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Exogenous Osmoprotectants Enhance Growth and Yield of Heat-Stressed Rice

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ABSTRACT

Heat stress is the crucial cause of yield loss due to the global shift in the ideal temperature caused by climate change. Maintenance of osmotic potential even under heat stress condition is vital to carry out normal growth activities and yield. Present study proved that application of osmoprotectants ameliorates deleterious effect of heat stress on rice cv. Mandakini sown in three separate sowing dates S_1, S_2 and S_3 (low, medium, and high heat stress). Osmoprotectants foliar spray has given at both vegetative and anthesis stage in all the three sowing dates. The periodic observations indicate that all osmoprotectants treatments significant in mitigating deleterious effects of heat stress, among which Salicylic acid 800 ppm and Salicylic acid 400 ppm are more effective in improvement of Plant height, Total tillers per plant, Effective tillers per plant, Panicle length, 1000-seed weight, Seed set percentage, Seed yield per plant and Seed yield per hectare. Whereas pollen viability express significant difference with the application of Salicylic acid 400 ppm and Ascorbic acid (10 ppm) + Citric acid (1.5%). The osmoprotectants effect more pronounced during S_2 and S_3 which are highly affected by heat stress as compared to S_1, the decrease of growth and yield attributing characters was observed in untreated control and delay of sowing date.

Keywords Mandakini, Salicylic acid, Citric acid, Ascorbic acid, KCl.

INTRODUCTION

Rice is the most significant food crop in the world. In India and many other countries, rice is a critical component of the long-term food production chain for more than 50% of the global population as a staple diet and source of nutritional security (Sandhu *et al*. 2017). Future crop production goals affected by changing climatic scenario by effecting crop productivity severely (Das *et al*. 2020). Projected increases in the average world temperature may have significant

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negative impact on crop productivity (Bailey *et al*. 2019, Prasad *et al*. 2008, Porter *et al*. 2005). Heat stress described as an increase in temperature that lasts for a prolonged length of time and permanently harms plant growth and improvement (Wahid *et al*. 2007). When the seasonal average temperature was increased by 1°C, it was discovered that grain yields in cereals decreased from 4.1% to 10.0% (Hasanuzzaman *et al*. 2013). Most of the rice is cultivated in areas where the temperature has already been close to what is ideal for rice farming; as a result, any further increases in mean temperature or sudden bursts of intense heat during crucial stages may be supra-optimal and result in decreased grain yield. The yield of rice expected to be 41% lower by the end of the twenty-first century (Ceccarelli *et al*. 2010, Shah *et al*. 2011).

Heat stress significantly affects rice spikelet fertility and grain quality during the flowering and grain-filling stages, flowering stage of rice is most susceptible to heat stress temperatures between 32-36°C result in high spikelet sterility (Satake and Yoshida 1978). In heat-susceptible rice plants, prolonged heat exposure of four days or more during the pilot stages of anther development promotes premature tapetum cell disintegration and programmed cell death (PCD), resulting in male sterility (Abiko *et al*. 2005, Oshino *et al*. 2007). Vapor pressure during elevated temperature stress leads to evaporative loss (moisture) from anther, thereafter, affected anther dehiscence which required for pollination (Matsui *et al*. 2000). Pollen water content reduction of 30–50% had a deleterious impact on cell differentiation as well as spikelet fertility (Liu *et al*. 2006). Inadequate assimilation and the nutritional competition among grains in high temperature conditions resulted in an increase in the rate of rice grain filling and a decrease in the duration of grain ripening (Kobata and Uemuki 2004). The crop development and the effective conversion of biomass into commercial yield are determined by the time of sowing. Compared to late sowing, which delivers lower grain and biological yields, normal sowing has a longer growing period, giving the opportunity to gain more yield (Singh and Usha 2003).

