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Dissertation for master's qualification presented to the Federal University of Lavras as part of the requirements of the Graduate Program in Agronomy/Plant Physiology, to obtain the Master's degree.

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PRIMING DE SEMENTES MELHORA A GERMINAÇÃO DE SEMENTES E O CRESCIMENTO DE MUDAS DE GIRASSOL EM CONDIÇÕES SALINAS

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DEDICATION

I dedicate this research work to Almighty ALLAH, who gave me the opportunity to achieve remarkable academic success.

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is an important oil seed crop that is produced mainly due to its high oil content that is consumed by humans in different ways. However, because of the high lipid content in sunflower, the seeds are more liable to reduce their quality during short-term storage as a result of oxidation due to temperature changes or moisture content. Additionally, salinity can hinder sunflower productivity. In this study, we evaluated the effect of seed halopriming on the germination of sunflowers after storage (manuscript one) and seedling establishment during the early growth stage under salinity conditions (manuscript two). The results from the first manuscript indicated that seed halopriming improved the germination of primed seeds compared to control seeds, and also increased antioxidant enzymatic activity in primed seeds compared to the control. The results of the second manuscript illustrate that seedlings from primed seeds were more tolerant to higher salinity concentrations by increasing their enzymatic activity. Altogether, it can be concluded that seed halopriming is a cost-effective, simple, and efficient technique for improving the germination and seedling growth of sunflowers under stressful conditions.

Keywords: Abiotic stresses. Short-term storage. Salinity halopriming. Enzymatic antioxidant system.

RESUMO

O girassol (*Helianthus annuus* L.) é uma importante oleaginosa produzida principalmente devido ao seu alto teor de óleo que é consumido pelo homem de diversas formas. No entanto, devido ao alto teor de lipídios no girassol, as sementes são mais propensas a reduzir sua qualidade durante o armazenamento a curto prazo como resultado da oxidação devido a mudanças de temperatura ou teor de umidade. Além disso, a salinidade pode prejudicar a produtividade do girassol. Neste estudo, avaliamos o efeito do halocondicionamento de sementes na germinação de girassóis após armazenamento (manuscrito um) e estabelecimento de plântulas durante o estágio inicial de crescimento em condições de salinidade (manuscrito dois). Os resultados do primeiro manuscrito indicaram que o halocondicionamento das sementes melhorou a germinação das sementes condicionadas em comparação com as sementes controle e também aumentou a atividade enzimática antioxidante nas sementes condicionadas em comparação com o controle. Os resultados do segundo manuscrito ilustram que mudas de sementes condicionadas foram mais tolerantes a concentrações de salinidade mais altas, aumentando sua atividade enzimática. Ao todo, pode-se concluir que o halopriming de sementes é uma técnica econômica, simples e eficiente para melhorar a germinação e o crescimento de mudas de girassóis sob condições estressantes.

Palavras-chave: Estresse abiótico. Armazenamento de curto prazo. Salinidade. Halopriming. Sistema antioxidante enzimático.

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SUMMARY

1. INTRODUCTION

Sunflower (*Helianthus annuus* L) is an oilseed crop that belongs to the family Asteraceae and is indigenous to North America. It is grown all over the world (YEGOROV et al., 2019) because it can acclimate to various climatic and soil conditions. This has improved its cultivation as an oilseed plant worldwide (FORLEO et al., 2018). As a source of high-quality oil and dietary fiber that considerably benefits human health, sunflower is one of the key oilseed crops cultivated worldwide (KHAN et al., 2015).

The demand for edible sunflower seeds, oil, and byproducts has increased in response to the ongoing rise in the human population, and to meet this demand, efforts must be intensified to boost sunflower production (TAHER et al., 2017). However, there is an extended duration of storage during which seed quality deteriorates before the seeds are utilized for germination or oil extraction. Therefore, it is essential to conduct comprehensive examinations of all changes that occur during storage (ARC et al., 2011)**.**

Seed storage typically causes seed aging, which manifests as permanent cellular and metabolic alterations such the oxidation of proteins, nucleic acids, and lipids. Enzyme inactivation, membrane disruption, and impairment of RNA and its production are further effects of seed aging (EL-MAAROUF-BOUTEAU et al., 2011; HU et al., 2012; KIBINZA et al., 2006). Given that oilseeds are more prone to seed deterioration than cereal grains, storage of oilseeds poses a major challenge.

Also, there are several abiotic stressors, such as salinity, cold, drought, flood, and high temperature, that have a significant impact on global agricultural productivity (SHRIVASTAVA; KUMAR, 2015). Soil salinity is one of these abiotic stresses that has the worst environmental effects, reducing the amount of arable area, crop productivity, and crop quality. Commonly, due to the accumulation of soluble salts in the soil, salinization of agricultural land occurs (BHARTI et al., 2016), especially the sodium (Na^+) and chloride (Cl^-) ions. As a result of higher Na^+ ion assimilation and a decrease in the Na^+/K^+ ratio as a result of decreased osmotic potential in plant roots, salinity stress results in osmotic stress and ion toxicity (ARIF et al., 2020).

Furthermore, these ionic imbalances impair critical plant processes and activities by interfering with the uptake and transport of other significant necessary ions in target cells (ARIF et al., 2020). Salinity lowers the viability of the seeds, hinders the establishment of the

seedlings, causes stunted plant growth, and poor reproductive development, and eventually lowers crop output (TURAN et al., 2009).

In order to minimize the harm caused by storage and salinity while preserving important resources for growth and reproduction, seeds, seedlings and mature plants have evolved unique mechanisms that enable them to sense precise environmental changes and respond to complicated stress circumstances. A range of plant responses is brought on by the perception of salt stress, including the activation of enzymatic and non-enzymatic antioxidant mechanisms (SUBRAMANYAM et al., 2012) to reduce ROS build-up and oxidative stress. Therefore, increasing plants' antioxidant capacity is a possible strategy to offset the negative effects of ROS-induced oxidative damage (KASOTE et al., 2015).

To reduce the negative effect storage and salinity on crop productivity, methods like seed priming has been adopted. Seed priming is the process of carefully hydrating seeds such that pre-germinative metabolic activity can continue but that real radicle emergence is halted. Seed priming allows pre-germinative physiological and biochemical changes to take place (SADDIQ et al., 2019). Seed priming enhances germination processes including defense, which give seeds the ability to withstand environmental challenges that arise during germination (FAROOQ et al., 2006, 2009).

To stimulate the seeds and reduce environmental stress, numerous kinds of seed priming techniques have been created and are currently being used successfully, these include hydropriming, halopriming, osmopriming, biopriming, hormonal priming, solid matrix priming, etc. with our main focus on halopriming technique (VENKATASUBRAMANIAN; UMARANI, 2007). Halopriming is imbibing seeds in salt solutions that are not organic, like NaCl or KCl. Halopriming encourages uniform germination and higher crop production even in unfavorable environmental conditions including temperature extremes and oxygen deprivation.

Halopriming boosts seed metabolism by generating osmolytes and/or signaling molecules, promoting germination and enhancing growth and yield capabilities (VENKATASUBRAMANIAN; UMARANI, 2007). Osmolytes are compatible solutes that build up inside plant cells and act as osmoticum to store water by reducing the osmotic potential, shielding macromolecules and cell membranes from oxidative stress and dehydration (FAHAD et al., 2017; TABASSUM et al., 2018; VERSLUES; JUENGER,

2011).

Sunflower is an important oilseed crop with a lot of health and economic benefits. Therefore, it is important to improve the quality of seeds and also minimize the negative effects of abiotic stresses. There is still a wide gap to be filled regarding the use of halopriming technique to mitigate the effect of abiotic stresses as a result of storage on the germination of sunflower seeds. The main objective of this study was to investigate the effect of seed halopriming on germination and seedling establishment of sunflowers after storage (manuscript one) or during early growth stage at salinity conditions (manuscript two). We hypothesize that halopriming procedure will improve the seed germination of sunflowers after storage, and we also hypothesize that seed halopriming technique will improve seed germination and seedling growth of sunflowers under saline conditions by enhancing the activities of enzymes and non-enzymatic antioxidants.

2. LITERATURE REVIEW

Sunflower is the third-most significant oilseed crop in the world (53.48 million metric tons), behind soybeans (337.48 million metric tons) and rapeseed (68.02 million metric tons), which are the most lucrative and economical (SHABANDEH, 2020). It is mainly produced for the creation of high-oil varieties by plant scientists under optimum conditions for maximum yield and productivity, which calls for fertile soil, sufficient rainfall, and ideal environmental conditions. Climate, edaphic, and management factors, along with nutrient-limiting environmental circumstances, can reduce the production of sunflower products such as seeds, oil content, and other goods. Sunflower output and quality may be affected by the addition of synthetic fertilizer and organic manure (ENEBE; BABALOLA, 2018). The availability of crucial micronutrients like potassium boosts crop output and increases a crop's resistance to salinity and other environmental stresses.

Sunflower crop yields fluctuate every year based on the climate and market prices. Sunflower plants thrive in a variety of soil types, making them a competitive alternative to other cereal grains like maize, sorghum, or cowpea. Sunflowers grow most vigorously in hot, dry climates compared to other cereal grains. The plant's long, deep taproot system conserves moisture, allowing it to recover from moisture loss quickly and to survive in stressful situations such as salinity and drought (HUSSAIN, Mubshar et al., 2018). Sunflower is a profitable and promising agricultural crop with numerous advantages for enhancing valuable

market goods, serving as a source of revenue, and reducing poverty (ERYILMAZ et al., 2016).

