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Enhancement of Rice Pollen viability under Heat Stress by Osmoprotectant Foliar Spray

GBN Jyothi[1*], S Mohanty[2], S. Das[3], D. Lenka[4], T.R. Mohanty[5], J.K. Beura[6] and A. Moharana[7]
[1]Ph.D. Scholar, Seed Science and Technology, OUAT, Bhubaneswar (Odisha), India.
[2]Assistant Seed Research Officer, Seed Technology Research,
AICRP on Seed (Crops), OUAT, Bhubaneswar (Odisha), India.
[3]Odisha State Seed and Organic Products Certification Agency, Bhubaneswar (Odisha), India.
[4]Professor & Head, Department of Seed Science & Technology,
College of Agriculture, OUAT, Bhubaneswar (Odisha), India.
[5]Agrometrologist, AICRP on Agricultural Metrology, OUAT, Bhubaneswar (Odisha), India.
[6]Technical Assistant, Seed Technology Research, AICRP on Seed (Crops), Bhubaneswar (Odisha), India.
[7]Ph.D. Scholar, Seed Science and Technology, OUAT, Bhubaneswar (Odisha), India.

(Corresponding author: GBN Jyothi*)

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ABSTRACT: Loss of pollen viability by heat stress is a major problem which directly affect the seed set and yield of the crop. To mitigate the deleterious effects of heats stress on viability of rice pollen a study conducted for the enhancement of pollen viability by exogenous osmoprotectants spray in the cropping year 2018-19 and 2019-20 on three rice varieties Hiranmayee, Naveen and Mandakini by sowing in three different dates (S_1, S_2 and S_3). Heat stress gradually reduced pollen viability with rise of heat stress and delay of sowing, higher pollen viability recorded during 1st date of sowing followed by 2nd and 3rd date of sowing. As a result of the osmoprotectant foliar spray, the third date of sowing observed percent higher pollen viability over untreated control, compared to 1st and 2nd dates of sowing. Foliar spray of osmoprotectants significantly improved pollen viability especially with treatments like Salicylic acid 400 ppm (68.78%)followed by Ascorbic acid (10 ppm) + Citric acid (1.5%) (67.93%) and Salicylic acid 800 ppm (67.09%). Significant reduction of pollen viability under heat stress was noted in untreated Control (59.39%).

Keywords: Pollen viability, Heat stress, Osmoprotectants, Foliar spray.

INTRODUCTION

Rice (*Oryza sativa* L.) is more diversely grown food crop with environment ranging from rainfed, upland, irrigated, to deep water, unfortunately several complications including climatic concerns limit rice productivity (Das *et al.,* 2014). Regarding production, acreage, and consumption rice is one of the main staple food crops and demand for rice is predicted to rise to 2000 million people as the world population quickly increases (Cho *et al.,* 2012). The anticipated worldwide climate change would make rice farming much more vulnerable to global median surface temperature which is rising by 1.4-5.8°C by 2100, and the potential for increasing variability around this mean are couple of the potential effects of worldwide climate change (IPCC, 2007). Even the results from irrigated field trials carried out at the farm of the International Rice Research Institute from 1992 to 2003 revealed that every 1°C increase in minimum temperature during summers resulted in decrease of yield by 10% (Peng *et al.,* 2004). Onset of heat stress during the reproductive phase drastically reduce grain yield by more than 50% and even result in absolute loss of harvest in rice (Zang *et al.,* 2018; Fu *et al.,* 2012), especially the anthers are highly sensitive to heat stress during early developmental stages which result in programmed cell death and finally male sterility (Abiko *et al.,* 2005; Oshino *et al.,* 2007; Liu *et al.,* 2013; Nath *et al.,* 2022). Temperature changes have a significant impact on rice flowering and grain filling, which also affects fertilization by lowering pollen viability, seed production, and subsequently rice seed yield (Das *et al.,* 2014; Lakshmi *et al.,* 2022).