These requirements for the use of several techniques to reduce heat stress by choosing suitable variety, ideal time to plant, and foliar applications of osmoprotectants. Based on their unique characteristics and functions, a variety of nitrogenous substances, inorganic salts, organic and inorganic plant growth regulators, and stress signaling molecules used to enhance the growth of grain, forage, and horticultural crops (Farooq *et al*. 2009). Applying exogenous osmoprotectants like salicylic acid, glycine betaine, ascorbic acid, citric acid and potassium chloride, which are able to bring longstanding heat tolerance in plants and can be helpful in mitigating the threats of yield reduction and are helpful in producing good quality grains, can help manage heat stress (Gitte *et al*. 2018 and Das *et al*. 2020). To develop a strategy

Table 1. Plant height of rice cv Mandakini grown in summer season as influenced by date of sowing and spray treatments (pooled over two years).

Treatments	Plant height (cm)			
	S_1	S_2	S_3	Mean
T_0	125.86	125.39	124.90	125.38
T_1	129.34	128.91	128.46	128.90
T_2	129.83	129.47	129.14	129.48
T_3	127.80	127.34	126.74	127.29
T_4	127.28	126.80	126.33	126.80
T_5	127.77	127.46	126.80	127.34
T_6	128.11	127.61	127.13	127.61
T_7	127.45	126.50	126.11	126.68
T_8	127.64	127.12	126.67	127.14
T_9	127.29	126.65	126.17	126.70
Mean	127.83	127.32	126.84	127.33
	S	T	S x T	
SEd (±)	0.191	0.448	8.087	
CD 5%	0.553	1.245	NS	

Table 2. Total tillers per plant of rice cv Mandakini grown in summer season as influenced by date of sowing and spray treatments (pooled over two years).

Treatments	Total tillers per plant			
	S_1	S_2	S_3	Mean
T_0	7.75	7.01	6.65	7.14
T_1	10.40	9.99	9.45	9.94
T_2	10.80	10.38	9.84	10.34
T_3	9.06	8.68	8.21	8.65
T_4	8.74	8.27	7.81	8.27
T_5	10.05	9.55	9.10	9.57
T_6	9.40	8.89	8.15	8.81
T_7	8.62	8.11	7.50	8.07
T_8	8.96	8.41	7.88	8.42
T_9	8.43	7.90	7.17	7.83
Mean	9.22	8.72	8.17	8.70
	S	T	S x T	
SEd (±)	0.052	0.043	0.793	
CD 5%	0.151	0.120	NS	

Table 3. Effective tillers per plant of rice cv Mandakini grown in summer season as influenced by date of sowing and spray treatments (pooled over two years).

Treatments	Effective tillers per plant			Mean
	S_1	S_2	S_3	
T_0	7.60	6.95	6.53	7.03
T_1	10.55	10.04	9.55	10.04
T_2	10.85	10.38	9.94	10.39
T_3	8.99	8.21	7.81	8.33
T_4	8.25	7.81	7.38	7.81
T_5	10.05	9.45	9.05	9.52
T_6	9.05	8.49	7.96	8.50
T_7	8.22	7.62	7.10	7.64
T_8	8.56	8.01	7.59	8.05
T_9	8.05	7.40	6.77	7.41
Mean	9.01	8.43	7.97	8.47
	S	T	S x T	
SEd (±)	0.067	0.062	1.139	
CD 5%	0.192	0.173	NS	

Table 4. Panicle length of rice cv Mandakini grown in summer season as influenced by date of sowing and spray treatments (pooled over two years).

Treatments	Panicle length (cm)			Mean
	S_1	S_2	S_3	
T_0	24.00	23.44	23.16	23.53
T_1	26.17	25.82	25.44	25.81
T_2	26.37	25.98	25.58	25.98
T_3	25.89	25.37	25.06	25.44
T_4	25.03	24.55	24.27	24.62
T_5	26.02	25.58	25.22	25.60
T_6	25.97	25.49	25.12	25.53
T_7	25.54	24.72	24.67	24.98
T_8	25.32	24.69	24.45	24.82
T_9	24.93	*24.24	24.13	24.43
Mean	25.52	24.99	24.71	25.07
	S	T	S x T	
SEd (±)	0.062	0.089	1.617	
CD 5%	0.178	0.248	NS	

Table 5. 1000-seed weight of rice cv Mandakini grown in summer season as influenced by date of sowing and spray treatments (pooled over two years).

