International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page:www.iscientific.org/Journal.html

Sunflower seedlings' biochemical and physiological reactions to cold stress are related to their growth characteristics

Sunil Kumar[1], Meenakshi Dheer[2], Anuradha Rohinkar[3]*

[1]*College of Agriculture Science, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India.*

[2]*Department of Agri-business Management, Vivekananda Global University, Jaipur, India.*

[3]*Department of Biochemistry, Parul University, PO Limda, Vadodara, Gujarat, India.*

Abstract

Extreme occurrences have grown increasingly common as a consequence of weather changes. April is the period for sunflower strewing. Numerous spring snowfalls or prolonged cold weather impair harvest development with degraded plant density per hectare. Once the sunflower production region spreads to remote locations with poor growing conditions, resistance to low temperatures is a crucial quality to possess. Investigating the physiological and biochemical aspects that complicated development revival afterward icy coverage at sunflower sprouts is a novel strategy for identifying resistant genotypes in breeding programs. This investigation looked at the biochemical and physiological responses of two different genotypes of sunflowers. Commercial hybrids Pampero (PM) as well as to Sierra (SA) are ice-covered for 96 hours at 5°C. At 0, 24, 48, and 72 hours after the application of cold, malondialdehyde content, growth performance, catalase enzyme activity, superoxide dismutase, chlorophyll content, and electrolyte leakage was evaluated. There were discovered various genotype-specific patterns. The sensitive genotype PM displayed decreased superoxide dismutase activity, lower membrane stability, and greater oxidative damage compared to the tolerant genotype. The consenting genotype SA showed a high capacity toward restoring resumed growth and chlorophyll content in contrast to the sensitive genotype, which only slightly raised chlorophyll content and delayed growth.

Keywords: Pampero (PM) and Sierra (SA), genotype

Full length article **Corresponding Author,* e-mail:sunilagro.chaudhary@gmail.com

1. Introduction

Due to its adaptability to a variety of soil and temperature conditions, the ornamental plant sunflower (Helianthus annuus) is often planted around the world. This one is often recognized as employing the very easy-going harvest capable of withstanding high heavy metal concentrations in soil. This is often grown up on deprived, grimy soils unsuitable for other crops [1]. Sunflower is one of the four most important annual edible oilseed crops farmed worldwide, along with soybean, peanut, and canola. Oil Bodies (OBs) and the intrinsic proteins linked to oleosins offer several pharmacological and biotechnological benefits for human health. 35 years ago, the research team was researching the importance of auxin/ethylene interactions in adventitious rooting (AR) from hypocotyl explants when they noticed variances in the expression of peroxidase isozymes [2]. Conventional oil primarily contains linoleic acid, while high oleic oil primarily contains oleic acid. The most dependable type of oil for use in industry is high oleic (Ho) oil. Furthermore, it has been demonstrated that employing HO oil in the production of food is healthy. “Trans fatty acids (TFA)”, must be replaced in the food business because they are frequently present in fried snacks and bakery goods and have been linked to health hazards. The best TFA alternative was determined to be HO sunflower oil, which had the best fatty acid profile and agronomic performance [3]. The manipulation of soap stock into a free fatty acid mixture with no lingering glycerides was optimized using an enzymatic method. Moreover, utilizing “High-Oleic Sunflower Oil (HOSO)” soap stock, which contains up to 70-79% oleic acid, azelaic and pelargonic acids were created by chemo-enzymatically breaking the CC double bond in oleic acid [4]. According to a study, the way various genotypes respond to cold temperatures in terms of germination varies. Both the cold-tolerant and cold-susceptible commercial cultivars Sierra HO and Pampero were used. The length of the sunflower cycle varies, but it typically lasts 125 days. Variables include temperature, genotypes, planting dates, latitude, and the availability of water and fertilizer. The ideal temperature for the growth of sunflower roots is around 8

°C. As a spring crop, sunflowers should be planted as soon as possible to reduce crop vulnerability to heat stress and water constraint during the more challenging summer months. However, to increase seed and oil yield, this early seeding management method frequently exposes plants to freezing temperatures. To enable early, productive seeding, sunflowers' early growth, and development must be promoted to increase their cold tolerance [5].

