Acclimation response of signalling molecules for high temperature stress on photosynthetic characteristics in rice genotypes

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ORIGINAL ARTICLE

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Abstract Three signalling molecules viz. salicylic acid, calcium (calcium chloride) and brassinosteroid (24-epi-brassinolide) were exogenously applied at pre-anthesis stage to investigate their role in ameliorating high temperature effects on CO_2 assimilation, chlorophyll fluorescence, photosynthesis pigments and their correlation with grain yield in two contrasting rice genotypes (Pusa Sugandh 5 and Nerica L 44). Three different concentrations of salicylic acid (SA) (0.1, 0.25 and 0.5 mM), calcium (Ca) (10, 50 and 100 mM of $CaCl_2$) and brassinosteroid (BR) (0.5, 1 and 1.5 ppm of 24-epibrassinolide) were applied thrice through foliar spray at pre-anthesis stage. After foliar spray, one set of plants was exposed to high temperature stress (36 ± 1.7 °C) using temperature tunnel for a period of 2 weeks. High temperature significantly decreased the net photosynthetic rate (P_N), photosynthetic water use efficiency (PWUE), stomatal conductance (g_s), total chlorophyll content as well as relative efficiency of PSII photochemistry (Fv/Fm) in both the genotypes. Transpirational water loss (E) and internal CO_2 concentration (Ci) increased under high temperature stress. Lower concentration of SA (SA_1 and SA_2) and Ca (Ca_1 and Ca_2) improved PWUE by enhancing P_N and reducing E as well as Ci. Similarly, all three concentrations of BR showed alleviation of high temperature stress effects on photosynthetic activity. The amelioration effect of signalling molecules for high temperature stress effects were more pronounced in PS 5 compared to Nerica L-44. Regression analysis indicated involvement of these molecules to nullify the effects of high temperature stress on P_N in relation with grain yield per plant in both rice genotypes and was significant in PS 5. The findings of the study conclude that application of above signalling molecules may negate the high temperature stress induced reductions in PSII efficiency, PWUE, chlorophyll pigments and gaseous exchange in rice at pre-anthesis stage.

Keywords High temperature · Photosynthesis · Chlorophyll fluorescence · Rice

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Introduction

It is well documented that global air temperature will increase by 1.4–5.8 °C by the end of this century (IPCC 2007). Rice (*Oryza sativa* L.), the world's most important staple food crop, is cultivated under wide range of environments (Khush 2005). Adverse effects of high temperature stress on growth, development, photosynthesis and yield are well known in various crops (Wheeler et al. 1997; Pushpalatha et al. 2008; Efeoglu and Terzioglu 2009; Allakhverdiev et al. 2008). Heat stress affects the metabolism, cell membrane integrity and many basic physiological processes such as photosynthesis, respiration and water relations (Wahid et al. 2007). Photosynthetic light reaction particularly photosystem II activity is highly thermo-liable and heat stress can inhibit the oxygen evolution and electron transport reaction (Allakhverdiev et al. 2008; De Ronde et al. 2004). Inhibition of CO_2 assimilation under high temperature stress has been attributed with differential solubility of CO_2 and O_2 as well as kinetic properties of RuBP carboxylase/oxygenase and increased photorespiration (Long et al. 2004; Pushpalatha et al. 2007). Higher concentration and synthesis of various signal

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molecules impart high temperature tolerance in plants. Larkindale and Knight (2002) reported induction of thermotolerance due to pre-treatment of plants with some endogenous signalling compounds. These molecules activate a range of signal transduction pathways and help the plants to overcome the stress effects (Table 1).

Elucidating such signal transduction pathways has been facilitated through exogenous application of these signal molecules and their response at the physiological and biochemical level in plants (Gong et al. 1997). Foliar application of salicylic acid (SA) showed significant increases in seedling growth and yield of spring wheat (Shakirova et al. 2000) and rice (Sivakumar et al. 2006). Ca^{2+} acts as stimulus response coupling the regulation of diverse cellular functions and plays crucial role in maintaining cell wall and membrane integrity (Bose et al. 2011). Positive effect of calcium treatments on photosynthesis, membrane stability and antioxidant system showed its immense potential for improving thermotolerance in plants (Kleinhenz and Palta 2002; Gong et al. 1997). Transient increase in the intracellular concentration of free calcium (Ca^{2+}) in tobacco (Gong et al. 1997), wheat (Liu et al. 2003), suspension-cultured *Arabidopsis* cells (Liu et al. 2006) and Moss plants (Saidi et al. 2009) has been reported under heat stress. Similarly, brassinosteroids (BRs), a family of 40 naturally occurring plant steroid hormones, has been shown to modulate plant response to environmental stress and pathogen infection (Nakashita et al. 2003; Bajguz and Hayat 2009). Divi et al. (2010) reported that BRs may have significant role in plant stress

