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# EFFECTS OF GLYCINE BETAINE AND SALICYLIC ACID FOLIAR SPRAY ON GROWTH OF LATE SOWN WHEAT

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**ABSTRACT**

A field experiment was conducted at College of Research, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, University in Faizabad, Uttar Pradesh, India during the year 2021-2022 to study the effect of glycine betaine and salicylic acid foliar spray on performance of wheat under late sown condition. The findings indicated that glycine betaine @100mM followed by salicylic acid @250ppm foliar application treatments significantly enhanced the growth parameters *viz.*, plant height (59.14 cm, 86.51 cm and 104.74 cm, respectively); leaf area index (3.20, 3.92 and 4.12, respectively) at 60DAS, 90DAS and at harvest. The number of tillers/ plant (7.85, 5.85), RWC (81.03%, 76.78%) at 60DAS and 90DAS) were also recorded higher in glycine betaine @100mM followed by salicylic acid @ 250ppm foliar application treatments. Phenological characters such as days to 50% flowering and days to physiological maturity were also recorded higher with foliar application of glycine betaine 100mM ($T_3$) followed by salicylic acid treatment 250ppm ($T_6$).

***Key words :*** Foliar application, Growth, Glycine betaine, Salicylic.

## Introduction

Wheat is the main staple food crop of the world and production is 778.6 MMT during the year 2021-22. According to Statistica (2022), the top producing nations of wheat worldwide are the United States (44,790 MT), Russia (75,500 MT), China (1,36,946 MT), India (1,09,520 MT), and the European Union (1,38,900 MT). It makes up a substantial portion of global food security. India is the second-largest producer of wheat in the world, contributing 14.0% of the total area and 13.6% of the output (Devi *et al.*, 2024). In the 2020–21 growing season, India's wheat production, yield, and area were 31,357 thousand hectares, 3.4 MT/hectare, and 107,860 thousand metric tons, respectively (USDA, 2022). It contributes significantly to the food security of the world. The Indian states of Uttar Pradesh, Madhya Pradesh, Punjab, Rajasthan and Haryana are the primary locations for wheat cultivation. Since wheat was first planted in the prehistoric era, all of the evidence that is currently available points to the North-East as the source of wheat cultivation. Wheat is an annual plant in the genus Triticum of the Poaceae (Gramineae) family. In India, the three most widely grown wheat varieties in terms of economic importance are *Triticum aestivum*, *Triticum durum* and *Triticum dicoccum*. The optimal temperature range for wheat is 10 to 15°C in the winter and 21 to 26°C in the summer. Plant physio-biochemical and molecular traits are negatively impacted by high temperatures, which hinders plant growth and development. Poor crop establishment leading to late sowing is the primary reason of low yield in India. A key component of crop establishment is a uniform emergence and an ideal plant population. The quality of the seed in terms of germination and seedling vigor is essential for proper crop establishment (Devi *et al.*, 2022). Lanting time, seed quality materialand supply of optimum nutrients during cropgrowth are some of the important parametersthat limit productivity. These elements decidecrop establishment, growth and development, which thus decide yielding capability of the harvest (Devi *et al.*, 2023a). Wheat has a wider planting window and is primarily planted during the *rabi* season. According to Devi *et al.* (2023b), the best period to sow in the northwestern plain zone of India is around the first to third week of November, with the second fortnight of October considered as early planting. Wheat productivity is estimated to decrease globally by 6% for every 1% increase in temperature because to its vulnerability to heat stress. Since many physiological, biological and biochemical processes in wheat are impacted by high temperatures, even a 1°C increase in mean temperature during the reproductive stage may result in higher grain output and reduced loss. In addition to deactivating the Rubisco enzyme, causing poor germination, shorter grain filling times, fewer grains overall, a decrease in photosynthetic capacity, a slower rate of assimilate translocation, early leaf senescence, a decrease in chlorophyll content, and a reduction in yield, heat stress also affects the starch and protein content of grains. Reactive oxygen species are produced when heat stress occurs, and these species also cause lipid peroxidation and protein oxidation, which alter the stability of membranes.