Sunflower oil is generally classified as a non-volatile obtained during the processing of sunflower seeds. It is frequently used as the main component in the preparation of food such as frying, and creation of cosmetics (emollient) (LAI et al., 2017). Sunflower oil mostly consists of linoleic acid (polyunsaturated lipid) and oleic acid (mono-saturated). The difference in the fatty acid and composition of sunflower oil may be caused by the type of plant used and the processing method used to produce it (AVNI et al., 2016).

The oil is known for its intriguing flavour and light amber colour. Vitamins and minerals are abundant in the oil. To meet consumer demand for high quality oil, the production and commercialization of sunflower oil can be improved with the use of modern technology (AVNI et al., 2016). The plants may thrive in colder or drier climates where other non-oilseed crops cannot because they are hardy and drought-resistant. Sunflower seeds are typically revealed in the apical section of the plant once they mature (ISLAM, R. T. et al., 2016)

Sunflower kernel which makes about 80% of the achene's total weight and covers the seeds, is enclosed within lignin and cellulolytic material shell. Raw sunflower seeds typically contain 25% oil, nut plant breeding has improved that to 40%. Cold extraction and hot pressing are two methods for obtaining the seed oil. Sunflower seeds are consumed by birds as they mature in the open fields because of the protein and lipids they contain (ISLAM, R. T. et al., 2016).

The need to supply the growing worldwide population, which is expanding at a pace of about 1.05% per year, with food is a significant problem for world agriculture (MODI, 2019). Seed storage and abiotic stressors have a significant negative impact on plant development, productivity, yield, and food quality (SINGH et al., 2018; WANG, Yanhong et al., 2017). Drought, salinity, temperature, and excessive rainfall are some of the abiotic stressors. Soil salinization is the most harmful of all abiotic stresses (DALIAKOPOULOS et al., 2016), and it is regarded as one of the major impediments to agricultural output and food security.

The two main factors that induce seed deterioration as a result of seed storage are a high moisture content and a high temperature during storage (KIBINZA et al., 2006). Seeds'

stored lipids degrade mostly as a result of (i) oxidation brought on by high temperatures and moisture content, (ii) hydrolysis, where fat is broken down into fatty acids, and (iii) contamination (ABDELLAH; ISHAG, 2012). One of the main variables contributing to seed degeneration is lipid peroxidation, according to numerous reports. Reactive oxygen species (ROS), a class of chemicals, are a significant source of these harmful alterations (ROACH et al., 2010). The seed deterioration is one of the challenges of food production.

Another important challenge of crop production around the world is the salinization. While the main cause of salinization of agricultural land is the buildup of salts in the soil (BHARTI et al., 2016; WANG, Yanhong et al., 2017), especially sodium (Na^+) and chloride (Cl^-) ions. Salinity has the biggest negative impact on the productivity and quality of crops by modifying physiological processes as well as plant biochemical processes. High Na+ buildup restricts soil porosity, aeration, and water conductivity, and the microbial diversity within and around plant roots is significantly impacted by soil salinity stress. Additionally, the rate of photosynthesis, stomatal conductance, and enzyme activity in seedlings and plants are all impacted by excessive salt concentrations (KUMAR et al., 2017).

Salinity stress and seed deterioration as a result of storage can both increase the likelihood of oxidative damage by reactive oxygen species formation (ROS), can cause harm to nucleic acids (DNA, RNA), proteins, lipids, and cell membranes, and may potentially result in programmed cell death. Due to the excessive accumulation of Na^+ and Cl^- ions, salinity also causes hypertonic stress (ZHANG et al., 2018). Salinity slows down the commencement of seed germination, lowers seedling development and dispersal of germination events, and decreases seedling metabolism, all of which harm plant growth and crop yield (SABAGH et al., 2019).

During salinity, the negative effect on most plants is apparent in the early stages of plant development, especially during seed germination and seedling establishment, as both stages are the most responsive and critical stages of plant development (HUSSAIN, S. et al., 2019). Osmotic stress, ionic imbalance, and oxidative stress are the three main causes that reduce crop production when there is salinity stress.

In plants lifecycle, seed germination begins with the imbibition of water is a dynamic and crucial stage. Seed germination commonly implies three distinct phases; consisting of Phase I: seed hydration process associated with passive imbibition of dry tissues associated

with water movement first occurring in the apoplastic spaces; Phase II: activation phase associated with the re-establishment of metabolic activities and repairing processes at the cell level; and Phase III: initiation of growing processes associated with cell elongation and leading to radical protrusion. Phases I and III both include an increase in water content, whereas Phase II maintains hydration. Germination is usually thought to be a reversible process before the completion of phase II (BEWLEY; BLACK, 2013).

Salinity reduces seed germination either by osmotic pressure or stress that hinders water absorption or results in ionic toxicity. These consequences as a whole prevent cell division and growth, and also modify the activity of some important enzymes; thus, decreasing the use of the seed reserves (EL-HENDAWY et al., 2019), therefore salinity has a detrimental impact on the process of germination by modifying the typical germination pathway that diminished plant development and growth, finally led to a fall in economic return.

Additionally, in whole plants, excessive salt reduces the photosynthetic efficiency, leaf area, and chlorophyll content of the leaves. During salinity stress, the activity of photosystem II (PSII), a crucial site of the electron transport chain (ETC), is inhibited (KALAJI et al., 2011; MEHTA et al., 2010). Salinity stress has an impact on several cellular enzymes, including RNAse, DNAse, proteases, and enzymes involved in nitrogen metabolism and amino acid synthesis (SIDDIQUI et al., 2008). The accumulation of ROS, such as singlet oxygen, superoxide radicals, and H_2O_2, is also indirectly triggered by salinity stress. Under salinity stress conditions, the ETC in the chloroplast and the mitochondria are the primary sources of ROS generation (GILL; TUTEJA, 2010).

There are several treatments that mitigate salt effect in seed germination and early growth. We can consider seed priming as one of them. Seed priming is the partial hydration of seeds until phase II and the attainment of appropriate metabolic activity that enables the seeds to commence crucial pre-germination processes. Phase II displays a steady level of hydration. Up until phase II, germination is still reversible allowing the seeds to be dried out once more, stored in viable state, and re-start germination in a suitable environment (FAROOQ et al., 2007). The primary alterations that occur during seed priming include membrane repair, DNA and RNA synthesis and repair, immature embryo growth, alterations to the endosperm tissues that surround the embryo, dormancy breakage, and enrichment of pre-germination metabolism (FAROOQ et al., 2007). Primed seeds have a higher germination

rate, early germination, uniformity in germination, superior growth qualities, faster emergence, and better stand establishment.

These enzymes are essential for breaking down macromolecules for the embryo's growth and development, which eventually leads to quicker and more vigorous seedling emergence (FAROOQ, et al., 2007). According to some research, priming makes it easier to repair chromosomal damage (SIVRITEPE; DOURADO, 1995), boosts RNA and de novo protein synthesis, enables early DNA replication and repair, and decreases metabolite leakage (BLACK; BEWLEY, 2000). Thus, seed priming significantly increased total seed protein, peroxidases, polyphenol oxidases, RNA, and de novo protein synthesis.

It is hypothesized that germination-related processes like respiration, energy metabolism, and mobilization of the initial reserve also take place during priming. To cover the energy pool and hasten germination, greater respiratory activity is needed. There has been a link between pre-sowing treatments and increased respiratory activity (FORTI et al., 2020). Seed priming raised the standard deviation of germination responses, decreased the oxygen time constant, and enhanced the respiratory activity of seeds (FORTI et al., 2020). Storage proteins must be activated during seed germination to support seedling growth since they provide a source of reduced nitrogen and inorganic minerals (LUTTS et al., 2016).

A crucial component of the physiology of primed seeds is the control of oxidative state. The antioxidant response is triggered by priming, and the seeds are modified for potential stressors like salinity (KIBINZA et al., 2011). Reactive oxygen species (ROS) are largely produced during the initial phases of seed ingestion and germination because of the respiratory activity of mitochondria, enzymes like NADPH oxidase, extracellular peroxidase, and oxalate oxidase, as well as activities in the β-oxidative pathway (JIN et al., 2016).

Antioxidants are crucial for effective germination because they reduce excessive ROS during early endocytosis, especially in stressful situations like salinity. Seed priming in tomato seeds exhibited increased activity of antioxidant enzymes such as ascorbate peroxidase, catalase, peroxidase, glutathione reductase, and superoxide dismutase (COOLBEAR; SLATER; BRYANT, 1990).

Halopriming has been shown to increase watermelon seed germination and seedling vigor (DEMIR; MAVI, 2004), rice (FAROOQ et al., 2006), maize (KUMARI et al., 2017), many other crops. Seed priming with various inorganic salts, including NaCl, KCl, and

$CaCl_2$, has a favorable effect on pepper germination when exposed to salinity stress by accelerating imbibition and enabling the seeds to perform metabolic processes more quickly (ALOUI et al., 2014). When primed with KCl and NaCl, the salt-sensitive and salt-tolerant wheat genotypes increased germination %, stimulated physiological traits like chlorophyll concentration, and decreased mean germination time (SADDIQ et al., 2019). Sesame seeds (*Sesamum indicum* L.) primed with $CaCl_2$ boosted germination by up to 88.75% and improved vigor and yield (TIZAZU et al., 2019).