Table 1 Analysis of variance for physiological traits viz., P_N (net photosynthetic rate), g_s (stomatal conductance), Ci (internal CO_2 concentration), E (transpiration rate), PWUE (photosynthetic water use efficiency) and Fv/Fm (PS II efficiency) in rice plants of genotypes PS-5 and Nerica L 44, pretreated with SA, Ca and Br and exposed to high temperature at pre-anthesis stage

Source of variation	P_N (µmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	Ci (µmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	PWUE (µmol $mmol^{-1}$)	PSII efficiency Fv/Fm
Salicylic acid						
PS-5						
C	187.47***	97.13***	59.05***	21.07***	41.98***	96.92***
T	134.11***	0.05^{ns}	11.00**	136.27***	303.56***	252.05***
C * T	36.62***	5.60**	59.27***	5.58**	19.48***	50.20***
Nerica L-44						
C	197.01***	17.06***	37.19***	17.68***	23.10***	98.77***
T	1211***	14.27***	338.72***	1.82^{ns}	102.45***	711.78***
C * T	87.13***	2.477^{ns}	35.70***	3.01*	10.45***	64.18***
Calcium chloride						
PS-5						
C	161.05***	36.05***	21.95***	61.84***	206.75***	143.55***
T	463.17***	116.25***	2.72^{ns}	1.23^{ns}	312.59***	432.78***
C * T	12.70***	17.47***	14.74***	51.22***	49.70***	75.61***
Nerica L-44						
C	92.15***	60.30***	9.77***	35.94***	21.64***	243.70***
T	87.45***	28.87***	6.91*	7.27*	51.89***	632.21***
C * T	3.91*	15.90***	32.26***	9.94***	19.62***	143.11***
24-Epibrassinolide						
PS-5						
C	84.42***	11.50***	13.28***	2.47^{ns}	31.11***	500.13***
T	112.45***	9.09**	0.003^{ns}	4.24^{ns}	49.97***	1338.00***
C * T	22.55***	0.51^{ns}	31.97***	16.16***	22.63***	406.51***
Nerica L-44						
C	124.73***	37.22***	8.75***	4.19*	34.95***	44.17***
T	67.35***	0.43^{ns}	15.86***	0.40^{ns}	16.20***	167.37***
C * T	84.50***	26.18***	11.38***	1.37^{ns}	19.52***	39.38***

C chemical, *T* temperature, *C * T* chemical * temperature, *ns* indicates non-significant results at $P \leq 0.05$

*, **, *** Significant at the 0.05, 0.01 and 0.001 levels of probability, respectively

alleviation. Keeping in view, a pot study was conducted to analyse the role of three signalling molecules viz. salicylic acid, calcium and brassinosteroid to acclimatise the plants and ameliorate the effects of high temperature stress on gaseous exchange, chlorophyll fluorescence, photosynthetic pigments and grain yield in two rice genotypes.

Materials and methods

Plant material and growth environment

Pot experiments were conducted during 2010–2011 and 2011–2012 at Indian Agricultural Research Institute, New Delhi with two contrasting rice genotypes viz., Pusa Sugandh 5 (PS 5) (high temperature sensitive) and Nerica L 44 (high temperature tolerant), raised under ambient temperature environment and exposed to high temperature stress (36 ± 1.7 °C) at pre-anthesis stage (1 week before anthesis). Three different concentrations of salicylic acid (SA) (0.1, 0.25 and 0.5 mM), calcium (Ca) (10, 50 and 100 mM of $CaCl_2$) and brassinosteroid (BR) (0.5, 1 and 1.5 ppm of 24-epibrassinolide) were foliar sprayed twice before exposure of plant to high temperature stress. Distilled water sprayed plants served as control. All recommended agronomic practices were followed and optimum doses of fertilizers in the form of urea, SSP and MOP were applied.

Gas exchange and chlorophyll fluorescence measurements

Gas exchange parameters were measured using a portable photosynthesis system LI-6400xt (LI-COR Inc., Lincoln, NE, USA) in uppermost fully expanded leaf between 10.00 and 11.30 AM. Above measurements were recorded in control and exposed plants of both the rice genotypes. Leaf net photosynthetic rate (P_N) and internal CO_2 concentration (Ci) were expressed as μmol m^{-2} s^{-1}, transpiration rate (E) and stomatal conductance (g_s) were expressed in mmol m^{-2} s^{-1} and mol m^{-2} s^{-1}, respectively. Photosynthetic water use efficiency (PWUE) was computed as ratio of P_N/E. Chlorophyll fluorescence (PS II efficiency, F_v/F_m) was measured simultaneously in dark adapted leaves (30 min).

Estimation of photosynthetic pigments

Chlorophyll content was estimated in the leaves following non-maceration technique of Hiscox and Israelstam (1979). Absorbance was measured at 663 and 645 nm and chlorophyll content was calculated using the formula given by Arnon (1949).

