Research Paper

Volume : 4 | Issue : 6 | June 2015 • ISSN No 2277 - 8179

Physiology of Cell Membranes, Stomata And Photosynthetic Pigments of Rice (*Oryza Sativa* L.) Under High Temperature.

Botany

KEYWORDS : MTS, stomata, pigments

Mr. Hilali Saleh — Department of Botany, Punjab Agricultural University, Ludhiana.

Dr. S. K. Thind — Department of Botany, Punjab Agricultural University, 141004, Ludhiana, India.

ABSTRACT

Twenty genotypes of rice were evaluated for their physiological tolerance of cellular membrane, stomata and photosynthetic pigments under high temperature stress. The selected genotypes were grown at two sowing dates viz; normal and stress environment. High temperature negatively affected these parameters and genotypic variation was observed. Photosynthetic pigments were significantly reduced under stress, while the number of stomata increased. Membrane thermal stability (MTS) decreased with increasing temperature exposure; hence 30oC recorded highest while 55oC recorded lowest MTS. Genotype Ganjay had highest chlorophyll a with lowest number of stomata in lower epidermis under stress. The highest MTS values were recorded for genotypes PR121, IR6 and PR122. These four genotypes can be recommended for growing under high temperature stress.

INTRODUCTION

Rice (*O. sativa* L.) is among the world's most important and second most produced crop worldwide (FAO 2015). It is the only major cereal crop which is consumed almost exclusively by humans (Khush and Virk 2000). The major challenge facing rice production ever in history is the threat imposed by rising of air temperature (global warming). Though, rice is the most diverse cereal crop in the world (FAO 2004), it is poorly adapted to high temperature, and flowering (anthesis) is the most heat sensitive stage (Farrell *et al* 2006).

Exposure of rice crop to heat stress at least during a part of its growth stages may drastically reduce the yield. It was reported that high temperature negatively affects physiological processes in plants; it may increase stomatal conductances (Yang and Heilman 1991) which leads to dehydration. High temperature also reduces photosynthetic pigments (e.g. chlorophyll content) which were mostly observed in thermo-sensitive genotypes (Zhou et *al* 2012). Furthermore, high temperature leads to cellular membranes injury which negatively affects crop productivity (Krishnan *et al* 2011). Ceccarelli *et al* 2010) reported that the yields of rice are estimated to be reduced by 41% due to global warming by the end of the 21st Century. Thus, the research should be conducted to improve physiological and yield performance of rice under high temperature. The objective of the present study was to find out the effect of high temperature on the physiology of stomata, cellular membranes and photosynthetic pigments of some selected rice genotypes.

MATERIALS AND METHODS

Twenty genotypes viz; Balilla, Cuiabana, Ganja Rangwala, Ganjay, IR6, IR 61250-3B-7-1-2, IR 65192-4B-17-3, IR 2006-P-12-12-2-2, IR 2307-247-2-2-3, IR28, IR50, IR72, Sadri, Sahel 108, Tak Ratia, Punjab rice (PR121, 122 and 123), Pusa Basmati (PB1121) and Pusa Punjab Basmati (PPB1509) were selected for the present study. The genotypes were tested under two different sowing dates (growing environments); early sowing on 06th March (stress) and timely sowing on 21st May 2014 (normal). All genotypes were planted in three replications in Randomized Complete Block Design in plot area of 1.44m^2, with 8 rows per plot covering the row and plant spacing of 20cm and 15cm respectively. Samples of leaves at respective stages were collected for measuring their cell membranes, stomata and phosynthetic pigments physiology.

Membrane Thermal Stability (MTS)

Random sample of two uppermost leaves were sharp cut during flowering stage in both sowing dates. Six leaf disks were cut from these, washed three times with distilled water and then placed in a test tube containing 10 ml of distilled water and finally placed in a water bath at 30°C. Additional five samples were similarly treated and placed at 35, 40, 45, 50 and 55°C for one hour respectively. The samples were cooled in growth chamber overnight at 25-27°C and then the leakage of electrolytes (conductance) was measured using electrical conductivity meter (Systronics Conductivity meter 304) according to Farooq *et al* (2011). Each sample was then boiled (100°C) for one hour, cooled in a growth chamber (25-27°C) overnight and conductance was measured again. MTS as an indication of cell membrane injury was calculated using the following formula: MTS = (1-T1/T2) x 100, where: T1, T2 refers to conductivity readings after heat treatment in water bath and after boiling, respectively.