Treatments	1000-seed weight (g)			Mean
	S_1	S_2	S_3	
T_0	24.46	23.49	22.32	23.42
T_1	26.55	25.79	24.77	25.70
T_2	26.61	25.89	24.89	25.79
T_3	26.30	25.50	24.47	25.42
T_4	26.08	25.26	24.18	25.17
T_5	26.47	25.70	24.68	25.62
T_6	26.40	25.60	24.60	25.53
T_7	25.94	25.10	24.05	25.03
T_8	26.20	25.38	24.34	25.30

Table 5. Continued.

Treatments	1000-seed weight (g)			Mean
	S_1	S_2	S_3	
T_9	25.83	24.97	23.90	24.90
Mean	26.08	25.27	24.22	25.19
	S	T	S x T	
SEd (±)	0.272	0.079	1.666	
CD 5%	0.787	0.220	NS	

Table 6. Pollen viability (%) of rice cv Mandakini grown in summer season as influenced by date of sowing and spray treatments (pooled over two years). * Values in braces are square root transformation values.

Treatments	Pollen viability (%)			Mean
	S_1	S_2	S_3	
T_0	56.68	55.34	51.50	54.51
	(48.85)	(48.07)	(45.86)	(47.59)
T_1	65.67	64.67	61.37	63.90
	(54.14)	(53.53)	(51.58)	(53.08)
T_2	63.95	62.89	59.74	62.19
	(53.11)	(52.48)	(50.62)	(52.07)
T_3	61.29	60.22	56.72	59.41
	(51.53)	(50.90)	(48.86)	(50.43)
T_4	61.86	60.78	57.38	60.00
	(51.87)	(51.23)	(49.25)	(50.78)
T_5	64.78	63.74	60.64	63.05
	(53.61)	(52.98)	(51.15)	(52.58)
T_6	63.19	62.11	58.89	61.40
	(52.65)	(52.02)	(50.13)	(51.60)
T_7	60.77	59.71	56.13	58.87
	(51.22)	(50.60)	(48.52)	(50.12)
T_8	60.30	59.25	55.61	58.39
	(50.95)	(50.33)	(48.23)	(49.84)
T_9	62.49	61.41	58.10	60.67
	(52.25)	(51.64)	(49.67)	(51.19)
Mean	56.68	55.34	51.50	54.51
	(48.85)	(48.07)	(45.86)	(47.59)
	S	T	S x T	
SEd (±)	0.151	0.206	3.738	
CD 5%	0.435	0.572	NS	

that uses osmoprotectants to boost development and productivity of rice crop sown in different sowing dates (Normal, medium and late sown) under heat stress, a necessary study was formulated to determine the role of osmoprotectants in mitigating injurious outcome of heat stress in rice crop.

MATERIALS AND METHODS

A field experiment organized in the summer season of 2018-19 and 2019-20 in the Department of Seed Science and Technology, College of Agriculture, OUAT, Bhubaneswar. Experimentation laid out in Split Plot

Table 7. Seed set (%) of rice cv Mandakini grown in summer season as influenced by date of sowing and spray treatments (pooled over two years). * Values in braces are square root transformation values.

Treatments	Seed set (%)			Mean
	S_1	S_2	S_3	
T_0	83.70 (9.15)	77.55 (8.81)	76.98 (8.77)	79.41 (8.91)
T_1	89.68 (9.47)	88.19 (9.39)	85.99 (9.27)	87.95 (9.38)
T_2	90.74 (9.53)	89.56 (9.46)	87.06 (9.33)	89.12 (9.44)
T_3	88.90 (9.43)	87.65 (9.36)	83.91 (9.16)	86.82 (9.32)
T_4	88.21 (9.39)	86.81 (9.32)	83.60 (9.14)	86.20 (9.28)
T_5	89.68 (9.47)	88.19 (9.39)	85.98 (9.27)	87.95 (9.38)
T_6	89.02 (9.43)	87.99 (9.38)	85.40 (9.24)	87.47 (9.35)
T_7	86.95 (9.32)	84.94 (9.22)	82.61 (9.09)	84.83 (9.21)
T_8	88.20 (9.39)	86.82 (9.32)	83.60 (9.14)	86.21 (9.28)
T_9	86.95 (9.32)	84.95 (9.22)	82.61 (9.09)	84.83 (9.21)
Mean	88.20 (9.39)	86.26 (9.29)	83.77 (9.15)	86.08 (9.28)
	S	T	S x T	
SEd (±)	0.008	0.009	0.167	
CD 5%	0.023	0.025	0.487	