High temperatures can have a significant negative impact on pollen cells and microspores, which can lead to male sterility (Freeha *et al.,* 2008). Pollen germination and pollen tube growth are hampered by high temperatures, which lowers the viability of the pollen (Amrutha *et al.,* 2021). Temperatures above 35°C negatively impact the opening spikelets, flowering duration, and panicle expansion, and significantly decrease pollen viability, tube length, poor anther dehiscence, and lesser pollens on the stigma (Prasad *et al.,* 2006). Sterility in rice was reportedly caused by temperatures over 35°C at anthesis that persisted for longer than one hour (Jagadish *et al.,* 2010) and temperatures over 35°C for exactly 5 days during flowering leads entire floral sterility (Rang *et al.,* 2011). The flag leaf sheath's excess heat may have contributed to the spikelet's full sterility under high temperatures by preventing open air passage under the leaf sheath (Das *et al.,* 2014).

The generation of viable pollen from anthers is one of the crucial steps in plant sexual reproduction (Feng *et al.,* 2018). Anthers lose moisture due to evaporative stress caused by high temperatures, which further affects anther dehiscence, a necessary process for pollination (Matsui *et al.,* 2001). Pollen water content loss by 30–50% had a deleterious impact on cell differentiation as well as spikelet fertility (Liu *et al.,* 2006). Pollen grains swelled poorly in high temperatures resulting in improper anther dehiscence (Matsui *et al.,* 2001) and poor stigmatic germination, stigma should essentially receive 20 viable pollen grains for successful pollination (Matsui and Kagata 2003). But decline of pollen grain receptivity by stigmatic surface is direct cause of yield reduction under heat stress (Fahad *et al.,* 2016).

Rice crops adaption to temperature and climatic changes depends upon the carefully selection of varieties, crop duration and planting date. Hence, pollen viability study was conducted on both lowland and upland rice by growing in different six different temperature regimes (Das *et al.,* 2014). The present experiment on rice pollen viability study under heat stress was conducted on three rice varieties by taking three dates of sowing, so that at least one date of sowing escapes from heat stress and at least one date of sowing is exposed to heat stress, to compare the effect of heat stress on pollen viability in untreated control and by osmoprotectant foliar spay.

METHODOLOGY

The experiment was carried out during summer season of 2018-19 and 2019-20 in the Department of Seed Science and Technology, College of Agriculture, OUAT, Bhubaneswar. The experiment designed in Split-splitplot design with three replications, main plot factor being three varieties (Hiranmayee, Naveen and Mandakini), sub-plot factor being three dates of sowing (S_1 - 1st December, S_2 - 21st December and S_3 - 10th January) and sub-sub plot factor being foliar spray of osmoprotectants at vegetative and anthesis stage, *viz.,* T_0 - untreated Control, T_1 - Salicylic acid 400 ppm, T_2 - Salicylic acid 800 ppm, T_3 - Ascorbic acid 10 ppm, T_4 - Citric acid 1.5%, T_5 - Ascorbic acid 10 ppm + Citric acid 1.5%, T_6 - KCl 1%, T_7 - Thiourea 400 ppm, T_8 - Cycocel 1000 ppm and T_9 - Glycine betaine 600 ppm.

For pollen viability assessment in each treatment, ten individual florets from ten distinct random plants were collected at flowering stage during early hours of the day (7:00-8:00 AM), right before anthesis. The spikelets were delicately opened with a needle and forceps, and the anthers were carefully sliced and brushed onto a glass slide before being smeared with a drop of 2% Lugol solution and then gently crushed and examined under microscope. Pollen grains that were deeply stained were regarded viable. The number of stained pollen grains divided by the total number of pollen grains was used to calculate pollen viability, expressed as percentage.

