Andhra Pradesh J Agril. Sci : 3(1): 69-74, 2017

INFLUENCE OF HEAT AND DROUGHT STRESS ON FLORAL ANATOMY AND FLOWERING BEHAVIOR OF RICE (*Oryza sativa* L.) GENOTYPES

A.R. NIRMAL KUMAR*, C. VIJAYALAKSHMI AND D. VIJAYALAKSHMI

Department of Crop Physiology, RARS, ANGRAU, Tirupati - 517 502, Chittoor Dt., A.P.

Date of Receipt: 28-12-2016

Date of Acceptance: 24-02-2016

ABSTRACT

Floral anatomy, which determines the yield in rice, is greatly affected by heat and drought stresses during reproductive phenophase. Trials under controlled environmental conditions and field experiments under rain out shelter facility were carried out to elucidate information on floral behavior of rice genotypes to combined heat and drought stresses. Three rice (*Oryza sativa* L.) genotypes namely ADT 43, TKM 9 and N 22 which differed in their tolerance behavior to heat and drought stress but had similar phenology were taken and exposed to combined stresses of heat and drought at panicle initiation (PI) and anthesis stages. Exposure of plants to combined stresses at anthesis resulted in early flowering, reduced anther length, width, pollen viability and stigma receptivity. Stress imposed at anthesis caused greater reduction in anther length compared to stress imposed at panicle initiation. The genotype N 22 was observed to maintain larger anther length and width compared to other genotypes. Pollen viability and stigma receptivity were assessed microscopically and it was found that N 22 registered more number of germinated pollen on stigma while it was less in ADT 43. The genotype N 22 was found to be tolerant with superior floral characters.

KEYWORDS: Anther, Drought, Heat, *Oryza sativa,* Pollen and Stigma

INTRODUCTION

Heat stress combined with drought, is one of the major limitations to food production worldwide. As the world population continues to grow, and water resources for the crop production decline and temperature increases, the development of heat and drought tolerant cultivars is an issue of global concern. Nearly half of the world's population depends on rice and an increase in rice production by 0.6-0.9 per cent annually until 2050 is needed to meet the demand (Carriger and Vallee, 2007). As a result, rice (*Oryza sativa* L.) is increasingly cultivated in more marginal environments that experience warmer temperatures where day/night temperatures average 28/22°C (Prasad *et al.,* 2006). In these environments, day temperatures frequently exceed the critical temperature of 33°C for seed set, resulting in spikelet sterility and reduced yield. Hence, in the future, rice will be grown in much warmer with a greater likelihood of high temperatures coinciding with heat sensitive processes during the reproductive stage.

Although rice has been used as a model plant for many years, the responses of rice genotypes to combined high temperature and drought is still poorly understood. Rice responses to high temperature differ according to the developmental stage, with the highest sensitivity recorded at the reproductive stage. Temperatures more than 35°C at anthesis and lasting for more than 1 hour can lead to high sterility in rice (Jagadish *et al*., 2007). High temperature stress induced increase in spikelet sterility was attributed to abnormal anther dehiscence, impaired pollination and pollen germination (Jagadish *et al.,* 2010). Moreover, high temperature of 39°C given a day before flowering resulted in poor anther dehiscence during subsequent anthesis.

Rice is sensitive to drought stress particularly during flowering stage, resulting in severe yield losses. The physiological processes during the sensitive flowering stage, negatively affect spikelet fertility under water stress. Effect on anther dehiscence and pollen germination were similar to high temperature stress (Jagadish *et al.,* 2010). Additionally, panicle exsertion and peduncle length were partly responsible for increased sterility under water stress.

Impacts of climate change demands adjustments in our rice production methods and development of new rice strains that can withstand higher temperatures, growing multiple stress tolerant varieties that can integrate in future climate change situations. The simultaneous occurrence

---

*Corresponding author, E-mail: nirmalr035@gmail.com of multiple abiotic stresses rather than one particular stress is commonly noticed under field conditions (Mittler, 2006). The combination of high temperature and water stress represents an excellent example of multiple abiotic stresses occurring concomitantly in the field. But, relatively little information is available with reference to combined high temperature and water stress in general and at the most sensitive flowering stage in particular in rice. Experiments were therefore carried out with the objective to study the flowering behaviour *viz*., anther length, anther width, pollen viability, stigma receptivity of rice genotypes under combined heat and drought stress.

MATERIALS AND METHODS

Three rice (*Oryza sativa* L.) genotypes namely ADT43, TKM9 and Nagina 22 (N22) which are differed in their tolerance behaviour to heat and drought stress but had similar phenology were taken for the study.

