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Short Research Article

Osmolyte Accumulation, Membrane Stability and ABA Profiles in Rice Genotypes Exposed to Heat and Drought Stress

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Abstract

The effect of combined heat and drought stress on proline, chlorophyll stability index, membrane thermal stability and abscisic acid (ABA) contents were investigated to screen and study the physiological basis of heat and drought tolerance in three rice genotypes (ADT 43, TKM 9 and N 22). The stresses were imposed at panicle initiation (PI) and anthesis stages of crop growth. The genotype N 22 was found to be the tolerant followed by TKM 9 while ADT 43 was observed to the susceptible genotype based on the above physiological and biochemical traits. Irrespective of genotypes, anthesis stage stress brought about accumulation of osmolyte and ABA contents with membrane stability changes compared to stress at PI stage. ADT 43 recorded low proline content of (201 μg g^{-1}), CSI of (52.5%), MTS of (24.39%) and ABA content of (0.587 μg g^{-1}).While, N 22 recorded higher proline content of (252.6 μg g^{-1}), CSI of (89.8%), MTS of (33.08%) and ABA content of (0.685 μg g^{-1}). Higher proline and ABA contents with improved membrane stability was clearly demonstrated in the tolerant genotype (N 22) compared to susceptible genotype (ADT 43). This conforms that these biochemical traits can be used to screen rice genotypes to combined stresses and understand the mechanism underlying stress tolerance.

1. Introduction

Rice is a major staple cereal grown in irrigated cropping systems of South and Southeast Asia, with maximum day temperatures either close to or higher than the critical threshold ranging between 33-35°C (Nakagawa et al., 2002). Recent global climate models predict an increase in mean temperature by 2-4.5°C and the rice area affected by water stress to double by the end of this century (IPCC, 2007). It is estimated that more than 50% of the world rice production area is affected by drought and high temperature (Bouman et al., 2005). Higher temperature of 45-48°C at flowering and grain formation stages is the most alarming factor that determines the crop growth and ultimate yields. Water limited condition (also referred to as drought), affecting 23 m ha of rice regularly (Pandey et al., 2007) is a condition related to insufficient soil moisture available to support average crop production. Rice is sensitive to drought stress particularly during flowering stage, resulting in severe yield losses (Liu et al., 2006).

Proline is one of the protective molecules that can unite oxygen and free radicals caused by stress. Proline acts as a free radical scavenger and may be more important in overcoming stress than in acting as a simple osmolyte (Reddy et al., 2004).The abscisic acid (ABA) hormone is synthesized in the plastids, and is linked to the stomatal mechanism (Kirda et al., 2007) and quickly responds to water deficiency (Liu et al., 2005). The ABA can be produced in the roots and/or shoots, but this hormone is usually synthesized under water deficiency in the roots and translocate to leaves in order to improve stomatal control. In field conditions where water shortage and high-temperature stresses frequently occur simultaneously, ABA production may also be an important component of thermo tolerance (Gong, Li&Chen 1998). The effect of drought stress was also investigated on ABA (abscisic acid) and proline in different *Zea mays* species and close correlation was found between proline accumulation and ABA with drought stress (Heidari and Moaveni, 2009). Proline accumulation is maximum during the flowering stage and minimum at vegetative stage.

Chlorophyll Stability Index (CSI) is a measure of integrity of membrane or heat stability of the pigments under stress conditions (Kaloyereas, 1958). The CSI is the single parameter used to measure frost or drought resistance of a plant. Both drought and temperature stress decreased membrane stability,

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chlorophyll content and Chlorophyll stability index in all the wheat genotypes (Sairam et al., 1996). The high chlorophyll stability indices help the plants to with stand stress through better availability of chlorophyll. This leads to increased photosynthetic rate and more dry matter production (Madhan Mohan et al., 2000).

The impacts of environmental stresses on crop physiology and yield particularly drought and heat on crop physiology and yield have been studied independently. However, under field conditions, both of these stresses often occur in combination. Hence, overcoming the effects of high temperature and water stress on rice production is essential for food security in the future. Hence, the present study was aimed to evaluate the impact of the heat and drought stress in combination on osmolyte accumulation, membrane stability and ABA contents to draw inferences on the major physiological processes and biochemical constituents enhancing stress tolerance in rice.

