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## Physiological and biochemical responses to cold stress in sesame (*Sesamum indicum* L.) during the early growth stage

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**Abstract:** Cold stress significantly impacts sesame during its early growth stages, with varying responses observed among different genotypes. Ten genotypes were evaluated for phenotypic response to various temperatures during germination. Cold stress at 10, 12, 14, and 16 °C inhibited germination, with zero germination at 10 °C. At 14 °C, genotypes showed significant germination variation, and it was selected as the threshold temperature for assessing cold tolerance in sesame. Four genotypes were grouped into two, and each group with extreme germination responses (high and low) were selected for further biochemical and physiological studies. Genotypes V5 and V7 exhibited higher cold tolerance, better germination percentage, and seedling parameters under low temperatures, while V8 and V9 showed significant reductions, indicating cold sensitivity. Biochemical analyses revealed that cold-tolerant genotypes had enhanced activities of antioxidant enzymes, including catalase, superoxide dismutase, and peroxidase, as well as higher proline accumulation compared to sensitive genotypes. These antioxidants played a crucial role in mitigating the oxidative stress induced by cold, as evidenced by lower levels of hydrogen peroxide and malondialdehyde in the tolerant genotypes. Cold-tolerant genotypes also accumulated higher soluble sugars and protein levels, contributing to osmotic regulation and membrane stability. The findings highlight the importance of enzymatic and non-enzymatic antioxidants in cold stress tolerance, suggesting these biochemical markers could be used to identify and develop cold-resistant sesame cultivars. The results offer valuable insights into the mechanisms underlying cold tolerance and provide a foundation for breeding efforts to improve sesame cold resistance.

**Keywords:** colder climate; low temperature stress; oilseed crop; seed germination; soluble protein

Sesame (*Sesamum indicum* L.,  $2n = 2x = 26$ ), oldest cultivated oilseed crops, domesticated over a member of the Pedaliaceae family, is one of the 5 000 years ago from its wild ancestor *S. malabaricum*

in the Near East, Asia, and Africa (Bedigian 2006). Known for its resilience to harsh climates, high oil yield, and unique antioxidant properties, sesame has become a vital source of high-quality edible oils and proteins (Ashri 1998). Nutritionally, sesame seeds contain 50–60% oil, enriched with natural antioxidants such as sesamol, sesamin, and sesamol, contributing to the oil's stability and extended shelf life (Uzun 2010). About 70% of sesame is processed into oil and meal, while the remaining 30% is used in food and confectionery products. With the growing global demand for vegetable oil due to the increasing population, ensuring sustainable oil production has become a challenge that can be addressed through effective planning and management (Corley 2009).

Environmental factors, including low temperature, drought, and salinity, can significantly restrict or influence plant growth and development (Kefale et al. 2024). Among these, low temperature is a major factor limiting the productivity and geographical distribution of many crops, including sesame. Seed germination, seedling growth, and seed fertility are all adversely affected by low (non-freezing) temperatures (Yan et al. 2006). Low-temperature stress is typically classified into two categories: cold stress (0–16 °C) and freezing stress (< 0 °C) (Lyons 1973). Seed germination is the first stage of the plant life cycle, and temperature is a major environmental stress for the inhibition of seed germination in plants. Temperatures lower than 15 °C are often unfavourable for germination in field conditions (Thapa et al. 2020). In the early stages of growth, cold stress can impair and delay germination, as well as have adverse effects on seedling growth, causing poor seedling establishment and non-uniform crop maturation (Bosetti et al. 2012).

Plant physiology, biochemistry, and molecular processes are impacted by cold stress (Xiong et al. 2002, Shinozaki et al. 2003). Cell membranes are severely damaged by cold stress, both directly and indirectly, resulting in reduced organelle function due to electrolyte leakage and deterioration of the cellular environment (Aroca et al. 2012, Chen et al. 2014). Cold stress induces the production of reactive oxygen species (ROS), including hydrogen peroxide ( $H_2O_2$ ), superoxide anion ( $O_2^-$ ), and hydroxyl radical ( $HO^\cdot$ ), thereby disrupting the balance between ROS and their scavenging systems, which leads to oxidative damage in plants (Corpas et al. 2001, Considine and Foyer 2021, Raza et al. 2022a, Soualiou et al. 2022, Saeed et al. 2023). A notable ROS is hydrogen peroxide ( $H_2O_2$ ), which acts in signalling and encourages the

production of other harmful ROS (Witlekens et al. 1995, Kazemi-Shahandashti and Maali-Amiri 2018). These ROS hinder photosynthesis and chlorophyll fluorescence at low temperatures, damage chloroplast membranes, and oxidatively alter lipids, proteins, and DNA (Apel and Hirt 2004). Malondialdehyde (MDA), a toxic byproduct of unsaturated fatty acid peroxidation in phospholipids, harms cell membranes under cold stress conditions (Wang et al. 2018).

Diverse types of plants have varying abilities to withstand temperatures. Temperate plants are more tolerant to colder climates compared to tropical plants (Dhingra 2015). Since plants cannot move, they rely on strategies to deal with various stresses, such as low and high temperatures, salt, and drought. These strategies impact crop growth and yield (Gill et al. 2003). Therefore, it is imperative to select genotypes that exhibit tolerance to these stresses to ensure optimal plant growth and productivity. Sesame is traditionally cultivated in tropical and subtropical regions. However, its expansion into temperate and high-altitude areas is limited by low-temperature stress, particularly during the early stages of germination and seedling development. Although sesame holds significant agronomic and economic importance, its physiological and biochemical responses to low-temperature stress remain insufficiently explored. While cold tolerance mechanisms have been extensively studied in other crops, such investigations in sesame are still scarce, especially in terms of identifying biochemical and antioxidant markers associated with tolerance. This lack of knowledge hampers the development of cold-resilient cultivars through breeding programs. Therefore, the present study aims to evaluate and compare the physiological and biochemical responses of cold-tolerant and cold-sensitive sesame genotypes under low-temperature conditions to identify reliable stress indicators that can guide future breeding efforts toward improved cold tolerance.

## MATERIAL AND METHODS

### Cold stress treatment at the germination stage.

Ten sesame genotypes (Table 1) were selected for germination at seven temperature treatments: 28 (control), 10, 12, 14, 16, 18, and 20 °C based on the modified approach described by Carvalho et al. (2001). All the seeds were mature, full, and undamaged, carefully selected to ensure uniformity. The experiment was conducted in a completely randomised design with four biological replicates per genotype

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Table 1. List of sesame genotypes used for cold stress temperatures at early growth stage

| No. | Gen code | Genotype name | Origin              |
|-----|----------|---------------|---------------------|
| 1   | SG046    | V1            | Xinjiang, China     |
| 2   | SG118    | V2            | Shanxi, China       |
| 3   | SG153    | V3            | Yunnan, China       |
| 4   | SG212    | V4            | Hubei, China        |
| 5   | SG277    | V5            | Guangxi, China      |
| 6   | SG343    | V6            | Hainan, China       |
| 7   | SG070    | V7            | Henan, China        |
| 8   | SG312    | V8            | Heilongjiang, China |
| 9   | SG297    | V9            | Liaoning, China     |
| 10  | SGH16    | V10           | Hubei, China        |

per temperature. Each biological replicate consisted of 50 seeds, placed in a 9 mm plastic box lined with two layers of Whatman filter paper and moistened with 10 mL of distilled water.