As a suitable osmolyte, glycine betaine probably has a major function in osmoregulation in plants exposed to harsh environmental stimuli, such as stress from high temperatures. Furthermore, it's possible that glycine betaine stimulates the activation of signaling molecules including heat-shock transcription factor (HSF) and calcium-dependent protein kinases (MAPKs) (Hemantarajan *et al.*, 2014), which in turn may stimulate the activation of stress-responsive and HSF genes. By increasing the activities of enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), the activated stress-responsive genes may strengthen the body's defenses and lessen the damaging effects of unchecked ROS that cause oxidative damage brought on by heat stress. Reducing or eliminating ROS may preserve biological membrane integrity. Moreover, HSP production and activation may result from active HSF genes. According to Jacob *et al.* (2017), the majority of HSPs can also function as chaperones, which can stop heat-induced protein aggregation. Numerous thorough investigations have clarified the function of HSPs in plant thermotolerance. When glycine betaine is applied exogenously, it counteracts the metabolic maladjustments generated by HS, improving the development and yield of many plants and improving their thermotolerance. For instance, Li *et al.* (2014) observed increased heat-shock protein 70 (HSP70) accumulation, heat-shock gene expression and seed germination when evaluating the impact of exogenous glycine betaine administration on heat-stressed tomato plants. Similarly, in heat-stressed plants, exogenous glycine betaine supplementation probably regulates numerous other important metabolic processes.

Salicylic acid (SA) is an endogenous phenolic plant hormone that is essential for plant development, ion absorption, and transport. It has an aromatic ring with a hydroxyl group (Laishram *et al.*, 2023; Klessig *et al.*, 2018). It functions as an endogenous signaling molecule and is crucial for controlling a plant's growth and development. It also interacts with other organisms and helps the plant respond to a variety of abiotic challenges. Along with thermogenesis, flower initiation, food uptake, ethylene production, stomatal movements, photosynthesis, and enzyme activity, it performs a variety of physiological functions in plants. It improves a plant's ability to withstand intense heat in various environments. It enhances root growth by increasing cell division in apical meristems in wheat seedlings, which help in better growth of plants under stress condition (Kumar *et al.*, 2021). Major abiotic stress tolerance mechanisms have been found to be induced in stressed plants by exogenously given salicylic acid using a variety of techniques (Horvath *et al.*, 2007; Khan *et al.*, 2014; Anwar *et al.*, 2013). A study conducted by He *et al.* (2002), Wang *et al.* (2010), and Khan *et al.* (2013) examined its ability to mediate tolerance to heat stress.

## Materials and Methods

### Experimental site of the study

A field experiment was conducted during *rabi* season of 2021-22 at College of Research, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya University in Faizabad, Uttar Pradesh, India (Fig. 1). The experimental plot is situated at latitude of $24.4^0$ to $26.56^0$ N and longitude of $82.12^0$ to $83.98^0$E on an elevation of 113 meters in the gangetic alluvium of eastern Uttar Pradesh.

### Weather conditions during the crop season

The meteorological data on weather conditions prevailing during *rabi* season of year 2021-22 have been illustrated in Fig. 2.

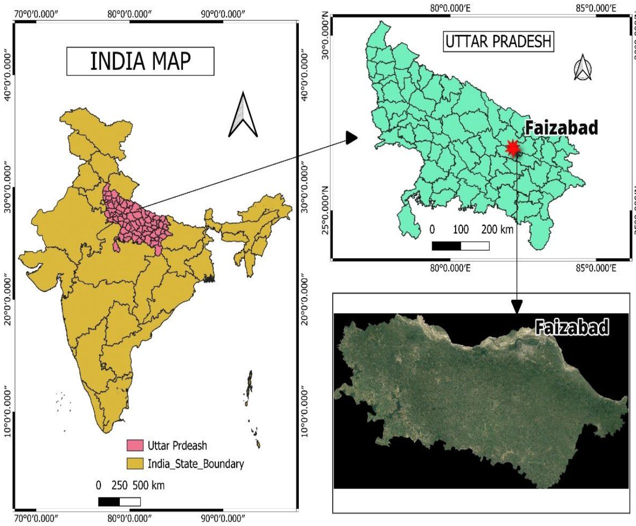


**Fig. 1 :** Map of the experimental area, Faizabad, Uttar Pradesh, India.

$T_6$ (SA @ 250ppm)