Sesame seeds and *Vigna mungo* L. were found to have an enhanced salt tolerance index (STI) based on growth factors such as shoot length, root length, and relative water content in NaCl primed seeds(BISWAS; SAHA, 2021; MOHAMMADI et al., 2013). When tomato (*Solanum lycopersicum* cv. 'Ro Grande) and fenugreek seeds (*Trigonella foenum-greacum*) were primed with NaCl, the harmful effects of salt stress were greatly reduced (GONZÁLEZ-GRANDE; SUÁREZ; MARÍN, 2020; MOHAMMADI et al., 2013).

After halopriming, CAT, POD, and APX antioxidant enzyme activities of the salt-tolerant Lu26s and salt-sensitive Lasani-06 wheat (*Triticum aestivum* L.) cultivars considerably increased (ISLAM, F. et al., 2015). The antioxidant activity in chicory was considerably boosted by halopriming with KCl (SADEGHI; ROBATI, 2015). Halopriming significantly raised the SOD, CAT, APX, and GPOX (guaiacol peroxidase) activities in primed seeds compared to non-primed seeds of various Oryza sativa cultivars (SEN; PUTHUR, 2021). Therefore, the use of priming as seed treatment technique, can improve seed germination and all physiological related metabolism even in seeds or in seedlings.

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Article 1: Seed halopriming before storage help maintain seed quality and improve germination of sunflower

Manuscript in the journal's rules Journal of Seed Science (Impact factor: 1,4)

SEED HALOPRIMING BEFORE STORAGE HELP MAINTAIN SEED QUALITY AND IMPROVE GERMINATION OF SUNFLOWER

ABSTRACT: Sunflower (*Helianthus annuus* L.) is an important oil seed crop mainly due to its high oil content, which is consumed by humans in different ways. However, because of the high lipid content in sunflower, the seeds are more liable to reduce their quality during short-term storage because of oxidation due to temperature changes or moisture content. We aimed to evaluate the effect of seed halopriming on the germination, reactive oxygen species, and antioxidant enzymes of sunflower hybrid seeds during storage (0, 7, 14, and 30 days). Priming improved germination by enhancing germination percentage (G%), germination speed index (GSI), and time taken for 50 percent germination (T50%), even in stored seeds. In non-primed seeds, storage time decreased all these parameters. Primed seeds had a lower hydrogen peroxide content than non-primed seeds at all storage durations. However, increased levels of the lipid peroxidation marker (MDA) were observed in primed seeds at 14 and 30 days of storage, even though there was no signal of oxidative stress. The superoxide dismutase enzyme activity was higher in control seeds than in primed seeds at 0 and 7 days of storage, while at 14 and 30 days, the activity was the same between control and primed seeds. Catalase and ascorbate peroxidase enzyme activities were higher in the control seeds than in the primed seeds across all storage durations. Therefore, it can be concluded that: (I) sunflower seeds can be sensitive to short-term storage, and halopriming can increase seed germination parameters even in stored seeds; (II) halopriming maintained controlled levels of reactive oxygen species, and anticipated lipid storage reserve mobilization with respect to MDA levels.

Keywords: Seed germination, oxidative damage, seed storage, oilseed, antioxidant enzymes

1. INTRODUCTION

Sunflower (*Helianthus annuus* L) is an oilseed crop that belongs to the family Asteraceae and is indigenous to North America. It is grown all over the world (YEGOROV et al., 2019) because it can acclimate to various climatic and soil conditions. This has improved its cultivation as an oilseed plant worldwide (FORLEO et al., 2018). The cultivation of these species is considered to have a short life cycle with extensive acclimatization to variations in altitude and photoperiod, in addition to being resistant to cold and high temperatures (CARVALHO et al., 2016). However, there is an extended duration of storage during which seed quality deteriorates before the seeds are utilized for germination or oil extraction. Therefore, it is essential to conduct comprehensive examinations of all changes that occur during storage (ARC et al., 2011)**.**

Seed storage typically causes seed aging, which manifests as permanent cellular and metabolic alterations such as the oxidation of proteins, nucleic acids, and lipids. Enzyme inactivation, membrane disruption, and impairment of RNA and its production are further effects of seed aging (EL-MAAROUF-BOUTEAU et al., 2011; HU et al., 2012; KIBINZA et al., 2006). Given that oilseeds are more prone to seed deterioration than cereal grains, storage of oilseeds poses a major challenge. Seeds' stored lipids degrade mostly as a result of (i) oxidation brought on by high temperatures and moisture content, (ii) hydrolysis, where fat is broken down into fatty acids, and (iii) contamination (ABDELLAH; ISHAG, 2012). One of the main variables contributing to seed degeneration is lipid peroxidation, according to numerous reports. Reactive oxygen species (ROS), a class of byproducts, are a significant source of these harmful alterations (ROACH et al., 2010).

Antioxidants found in plants act as a line of defense against dangerous chemicals. Numerous enzymes in the enzymatic antioxidant system regulate the amount of ROS and antioxidants in the body, removing any excess ROS. Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) are a few of the key antioxidant enzymes in plants that scavenge free radicals that are harmful to seed viability, according to research by (BAILLY, 2004). These antioxidant enzymes become compromised by prolonged seed storage or synthetic aging. To minimize the negative effect of abiotic stresses caused by storage on seeds, methods like seed priming have been adopted.

Seed priming enhances germination processes including defense, which give seeds the ability to withstand environmental challenges that arise during germination (FAROOQ et al.,

2006, 2009). In primed seed, pre-germination metabolic processes include structural (membrane protection during imbibition) and genetic repair, RNA and protein synthesis, and antioxidant mechanisms (SADDIQ et al., 2019). These processes guarantee appropriate seed germination and seedling development (SADDIQ et al., 2019). To stimulate the seeds and reduce environmental stress that can be caused due to seed storage, numerous kinds of seed priming techniques have been created and are currently being used successfully, these include hydropriming, halopriming, osmopriming, biopriming, hormonal priming, solid matrix priming, etc. with our main focus on halopriming technique (VENKATASUBRAMANIAN; UMARANI, 2007).

Halopriming is imbibing seeds in salt solutions that are not organic, like NaCl or KCI. Halopriming encourages uniform germination and higher crop production even in unfavorable environmental conditions including temperature extremes and oxygen deprivation. Halopriming is an easy and affordable priming method that ensures better emergence and crop stand synchronization under a variety of environmental situations(KUMARI et al., 2017).

There is still a wide gap to be filled regarding the use of halopriming technique to mitigate the effect of abiotic stresses as a result of storage on the germination of sunflower seeds. The main objective of this study is to investigate the effect of seed halopriming on seed germination of sunflowers seeds after storage. For this, we hypothesis that halopriming procedure will improve the seed germination of sunflowers after storage conditions by enhancing the activities of enzymatic and non-enzymatic antioxidants.

2. MATERIALS AND METHODS

2.1 Plant Material

The experiment and assays were performed at the Plant Growth and Development Lab and the Seed Central Lab at the Federal University of Lavras (UFLA), located in the state of Minas Gerais. Seeds were obtained commercially for the experiment. The sunflower seeds used were hybrid species produced in 2021 by a company named Sakata, Brazil.

2.2 Priming Procedure

For the imbibition curve, 20 seeds per replicate (n = 4) were used. The seeds were placed in germination boxes with paper moistened with water. The seed mass was weighed every hour to determine phases I and II of imbibition.

For the dry curve, the seeds were washed with distilled water and placed in an oven at 27 °C to dry back to their initial mass, which was determined prior to priming. The seeds took approximately 4 hours to dry back to their initial masses. Seeds were primed with 50 mM NaCl (BISWAS; SAHA, 2021).

2.3 Storage Conditions

After priming, both non-primed and primed seeds were divided into four groups and stored for different periods (0, 7, 14, and 30 days). Seeds were placed into paper bags, sealed with a stapler, and labelled according to each storage period. Each paper bag was placed in polyethylene bags, and the bags were kept in a refrigerator (5-8^{o}C). Non-primed seeds were used for the germination experiment immediately after the priming procedure.

2.4 Germination Parameters

Seeds were placed in a gearbox containing germination paper that had been moistened with sterile water. Afterwards, seeds were kept in a germination chamber at 25 °C with a 12 hours photoperiod. Seeds were considered germinated at 2mm radicle protrusion and germination was checked daily for 7 days to reach a constant count and result was expressed as percentage (germination %) based on seed analysis rules (BRASIL, 2009)

The germination speed index (GSI) was determined according to (SCOTT; JONES; WILLIAMS, 1984): GSI = $\sum T_i N_i / S$. The time for 50% germination ($T_{50\%}$) was also

calculated according to ((SCOTT; JONES; WILLIAMS, 1984): $T_{50\%}$ is given by the following formula;

$$T_{50} = t_i + [(N / 2) - - n_i] (t_j - t_i)/ (n_j - n_i)$$

2.5 Sampling

For the biochemical analysis, the seeds were collected after 7 hours of which marked the beginning of phase II of germination. After collection, seeds were macerated using liquid nitrogen and prepared for biochemical analysis.