Seed surface sterilisation was performed using 75% ethanol for 30 s, followed by three rinses with sterile deionised water. Only intact sesame seeds with undamaged seed coats and uniform appearance were selected for testing. All plastic boxes were incubated in SANYO growth chambers (MLR-351H, SANYO Electric Co., Ltd., Osaka, Japan) set at  $60 \pm 2\%$  relative humidity and darkness to mimic underground conditions. The germination experiments lasted 15 days for all temperatures except the control (28 °C), which concluded after 7 days. Daily observations of germinated seeds were based on a 2 mm radicle protrusion.

The number of seeds germinated was recorded daily and the following parameters were calculated: Final germination percentage (GP) was calculated 15 days and seven days after sowing following Hernández-Herrera et al. (2014) recommendation for cold stress and control treatments; respectively (Hernández-Herrera et al. 2014):

$$GP = \frac{\text{germinated seeds count}}{\text{total seed count}} \times 100 \quad (1)$$

**Germination energy (GE).** This parameter represents the germination percentage four days after sowing (Farooq et al. 2005) (2).

**Germination index (GI):**

$GI = (15 \times n_1) + (14 \times n_2) + \dots + (1 \times n_{15})$  (3)  
where:  $n_1, n_2, \dots, n_{15}$  signify the quantity of seeds germinated daily from day 1 to day 15. The coefficients (15, 14, ..., 1) emphasise earlier germination, reflecting both speed and percentage. Higher germination rates and shorter germination periods result in elevated GI values (Benech Arnold et al. 1991).

**Mean germination time in days:**

$$MGT = \frac{\sum(D \times n)}{\sum n} \quad (4)$$

where:  $n$  – newly germinated seeds on day  $D$ ;  $D$  – denotes the number of days since test commencement (Hernández-Herrera et al. 2014).

**Cold stress treatment at the early seedling stage.**

Four sesame genotypes (two with high and two with low germination percentages from the low-temperature germination experiments) were exposed to low temperatures following normal germination (28 °C for 24 h, when the radicle apex penetrates the seed coat). The treatments included 28 °C (control) and 14 °C (cold stress temperatures). The experiment was arranged in a completely randomised design with four biological replicates per treatment. Each replicate comprised 10 uniform seedlings sown in a plastic box. The seedling experiment at 14 °C was terminated after 15 days, while those at 28 °C concluded after 7 days.

Radicle and plumule length were measured using a graduated ruler. For the fresh weight measurement, the samples were gently blotted with a tissue to remove excess water and then weighed using a sensitive balance at the end of the respective treatment periods.

**Physiological and biochemical parameters measurements.** The identical four sesame genotypes (two cold-tolerant and two cold-sensitive, selected based on germination percentage under low temperatures) were used. Seeds were initially germinated at 28 °C for 24 h to allow radicle emergence. Subsequently, seedlings were subjected to two temperature regimes: 28 °C (control) and 14 °C (cold stress). Seedlings exposed to control conditions were harvested at 7 days, whereas those under cold stress were collected at 15 days.

Whole seedlings were used for all physiological and biochemical analyses. For each temperature treatment, four biological replicates were assessed per genotype. Each biological replicate consisted of a pooled sample of 30 uniformly grown seedlings for biochemical analysis. Samples from four sesame genotypes were promptly frozen in liquid nitrogen and stored at –80 °C until further processing. The frozen samples were ground in liquid nitrogen to obtain fine powder for biochemical assays. All measurements were performed with four technical replicates per biological replicate to ensure the accuracy and reliability of the results. Eight physiological and biochemical parameters, including total superoxide dismutase (SOD) activity, peroxidase (POD) activity,

catalase (CAT) activity, soluble protein (SP), proline content (PRO), soluble sugars content (SS), hydrogen peroxide concentration ( $\text{H}_2\text{O}_2$ ) and malonaldehyde content were then determined. The activities of SOD, POD, and CAT were detected by SOD Assay Kit (YH1201, Angle Gene), POD Assay Kit (YH1210, Angle Gene), and CAT Assay Kit (YH1208, Angle Gene), respectively. The content of SP, PRO, and SS were detected by Protein Content Assay Kit (DB7120, Angle Gene), Proline Content Assay Kit (YH1231, Angle Gene), and Plant Soluble Sugar Content Assay Kit (DX3206, Angle Gene), respectively. The levels of MDA and  $\text{H}_2\text{O}_2$  were determined using the MDA Assay Kit (YH1217, Angle Gene) and the  $\text{H}_2\text{O}_2$  Assay Kit (YH1212, Angle Gene), respectively. All the kits were supplied by Nanjing Aoking Biotechnology Co., and all the operations were performed according to the manufacturer's instructions.

**Statistical analyses.** Each experiment was replicated four times, with results presented as means  $\pm$  standard deviations derived from four separate replicates. The analysis of all data was performed using R package software (R 4.3.2. version, Vienna, Austria). Tukey's test was employed to make comparisons between groups. To evaluate the association between various germination and seedling parameters, we computed correlation coefficients that quantify the intensity and orientation of the linear association between pairs of variables. Graphs were constructed using GraphPad Prism 8.0.2 (San Diego, USA).

## RESULTS

### Genotypes screening of sesame under artificial conditions

The results revealed a clear difference in how various genotypes responded to low-temperature treatment (Figure 1A). No germination occurred for any genotype at 10 °C of cold stress. Some genotypes germinated at 12 °C, while others did not. At 14 °C, germination was observed in all genotypes, with significant variation among genotypes, suggesting that this is an optimal temperature for cold stress in sesame (Figure 1B). Based on this differential germination among genotypes, two accessions (V5 and V7) were selected for cold stress tolerance, with minimal observable changes (Figure 1). Conversely, two other accessions (V8 and V9) displayed negative changes under cold stress compared to control conditions, suggesting their susceptibility to cold stress (Figure 1).

### Effects of cold stress on germination and seedling parameters of sesame genotypes

**Germination percentage.** The germination percentages of four genotypes (V5, V7, V8, and V9) showed differential response to temperature (14, 16, and 28 °C) (Figure 2A). Two genotypes, V5 and V7, recorded 98.5% and 98% germination percentages at 14 °C and 100% at 16 °C and 28 °C, respectively.