$T_7$ (SA @ 350ppm)

**Factor B: Variety**

$V_1$ (PBW-343)

$V_2$ (Halna)

## Soil fertility management

Through the use of urea, DAP, and muriate of potash, respectively, 120, 60 and 40 kg/ha of nitrogen, phosphorus, and potash were added. Prior to seeding, a basal dose of half of the nitrogen, total phosphorus and total potash were added. Two equal split doses of the remaining nitrogen were applied, one during the tillering stage and the other during the flowering stage. For regular plant growth, standard cultural practices were implemented.

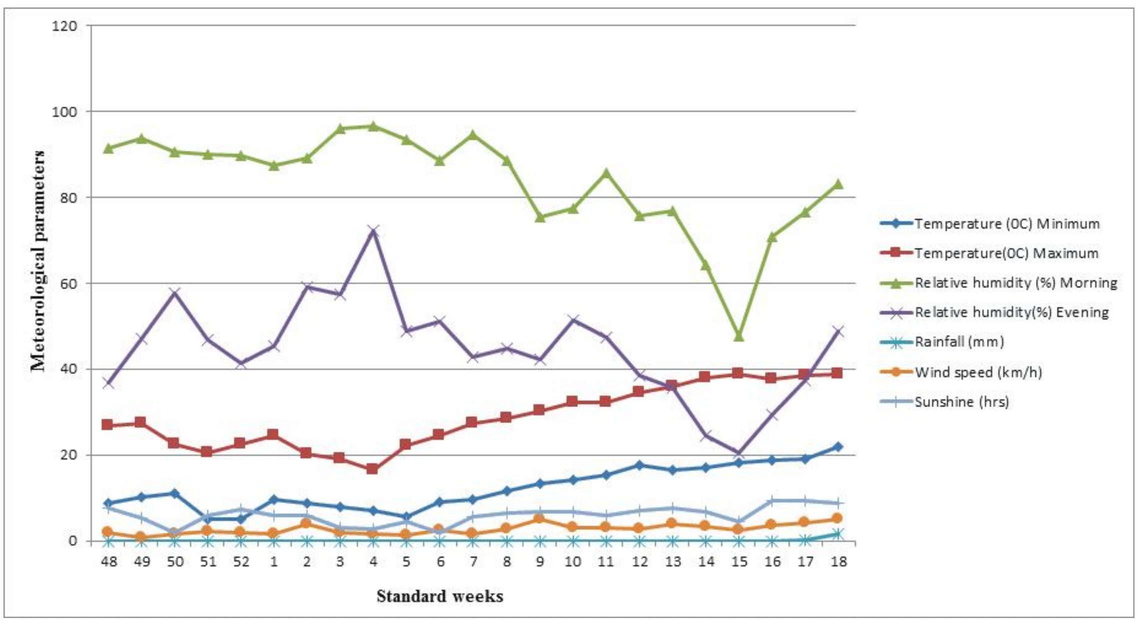


**Fig. 2 :** Meteorological data during the crop season (2021-22).

## Experimental details

The experiment was conducted in field containing silt loam soil with two wheat varieties PBW-343 and Halna. Three replications of the experiment were conducted using a factorial randomized block design (RBD) (Rangaswamy, 1995). The study comprised of following treatments as below:

**Factor A: Foliar spray treatments**

$T_1$ (Control)

$T_2$ (glycine betaine @50milli molar)

$T_3$ (glycine betaine @100milli molar)

$T_4$ (glycine betaine @150milli molar)

$T_5$ (SA @ 150ppm)

## Sowing

On December 20, 2020, wheat cultivars PBW-343 and Halna were sowed at a depth of 5-7 cm in the soil and 20 cm apart. The seeds were obtained from the Crop Physiology Department, ANDUA&T, Kumarganj, Ayodhya (Uttar Pradesh).