The research [6] investigated whether magnetic seed stimulation could be used to promote sunflower germination, growth, and yield. Studies on emergence and germination were carried out to improve the magnetic field's strength for sunflower seed production. The study [7] analyzed the biochemical and physiological characteristics of the Hysun-336 sunflower hybrid produced in a greenhouse on sandy soil as well as the advantages of soaking seeds in maize grain extract to regulate development, fatty acid profile, yield performance, as well as seed oil. The study [8] showed how vulnerable sunflower seedling roots are to salt stress due to nitrite buildup. In reaction to salt stress, S-nitroso glutathione reductase (GSNOR) activity sharply declines. The restoration of GSNOR activity with dithioerythritol shows that the enzyme's 120 mM NaCl-induced inhibition is reversible. At the cell, plant, and crop levels, the effects of drought on sunflower achene yield and oil quality are discussed, and practical management techniques to lessen the severity of the drought stress are recommended [9]. Sunflower seeds were exposed to low-power continuous wave He-Ne laser irradiation at energies ranging from 0 to 250, 450, and 650 mJ to determine the effect on biochemical, physiological, growth, and yield features. The study [10] was performed in a greenhouse setting and had a Fully Randomized Design (CRD) with four repetitions. drought stress's effects on sunflower seed germination. After applying several drought stress conditions to sunflower inbred lines with hybrids during seed development, the seed germination of the offspring was evaluated in [11]. Sunflowers can be adapted to abiotic stresses in a variety of ways, including characteristics, genetic resources, and methods for introducing them into farmed sunflowers to make them resilient to harsh climatic circumstances, as described in [12]. In East Azerbaijan, Iran, a three-year study was conducted on the effects of water deprivation stress on a range of quantitative and qualitative characteristics of sesame (Sesamum indicum L.) sunflower (Helianthus annuus L.), along with the safflower (Carthamus tinctorius L.) [13]. The research [14] examined the physiological impacts of feeding European seabass a pre-treated “Plant Feedstuff Mixture (PFM)” that had undergone “Solid-State Fermentation (SSF)”. The research [15] discovered that increasing sunflower hyperaccumulation capability by mixing Ethylene Diamine Tetra-Acetic acid (EDTA) and Indole acetic acid (IAA). Sunflowers were grown in a range of Cu concentrations and were either treated with IAA or EDTA alone.

2. Materials and methods

2.1 Substances and environments for plant development

Two hybrids of commercial sunflowers, Sierra (SA) High-Oleic and Pampero (PM), were employed. In prior studies, the substances remained categorized by way of being both cold-tolerant in addition to cold-sensitive. These hybrid seeds were planted in pots with a 9 cm diameter, an 11 cm height, and a sand substrate. Following sowing, pots were housed in a growth chamber for 15 days, where they received regular watering and were maintained at a temperature of 20°C at night and 30°C during the day, with an 8-hour darkness and 16-hour light cycle. After the seedlings had two leaves, treatments started.

2.2 The control procedure

The control treatment (C), which is kept in the growth chamber under predetermined circumstances, uses 40 pots of each hybrid sunflower. In a laboratory setting with a 96-hour photoperiod of 16 hours of light and 8 hours of darkness and a constant temperature of 5°C, 40 pots underwent the Cold Treatment (CT). After the CT, the pots were left in carefully monitored treatment conditions for 25 days so they could cool. Each of the 8 plants had their leaves sampled at 0, 24, and 72 hours following the application of cold. These samples' physiological and biochemical properties were evaluated. 8 plants from each hybrid and each treatment were collected at 15 and 25 days to measure growth characteristics.