2.6 Biochemical Analysis:

2.6.1 Hydrogen Peroxide (H_2O_2)

The levels of H_2O_2 were measured according to (VELIKOVA; YORDANOV; EDREVA, 2000), in which 100mg of the seeds sample was grinded in liquid nitrogen and then homogenized with 1 mL of 0.1% Trichloroacetic Acid (TCA). After centrifuging the samples, the reactions were carried out with 10 mM potassium phosphate buffer pH 7 and 1M potassium iodide (KI). The samples were read using a spectrophotometer at 390 nm, and a standard curve were used to calculate the quantities of H_2O_2 in the samples.

2.6.2 Lipid Peroxidation

Lipid peroxidation estimates was performed by quantifying Malondialdehyde (MDA) as described by (DU; BRAMLAGE, 1992), 100mg of the seed sample was grinded in liquid nitrogen and then homogenized with 1 mL of 80% ethanol (three times). The reaction was carried out using 1 mL of the extraction and 1 mL of % TCA, 0.65 % thiobarbituric acid (TBA), and 0.1 percent beta-hydroxytoluene (BHT). At wavelengths of 600, 440, and 532 nm, a spectrophotometer was used to measure the estimation of lipid peroxidation.

2.6.3 Antioxidant Enzymatic System Analysis

The enzymatic antioxidant system activity (SOD, CAT, and APX) were quantified. The extracts performed according to BIEMELT; KEETMAN; ALBRECHT, (1998) in which 100mg of seed sample was grinded in liquid nitrogen with PVPP (Polyvinylpyrrolidone) added to 1.5 mL of extraction buffer composed of 400 mM potassium phosphate (pH 7.8), 10

mM EDTA, and 200 mM ascorbic acid. The solution was centrifuged at 13.000g for 10 minutes at 4°C and the supernatant collected to quantify enzyme activities.

SOD activity was based on the enzyme capacity of inhibiting the photoreduction of nitro blue tetrazolium (NBT) (GIANNOPOLITIS; RIES, 1977). Absorbance readings was made at 560 nm. A unity of SOD corresponds to the number of enzymes able to inhibit 50% of NBT photoreduction in the assay conditions.

HAVIR; MCHALE, (1987) findings were used to determine the CAT activity. Based on the hydrogen peroxide consumption and the decrease in absorbance at 240nm, every 15 seconds for 3 minutes, this enzyme activity was estimated. We assumed a molar extinction coefficient of 36 mM^{-1} cm^{-1} (AZEVEDO et al., 1998). According to NAKANO; ASADA, (1981), APX activity was examined using ascorbate oxidation at 290 nm and a molar extinction coefficient of 2.8 mM^{-1} cm^{-1}.

2.7 Statistical Analysis

The statistical analysis were performed with the software R-Bio (BHERING, 2017). Data was subjected to two-way ANOVA and, in case of normal distribution, Tukey means test was selected at 5% significance. Data without a normal distribution was evaluated by a GLM analysis, assuming normality by observation of the qq- plot graphs and then applying the Tukey test at 5% of significance.

3. RESULTS AND DISUSSIONS

3.1 Effect of Priming on Germination of Sunflower seeds

For the germination results, non-primed seeds at zero and 7 days of storage were more synchronized, uniform, and had better germination percentage, while there were delays and reductions in the number of germinated seeds at 14 and 30 days of storage (Fig. 1A). Primed seeds from 14 and 30 days of storage presented a delay in germination in the first 24 - 72 hours, but they reached the same germination percentage as primed seeds d from zero and 7 days of storage.

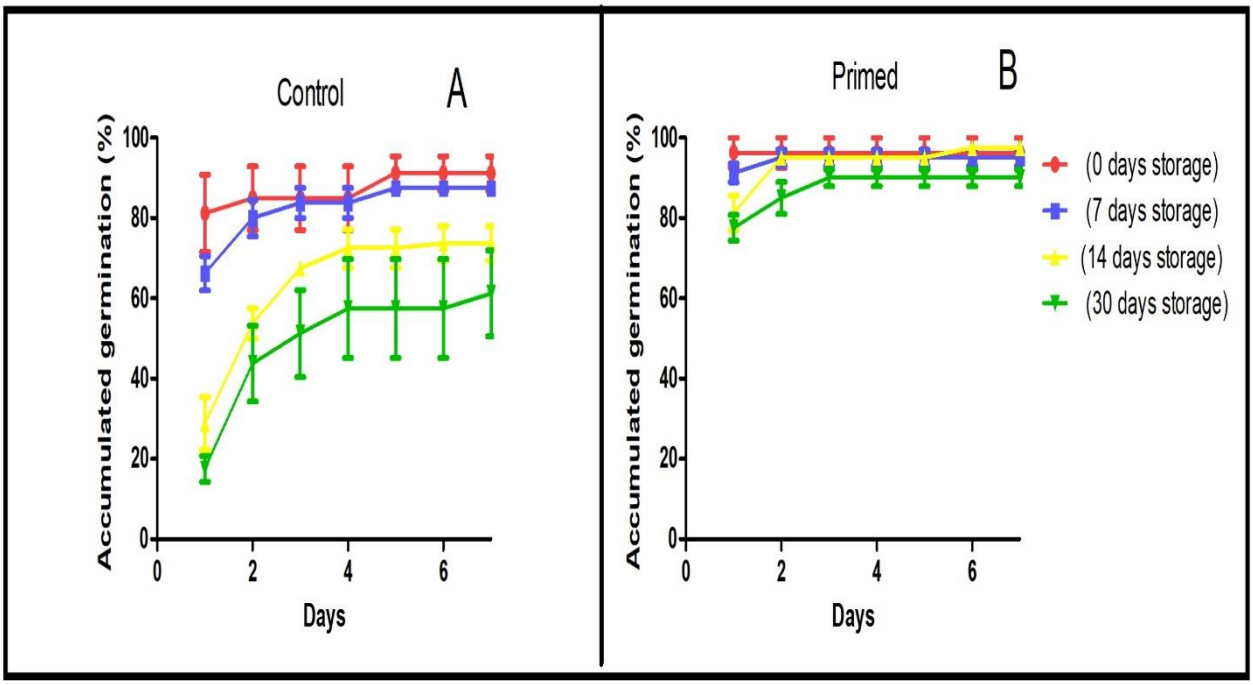
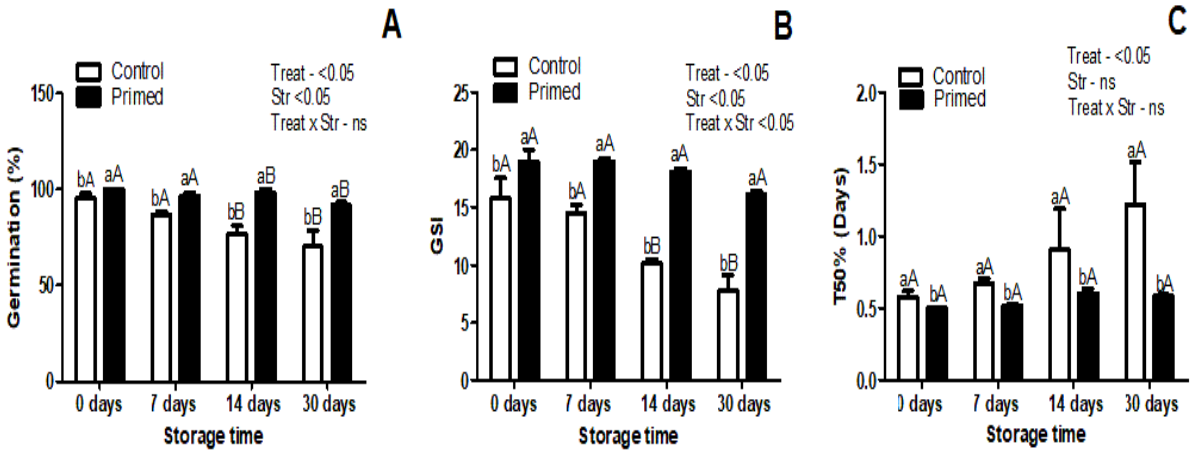


Fig. 1. Cumulative germination of control seeds (**A**) and (**B**) in primed seeds. Bars are means ± standard error (n = 4).

In general, priming increased seed germination, speed index, and time to reach 50% germination, even in stored seeds compared to non-primed seeds (Fig. 2). The results showed that the overall seed germination percentage (G%) in primed seeds did not differ with increasing storage time when compared to control seeds, which had a reduction in overall germination percentage with increasing storage time. Primed seeds had a better overall germination percentage than the control (non-primed) seeds at each storage time (Fig. 2A).

The germination speed index (GSI) was significantly different between treatments and storage times. Seeds with no storage had the highest germination speed index (GSI). GSI decreased with increasing storage time for both primed and non-primed seeds.

