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Impact of Anti-frost Agents on Growth Parameters of Papaya in the Punjab Region

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ABSTRACT

Papaya is sensitive to low-temperature stress, which limits its commercial cultivation in subtropical regions like Punjab. Anti-frost agents such as glycine betaine and salicylic acid can help plants withstand cold stress. The current study evaluated the effectiveness of different concentrations (2 and 3 mM) of these agents in preventing frost damage. Growth parameters were measured at: pre-frost (October, November and December) and post-frost (January, February and March). Among both agents, glycine betaine was most effective in preventing frost damage and helped to maintain the highest average plant height (89.19 cm), average petiole length (29.67 cm), leaf number (9.87) and stem girth (59.14, 41.09, and 30.74 mm, respectively, at lower, middle and upper girth) after frost. The most effective concentration of glycine betaine was 3 mM, which showed the highest effectiveness, followed by salicylic acid at 3 mM and glycine betaine at 2 mM.

Key words: Papaya, glycine betaine, salicylic acid, cold stress, frost damage

INTRODUCTION

Papaya (*Carica papaya* L.) is a tropical fruit crop with high value that is cultivated intensively in the Punjab region for its nutritional and economic value (Koul *et al.,* 2022). However, the frost factor during winter severely hampered papaya growth and production in this region (Burns *et al.,* 2022). Papaya is naturally susceptible to low temperature stress; even short-term exposure to frost causes leaf necrosis, stem cracks, stunted growth and even death of the plants in the worst conditions (Cabrera *et al.,* 2021). This abiotic stress not only decreases the yield and quality of the fruits, but it also threatens the source of income of the farmers who rely only on the cash crop.

As a possible solution to this issue, applying exogenous protectants such as glycine betaine and salicylic acid has become a promising area of investigation. These compounds help plants to recover from various abiotic stresses like cold and drought (Khalid *et al*., 2022). As an osmoprotectant, glycine betaine safeguards proteins and maintains membrane stability under stress. It preserves cellular osmotic integrity and protects the photosynthetic apparatus, enabling protective physiological mechanisms to lessen the impact of low-temperature stress. Research by Min *et al*. (2021) has shown that exogenous glycine betaine treatment increases antioxidant activities, including ascorbate peroxidase, catalase and superoxide dismutase, which are crucial for scavenging reactive oxygen species (ROS) produced during frost events. The antioxidant protection activity reduces membrane lipid peroxidation and cell damage, enhancing plant survival and vigour under low-temperature stress. Additionally, glycine betaine was observed to regulate hormones such as abscisic acid, which otherwise contributes to improved cold resistance in tomato (Dai *et al.,* 2024).

Salicylic acid, however, is one of the phyto-hormones that would help to regulate the plant defense system by enhancing the enzyme activity of peroxidase and catalase, reducing the oxidative damage, and boosting the deposition of defense metabolites like proline and soluble sugars (Wang *et al.,* 2023). These

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physiological adaptations maintain the cellular structure and metabolic processes under freezing stress. It has been proven in experimentation that foliar application of salicylic acid can enhance the ability of freezing tolerance in several crops successfully, reducing the electrolyte leakage and preserving plant turgidity after cold treatment (Saleem *et al.,* 2021). Since salicylic acid acts additively by enhancing the antioxidant defense and metabolic adjustment, it is valuable for protecting against frost damage in susceptible crops such as papaya.

The existing research on glycine betaine and salicylic acid is focused on the model plants or temperate species, and experimentation on their efficacy on tropical fruits like papaya is limited. In the current study, treatments were applied as foliar sprays earlier than expected frosts to promote direct absorption and rapid physiological response. The scientific rationale behind this approach was to utilize the protective effects of glycine betaine and salicylic acid in enhancing the natural defense against oxidative stress caused by cold, destabilizing membranes and disrupting plant metabolism (Jain *et al.,* 2021). The main goal of the research was to compare the effectiveness of glycine betaine and salicylic acid in reducing frost damage and increasing the resilience of papaya plants under field conditions in Punjab. The researchers aimed at evaluating foliage growth and damage control by applying different concentrations of these substances to papaya plants, which could be recommended to farmers for sustainable papaya production in frost-prone areas. Additionally, the study seeked to support the broader objective of achieving more stable and higher crop yields under increasingly unpredictable climatic conditions. The exogenous application of these compounds was hypothesized to synergistically reduce the damage to the vegetative parts of the papaya plants by modulating osmolyte accumulation and antioxidant defense.

