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Physiological and biochemical changes in rice associated with high night temperature stress and their amelioration by exogenous application of ascorbic acid (vitamin C)

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Abstract

Significant efforts are required to enable rice plant to cope with the menace of high night temperature (HNT) stress. As an immediate solution, use of plant growth hormones can be targeted to maximize plant tolerance against HNT stress. Ascorbic acid (AsA) is a small water soluble molecule which acts as an antioxidant and can directly detoxify active oxygen species. In this study, the potential role of AsA has been investigated for the alleviation of HNT related problems by improving the overall anti-oxidant status and physiological performance of rice. In this regard, the current research was carried out in controlled environment to study the interactive effect of HNT and foliar application of AsA on physiological traits of rice; mainly focusing on its anti-oxidative and nutrient status. Two tested genotypes showed considerable similarity in response to both HNT and AsA. HNT increased the malondialdehyde (MDA) content of both varieties and exogenous AsA reduced it almost to the level of control night temperature (CNT). Similarly, the HNT associated increases in Na^+ and K^+ in both root and shoot were also lowered by exogenous AsA. The anti-oxidative enzymes catalase (CAT) and peroxidase (POD) were also increased under HNT. While application of AsA led to reductions in their concentrations. POD was more sensitive to HNT than CAT and AsA also showed more pronounced effect on POD compared to CAT. Although preliminary results for the acclamatory role AsA under HNT are favorable, future detailed studies including diverse genotypes and different levels of AsA applied at various stages are imperative.

Keywords:Anti-oxidants; Catalase; Malondialdehyde; Sodium; *Oryza sativa*; Peroxidase; Potassium; Stomatal conductance.
Abbreviations: AOS-Active oxygen species; AsA-Ascrorbic acid; CAT-Catalase; CNT-Controlled night temperature; DACHT-Days after initiation of chemical and heat treatments; HNT-High night temperature; HNT + AsA-High night temperature + ascorbic acid; MDA-Malondialdehyde; gs-Stomatal conductance; POD-Peroxidase; ROS-Reactive oxygen species; SOD-Superoxide dismutase.

Introduction

Rice production is facing many problems around the world due to many biotic and abiotic stresses against rice plant (Sabouri et al., 2011). The projected rise in global warming is expected to have a tremendous impact on rice (Matsui et al., 2007), which is grown in wide climates already experiencing increases in day and night temperatures (Mohammed and Tarpley, 2009a). Moreover, periodic episodes of heat stress and seasonally HNT are predicted to occur more frequently in the near future (Mohammed and Tarpley, 2011). Nighttime temperature not only has been reported to increase rapidly (Peng et al., 2004), but has also been argued as to have more devastating effects compared to daytime or mean daily temperatures (Shah et al., 2011). It is one of the probable causes that most of the current researches focus more on night temperature stress. Several reasons have been proposed for the yield reduction of plants exposed to HNT. Besides yield reduction, changes in growth temperature also induce morphological responses and alterations in biomass allocation (Atkin et al., 2006). The agronomic response to environmental stress is based on physiological and biochemical response of a plant. Heat sensitive plants show a great deal of lability of the cellular membranes, thereby disrupting the vital cellular phenomena (Rasheed et al., 2011). The severity of reactive oxygen species (ROS)-induced damage depends on the anti-oxidant status of the plant (Mohammed and Tarpley, 2009b). Under environmental stress the production of active oxygen species (AOS) can increase and the protective activity of anti-oxidants (which normally control these AOS) is affected (Shalata and Neuman, 2001). Thus along with several other factors, oxidative stress damage caused by high night temperature disrupts rice growth and development of rice plant (Mohammed and Tarpley, 2011). One approach for inducing oxidative stress tolerance would be to increase the cellular level of enzyme substrates such as ascorbic acid (vitamin C, AsA). AsA is a small, water-soluble anti-oxidant molecule which acts as a primary substrate in the cyclic pathway of enzymatic detoxification of hydrogen peroxide (Beltagi, 2008). In rice leaves, it is found in milli-molar concentration which plays an important role as a component of anti-oxidant system (Noctor and Foyer, 1998). AsA is also vital for regulation of photosynthesis, cell expansion, root elongation and is involved in some enzymatic reactions (Hager and Holocher, 1994). It has been reported that AsA protects the rice plant against drought and chilling (Guo et al., 2005), and mungbean against heat stresses (Kumar et al., 2011) by enhancing its anti-oxidative capacity. Further research found that AsA eliminates ROS through coordination with the production of reduced glutathone in ascorbate glutathione cycle (Noctor et al., 2002). Due to its vital role in anti-oxidant status and metabolic functions, AsA is important for plants. The increased production of AsA in plants will enhance their nutritional value and ability to tolerate stresses (Wheeler et al., 1998). Increased resistance in response to exogenous application of various organic compounds has been achieved in some plants; an approach which may significantly contribute to crop production in stressed environments (Ashraf and Foolad, 2007). Though the possible role of AsA has been studied for some other stresses, but limited information is available about AsA in relation to high night temperature stresses. It is hypothesized here that exogenous AsA application may alleviate the oxidative damage caused by HNT stress by inducing tolerance through its positive influence on the activities of anti-oxidant enzymes. Moreover, the present study was aimed to explore some physiological implications of exogenous AsA on the attributes which seem to be negatively affected by HNT stress in two rice genotypes.