Estimation of chlorophyll contents

Random samples of uppermost three fresh leaves were cut from the rice plants during vegetative stage. Each 25mg of leaf disks were rinsed with distilled water and then put in test tubes. 10 ml of dimethyl sulphoxide (DMSO) was poured into each test tube containing 25mg of leaf disks. The samples were then incubated at 65°C for five hours to extract chlorophyll contents. The samples were then cooled for 4 hours and finally the concentrations of these pigments were recorded with Systronics Spectrophotometer 106 from the absorbance values at 663, 645 and 480 nm as per Hiscox and Israelstam (1979).

Number of stomata

Random samples of three fully expanded uppermost leaves were taken during flowering and leaf impressions (about 1cm^2) were made at the central portions of adaxial (Upper) and abaxial (Lower) surface using quick fix gel. After 3-5 minutes of drying, the imprint (epidermis) was peeled from the leaf and mounted on a glass slide (Dhillon *et al* 1997). The number of stomata were counted under Microscope (Nikon eclipse 90i) fitted with camera and computer under X 20 Magnification.

The data were statistically analyzed using SAS-V8 software using LSD test at 5% probability level.

RESULTS AND DISCUSSION

Effects of growing environment on plant pigments of rice genotypes

Rice genotypes had significantly ($P< 0.05$) higher amount of chlorophyll a (chl a), chlorophyll b (chl b) and total chlorophyll (tot chl) contents under normal environment than under stress environment (Table 1). The contents of pigment were reduced significantly under high temperature possibly due to increased activity of active oxygen species which accelerated oxidation of membranes. Similar results were reported by Xie *et al* (2011) and Zhou et *al* (2012) in other genotypes. Significantly higher value of chl a was observed in genotypes PR121 and Ganja Rangwala

under normal and Ganjay under stress environment. It was observed that chl b of Ganja Rangwala was less affected under stress environment. However, genotype IR50 showed lowest chl b content under stress environment. Total chl among tested genotypes ranged from 6.9 to 1.1 mg/g fresh weigh and highest value was recorded in genotypes IR72 and Ganja Rangwala under normal and stress environment respectively. This genotypic variation in the reduction of photosynthetic pigments under high temperature was also observed earlier in other genotypes (Zhou *et al* 2012).

Effects of high temperature on MTS

The results revealed that MTS was significantly (P<0.05) higher at 30ºC as compared with other temperature treatments. It decreased considerably with increasing temperature exposure whereas at 55ºC lowest MTS was recorded (Table 2). The highest MTS at 30ºC indicated that this temperature provided good conditions for functioning of cellular membranes (Krishnan *et al* 2011). The lowest MTS at 55ºC revealed that highest damage occurred to cellular membranes at this temperature. Similar result was reported earlier in other genotypes (Mohammed and Tarpley 2009).

In average of both growing environments, the highest MTS at 35ºC, 40ºC and 45ºC was recorded in genotypes PR121, IR6 and PR122 respectively while lowest MTS was recorded in Sadri, PB1121and PB1121respectively.

Effects of growing environments on the numbers of stomata and stomatal ratio in lower and upper epidermis of rice leaves

The number of stomata in lower and upper epidermis increased significantly (P< 0.05) in genotypes subjected to stress environment (Table 3). The numbers of stomata in a particular area is termed as stomatal density and is positively correlated with stomatal conductance (Liu *et al* 2012). Genotype Ganjay recorded the lowest number of stomata in lower epidermis in both growing environments while PR123 recorded highest number of stomata in lower epidermis under stress environment. Furthermore, it was observed that IR2307 and IR65192 had the highest number of stomata in their upper epidermis under stress environment; however, the stomatal ratio was not significantly affected. Significant negative correlation was observed between chl a (r = -0.3) and total chl (r = -0.2) with number of stomata in lower epidermis. Similarly between chl a (r = -0.2) and number of stomata in upper epidermis, indicating that the plants with high number of stomata experienced more injury at high temperature.