2.3 Biochemical measurement

100 mg of leaves per sample were used to test oxidative damage (MDA concentration) with antioxidant enzyme activity (SOD and CAT) at 0, 24, and 72 hours. By calculating the MDA concentration, Heath and Packer described how to measure lipid peroxidation in leaves. The materials were handled following the technique described to assess the activity of antioxidant enzymes. The method described was used to estimate SOD activity. To measure enzymatic activity, SOD units per mg of protein were utilized. The Bradford technique was used to determine the enzyme extracts' protein content. CAT activity was assessed using the absorbance at 240 nm, which displays the consumption of H_2O_2. The quantity of enzyme needed to catalyze the conversion of 1 mol of H_2O_2 into the water once every minute was used to define one unit of CAT activity. The data were given in milligrams of protein and mol H_2O_2 extinct per minute.

2.4 Electrolyte leakage

At 0, 24, and 72 hours after the end of the low-temperature exposure, six leaf discs from each treatment were collected, splashed, and then flooded in 45 ml of demineralized water. The unique conduction (initial Ω) of the cleaning water was measured after the discs had been incubated for 24 hours at 25 °C. The discs underwent a 30-minute autoclave at 100°C to assess the maximum conduction of injured soft tissues. The electrolyte leakage is estimated as a percentage of TM = (initial Ω/maximum Ω) x 100. By utilizing a UV-120 spectrophotometer to analyze

extracts that were cooked in an 80% ethanol solution, the amount of chlorophyll was determined.

2.5 Growth performance evaluation

Using eight plants per hybrid and treatment, Total Dry Weight (TDW) remained calculated 21 and 31 days after the cold treatment terminated. Using a burner set at 60 °C, the collected samples were dried to a consistent weight. "LDW (Leaves Dry Weight), SDW (Stem Dry Weight), and RDW were combined to create TDW (Roots Dry Weight)".

2.6 Statistical investigation

All variables were determined using a randomized method by 3 recurrences per conduct per the genetic constitution. To compare average values, an ANOVA was employed, along with generalized linear mixed models. CAT and SOD enzyme levels, chlorophyll content, and electrolyte leakage were examined concerning each other utilizing three-way interactions.

The InfoStat statistical program was used to perform the Fisher test at the 7% parallel in consequence ($P \leq 0.05$). In all figures, the standard error was plotted.

The differences between treatments for all measured parameters are intended using the method below: change (%) = ([z_(d) z_p])/z_d , where z_(d)is the average rate of the controller plant life and z_p is denoted by sample size. The average value of stressed plants is described.

3. Results and Discussions

Substantial differences in three-way interactions were seen in the activity of both SOD and CAT, two antioxidant enzymes (P 0.05). After the CT, both genotypes (PM and SA) displayed higher enzyme activity than following the Control Treatment (C). The SA genotype showed higher rise values than the PM genotype, and varied reactions between genotypes were also found (Fig. 1). It was demonstrated that following the CT, the SA genotype's SOD enzyme activity rose and stayed high for three days. Yet only 24 hours after CT did the genotype (PM) show an increase in SOD activity (Fig. 1a).

Both genotypes' CAT movement was high after cold therapy. Yet, when it came to CAT activity values, the SA genotype greatly outperformed the PM genotype (Fig. 1b).

Both genotypes experienced significant oxidative damage following CT, as indicated by the quantity of malondialdehyde (Fig. 2a). MDA content stayed greater in the PM genotype than in the SA genotype (99% higher) through the factor of 210 over the SA genotype. On the other hand, the electrolyte leakage parameter displayed a similar response (Fig. 2b). While the PM genotype had larger electrolyte leakage, the SA genotype had fewer increases.

Both genotypes' total chlorophyll concentration decreased after exposure to cold. The lowest content (53% less than their control) was seen in the PM genotype. Although both genotypes displayed increased chlorophyll content 72 hours after CT, the SA genotype exhibited the greatest levels in contrast to the modification treatment (figure 3).Both genotypes performed worse in terms of growth than the CT in terms of total dry weight (Figure. 4). At the end of the CT, the SA genotype had 12% less TDW than the control condition, albeit this difference reduced during the following 25 days. The PM genotype, on the other hand, lost 25% of its TDW; the distinction became even more apparent after an additional 25 days. Hence, whereas the PM genotype was more adversely affected and developed more slowly, the SA genotype was able to endure and recover from CT. Similar patterns can be seen in the DWR, DWS, as well as (DWL).

