Seed Research, Vol. 44(1): 47-55, 2016

Influence of High Temperature on Seed Yield of Popular Tamil Nadu Rice Cultivars

LAKSHMIPRASANNA* KATA AND M BHASKARAN

Department of Seed Science and Technology, TNAU, Coimbatore 641 003
*prasanna.agrico@gmail.com

ABSTRACT: Rice cultivation will be subjected to much warmer environments in near future. In the present study four popular rice cultivars (ADT 46, CO (R) 50, CORH 4 and TKM 9) of Tamil Nadu were exposed to elevated temperature *i.e.*, ambient + 5°C in a Climate Control Chamber from transplanting to harvesting, to quantify its effect on pollen dispersal, viability, pollen tube growth, seed set and seed yield. Exposure of plants to high temperature resulted in early flowering in all the cultivars which were tested. Microscopic analysis revealed that all the pollen related parameters like pollen viability, pollen germination and also pollen tube growth were adversely affected to an extent of 22-47 %, 4-13 % and 4-12 % respectively by the heat stress. Scanning Electron Microscopic analysis revealed the shrivelling of pollen grains under heat stress. In addition to viability, tube growth was also very much affected, as a result the tube could not able to reach the egg cell to fertilize it, causing spikelet sterility (24-72 %). Total number of spikelets per panicle (9-17 %) and number of filled seed per panicle (21-68 %) were reduced under high temperature conditions which resulted in reduced seed yield/plant (29-63 %) and also harvest index (13-33 %). High temperature negatively influenced root and shoot lengths. Among the four cultivars TKM 9 was found to be more tolerant to heat stress compared to other three cultivars.

Key words: Rice, High temperature (ambient + 5°C), Flowering, Pollen viability, Pollen germination, Pollen tube growth, Seed set, Seed yield

INTRODUCTION

Rice is the staple food for most of the Indians and plays a major role in Indian economy. Nearly half of the world's population also depends on rice for their staple diet and an increase in rice production by 0.6-0.9 % annually until 2050 is needed to meet the demand [1]. Since there is not much scope to increase the area of rice cultivation there is a need to increase the productivity of rice. Even though rice originated from tropics it cannot tolerate temperature more than 35°C. Rice seed production has to go through a number of well differentiated stages like uniform germination and plant stand, with high level of productive plants, timely flowering, complete development of flower primordia, pollen and egg fertility, pollination followed by double fertilization, embryo formation and endosperm development, proper transportation of nutrients from source to sink, seed maturation, timely harvest and post harvest handling operations. Every stage of this long chain is susceptible to abiotic stress, especially to temperature [2-4]. Mean surface air temperature has increased globally by about 0.5°C in the 20th century and is projected to increase by 1.4 to 5.8°C in this century [5]. Any further increase in mean temperatures (*i.e.*, more than 35°C) or of short episodes of high temperatures during sensitive stages, may be supra optimal and will reduce rice yield [6].

A number of plant developmental and physiological processes are negatively affected by heat stress. When the stress occurs at key developmental stages such as reproduction, this becomes one of the major constraints of plant

Received : February 2016 Revised : April 2016 Accepted : April 2016

adaptation to a changing environment [7]. Phenological stages differ in their response to high temperature. Heat stress at vegetative stage reduces the number of tillers, total biomass and photosynthetic rate [8-9]. Moderate stress of heat often accelerates flowering which results in the occurrence of reproductive phase prior to the sufficient accumulation of resources in the vegetative stage [10]. Male reproductive organs are the most affected ones, right from the pollen production, anther dehiscence, pollen viability, germination of pollen grains and tube growth are affected [11-12]. The main reason for reduced pollen viability under heat stress is poor carbohydrate accumulation in pollen grains which in turn results in spikelet sterility and low seed yield [13]. Normal seed development is disrupted when high temperatures coincides with seed filling, which increases the proportion of seeds that are shrivelled, abnormal and are of lower quality [14]. The goal of present experiment was to evaluate the yield and yield components of four popular rice cultivars (ADT 46, CO (R) 50, CORH 4 and TKM 9) of Tamil Nadu under heat stress conditions.

MATERIALS AND METHODS

Crop husbandry and chamber conditions

Four rice cultivars namely ADT 46, CO (R) 50, CORH 4 and TKM 9 were subjected to elevated temperature and changes in crop phenology and reproductive parameters were observed. Experiments were carried out in Climate Control Chamber (CCC), established at Agro Climate Research Centre, Tamil Nadu Agricultural University, which is situated at 11°N latitude, 77°E longitude and at an elevation of 427 m above the mean sea level. Temperature of CCC was controlled using heater, fogger and a mist fan. Temperature was continuously recorded by the sensor which is connected to a data logger, at fixed intervals. The temperature maintained inside the chamber was ambient + 5°C (Fig. 1). The plants of the varieties raised outside the chamber served as control. Seeds of selected varieties were sown in trays and after 21 days, the seedlings were transplanted in iron tubs (2 m x 1m) which were filled with clay loam soil obtained from rice growing area.