Statistical analysis

Photosynthetic rate was regressed against grain yield under high temperature for different chemical treatments using linear regression equations to know the relation of chemical treatments under high temperature in contrasting rice genotypes. The data was statistically analyzed using analysis of variance (ANOVA) using SPSS v.10 computer package (SPSS Inc. USA).

Results and discussion

Plants have evolved various mechanisms to protect themselves from heat stress like expression of HSPs and enhanced activities of antioxidant enzymes. Some signal molecules like calcium, salicylic acid and brassinosteroids have been shown to impart tolerance in plants against heat stress (Kleinhenz and Palta 2002; Vlot et al. 2009; Hayat et al. 2010). This study reports the role of above signal molecules/growth regulators in ameliorating the effects of high temperature stress in rice genotypes during anthesis stage.

In this study, high temperature decreased net photosynthesis rate (P_N) in both the rice genotypes. PS-5 showed more reduction in P_N (31.2 %) while in Nerica L-44 it reduced by 28.4 % under high temperature stress. On the other hand, plants pre-treated with signal molecules showed a significant gain in P_N under high temperature stress. The pre-treatment of salicylic acid showed maximum gain in P_N with SA_1 (62 % higher) followed by SA_2 (35.8 %) in PS 5, while in Nerica L-44, SA_1 applications showed 18 % enhancement in P_N under high temperature stress (Fig. 1a). For calcium concentration application, only Ca_1 showed 16.4 % increase in P_N in PS-5, while higher concentrations did not have any amelioration effects and P_N reduced due to high temperature stress. In Nerica L-44, both Ca_1 and Ca_2 showed gain in P_N by 37–22 %, while Ca_3 application showed reduction under high temperature stress compared to control (Fig. 2a). BR pre-treatment showed similar effects and enabled the plants to maintain higher P_N in both the rice genotypes under high temperature environment (Fig. 3a). Above findings suggest that pre-treatment of signalling molecules enabled the plants to maintain higher P_N compared with high temperature stress. In the light reaction of photosynthesis, PSII is considered most sensitive to all abiotic stresses including high temperatures

