Elevated temperature modulates rice pollen structure: a study from foothill of Himalayan agro-ecosystem in India

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Abstract

Rice cultivation is practiced in various agro-climatic zones ranging from tropical to temperate. Anthesis is a critical stage for deciding productivity which is controlled by numerous biotic and abiotic stresses. Elevated temperature is one of the key abiotic stresses that adversely modulate the pollen formation and structure, i.e., infertility, results in productivity decline. The present study was conducted to investigate the effects of elevated temperature on morphological changes in pollen grains of rice grown in temperature gradient tunnel (TGT) constructed in filed condition. The ambient and TGT temperature varied from 31.84 °C_(max) to 20.56 °C_(min) and 41.91 °C_(max) to 22.37 °C_(min), respectively, during study period. The scanning electron and light microscopic study showed extreme morphological abnormalities viz. shape and size in pollen grains of rice exposed to elevated temperature as compared to ambient. These abnormalities induced by elevated temperature may drastically decline grain filling potential which ultimately reduces grain yield in future climate change. This study will enlighten rice researchers for further screening of rice genotypes thermotolerant to foothills of Himalayan agro-ecosystem.

Keywords Rice · Elevated temperature · Pollen behavior · Climate change · Himalayan foothills

Introduction

Rice is a semi-aquatic crop among the cereals, which is a staple food for half of the population (3.5 billion people) of the world. Various abiotic and biotic factors control growth and development of rice, ultimately productivity (Singh et al. 2010a, b, 2013, 2014; Singh and Verma 2013a, b). Cultivation of rice required temperature ranges from 27 to 35 °C, high humidity and the well-distributed precipitation of about 100 cm for its proper growth and development. Rice production is intensified by climate change, particularly threatened by elevated temperature (Kilasi et al. 2018). The episode of elevated temperatures is often directly proportional to the rainfall. In addition, global mean temperature

rises from 1906 to till date it is about $>0.6 \pm 0.2$ °C (IPCC 2007). By the end of the twenty first century, a further temperature increase of 1.8–4.0 °C is predicted (IPCC 2014). With this variation in the elevated temperature, rice production cannot be stable as the elevated temperature causes a cumulative linear decline in spikelet fertility particularly night temperature rises by 1 °C might reduce rice yields up to 10%. Apart from that higher respiration loss is the factor which linked to elevated temperature also makes rice less productive and reduction in the rice yields by 0.3 tons per hectare (IPCC 2007; Kumar et al. 2015).

Moreover, elevated temperature stress during anthesis leads to poor germination of pollen grain for about 65% and reduction in rice productivity and quality (Shi et al. 2016). During elevated temperature, water deficit condition also occurs in the pollen grain which causes dehydration of the pollen and inactivation of the metabolic process resulting in limited grain filling and reduced yields (Pressman et al. 2002; Tang et al. 2008). To understand the morphological behavior of the pollen germination during anthesis stage under elevated temperature, an investigation was carried out on effects of increasing mean temperature on terminal stage of rice.

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Fig. 1 Weekly average ambient and TGT temperature during study period of 2013

Materials and methods

Experimental details

The experimental was conducted in kharif season from June to November, 2013. The investigation has been done with eleven rice genotypes obtained from the Directorate of Rice Research, Rajendranagar, Hyderabad was chosen for the study. The selected inbred lines (i.e. IET 21404, IET 21405, IET 21411, IET 21415, IET 21577 and IET 21582) and hybrids (i.e. KRH-2, PA 6129, PA 6201, PA 6444 and PHB 71) at the Norman E. Borlaug Crop Research Centre, G. B. Pant University of Agriculture and Technology Pantnagar, U.S. Nagar (Uttarakhand), India. Out of these, only one genotype, i.e., IET 21405 was selected for microscopic studies (Kumar et al. 2015, 2017). Treatment was given at the commencement of the flowering stage (~60 days). For raising temperature, a temperature gradient tunnel (TGT) was constructed using 0.1 mm thick UV transparent PVC film with the tunnel size of $2 \times 2 \times 25$ m (width $\times$ height $\times$ length) in field condition at reproductive stage (Kumar 2014). Maximum and minimum temperature was recorded daily with the help of an automatic thermometer inside and outside the TGT (Fig. 1).

