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Growth, Yield and Physiology of Wheat Influenced by Osmoprotectants Application and Different Sowing Dates

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ABSTRACT

Background: With climate change intensifying heat episodes, developing heat-tolerant wheat varieties and adopting heat-mitigating agronomic practices such as optimal sowing dates and use of osmoprotectants has become essential for sustaining wheat production.

Methods: The field experiment was conducted during *Rabi* season 2022-23 at Research farm Department of Agriculture, Guru Nanak Dev University, Amritsar to study the effect of sowing dates and foliar application of osmo-protectants on growth, physiology and yield of wheat (*Triticum aestivum* L.). The experiment was conducted using a split-plot design, with sowing dates (October-20, November 20 and December 20) assigned to the main plots and foliar application (Control, water spray, potassium nitrate @ 2%, salicylic acid (100 ppm), sodium nitroprusside @ 800 µg/ml, zinc sulphate heptahydrate @ 1%) to the sub plots. The experiment had three replications.

Result: Growth parameters including plant height, leaf area index and dry matter accumulation were significantly higher in early sowing (October 20), while maximum emergence count and yield were recorded under November 20 sowing. Late sowing (December 20) resulted in reduced pollen viability, membrane stability index, relative water content and yield, primarily due to exposure to higher temperatures at the reproductive stage. Among osmo-protectants, sodium nitroprusside (SNP @ 800 µg/ml) and salicylic acid (100 ppm) significantly enhanced pollen viability, proline accumulation, relative water content and grain yield compared to control and other treatments.

Key words: Grain yield, Heat stress, Osmo-protectants, Sowing dates, Wheat.

INTRODUCTION

In India, most of the wheat growing areas falls under the tropical and subtropical regions, the climate change transforming them into heat stressed and short season environments (Beta and Gerats, 2013). The recent studies on the climate change reported that the earth's average global temperature is projected to rise by about 1.5°C over the next two decades (IPCC, 2021). Recent assessments by scientific organizations, including the Goddard Institute for Space Studies (GISS), have reported that the planet's mean temperature increased by approximately 1.04°C between 1880 and 2019, according to National Oceanic and Atmospheric Administration (2020) (Yadav *et al.,* 2022). In wheat, increase in the temperature beyond the optimum range 12-22°C, causing terminal heat stress and led to yield loss of wheat (18-34%) (Dwivedi *et al*., 2017). Wheat is comparatively more susceptible to the effects of extreme weather conditions, such as temperature increase and drought periods (Daryanto *et al*., 2016; Akter and Islam, 2017). The occurrence of heat stress results into the changes in physiological and molecular processes, including changes in photosynthesis, accumulation of lipids and transcript expression, which further effect the reproductive traits and yield determination (Prasad *et al.,* 2011; Mirosavljevi *et al.,* 2021). Heat stress commonly initiates the generation of reactive oxygen species (ROS) that can cause cellular damage (Šebela *et al.,* 2020).

Agronomic practices, such as sowing-date manipulation, mulching, ethylene-production inhibitors, growth regulators and osmo protectants, have been proposed to improve wheat tolerance to terminal heat stress (Wahid *et al.,* 2007). However, there is still a need to integrate cultural (Sowing date) and physiological (Growth regulators) manipulations to improve wheat performance under field conditions.

In case of Late-sowing, wheat could be primed against heat stresses with the application of osmoprotectants and signaling molecules (*e.g*., stress hormones) (Jatana *et al.,* 2022). Among the various methods to reduce the impact of terminal heat stress, foliar and seed priming with inorganic salts (Potassium chloride and potassium nitrate), osmoprotectants (proline) and signaling molecules have been proposed (Wahid *et al.,* 2007). Foliar applications of sodium nitroprusside (Bavita *et al*., 2012), potassium nitrate (Bardhan *et al*., 2007) was reported to