OBSERVATIONS

Vegetative phase

The height of plants in each hill was measured from the crown region to the tip of the main stem at flowering stage and the mean was expressed in cm. Total number of tillers per plant was counted and recorded at maximum tillering stage. The number of ear bearing tillers per hill

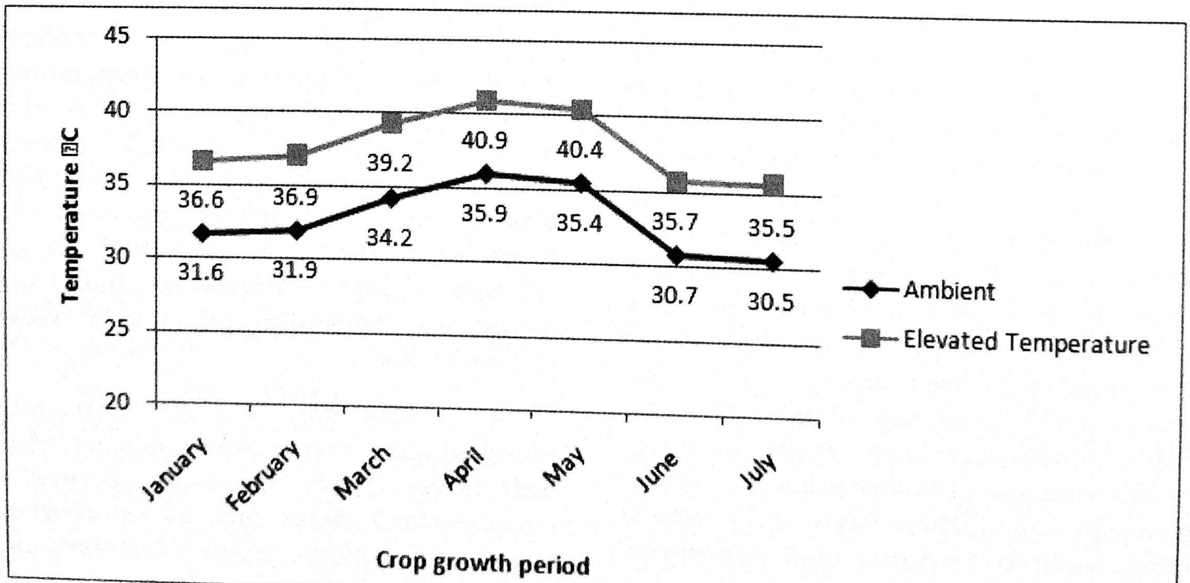


Fig. 1. Temperature curve of Coimbatore, Tamil Nadu for the year 2013 (January-June) January – March : Vegetative phase, April – May – Reproductive phase

at maturity and the number of days the plants took to flower from sowing were counted and recorded.

Leaf area : From each hill, five random leaves were selected and length and width of those leaves were measured and the leaf area was calculated by the following formula [15].

Leaf area (cm^2) = L x B x K (0.75) x n

L = Length; B = Breadth; K = Extinction coefficient (0.75 for rice)

n = Number of leaves/plant

Stomata number : Stomata counts were recorded on the middle portion of the five random terminal leaves by nail polish on abaxial and adaxial surfaces and expressed as number per mm^2.

Chlorophyll index : It was observed at maximum tillering stage using chlorophyll meter (Model CCM 200 plus).

Microscopic observations

Anther length and width : Randomly selected three florets (18 anthers) in each cultivar were used for measurement of anther length and width by using a micrometer under binocular microscope and expressed in mm.

Pollen morphology : Pollen grains of each cultivar were selected randomly and the morphology was observed under scanning electron microscope at a magnification of 3000x, 1000x and 400x.

Pollen viability : 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). Observations on the staining were done using a microscope. The pollen which stained dark blue immediately were counted as viable and the pollen which remained light yellow were counted as non-viable. The viability percentage was calculated from the mean of three microscopic field counts for each variety [16]. Images were taken with a Leica camera attached to the stereo binocular microscope.

Pollen germination : The spikelets were collected after pollination, at the stage of closed lemma and palea and immediately fixed in one part of glacial acetic acid and three parts of absolute alcohol (1:3 glacial acetic acid: absolute alcohol) and allowed to remain as such for overnight. The fixed spikelets were dissected to isolate the pistils. The pistils were transferred to 8N NaOH for four hours and subsequently washed in distilled water and then placed overnight in 0.1% aniline blue in 50 mol m^{-3} NaH_2PO_4 (pH 8.2). Later, the stained pistils were mounted on microscope slide in a drop of 50% glycerine and observed for pollen germination on the stained pistils[17]. After the germination of pollen grains, pollen tube length was measured with a micrometer and expressed in µm.

Seed yield and yield parameters

The total number of spikelets and number of filled seeds in the panicle of main tillers was counted.

Seed set % : The filled seeds in the main tiller of 10 randomly selected plants were counted and seed set per cent was calculated using the following formula

1000 seed weight : Thousand seeds were counted in eight replicates and the weight was recorded using an electronic weighing balance and expressed in g.