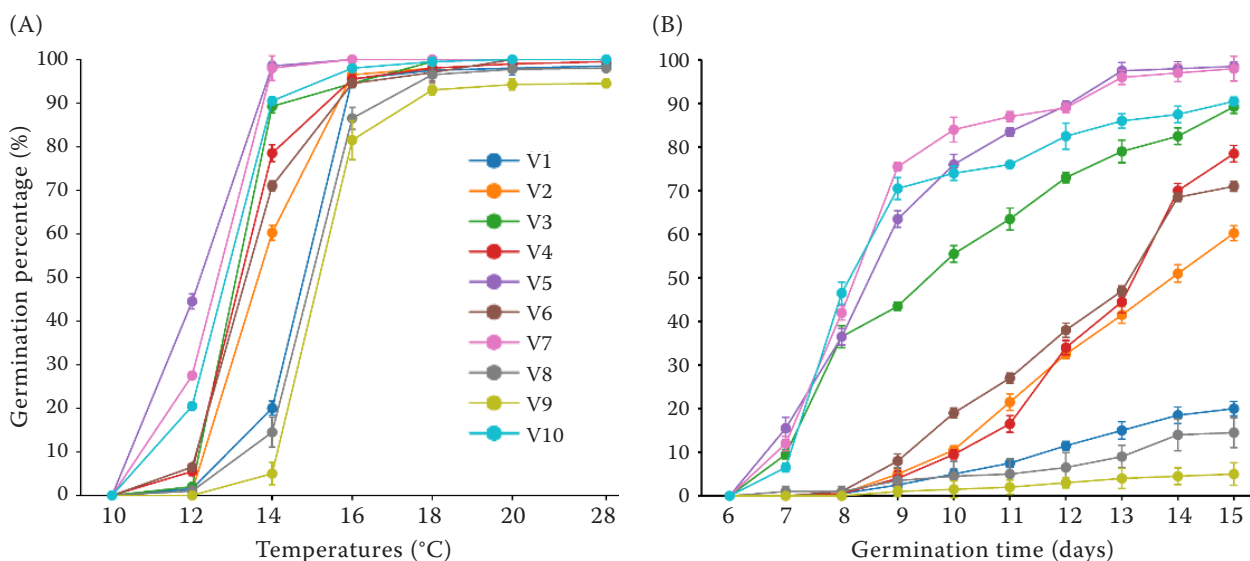


Figure 1. Impact of low temperature on the germination of sesame seeds. (A) Germination percentage of 10 sesame genotypes after 15 days of germination at different temperatures, and (B) daily germination percentages over 15 days under cold stress at 14 °C showed significant variation among 10 sesame genotypes. Data represent mean  $\pm$  standard deviation ( $n = 4$ )



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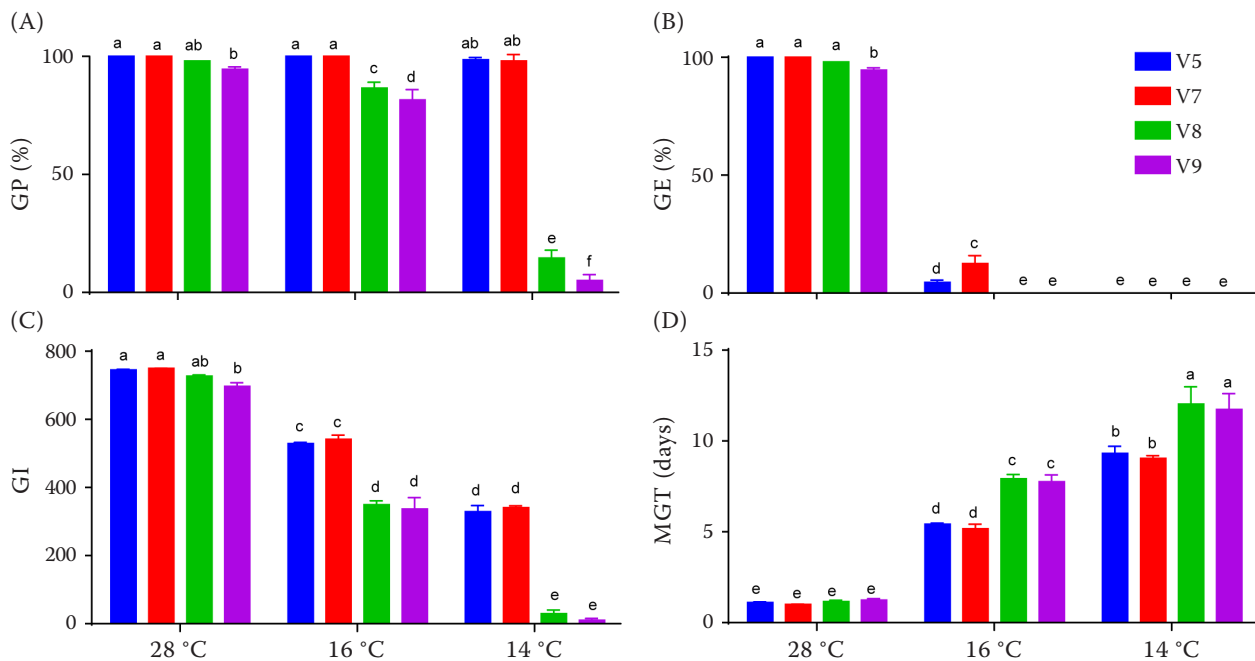


Figure 2. Impact of cold stress on the germination parameters. Measurement of (A) germination percentage (GP); (B) germination energy (GE); (C) germination index (GI), and (D) and mean germination time (MGT) of 4 genotypes of sesame under 14 °C and 16 °C treatments. Data represent mean  $\pm$  standard deviation ( $n = 4$ ). Different letters indicate significant differences ( $P < 0.05$ ) among the genotypes within each parameter using Tukey's test

Genotype V8 recorded 14.5, 86.5, and 98%, while V9 had 5, 81.5, and 94.5% at 14, 16, and 28 °C, respectively, indicating that the effect of low temperature on germination was greater in V8 and V9 than in V5 and V7.

**Germination energy.** At 14 °C, a severe cold condition, the germination energy of all four genotypes (V5, V7, V8, and V9) was zero on the fourth day, as illustrated in Figure 2B. When the temperature was raised to 16 °C, V8 and V9 continued to exhibit no germination energy, while V5 and V7 demonstrated improved performance, with germination energies of 4.5 and 12.5, respectively. Under the control condition of 28 °C, V5 and V7 achieved 100% germination, highlighting their robust germination capacity in normal circumstances. In contrast, V8 and V9 displayed slightly lower rates, with V8 reaching approximately 98% germination energy and V9 about 94.5%.