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be effective for enhancing the productivity of wheat under adverse environmental conditions. Hormones at low levels (ppm), such as salicylic acid, prime the plant to defend against terminal heat stress (Jatana *et al.,* 2022). Salicylic acid has been reported to be involved in various plant stress tolerance mechanisms and plays an important role in abiotic stress signaling (Wahid *et al.,* 2007; Sathishkumar *et al.,* 2020). Under stress, foliar application of salicylic acid regulates physiological activities, such as photosynthetic rate, stomatal conductance, nutrient-uptake rate, protein synthesis, proline content and endogenous content of stress-related enzymes (Wang *et al*., 2014; Khamseh *et al*., 2013; Khan *et al*., 2013). These osmo protectants are also known for maintaining the redox state of the cell and its proper functioning under heat stress (Nathawat *et al*., 2007). As potassium is essential for enzyme activation, it may act as osmoregulator during stress for increased active update of potassium ion by the guard cells and stomatal regulation. It has proved to be very effective in improvement of relative water contents, photosynthesis, oxidant and antioxidants status, gas exchange characteristics and many other processes needed for osmotic stress mitigation in wheat seedlings (Tian and Lei, 2006). Foliar applied fertilizers (acting as osmoprotectants under environmental stress) often show a better efficacy which may help to reduce the required fertilizer dose (Suryavanshi *et al.,* 2016). Zinc, by its various roles in cell metabolism, especially enzymatic reactions and carbohydrate metabolism, improved the quantity and quality of yield under normal and heat stress conditions (Mosavian *et al.,* 2021; Singh *et al.,* 2024). However, the current knowledge about the cost effectiveness of foliar application of these Osmo protectants in the wheat sown across a wide sowing window is still limited. This led us to conduct a field study to evaluate the effect of foliar application of potassium nitrate, salicylic acid, SNP and zinc sulphate heptahydrate on the growth, proline content, pollen viability, yield and yield contributing characters of wheat sown at different dates.

MATERIALS AND METHODS

Location and experimental treatments

The field experiment was conducted during *Rabi* season 2022-23 at Research farm Department of Agriculture, Guru Nanak dev University Amritsar (31°.63′N, 74°.82′E, 219 m elevation). The mean maximum and minimum temperatures and relative humidity were: 27.2°C, 13.2°C and 53.18% during 2022-23 (Fig 1) and total rainfall received during the crop season was 155.2 mm. The soil of the experimental field had a pH of 8.1, EC-0.29 mhos cm^2, sandy loam texture and 195, 18 and 263 kg/ha of available N, P and K content, respectively. The field soil was low in organic carbon (0.37%), zinc (.44 mg/kg). Wheat variety 'PBW-826' was sown at a recommended seed rate of 100 kg seed/ha, using a row spacing of 20 cm. Nitrogen in the form of Urea and phosphorus in form of DAP were applied at the rate of 275 and 137.5 kg ha^{-1}, respectively. The experiment was conducted using a split-plot design, with sowing dates (October-20, November 20 and December 20) assigned to the main plots and foliar application (Control, water spray, potassium nitrate @ 2%, salicylic acid (100 ppm), sodium nitroprusside @ 800 µg/ml, zinc sulphate heptahydrate @ 1%) to the sub plots. The experiment had three replications. Potassium nitrate, salicylic acid, sodium nitroprusside and zinc sulphate heptahydrate applied as a foliar spray (at 2 g l^{-1}, 100 mg l^{-1}, 800 µg/ml and 1 g l^{-1} respectively) twice, once at booting and then at anthesis, using a solution volume at a rate of 500 l ha^{-1}.

Pollen viability and proline content

Pollen viability was determined using the method proposed by Chang *et al*. (2014). At anthesis stage, randomly selected early in the morning, immediately analyzed for pollen viability. Anthers from 10 to 12 flowers were dissected and prefixed in 70% ethyl alcohol. The flowers were placed on a glass slide in one drop of water and anthers were opened by crushing to release the pollen grains. The debris was removed with the help of dissecting needle. To pollen grains on the slides were stained with one or two drops of potassium iodide solution and incubated for 5 min. A coverslip was placed on each slide, which was then viewed under a compound microscope at 10× resolution. Pollen grains appearing black were counted as viable and orange- coloured ones were counted as non-viable. Pollen viability was expressed as per cent viable pollen grains using the following formula:

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

Proline content was measured from the frozen fresh leaves according to the method of (Bates *et al*., 1973).