Collection of pollen grains

Rice pollen is short lived which can lose its viability within 5 min under elevated temperature. For maintaining the viability, panicle was sampled early in the morning (between 08:00 and 09:00 h) from the newly opened anther under ambient as well as elevated temperature and brought to the laboratory.

Examination of pollen using light microscopic method

The number of pollens were estimated from a single anther placed on the glass slide with grid followed by squashed by a needle for the dispersal of pollen on the slide and stained with 1% Iodine + Potassium Iodide ($I_2 + KI$) solution to examine the viability and counting was done using light microscope (Kumar et al. 2017).

Scanning electron microscopy (SEM) of pollen grains

The pollens of rice were also examined under SEM to ascertain the changes on the morphological behavior of pollens under ambient and elevated temperature condition. For examination of samples microscope stubs were used and prepared by dusting pollen from open flowers on double-sided carbon tape affixed to the stub. Samples were allowed to dry overnight and coated with approximately 100 Å gold in a vacuum sputter instrument. These samples were examined using a JEOL JSM-6610LV scanning electron microscope under high vacuum at 5.00 kV (Kumar et al. 2015).

Results and discussion

Light microscopic analysis

Light microscopic examination of rice pollen grain revealed that there was significant variation in the pollen viability under ambient and elevated temperature. The observation exhibited that pollen grains under ambient temperature appeared dark black, which indicates as fertile pollen and light colour under temperature stress indicate sterile pollen grains (Fig. 2). The reduction percentages of the pollen fertility might be due to less moisture content during anthesis under treatments as well as the decreased ability of the pollen grains to swell, resulting in poor dehiscence (Singh et al. 2013; Fang et al. 2010; Prasad et al. 2006; Matsui et al. 2009; Endo et al. 2009).

Scanning electron microscopic analysis

Significant temperature fluctuations from minimum to maximum disturb the stage of flowering in rice which leads to decreases in viability and tube length of pollens, poor anther dehiscence and reduced number of pollens on stigma. In scanning electron microscopic studies pollen from control plants appeared normal round shaped, while the diameter of the pollen decreased by 50% as compared to the control under temperature stress (35/25 °C). It might be due to low

Fig. 2 Examination of rice pollen under light microscope **b, d** shows viable pollen, **a, c** shows non-viable pollen

Fig. 3 Studies of rice pollen grains under the Scanning electron microscope **a, c** shows viable pollen, **b, d** shows non-viable pollen under heat stress condition

moisture content and drought conditions arising in rice inflorescence. Therefore, elevated temperature decreases the ability of pollen grain to swell and reduce the size (Fig. 3). The findings of present study are in conformity with previous

studies by Kumar et al. (2015); Das et al. (2014); Zhou et al. (2011).

Conclusion

Atmospheric temperature beyond the threshold terminates flowering stage, a phenophase of rice which determined economic yield. Rice pollens could not tolerate elevated temperature that leads to sterility and finally causes a decline in grain yield. The present study observed economic loss in terms of grain yield due to elevated temperature. This loss is the results of abnormality (shrinkage) in pollen morphology that induces a reduction in the grain filling process. Our findings of light and scanning electron microscopy showed significant changes in the shape of rice pollen in ambient and elevated temperature, i.e., heat treated plants. Therefore, this study will provide an insight into the selection of best thermotolerance rice genotype in changing climate scenario.

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Compliance with ethical standards

Conflict of interest The authors declared there is no conflict of interest in this manuscript.