Design with three replications, main plot factor being three dates of sowing (S_1-1st December, S_2- 21st December and S_3- 10th January) and sub-plot factor being foliar spray of osmoprotectants at vegetative

Table 8. Seed yield per plant of rice cv Mandakini grown in summer season as influenced by date of sowing and spray treatments (pooled over two years).

Treatments	Seed yield (g/plant)			Mean
	S_1	S_2	S_3	
T_0	13.25	10.98	9.26	11.16
T_1	15.51	14.41	12.31	14.07
T_2	15.55	14.48	12.38	14.14
T_3	15.15	14.05	11.93	13.71
T_4	15.04	13.84	11.87	13.58
T_5	15.43	14.29	12.10	13.94
T_6	14.97	13.57	11.47	13.34
T_7	15.04	14.07	12.00	13.70
T_8	15.07	14.01	11.82	13.63
T_9	14.75	13.65	11.52	13.31
Mean	14.98	13.73	11.66	13.46
	S	T	S x T	
SEd (±)	0.059	0.052	0.960	
CD 5%	0.172	0.145	2.826	

Table 9. Seed yield per hectare of rice cv Mandakini grown in summer season as influenced by date of sowing and spray treatments (pooled over two years).

Treatments	Seed yield (q/ha)			Mean
	S_1	S_2	S_3	
T_0	41.08	35.73	32.98	36.60
T_1	47.20	44.37	43.02	44.86
T_2	48.97	46.77	44.33	46.69
T_3	45.62	42.51	40.24	42.79
T_4	43.59	39.58	37.03	40.06
T_5	46.13	42.43	40.22	42.93
T_6	45.55	41.69	39.33	42.19
T_7	42.95	37.79	35.81	38.85
T_8	44.16	40.39	39.39	41.31
T_9	41.98	37.56	34.98	38.18
Mean	44.72	40.88	38.73	41.44
	S	T	S x T	
SEd (±)	0.180	0.197	3.583	
CD 5%	0.519	0.546	10.485	

and anthesis stage along with one untreated control. Treatments like 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), T_9-Glycine betaine (600 ppm) and T_{10}-Control were used, and rice variety Mandakini (105 days duration) was selected for the experiment. The observations like Plant height, Total tillers per plant, Effective tillers per plant, Panicle length,1000-seed weight, Pollen viability, Seed set percentage, Seed yield per plant and Seed yield per

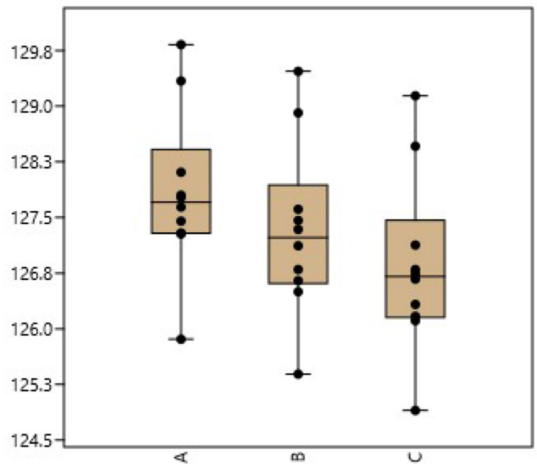

Fig. 1. Depicts the impact of exogenous osmoprotectants on plant height. The sowing timings were displayed on the X axis together with the treatments values on the Y axis. T_0 -Lowest, T_2 -Highest.