**Germination index.** The germination index results shown in Figure 2C revealed a notable distinction between cold-tolerant genotypes (V5, V7) and cold-sensitive genotypes (V8, V9) when subjected to cold stress temperatures of 14 °C and 16 °C, as compared to the control temperature of 28 °C. At 14 °C, cold-tolerant genotypes exhibited superior GI maintenance. V7 recorded a GI of 340.75, representing

a 54.54% reduction, while V5 recorded 329.25, a 55.79% decrease relative to the control. Conversely, cold-sensitive genotypes experienced a drastic GI decline, with V8 recording 29.50 (a 95.94% reduction) and V9 recording 10.50 (a 98.49% reduction). The GI performance marginally improved across all genotypes at 16 °C. V5 recorded 529, indicating a 28.97% decrease compared to the control (28 °C), while V7 registered 541.50, showing a 27.75% reduction. Among the sensitive genotypes, V8 measured 349.50 (a 51.94% reduction), and V9 recorded 336.75 (a 51.69% reduction) relative to the control.

**Mean germination time in days.** There were differential reactions to cold stress at 14 °C and 16 °C among the four genotypes (V5, V7, V8, and V9) (Figure 2D) concerning mean germination time (MGT). At 14 °C, all genotypes recorded a significant increase in the MGT, with a greater impact on sensitive genotypes. The V8 recorded the longest MGT (12.03), closely followed by the V9 (11.73), reflecting these genotypes' sensitivity to cold stress. On the other hand, V5 (9.31) and V7 (9.04) genotypes had the shortest MGT, indicating a relative ability to germinate faster under low temperatures. At 16 °C, the sensitive genotypes showed a significant increase in MGT, with MGT for V5 being 5.42 and for V7 being 5.17, while at

28 °C it was 1.11 and 1.01, respectively. For V8 and V9, the MGT increased by 583.61% and 520.47%, respectively, at 16 °C, compared to the control temperature.

**Radicle length.** The genotypes showed differential responses to cold temperature treatment with regard to radicle length, with tolerant genotypes showing better root development under stressful conditions (Figure 3A). Genotypes V8 and V9 had average radicle lengths of 0.67 cm and 0.59 cm, respectively, at 14 °C. Conversely, genotypes V5 and V7 had mean radicle lengths of 1.23 cm and 1.38 cm, respectively, at 14 °C.

**Plumule length.** Cold stress significantly reduced plumule length compared to the control (Figure 3B). The average plumule lengths of V8 and V9 were 0.38 cm and 0.35 cm, respectively, at 14 °C. On the other hand, under stress conditions at 14 °C, the average plumule lengths of the tolerant genotypes V5 and V7 were 0.83 cm and 1.18 cm, respectively.

**Seedling fresh weight.** At 14 °C, the fresh weight of the genotypes V5, V7, V8, and V9 decreased by 49.13, 36.45, 71.44, and 72.18%, respectively, compared to

the control, after being exposed to cold stress for 15 days) (Figure 3C).

### Effects of cold stress on biochemical traits of sesame genotypes

#### Effects of cold stress on antioxidant enzymes.

There was a significant increase in the activities of SOD, POD, and CAT under cold stress conditions (Figure 4). V5 exhibited the highest SOD (71.26%), POD (4 845.5), and CAT (66.71%) at 14 °C compared to other genotypes. Susceptible genotypes (V8 and V9) demonstrated the weakest SOD, POD, and CAT response at 14 °C (Figure 4).

**Effect of cold stress on soluble sugar, soluble protein, and free proline (PRO) levels.** Cold-tolerant genotypes (V5 and V7) exhibited a more pronounced response of soluble sugar (SS), soluble protein (SP), and free proline (PRO) to cold stress compared to their susceptible counterparts (V8 and V9). Notable V5 for SS and V7 for SP and PRO highlights the significant variation in cold stress compared to the sen-

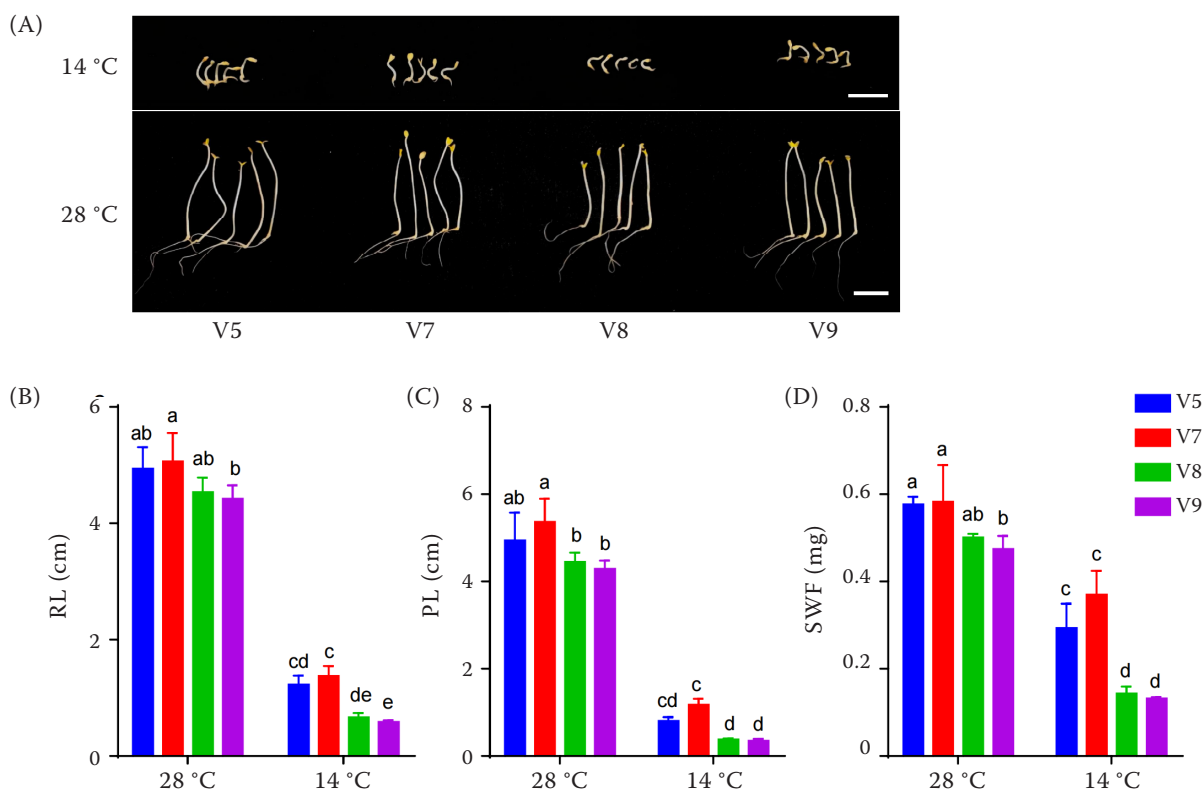


Figure 3. Impact of cold stress on the seedling parameters. (A) Visual representation of radicle and plumule of 4 sesame genotypes at 14 °C for 15 days. The bar indicates 2 cm. Measurement of (B) radicle length (RL); (C) plumule length (PL), and (D) seedling fresh weight (SWF) of 4 sesame genotypes under 14 °C treatment. Data represents mean  $\pm$  standard deviation ( $n = 10$ ). Different letters indicate significant differences ( $P < 0.05$ ) among the genotypes within each parameter using Tukey's test