Relative water content and membrane stability index

Relative water content was calculated by taking Fresh weight of the leaf samples and then kept in distilled water for 4 hours to obtain turgid weight. The turgid weight was recorded after blotting the excess water on the surfaces of the samples. Dry weight was obtained after drying the samples in oven at 60°C till constant weight obtained. The relative water content was then calculated as:

$$\text{RWC (\%)} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

For membrane stability index, flag leaves were sampled randomly from each plot at grain filling stage. from 0.1 g fresh leaf tissue 1 cm^2 leaf disks were cut. Leaf tissues were rinsed in double distilled water to remove surface adhered ions and placed in test tubes filled with 10 ml of deionized distilled water and incubated at 40°C for 30 minutes. After 30 minutes their electrical conductivity was recorded by the conductivity meter (C1). Subsequently, the same samples were placed in a water bath having

boiling water (100°C) for 10 minutes only. After 10 minutes, their electrical conductivity was recorded as above (C2). The membrane stability index (MSI) was calculated by the formula used by Sairam *et al*. (1997) as follows:

$$\text{Membrane stability index} = \frac{1 - C1}{C2} \times 100$$

Statistical analyses

Data obtained from the experiment conducted under split plot design were statistically analysed by analysis of variance (ANOVA) with help of statistical tool 'OPSTAT', least significant difference (LSD) values were computed at 5 per cent level of significance for making comparisons among the treatment means.

RESULTS AND DISCUSSION

Growth parameters

The maximum emergence count (m^{-2}) was recorded in the crop sown on November 20 (Table 1), which was statistically at par with the crop sown on October 20 but significantly higher than the crop sown on December 20. The lower emergence in late sowing (December) could be due to low temperature during month of December. The mean temperature of 18-25°C is required for good emergence in wheat (Gupta *et al.,* 2013). Plant height was significantly influenced by dates of sowing. At maturity, maximum plant height was observed in the October 20 sowing which was significantly higher than the November 20 and December 20 sowing (Table 1). Reduced plant height of wheat in late sown conditions was also reported by (Yajam and Madani, 2013). High temperature during early sowing might be produce the taller plants in wheat. As foliar spray of osmo protectants was done at anthesis stage, it did not affect the initial growth of plants. Leaf area index of crop sown on October 20 was significantly higher than the November 20 and December 20 sown crop (Table 1). Higher leaf area index in early sowing is due to the taller plants in the early sowing. Decrease in leaf area index in late sowing was also reported by (Suleiman *et al.,* 2014). Similarly, maximum dry matter accumulation was recorded in October 20 sown crop which was significantly higher than the December 20 sown crop but statistically at par with November 20 sown crop (Table 1). Higher dry matter accumulation in early

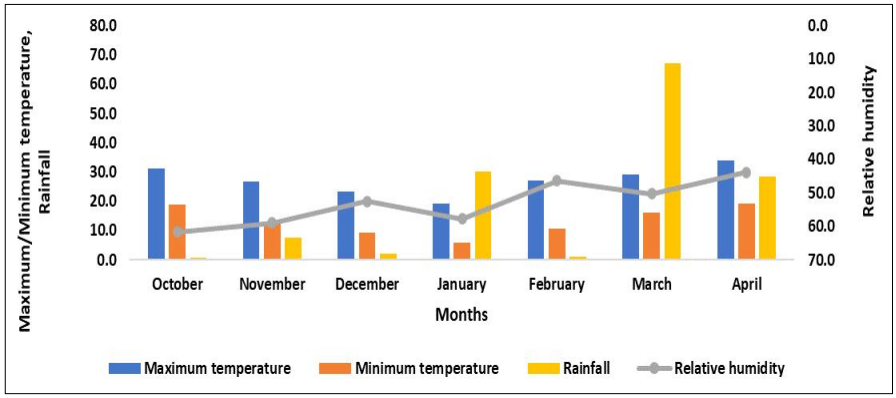


Fig 1: Mean meteorological data of *Rabi* 2022-23.

Table 1: Growth of wheat as affected by sowing date and osmo protectants treatment.