3.1 Discussion

Two genotypes of sunflowers were examined to determine how a CT affected their physiological, biochemical, and growth factors. A variety of morphological, cellular, physiological, biochemical, and molecular adaptations have been produced by plants to protect them from abiotic stress.On the way to examine the pattern of antioxidant defenses, the activity of important antioxidant enzymes including total SOD and CAT was assessed. Some researchers, however, reported that the SOD activity was much higher in tolerant cultivars and lower in sensitive cultivars when they used a variety of plant species. The tolerant genotype of the sunflower in this study had elevated CAT and SOD activities. The enzyme activity measured following the CT was higher than it was following the C aimed at together genotypes PM and SA. Moreover, there were differences in the responses between the two genotypes, with the SA genotype showing increased values than the PM genotype. This information suggests that even after the stress has receded, cold still affects how the antioxidant system and oxidative metabolism are managed. Many studies have examined how stress affects CAT activities, such as how it induces, decreases, or stabilizes them. In the current study, the tolerant genotype had significantly greater overall CAT activity, whereas the sensitive genotype had somewhat higher overall CAT activity. The responses to CAT activity varied between the genotypes of sunflowers analyzed.

Numerous studies have found that tolerant genotypes have superior oxidative damage regulation than sensitive genotypes. Several crops have employed MDA as a suitable biomarker for the selection of resistant genotypes as a signal of oxidative damage. We discovered during our experiment that MDA levels varied between genotypes. In comparison to the tolerant genotype (SA), the susceptible genotype (SA) displayed greater oxidative damage (PM). The electrolyte leakage measurement may display a similar trend. Moreover, these two metrics are linked because membrane degradation causes electrolyte leakage to increase and membrane lipid peroxidation causes an increase in MDA concentration. The SA genotype could lessen oxidative damage, indicating that it was more resistant to the effects of cold therapy than the PM genotype. In our investigation, after exposure to cold, both genotypes showed reduced total chlorophyll contents. The sensitive genotype (PM) was more susceptible to cold stress and had a reduced chlorophyll content, while the tolerant genotype (SA) recovered from it more quickly.