Results and discussion

The study investigated acclamatory role of exogenous AsA in two rice cultivars under HNT stress. Abiotic stress variation has earlier been reported for the same varieties i.e. Kasalath and Nipponbare (Frei et al., 2008). Existence of such variation in response to HNT stress will serve as a source for genetic exploitation in breeding programs and may help in coping HNT stresses. Foliar application of AsA showed positive effects in mitigating HNT stress. The possible mechanisms of positive effects of AsA in mitigating HNT stress are discussed below.

Lipid peroxidation and anti-oxidant enzymes

Increase production of MDA during peroxidation of membrane lipids is often used as an indicator of oxidative damage. Data regarding MDA content displayed higher levels of MDA in Nipponbare than Kasalath under all treatments (Fig.1a). Understandably, HNT increased the MDA content compared to CNT for both the genotypes and at both stages. In terms of chemical treatment, AsA reduced MDA content of the plants grown under HNT. The increased MDA level in the leaves under HNT stress shows that the production of ROS was greater than CNT. Application of AsA mitigated the increase in MDA level brought by HNT stress. While comparing the MDA level at two stages our findings imply that the effect of both HNT and AsA was more pronounced at the later stage; indicating an increase in the level of lipid peroxidation when the plant continues to grow under stress condition. Nipponbare was more responsive to AsA application than Kasalath (which was slightly affected by AsA and temperature treatments). These findings confirm the effective role of AsA as ROS scavenger, thus leading to increased thermal stability by sustaining the integrity of membranes under HNT stress. Similar role of some other anti-oxidants has already been reported by Foyer and Noctor (2005). For scavenging ROS two types of anti-oxidant systems i.e. enzymatic and non enzymatic are employed in plants (Hu et al., 2009). POD and CAT are two of the key enzymes involved along with SOD and others. The POD activity of both genotypes showed almost the same response to both HNT and AsA treatments at the same level. Plants grown under CNT treatment showed the lowest activity of POD which was markedly increased under HNT. The increased POD activity was reduced by the application of AsA under HNT. POD activity also showed positive correlation with stomatal conductance which was more pronounced in Kasalath than Nipponbare (Fig.4b). Likewise increase in POD activity in two creeping bentgrass cultivars was observed by Liu and Huang (2000) under high temperature stress. Previously, Khan et al. (2006) also reported similar variations in response of two different wheat varieties to AsA under salinity stress. In current findings, the CAT activity of both genotypes was higher under HNT than CNT, and AsA did not show any visible effect in Kasalath and mildly reduced its activity in Nipponbare (Fig.3a). This increase in CAT activity under HNT is consistent with the findings of Demiral and Turkan (2004), who found an increase in CAT activity under sodium stress in a tolerant cultivar Pokkali. In contrast, Hu et al. (2009) documented a reduction in catalase activity under cadmium stress in rice roots and shoots. The increase in enzyme activities shows that heat stress increases production of ROS, which in turn resulted in the up-regulation of anti-oxidant enzymes activities. It seems likely that foliar applied exogenous AsA may have hampered ROS-based damages in rice seedlings and led to lower activities of CAT enzyme.