## Irrigation

All the plots were irrigated uniformly as and when irrigation was required.

## Plant protection

Suitable plant protection measures were adopted in order to keep the plants free from insects and diseases.

### Preparations of salicylic acid and glycine betaine solutions

Salicylic acid was prepared in the appropriate volume of water at different concentrations *viz*; 150 ppm, 250 ppm and 350 ppm, respectively. Sticky agent teepol was added to the spray solution at a rate of 0.5 milliliters per litre to enhance spray retention. One liter of water was mixed with varying concentrations of glycine betaine to obtain 50 mM, 100 mM and 150 mM solutions. Foliar spraying was carried out at fifty days after sowing, with the help of backpack sprayer.

### Observations

#### Plant height (cm)

Plant height was measured in cm from soil surface up to tip of the plant at different growth stages.

#### Number of tillers/plant

Number of tillers per plant was recorded by counting tillers of five plants at various stages of crop and average number of tillers per plant was calculated.

#### Leaf Area Index

Leaf area from five plants was measured at 30, 60 days after sowing and at maturity using LI-COR model LI-3100 leaf area meter with transparent conveyer belt having electronic digital display and expressed in $cm^2$. Leaf area index was calculated by dividing the total leaf area by corresponding land area as suggested by Watson (1952).

LAI (%) = Total leaf / unit land area

#### Relative water content (%)

The relative water content (RWC) was determined by method described by Barrs and Weatherly (1962). Leaf discs were cut from the leaves, weighed and saturated by floating on distilled water in petri dishes for four hours. The discs were surface dried and weighed. After that disc were kept in oven at $80^oC$ for 24 hours. After drying, weight of discs was taken with the help of electronic balance.

RWC (%) = (Fresh weight-Dry weight)/(Turgid weight-Dry weight) × 100

Where, FW = Fresh weight, DW = Dry weight, TW = Turgid weight.

#### Days to 50% flowering

The days to 50% flowering was recorded by counting the number of days taken from the date of sowing to appearance of flower on 50% plant population.

#### Days to physiological maturity

The maturity duration of the crop for each treatment was assessed by visual appearance of grains and color of leaves particularly flag leaf. At maturity, flag leaf becomes yellowish and remaining half of the leaves also become yellowish.

### Statistical analysis

Data recorded on various growth and yield attributes were subjected to statistical analysis by Fisher method of analysis of variance (Fisher and Yates, 1949). The significance of various treatments was judged by comparing calculated, F' value with Fisher's, F' value at 5 percent level, incorporate in tables, were also calculated to compare the relative performance of various treatments by using the following formula

$$SEm\pm = \sqrt{\frac{EMS}{N}}$$

Where,

EMS is mean sum of square of error

N = total number of experimental units

Level of factors

$$CD = \sqrt{\frac{2EMS}{N}} \times t\,(5\%)$$

Where,

Value of 't' from Fisher's table at error degree of freedom on 5% level of significance.

## Results and Discussion

### Plant height (cm)

The data pertaining to plant height as presented in Table 1 revealed that the maximum plant height at 60DAS, 90DAS and harvest stage (59.14 cm, 86.51 cm and 104.74 cm, respectively) was recorded with foliar application of glycine betaine @100mM ($T_3$) followed by foliar application of SA @ 250 ppm ($T_6$) as compared to rest of treatments.