2. Materials and Methods

2.1. *Stress treatments and measurements*

Three rice (*Oryza sativa* L.) genotypes namely ADT 43, TKM 9 and Nagina 22 (N 22) differing in their tolerance behaviour to heat and drought stress but had similar phenology were taken for the study during the period January to May 2013. Nursery was raised at Paddy Breeding Station of Tamil Nadu Agricultural University, Coimbatore. Twenty one days old seedlings at one seedling hill^{-1} were transplanted with a spacing of 20×10 cm in the Rain Out Shelter (ROS) facility of the Department of Crop Physiology. Stress treatments were imposed in the ROS, while a similar area of control was maintained adjacent to the ROS facility. The dimensions of the Rain Out Shelter and the Control were 21 m long and 6 m wide. Prior to transplanting, the land inside ROS and the area which is parallel outside the ROS (Control) were puddled, levelled and incorporated with recommended dosage of basal fertilizer 150:50:50 N, P_2O_5, K_20 kg ha^{-1}. The land was divided into 12 plots with 2 m^2 plot^{-1}. The experiment consist three treatments and four replications. The treatment details are as follows T_1:(Control) well watered throughout the life cycle, T_2:Drought and Natural High temperature stress at Panicle Initiation (PI) stage in which water was withheld for 2 weeks and the temperature ranged from 36.4 to 37.8^{0}C at the time of panicle initiation, T_3:Drought and Natural high temperature stress at Anthesis stage in which water was withheld for 2 weeks and the temperature ranged from 36.4 to 36.6^{0}C at the time of Anthesis.

The time of sowing in the selected genotypes were staggered such that their PI and Anthesis coinciding the natural high temperature around the early of April for panicle initiation stress and May to June for anthesis stress. Temperature for the entire experiment period was monitored by installing the log stick data logger (Model. LS350-TH Japan) and an automated weather station inside the ROS and control area. Drought stress treatments were administrated and monitored by measuring the soil water potential using the tensiometers installed at 30 cm depth in each plot. Water was completely withheld for 2 weeks during the stress period. Plants were re watered when the tensiometers registered soil water tension of-50 to-70 kpa.

2.2. *Determination of proline content*

The proline content in the leaves were estimated one week after imposing the stress treatment on fully expanded leaf based on the method described by Bates et al. (1973) 100 mg of rice leaves was ground with 5 ml of 3% sulfosalicylic acid, and the mixture was then filtered. To 2 ml of the filtered mixture in a test tube, 2 ml of acidninhydrin and 2 ml of glacial acetic acid were added. The mixture was mixed with a Vortex mixer and boiled at 100°C for 1 h. The mixture was then frozen in ice and combined with 4 ml of toluene, mixed, and then left to stand for 5-10 min. Absorbance of the reddish pink upper phase was recorded at 520 nm against a toluene blank.

2.3. *Measurement of chlorophyll stability index (CSI)*

250 mg of leaf sample was homogenized using 80% acetone. The sample was then centrifuged at 3000 rpm for 10 min. The supernatant was collected and made up to 25 ml. The OD value was measured at 652 nm. The protocol of (Koleyoras, 1958) is followed to estimate CSI and expressed in terms of percent.

2.4. *Measurement of membrane thermal stability (MTS)*

Leaf membrane thermal stability was measured by an electrolytic leakage technique (Agarie et al., 1995) during panicle initiation and anthesis stage. Leaf cell membrane thermal stability (CMTS) was estimated using following equation

$$\text{CMTS}\ (\%) = (1-T_1/\ T_2)/\ (1-\ C_1/\ C_2)100$$

Where, T and C refer to conductivity in control and heat-treated samples and subscripts 1 and 2 refer to conductance before and after autoclaving, respectively.

2.5. *Quantification of hormone-ABA*

Endogenous levels of ABA were determined by the method of (Krochko et al.,1998). Leaf samples were extracted using 80% chilled methanol following series of steps and finally partially purified methanolic extracts were filtered through 0.52 µm Millipore filters and injected into 20 µl injector loop fitted over the Cyber lab RP protected by guard column. A volume of 20 µl of sample was injected into HPLC. The elution was carried out by a binary gradient of 60% HPLC grade acetonitrile for 10 minute at the flow rate of 1 ml min^{-1}. The column elutes were passed through an UV detector set at 254 nm and the ABA were estimated measuring the peak area and comparing with standard curve of hormones. The peak areas were measured and ABA concentration quantified using the standard curve obtained from ABA.