Table 1: Mean Chlorophyll contents (mg/g of fresh weight) of flag leaves of rice genotype

Table 1: Mean Chlorophyll contents (mg/g of fresh weight) of flag leaves of rice genotype

	Normal environment			Stress environment		
Genotype	Chl a	Chl b	Tot Chl	Chl a	Chl b	Tot Chl
Balilla	2.657	0.493	3.561	1.956	1.490	3.857
Cuiabana	2.141	0.423	2.898	1.652	1.554	3.582
G Rangwala	3.212	0.549	4.252	1.386	4.926	6.979
Ganjay	1.830	0.321	2.432	2.513	1.574	4.583
IR6	2.002	2.953	5.514	2.005	1.458	3.877
IR61250	2.534	0.547	3.479	2.047	1.565	4.043
IR65192	3.086	0.663	4.235	1.774	0.299	2.345
IR2006	2.764	0.544	3.739	1.502	0.180	1.905
IR2307	2.973	1.925	5.491	0.645	0.437	1.134
IR28	2.522	1.964	5.021	0.921	0.114	1.172
IR50	2.764	0.689	3.898	1.178	0.109	1.458
IR72	1.672	3.433	5.666	1.727	0.951	3.006
Sadri	2.164	1.167	3.739	1.803	1.595	3.799
Sahel 108	2.491	0.844	3.757	1.493	1.397	3.229
Tak Ratia	2.866	0.628	3.947	1.759	1.546	3.695
PR 121	3.400	0.783	4.724	1.121	0.188	1.481
PR 122	3.123	0.806	4.433	1.350	0.137	1.685
PR 123	2.196	0.855	3.434	1.324	0.409	1.716
PB1121	2.572	1.355	4.409	1.126	0.238	1.540
PPB1509	1.993	0.403	2.707	1.248	0.242	1.684
LSD at 5%	0.0172	0.0348	0.0338	0.0338	0.0547	0.0411

Table 2: Mean Percentage values of MTS of the rice leaves of genotypes at flowering stage

Genotype	30°C	35 °C	40 °C	45 °C	50 °C	55 °C
Balilla	61.6	57.2	51.1	39.8	33.2	15.2
Cuiabana	64.8	58.8	53.9	46.6	34.6	16.8
G Rangwala	69.4	59.2	55.7	52.3	51.3	25.7
Ganjay	67.7	64.7	62.2	58.5	38.2	15.9
IR6	71.2	69.5	65.2	59.0	39.4	22.0
IR61250	66.0	64.0	55.1	46.9	24.8	13.5
IR65192	66.0	58.9	54.0	47.1	22.9	09.9
IR2006	73.8	67.0	62.2	53.2	36.2	22.1
IR2307	57.0	52.2	47.0	42.9	33.0	17.7
IR28	67.2	63.6	59.6	52.5	46.2	21.2
IR50	67.8	59.5	55.4	49.3	27.4	17.2
IR72	65.5	60.8	56.6	50.6	35.4	15.4
Sadri	67.5	46.0	58.1	54.4	52.0	15.1
Sahel 108	67.6	62.6	53.8	47.8	18.0	11.0
Tak Ratia	67.1	58.0	54.3	51.9	45.4	19.6
PR 121	78.4	71.1	63.3	54.3	35.2	15.1
PR 122	70.0	65.5	64.8	63.5	40.7	27.0
PR 123	67.3	65.7	50.2	42.0	21.5	17.4
PB1121	55.4	49.8	46.0	38.1	29.7	21.7
PPB1509	59.9	58.1	51.5	46.1	40.9	15.1
LSD at 5%	10.4	10.7	15.2	16.6	17.5	21.4

Volume : 4 | Issue : 6 | June 2015 • ISSN No 2277 - 8179

Table 3: Average number of stomata and stomatal ratio in lower and upper epidermis of rice leaves.