As for varieties, $V_2$-Halna was recorded significantly higher plant height at 60DAS, 90DAS and harvest stage (61.05cm, 86.26cm and 101.51cm, respectively) as compared to $V_1$-PBW-343 (52.70cm, 78.22cm and 99.01cm, respectively). The interaction effect was found non-significant between varieties and treatments at all stages of growth. Increase in plant height in all stages of late sown wheat may be due to optimum concentration of glycine betaine and SA used during foliar application. The results are in accordance with Shahbaz *et al.* (2011), Sujatha *et al.* (2001). This may be due to different

**Table 1 :** Effect of foliar application of Glycine betaine and Salicylic acid on plant height (cm) at various stages of late sown wheat varieties.

| Treatments | Plant height at 60DAS | | Plant height at 90DAS | | Plant height at harvest | |
|---|---|---|---|---|---|---|
| | $V_1$ | $V_2$ | $V_1$ | $V_2$ | $V_1$ | $V_2$ |
| $T_1$ | 51.10 | 58.98 | 74.25 | 82.30 | 95.00 | 97.25 |
| $T_2$ | 52.27 | 60.25 | 75.35 | 84.25 | 98.25 | 99.33 |
| $T_3$ | 54.58 | 63.70 | 83.45 | 89.57 | 103.12 | 106.35 |
| $T_4$ | 52.50 | 59.75 | 79.50 | 85.33 | 98.75 | 102.31 |
| $T_5$ | 52.57 | 60.59 | 78.60 | 87.40 | 99.11 | 100.25 |
| $T_6$ | 53.21 | 63.10 | 80.00 | 88.39 | 100.53 | 103.33 |
| $T_7$ | 52.70 | 61.00 | 76.40 | 86.60 | 98.32 | 101.75 |
| Mean | 52.70 | 61.05 | 78.22 | 86.26 | 99.01 | 101.51 |
| Factors | SE(m) | C.D. | SE(m) | C.D. | SE(m) | C.D. |
| Variety | 0.27 | 0.79 | 0.78 | 2.28 | 0.42 | 1.25 |
| Treatments | 0.51 | 1.49 | 1.46 | 4.26 | 0.80 | 2.34 |
| VxT | 0.72 | NS | 2.065 | NS | 1.13 | NS |

**Table 2 :** Effect of foliar application of glycine betaine and salicylic acid on leaf area index at various stages of late sown wheat varieties

| Treatments | Leaf area index at 60 DAS | | Leaf area index at 90 DAS | | Leaf area index at harvest | |
|---|---|---|---|---|---|---|
| | $V_1$ | $V_2$ | $V_1$ | $V_2$ | $V_1$ | $V_2$ |
| $T_1$ | 2.20 | 2.13 | 2.80 | 2.72 | 2.94 | 2.85 |
| $T_2$ | 3.01 | 2.92 | 3.82 | 3.71 | 4.01 | 3.89 |
| $T_3$ | 3.27 | 3.14 | 3.98 | 3.86 | 4.18 | 4.05 |
| $T_4$ | 2.83 | 2.75 | 3.59 | 3.48 | 3.77 | 3.66 |
| $T_5$ | 3.15 | 3.02 | 3.16 | 3.12 | 4.02 | 3.85 |
| $T_6$ | 3.21 | 3.16 | 3.95 | 3.81 | 4.16 | 4.01 |
| $T_7$ | 2.74 | 2.66 | 3.48 | 3.38 | 3.65 | 3.54 |
| Mean | 2.92 | 2.83 | 3.54 | 3.44 | 3.82 | 3.69 |
| Factors | SE(m) | C.D. | SE(m) | C.D. | SE(m) | C.D. |
| Variety | 0.02 | 0.07 | 0.03 | 0.09 | 0.03 | 0.10 |
| Treatments | 0.04 | 0.14 | 0.06 | 0.18 | 0.06 | 0.18 |
| VxT | 0.06 | NS | 0.088 | NS | 0.09 | NS |

mechanisms such as osmotic adjustment, maintaining activity of aquaporins and hence water uptake, cell elongation, promotion of root growth, cell membrane stability, stomatal regulation as well as detoxification of reactive oxygen species and thereby increasing availability of water to plants. Laishram *et al.* (2020) also found higher plant height for the plots where 200 ppm of salicylic acid has been applied. The increased in plant height under increasing levels of salicylic acid might be due to water soluble antioxidant compound of salicylic acid which regulate the plant growth (Laishram *et al.*, 2020; Wangkheirakpam *et al.*, 2020).