The time taken for 50 percent of germination to occur (T50%) differed between primed and non-primed seeds. There was no difference in the T50% of primed seeds with increasing storage time. At zero and 7 days of storage, non-primed seeds showed a decrease in T50% compared to 14 and 30 days of storage. Primed seeds had a lower T50% than non-primed seeds during each storage period. The results G%, GSI and T50% illustrate physiological effect of priming was more evident in GSI as priming reduces the time for

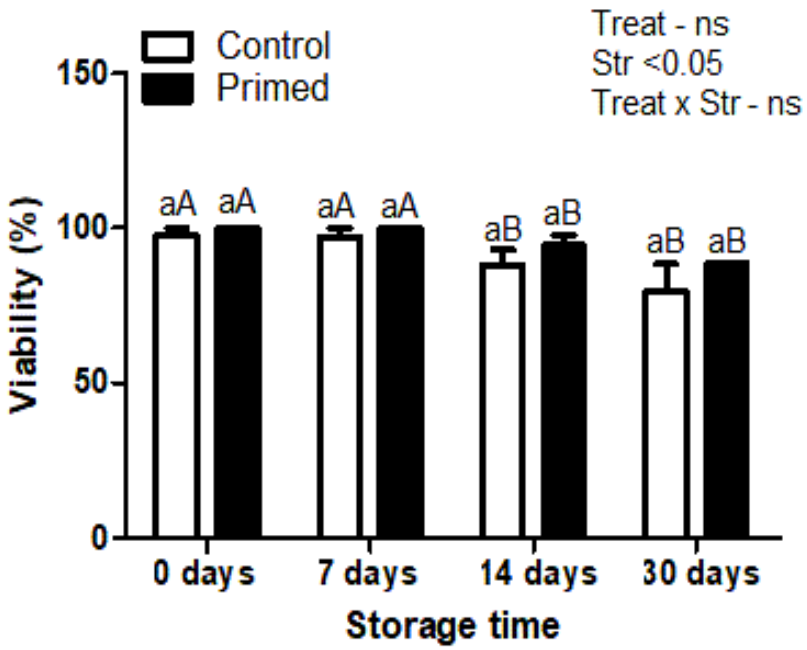


imbibition, hasten germination and enhance respiratory activity of seeds (FORTI et al., 2020)

Fig. 2 Germination percentage (**A**); germination speed index (**B**); and time taken for fifty percent germination (**C**) of seeds of *Helianthus annuus* (control and primed). Bars are means ± standard error (n = 4). Capital letters compare differences among storage time and lower letters compare differences between treatments (control and primed). The same letters or ns (not significant) do not show any significance difference by Tukey's test at 5% significance.

3.2 Effect of Priming on Viability of Sunflower Seeds

The results showed that the viability of sunflower seeds was maintained in both control and primed seeds at zero and seven days of storage (Fig. 3). At 14 and 30 days, the viability of sunflower seeds was reduced by 12% and 22% in control seeds and by 6% and 11% in primed seeds, respectively. The reduction in seed viability observed at 14 and 30 days of storage was due to the storage effect and not priming. Primed seeds also had almost 100% germination after 30 days of storage, which shows that priming increased the germination

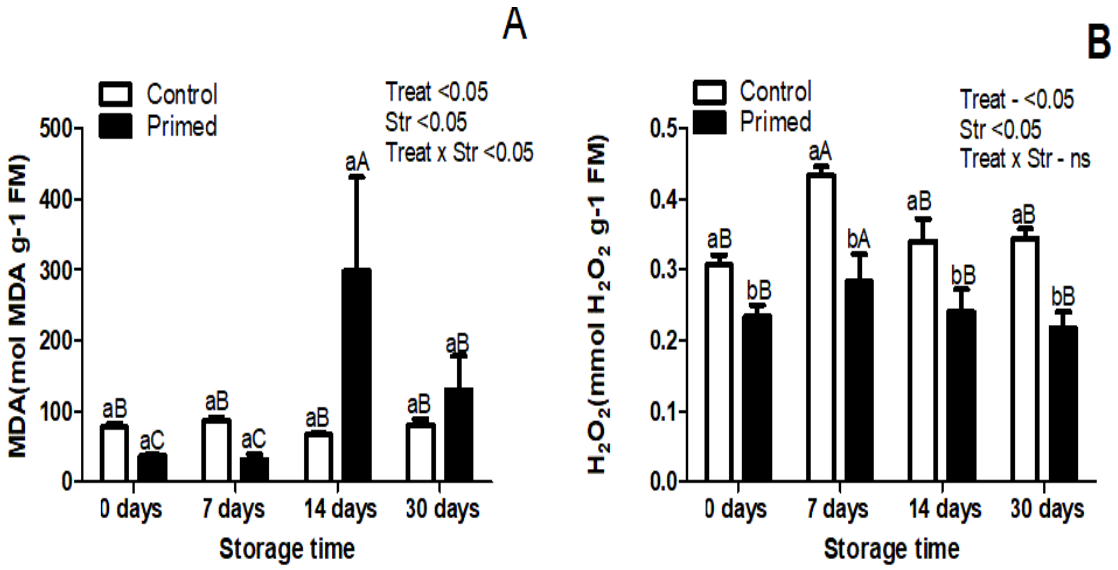


capacity of sunflower seeds (ZULFIQAR, 2021).

Fig. 3 Viability of seeds of *Helianthus annuus* (control and primed). Bars are means ± standard error (n = 4). Capital letters compare differences among storage time and lower letters compare differences between treatments (control and primed). The same letters or ns (not significant) do not show any significance difference by Tukey's test at 5% significance.

3.4 Effect of Priming on Oxidative stress of Sunflower Seeds

Lipid peroxidation (MDA) and hydrogen peroxide levels showed no evidence of oxidative stress as a result of seed storage (Fig. 4). The results indicated that the concentration of MDA in primed seeds was lower than that in non-primed seeds at zero and seven days of storage (Fig. 4A). At 14 and 30 days of storage, primed seeds had a higher concentration of MDA than non-primed seeds. This MDA increase is probably due to the process acceleration of lipid storage mobilization that was meant to occur during phase III of germination and was brought forward to phase II. This possibility has been previously discussed in an oil seed species in which increasing levels of MDA followed imbibed and



early germinated seeds (BICALHO et al., 2015).

Fig. 4 Estimated levels of lipid peroxidation (**A**); and hydrogen peroxide (**B**) of *Helianthus annuus* seeds. Bars are means ± standard error (n = 4). Capital letters compare differences among storage time and lower letters compare differences between treatments (control and primed). The same letters or ns (not significant) do not show any significance difference by Tukey's test at 5% significance.

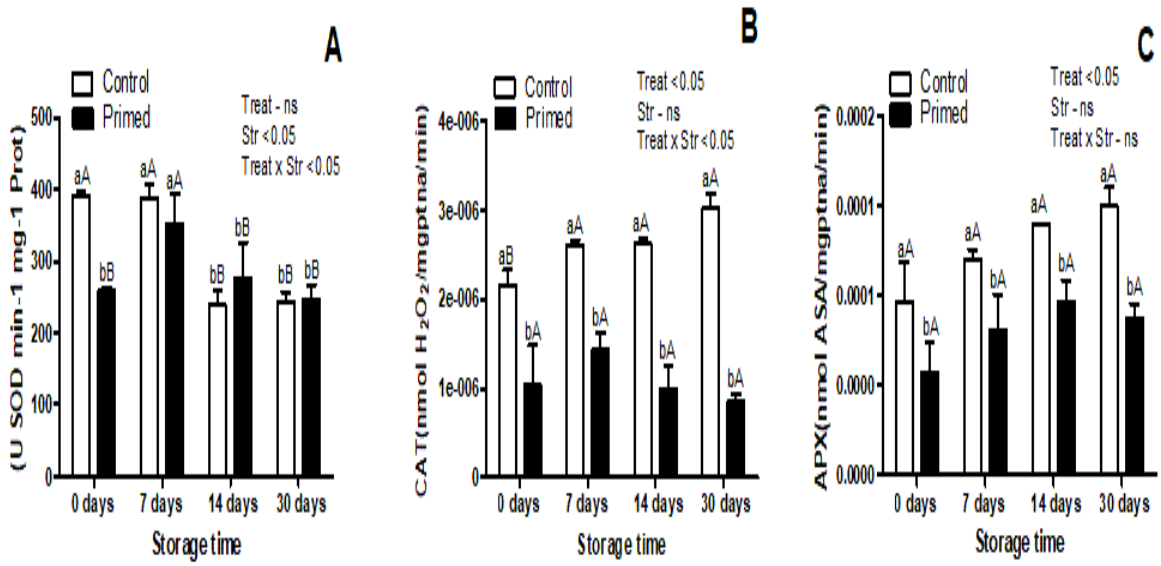
We may conclude there was no evidence of oxidative stress in the seeds, as the level of H_2O_2 was relatively controlled by both primed and non-primed seeds. The result for hydrogen peroxide (H_2O_2) differed from that of MDA. Primed seeds had a lower H_2O_2 concentration than non-primed seeds at all four storage times. The results show the importance of priming, as priming of seeds helps to control the formation of reactive oxygen

species (RAHIMI, 2013). The low level of H_2O_2 in primed seeds also explains why primed seeds had better G%, GSI, and T50 values than non-primed seeds (Fig. 4B).

3.5 Effect of Priming on the Enzyme activity of the Antioxidant System of Sunflower Seeds

Among the specific activities of the enzymes of the antioxidant system, it was observed that superoxide-dismutase (SOD) had higher enzymatic activity in non-primed seeds than in primed seeds at zero and 7 days of storage. The results showed a different dimension at 14 days of storage, where SOD activity was higher in primed seeds than in non-primed seeds (Fig. 5A) For the enzymatic activity of catalase (CAT) and ascorbate peroxidase (APX), the results illustrated that non-primed seeds had higher CAT and APX activity than primed seeds at all four storage times (Fig. 5B&C).