RESULTS AND DISCUSSION

Observation of pollen viability recorded in both the planting season 2018-19 and 2019-20 and pooled data have been presented in Table1 and Fig. 1. The microscopic photographs of pollens have been presented in Plates 1 to 4. Among the three varieties used in the study Hiranmayee recorded higher mean pollen viability of 70.06%, followed by Naveen 65.31% and Mandakini 60.24%. In all three varieties, it was observed that pollen viability gradually decreased with delay of sowing date and increase in heat stress. First date of sowing (1st December) produced highest mean pollen viability of 66.98%, followed by second date of sowing (21st December) (65.60%) and third date of sowing (10th January) (63.02%). Das *et al.* (2020) reported similar findings that there was gradual decrease of pollen viability with delay of sowing time and maximum percent pollen viability observed during 1st sowing time than 2nd and 3rd dates of sowing time. In rice crop 78.8 % decrease of pollen viability under heat stress (Feng *et al.,* 2018). Sakata *et al.* (2010) stated that pollen development arrested when the crop grown under 30-35°C.

All the osmoprotectant foliar spray treatment were influential in improving pollen viability under heat stress in all the three varieties and three sowing dates, the percent increase over Control varies among treatments. Salicylic acid 400 ppm (T_1) recorded 68.78% pollen viability which is of 15.88% higher viability over untreated Control and the same treatment during 3rd date of sowing shown 66.64% pollen viability which is 17.91% higher over untreated Control, followed by Ascorbic acid 10 ppm + Citric acid 1.5% (T_5) (67.93%) recorded 14.45% increase of viability over untreated Control and Salicylic acid 800 ppm (T_2) also improved pollen viability by 67.09% which is 13.44% higher over Control. Least percent pollen viability found under untreated Control of 59.39%. Similar results were also noted by Das *et al.* (2020) that significant higher pollen viability of rice recorded with Salicylic acid 400 ppm osmoprotectant foliar spray treatment during 1st, 2nd and 3rd date of sowing times. Zhang *et al.* (2018) also noted that rice plants sprayed with different concentrations of Salicylic acid (SA) 0.01, 0.1, 1.0, 10- and 50-mM during pollen meiotic stage and found SA 0.1- and 1.0-mM treatments significantly improved the pollen viability under heat stress over no SA treatment under heat stress and noted that the decrease of pollen viability under HT in SA treated plants was 58.6%, 45.6% and 47.9% while 78.8% decrease noted from no SA treatment (Feng *et al.,* 2018). Exogenous SA application reduced the accumulation of ROS production and stopped programmed cell death. A positive effect of exogenous application 400 mM Ascorbic acid treatment was observed on pollen viability under HS enhances the viability of pollen (Kumar *et al.,* 2013).

Table 1: Pollen viability of three rice varieties grown in summer season (pooled over two years) as influenced by date of sowing and spray treatments.