MATERIALS AND METHODS

An experiment was conducted on the papaya (*Carica papaya* L.) variety Red Lady at the Agricultural Research Farm of Lovely Professional University in Jalandhar, Punjab, during 2023 to 2024. The coordinates for the location were latitude 31°14’30.5'' N and longitude 75°41’52.1'' E. A total of five treatments were applied in a randomized complete block design, with three replications. The treatments were: T_1 (Control), T_2 (salicylic acid 2 mM), T_3 (Salicylic acid 3 mM), T_4 (Glycine betaine 2 mM) and T_5 (Glycine betaine 3 mM). Planting was done in June 2023, where plant-to-plant distance was kept at 2.5 meters, and row-to-row distance was 3 meters. The treatments were applied through foliar spray on leaves in the first week of October, November and December.

The climatic data were taken on temperature (maximum and minimum), relative humidity (maximum and minimum), wind speed, average monthly rainfall, evaporation and sunshine. The observations were recorded through an automated weather station at the agricultural farm of the university. A sudden decrease in average daily maximum ($T_{(max)}$: 12.86°C) and daily maximum ($T_{(min)}$: 6.04°C) temperature occurred in January. However, the average relative humidity was higher in January (maximum: 94.36% and minimum: 79.50%). The month was characterized by the lowest wind speed (3.53 km/h), no rainfall, the lowest evaporation (0.74 mm), and minimum daily sunshine (5.93 h).

This study was carried out to evaluate the effect of various antifrost agents on the vegetative growth of papaya plants under low temperature stress in the field conditions. The observations on various parameters were recorded every 30 days after transplantation. Important parameters studied were average plant height (cm), average petiole length (cm), number of leaves per plant and stem girth (mm). Average plant height was determined using a calibrated wooden scale from the base of the stem to the shoot apex. Average petiole length was determined by measuring the largest dimensions of the largest leaf and its corresponding petiole on each plant. Stem diameter was measured from three sections of the stem: The lower stem girth at soil surface level, the middle portion and the upper portion, using a digital versnier caliper for improved accuracy.

The observations recorded on various parameters were analyzed in a randomized block design to evaluate the statistical significance of variation due to treatment. The F-test was applied to the recorded means of all parameters for each replication to determine the significance of variation among treatments. The used treatments were grouped at Pd"0.05 using Duncan's Multiple Range test (DMRT).

RESULTS AND DISCUSSION

The average plant height of papaya was significantly affected by salicylic acid and glycine betaine application at all growth stages (Table 1). The observations revealed that maximum average plant height was recorded under T_5 (89.19 cm), followed by T_4 (87.88 cm) and T_3 (87.20 cm) at 270 days of transplanting (March 2023). These treatments (T_5, T_4 and T_3) were reported to be at par with each other on all days of observations, reflecting the positive influence of glycine betaine at 2 or 3 mM concentration. In comparison, salicylic acid was effective at a 3 mM concentration. Both of the treatments, glycine betaine and salicylic acid, had a substantial effect on the average plant height at pre-frost (October-December) and post-frost (January-March) stages of papaya; however, this effect was more noticeable after application of glycine betaine, even at a lower concentration (2 mM). The growth-promoting effect of exogenous application of glycine betaine and salicylic acid might be associated with enhanced photosynthetic and tissue expansion activities under cold stress, as reported by Wang *et al.* (2021) in wheat and Mahouachi *et al.* (2023) in papaya. In plants, glycine betaine occurs mainly in the chloroplast, accumulates in younger leaf tissues, and acts as a protective agent to the thylakoid membrane, thereby maintaining the efficiency of photosynthesis and the elevation of antioxidant enzyme activity. According to Tisarum *et al.* (2020), exogenous application of glycine betaine as a foliar spray increased its level in the cytoplasm of leaf cells to avoid the breakdown of photosynthetic apparatus and chloroplast structure, leading to the growth of net photosynthetic rate. In addition, it ensured the higher nutrient uptake and stabilization of the photosynthetic pigment, net photosynthetic rate and chlorophyll fluorescence, as well as the enhancement of the growth performance in *Brassica oleracea* (Ahmad *et al.*, 2020).