The difference in SOD enzymatic activity between primed and non-primed seeds at zero and 7days storage could be a result of an increase in superoxide anion ($O_2^{\cdot-}$) oxidation in primed seeds. The difference at 14 days may be due to the higher catalysis of superoxide anions ($O_2^{\cdot-}$) by SOD (WANG Ying et al., 2018). The increase in CAT and APX in non-



primed seeds shows there was no evidence of oxidative stress and may also indicate non-primed seeds regulated the level of H2O2 and this process involves the conversion of H2O2 by CAT and APX into water (WANG, Ying et al., 2018)

Fig. 5 Specific superoxide dismutase activities, SOD (**A**); catalase, CAT (**B**) and ascorbate peroxidase, APX (**C**) in control and primed seeds of *Helianthus annuus*. Bars are means ± standard error (n = 4). Capital letters compare differences among storage time and lower letters compare differences between treatments (control and primed). The same letters or ns (not significant) do not show any significance difference by Tukey's test at 5% significance.

4. CONCLUSIONS

This study demonstrated that haloprimed sunflower seeds had a better germination performance when compared to non-primed seeds. Furthermore, it was also observed that the side effect of storage was more evident in control seeds with increasing storage time than in primed seeds.

Short-term storage also reduced the viability of sunflower seeds at 14 and 30 days in both treatments, but halopriming helped improve germination capacity.

The results indicated that physiological sunflower seeds were able to tolerate the negative effects of short-term storage. We may conclude that there was no evidence of oxidative damage in either the control or primed seeds.

Enzymatic activities were also observed to be high in the control seeds, which also proved that the level of oxidative stress was relatively controlled by the seeds, especially in primed seeds.

With more studies, halopriming technique can be used to improve the germination rate of sunflower seeds after short-term storage, as it was evident in this study.

Farmers and consumers can also adopt halopriming of sunflower seeds as it is a simple, cost-effective, and efficient technique.

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Article 2: Seed halopriming improves seedling growth of sunflower under saline conditions

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SEED HALOPRIMING IMPROVES SEEDLING GROWTH OF SUNFLOWER UNDER SALINE CONDITIONS

ABSTRACT: The maximum production of sunflowers (*Helianthus annuus* L.) can be limited by salinity stress by reducing germination, early seedling growth, and overall quality. In this study, we evaluated the effects of seed halopriming on seedling growth, pigments, antioxidant enzymes, oxidative stress, osmolytes, proteins, and sugars in sunflower hybrids under saline conditions (0mM, 60mM, 120mM and 240mM). Seedlings from primed seeds had higher enzymatic activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX) and higher protein and chlorophyll a content than the control at higher salinity concentrations. Priming also improved root length and root dry matter at higher salinity concentrations than in the control. The reducing sugar concentration was also improved by priming at higher salinity concentrations than the control. It can be concluded that priming technique is useful for increasing salinity tolerance in sunflower seedlings by the recruitment or permanency of some osmolytes, such as soluble sugars, and is capable of increasing antioxidant capacity by enhancing SOD and APX activity under high salinity.

Keyword: Helianthus annuus, salinity, halopriming, enzymatic antioxidant system, osmolyte

1. INTRODUCTION

Sunflower (*Helianthus annus*) is one of the most important oilseed crops grown worldwide because it provides high-quality oil and dietary fiber that significantly improves human health (KHAN et al., 2015). Several abiotic stressors, such as salinity, cold, drought, flood, and high temperature, have a significant impact on global agricultural productivity (SHRIVASTAVA; KUMAR, 2015). Commonly, due to the accumulation of soluble salts in the soil, salinization of agricultural land occurs (BHARTI et al., 2016), especially the sodium (Na^+) and chloride (Cl^-) ions. As a result of higher Na^+ ion assimilation and a decrease in the Na^+/K^+ ratio as a result of decreased osmotic potential in plant roots, salinity stress results in osmotic stress and ion toxicity (ARIF et al., 2020).

During the early stages of salinity effects, an osmotic imbalance causes roots' capacity to absorb water to drop and their transpiration rate to increase, which results in hyperosmotic stress (MUNNS, 2005; YANG; GUO, 2018). Additionally, these ionic imbalances interfere with the uptake and transport of other important and essential ions in target cells, which negatively impacts crucial plant processes and activities (ARIF et al., 2020). Salinity also damages the photosynthetic system, the cell's ultrastructure, and the membrane structure (HASANUZZAMAN et al., 2014) by increasing the production of reactive oxygen species (ROS), which oxidative stress enables to be harmful to cell membranes, proteins, lipids, and nucleic acids (DNA, RNA).

Several techniques have been used to decrease the detrimental impact of salt and other abiotic stressors on crop productivity. Seed priming is the process of hydrating seeds until phase II, drying before radicle emergence for sowing them. Seed priming allows pre-germinative physiological and biochemical changes to take place and improve germinative parameters (HUSSAIN, M; FAROOQ; LEE, 2017). Among seed priming, the halopriming is imbibing seeds in salt solutions that are not organic, like NaCl or KCI. Halopriming promotes uniform germination and higher crop production even in unfavorable environmental conditions including temperature extremes and oxygen deprivation. Halopriming is an easy and affordable priming method that ensures better emergence and crop stand synchronization under a variety of environmental situations (KUMARI et al., 2017).

There is still a wide gap to be filled regarding the use of halopriming technique to mitigate the negative effect of salinity on seed germination and seedling growth of sunflowers. The objective of this study is to investigate the effect of seed halopriming on

seedling growth of sunflowers under saline conditions. For this, we hypothesize that halopriming procedure will improve the seedling growth of sunflower under saline conditions by enhancing the activities of enzymatic and non-enzymatic antioxidant.

2. MATERIALS AND METHODS

2.1 Plant material and Priming Procedure

The experiment and assays were performed at the Plant Growth and Development Lab at the Federal University of Lavras (UFLA) located in the state of Minas Gerais. The seeds of sunflower (Helianthus annus L.), obtained commercially (Sakata, Brazil), were used for the experiment. The seeds were imbibed for 7 hours (phase II of the imbibition curve) in 50mM of NaCl according to (BISWAS; SAHA, 2021) and dried in an oven at 27°C until they returned to their initial mass, which was determined prior to priming. 20 seeds in four replicates were used for this procedure. After the priming procedure, the seeds were sowed in 7 L pots containing oxisoil for greenhouse experiments.

2.2 Preparation of Salt Concentrations and Soil Incubation

Thirty-two pots (7 L) were used, with 16 pots for control seeds and 16 pots for primed seeds. The pots were transferred to a greenhouse after they were filled with oxisoil. NaCl was used to prepare four concentrations - 0mM, 60mM, 120mM and 240mM - (KAIWEN et al., 2020) of salinity that were used for the experiment. Salt solutions were added to pots containing soil, and each concentration of salinity had four replicates. The soil was left for a period of twenty-one days to allow for proper incubation of the salts into the soil and to avoid damage to the seeds during sowing (YAN et al., 2015)

2.3 Experimental Conditions

After twenty-one days of soil incubation, five primed seeds or control seeds were sown in each pot. The experiment consisted of a factorial 2x4, of two treatments (control and primed) seeds and four concentrations of salinity (0mM, 60mM, 120mM and 240mM). Irrigation was performed regularly, and seedlings were thinned into three seedlings per pot after 14 days of sowing.

2.4 Sampling

The Seedlings were collected after the development of the first four expanded leaves, which took approximately twenty-nine days after sowing. Seedling roots were washed to remove the soil. One seedling from each replicate was used to determine growth parameters, such as shoot length, root length, leaf area, and dry mass. The other two seedlings were macerated using liquid nitrogen and prepared for biochemical analysis.

2.5 Growth Parameters

The seedlings were separated into roots and shoots. The dry matter content of the shoots and roots was calculated using the formula by (SHIPLEY; VU, 2002). Image-J software (SCHNEIDER; RASBAND; ELICEIRI, 2012) was used to determine the leaf area and plant height (shoot and root).

2.6 Biochemical Analysis:

2.6.1 Hydrogen Peroxide (H_2O_2)

The levels of H_2O_2 was measured according (VELIKOVA; YORDANOV; EDREVA, 2000) approach. In brief, 100mg of the seedling sample was grinded in liquid nitrogen and then homogenized with 1 mL of 0.1% Trichloroacetic Acid (TCA). After centrifuging the samples, the reactions were carried out with 10 mM potassium phosphate buffer pH 7 and 1M potassium iodide (KI). The samples were read using a spectrophotometer at 390 nm, and a standard curve were used to calculate the quantities of H_2O_2 in the samples.

2.6.2 Extension of Lipid Peroxidation (MDA content)

Lipid peroxidation was determined by malondialdehyde (MDA) quantification (HODGES et al., 1999). Samples of 100mg of seedlings were grinded in liquid nitrogen and homogenized in 1 mL of 80% ethanol, centrifuged, and the supernatant collected. This step was performed three times, totaling 3 ml. The reaction with 1ml of extract and 1 mL of 20% TCA, 0.65% tiobarbituric acid (TBA) and 0.01% butylated hydroxytoluene (BHT) was carried. The estimate lipid peroxidation was calculated using the absorbance reading in a spectrophotometer at 440, 532, and 600 nm.