Seed yield/plant : Seeds were threshed from each plant separately, dried to 12 per cent moisture content, processed, weighed and expressed in g per plant.

Harvest index (HI) : HI was recorded as the ratio of grain weight to total plant weight.

Germination test was conducted on the resultant seed as outlined by ISTA (2004) except on CORH 4 (hybrid) as the seed becomes F2 and it segregates. Shoot and root lengths of the seedlings were also recorded.

RESULTS

Plants grown under high temperature exhibited lean and lanky appearance and recorded an increased plant height (Fig. 2), which was very significant in the cultivar TKM 9 (86.74 to 92.28 cm). However, no significant difference was observed in other vegetative growth parameters like total number of tillers per hill, number of productive tillers per hill, leaf area and chlorophyll index compared to the counterparts grown under ambient conditions. Stomata number on adaxial surface of the leaves was reduced under elevated temperature in all the cultivars except in CO(R) 50. Interaction effect revealed that all the varieties used in the experiment took significantly lesser number of days to flower under high temperature conditions compared to ambient temperature. Under ambient conditions, CO(R) 50 and ADT 46 took 90 days to flower where as under elevated temperature CO(R) 50 required only 84 days and ADT 46 took 87 days to initiate flowering. Elevated temperature reduced the duration of vegetative growth in all the four varieties, as compared to ambient temperature. Significant influence of high temperature was observed on anther development in the cultivar ADT 46, wherein reduction in anther width was observed (4.5 % *i.e.*, from 0.45 mm to 0.43 mm). In the cultivars CORH 4 and TKM 9 elevated temperature did not show any affect on anther size (0.46 mm and 0.49 mm respectively under both the temperature conditions).

SEM analysis

Elevated temperature had significant effect on pollen morphology. Higher number of shrivelled pollen was observed in plants grown under high temperature (Fig. 3). Under high temperature, the swelling of the pollen grains was inhibited, which lead to indehiscence of the theca and lesser number of pollen grains on stigma. Good number of pollen grains on stigma was recorded by ADT 46 and TKM 9, both under control (44, 43) and elevated temperatures (42, 40).

Pollen viability

When the anthesis time coincided with elevated temperature, it significantly affected the fertility of pollen grains. Maximum per cent of pollen viability reduction (46.89 %) was observed in case of CORH 4. Hybrid vigour did not help CORH 4 in overcoming the adverse effects of high temperature on pollen viability % as observed in the low level of pollen viability (48.24 %) (Fig. 4). Among the varieties, pollen grains of TKM 9 recorded the lowest level of reduction in pollen viability (22 per cent *i.e.*, from 89 to 70 %) when exposed to elevated temperature which was less compared to the other three cultivars under study.

Elevated temperature impact on pollen germination

Under elevated temperature, increase in number of sterile pollen could have resulted in less

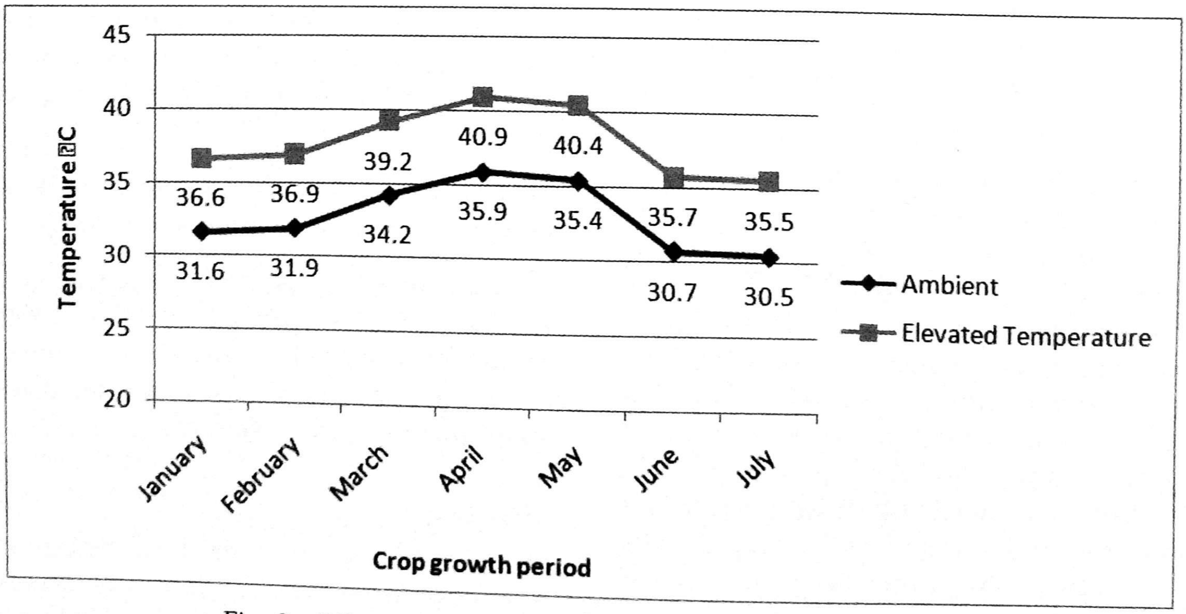


Fig. 2 : Effect of elevated temperature on plant height (cm)