The observation revealed that salicylic acid and glycine betaine application significantly affected the petiole length of papaya at all growth stages under cold stress (Table 2). The maximum average petiole length was recorded under T_5 (48.47 cm), followed by T_4 (46.21 cm) and T_3 (44.91 cm) at 240 days of transplanting (February 2023). However, it was substantially

Table 1. Effect of salicylic acid and glycine betaine on the average plant height (cm) of papaya

Treatment	October 2023	November 2023	December 2023	January 2024	February 2024	March 2024
T_1	44.82^{b}	56.74^{c}	66.64^{b}	67.90^{b}	67.82^{c}	68.92^{c}
T_2	51.78^{ab}	68.44^{bc}	74.92^{ab}	77.01^{ab}	76.99^{bc}	77.87^{bc}
T_3	57.62^{a}	80.31^{a}	83.01^{a}	85.09^{a}	85.78^{ab}	87.20^{ab}
T_4	60.52^{a}	81.54^{a}	84.25^{a}	86.33^{a}	87.66^{a}	87.88^{ab}
T_5	58.28^{a}	77.27^{ab}	82.39^{a}	83.78^{a}	87.98^{a}	89.19^{a}
C. D. (P=0.05)	10.178	11.762	10.355	10.205	9.249	10.032
C. V.	12.099	10.479	8.590	8.278	7.389	7.921

Similar superscripts represent the non-significant variation between observations.

Table 2. Effect of salicylic acid and glycine betaine on the average petiole length (cm) of papaya

Treatment	October 2023	November 2023	December 2023	January 2024	February 2024	March 2024
T_1	27.08^{a}	34.54^{b}	36.63^{b}	36.68^{b}	33.93^{b}	18.13^{d}
T_2	32.43^{a}	41.37^{ab}	43.34^{ab}	40.81^{b}	38.31^{b}	20.17^{cd}
T_3	33.73^{a}	45.45^{a}	47.40^{a}	47.15^{a}	44.91^{a}	22.69^{bc}
T_4	34.37^{a}	45.96^{a}	47.37^{a}	47.52^{a}	46.21^{a}	24.69^{b}
T_5	34.89^{a}	46.19^{a}	47.34^{a}	47.31^{a}	48.47^{a}	29.67^{a}
C. D. (P=0.05)	NS	7.095	7.323	6.196	6.449	3.781
C. V.	15.087	10.785	10.701	9.162	9.881	10.638

The similar superscripts represent the nonsignificant variation between observations. NS–Not Significant.

reduced due to damage to larger leaves after severe frost in February. Among the various treatments, the greater retention of leaves was observed after application of glycine betaine at 3 mM. Before frost, T_5, T_4 and T_3 were reported to be at par with each other on all days of observations, reflecting the positive influence of glycine betaine at 2 or 3 mM concentration. In comparison, salicylic acid was effective at a 3 mM concentration. Average petiole length was also significantly improved after glycine betaine (3 and 2 mM) and salicylic acid (3 mM) application. These treatments retained more leaves, resulting in a greater petiole length after frost damage.