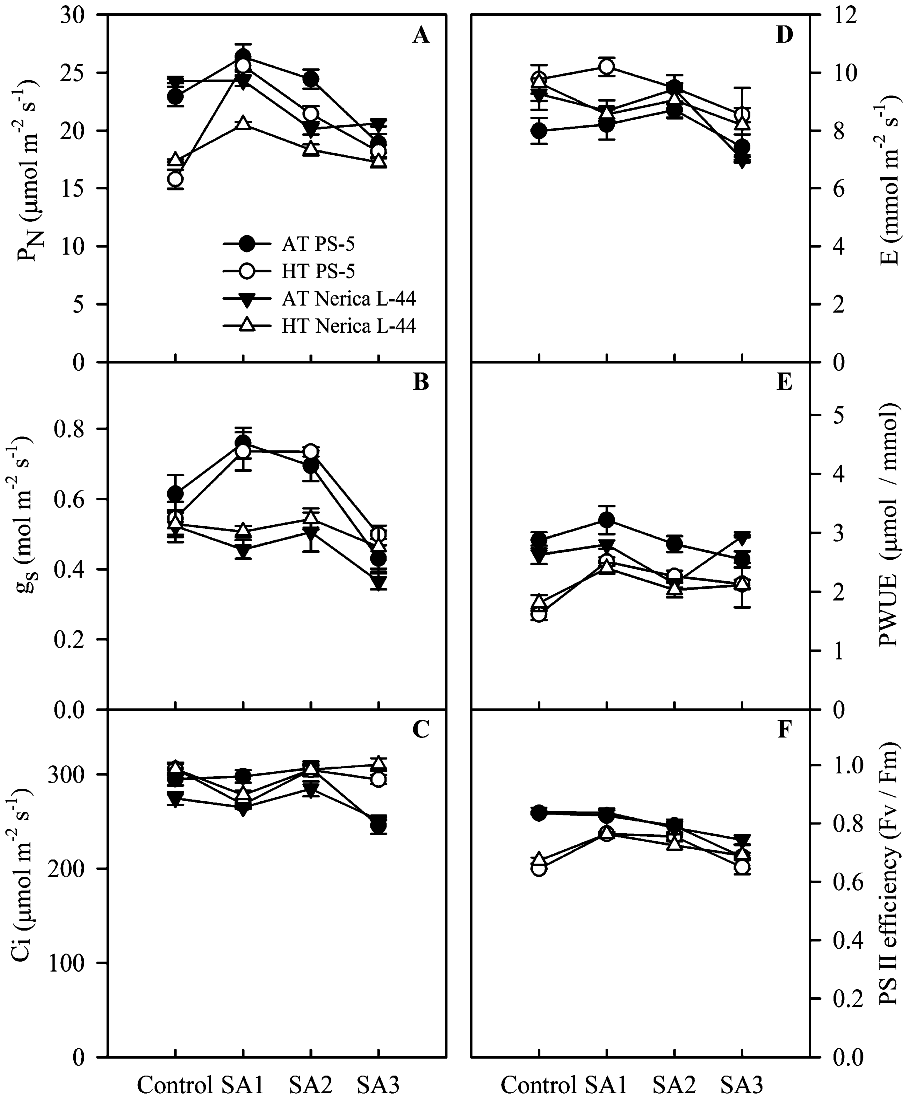

Fig. 1 Physiological parameters of rice genotypes pre-treated with different concentrations of salicylic acid (SA) (0.1, 0.25 and 0.5 mM i.e. SA_1, SA_2 and SA_3) at pre-anthesis stage and exposed to ambient temperature (AT, *closed circles*) and high temperature (HT, *open circles*) environment. Values shown here are mean ± SE of five replicates. (P_N net photosynthetic rate; g_s stomatal conductance; *Ci* internal CO_2 concentration; *E* transpiration rate; *WUE* photosynthetic water use efficiency and Fv/Fm-PS II efficiency)

(Lu and Zhang 2000). In this study we estimated PSII efficiency as Fv/Fm ratio. High temperature stress significantly decreased PSII efficiency (Fv/Fm) in both the rice genotypes with higher reduction in PS-5. SA pre-treatment maintained high Fv/Fm in both the genotypes under high temperature treatment and the response was better with pre-treatment of lower concentrations (Fig. 1f). Wang et al. (2010) have reported similar role of SA in protecting PS II in grapevines under heat stress.

Lower concentrations of calcium pre-treatment improved Fv/Fm in both PS-5 and Nerica L-44 plants exposed to high temperature, while Ca_3 concentrations caused further reductions showing additive stress (Fig. 2f). Brassinosteroids didn't show any concentration dependent effects on Fv/Fm as pre-treatment of BRs significantly enhanced Fv/Fm at all concentrations. Its application enhanced Fv/Fm by 25, 26 and 27 % for BR_1, BR_2 and BR_3, respectively, in PS 5, while in Nerica L-44, it enhanced Fv/Fm by 22, 26 and 27 % with above concentrations (Fig. 3f). Similar alleviation of heat stress induced reduction in photosynthesis and enhancement in antioxidant enzyme activity occurred through pre-treatment of EBR in tomato (Ogweno et al. 2008). EBR pre-treatment has been shown to protect the plants against high temperature stress in Brassica (Kagale et al. 2007) and tomato (Singh and Shono 2005).

Although P_N, g_s and E are equally affected by high temperature, it has been suggested that increase in g_s and E lowers the leaf surface temperature and thus helps plants to avoid high temperature stress effects (Aien et al. 2011; Leigh et al. 2012). However, at high temperature decrease in P_N is independent of g_s and E. In the present study, high temperature treatment increased the transpiration rate in both the rice genotypes and highest enhancement was found in PS-5 (22 %) compared to Nerica L 44 (4 %). Pre-treatment of plants with salicylic acid reduced transpiration