Sowing dates	Emergence count (m^{-2})	Plant height (cm)	Leaf area index (LAI)	Dry matter accumulation (q ha^{-1})
October 20	164.1	99.2	4.54	138.6
November 20	169.3	94.2	4.22	136.4
December 20	146.4	87.5	3.83	128.8
CD (p=0.05)	10.6	3.6	0.24	5.6
Osmo protectants				
Control	160.5	93.6	4.15	134.1
Water spray	159.6	93.6	4.21	134.6
Potassium nitrate @ 2%	161.8	93.2	4.17	134.9
Salicylic acid (100 ppm)	160.0	93.6	4.23	134.4
Sodium nitroprusside @ 800 µg/ml	159.6	94.0	4.19	134.8
Zinc sulphate heptahydrate @ 1%	157.6	93.2	4.16	134.9
CD (p=0.05)	NS	NS	NS	NS
Interaction	NS	NS	NS	NS

sowing was due to the higher plant height, leaf area index in the early sown conditions. Delayed sowing resulted in lower dry accumulation (Alam *et al.,* 2013).

Phenology

Date of sowing had significant effect on days taken to emergence, December sown crop took longer time (11 days) for emergence (Table 2) than crop sown in October (7) and November (9). Delayed sowing resulted into the higher number of days to emergence due to low temperature in late sown conditions (Hussian *et al.,* 2013). October 20 sown crop took minimum days (21) to crown root initiation as compared to November 20 (23) and December 20 (25) sown crop. Delay in crown root initiation in late sown wheat was also observed by (Amrawat *et al.,* 2013). For booting, November 20 sown crop took maximum number of days (95) which were significantly higher than October 20 (93) and December 20 (89) sown crops. November sown wheat took maximum days for booting was also reported by Mumtaz *et al.* (2015). Similarly, November sown crop took maximum number of days for earing and anthesis followed by the October and December sown crop. December 20 sown took minimum days for earing and anthesis. Early occurrence of anthesis under late sown conditions was due to high temperature, it was also reported by (Hossain *et al.,* 2015). However, for milking, dough and physiological maturity maximum number of days were taken by October sown crop followed by the November and December sown crop. Due to more favourable conditions, early sown crop took maximum days to milking, dough and physiological maturity was also reported by Basu *et al.* (2014); Amrawat *et al.* (2013).

As osmo protectants were applied at anthesis stage, so it did not effect the initial growth stages but application of potassium nitrate, salicylic acid and sodium nitroprusside extends the duration of grain filling period as compared to foliar application of zinc sulphate, water and control. Salicylic acid application increases the grain filling duration was also reported by Jatana *et al.* (2022).

Pollen viability and proline content

Date of sowing significantly affected the pollen viability in wheat (Table 3). Maximum pollen viability was recorded in November sown wheat which was statistically at par with the October sowing but significantly higher than the December sown wheat. Lower pollen viability under late sown condition was due to comparatively high temperature during anthesis (Jatana *et al.,* 2022). Foliar application of sodium nitroprusside resulted in higher pollen viability in wheat which is statistically at par with salicylic acid and significantly higher than the foliar application of potassium nitrate, Zinc sulphate, water spray and control.

Proline production was also significantly influenced by the different dates of sowing (Table 3); highest proline content was recorded in December sown wheat which was significantly higher than the October and November sown wheat. Laghari *et al*. (2021) also found increase in proline content in the wheat, upon delaying the sowing of wheat from November to December and consequent rise in temperature at that crop growth stage. Osmo protectants also increase the proline production in plants; maximum proline content was recorded with foliar application of sodium nitroprusside which was closely followed by the salicylic acid application which were significantly higher than the foliar application of potassium nitrate, zinc sulphate, water spray and control. Proline content generally increases under stress conditions but application of sodium

Table 2: Phenology of wheat as affected by sowing date and osmo protectant treatment.