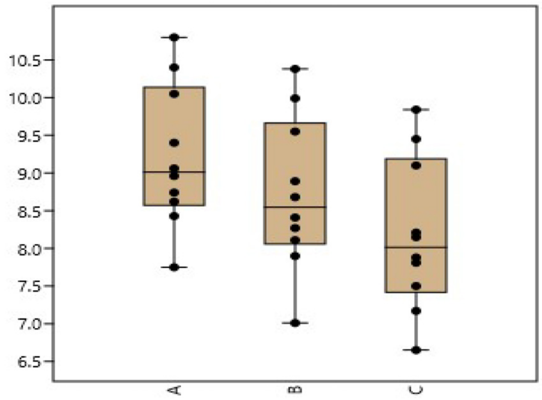

Fig. 2. Depicts the impact of exogenous osmoprotectants on total tillers palnt[-1]. The sowing timings were displayed on the X axis together with the treatments values on the Y axis. T_0 -Lowest, T_2- Highest.

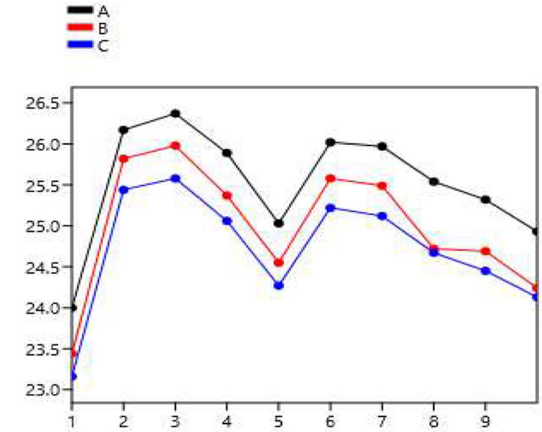

Fig. 4. Depicts the impact of exogenous osmoprotectants on Panicle length. The sowing timings were displayed on the X axis together with the treatments values on the Y axis. T_0 -Lowest, T_2- Highest.

hectare recorded.

RESULTS AND DISCUSSION

Observation on growth and yield attributing parameters along with seed yield were taken, and pooled analysis data of two planting years have been presented in Tables 1 - 9 and Figs. 1 - 9. The results indicated the growth and yield attributing parameters decrease with delay of sowing date because late sowing met with more heat stress compared to normal sowing, significantly higher Plant height, Total tillers per plant, Effective tillers per plant, Panicle length, 1000-seed weight, Pollen viability, Seed set percentage, Seed yield per plant and Seed yield per hectare were recorded from 1st date of sowing (1st December) followed by 2nd date of sowing (21st December) and 3rd date of sowing (10th January). Similar findings were noted by Gitte *et al*. (2018) wheat cv NLAW-3096 sown in three different durations 25th November, 15th December and 5th January and maximum seed yield

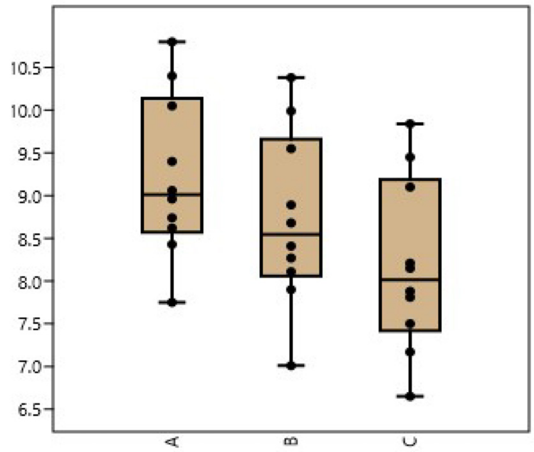

Fig. 3. The effect of exogenous osmoprotectants on effective tillers palnt[-1] is displayed in Fig. 3. The X axis showed the sowing dates, while the Y axis showed the treatments' values. T_2 - Highest, T_0 - Lowest.