Glycine betaine and salicylic acid acted as osmoprotectants and maintained the physiological and biochemical reactions. They elevated the level and activity of Calvin cycle crucial enzymes and increased antioxidant enzyme activities (SOD, CAT and POD) that protected the thylakoid membrane and prevented the plant tissues from oxidative damage (Niu *et al.,* 2023). It increased retention of chlorophyll, leaf thickness and photosynthesis, leading to better growth and leaf expansion even in low temperatures (Wang *et al.,* 2020).

Application of salicylic acid and glycine betaine significantly affected the number of leaves in papaya at 150, 180, 240 and 270 days of planting (Table 3). The observations revealed that the average number of leaves was under T_5, T_4 and T_3 on all days of observation. After severe frost in January and February, the number of leaves was substantially retained under these treatments (T_5, T_4 and T_3). Among these treatments, T_5 reflected a greater ability to protect the plants against cold stress. Leaves were the most sensitive part of papaya plants, which were severely affected by cold stress. In the current study, the leaves were severely affected; however, application of glycine betaine (3 or 2 mM) and salicylic acid (2 mM) had protected the leaves and papaya plants from severe damage. It resulted in substantial retention of leaves per plant. Glycine betaine and salicylic acid were very efficient stabilizers of the quaternary structure of enzymes and complex proteins under abiotic stress and protected different components of the photosynthetic apparatus, including ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO) and photosystem II (PSII), and preserved the highly ordered state of membranes at non-physiological temperatures. Salicylic acid was generally considered and involved in the normal growth and development of plants. Salicylic acid signalling pathway enhanced the chlorophyll content and improved the photosynthetic activity in plants, which was directly linked to plant growth and health (Song *et al.,* 2023).

The observations revealed that application of salicylic acid and glycine betaine had a significant effect on the lower stem girth (LSG) of papaya plants at 180, 210, 240 and 270 days after transplanting; however, at all these growth stages T_5, T_4 and T_3 were at par with each other (Table 4). The middle stem girth (MSG) of papaya plants was significantly affected by various treatments at 150, 180, 240 and 270 days after transplanting (Table 5). Like LSG, treatments T_5, T_4 and T_3 had an equal effect on the MSG of papaya plants. A significant effect of salicylic acid and glycine betaine application on upper stem girth (USG) was observed at all growth stages except 120 days of transplantation (Table 6). However, the T_5 was observed with the highest USG (30.74 mm) at 270 days of transplanting, followed by T_3 and T_4, which were at par. Though the stem girth of papaya before frost was not noticed to be influenced by the application of glycine betaine and salicylic acid, the post-frost impact was significantly visible and the plants provided

Table 3. Effect of salicylic acid and glycine betaine on the average number of leaves per plant

Treatment	October 2023	November 2023	December 2023	January 2024	February 2024	March 2024
T_1	16.67^a	17.39^b	13.27^c	15.19^a	7.94^d	5.67^d
T_2	17.11^a	19.70^{ab}	14.84^{bc}	17.25^a	9.17^{cd}	7.56^c
T_3	19.25^a	21.65^a	17.42^a	18.65^a	10.92^{ab}	9.09^b
T_4	16.45^a	20.18^a	15.78^{ab}	18.80^a	10.09^{bc}	8.32^c
T_5	17.01^a	20.61^a	16.77^{ab}	18.58^a	11.79^a	9.87^a
C. D. (P=0.05)	NS	2.335	2.123	NS	1.444	0.766
C. V.	20.864	7.614	8.825	11.016	9.392	6.136

The similar superscripts represent the non-significant variation between observations. NS–Not Significant.

Table 4. Effect of salicylic acid and glycine betaine on the lower stem girth (mm) in papaya

Treatment	October 2023	November 2023	December 2023	January 2024	February 2024	March 2024
T_1	25.56[a]	37.70[a]	44.66[b]	45.91[b]	47.92[b]	46.29[b]
T_2	31.18[a]	43.16[a]	49.70[ab]	50.54[ab]	51.42[b]	49.74[b]
T_3	33.76[a]	49.62[a]	57.18[a]	57.70[a]	60.72[a]	59.21[a]
T_4	32.49[a]	49.84[a]	57.17[a]	58.05[a]	61.18[a]	56.95[a]
T_5	30.69[a]	48.40[a]	55.26[a]	57.30[a]	63.72[a]	59.14[a]
C. D. (P=0.05)	NS	NS	9.076	8.769	7.721	6.927
C. V.	20.164	13.837	11.159	10.560	8.793	8.286

The similar superscripts represent the non-significant variation between observations. NS–Not Significant.