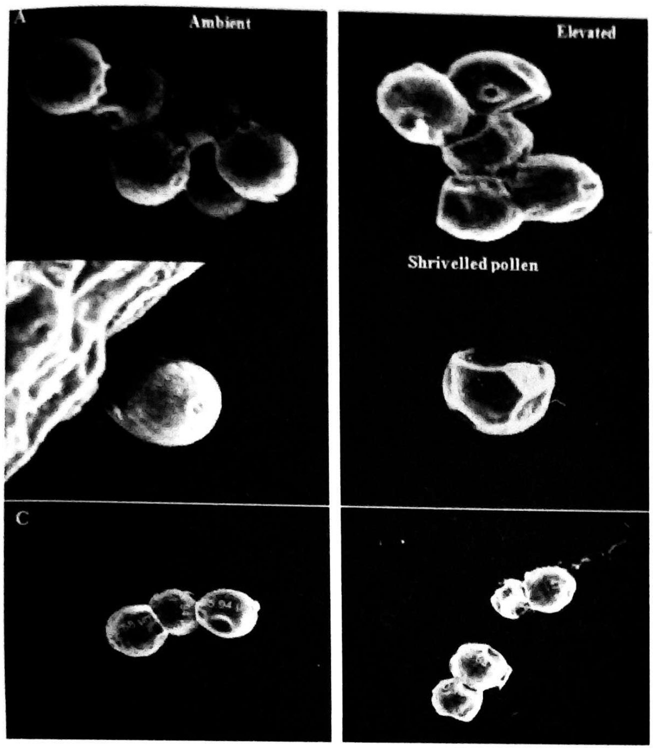


Fig. 3: Morphology of the rice varieties as affected by the elevated temperature. A-400X magnification B-3000 x magnification C-1000X magnification

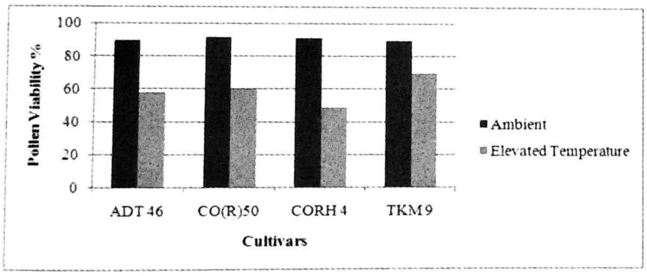


Fig. 4 : Effect of elevated temperature on pollen viability % of rice varieties

number of germinated pollen on stigma. Maximum number of germinated pollen was observed in case of TKM 9 (Fig. 5). A negative correlation was recorded between elevated temperature and pollen germination. No significant interaction was found among the varieties and temperature for pollen germination %. The highest reduction in tube length was observed in the cultivar, ADT 46 (12.4 %) while lowest reduction was observed in case of TKM 9 (4 %) (Fig. 6).

Seed yield and related parameters

Number of filled seed per panicle was found to be significantly lower under elevated temperature (Fig. 7). Interaction effect revealed that the reduction was higher in case of ADT 46 (68 %) and CORH 4 hybrid (68 %) and lower in TKM 9 (21 %). The number of filled seed per panicle positively correlated with pollen viability % (r=+0.85). The interactional effect for seed set revealed that the highest reduction was observed in case of ADT 46 (51.8 % reduction) and lowest reduction was observed with TKM 9 (4.17 % reduction) (Fig. 8). Thousand seed weight determined from the filled seed revealed that only in case of ADT 46 it was reduced significantly (4 % reduction) while in other cultivars not much effect of elevated temperature was found on thousand seed weight. Seed yield/ plant was found to be reduced significantly under HT (Fig. 9). The reduction was greater in case of CORH 4

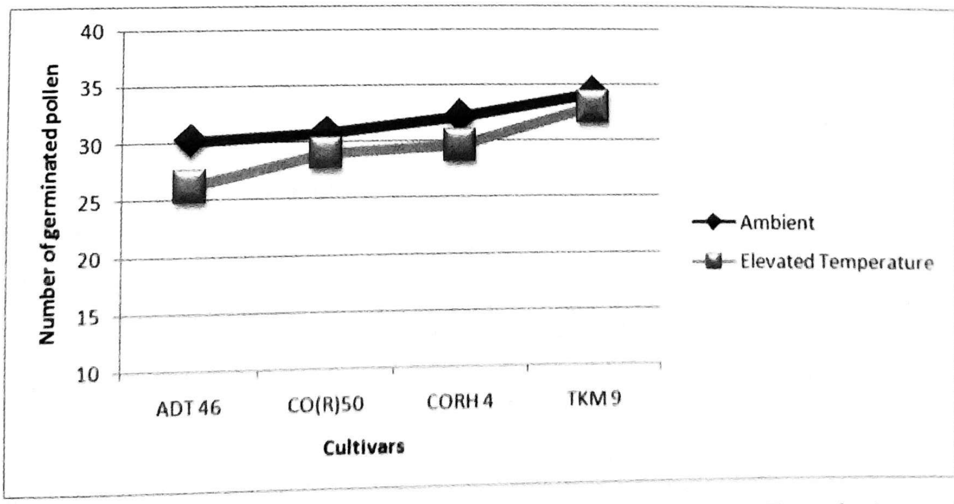


Fig. 5 : Effect of elevated temperature on Number of germinated pollen of rice varieties