2.6. Statistical analysis

Factorial Randomized Block Design (FRBD) analysis was carried out on various parameters as per the procedure suggested by (Gomez and Gomez, 1984). Wherever the treatment differences are found significant, critical differences were worked out at 5% probability level and the values are furnished.

3. Results and Discussion

The proline contents in all the cultivars under control and stress conditions were significantly different. The proline content of the leaf showed an increasing trend as the growth stage advanced from PI to anthesis both under control and combined stresses.Among the genotypes N 22 accumulated greater proline content at PI (150.63 µg g^{-1}) (Table 1) and at anthesis (252.3 µg g^{-1}) with 26% increase over control under combined stresses. The low proline content was registered by ADT 43 at PI (103.22 µg g^{-1}) and at anthesis (201.06 µg g^{-1}) respectively. Osmotic adjustment (OA) is a key mechanism by which plants adapt to water shortages resulting from an increased solute concentration of cells in order to maintain the water potential gradients needed to ensure continued uptake of water during the stress period. In addition, osmotic adjustment allows cell to maintain the turgor, which isessential for plant growth and various other physiological processes (Nahar et al., 2011). Proline might regulate the osmotic balance of the cell thus relieving the negative effect of stress (Reddy et al., 2004). (Abdellah et al., 2011) reported that, threefold increase in proline content was observed in tolerant wheat cultivar by water stress over control. In wheat, it has been found that prolineconcentration was higher in stress-tolerant cultivars than in sensitive cultivars (Nayyar and Walia, 2003). It corroborates with present findings.

The chlorophyll stability index is an indicative of the maintenance of physiological processes under stress situation and an index to measure stress resistance. Stress imposed at PI and anthesis stages invariably showed reduction in CSI. Among the genotypes N 22 maintained a good CSI and membrane thermo stability value of (87.9%, 34.73% and 89.8%, 33.08%) at PI and anthesis stages respectively. TKM 9 was found to be moderately tolerant to combined stresses. The CSI and MTS values were (82.9% and 30.94%) at PI and (85.03% and 29.04%) at anthesisrespectively **(**Figure 1). The susceptible genotype ADT 43, showed the lowest CSI and MTS values of (55.36%, 25.89%) and (52.50%, 24.39%) at PI and anthesis stages respectively (Table 1). The high CSI help the plant to with stand stress through better availability of chlorophyll contents. This leads to increased photosynthetic rate and more dry matter production (Madan Mohan et al., 2000). In line with the findings of our present study, (Kilen and Andrew, 1969) observed, a high correlation between CSI and drought tolerance in corn. Membrane stability index in susceptible wheat varieties decreased significantly under water stress (Gupta et al., 2000). While, (Beena et al., 2012) reported that, high membrane stability index and chlorophyll stability index were recorded in tolerant inbred lines of rice than in susceptible lines under water stress condition.

ABA is a "stress hormone." It's most conspicuous function is in controlling water relations by regulating stomatal conductance and CO$_2$ uptake (Kim et al., 2010). The present study showed a significant increment in ABA contents in leaf under combined stresses. The increment of ABA content under drought condition was reported by several workers (Abdellah et al., 2011; Unyayar et al., 2004). Accumulation of ABA under drought condition is a favourable mechanism for drought tolerance through reducing transpiration rate by closing of stomata. The ABA content of the leaf showed an increasing trend as the growth stage advanced from PI to anthesis both under control and combined stresses.Among the genotypes, the elevation in ABA was less in ADT 43 (0.254 µg g^{-1}) with 27.5% increase over control at PI stage and (0.587 µg g^{-1}) 54.85% increase over control at anthesis stage under combined heat and drought stress (Figure 2**)**. N 22 showed higher ABA

Table 1: Proline content (µg g^{-1}) in rice genotypes subjected to heat and drought stress at reproductive stages of plant growth

Varieties	Panicle initiation		Anthesis		Panicle initiation		Anthesis	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
ADT 43	80.9±0.38	103.2±0.43	141.8±1.05	201.0±0.64	66.29±0.18	25.89±0.073	71.04±0.025	24.39±0.14
TKM 9	77.5±0.49	80.3±0.74	199.6±1.36	204.7±0.57	68.72±0.28	30.94±0.091	73.49±0.25	29.04±0.30
N 22	138±0.68	150.6±0.85	186.3±1.11	252.6±1.40	69.55±0.032	34.73±0.38	75.73±0.26	33.08±0.16
Variety	1.42**		2.34**		0.48**		0.43**	
Treatment	1.1 6**		1.95**		0.39**		0.35**	
V*T	2.02**		3.38**		0.68**		0.61**	

p=0.05 Significance and the values are mean of 4 replications.