Leaf stomatal conductance (gs)

At the same leaf nitrogen content the stomatal conductance gs rate is responsible for varietal variation in photosynthetic rates (Hirasawa et al., 2010). Data regarding leaf stomatal conductance showed that the overall stomatal conductance of Kasalath was higher than that of Nipponbare measured at two growth stages (Fig.3b). The initial stage conductance of both cultivars was higher than the later. While comparing the effect of HNT and AsA with CNT, the data showed that HNT increased the conductance of both genotypes and at both stages compared to CNT. Exogenous AsA application reduced the conductance under HNT. An increase in stomatal conductance has been observed by Lu et al. (1998) in cotton and wheat under supra-optimal temperatures. They argued that higher stomatal conductance under supra-optimal temperature lowers leaf and canopy temperature and thus acts as an avoidance type of resistance in irrigated crops.

Sodium and potassium ions content

Heat stress is reported to increase the concentration of K^+ in shoots and Na^+ in both shoots and roots of wheat (Dias et al., 2009), and Na^+ in shoot of sunflower by Quintero et al. (2007). Ashraf and Hafeez (2004) found a significant increase for K^+ in pearl millet and maize. For Na^+ they observed no effect and a decrease in uptake in pearl millet and maize, respectively, when grown under high temperature. In view of these previous studies, current findings demonstrate that heat stress can increase the uptake of Na^+ and K^+ in most parts of the rice plants. Leaves of Nipponbare showed higher concentration of Na^+ than Kasalath at all treatment combinations, while the total (root + shoot) amount was higher in Kasalath. In stem tissues no significant variations for Na^+ were observed among the

Table 1. Results of a three way analysis of variance (ANOVA) of variety, temperature, chemical and their interaction for Potassium and Sodium contents

Dependant Variable	Independent Variable			
	Var (V)	Temp (T)	AsA (A)	V x T x A
Leaf K$^+$ content	*	*	*	*
Stem K$^+$ content	*	*	*	**
Root K$^+$ content	*	ns	ns	**
Leaf Na$^+$ content	*	ns	ns	ns
Stem Na$^+$ content	ns	*	ns	ns
Root Na$^+$ content	ns	ns	ns	ns

* Significant at P<0.05, ** Significant at P< 0.01 and ns = non significant.