Treatments	Pollen viability (%)															
	Hiranmayee (V_1)				Naveen (V_2)				Mandakini (V_3)				Mean S_1T	Mean S_2T	Mean S_3T	Mean T
	S_1	S_2	S_3	Mean V_1T	S_1	S_2	S_3	Mean V_2T	S_1	S_2	S_3	Mean V_3T				
T_0	67.57 (55.29)	64.26 (53.31)	59.83 (50.67)	**63.88 (53.09)**	61.85 (51.89)	59.25 (50.33)	58.22 (49.74)	**59.77 (50.66)**	56.68 (48.85)	55.34 (48.07)	51.50 (45.86)	**54.51 (47.59)**	62.03 (52.01)	59.61 (50.57)	56.51 (48.76)	**59.39 (50.45)**
T_1	75.67 (60.46)	74.57 (59.78)	70.72 (57.25)	**73.65 (59.16)**	69.78 (56.67)	68.74 (56.02)	67.82 (55.45)	**68.78 (56.05)**	65.67 (54.14)	64.67 (53.53)	61.37 (51.58)	**63.90 (53.08)**	70.37 (57.09)	69.33 (56.44)	66.64 (54.76)	**68.78 (56.10)**
T_2	73.94 (59.31)	73.07 (58.77)	68.83 (56.09)	**71.95 (58.06)**	68.36 (55.78)	66.84 (54.86)	66.25 (54.50)	**67.15 (55.05)**	63.95 (53.11)	62.89 (52.48)	59.74 (50.62)	**62.19 (52.07)**	68.75 (56.07)	67.60 (55.37)	64.94 (53.74)	**67.09 (55.06)**
T_3	71.26 (57.61)	70.14 (56.89)	66.55 (54.68)	**69.32 (56.39)**	65.93 (54.30)	64.02 (53.15)	63.69 (52.95)	**64.54 (53.47)**	61.29 (51.53)	60.22 (50.90)	56.72 (48.86)	**59.41 (50.43)**	66.16 (54.48)	64.79 (53.65)	62.32 (52.16)	**64.42 (53.43)**
T_4	71.84 (57.96)	70.80 (57.30)	67.27 (55.12)	**69.97 (56.79)**	66.55 (54.68)	64.60 (53.50)	64.27 (53.30)	**65.14 (53.83)**	61.86 (51.87)	60.78 (51.23)	57.38 (49.25)	**60.00 (50.78)**	66.75 (54.84)	65.39 (54.01)	62.97 (52.56)	**65.04 (53.80)**
T_5	74.76 (59.88)	73.94 (59.34)	69.76 (56.67)	**72.82 (58.63)**	69.08 (56.23)	67.75 (55.41)	66.95 (54.92)	**67.93 (55.52)**	64.78 (53.61)	63.74 (52.98)	60.64 (51.15)	**63.05 (52.58)**	69.54 (56.57)	68.48 (55.91)	65.78 (54.24)	**67.93 (55.58)**
T_6	73.19 (58.84)	72.27 (58.25)	68.91 (56.14)	**71.45 (57.74)**	67.71 (55.39)	66.00 (54.35)	65.60 (54.11)	**66.43 (54.61)**	63.19 (52.65)	62.11 (52.02)	58.89 (50.13)	**61.40 (51.60)**	68.03 (55.63)	66.79 (54.87)	64.46 (53.46)	**66.43 (54.65)**
T_7	70.71 (57.26)	69.55 (56.52)	65.88 (54.27)	**68.71 (56.01)**	65.43 (53.99)	63.49 (52.83)	63.28 (52.71)	**64.06 (53.18)**	60.77 (51.22)	59.71 (50.60)	56.13 (48.52)	**58.87 (50.12)**	65.64 (54.16)	64.25 (53.31)	61.76 (51.83)	**63.88 (53.10)**
T_8	70.23 (56.95)	69.01 (56.20)	65.31 (53.93)	**68.18 (55.69)**	64.93 (53.70)	63.03 (52.56)	62.65 (52.34)	**63.53 (52.86)**	60.30 (50.95)	59.25 (50.33)	55.61 (48.23)	**58.39 (49.84)**	65.15 (53.87)	63.76 (53.03)	61.19 (51.50)	**63.37 (52.80)**
T_9	72.49 (58.37)	71.51 (57.75)	68.07 (55.61)	**70.69 (57.24)**	67.10 (55.01)	65.26 (53.90)	64.81 (53.64)	**65.72 (54.18)**	62.49 (52.25)	61.41 (51.64)	58.10 (49.67)	**60.67 (51.19)**	67.36 (53.87)	66.06 (54.43)	63.66 (52.97)	**65.69 (54.20)**
Mean VS	**72.16 (58.19)**	**70.91 (57.41)**	**67.11 (55.04)**	**70.06 (56.88)**	**66.67 (54.76)**	**64.90 (53.69)**	**64.35 (53.37)**	**65.31 (53.94)**	**62.10 (52.02)**	**61.01 (51.38)**	**57.61 (49.39)**	**60.24 (50.93)**	**66.98 (54.99)**	**65.60 (54.16)**	**63.02 (52.60)**	**65.20 (53.92)**
	V	**S**	**T**	**V × S**	**S × V**	**V × T**	**T × V**	**S x T**	**T x S**	**T x VS**	**S x VT**	**V x ST**				
SEd(±)	**0.129**	**0.151**	**0.206**	**0.611**	**0.261**	**0.965**	**0.357**	**3.738**	**0.357**	**0.618**	**1.724**	**2.956**				
CD 5%	**0.407**	**0.435**	**0.572**	**1.907**	**0.783**	**NS**	**1.009**	**NS**	**1.009**	**1.747**	**5.014**	**8.749**				