## Leaf area index

The data with respect to leaf area index presented in Table 2 depicted that the maximum leaf area index at 60DAS, 90DAS and harvest stage (3.20, 3.92 and 4.12, respectively) was recorded with foliar application of glycine betaine @100mM ($T_3$) followed by foliar application of SA @ 250ppm ($T_6$) against rest of treatments at all stages of growth.

The variety $V_1$-PBW343 (2.92, 3.54 and 3.82, respectively) showed significantly higher leaf area index as compared to $V_2$-Halna (2.83, 3.44 and 3.69, respectively) at 60DAS, 90DAS and harvest stage. The interaction effect was found non-significant between varieties and treatments at all stages of growth. Increase in leaf area index in all stages of late sown wheat may be due to optimum concentration of glycine betaine and SA used during foliar application. These findings are similar with Shemi *et al.* (2021), Sujatha *et al.* (2001). The reason

**Table 3 :** Effect of foliar application of glycine betaine and salicylic acid on number of tillers and relative water content (%) at various stages of late sown wheat varieties.

| Treatments | Number of tillers/plant at 60 DAS | | Number of tillers/plant at 90 DAS | | Relative water content at 60 DAS | | Relative water content at 90DAS | |
|---|---|---|---|---|---|---|---|---|
| | $V_1$ | $V_1$ | $V_1$ | $V_2$ | $V_1$ | $V_2$ | $V_1$ | $V_2$ |
| $T_1$ | 5.50 | 5.50 | 4.60 | 4.50 | 70.60 | 73.60 | 67.60 | 70.44 |
| $T_2$ | 6.30 | 6.30 | 4.80 | 5.10 | 71.40 | 76.37 | 71.64 | 73.55 |
| $T_3$ | 7.60 | 7.60 | 5.60 | 6.10 | 76.35 | 85.70 | 75.34 | 78.21 |
| $T_4$ | 6.60 | 6.60 | 5.10 | 5.60 | 72.10 | 79.50 | 73.83 | 76.94 |
| $T_5$ | 6.80 | 6.80 | 5.00 | 5.50 | 74.00 | 78.00 | 73.00 | 75.65 |
| $T_6$ | 7.50 | 7.50 | 5.30 | 6.00 | 75.25 | 81.40 | 74.40 | 76.49 |
| $T_7$ | 7.10 | 7.10 | 4.80 | 5.30 | 73.20 | 80.10 | 72.79 | 74.06 |
| Mean | 6.77 | 6.77 | 5.03 | 5.44 | 73.27 | 79.24 | 72.66 | 75.05 |
| Factors | SE(m) | SE(m) | SE(m) | C.D. | SE(m) | C.D. | SE(m) | C.D. |
| Variety | 0.06 | 0.06 | 0.05 | 0.14 | 0.71 | 2.08 | 0.56 | 1.65 |
| Treatments | 0.12 | 0.12 | 0.09 | 0.27 | 1.33 | 3.89 | 1.06 | 3.08 |
| VxT | 0.17 | 0.17 | 0.13 | NS | 1.88 | NS | 1.49 | NS |

**Table 4 :** Effect of foliar application of glycine betaine and salicylic acid on days to 50%flwering and days to physiological maturity at various stages of late sown wheat varieties.

| Treatments | Days to 50% flowering | | Days taken to physiological maturity | |
|---|---|---|---|---|
| | $V_1$ | $V_2$ | $V_1$ | $V_2$ |
| $T_1$ | 75.55 | 77.26 | 103.00 | 106.82 |
| $T_2$ | 77.25 | 79.54 | 107.73 | 114.00 |
| $T_3$ | 83.06 | 85.10 | 110.40 | 116.60 |
| $T_4$ | 81.50 | 83.22 | 106.30 | 113.10 |
| $T_5$ | 80.57 | 81.28 | 107.01 | 114.80 |
| $T_6$ | 82.53 | 84.52 | 109.70 | 115.50 |
| $T_7$ | 79.00 | 81.67 | 107.69 | 113.47 |
| Mean | 79.92 | 81.80 | 107.41 | 113.47 |
| Factors | SE(m) | C.D. | SE(m) | C.D. |
| Variety | 0.32 | 0.94 | 0.49 | 1.44 |
| Treatments | 0.60 | 1.76 | 0.92 | 2.69 |
| VxT | 0.85 | NS | 1.30 | NS |

may be due to increase in cell elongation and increase in photosynthetic capacity, thus higher the leaf area index.