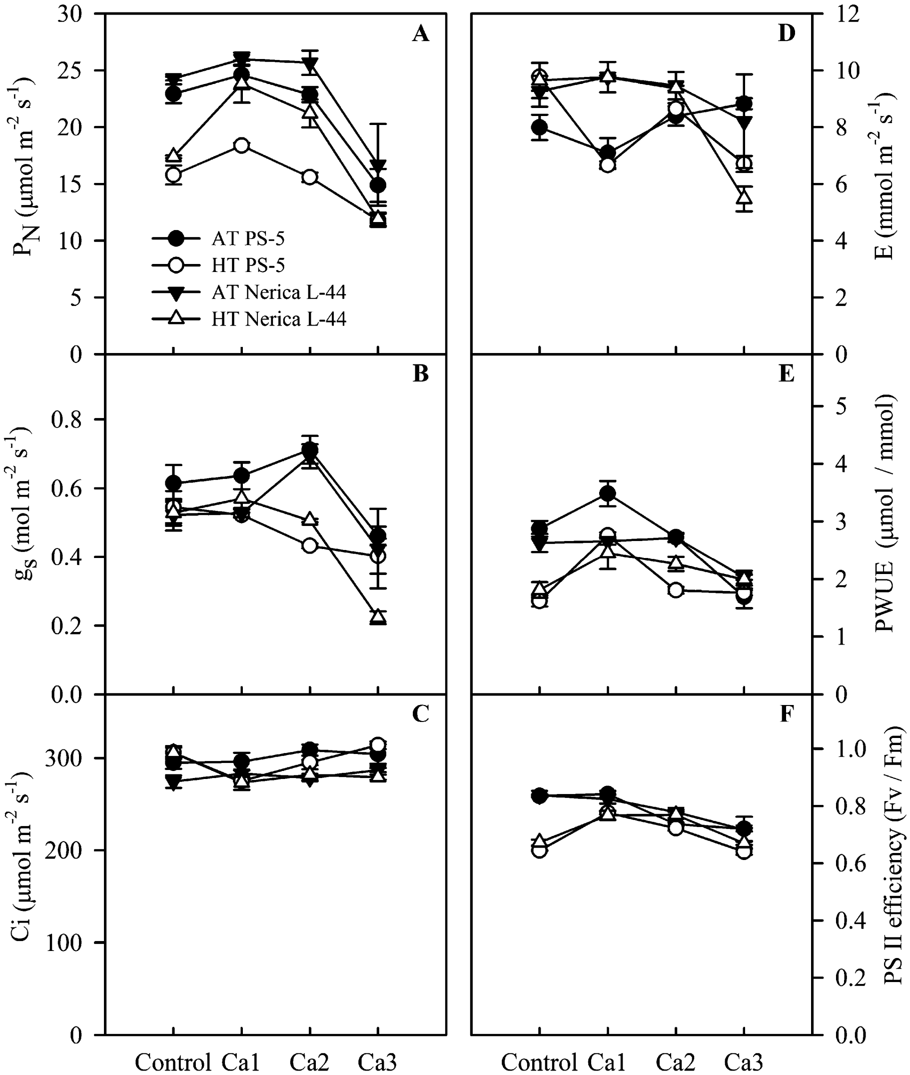

Fig. 2 Physiological parameters of rice genotypes pre-treated with different concentrations of $CaCl_2$ (10, 50 and 100 mM i.e. Ca_1, Ca_2 and Ca_3) at pre-anthesis stage and exposes to ambient temperature (AT, *closed circles*) and high temperature (HT, *open circles*) environment. Values shown here are mean ± SE of five replicates. (P_N net photosynthetic rate; g_s stomatal conductance; *Ci* internal CO_2 concentration; *E* transpiration rate; *PWUE* photosynthetic water use efficiency and Fv/Fm-PS II efficiency)

rate in PS-5 plants (SA_2 3 %; SA_3 12.6 %) in high temperature exposed plants. In Nerica L-44, the SA pre-treatment reduced transpiration rate under all concentrations in high temperature exposed plants (Fig. 1d). For calcium pre-treatment, all concentrations showed reduction in transpiration rate compared to control (H_2O + high temperature) in PS 5, whereas in Nerica L-44 only Ca_3 caused a reduction of 43 % in transpiration rate and Ca_1 and Ca_2 showed no significant effects (Fig. 2d). Pre-treatments of high temperature exposed rice plants with three concentrations of BR showed similar response (Fig. 3d). Dat et al. 2000 have reported improved heat tolerance in mustard with pre application of lower concentrations of SA (0.01–0.1 mM). Role of SA to induce heat tolerance has been confirmed in above study using inhibitors of SA synthesis. Similarly, positive effects of exogenous Ca application have been shown on photosynthesis recovery and lower lipid peroxidation and improved acquired high temperature tolerance (Jiang and Haung 2001). In this study, we observed lower g_s in rice plants exposed to high temperature and pre-treated with SA, Ca and BR. There was a reduction of 11 % in PS 5 under high temperature stress, while Nerica L-44 showed slightly higher g_s. Salicylic acid pre-treatment, controlled the reduction in g_s in PS-5 (SA_1-33.7 % and SA_2-33.5 %) (Fig. 1b). Calcium pre-treatment did not show any changes in g_s at any concentration in PS-5, while in Nerica L-44, only Ca_1 enhanced the g_s by 8 % (Fig. 2b). BR pre-treatment showed lower reduction of g_s under high temperature stress. All BR concentrations showed enhancement in g_s in PS-5 (up to 24 %) and in Nerica L-44, enhancement in g_s ranged between 14–21 % with pre-treatments of all three concentrations of BR (Fig. 3b). High temperature increased the internal CO_2 concentration in both genotypes, and the increase was more in Nerica L-44 (11.35 %) than PS-5 (3.7 %) without pre-treatment of any signalling molecules.