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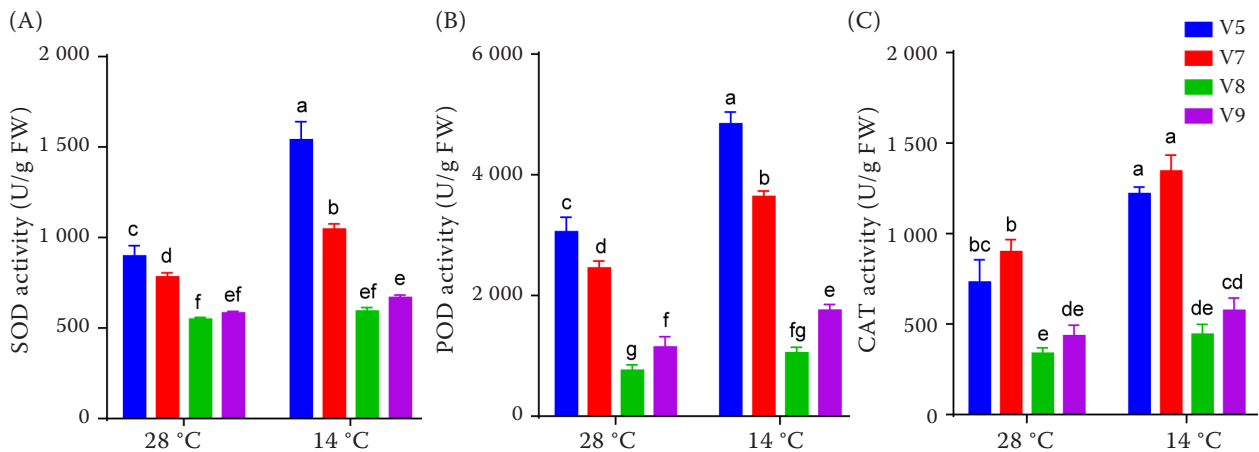


Figure 4. Impact of cold stress on the antioxidant enzymes: measurement of (A) superoxide dismutase (SOD); (B) peroxidase (POD), and (C) catalase (CAT) of 4 sesame genotypes under 14 °C treatment. Data represent mean  $\pm$  standard deviation ( $n = 4$ ). Different letters indicate significant differences ( $P < 0.05$ ) among the genotypes within each parameter using Tukey's test. FW – fresh weight

sitive genotypes (Figure 5). Amongst the genotypes studied, V7 exhibited the highest concentrations of PRO, and SP, with V5 following closely behind. V9 and V8 ranked third and fourth, respectively (Figures 5B–C). For SS (Figure 5A), V5 demonstrated the peak concentration at 14 °C. Concerning SP, V7 displayed the highest value at 14 °C, succeeded by V5, V9 and V8, respectively (Figure 6B). Regarding PRO analyses revealed a marked increase across all genotypes under cold stress relative to the control (28 °C) (Figure 5C).

#### Effects of cold stress on $H_2O_2$ and MDA content.

There were significant differences in  $H_2O_2$  content between the different genotypes and temperatures, with V8 and V9 recording the highest content com-

pared to V7 and V5 at lower temperatures (Figure 6A). At 14 °C, the  $H_2O_2$  content was highest in V8 and V9, respectively, followed by V7 and V5. Regarding the MDA (Figure 6B), genotypes V8 and V9 recorded higher values than V7 and V5 at cold stress. At 14 °C, the highest MDA content was in V8, which reached 15.91  $\mu\text{mol/g}$ , followed by V9. V7 and V5 recorded lower contents, respectively (Figure 6B).

#### Association of germination, seedling, and biochemical parameters

Significant correlations were among and between parameters measured across all measured temperatures (Figure 7). Notably, a strong positive correlation was

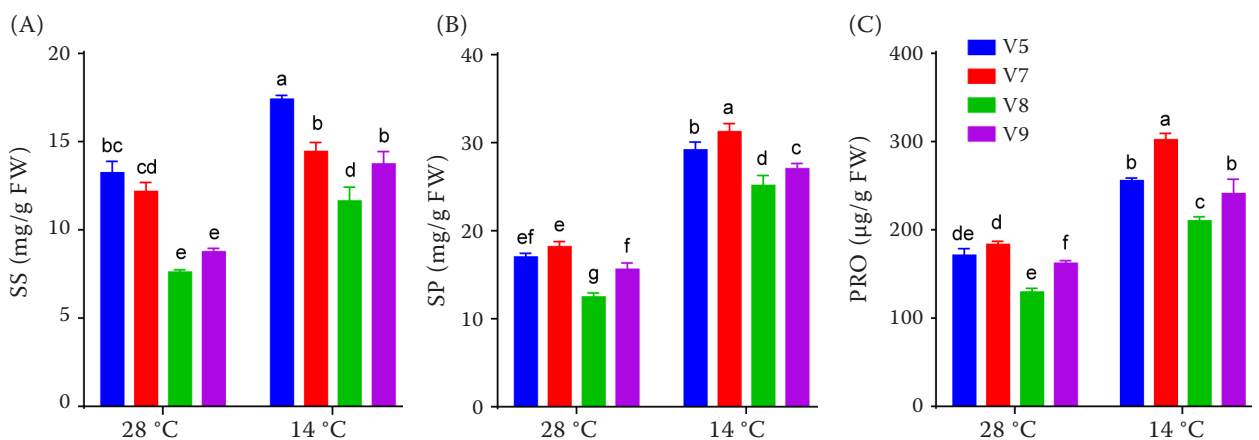


Figure 5. Impact of cold stress on the osmotic adjustment substance: (A) measurement of soluble sugars (SS); (B) soluble proteins (SP), and (C) proline (PRO) of 4 sesame genotypes under 14 °C treatment. Data represent mean  $\pm$  standard deviation ( $n = 4$ ). Different letters indicate significant differences ( $P < 0.05$ ) among the genotypes within each parameter using Tukey's test. FW – fresh weight