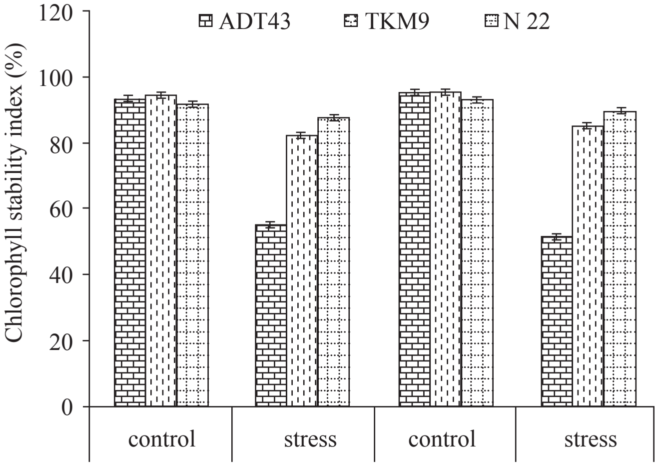

Figure 1: Chlorophyll stability index (%) in rice genotypes subjected to heat and drought stress at reproductive stages

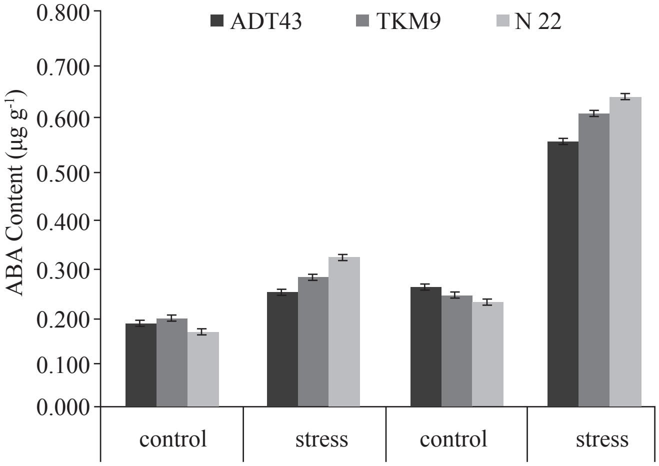

Figure 2: ABA content (µg g^{-1}) in rice genotypes subjected to heat and drought stress at reproductive stages

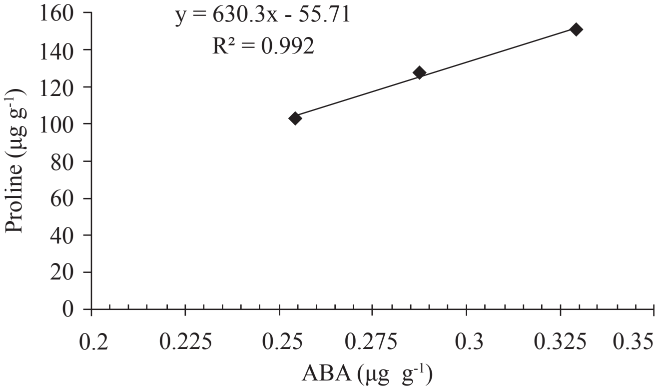

Figure 3: Correlation between ABA (µg g^{-1}) and proline(µg g^{-1}) contents in rice genotypes subjected to heat and drought stress at panicle initiation stage

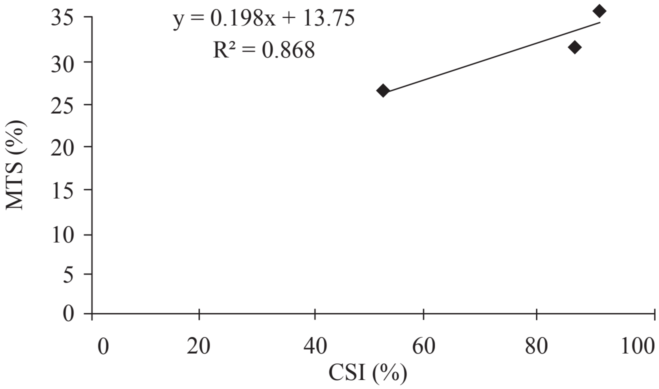

Figure 4: Correlation between CSI (%)and MTS (%) in rice genotypes subjected to heat and drought stress at anthesis stage