Sowing dates	Emergence	Crown root initiation	Booting	Earing	Anthesis	Milking	Dough	Physiological maturity
Sowing dates								
October 20	6.9	21.4	93.4	102.4	109.3	120.2	134.8	150.3
November 20	8.9	23.2	95.2	104.2	111.6	118.0	130.0	144.2
December 20	11.1	25.6	89.2	95.0	98.4	105.3	115.3	130.2
CD (p=0.05)	0.9	1.2	1.8	2.2	1.8	1.2	2.6	1.8
Osmo protectant								
Control	8.9	23.4	92.9	100.7	106.3	114.4	125.6	140.8
Water spray	8.9	23.2	92.3	100.5	106.2	114.7	125.8	140.7
Potassium nitrate @ 2%	8.9	23.5	92.5	100.2	106.6	114.1	127.3	142.1
Salicylic acid (100 ppm)	8.9	23.1	92.7	100.8	106.1	114.2	127.6	142.3
Sodium nitroprusside @ 800 µg/ml	8.9	23.6	92.5	100..4	106.4	114.9	127.8	142.7
Zinc sulphate heptahydrate @ 1%	8.9	23.6	92.4	100.6	106.5	114.7	126.6	141.2
CD (p=0.05)	NS	NS	NS	NS	NS	NS	0.50	0.60
Interaction	NS	NS	NS	NS	NS	NS	NS	NS

nitroprusside as osmo protectant also increases the proline accumulation in wheat (Suryavanshi and Buttar, 2018).

Membrane stability index and relative water content

Different sowing dates significantly influence the membrane stability index (MSI) in wheat (Table 3). Highest MSI was obtained in the early sowing *i.e*. October which was significantly higher than MSI obtained in December sowing and statistically at par with the November sown wheat. Reduction of MSI in wheat under late sown conditions was observed by the Pal *et al.* (2024). Foliar application of osmo protectants also influenced the MSI in wheat. Highest MSI was obtained with the foliar application of potassium nitrate which was closely followed by the SNP and salicylic acid treatments and significantly higher than the zinc sulphate, water spray and control treatments.

Relative water content was also influenced by the different sowing dates and foliar application of osmo protectants in wheat (Table 3). Higher relative water content was observed in the October sown wheat which was closely followed by November sown wheat but significantly RWC was observed in the December sown wheat. lower RWC under delayed sowing in wheat was also reported by Raj *et al.* (2024). Under osmo protectants, higher RWC was observed in the foliar application of potassium nitrate which was statistically at par with the SNP and salicylic acid treatments but significantly higher than the zinc sulphate, water spray and control treatments. Similar findings of higher RWC in wheat with osmo protectants application were given by Suryavanshi and Buttar (2018).

Yield contributing characters and yield

Effective tillers were significantly influenced by different sowing dates (Table 4). Maximum number of effective tillers were recorded in October sown which was significantly higher than the November and December sown wheat. Reduction in no. of effective tillers under late sown condition was also observed by Mehta *et al.* (2020). Maximum ear length, no of grains ear^{-1} and test weight were recorded in the October sown wheat which were

Table 3: Effect of sowing date and Osmo protectants on pollen viability, proline content, membrane stability index and relative water content of wheat.

Sowing dates	Pollen viability (%)	Proline content (µg/g)	Membrane stability index	Relative water content (%)
October 20	74.6	31.6	80.2	82.2
November 20	78.2	37.4	76.5	80.4
December 20	65.4	42.3	62.6	74.3
CD (p=0.05)	5.2	3.4	4.5	3.4
Osmo protectants				
Control	68.9	29.0	62.0	74.0
Water spray	70.1	31.2	67.8	76.4
Potassium nitrate @ 2%	72.3	36.0	79.0	82.2
Salicylic acid (100 ppm)	75.5	43.2	76.1	80.0
Sodium nitroprusside @ 800 µg/ml	77.1	48.0	78.8	81.8
Zinc sulphate heptahydrate @ 1%	72.1	34.9	70.8	78.6
CD (p= 0.05)	4.2	5.2	3.8	2.3
Interaction	NS	NS	NS	NS

Table 4: Yield attributes of wheat as affected by sowing date, varieties and osmo protectants treatment.