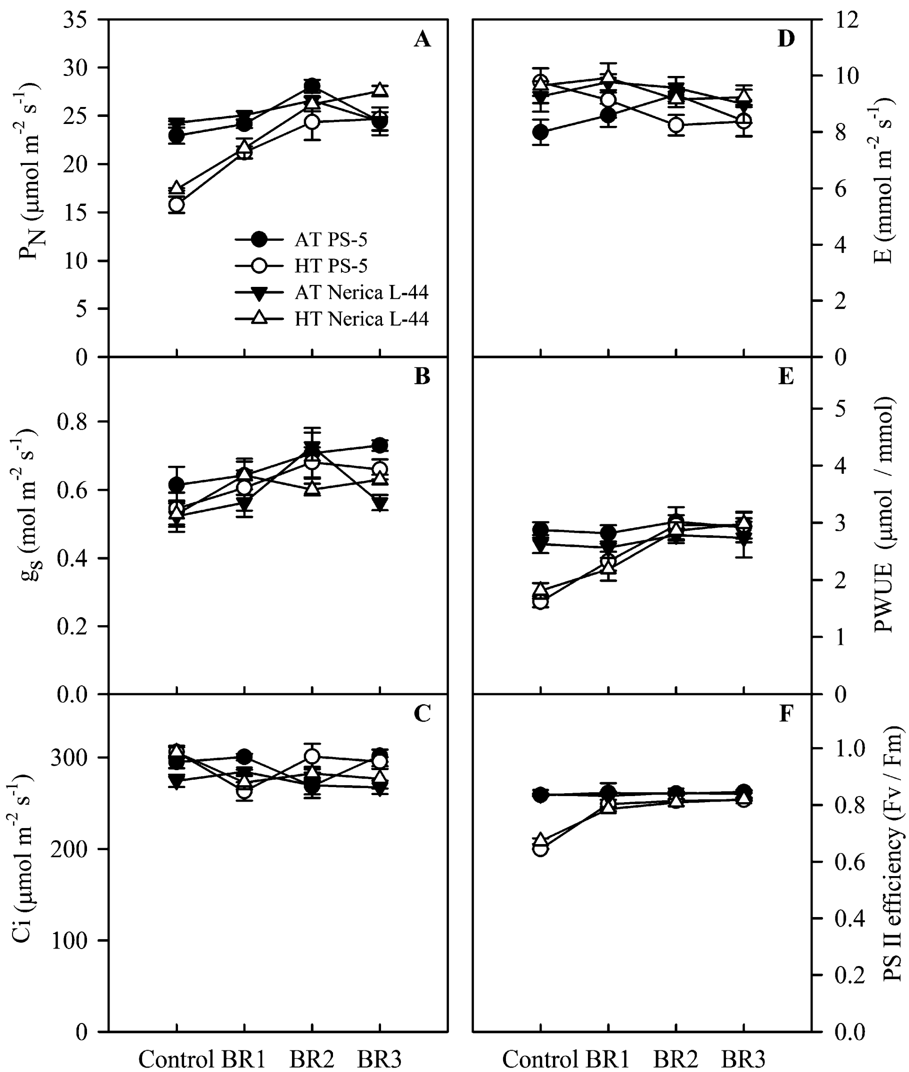

Fig. 3 Physiological parameters of rice genotypes pre-treated with different concentrations of 24-epibrassinolide (0.5, 1.0 and 1.5 ppm i.e. BR_1, BR_2 and BR_3) at pre-anthesis stage and exposed to ambient temperature (AT, *closed circles*) and high temperature (HT, *open circles*) environment. Values shown here are mean ± SE of five replicates. (P_N net photosynthetic rate; g_s stomatal conductance; *Ci* internal CO_2 concentration; *E* transpiration rate; *WUE* photosynthetic water use efficiency and Fv/Fm-PS II efficiency)

In general, pre-treatment of signal molecules caused reduction in Ci of both the genotypes under high temperature conditions compared to control plants (Figs. 1, 2, 3c).

In this study high temperature stress affected the concentration of photosynthetic pigments. In general, there was reduction in total chlorophyll content in both genotypes under high temperature stress and PS 5 showed more reductions (11.3 %) compared to Nerica L-44 (8 %). Pre-treatment of plants with lower concentration of signal molecules (SA_1, Ca_1 and BR_1) enabled the plants of PS 5 genotypes to maintain higher chlorophyll under high temperature stress. On the other hand pre-treatment with higher concentration of SA and Ca caused further reductions in chlorophyll content due to their additive stress effects on pigments concentration (Fig. 4). High temperature exposed plants of the both the genotypes pre-treated with three concentrations of BR maintained higher chlorophyll content (Fig. 4). Similarly, foliar applications of $CaCl_2$ (10 mM) have been reported to enhance the chlorophyll content under high temperature stress in cool season grass species (Jiang and Haung 2001).

The findings of this study showed reductions in PWUE in both the rice genotypes due to lower P_N under high temperature. PWUE in PS-5 reduced by 44 % compared to Nerica L-44 (31.2 %) in high temperature exposed plants. Rice plants pre-treated with signal molecules showed significant gain in their PWUE. Application of SA enhanced PWUE by 55, 40 and 32 % (SA_1, SA_2 and SA_3, respectively) in PS 5, while in Nerica L-44, it increased by 33, 12 and 17 % for above treatments (Fig. 1e). Pre-treatment with lower concentrations of Ca (Ca_1 and Ca_2) showed significant gain in PWUE in both the rice genotypes (Fig. 2e). BR pretreatment showed similar response and enhanced PWUE in rice genotypes exposed to high temperature stress, especially with BR_2 and BR_3 concentration (Fig. 3e). Enhanced PWUE in rice