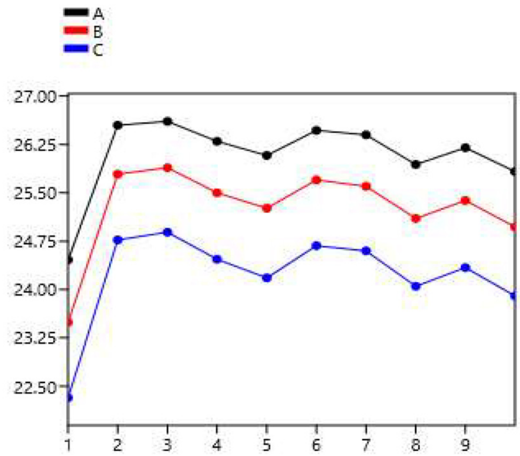

Fig. 5. Depicts how exogenous osmoprotectants affected the weight of 1000 seeds. The seed weight values were shown on the Y axis with the treatment values (X axis, 1- to 10). T_0 - Lowest, T_2 – Highest.

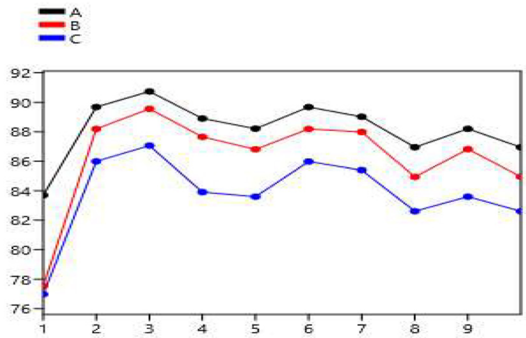

Fig. 6. Depicts how exogenous osmoprotectants affected the seed set %. The seed weight values were shown on the Y axis with the treatment values (X axis, 1- to 10). T_0 - Lowest, T_2 - Highest

and seed set percentage were noted from 1st date of sowing (25th November). Das *et al.* (2020) experimented on rice cv Naveen by planting in three dates of sowing D_1- 30th November, D_2- 15th December and D_3- 30th December, and found that among the three dates of sowing D_1 (26.32 g) recorded maximum 1000-seed weight and yield over D_2 and D_3 (20.27g and 19.36g, respectively).

Foliar spray of osmoprotectants found influential in mitigating heat stress by improving overall growth and yield among which T_2-Salicylic acid 800 ppm significantly improved all growth and yield attributing parameters like Plant height, Total tillers per plant,

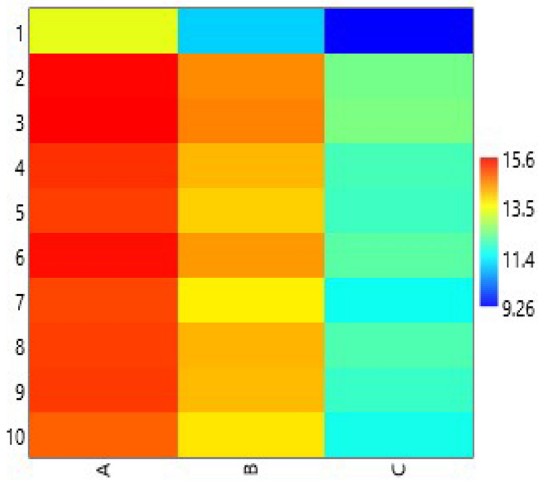

Fig. 7. Matrix plot depicted seed yield plant-1 dependent on color brightness it showed the effect and yield values highest values showed brighter color while lower values showed lower brightness. T_0 - Lowest, T_2 - Highest.

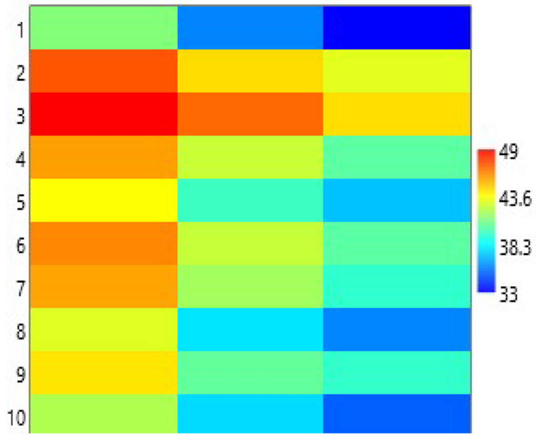

Fig. 8. Shows a matrix plot of the relationship between seed yield and color brightness. Displayed the results and values lower numbers displayed less brightness while the highest levels displayed brighter colors. T_0: The lowest, T_2: The highest (x- Treatments, X- Sowing times).