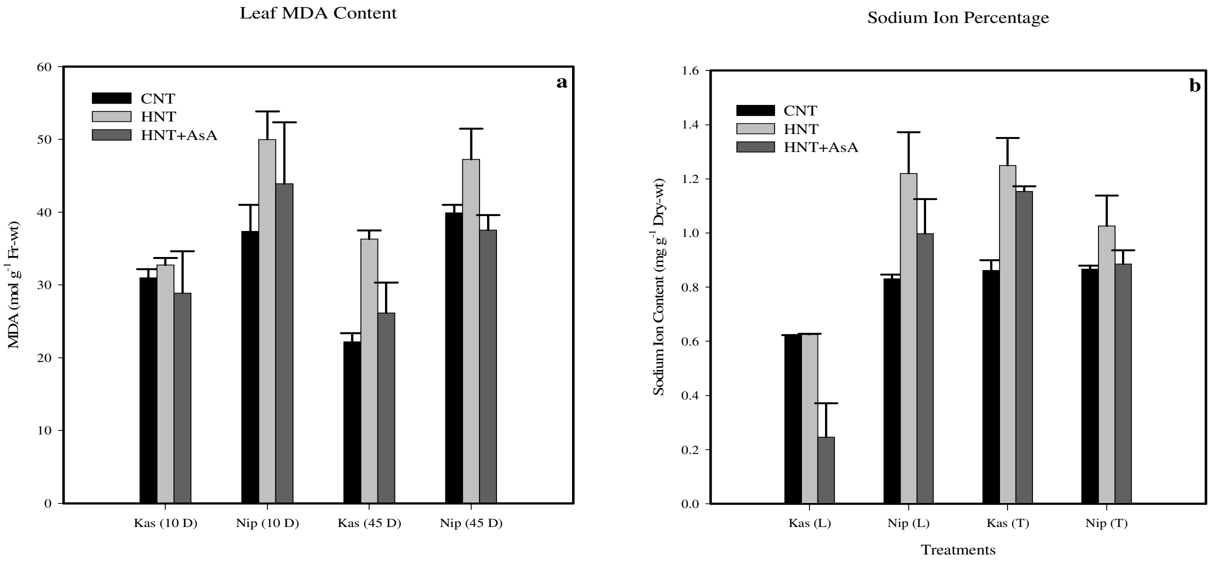

Fig 1. MDA content measured at two intervals (a) and Na$^+$ content of leaf and total (root + shoot) of the two varieties (b). Kas and Nip stand for Kasalath and Nipponbare, respectively. While 10 D and 45 D represent the two intervals at which MDA was measured CNT, HNT and HNT + AsA represent plants grown under control, high night temperature without AsA and high temperature plus AsA, respectively. Vertical bars indicate standard errors of means. Vertical bars indicate standard errors of means.

various treatment combinations (data not shown). Thus, an overall increase in total uptake (shoot + root) of Na$^+$ was observed under HNT for both genotypes. Foliar application of AsA reduced the concentration of Na$^+$ under HNT. Analysis of variance (Table 1) showed significant effect of variety, temperature and AsA on leaf and stem Na$^+$ content while in case of root its content was only significant for variety. Both varieties mostly showed an increase in K$^+$ content of leaves, stems and roots under HNT stress (except stem of Nipponbare). But when this HNT stress treatment was accompanied with the application of exogenous AsA, reduction in K$^+$ content was observed. The effect of HNT on K$^+$ content was dependant on the type of plant tissues in which they were measured. The responsiveness of both genotypes to AsA in terms of K$^+$ content also varied with the plant part in which K$^+$ content was measured. Previously, in a tolerant maize cultivar a similar increase in K$^+$ with high temperature stress has been found (Mahmood et al., 2010), while, an increase of K$^+$ content in shoot and root of sweet pepper has been reported in response to AsA under normal and saline conditions. Results demonstrated that except the leaf, concentration of K$^+$ in most of the plant parts was higher in Kasalath than Nipponbare. The total (root + shoot), leaf and root K$^+$ concentrations were higher under HNT than CNT for both genotypes. The same trend was found only in stem of Kasalath plants treated with HNT without AsA application. AsA decreased the concentration of Na$^+$ and K$^+$ for majority of the treatments except K$^+$ content in stem of Nipponbare. In terms of partitioning of Na$^+$ and K$^+$ to different plant tissues, clear distinction among the treatments was observed in the ability to transport these ions to the leaf, stem and root.