2.6.3 Antioxidant Enzymes

The enzymatic antioxidant system activity (SOD and APX) was quantified. The enzymatic extracts were obtained according to BIEMELT; KEETMAN; ALBRECHT, (1998) in which 100mg of seedling sample was grinded in liquid nitrogen with PVPP (Polyvinylpyrrolidone) added to 1.5 mL of extraction buffer composed of 400 mM potassium phosphate (pH 7.8), 10 mM EDTA, and 200 mM ascorbic acid. The solution was centrifuged at 13.000g for 10 minutes at 4°C and the supernatant collected to quantify enzyme activities.

SOD activity was based on the enzyme capacity of inhibiting the photoreduction of nitro blue tetrazolium (NBT) (GIANNOPOLITIS; RIES, 1977). Absorbance readings was made at 560 nm. A unity of SOD corresponds to the number of enzymes able to inhibit 50% of NBT photoreduction in the assay conditions. According to (NAKANO; ASADA, 1981) APX activity was examined using ascorbate oxidation at 290 nm and a molar extinction coefficient of 2.8 mM^{-1} cm^{-1}.

2.6.4 Proline Quantification

The concentration of proline was determined using (BATES; WALDREN; TEARE, (1973). 100mg of the seedling sample was grinded in liquid nitrogen, and 25mg of ninhydrin was dissolved in 13.8% (w/v) phosphoric acid and 60% (v/v) acetic acid. Samples were combined with an equal volume of both glacial acetic acid and ninhydrin solution was heated to 100ºC for 60 minutes. The generated chromophore was removed with 200L of toluene by vortexing several times after cooling. The organic phase was separated by centrifugation at 10,000g for 3 minutes, and an ultrospec 1100 pro spectrophotometer was used to read the results in quartz or PMMA cuvettes.

2.6.5 Determination of Pigment Content

The procedure was carried out at 4 °C and dark and followed the methodology described by LICHTENTHALER; BUSCHMANN, (2001):. A leaf sample (25mg) was mashed in a mortar and pestle with 80% acetone (v/v), the extract was filtered through two layers of nylon and centrifuged in sealed tubes at 15000*g* for 5 min. The supernatant was collected and read at 663 and 647 nm for chlorophyll *a* and chlorophyll *b*, respectively, and at 470 nm for carotenoid content. The concentrations for Chlorophyll *a*, Chlorophyll *b*, and the sum of leaf carotenoids (xanthophylls and carotenes) were given in μg ml^{-1} extract solution.

2.6.6 Determination of Reducing Sugars Content

Reducing sugars was also quantified with the same procedure as total soluble sugars. Reducing sugar content was done according to (SOMOGYI, 1952). In a test tube, 100 µL of the extract was diluted to 2 mL with water and one mL alkaline copper tartrate was added, then placed in a boiling water bath for 30 min. After samples had cooled down, 1 mL of arsenomolybdolic acid reagent was added. The mixture was diluted with a final volume of 10 mL with MPW and after 10 min absorbance was read at 620 nm in UV–Vis (PerkinElmer Lambda 14 UV/Vis spectrometer), using the same calibration curve as total sugar.

2.6.7 Determination of Total Soluble Sugars Content

Total soluble sugar (mg/g fresh weight) was determined based on the method given by DUBOIS et al. (1956). 100 mg of oven dried seedling sample were added with 5 ml of 80% ethanol to test tubes, placed in water bath and heated for 1 h at 80°C. Then 1 ml of the sample extract was taken in another set of test tubes and mixed with 1 ml each of 18% phenol and distilled water, and then allowed to stand at room temperature for 1 h. Finally, 5 ml of sulphuric acid was added and the whole mixture was vortexed. Absorbance was read at 490 nm wavelength on the UV spectrophotometer. 80% Ethanol was used as blank of sample.

2.6.8 Quantification of Proteins

Protein extracts were prepared by macerating the seedling samples into fine powder and dissolving 100 mg of the powder in the extraction buffer. Extraction buffers tested included sodium phosphate (50 mM) or Tris–HCl (100 mM), both at pH 7, with or without SDS (1–2%) or glycerol (10%), and with or without the following reducing agents: 2-mercaptoethanol (20–200 mM), DTT (5–100 mM), or reduced glutathione (5–10 mM). The extracts were then centrifuged for 10 min at 12 000 g and the supernatant subjected to SDS–PAGE for staining, or to protein gel blots as described previously by (MITTLER et al., 2001)

2.7 Statistical Analysis

The statistical analysis were performed with the software R-Bio (BHERING, 2017). Data was subjected to two-way ANOVA (2x4; two seeds treatment – control and primed, and four salt concentrations) and, in case of normal distribution, Tukey means test was selected at 5% significance. Data without a normal distribution was evaluated by a GLM analysis,

assuming normality by observation of the qq- plot graphs and then applying the Tukey test at 5% of significance.

3. RESULTS

The salt treatments did not influence the length of shoots but did affect the root length and mass of sunflower seedlings. Root length was greater in seedlings that emerged from primed seeds than control at 0mM, 120mM and 240mM respectively. Root length was observed to be the same among treatment at 60mM salinity concentration (Fig. 1A&B). At 240mM salinity, the shoots had a higher dry mass when compared to other salinity concentrations, independent of the priming treatment. For root dry mass, that of 0mM and 60mM salinity concentrations resulted in lower root dry mass when compared to 120mM and 240mM. The leaf area results showed that there was no difference in leaf area between treatments at 0mM, 60mM, and 120mM salinity. However, at 240mM of salinity, control seedlings had a relatively higher leaf area than seedlings that emerged from primed seeds (Fig. 1C─D).

There was no difference in chlorophyll a content between the priming treatments. However, an increase in chlorophyll a content was observed with increasing salinity, independent of the treatments. No differences were observed in the chlorophyll b and carotenoid contents (Fig. 2A─C).

The levels of reducing sugars (RS) in seedlings that emerged from primed seeds were higher than those in the control across all salinity concentrations. However, the total soluble sugars (TSS) results were different. The concentration of TSS was the same between treatments at 60mM and 240mM salinity, but seedlings at 240mM salinity concentration had a relatively higher TSS than seedlings at 60mM concentration of salinity. Seedlings that emerged from primed seeds had higher TSS concentrations than the control at 0mM and 120mM salinity concentrations (Fig. 3A&B).

The proline content was the same between treatments. At salinity concentrations of 60mM, 120mM and 240mM, the proline concentration was the same between treatments, but lower than the 0mM concentration of salinity. The protein results showed that there was no difference in protein content between treatments across all salinity concentrations, except

240mM where control seedlings had a relatively higher protein content than seedlings that emerged from primed seeds (Fig. 4A─B).

The extent of lipid peroxidation (MDA) showed that there was no difference in MDA concentration between treatments at 0mM and 60mM salinity concentrations. However, at 120mM and 240mM salinity concentration, seedlings that emerged from primed seeds had lower concentrations of MDA than the control at 120 and 240mM. Hydrogen peroxide (H_2O_2) results indicated that there was no difference in H_2O_2 concentrations between treatments across all concentrations of salinity (Fig. 5A&B). The enzymatic activity of ascorbate peroxidase (APX) indicated that control seedlings had more APX activity than seedlings that emerged from primed seeds at 0mM, 60mM and 240mM salinity concentrations. At 120mM concentration of salinity, seedlings that emerged from primed seeds had higher APX activity than the control. Superoxide dismutase (SOD) enzymatic activity was higher in seedlings that emerged from primed seeds than control at 0mM, 120mM and 240mM. However, at 60mM salinity concentration there was no difference in SOD activity between the treatments (Fig 5C&D).