References

- Das S, Krishnana P, Nayak M, Ramakrishnan B (2014) High temperature stress effects on pollens of rice (*Oryza sativa* L.) genotypes. *Environ Exp Bot* 101:36–46
- Endo M, Tsuchiya T, Hamada K, Kawamura S, Yano K, Ohshima M, Higashitani A, Watanabe M, Kobayashi MK (2009) High temperatures cause male sterility in rice plants with transcriptional alterations during pollen development. *Plant Cell Physiol* 50:1911–1922
- Fang X, Turner NC, Tan G, Li F, Siddique KHM (2010) Flower numbers, pod production, pollen viability and pistil function are reduced and flower and pod abortion increased in chickpea under terminal drought. *J Exp Bot* 61:335–345
- IPCC (2007) Intergovernmental panel on climate change. Fourth assessment report of the intergovernmental panel on climate change: the impacts, adaptation and vulnerability. Cambridge University Press, New York
- IPCC (2014) Climate change 2014: synthesis report. In: Pachauri RK, Meyer LA (eds) Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change. IPCC, Geneva, p 151
- Kilasi NL, Singh J, Vallejos CE, Ye C, Jagadish SVK, Kusolwa P, Rathinasabapathi B (2018) Heat stress tolerance in rice (*Oryza sativa* L.): identification of quantitative trait loci and candidate genes for seedling growth under heat stress. *Front Plant Sci* 9:1578
- Kumar N (2014) PhD thesis: physiological, biochemical and molecular characterization of some rice (*Oryza sativa* L.) genotypes in response to terminal heat stress submitted to GBPUA&T, Pantnagar, p 180
- Kumar N, Kumar N, Shukla A, Shankhdhar SC, Shankhdhar D (2015) Impact of terminal heat stress on pollen viability and yield attributes of rice (*Oryza sativa* L.). *Cereal Res Commun* 43(4):616–626
- Kumar N, Suyal DC, Sharma IP, Verma A, Singh H (2017) Elucidating stress proteins in rice (*Oryza sativa* L.) genotype under elevated temperature: a proteomic approach to understand heat stress response. *3 Biotech* 7:205
- Matsui T, Manigbas NL, Redona E (2009) Heat-induced floret sterility in rice: mechanisms of occurrence and tolerance. *Gamma Field Symp* 48:43–49
- Prasad PVV, Boote KJ, Allen JLH, Sheehy JE, Thomas JMG (2006) Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high-temperature stress. *Field Crop Res* 95:398–410
- Pressman E, Peet MM, Pharr DM (2002) The effect of heat stress on tomato pollen characteristics is associated with changes in carbohydrate concentration in the developing anthers. *Ann Bot* 90:631–636
- Shi PH, Zhu Y, Tang L, Chen JL, Sun T, Cao WX, Tian YC (2016) Differential effects of temperature and duration of heat stress during anthesis and grain filling stages in rice. *Environ Exp Bot* 132:28–41
- Singh H, Verma A (2013a) Characterization and screening of high nitrogen efficient rice genotype to curtail environmental pollution. *Int J Agric Environ Biotechnol* 6:23–30
- Singh H, Verma A (2013b) Physiological responses of rice cultivars to various nitrogen levels. *Int J Agric Environ Biotechnol* 6:383–388
- Singh H, Verma A, Krishnamoorthy M, Shukla A (2010a) Consequence of diverse nitrogen levels on leaf pigments in five rice genotypes under field emergent circumstance. *Int J Bio-resour Stress Manag* 1:189–193
- Singh H, Verma A, Shukla A (2010b) Comparative study of yield and yield components of hybrid and inbred genotypes of rice (*Oryza sativa* L.). *Int J Agric Environ Biotechnol* 3:355–360
- Singh DK, Agnihotri RK, Ganie SA, Singh G, Sharma R (2013) Pollen fertility and scanning electron microscopic studies of *Sida cordifolia* L. *Indian Res J Genet Biotechnol* 5:98–104
- Singh H, Verma A, Ansari MW, Shukla A (2014) Physiological response of rice (*Oryza sativa* L.) genotypes to elevated nitrogen applied under field conditions. *Plant Signal Behav* 9:e29015. <https://doi.org/10.4161/psb.29015>
- Tang RS, Zheng JC, Jin ZQ, Zhan DD, Huang YH, Chen LH (2008) Possible correlation between high temperature induced floret sterility and endogenous levels of IAA, Gas and ABA in rice (*Oryza sativa* L.). *Plant Growth Reg* 54:37–43
- Zhou S, Wang Y, Li W, Zhao Z, Ren Y, Wang Y, Gu S, Lin Q, Wang D, Jiang L, Su N, Zhang X, Liu L, Cheng Z, Lei C, Wang J, Guo X, Wu F, Ikehashi H, Wang H, Wana J (2011) Pollen semi-sterility1 encodes a kinesin-1-like protein important for male meiosis, anther dehiscence, and fertility in rice. *Plant Cell* 23:111–129