Figures in the parentheses are arc sine transformed values

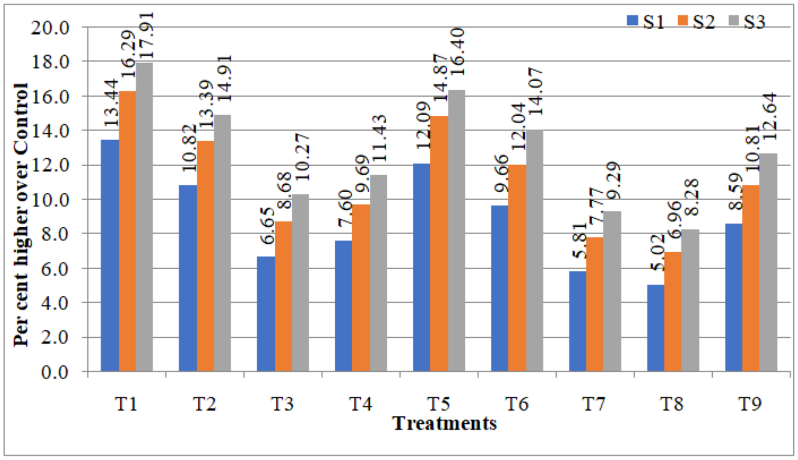
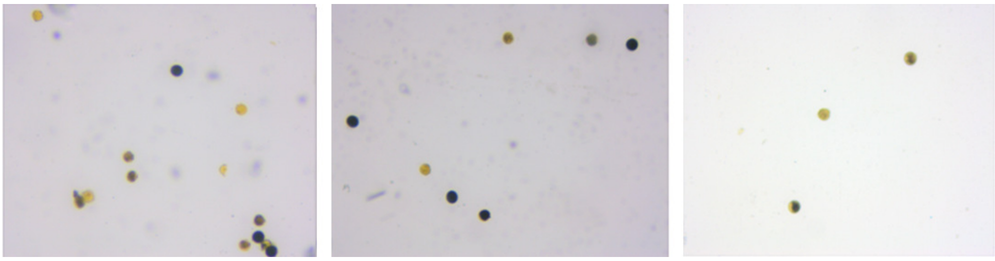
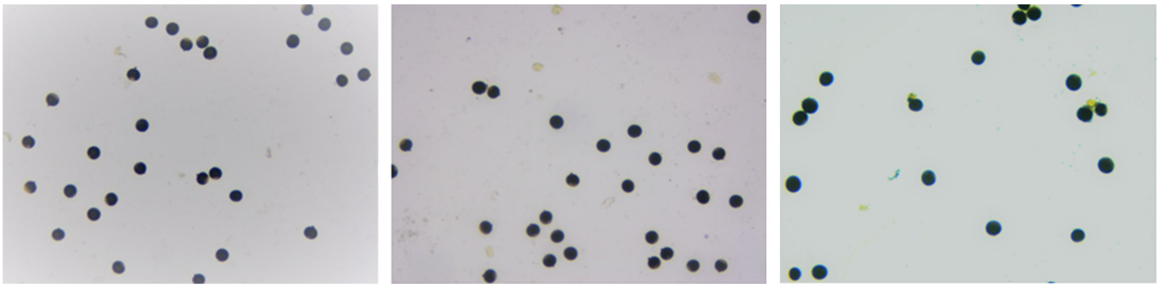