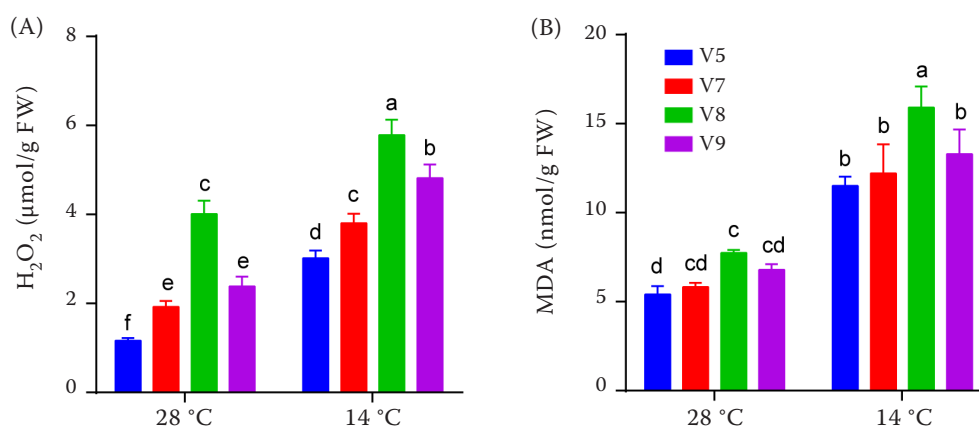


Figure 6. Impact of cold stress on the markers of reactive oxygen species (ROS) and lipid peroxidation: measurement of (A) hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and (B) malondialdehyde (MDA) of 4 sesame genotypes under 14 °C treatment. Data represent mean  $\pm$  standard deviation ( $n = 4$ ). Different letters indicate significant differences ( $P < 0.05$ ) among the genotypes within each parameter using Tukey's test. FW – fresh weight

observed among germination parameters, particularly between GP and GI at 14 °C and 28 °C. Conversely, MGT exhibited a negative correlation with all antioxidant, germination, and seedling parameters while showing a positive correlation with H<sub>2</sub>O<sub>2</sub> and MDA across 14 °C and 28 °C (Figure 7). Interestingly, RL demonstrated a significant positive correlation with PL ( $P = 0.05$ ) and SFW ( $P = 0.01$ ) at 14 °C and 28 °C (Figure 7). The antioxidant POD displayed a significant negative cor-

relation with H<sub>2</sub>O<sub>2</sub> ( $P = 0.05$ ) and a significant positive correlation with SOD at 14 °C (Figure 7B). The seedling parameter PL exhibited a significant positive correlation with the antioxidant CAT ( $P = 0.05$ ) at 28 °C while showing a strong negative correlation with MGT (0.05) at 28 °C (Figure 7A). Under stress temperatures (14 °C) (Figure 7B), GI and GP demonstrated strong positive correlations with most seedling parameters (PL and SFW) and significant negative correlations with MGT. Notably,

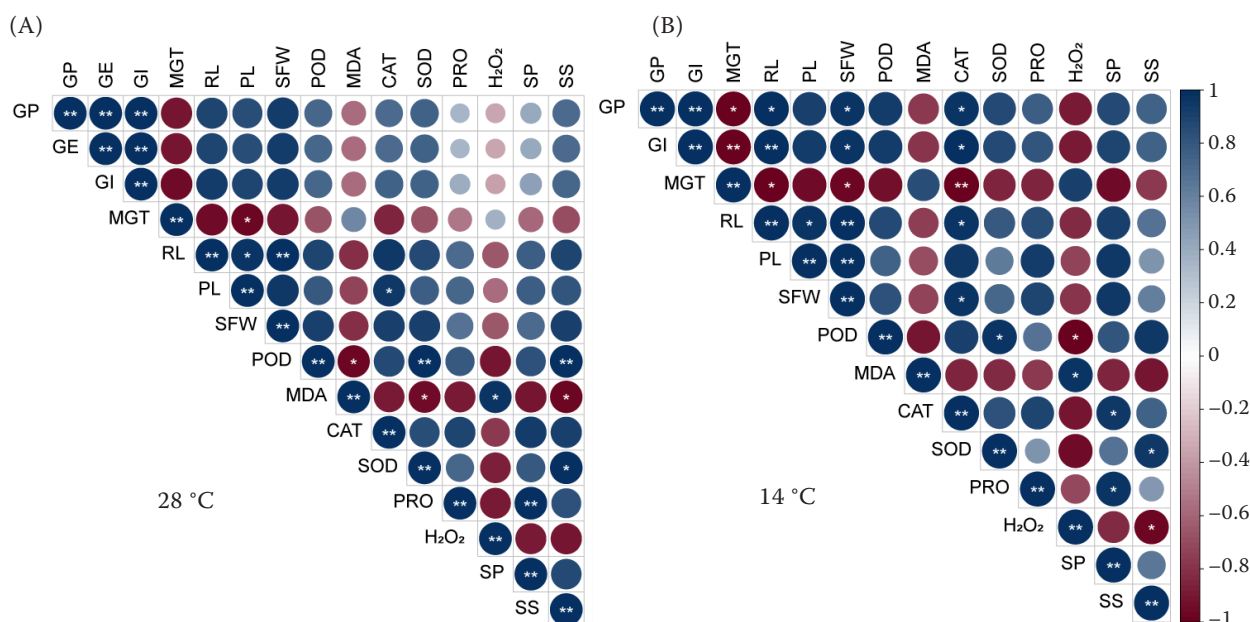


Figure 7. Correlation analysis between morphological, physiological, and biochemical traits in sesame under two temperature conditions: (A) optimum (28 °C) and (B) cold stress (14 °C). GP – germination percentage; GE – germination energy; GI – germination index; MGT – mean germination time; RL – radicle length; PL – plumule length; SFW – seedling fresh weight; POD – peroxidase; MDA – malondialdehyde; CAT – catalase; SOD – superoxide dismutase; PRO – proline; H<sub>2</sub>O<sub>2</sub> – hydrogen peroxide; SP – soluble proteins; SS – soluble sugars



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at 14 °C, CAT showed significant positive ( $P = 0.05$ ) associations with many traits, including GP, GI, RL, SFW, and SP, and significant negative association ( $P = 0.05$ ) with MGT.

## DISCUSSION

The screening of sesame genotypes for cold stress conditions revealed significant variation in how different genotypes responded to temperature fluctuations. No germination was observed for any genotype at 10 °C, indicating a cold stress threshold below which sesame seeds cannot germinate. This finding is consistent with the general understanding that low temperatures can inhibit seed germination in many plant species. At 12 °C, some genotypes began to germinate, while others did not, demonstrating variability in cold tolerance among the sesame genotypes. This differential response could reflect inherent genetic differences in cold-stress adaptability, with certain genotypes possibly harbouring cold-tolerant traits that allow them to overcome mild cold stress. At 14 °C, germination was observed in all genotypes, suggesting that sesame seeds can tolerate moderate cold stress, but that lower temperatures significantly impair germination success.