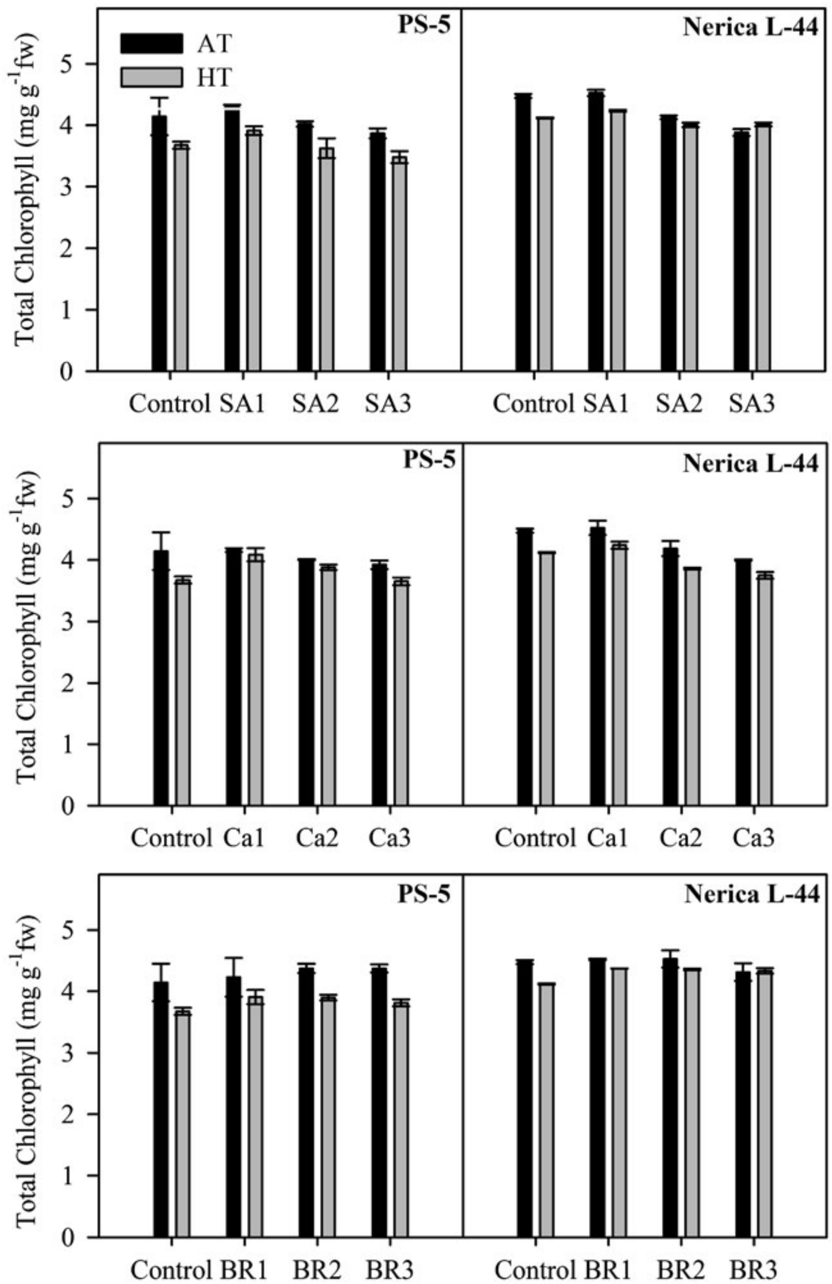

Fig. 4 Total chlorophyll content (mg g^{-1} fw) of rice genotypes pre-treated with different concentrations of signalling molecules viz. Salicylic acid (SA) (0.1, 0.25 and 0.5 mM i.e. SA_1, SA_2 and SA_3), $CaCl_2$ (10, 50 and 100 mM i.e. Ca_1, Ca_2 and Ca_3) and 24-epibrassinolide (0.5, 1.0 and 1.5 ppm i.e. BR_1, BR_2 and BR_3) and exposed to ambient temperature (AT) and high temperature (HT) environments. Values shown here are mean ± SE of five replicates

plants exposed to high temperature and pre-treated with signal molecules could be the result of maintaining higher P_N and lower E. Higher rate of photosynthesis was accompanied with enhanced E, membrane stability and total biomass in potato pre-treated with Ca and exposed to high temperature (Tawfik et al. 1996). In plant pre-treated with signalling molecules, relationship of photosynthetic rate with grain yield under high temperature was stronger for PS 5 compared to Nerica L-44. Among three signalling molecules, it was strongest ($r^2 = 0.51$) in BR treatment for PS 5 (Fig. 5a), followed by SA ($r^2 = 0.33$, Fig. 5b) and Ca ($r^2 = 0.23$, Fig. 5c). In Nerica L-44 relationship in P_N and grain yield was appreciable ($r^2 = 0.22$) only under BR pretreatments and high temperature exposure (Fig. 5f).