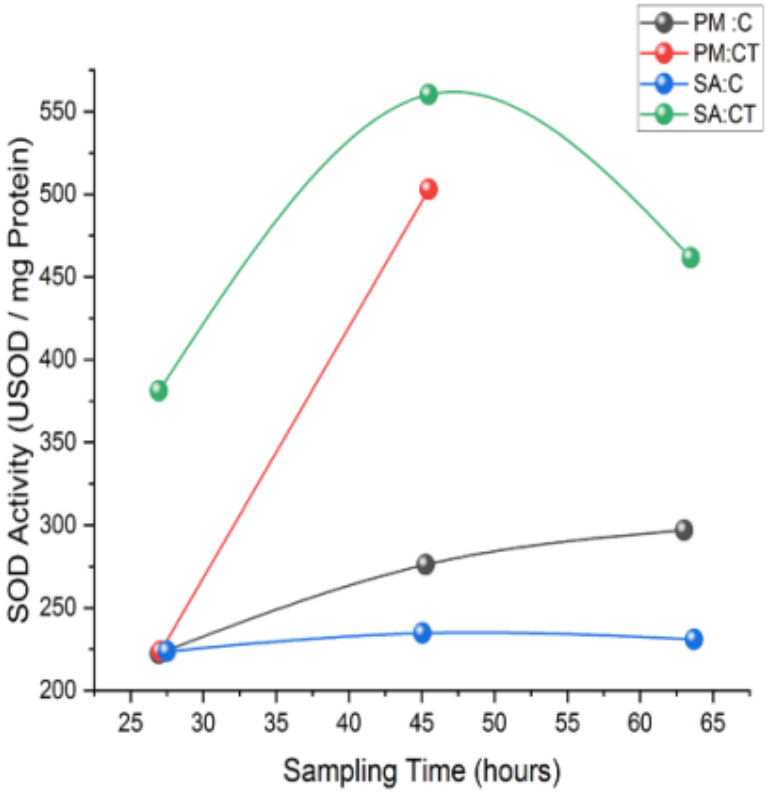

Figure a. Enzyme activity in SA genotype

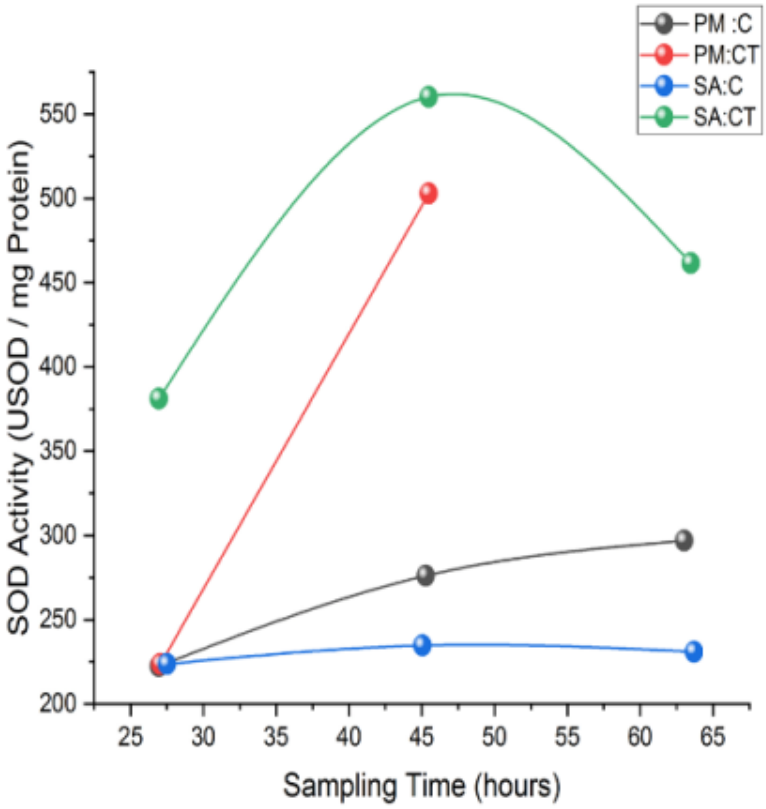

Figure b. Enzyme activity in PM genotype

Figure 1 shows the enzyme activity in the SA and PM genotypes of sunflowers under control (C) and cold treatment conditions (CT) Superoxide dismutase (SOD) activity; catalase activity (CAT) Significant deviations are denoted by different letters (p.o.5). The error bars show the standard deviation (n=8). In comparison to the control treatment, the rise was shown with cold therapy.