### Effects of cold stress on germination parameters of four sesame genotypes

Cold stress at 14 °C and 16 °C on sesame genotypes distinguished cold-tolerant from cold-sensitive genotypes. Cold-tolerant genotypes exhibited higher germination percentages and indices than cold-sensitive ones, aligning with studies showing a positive correlation between higher germination success and cold stress tolerance (Almodares et al. 2007, Yousefi et al. 2020). Conversely, cold-sensitive genotypes showed significant reductions in these parameters, suggesting a lack of necessary physiological mechanisms to cope with low temperatures. At the control temperature of 28 °C, all genotypes had higher germination percentages and indices, indicating optimal conditions for sesame germination. At 14 °C, all genotypes had no germination energy after four days, indicating this temperature is too low to support germination, even in tolerant genotypes, highlighting the detrimental effects of cold stress on seed germination. At 16 °C, cold stress significantly impacted germination energy; however, tolerant genotypes maintained some germination energy, 4.5 and 12.5, respectively, while sensitive genotypes remained at zero. These results support the hypothesis that

tolerant genotypes have physiological mechanisms enabling better cold stress resistance, consistent with research showing cold-tolerant plants maintain higher germination rates under stress (Mavi et al. 2010, Ping et al. 2015).

The mean germination time was negatively correlated with germination percentage, energy, and index, with sensitive genotypes showing longer MGT than tolerant genotypes. Seeds exposed to cold stress needed more time to germinate effectively due to the adverse effects of cold on metabolic processes, especially in sensitive genotypes where cellular repair mechanisms activate more slowly. Studies have documented similar delays in germination under cold or environmental stresses due to the time needed for genetic and enzymatic repair (Ellis and Roberts 1980, Bradford et al. 1990, Demir and Mavi 2008, Wang et al. 2018). Our findings align with previous research on *Brassica* and *Capsicum* species, where cold stress increased MGT, indicating that repair mechanisms take longer to address low-temperature damage. Conversely, the cold-tolerant genotypes had significantly lower MGT, suggesting more efficient cellular repair and quicker germination initiation. This rapid response may be linked to better preservation of proteins and DNA during stress, reducing repair time (Mavi et al. 2010).

Based on these findings, we conclude that MGT serves as a valuable indicator of seed vigour and cold stress tolerance. Seeds that exhibit more efficient repair mechanisms and a shorter MGT are likely to be more resilient to cold stress, as evidenced by the cold-tolerant genotypes in this study. These results further support the hypothesis that MGT can be utilised as a proxy for seed quality and stress resilience in sesame, as has been demonstrated in other species under various stress conditions (Ellis and Roberts 1980, Bradford et al. 1990, Demir and Mavi 2008, Wang et al. 2018).

### Effects of cold stress on seedling parameters of four sesame genotypes

Cold stress significantly impacted seedling parameters, particularly radicle length and fresh weight, highlighting differences between cold-tolerant and cold-sensitive sesame genotypes. Cold stress at 14 °C inhibited radicle growth in all genotypes, indicating that low temperatures impair root development processes. Cold-sensitive genotypes showed a greater reduction in root length compared to cold-tolerant

ones, suggesting the latter have mechanisms to mitigate cold stress effects. This aligns with studies emphasising root growth's role in cold tolerance due to its importance for nutrient and water uptake under adverse conditions (Landi et al. 2001, Hund 2010). The significant reduction in root length in sensitive genotypes under cold conditions indicates their poor coping ability, while tolerant genotypes maintained better root growth.

Similarly, cold stress at 14 °C significantly reduced the fresh weight of sesame seedlings compared to the control temperature of 28 °C. This reduction likely reflects cold stress's negative impact on metabolic activity and water absorption, essential for seedling biomass. Cold stress impairs metabolic efficiency and water uptake, affecting overall growth and development. These findings are consistent with El-Mahdy et al. (2018) who reported a 25% and 22% reduction in fresh weights of Grand Nain and Williams cultivars under cold treatment compared to controls, indicating that cold stress hampers both root development and overall seedling growth and physiological functioning.

#### **Comprehensive impact of cold stress on antioxidant systems and stress indicators in four sesame genotypes**

Cold stress triggers the production of reactive oxygen species (ROS), leading to oxidative harm in plants and requiring a robust antioxidant defence mechanism. Among these ROS, hydrogen peroxide plays a central role in mediating oxidative damage under cold stress. Its excessive accumulation has been widely associated with stress sensitivity. In agreement with our findings, Zhang et al. (2022) also reported increased H<sub>2</sub>O<sub>2</sub> accumulation in cold-stressed peanut plants, especially in the sensitive genotype. These results support the notion that excessive ROS accumulation is negatively associated with cold tolerance.

Hydrogen peroxide and malondialdehyde concentrations, which serve as indicators of oxidative stress and cellular damage, were elevated in cold-sensitive genotypes, demonstrating their inability to resist ROS-induced harm (Sairam et al. 2008, İşeri et al. 2013). This supports the findings of Gadjev et al. (2008), highlighting the importance of efficient antioxidant activity in preserving cellular stability and protecting against oxidative injury. The inverse relationship between H<sub>2</sub>O<sub>2</sub> and MDA levels and antioxidant enzyme activity in sensitive genotypes underscores the significance of these enzymes in mitigating oxidative damage and enhancing stress

tolerance. The accumulation of reactive oxygen species, particularly hydrogen peroxide, and the concomitant increase in malondialdehyde levels, a marker of lipid peroxidation, underscore the oxidative stress induced by cold exposure. This accumulation highlights the importance of antioxidant defence systems in mitigating oxidative damage (Ozkur et al. 2009). Therefore, our research revealed significant changes in both enzymatic and non-enzymatic antioxidants in response to cold stress, which were closely associated with the cold hardiness of the examined sesame genotypes. These results align with earlier studies on the critical role of antioxidants in plant stress resilience (Apel and Hirt 2004, Ahmad et al. 2010, Turan and Ekmekci 2016). The comprehensive evaluation of antioxidant enzyme activity, proline buildup, and cellular damage markers (H<sub>2</sub>O<sub>2</sub> and MDA) sheds light on how sesame genotypes manage cold-induced oxidative stress.

Catalase is a crucial enzyme in oxidative stress conditions, including cold stress, while superoxide dismutase is the sole antioxidant enzyme that neutralises superoxide radicals (Kwon et al. 2001). Where superoxide radical is converted to H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> is processed by SOD, followed by POD, CAT, and APX, which detoxify H<sub>2</sub>O<sub>2</sub> through various antioxidant cycles (Ozkur et al. 2009).