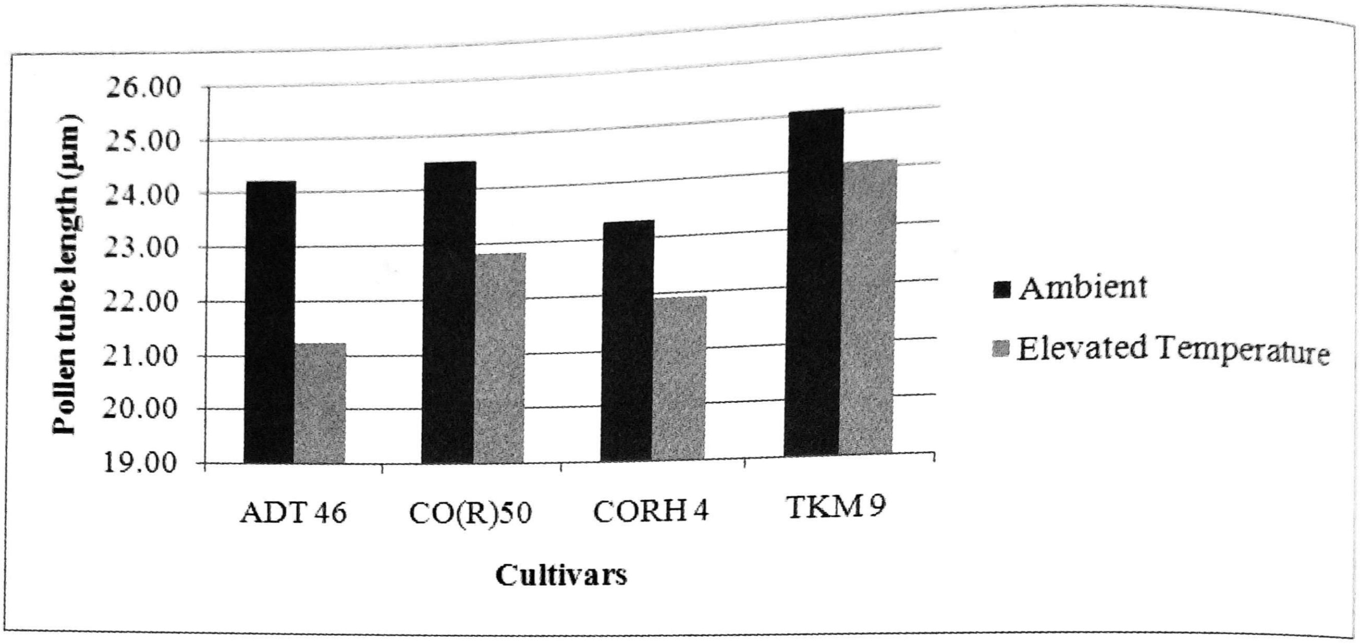


Fig. 6 : Effect of elevated temperature on pollen tube growth of rice varieties

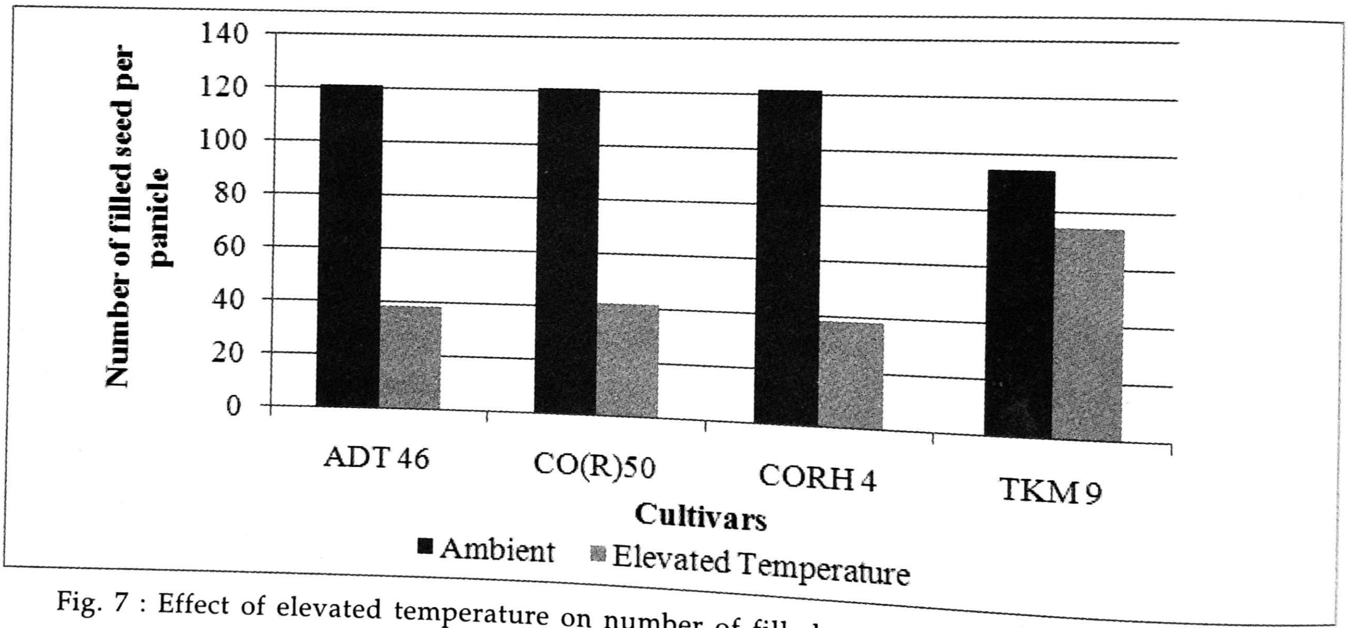
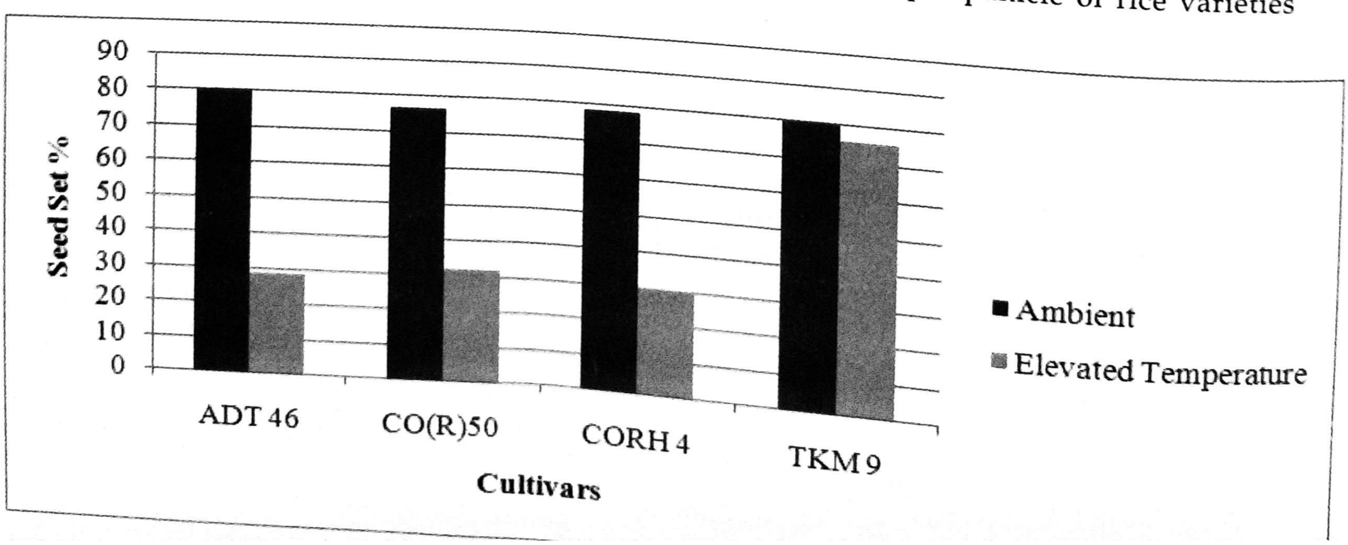