Materials and methods

Plant growth

Two rice (*Oryza sativa* L.) model varieties viz. Kasalath (*indica*) and Nipponbare (*japonica*) with different plant architecture and response to temperature were used in the experiment conducted at Huazhong Agricultural University Wuhan China, during 2010. Nipponbare is a short stature variety which is sensitive to high temperature (She et al., 2010), while Kasalath is a long stature variety with no clear information about its response to high temperature stress. Moreover, based on difference in varietal class Nipponbare is grouped with “advanced or modern type” varieties (Jahn et al., 2011), while Kasalath is placed in “landrace or traditional type” (Frei et al., 2008; Madoka et al., 2008). Germinated seeds of

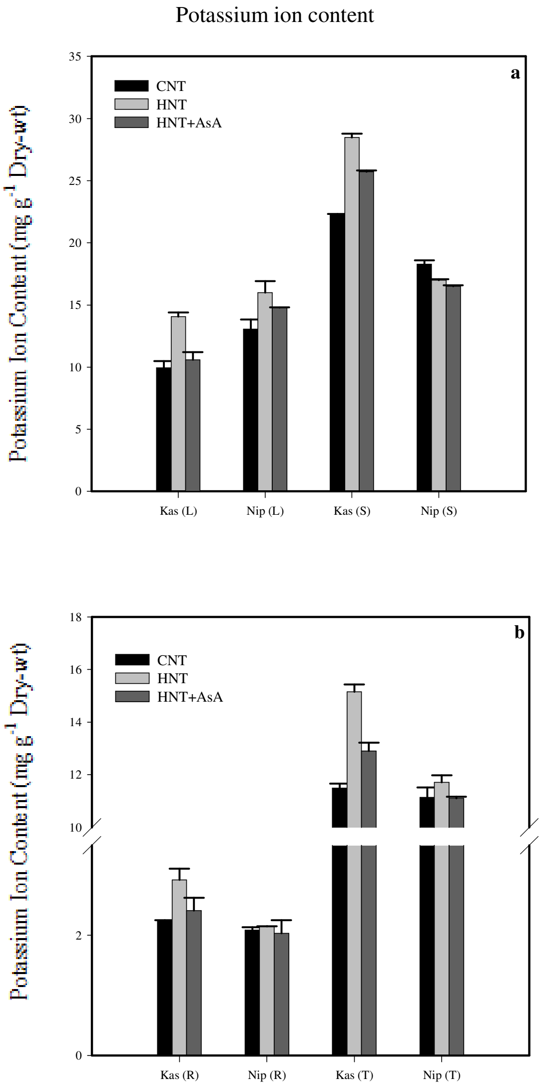

Fig 2. Potassium ion (K^+) content measured in leaf and stem (a), and root and total (root + shoot) (b) Kas and Nip stand for Kasalath and Nipponbare, respectively, while L, S, R and T represent leaf, stem, root and total, respectively. CNT, HNT and HNT + AsA represent plants grown under control, high night temperature without AsA and high night temperature plus AsA, respectively. Vertical bars indicate standard errors of means.