The study concludes that pre-treatments of plants with lower concentrations of SA, Ca and BR can ameliorate the effects of high temperature stress on net photosynthetic rate (P_N), photosynthetic water use efficiency (PWUE), stomatal conductance (g_s), total chlorophyll content as well as relative efficiency of PSII photochemistry (Fv/Fm) at anthesis stage in rice. However, application with higher concentrations may cause additive stress. These findings warrants further studies using wide range of concentration of above signal molecules to confirm their role in amelioration of high temperature stress effect in rice.

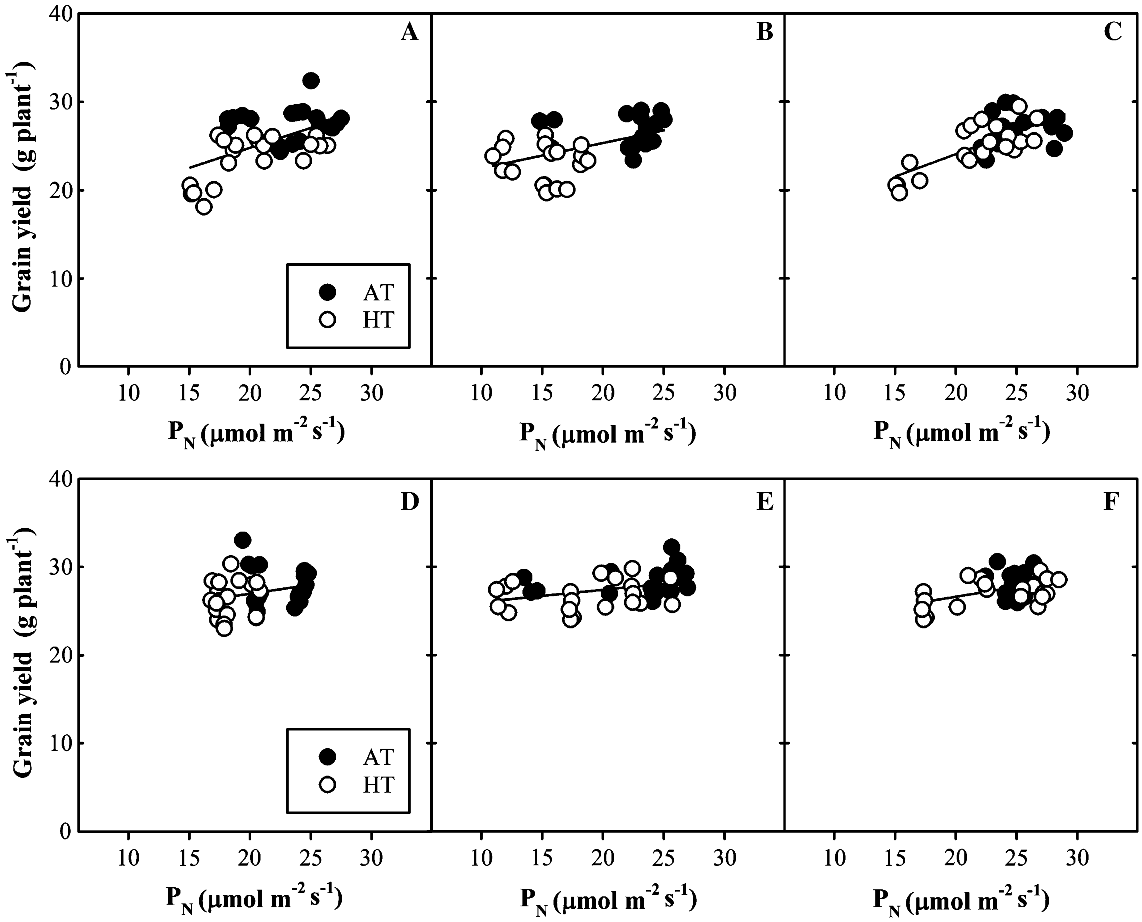

Fig. 5 Relationship between leaf net photosynthesis and grain yield of rice genotypes PS-5 (**a**–**c**) and Nerica L-44 (**d**–**f**) pre-treated with salicylic acid (**a**, **d**), $CaCl_2$ (**b**, **e**) and 24-epibrassinolide (**c**, **f**). *Open symbols* are values from plants grown at ambient temperature and *solid symbols* are for plants grown at high temperature. Regression equations: (a) $y = 0.45x + 15.75$; $r^2 = 0.33$; $n = 20$; (b) $y = 0.29x + 19.63$; $r^2 = 0.23$; $n = 20$; (c) $y = 0.51x + 13.90$; $r^2 = 0.51$; $n = 20$; (A) $y = 0.20x + 22.94$; $r^2 = 0.06$; $n = 20$; (B) $y = 0.14x + 24.62$; $r^2 = 0.15$; $n = 20$; (C) $y = 0.22x + 22.14$; $r^2 = 0.22$; $n = 20$

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