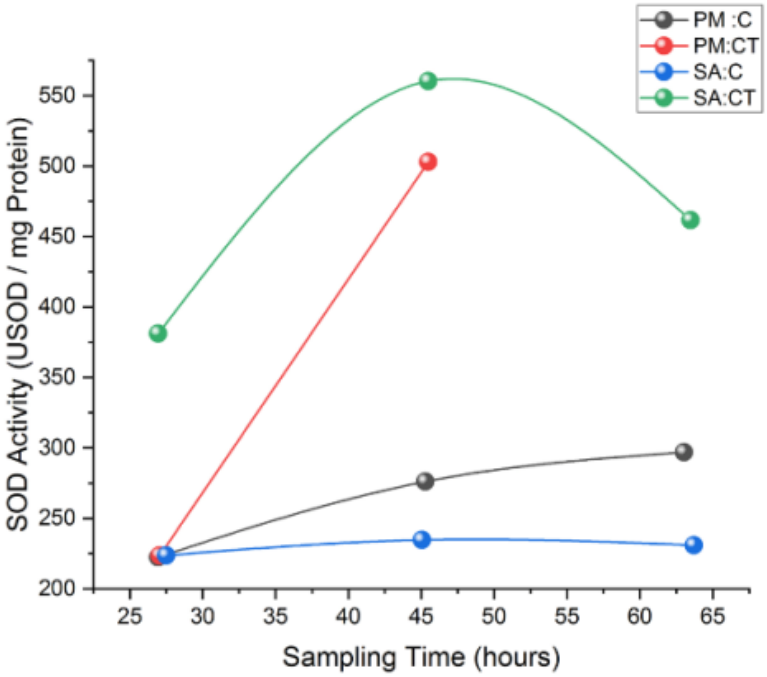

Figure a. Level of malondialdehyde (MDA)

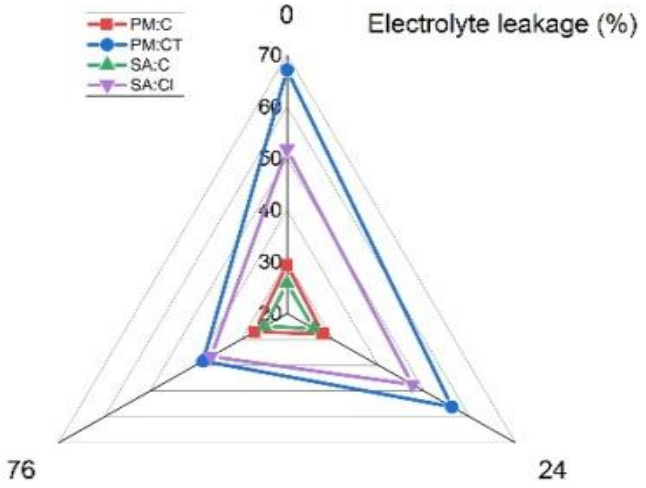

Figure b. Electrolyte leakage EL

Figure 2: Assessment of membrane stability and oxidative damage in (SA) and PM genotypes under control circumstances (c) and control treatment (CT). a) Level of malondialdehyde (MDA). b) Electrolyte leakage (EL). A significant change is indicated by a different letter (p0.05). The standard deviation is represented by the error bars (n-8). The percentage figure shows how much more cold therapy is being used for each genotype when compared to the control treatment.