### Number of tillers/plant

The data with respect to number of tillers/ plant presented in Table 3 showed that the maximum number of tillers/plant at 60DAS and 90DAS (7.85, 5.85) was recorded with foliar application of glycine betaine 100mM ($T_3$) followed by foliar application of SA 250ppm ($T_6$) than rest of treatments at all stages of growth.

As for varieties were concerned the variety $V_2$-Halna (7.54, 5.44) showed significantly increased number of tillers as compared to V1-PBW343 (6.77, 5.03) at 60 DAS and 90DAS. The interaction effect was found non-significant between varieties and treatments at all stages of growth. Increase in number of tillers in all stages of late sown wheat may be due to optimum concentration of glycine betaine and SA used during foliar application. The results are promising with findings of Raza *et al*. (2014) and Mervat *et al*. (2015). This may be due to different mechanisms such as osmotic adjustment, maintaining activity of aquaporins and hence water uptake, cell elongation, cell membrane stability.

### Relative water content (%)

The data with respect to relative water content presented in Table 3 revealed that maximum relative water content at 60DAS and 90DAS (81.03, 76.78) was recorded with foliar application of Glycine betaine 100mM ($T_3$) followed by foliar application of Salicylic acid 250ppm ($T_6$) than rest of treatments at all stages of growth.

As for varieties were concerned the variety $V_2$-Halna (79.24, 75.05) showed significantly increased relative water content as compared to $V_1$-PBW343 (73.27, 72.66) at 60DAS and 90DAS. The interaction effect was found non-significant between varieties and treatments at all stages of growth. Increase in relative water content in all stages of late sown wheat may be due to optimum concentration of Glycine betaine and Salicylic acid used during foliar application. The results are in line with Rao *et al*. (2012) and Gupta *et al*. (2015) found that foliar application of osmolytes had significant effect on relative water content as they help to maintain water potential under stress condition.

### Phenological characters

### Days to 50% flowering

The data with respect to days taken to 50% flowering presented in Table 4 revealed that the days taken to 50% flowering was recorded higher (84.08) with foliar application of Glycine betaine 100mM ($T_3$) followed by foliar application of Salicylic acid 250ppm ($T_6$) than rest of treatments.

As for varieties were concerned the variety $V_2$-Halna (81.80) showed higher days to 50% flowering as compared to $V_1$-PBW343 (79.92). The interaction effect was found non-significant between varieties and treatments at all stages of growth. Increase in days to 50% flowering of late sown wheat may be due to optimum concentration of Glycine betaine and Salicylic acid used during foliar application.

### Days taken to physiological maturity

The data with respect to days taken to physiological maturity presented in Table 4 and revealed that the days taken to physiological maturity was recorded higher (113.50 days) with foliar application of glycine betaine 100mM ($T_3$) followed by foliar application of salicylic acid 250ppm ($T_6$) than rest of treatments.

As for varieties were concerned the variety $V_2$-Halna (113.47) showed higher days to 50% flowering as compared to $V_1$-PBW343 (107.41). The interaction effect was found non-significant between varieties and treatments at all stages of growth. Increase in days to 50% flowering of late sown wheat may be due to optimum concentration of glycine betaine and salicylic acid used during foliar application.

## Conclusion

The significant improvement in plant growth by applying glycine betaine topically 100mM, followed by salicylic acid 250ppm over rest of treatments was found in the present study. This study highlights the pivotal role played by osmolytes and PGRs on growth and phonological characters. Thus, exogenous foliar treatment of 250 ppm salicylic acid and 100 mM glycine betaine may be a possibility for enhancing late-sown wheat's heat tolerance and grain output.

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