Nursery was raised at Paddy breeding station of Tamil Nadu Agricultural University, Coimbatore. Twenty one days old seedlings and one seedling per hill 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_2O kg ha^{-1}. The land was divided into 12 plots with each plot measuring 2 m^2. The experiment was conducted in FRBD comprised of three treatments three varieties and four replications. The treatment details were as follows T_1 - (Control) well-watered throughout the crop growth period T_2 - Drought and natural high temperature stress at Panicle Initiation (PI) stage in which water was withheld for 2 weeks with a moisture stress of -50 to 70 kpa and the temperature ranged from 34.2 to 37.8°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 with soil water tension of -50 to -70 kpa and the temperature ranged from 33.5 to 36.6°C at the time of anthesis.

The time of sowing in the selected genotypes were staggered such that their PI and anthesis stages coincided the natural high temperature around early April for panicle initiation stage stress and May to June for anthesis stage 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.

Microscopic studies

Anther length and width

Anthers were collected from the spikelets early in the morning before anthesis. These anthers were fixed in a fixative FAA (50% absolute ethanol, 5% acetic acid, 27% formaldehyde, and 18% sterilized water) following the procedure of Jagadish *et al.* (2010). These anthers were observed under a compound microscope. Photographs were taken with SONY 12.1 megapixel camera to measure the anther length and width. The length and width was measured using an image analyser and the measurements were taken using the capture pro software version 4.1. The length of about 3 anthers were measured and their average was taken for analysis.

Pollen viability test

The pollen grains from anthers of randomly selected spikelets were collected and taken on cavity slides and stained with Iodine-potassium iodide solution (0.44 g Iodine + 20.08 g potassium iodide in 500 ml of 70% alcohol). The viable pollen stained immediately dark blue and the non viable ones remained as light yellow. The number of viable and non-viable ones were counted using microscope. The viability percentage was calculated from the mean of three microscopic field counts for each genotype (Jensen, 1962).

$$\text{Viability (\%)} = \frac{\text{Number of viable pollen grains}}{\text{Total number of pollen grains}} \times 100$$

Stigma receptivity

Fifteen to 20 spikelets were randomly sampled between 10.30 hours and 12.00 hours from the treatments. For this, spikelets about to flower on the main tiller were marked using acrylic paint, and, after pollination (around

30 min after the spikelet closed) marked spikelets were collected into vials filled with FAA fixative following the protocol by Jagadish *et al.* (2010). Anthers separated from the fixed spikelets were used to record per cent anther dehiscence. Spikelets were washed in deionized water before dissecting under a stereo-microscope (Olympus SZX7, Olympus Corp., Japan). Isolated stigmas were cleared in 8 N NaOH for 3 -5 hours at room temperature and stained with aniline blue dissolved in 0.1 M K_2HPO_4 for 5-10 minutes and number of germinated pollen on the stigma were recorded.

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 five per cent probability level and the values are furnished.

RESULTS AND DISCUSSION

The reduction in anther length was highly significant among the genotypes with the stress treatments. Lengthier anther were registered in case of N 22 (1.78) followed by ADT 43(1.64) and comparatively shorter anthers in TKM 9 (1.53) in control (Table 1). Heat and drought stress during PI caused reduction in anther length in ADT 43 (1.51 mm) whereas, no much variation was observed in TKM 9 (1.45) and N 22 (1.76). When the stress was imposed at anthesis all the genotypes showed reduction in anther length. The reduction in length was highly significant in ADT 43 (from 1.64 to 1.38) less reduction was observed in N 22 (from 1.78 to 1.74) followed by TKM 9 (from 1.45 to 1.32). Stress imposed at anthesis caused greater reduction in anther width compared to stress at PI in both the trials. There was no significant difference in ADT 43 for anther width (Table 2). Whereas, in control anther width was highest (0.73) in TKM 9 followed by N 22 (0.56) and stress at anthesis caused greater reduction in anther width in ADT 43 (from 0.31 to 0.28). Matsui and Omasa (2002) reported that cultivars with large anthers are tolerant to high temperature at the flowering stage and they further suggested that cultivars with large anthers were tolerant to heat and drought stress because of large number of pollen grains per anther, which compensates for the reduction in the number of pollen grains that germinate under high temperature. Contradictory, to our finding Jagadish *et al.,* (2007) reported that drought and heat stresses did not affect the anther size.