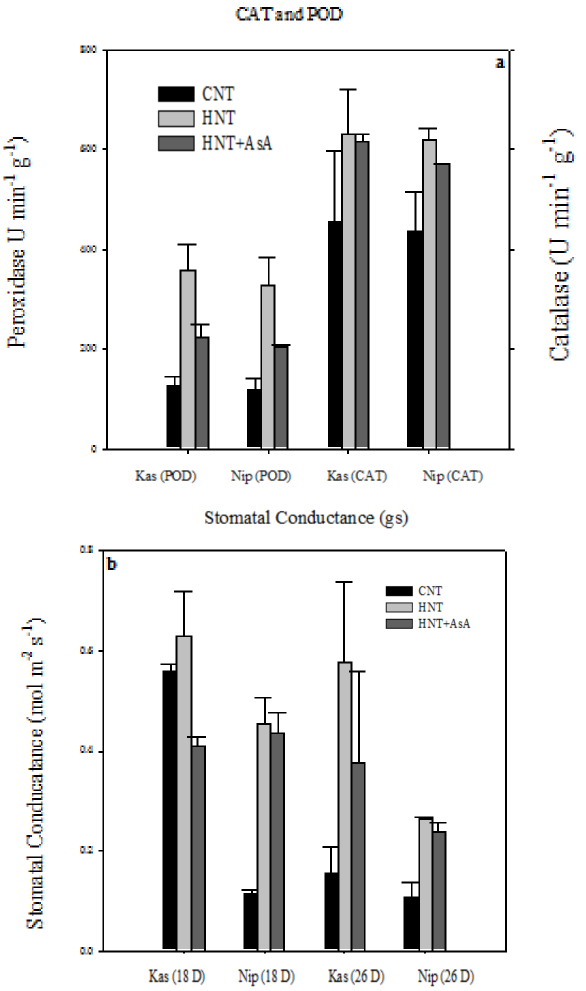

Fig 3. Effect of HNT stress and AsA on the POD and CAT content measured in leaves (a) and Stomatal conductance (gs) measured at two intervals i.e. 18 and 26 days after initiation of heat and AsA treatment (b). Kas and Nip stand for the two varieties Kasalath and Nipponbare, respectively. CNT, HNT and HNT + AsA represent plants grown under control, high night temperature without AsA and high night temperature plus AsA, respectively. Vertical bars indicate the standard errors of means.

both varieties were planted in different seedling trays (2 seeds per cell). Three weeks after sowing, two seedlings were transplanted to plastic pot (13 cm lower inside diameter, 17.3 cm upper inner diameter, 14.3 cm height and 0.3 cm thickness) filled with 2.4 kg of air-dried soil and sand in 2:1 ratio. A compound fertilizer (NPK) was added at the rate of 2.8 gm per pot. Standard practices suiting pot experiments were followed and no pest or disease problem was found during the experimental period.

Ascorbic acid plus temperature treatments

Ascorbic acid (Sigma-Aldrich, Shanghai, China) was first dissolved in de-ionised water to prepare 5 mM solution. Half (eight) of the pots were sprayed with AsA solution, while the remaining half were sprayed with equal volume of water. Plants were transferred to growth chambers for temperature treatments on the same day after spray. For temperature treatments two indoor controlled environment growth chambers (Climatrons, Southeast Ningbo Instruments Ltd, Zhejiang, China) already set at two different night temperatures i.e. HNT (high night temperature of 30°C) and CNT (control/ optimum night temperature 23°C) were provided. Night temperatures started at 7 PM and lasted for 11 hours daily. Daytime temperature was kept same for both treatments (33°C) for 13 hours starting from 6 AM. Humidity was kept constant at 75% throughout the experimental period.

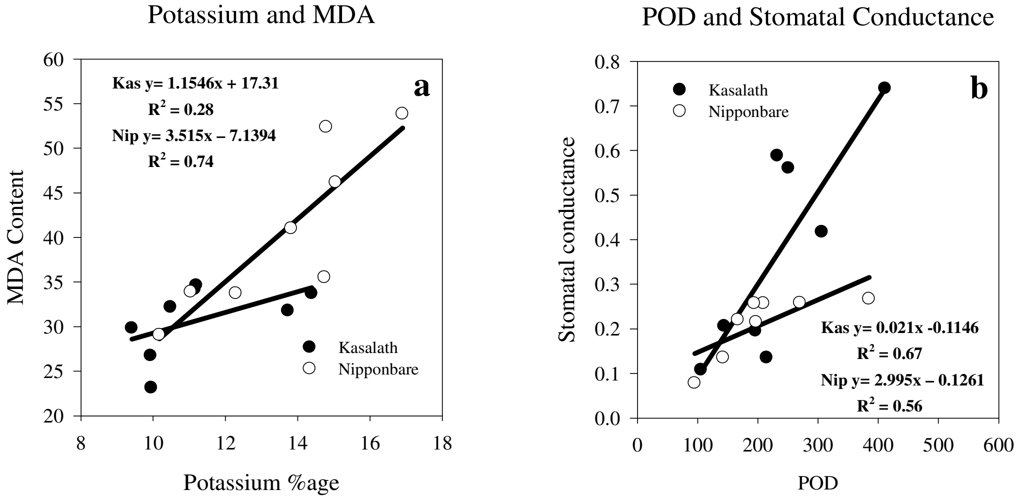

Fig 4. Relationship between MDA and Potassium content of leaf (a), and POD activity and stomatal conductance (b) of the two varieties. R^2 values indicate the coefficient of determination.