Notably, cold treatment enhanced SOD and CAT activities in the cold-induced sesame genotypes, in contrast to the control group. A similar trend was reported by Zou et al. (2007) who demonstrated that canola SOD isoforms were enhanced during cold treatment. Cold-tolerant genotypes exhibited markedly higher levels of antioxidant enzyme activities compared to cold-sensitive genotypes. The enhanced antioxidant enzyme activities in tolerant genotypes indicate their crucial function in reducing ROS-induced damage during cold stress. In particular, the increased CAT activity was observed in tolerant genotypes. This corroborates previous findings reported in oilseed crops and other crop species (Slesak et al. 2008, Rajabi et al. 2012, Skyba et al. 2012, Moieni-Korbekandi et al. 2014, Lei et al. 2019) emphasizing the importance of catalase in neutralising hydrogen peroxide.

Catalase is unique among many ROS detoxification enzymes in that it does not require a supply of reducing equivalents and, therefore, does not reduce energy reserves in plants (Ozkur et al. 2009). It is a significant advantage, particularly in stress settings where the photosynthesis rate slows and the plant's reservation of energy gets starved. Consequently, a robust link exists between CAT activity and stress tolerance in plants (Juhnke et al. 1996).

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The synchronised increase in CAT, SOD, and POD activities under cold stress suggests a well-regulated antioxidant defense system that enhances ROS neutralisation and cellular protection (Javadian et al. 2010). In contrast, cold-sensitive genotypes showed lower antioxidant enzyme activity, corresponding to their diminished ability to manage oxidative stress. This supports earlier research indicating that efficient antioxidant defences are vital for stress tolerance, as robust systems are better equipped to handle ROS accumulation (Desikan et al. 2003).

In addition to the enzymatic responses, several genes have been identified as primary molecular regulators of cold stress resilience. In oil palm, it was revealed that genes such as CBF1, CBF2, and ICE1/ICE2 played key roles in molecular expressions and antioxidant enzymatic activities under cold stress conditions (Li et al. 2019). These molecular regulators enhance the plant's defence mechanisms by activating downstream genes involved in ROS detoxification, thereby contributing to improved cold tolerance. The observed increase in enzyme activities confronted with cold stress further confirms their protective role in response to cold-induced oxidative stress. Such elevated enzymatic responses may indicate the upregulation of these cold-responsive genes, highlighting a strong link between molecular regulation and physiological defense mechanisms under low-temperature stress.

Non-enzymatic antioxidants, especially proline, play a significant role in safeguarding cold-stressed sesame plants by functioning as an osmoprotectant and ROS scavenger, thereby stabilising cellular structures (Banu et al. 2009, Kaul et al. 2008, 2011). Our study showed higher proline accumulation in cold-stressed, cold-tolerant genotypes, consistent with research demonstrating proline as a common response to cold stress across various plant species (Ruiz et al. 2002, Verbruggen and Hermans 2008, Lukoševič et al. 2009). Proline helps stabilise proteins and membranes while scavenging ROS, which is crucial for maintaining cellular integrity. Cold-tolerant genotypes accumulated more proline, suggesting an adaptive mechanism for cold tolerance, consistent with similar results in Bermuda grass (Zhang et al. 2011). Conversely, cold-sensitive genotypes exhibited lower proline levels, indicating sensitivity to cold stress due to a weaker defence mechanism. The function of proline as a non-enzymatic antioxidant is emphasised as an indicator of cold tolerance.

The function of proline in stress adaptation is generally explained by its characteristic as an osmolyte

and its ability to balance water stress (Delauney and Verma 1993). However, adverse environmental conditions typically disturb intracellular redox homeostasis, necessitating mechanisms that also operate to control oxidative stress. Thus, proline protective mechanisms have also been postulated to entail the stability of proteins and antioxidant enzymes, direct scavenging of ROS, balancing of intracellular redox homeostasis (e.g., the ratio of NADP<sup>+</sup>/NADPH and GSH/GSSG), and cellular signalling facilitated by proline metabolism (Liang et al. 2013).

Soluble sugars and proteins significantly contributed to the cold stress response in sesame. Cold-tolerant genotypes showed higher levels of soluble sugars, essential for osmotic regulation and membrane stability, corroborating the findings of Li et al. (2019), who found that CBF expression is positively correlated with sucrose content under cold stress in oil palm. CBF, a key transcription factor, regulates cold-responsive genes and promotes the accumulation of osmoprotectants like sucrose, highlighting the importance of soluble sugars in stress adaptation under low temperatures. Additionally, soluble protein accumulation, crucial for membrane stabilisation and enzyme protection, was more pronounced in tolerant genotypes. Karimzadeh et al. (2006) indicated that soluble proteins involved in oxidative stress response are key adaptive strategies during cold stress. Soluble sugar and protein contents are strongly correlated to improved stress tolerance in plants (Ling et al. 2015). The accumulation of soluble sugars and soluble proteins maintains plant turgidity, enhances absorptive ability, and protects membranes and macromolecules during stress (Mielke et al. 2003, Zhang et al. 2010). Additionally, adjusting the levels of soluble substances (SS, SP) and regulating antioxidant enzyme activities have been reported to maintain osmotic balance and reduce membrane lipid peroxidation under stress (Dai et al. 2014, Gulhane and Kolekar 2014). Hence results of proposed study supports the above findings and it was observed that soluble proteins and sugars accumulated in cold-tolerant genotypes, likely to maintain the cellular function and boost its integrity.

### **Correlation among germination, seedling, and biochemical parameters**

The correlation patterns observed in this study suggest that the antioxidant defence system plays a crucial role in regulating germination and seedling

development under cold stress. The significant positive associations between antioxidant enzymes (e.g., CAT, SOD, POD) and germination traits (GP, GI) indicate that enhanced antioxidant activity creates a favourable metabolic environment that supports cellular homeostasis and seedling vigour under stress conditions. These findings are consistent with previous reports that showed a strong interplay between enzymatic antioxidants and osmotic adjustment substances in enhancing cold stress tolerance (Wu et al. 2013).

Moreover, significant negative correlations were observed between oxidative stress markers ( $H_2O_2$ , MDA) and antioxidant enzymes, key germination and seedling traits, supporting the hypothesis that cold-induced oxidative stress may impair early seedling establishment unless adequately mitigated by robust antioxidant activities. The findings of the current study align with the results reported by Raza et al. (2022b) and Zhang et al. (2022). They observed comparable trends in canola and peanuts under cold stress regimes for the mentioned attributes. Furthermore, Xia et al. (2020) and Raza et al. (2022a) reported a significant positive association between antioxidant enzymes and osmoprotectants, such as soluble protein and sugars under cold stress, suggesting an integrated defence mechanism. During the current study, it was observed that POD had a significantly negative correlation with  $H_2O_2$  whereas it positively correlated with SOD at 14 °C, proposing an integrated antioxidant response to maintain cell homeostasis. Moreover, a significant positive correlation was reported between germination percentage, germination index, radicle length, and plumule length while the studied traits exhibited a negative association with mean germination time. Therefore, findings depicted that better germination is associated with faster emergence and seedling vigor, seconded by the results of Ping et al. (2015) in canola under cold stress.

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