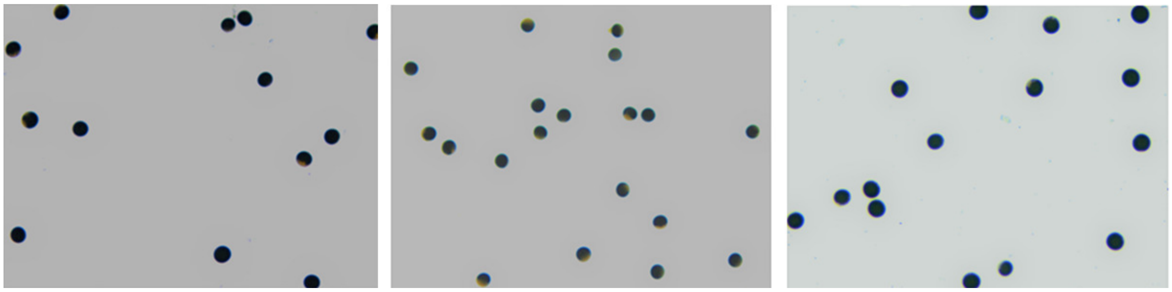
Fig. 1. Graph depicts the pollen viability percent higher over Control during three dates of sowing.



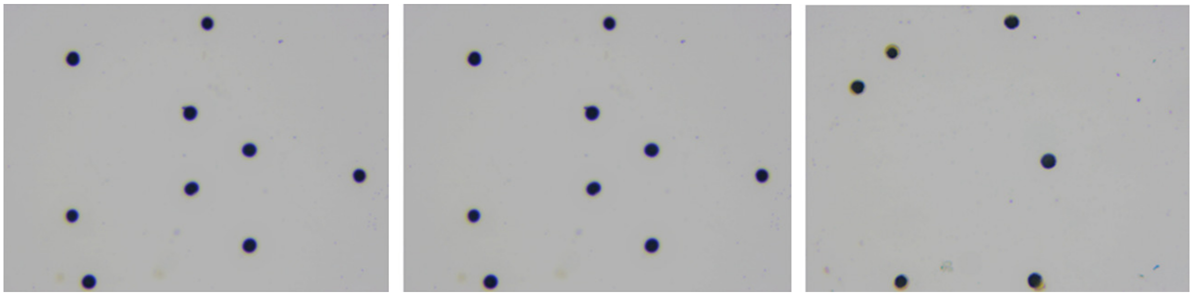
Plates 1: (a), (b) and (c) depict the pollen viability of Control from 1st, 2nd and 3rd dates of sowing.



Plates 2: (a), (b) and (c) depict the pollen viability of SA 400 ppm from 1st, 2nd and 3rd dates of sowing.



Plates 3: (a), (b) and (c) depict the pollen viability of Ascorbic acid (10 ppm) + Citric acid (1.5%) from 1st, 2nd and 3rd dates of sowing.



Plates 4: (a), (b) and (c) depict the pollen viability of SA 800 ppm from 1st, 2nd and 3rd dates of sowing.

CONCLUSION

Pollen grains are highly sensitive to heats stress because of desiccation, heat stress gradually reduce pollen viability which effect the pollination and fertilization of plant. Osmoprotectant foliar spray treatments maintained the viability of pollen grain even under heat stress condition among the treatments significant higher pollen viability recorded from the treatments Salicylic acid 400 ppm followed by Ascorbic acid (10 ppm) + Citric acid (1.5%) and Salicylic acid 800 ppm, whereas the lowest pollen viability of all treatments recorded from untreated Control.

FUTURE SCOPE

Pollen viability have a significant role in pollination and fertilization without which grain formation will not occur which reduces the yield, so pollen viability study can be undertaken while breeding the crop in all possible aspects such as genetical, physiological, morphological and biochemical study of the genotypes which helps in estimation of genotypes or varieties performance under various stress conditions.

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Conflict of Interest. None.

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