The photosynthetic photon flux density inside the growth chamber was maintained at 1000 µmol m^{-2} s^{-1}, while CO_2 inside the chamber was not measured. To minimize the variation caused by chambers, plant pots locations were regularly reshuffled between the growth chambers at 15 days interval after resetting temperature treatments; to provide uniform environmental conditions in growth chambers.

Lipid per oxidation and anti-oxidant enzymes

MDA content was measured to determine the level of lipid per oxidation in leaves. It was measured at two stages i.e. 10 DACHT (days after initiation of chemical and heat treatments) and at harvest (45 DACHT). MDA was determined by using the thiobarbituric acid reaction (Bai et al., 2009). MDA content was calculated from the absorbance at 532 nm and was corrected by subtracting the absorbance at 600 nm for non-specific turbidity. For enzymes extractions frozen leaves (0.5 g) were homogenized in 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM EDTA, 3 mM 2-mercaptoethanol, and 2% (w/v) polyvinylpolypyrrolidone in mortar and pestle. The homogenate was then centrifuged at 16,000 g for 30 min at 4°C and the supernatant was further used for assaying enzymes. POD and CAT activities were also assayed following the method described by Bai et al. (2009). The 3 ml reaction solution for POD contained 50 mM phosphate buffer (pH 7.8), 25 mM guaiacol, 200 mM H_2O_2, and 0.5 ml enzyme extract. One unit of POD activity was determined as 0.01 units/min change in absorbance. The CAT reaction solution (3 ml) contained 50 mM phosphate buffer (pH 7.0), 200 mM H_2O_2, and 50 µL enzyme extract. The reaction was initiated by adding the enzyme extract. Changes in absorbance of the reaction solution at 240 nm were read after every 30 s. Like POD, one unit of CAT activity was also defined as an absorbance change of 0.01 units/min.

Sodium and potassium ions determination and leaf stomatal conductance

The concentrations of sodium (Na^+) and potassium (K^+) in different tissues (leaves, stem and roots) were determined by flame photometer according to the method of Yoshida et al. (1976) with slight modifications. Leaf stomatal conductance (gs) was measured at the vegetative stage (18 and 26 DACHT) using a LI- 6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) between 1000 h and 1200 h according to Hirotsu et al. (2004). The photosynthetic photons flux density (PPFD) was set at 1500 µ mol quanta m $^{-2}$ s $^{-1}$. The temperature and CO_2 concentration in the leaf cuvette were set to 25°C and 360 ppm, respectively. A steady flow rate of 500 µ mol s $^{-1}$ was maintained in the leaf chamber. Stomatal conductance of three individual leaves (penultimate position) per plant was measured for each temperature × chemical treatment combination.

Statistical analysis

All data were analyzed using statistical analysis system (SAS) to test the significance of various treatments. Data were subjected to analysis of variance (ANOVA); the means were separated using least significant difference (LSD) at an alpha level of 0.05. The standard error of the mean was also calculated and presented in the graphs as error bar.

Conclusion

It is concluded from the current findings that HNT stress altered the physiological attributes of both genotypes and application of exogenous AsA partially alleviated the changes associated with high temperature stress. Further efforts to study a diverse group of rice genotypes and AsA concentrations at both lower and greater levels than the one applied in this study will help to determine existing variation in rice germplasm and the optimum level of AsA, which may be probably even more effective in ameliorating the effects of HNT stress.

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