Fig. 7 : Effect of elevated temperature on number of filled seed per panicle of rice varieties



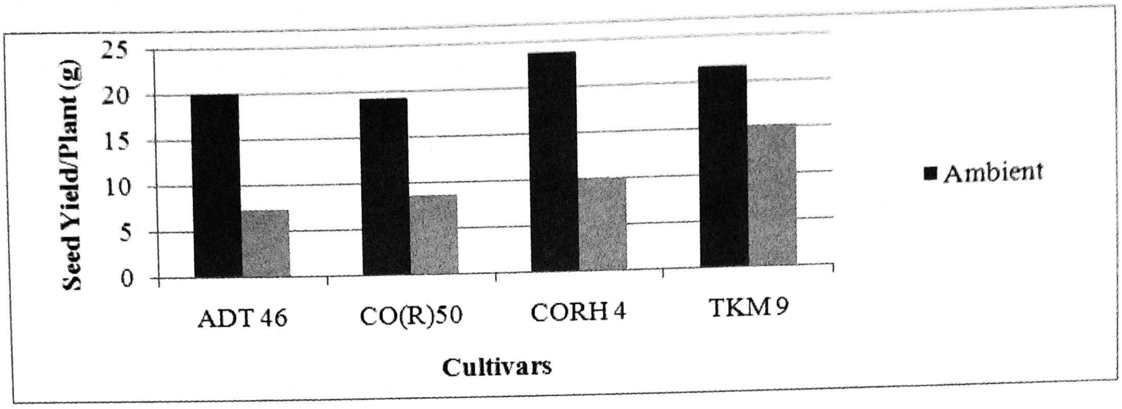


Fig. 9 : Effect of elevated temperature on seed yield per plant (g)