Table 2: Yield and yield components as influenced by heat and drought stress in rice

Varieties	Treatments	Length of primary panicle (cm)	Weight of primary panicle (g)	Total no. of panicles plant^{-1}	Total weight of panicle (g plant^{-1})	Total grain Weight (g plant^{-1})	1000 Grain weight (g)	TDMP (g plant^{-1})
ADT 43	Control	25.98 ±0.20	3.50 ±0.08	14.83±1.07	33.91±2.76	31.91±2.76	18 ±0.20	55.6±3.84
	Stress at PI	22.6 ±0.19	2.29 ±0.14	12.23±0.40	19.39±0.18	17.4±0.18	15.1 ±0.19	41.17±2.55
	Stress at anthesis	23.61 ±0.10	2.36 ±0.25	9.05±0.30	13.91±1.10	11.9±1.10	13.7±0.25	29.55±1.49
TKM 9	Control	25.43 ±0.19	4.38 ±0.11	13.13±0.75	30.12±2.59	28.13±2.59	22.9±0.23	55.17±3.95
	Stress at PI	23.27 ±0.24	2.96 ±0.09	12.40±0.72	22.84±0.62	20.85±0.62	19.40±0.10	46.32 ±2.25
	Stress at anthesis	24.79±0.15	2.95±0.21	9.93±0.62	18.87±2.45	16.87±2.45	18.30±0.97	39.57±1.19
N 22	Control	21.32±0.42	2.20±0.10	14.10±2.02	17.15±0.74	15.15±0.62	18.6±0.01	40.76±1.55
	Stress at PI	18.40±0.46	1.36±0.13	13.05±1.10	13.09±0.43	11.09±0.21	17.10±0.02	32.24±1.00
	Stress at anthesis	20.17±0.26	1.46±0.08	10.68±1.74	12.42±1.22	10.42±1.18	16.60±0.01	31.15±1.20
p=CD (0.05)	Variety	0.582**	0.26**	1.72	1.34**	2.78**	1.73**	3.94**
	Treatment	0.542**	0.29**	1.64**	1.69**	2.36**	1.82**	3.21**
	V×T	1.00	0.45	2.99	2.31*	4.82**	3.00	6.84*

content of (0.329 μg g^{-1}) with 50.15% increase over control at PI and (0.685 μg g^{-1}) 66.13% increase over control at anthesis stage followed by TKM 9 with an content of (0.287 μg g^{-1}) with 32.40% increase over control at PI and (0.651 μg g^{-1}) 62.05% increase over control at anthesis stage. Thus, the study clearly demonstrated ABA as a tress hormone by showing greater accumulation in tolerant genotype.

There was a highly significant varietal effect in yield and Total Dry matter production (TDMP). N 22 having significantly lower per cent of yieldand TDMP reduction (yield-31.22%,TDMP-23.57%) at anthesis stresswhereas,TKM 9 showed lower reduction (yield-25.87%,TDMP-16.04%) at PI stress. ADT 43 was most affected and recorded the highest percentage reduction in yield and TDMP atpanicle initiation (yield-45.7%, TDMP-25%) followed by stress at anthesis stage (yield-62%, TDMP-48.85%) relative to the control. Plants exposed to combined stress at anthesisstage were themost affected.The low yield might have been as a result of decreased filled grains per panicle caused by inhibition of sufficient translocation of assimilates to the grains asthe plants competed for moisture. Varieties with greater growth rate under water deficit conditions provide the highest grain yield (Bogale et al., 2011). As observed by (Bogale et al., 2011) favourable conditions during growth may permit an expansion of the last internodes as well as higher yield. Carbohydrates are also remobilized from the peduncle and flag leaf to the grain during grain filling period. Grain yield of rice may be limited by the supply of assimilates to the developing grain (source limitation) or by the capacity of the reproductive organ to accept assimilates (sink capacity).

4. Conclusion

ABA and proline contents under combined stresses can be used as physiological traits to screen rice genotypes. A high positive correlation of these traits($r=0.99$, $p< 0.05$; Figure 3) shows that up regulation of these osmolytes underlie stress tolerance in rice genotypes. CSI had a strong positive correlation ($r=0.86$, $p< 0.05$; Figure 4) with MTS (%). Tolerant genotypes with intact membranes under stress were able to maintain the chlorophyll contents and survive well under stress situations.

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