlling crop yields. Chlorophyll, however, is quite fragile, not very stable and is readily influenced by abiotic stress. The decrease in chlorophyll content under stress condition may be due to stress-induced disruption in biosynthetic pathways or in pigment degradation, loss of the chloroplast membrane, and increased lipid peroxidation thus resulting in production of reactive oxygen species (ROS) (Reddy *et al.*, 2004; Pravallika *et al.*, 2020; Vijayakumar *et al.*, 2021).

The mean Na⁺-K⁺ ratio of the control grown plants (0.42) was found to be lower than the mean of the plants subjected to the combined stress (0.57) of high temperature and salinity ($T_h \times S_h$). Among the

genotypes, MO-16 expressed higher Na⁺-K⁺ ratio of 0.72 and 0.69 compared to other genotypes under the stressed and control conditions, respectively. Under control condition, lowest Na⁺-K⁺ was recorded by NL-44 (0.29). But under combined stress condition, lowest Na⁺-K⁺ was maintained by N-22 (0.46). This is mainly attributed to the higher ionic imbalances resulting in passive accumulation of Na in root and shoot during salinity stress. Na⁺ is the principal poisonous ion in salinized soil and low Na⁺ /K⁺ ratio in the cytoplasm is essential to maintain a number of enzymatic reactions (Reshna and Beena, 2021).

The superoxide dismutase activity of combined stress treatment ($T_h \times S_h$) was 0.38 g⁻¹ min⁻¹ which was significantly higher than 0.26 g⁻¹ min⁻¹ under the control conditions. Among the genotypes, N-22 expressed significantly higher superoxide dismutase activity (0.42 g⁻¹ min⁻¹) followed by MO-16 (0.37 g⁻¹ min⁻¹) under the stress conditions whereas NL-44 expressed the highest superoxide dismutase activity (0.29 g⁻¹ min⁻¹) under the control conditions. However, the difference in the superoxide dismutase activity was found to be non-significant between the genotypes. The severe effects of heat stress are oxidative damage to cells by reactive oxygen species, as found with low temperature, drought, and salinity stress. Plants have developed enzymatic and nonenzymatic scavenging systems to quench active oxygen, and to eliminate the detrimental effects of active oxygen (Bowler *et al.*, 1992).

From this study, we found that N-22 had a high pollen viability, chlorophyll content, proline, cell membrane stability index, superoxide dismutase activity; less MDA content and Na⁺/K⁺ ratio. This is possible due to accumulation of osmo-protectant that may have enhanced the turgor by osmotic adjustment leading to stomatal opening and maintaining CO₂ level for efficient photosynthesis.

Authors' contribution

Conceptualization and designing of the research work (BR); Execution of field/lab experiments and data collection (ABS); Analysis of data and interpretation (ABS, BR, SK); Preparation of manuscript (BR,SK).

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