Pollen viability was significantly influenced by stress treatments. Stress at anthesis caused greater reduction in pollen viability. Viability (Table 3) of pollen was higher in control. N 22 recorded highest viability (90.7) followed by TKM 9 (89.8) and ADT 43 (88.6). Stress treatments exhibited significant negative effect on pollen viability. When the stress was imposed at PI maximum pollen viability was observed in N 22 (87.0) followed by TKM 9 (80.6) and least in ADT 43 (75.3). Stress imposed at anthesis caused greater reduction in pollen viability. The genotype N 22 recorded highest pollen viability (83.6) and the least was observed in ADT 43 (69.1). Similar results were also reported by Tao *et al*, (2008). Decreased longevity of pollen under heat and drought could be a result of disruption of carbohydrate accumulation in pollen grains and/or change in the ultra-structure of pollen grain at high temperature (Jain *et al*., 2007). Heat stress reduced carbohydrate accumulation in pollen grains and in the stigmatic tissue by might alter assimilate partitioning and change the balance between symplastic and apoplastic loading of the phloem (Taiz and Zeiger, 2006). In addition, the quick loss of moisture from pollen (Luna *et al*., 2001) due to high temperature and high vapour pressure deûcit could result in quick loss of viability. High temperature during flowering decreased the ability of the pollen grains to swell resulting in poor anther dehiscence. Pollen viability is considered as an important parameter of pollen quality. These studies unequivocally proved that heat and drought stress reduces pollen viability and genotypic variation exists for this trait and hence it is important to identify the genotypes with high pollen viability under combined stresses so as to use them in crop breeding programs as donors.

In the present study, stigma receptivity was assessed microscopically. It was found that N 22 registered more number of germinated pollen on stigma and less number was observed in ADT 43 (Fig 1). Stress imposed at anthesis caused greater reduction in stigma receptivity compared to stress at PI. N 22 showed good anther dehiscence. Under heat and drought stress, the swelling of the pollen grains is inhibited, which leads to indehiscence of the anther. The results are in agreement with Rang *et al*. (2011). Other factors that might influence pollen count on stigma was increased pollen stickiness which prevented pollen shedding even when the anthers were open.

From the controlled environment facility study it was inferred that early flowering genotype recorded higher

Table 1. Anther length (mm) in rice genotypes subjected to combined heat and drought stress under controlled environment facility

Genotypes	Control	Stress	
		Panicle Initiation	Anthesis
ADT 43	1.64 ± 0.012	1.51 ± 0.014	1.38 ± 0.001
TKM 9	1.53 ± 0.013	1.45 ± 0.011	1.32 ± 0.011
N 22	1.78 ± 0.001	1.76 ± 0.012	1.74 ± 0.012
P = CD (0.05) Variety		0.01**	
Treatment		0.01**	
V × T		0.03**	

Table 2. Anther width (mm) in rice genotypes subjected to combined heat and drought stress under controlled environment facility

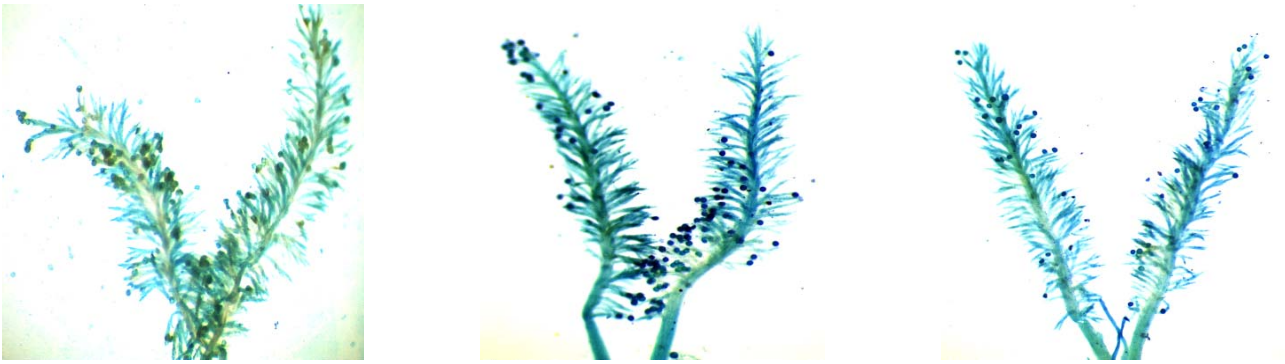
Genotypes	Control	Stress	
		Panicle Initiation	Anthesis
ADT 43	0.31 ± 0.003	0.29 ± 0.011	0.28 ± 0.013
TKM 9	0.73 ± 0.001	0.72 ± 0.011	0.71 ± 0.012
N 22	0.56 ± 0.002	0.56 ± 0.012	0.56 ± 0.001
P=CD(0.05) Variety		0.008**	
Treatment		0.006**	
V × T		0.015**	