hybrid (23.65 g to 10.03 g) and ADT 46 (20.03 g to 7.35 g) and lower in case of TKM 9 (21.55 g to 15.28 g). Among the cultivars CORH 4 recorded high harvest index values under ambient temperature conditions but when exposed to the elevated temperature the same cultivar recorded highest reduction in the harvest index value (39.13 % reduction). The reduction in HI was very low in the variety TKM 9 (8 % reduction). However, high temperature did not affect the germination percentage significantly (Table 1). Significant reduction in shoot and root lengths were observed. ADT 46 recorded highest reduction in root length from 19.22 cm to 16.45 cm (14.41 %) where as shoot length was highly reduced in CO (R) 50 (14. 37 %).

DISCUSSION

A number of experiments were conducted on effect of heat stress on paddy seed production but the current study focused at the tolerance of popular cultivars of Tamil Nadu to heat stress and whether the hybrid vigour helps to tolerate the elevated temperature or not. High temperature stress during vegetative phase altered only plant height which was significantly increased by high temperature. These results are similar to the reports of earlier researchers that plant height increases due to high daily temperature [18]. Plant height is the sum of the lengths of each of the internode. Environmental or genetic factors influence internode length. The effect of elevated temperature on internodal length is due to increased cell elongation than an increase in cell number. Lack of response of tiller number to the elevated temperature is mainly because the temperature of climate chamber during initial vegetative phase was 36°C. 1°C increase in temperature than optimum (35°C) during vegetative phase did not affect the vegetative growth parameters like tiller number, leaf area and chlorophyll index.

Irreversible effect of elevated temperature was observed on reproductive growth. Flowering is controlled by the developmental age of the plant and environmental signals. High temperature resulted in accelerated flowering in all the four cultivars (ADT 46, CO(R) 50, CORH 4 and TKM 9) tested with the maximum acceleration in CO(R) 50. As suggested by earlier researchers that cultivars with large anthers are tolerant to high temperature at the flowering stage [19] in the present experiment the variety TKM 9 with larger anther size was found to be tolerant to high temperature compared to the other cultivars. In one of the cultivar (ADT 46) even after proper anther dehiscence the pollen germination and seed set % was found to be lower. This can be attributed to the reduced pollen viability and tube growth under elevated temperatures [17, 20-21]. In the present experiment when the plants were exposed to 40°C at flowering stage there was a significant reduction in all the pollen related parameters. Decreased longevity of pollen at high temperature 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 [22]. In addition, the quick loss of moisture from pollen due to high temperature and high vapour pressure deûcit

Table 1: Seed quality characters of cultivars under normal and elevated temperatures

Variety (V)	Germination (%)			Root length (cm)			Shoot length (cm)		
	Ambient	Elevated	Mean	Ambient	Elevated	Mean	Ambient	Elevated	Mean
ADT 46	94(75.82)	91(72.54)	**93(74.66)**	19.22	16.45	**17.84**	11.88	11.01	**11.45**
CO(R) 50	97(80.03)	93(74.66)	**95(77.08)**	19.70	18.35	**19.03**	12.11	10.37	**11.24**
TKM 9	93(74.66)	92(73.57)	**93(74.66)**	16.30	15.38	**15.84**	6.73	6.67	**6.70**
Mean	95(77.08)	92(73.57)	93(74.66)	18.41	16.73	17.57	10.24	9.35	9.80
	V	T	VT	V	T	VT	V	T	VT
SEd	0.59	0.48	0.83	0.23	0.19	0.33	0.13	0.27	0.37
CD (P = 0.05)	NS	1.011	NS	0.49	0.40	0.67	0.25	0.58	0.79

results in quick loss of viability [23-24]. Further the pollen tube growth was very much affected under elevated temperature.

The significant interaction effect between the cultivars and temperature was observed for the parameters like pollen tube length, number of filled seed per panicle, seed set %, seed yield and harvest index. Among the cultivars seed yield of ADT 46 and CORH 4 was very much affected when the temperature was elevated by 5°C. Assimilates were divided among the less number of kernels which resulted in no significant difference in 1000 seed weight. Since there is no significant difference in weight of the kernel no significant effect on germination was observed but the vigour of the seedlings was reduced which was evident from shoot and root lengths under high temperature.

CONCLUSION

The present study has provided critical information regarding the sensitivity of paddy reproductive phase to the elevated temperature. During vegetative phase a temperature of 36°C exhibited no significant affect on the total tiller number and number of productive tillers. During reproductive phase the temperature above 35°C drastically reduced the seed yields by dehydrating of pollen (shrivelling of pollen), by impairing the viability and germination. Even the germinated pollen grains recorded, impaired tube growth which reduced the chance of fertilization and ultimately yield. These parameters may be critically evaluated while screening the germplasm for heat tolerance.