Table 5. Effect of salicylic acid and glycine betaine on middle stem girth (mm) in papaya

Treatment	October 2023	November 2023	December 2023	January 2024	February 2024	March 2024
T_1	18.78[a]	25.40[b]	28.46[c]	29.48[a]	28.26[b]	31.22[c]
T_2	23.29[a]	29.88[ab]	33.18[bc]	48.72[a]	32.98[b]	35.24[bc]
T_3	24.16[a]	33.59[a]	38.18[ab]	40.31[a]	39.61[a]	39.50[ab]
T_4	22.07[a]	32.48[a]	38.08[ab]	38.16[a]	41.01[a]	38.04[ab]
T_5	22.48[a]	33.89[a]	39.93[a]	39.98[a]	43.53[a]	41.09[a]
C. D. (P=0.05)	NS	6.019	6.022	NS	6.340	5.798
C. V.	19.426	12.582	10.991	26.285	11.099	10.167

The similar superscripts represent the non-significant variation between observations. NS–Not Significant.

Table 6. Effect of salicylic acid and glycine betaine on upper stem girth (mm) in papaya

Treatment	October 2023	November 2023	December 2023	January 2024	February 2024	March 2024
T_1	16.92[a]	22.79[c]	23.86[b]	22.89[c]	20.08[d]	20.99[c]
T_2	19.36[a]	27.35[bc]	29.31[a]	25.51[bc]	25.26[c]	23.81[bc]
T_3	19.82[a]	32.18[a]	31.26[a]	29.08[ab]	29.63[b]	26.73[b]
T_4	20.20[a]	26.68[bc]	31.71[a]	29.14[ab]	30.92[ab]	26.64[b]
T_5	20.86[a]	28.21[ab]	32.97[a]	30.75[a]	34.25[a]	30.74[a]
C. D. (P=0.05)	NS	4.646	4.467	4.488	3.887	3.911
C. V.	20.026	10.988	9.723	10.604	9.002	9.846

The similar superscripts represent the nonsignificant variation between observations. NS–Not Significant.

with glycine betaine (2 or 3 mM) and salicylic acid (3 mM) had minimum damage during cold stress. Glycine betaine and salicylic acid contributed to stabilizing the structural and functional integrity of RuBisCO, RuBisCO-activating enzyme (RCA), Fructose-1,6-bisphosphatase (FBPase), FBP aldolase, and PRKase (Niu *et al.,* 2023). These enzyme activities directly influence the chloroplast apparatus and help to maintain or improve the photosynthetic efficiency and plant growth. It can also scavenge the excess free radicals by increasing the activity of the ROS enzyme under cold stress. Glycine betaine up-regulated the expression of CaSOD, CaPOD and CaCAT, which increased the activity of antioxidant enzymes (SOD, POD and CAT), which could be responsible for protecting the PSI and PSII system under stress conditions (Delfani *et al.,* 2023). Li *et al.* (2025) had also reported improvement in plant growth and health in pepper after application of glycine betaine under low temperature stress.

CONCLUSION

Pre-frost application of glycine betaine at 2-3 mM concentration was an effective approach to mitigate the frost damage in papaya plants during cold stress in subtropical regions. This treatment prevented damage to vegetative parts of plants from cold stress, which was essential for photosynthetic activities and substantial growth to ensure better crop productivity. The current study recommends that the pre-frost application of glycine betaine during October-December will help the papaya plants to withstand severe frost occurring during January-February in the subtropical regions of Punjab.

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