Table 3. Pollen viability (%) in rice genotypes subjected to combined heat and drought stress under controlled environment facility

Genotypes	Control	Stress	
		Panicle Initiation	Anthesis
ADT 43	88.6 ± 0.75	75.3 ± 1.25	69.1 ± 0.28
TKM 9	89.8 ± 0.73	80.6 ± 1.16	78.9 ± 1.11
N 22	90.7 ± 0.15	87.0 ± 1.26	83.6 ± 0.47
P = CD (0.05) Variety		1.39**	
Treatment		2.13**	
VXT		2.41**	

i) ADT 43

(a) Control **(b) Stress at PI** **(c) Stress at anthesis**



ii) TKM 9

(a) Control **(b) Stress at PI** **(c) Stress at anthesis**

iii) N 22

(a) Control **(b) Stress at PI** **(c) Stress at anthesis**

Fig 1. Microscopic images of stigma receptivity in rice genotypes exposed to combined heat and drought

pollen viability coupled with better anther length and stigma receptivity. It was obviously observed that the susceptible genotype recorded less pollen viability mainly due to the loss in receptivity of stigma to allow the pollen tube growth and subsequent fertilization. Since N 22 is universal donar, early flowering under high temperature stress condition is an escape mechanism to avoid heat stress and yield normally. Similar to our reported results it was found that an increase in temperature of 4°C during the growing season of rice resulted in earlier maturation of the crop by five to six days for the wet and dry seasons, respectively (Ziska *et al*., 1997).

CONCLUSION

Stress imposed at anthesis caused greater reduction in anther length compared to stress imposed at panicle initiation. From this study, the genotype N 22 was emerged as an ideal donar for the future breeding programme for heat and drought stress.

REFERENCES

Carriger, S and Vallee, D. 2007. More crop per drop. *Rice Today*. 6: 10-13.

Gomez, K.A and Gomez, A.A. 1984. Statistical procedures for agricultural research. (2nd Ed.) John Wiley and Sons, New York, USA.

Jagadish, S.V.K., Craufurd, P.Q and Wheeler, T.R. 2007. High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). *Journal of Experimental Botany.* 58: 1627–1635.

Jagadish, S.V.K., Muthurajan, R., Oane, R., Wheeler, T.R., Heuer, S., Bennett, J and Craufurd, P.Q. 2010. Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). *Journal of Experimental Botany.* 61: 143–156.

Jain, M., Prasad, P.V.V., Boote, K.J., Hartwell, A.L and Chourey. P.S. 2007. Effects of season-long high temperature growth conditions on sugar- to-starch metabolism in developing microspores of grain sorghum (*Sorghum bicolor* L. Moench). *Planta.* 227: 67–79.

Jensen, W.A. 1962. Botanical Histo Chemistry, Freeman, San Fransisco.

Luna, S., Figueroa, J. Baltazar, B., Gomez, R., Townsend, R and Schoper, J.B. 2001. Maize pollen longevity and distance isolation requirements for effective pollen control. *Crop Science*. 41: 1551–1557.

Matsui,T. and Omasa, K. 2002. Rice (*Oryza sativa* L.) cultivars tolerant to high temperature at ûowering: anther characteristics. *Annuals Botany. 89:* 687-688.

Mittler, R. 2006. Abiotic stress, the field environment and stress combination. *Trends in Plant Science.* 11: 15–19.

Prasad, P.V.V., Boote, K.J., Allen, L.H., Sheehy, J.E and Thomas, J.M.G. 2006. Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Research*. 95: 398–411.

Rang, Z.W., Jagadish, S.V.K., Zhou, Q.M., Craufurd, P.Q and Heuer, S. 2011. Effect of heat and drought stress on pollen germination and spikelet fertility in rice. *Environment and Experimental Botany.* 70: 58-65.

Taiz, L and Zeiger, E. 2006. Stress physiology. In: (eds) Plant Physiology, pp 671–681. Panima publishing corporation, New Delhi.

Tao, L.X., Tan, H.J., Wang, X., Cao, L.Y., Song, J. and Cheng, S.H. 2008. Effects of high temperature stress on flowering and grain-setting characteristics for Guodao 6. *Acta Agronomica Sinica.* 34:669-674.

Ziska, L.H., Namuco, O., Moya, T and Quilang, J. 1997. Growth and yield response of field grown tropical rice to increasing carbon dioxide and air temperature. *Agronomy Journal*. 89: 45- 53.