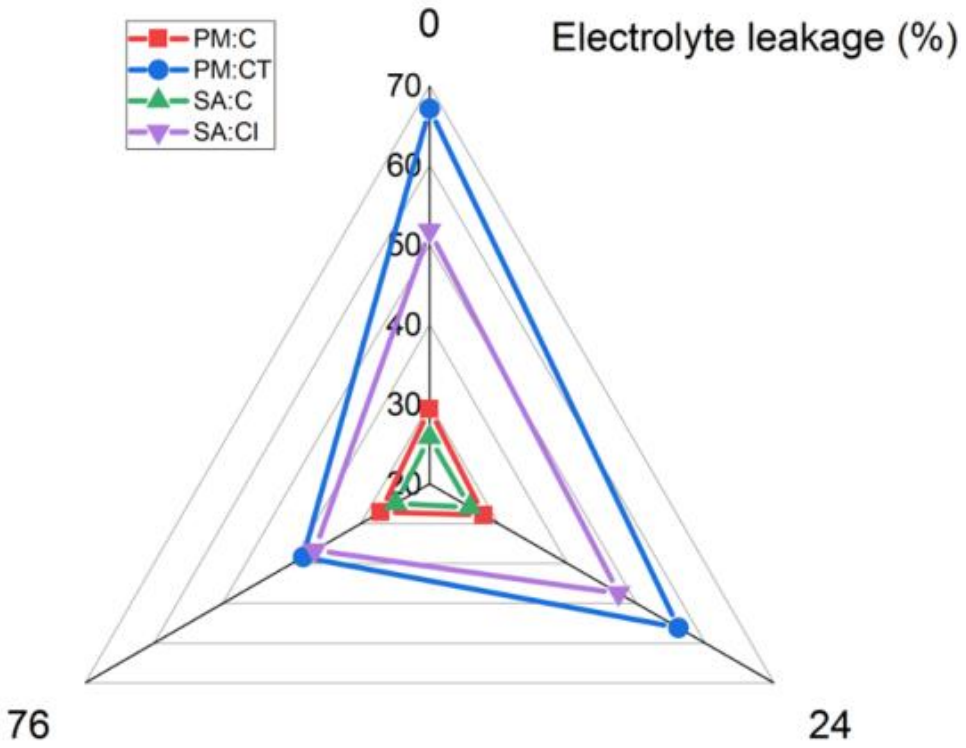

Figure 3. For genotypes (SA) and (PM), total chlorophyll content was assessed under control (C) and cold treatment (ct). A significant difference is indicated by different letters (p0.05). The standard deviation is represented by the error bars (n-8). The percentage value shows how much less cold therapy is used for each genotype as compared to the control treatment.

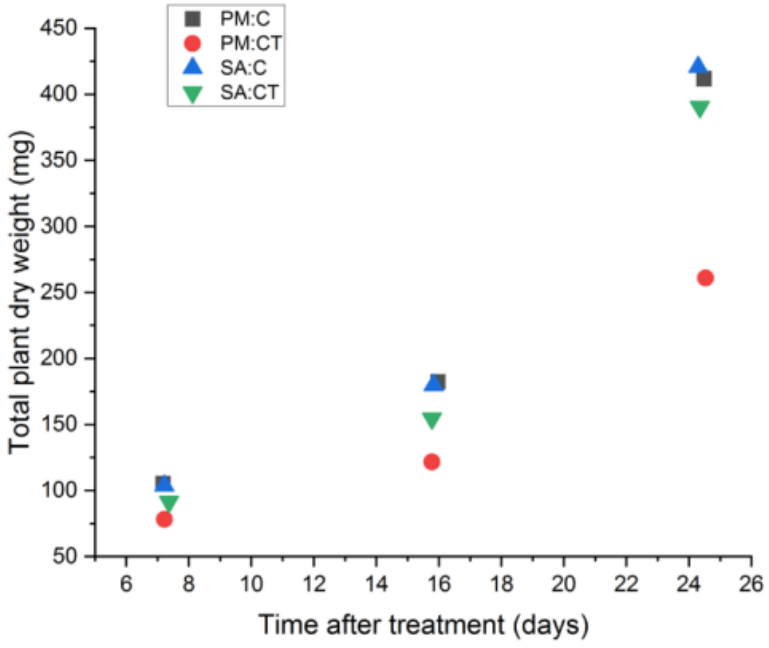

Figure 4. Total plant dry weight was measured on SA and PM genotypes under control (C) and cold treatment conditions (ct). A different letter indicates a statistically significant difference (p0.05). The error bars represent the standard error (n=8). For each genotype, the percentage number represents the decrease in cold therapy compared to the control treatment.

4. Conclusions

Cold temperatures disrupt oxidative metabolism in sunflower seedlings and result in biochemical, physiological, and growth problems. The stronger responsiveness to cold therapy during stress management and recovery is associated with the tolerant genotype and involves oxidative metabolism. As an additional indicator, this sensitive genotype presented decreased catalase and superoxide dismutase activity, weakened membrane stability, and increased oxidative damage. The accepting genetic constitution presented compared to the sensitive genotype, the potential to repair chlorophyll content and restore growth parameters, which developed at a much slower rate. The assay shown here may prove to be an essential tool for genotype characterization in a breeding program for sunflowers, enabling the creation of selection strategies for the tolerable genetic constitution by improved development next to the scattering phase at cold temperatures.

References

[1] N. Elloumi, M. Zouari, L. Chaari, F.B. Abdallah, S. Woodward, and M. Kallel, 2015. Effect of phosphogypsum on growth, physiology, and the antioxidative defense system in sunflower seedlings. Environmental Science and Pollution Research, 22: pp.14829-14840.