Sowing dates	Effective tillers (m^{-2})	Ear length (cm)	Number of grains per ear	1000 grain weight (g)
October 20	402.2	14.1	43.4	40.5
November 20	384.6	14.2	44.6	40.8
December 20	370.2	13.1	38.2	37.2
CD (p=0.05)	12.2	0.6	2.2	1.2
Osmo protectants				
Control	384.5	13.8	39.6	37.6
Water spray	385.1	13.8	40.2	37.9
Potassium nitrate @ 2%	385.7	13.8	42.0	40.0
Salicylic acid (100 ppm)	386.9	13.8	43.6	40.5
Sodium nitroprusside @ 800 µg/ml	385.5	13.8	44.8	40.9
Zinc sulphate heptahydrate @ 1%	384.9	13.8	41.6	40.0
CD (p=0.05)	NS	NS	2.6	NS
Interaction	NS	NS	NS	NS

Table 5: Effect of sowing date, varieties and osmo protectants on grain yield, straw yield, biological yield and harvest index.

Sowing dates	Grain yield (q/ha)	Straw yield (q/ha)	Biological yield (q/ha)	Harvest index (%)
October 20	54.6	77.8	132.2	41.5
November 20	56.4	80.2	136.4	41.2
December 20	49.2	69.4	119.3	41.1
CD (p=0.05)	2.4	5.2	5.2	NS
Osmo protectants				
Control	51.7	73.4	125.1	41.2
Water spray	52.1	74.6	126.7	41.2
Potassium nitrate @ 2%	52.7	75.9	128.6	41.0
Salicylic acid (100 ppm)	55.1	77.1	132.2	41.5
Sodium nitroprusside @ 800 µg/ml	56.1	77.4	133.5	41.9
Zinc sulphate heptahydrate @ 1%	52.5	76.0	128.5	41.0
CD (p=0.05)	2.1	NS	NS	NS
Interaction	NS	NS	NS	NS

statistically at par with November sown wheat but significantly higher than the December sown wheat, maximum ear length, grains per ear and test weight in early sowing of wheat was also reported by Yusuf *et al.* (2019). Osmo protectants application did not affect the effective tillers, ear length and test weight. However, maximum no. of grains per ear were recorded in SNP treatment which was statistically at par with salicylic acid treatment but significantly higher than the potassium nitrate, zinc sulphate, water spray and control treatments. Role of SNP in protecting the cell membrane, function against the destructive effect of reactive oxygen species, increase in chlorophyll content, enzyme activity and maintenance of water balance might be the reason of increase in no. of grains per ear (Suryavanshi *et al.,* 2016).

Grain, straw and biological yield were significantly influenced by the different sowing dates (Table 5). Maximum grain, straw and biological yield was recorded in the November sown wheat which was closely followed by the October sown crop but significantly higher than the December sown wheat. The late sown wheat is subjected to elevated temperatures at the reproductive stage in South Asia, which finally affects the productivity of wheat (Jatana *et al.,* 2020; Ram and Kaur, 2024). Harvest index was not significantly influenced by the dates of sowing. Among osmo protectants application, maximum grain yield was recorded with SNP application which was closely followed by the salicylic acid application but significantly higher than the potassium nitrate, zinc sulphate, water spray and control. SNP has its role in accelerating photosynthesis rate, maintaining RWC, scavenging ROS under heat stress conditions (Suryavanshi *et al.,* 2016). The increase in grain yield with SNP and salicylic acid application may also attributed to more translocation of photosynthates from straw to grain as their application extends the grain filling period (Table 2) in wheat. Straw yield, biological yield and harvest index was not significantly influenced by the Osmo protectants application.

CONCLUSION

From experimental findings it is suggested to ensure the sowing of wheat on recommended time, as late sown wheat is subjected to high temperature at reproductive stage and ultimately resulted in significant decline in grain yield. Foliar spray of sodium nitroprusside @ 800 µg/ml and salicylic acid (100 ppm) found suitable to enhance the grain yield in timely and late sown conditions.

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Disclaimers

The opinions and conclusions presented in this article are entirely those of the authors and do not necessarily reflect the positions of their affiliated institutions. While the authors have taken responsibility for ensuring the accuracy and completeness of the information, they disclaim any liability for direct or indirect losses arising from the use of this material.

Conflict of interest

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