Effective tillers per plant, Panicle length,1000-seed weight, Seed set percentage, Seed yield per plant and Seed yield per hectare over untreated Control, followed by T_1- Salicylic acid 400 ppm and T_5-Ascorbic acid (10 ppm) + Citric acid (1.5%). Whereas significant Pollen viability noted from T_1-Salicylic acid 400 ppm followed by T_5-Ascorbic acid (10 ppm) + Citric acid (1.5%) and T_2-Salicylic acid 800 ppm, improved pollen viability led to more seed set under heat stress, so pollen viability maintenance under heats stress is key factor for yield improvement under stress. The sowing and treatment interaction of Percent seed set, Seed yield per plant and Seed yield per hectare

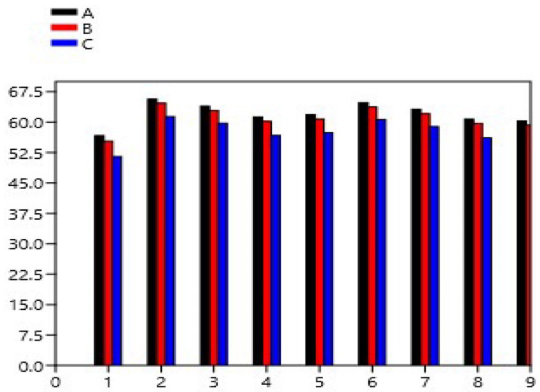

Fig. 9. Shows a graphical representation of pollen viability at 3 sowing times on X axis 1-10 treatments and Y axis represents pollen viability values. T_0: Lowest, T_2: Aighest.

found significant. Gitte *et al*. (2018) used chemical treatments like Glycine betaine-600ppm, Salicylic acid-400 ppm, Salicylic acid-800 ppm, Ascorbic acid (10 ppm) +Citric acid (1.3%), KCl 1% to mitigate heat stress of wheat and found that Ascorbic acid (10 ppm) +citric acid (1.3%) treatment significant in improved seed set percentage and seed yield. Das *et al*. (2019) also found that spraying osmoprotectants like Glycine betaine-600ppm, Salicylic acid-400 ppm, Salicylic acid-800 ppm, Ascorbic acid (10 ppm) +Citric acid (1.3%), KCl 1% are effective in mitigating high temperature stress of rice crop cv Naveen, among all treatments. Salicylic acid 800 ppm and Ascorbic acid (10 ppm) +Citric acid (1.3%) are significant in improving 1000- seed weight, pollen viability and yield over control. Citric acid 100 mg/L treatment improved plant height and number of tillers per plant compared to control in two dates of sowing (Sadak *et al*. 2015). Plant treatment with 50 ppm salicylic acid improved panicle length of wheat by 6.9% and 3.2% in both cropping year 2012 and 2013(Sandhu *et al*. 2017). With delay in sowing, there was drastic reduction in the seed yield. Among the treatments, T_2 and T_1 gave the highest seed yields (46.69 q/ha and 44.86 q/ha respectively), followed by T_5 and T_3 (42.93 q/ha and 42.79 q/ha, respectively). The seed yields from S_3T_0 and S2T0 (38.73 q/ha and 40.88 q/ha, respectively) were significantly lower than all the other treatments, as well as S_1T_0 (44.72 q/ha). When compared to the control (T_0), the increase in seed yields brought by the various treatments was more noticeable on the second and third dates of planting. Yield and yield attributing parameters enhanced by salicylic acid (100 mg/L) foliar spray (Amin *et al*. 2008).

CONCLUSION

In usually growth and yield of crop will reduce under heat stress and delay of sowing but spraying of Salicylic acid 400 ppm, Salicylic acid 800 ppm and Ascorbic acid (10 ppm) + Citric acid (1.5%) treatments improved growth, yield attributing characters and grain yield. This may be due to boosting of osmotic potential, activation of antioxidants to counter ROS and enhanced photosynthesis of rice crop by exogenous salicylic acid application which helps in heat stress mitigation.

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