[2] S.C. Bhatla, M. Gogna, P. Jain, N. Singh, S. Mukherjee, and G. Kalra, 2021. Signaling mechanisms and biochemical pathways regulating pollen-stigma interaction, seed development, and seedling growth in sunflowers under salt stress. Plant Signaling & Behavior, 16(11): p.1958129.

[3] M. Barrio-Conde, M.A. Zanella, J.M. Aguiar-Perez, R. Ruiz-Gonzalez, and J. Gomez-Gil, 2023. A Deep Learning Image System for Classifying High Oleic Sunflower Seed Varieties. Sensors, 23(5): p.2471.

[4] E. Brenna, V. De Fabritiis, F. Parmeggiani, F. Tentori, and D. Tessaro, 2023. Lipase-Mediated Synthesis of Oleoyl Ethanolamide Starting from High-Oleic Sunflower Oil Soapstock. ACS Sustainable Chemistry & Engineering.

[5] A. Haj Sghaier, H. Khaeim, A. Tarnawa, G.P. Kovács, C. Gyuricza, and Z. Kende, 2023. Germination and Seedling Development Responses of Sunflower (Helianthus annuus L.) Seeds to Temperature and Different Levels of Water Availability. Agriculture, 13(3): p.608.

[6] I. Afzal, S. Saleem, M. Skalicky, T. Javed, M.A. Bakhtavar, Z. ul Haq, M. Kamran, M. Shahid, M. Sohail Saddiq, A. Afzal, and N. Shafqat, 2021. Magnetic field treatments improve sunflower yield by inducing physiological and biochemical modulations in seeds. Molecules, 26(7): p.2022.

[7] H. ur Rehman, H.F. Alharby, Y. Alzahrani, and M.M. Rady, 2018. Magnesium and organic biostimulant integrative application induces physiological and biochemical changes in sunflower plants and their harvested progeny on sandy soil. Plant Physiology and Biochemistry, 126: pp.97-105.

[8] P. Jain, C. von Toerne, C. Lindermayr, and S.C. Bhatla, 2018. S-nitrosylation/denitrosylation as a regulatory mechanism of salt stress sensing in sunflower seedlings. Physiologia Plantarum, 162(1): pp.49-72.

[9] M. Hussain, S. Farooq, W. Hasan, S. Ul-Allah, M. Tanveer, M. Farooq, and A. Nawaz, 2018. Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. Agricultural water management, 201: pp.152-166.

[10] R. Perveen, Y. Jamil, M. Ashraf, Q. Ali, M. Iqbal, and M.R. Ahmad, 2011. He-Ne laser-induced improvement in biochemical, physiological, growth, and yield characteristics in sunflower (Helianthus annuus L.). Photochemistry and Photobiology, 87(6): pp.1453-1463.

[11] B. Vancostenoble, N. Blanchet, N.B. Langlade, and C. Bailly, 2022. Maternal drought stress induces abiotic stress tolerance to the progeny at the germination stage in sunflowers. Environmental and Experimental Botany, 201: p.104939.

[12] C. Kole, ed., 2022. Genomic Designing for Abiotic Stress Resistant Pulse Crops. Springer International Publishing.

[13] E. Ebrahimian, S.M. Seyyedi, A. Bybordi, and C.A. Damalas, 2019. Seed yield and oil quality of sunflower, safflower, and sesame under different levels of irrigation water availability. Agricultural Water Management, 218: pp.149-157.

[14] D. Amaral, D.M. Filipe, T.F. Cavalheri, L. Vieira, R.P. Magalhães, I. Belo, H. Peres, and R.O.D.A. Ozório, 2023. Solid-state fermentation of plant feedstuff mixture affected the physiological responses of European seabass (Dicentrarchuslabrax) reared at different temperatures and subjected to salinity oscillation. Animals, 13(3): p.393.

[15] N. Shah, M. Irshad, A. Hussain, M. Qadir, W. Murad, A. Khan, M. Awais, A.F. Alrefaei, and S. Ali, 2023. EDTA and IAA Ameliorates Phytoextraction Potential and Growth of Sunflowers by Mitigating Cu-Induced Morphological and Biochemical Injuries. Life, 13(3): p.759.