ACKNOWLEDGEMENTS

The first author is thankful to Department of Science and Technology, TNAU, Govt. of India for providing financial assistance under Innovation in Science Pursuit for Inspired Research (INSPIRE) programme and also to the members of advisory committee for their valuable guidance.

REFERENCES

1. CARRIGER S AND D VALLEE (2007). More crop per drop. *Rice Today*, **6**: 10

2. ELLIS RH (2011). Rice seed quality development and temperature during late development and maturation. *Seed Sci. Res.*, **21**: 95

3. KHANDURI VP (2011). Variation in anthesis and pollen production in plants. *American-Eurasian J. Agric. & Environ. Sci.*, **11**(6): 834

4. MAITY A, SK CHAKRABARTY AND JB YADAV (2012). Standardization of hybrid seed production technology of first Indian mustard (*Brassica juncea*) hybrid NRCHB 506. *Indian J. Agric. Sci.*, **82**(9): 753

5. WASSMANN R AND A DOBERMANN (2007). Climate Change Adaptation through Rice Production in Regions with High Poverty Levels.

J. ICRISAT Agric. Res., **4**(1): 1

6. SHAH F, J HUANG, K CUI, L NIE, T SHAH, C CHEN AND K WANG (2011). Impact of high-temperature stress on rice plant and its traits related to tolerance. *J. Agr. Sci.*, **1**: 1

7. HALL AE (2001). Crop responses to environment. Boca Raton. CRC Press pp 232

8. MITRA R AND CR BHATIA (2008). Bioenergetic cost of heat tolerance in wheat crop. *Curr. Sci.*, **94**: 1049

9. HURKMAN WJ, WH VENSEL, CK TANAKA, L WHITEH AND SB ALTENBACH (2009). Effect of high temperature on albumin and globulin accumulation in the endosperm proteome of the developing wheat grain. *J. Cereal Sci.*, **49**: 12

10. PUTTERILL J, R LAURIE AND R MACKNIGHT (2004). It's time to flower: the genetic control of ûowering time. *Bioessays*, **26**: 363

11. WASSMANN R, SVK JAGADISH, K SUMFLETH, H PATHAK, G HOWELL AND A ISMAIL (2009). Regional vulnerability of climate change impacts on Asian rice production and scope for adaptation. *Adv. Agron.*, **102**: 99

12. HARSANT J, L PAVLOVIC, G CHIU, S SULTMANIS AND TL SAGE (2013). High temperature stress and its effect on pollen development and morphological components of harvest index in the C_3 model grass *Brachypodium distachyon*. *J. Exp. Bot.*, **64**(10): 2971

13. TAIZ L AND E ZEIGER (2006). "Stress physiology," in Plant Physiology, 4th edition (Sinauer Associates, Inc.) pp. 671

14. THOMAS JMG, PVV PRASAD, KJ BOOTE AND LH ALLEN (2009) Seed composition, seedling emergence and early seedling vigour of red kidney bean seed produced at elevated temperature and carbon dioxide. *J. Agron. Crop Sci.*, **195**: 148

15. PALANISWAMY KM AND KA GOMEZ (1974) Length-width method for estimating leaf area of rice. *Agron J.*, **66**(3): 430

16. JENSEN WA (1962). Botanical Histochemistry, Freeman, Sanfransisco. pp. 201

17. JAGADISH SVK, R MUTHURAJAN, R OANE, TR WHEELER, S HEUER, J BENNETT AND PQ CRAFURD (2010). Physiological and proteomic approaches to dissect reproductive stage heat tolerance in rice (*Oryza sativa* L.). *J. Exp. Bot.*, **61**: 143-156

18. OH-E I, K SAITOH AND T KURODA (2007). Effects of high temperature on growth, yield and dry-matter production of rice grown in the paddy field. *Plant Prod. Sci.*, **10**(4): 412

19. MATSUI T AND K OMASA (2002). Rice (*Oryza sativa* L.) cultivars tolerant to high temperature at ûowering: anther characteristics. *Ann. Bot.*, **89**: 683

20. PRABA ML, JE CAIRNS, RC BABU, AND HR LAFITTE (2009). Identification of physiological traits underlying cultivar differences in drought tolerance in rice and wheat. *J. Agron. Crop Sci.*, **195**: 30

21. RANG, ZW, SVK JAGADISH, QM ZHOUA, PQ CRAUFURD AND S HEUER (2011). Effect of High temperature and water stress on pollen germination and spikelet fertility in rice. *Environ. and Exp. Bot.*, **70**: 58

22. JAIN M, PVV PRASAD, KJ BOOTE, LH. Jr. ALLEN AND PS CHOUREY (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

23. BUITINK J, C WALTER-VERTUCCI, FA HOEKSTRA AND O LEPRINCE (1996). Calorimetric properties of dehydrating pollen: analysis of desiccation-tolerant and an intolerant species. *Plant Physiol.*, **111**: 235

24. LUNA S, J FIGUEROA, B BALTAZAR, R GOMEZ, R TOWNSEND AND J B SCHOPER (2001). Maize pollen longevity and distance isolation requirements for effective pollen control. *Crop Sci.*, **41**: 1551.