Genotypes	Normal environment			Stress environment		
	Upper epidermis	Lower epidermis	Ratio of lower/ upper	Upper epidermis	Lower epidermis	Ratio of lower /upper
Balilla	16	31	2	22	36	4
Cuiabana	13	27	2	19	32	2
G Rangwala	17	46	3	13	39	3
Ganjay	11	17	2	12	15	2
IR6	18	26	2	19	37	2
IR61250	16	26	2	18	28	2
IR65192	19	20	1	31	35	1
IR2006	14	40	3	16	56	4
IR2307	18	48	3	45	71	2
IR28	16	28	2	17	45	3
IR50	10	31	5	11	21	2
IR72	23	52	2	21	25	1
Sadri	30	45	2	19	51	3
Sahel 108	23	31	1	19	29	2
Tak Ratia	16	25	2	14	24	2
PR 121	13	35	3	18	28	2
PR 122	16	23	2	17	26	2
PR 123	17	30	2	19	77	4
PB1121	19	27	1	13	45	5
PPB1509	10	53	5	21	66	3
LSD at 5%	6.5905	18.925	1.6117	11.569	22.107	2.6694

CONCLUSION

Thus, it can be concluded that PR121and Ganjay recorded highest photosynthetic pigments among the tested genotypes. The number of stomata was significantly increased under stress with genotypes PR123, IR2307 and IR65192 recording highest numbers. A significant decrease in MTS with increasing temperature exposure was recorded with highest MTS observed at 30°C and the lowest at 55°C for all genotypes. The highest MTS was recorded in PR121, IR6 and PR122 which were able to withstand high temperature stress.

REFERENCE

[1] Ceccarelli, S., Grando. S., Maatougui, M., Michael, M., Slash, M., Haghparast, R., Rahmanian, M., Taheri, A., Al-yassin, A., Benbelkacem, A., Labdi, M., Mimoun, H., and Nachit, M. (2010), Plant breeding and climate changes. J Agric Sci Cambridge 148: 627–637. | [2] Dhillon, M., Kaur, R., and Basra, K. (1997) A rapid method for obtaining leaf impression for studying leaf epidermal system. J Pl sci res 13:7-10. | | [3] FAO (2004) Rice conference: http://www.fao.org/rice2004/en/pdf/khush.pdf | [4] FAO (2015) World Food Situation: http://www.fao.org/worldfoodsituation/csdb/en/ | [5] Farrell, T., Fox, M., Williams, L., & Fukai, S. (2006), Genotypic variation for cold tolerance during reproductive development in rice: screening with cold air and cold water. Field Crop Res 98: 178–194. | [6] Farooq, J., Khaliq, I., Ali, M., Kashif, M., Ali, Q., Rehman, A., Naveed, M., Nazeer, W., and Farooq, A. (2011) Inheritance pattern of yield attributes in spring wheat at grain filling stage under different temperature regimes. J Expt Bot 10: 1093-216 | [7] Hiscox, J., and Israelstam, F. (1979) A method for the extraction of chlorophyll from leaf tissue without Maceration. J Bot 57: 1332-34. | [8] Khush, S. G., and Virk, S. P. (2000), Rice breeding achievements and future strategies. Crop Improv 27(2): 115-144 | [9] Krishnan, P., Ramakrishna, B., Raja, K., and Reddy, V. (2011), High Temperature Effects on Rice Growth, Yield, and Grain Quality. In: Sparks D (ed). Adv in Agron, Vol. 111, pp 87-206, Burlington Academic Press. | [10] Liu, J., Zhang, F., Zhou, J., Chen, F., Wang, B., and Xie, X. (2012), Phytochrome B control of total leaf area and stomatal density affects drought tolerance in rice. Pl Mol Bio 78: 289–300 | [11] Mohammed, A., and Tarpley, L. (2009), Impact of high nighttime temperature on respiration, membrane stability, antioxidant capacity, and yield of rice plants. Crop Sci 49: 313–322. | [12] Xie, X., Shen, H., Li, X., Zhao, Y., Li, B., and Xu, F. (2011), Effect of photosynthetic characteristic and dry matter accumulation of rice under high temperature at heading stage. Afric J of Agric Res 6(7): 1931-40. | [13] Yang, C., and Heilman, L. (1991), Short-term High Temperature Effect on Stomatal Behaviors of Rice Plants II Occurring at the Grain-filling stage. J Agric Res China 40(2): 243-47 | [14] Zhou, H., Xue, D., & Zhang, G. (2012), Identification and physiological characterization of thermo-tolerant rice genotypes. J Zhej Univ 38: 1–9.