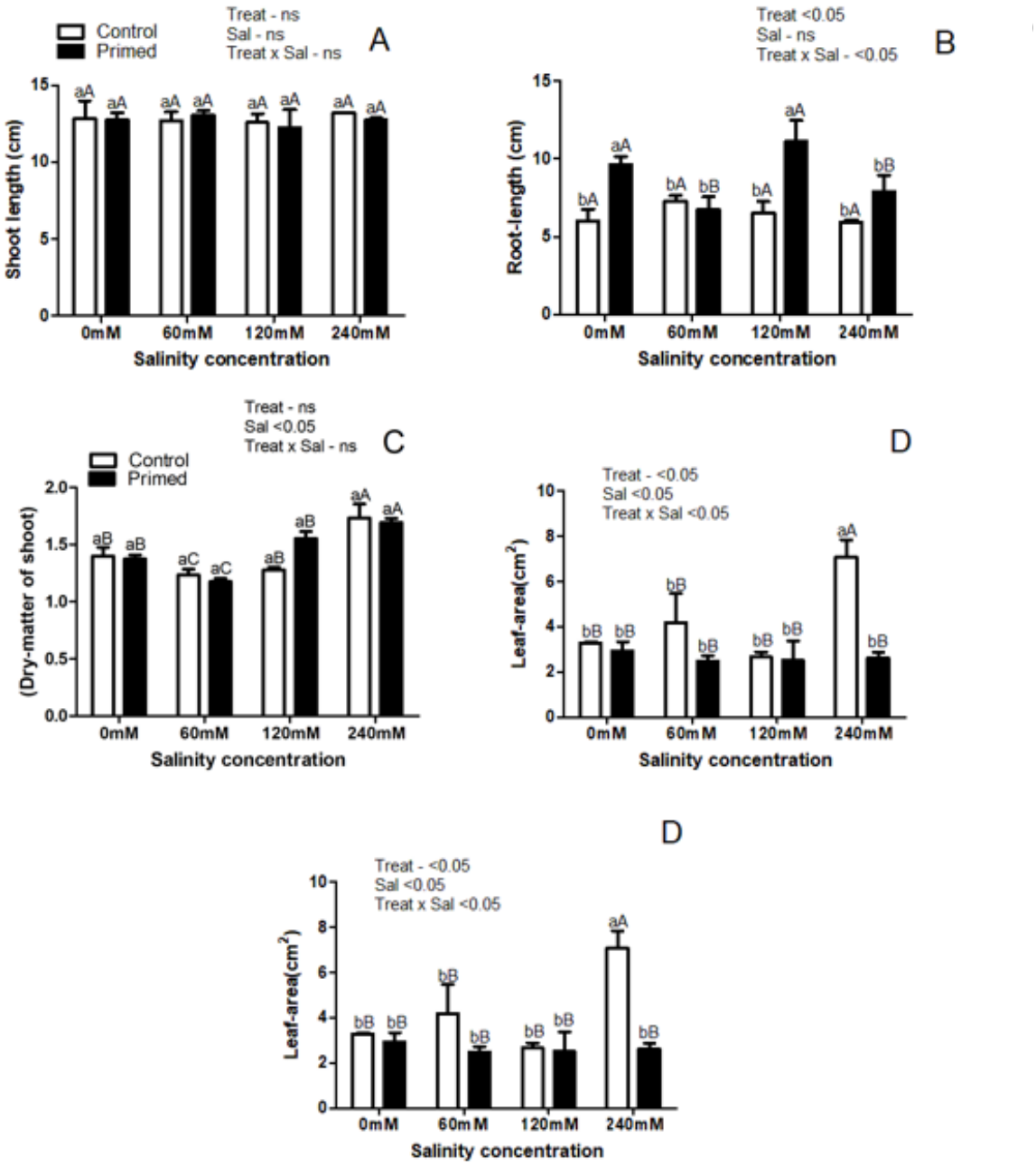


Fig. 1 Length of shoot (**A**); length of root (**B**); dry mass of shoot (**C**); dry mass of root(**D**); and leaf area (**E**) of *Helianthus annuus* seedlings. Bars are means ± standard error (n = 4). Capital letters compare differences among salinity concentrations and lower letters compare differences between treatments (control and primed). The same letters or ns (not significant) do not show any significance difference by Tukey's test at 5% significance.

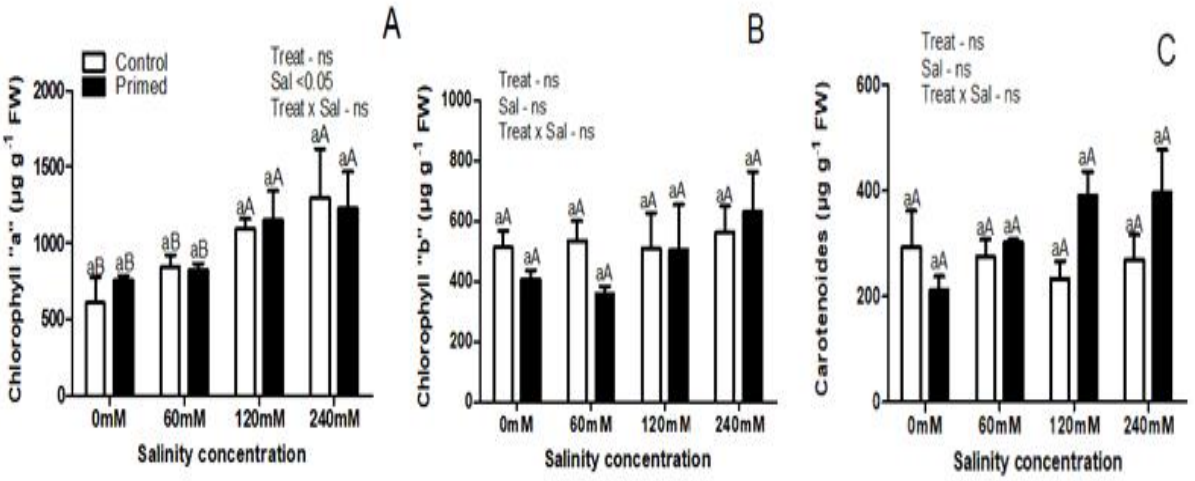
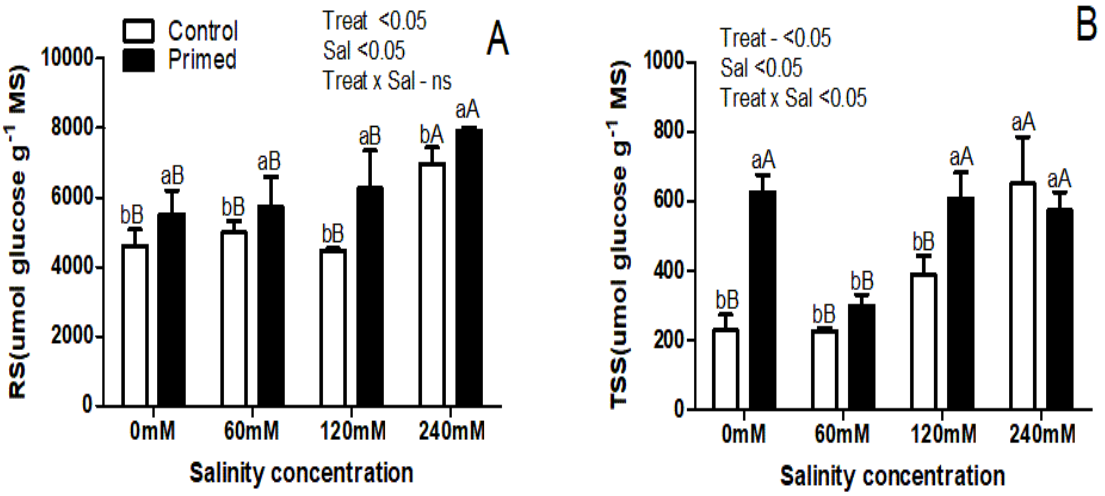


Fig. 2 Estimated levels of chlorophyll a (**A**); chlorophyll b (**B**); carotenoids (**C**) of *Helianthus annuus* seedlings. Bars are means ± standard error (n = 4). Capital letters compare differences among salinity concentrations and lower letters compare differences between treatments (control and primed). The same letters or ns (not



significant) do not show any significance difference by Tukey's test at 5% significance.

Fig. 3 Estimated levels of reducing sugars (**A**) and total soluble sugars (**B**) of *Helianthus annuus* seedlings. Bars are means ± standard error (n = 4). Capital letters compare differences among salinity concentrations and lower letters compare differences between treatments (control and primed). The same letters or ns (not significant) do not show any significance difference by Tukey's test at 5% significance.

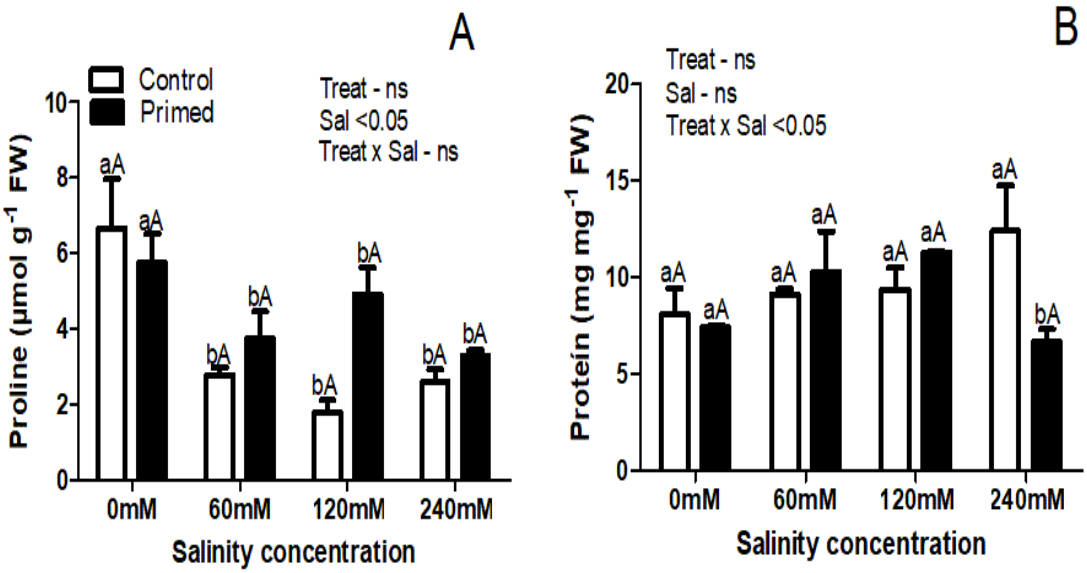


Fig. 4 Estimated levels of proline (**A**) and proteins (**B**) of *Helianthus annuus* seedlings. Bars are means ± standard error (n = 4). Capital letters compare differences among salinity concentrations and lower letters compare differences between treatments (control and primed). The same letters or ns (not significant) do not show any significance difference by Tukey's test at 5% significance.

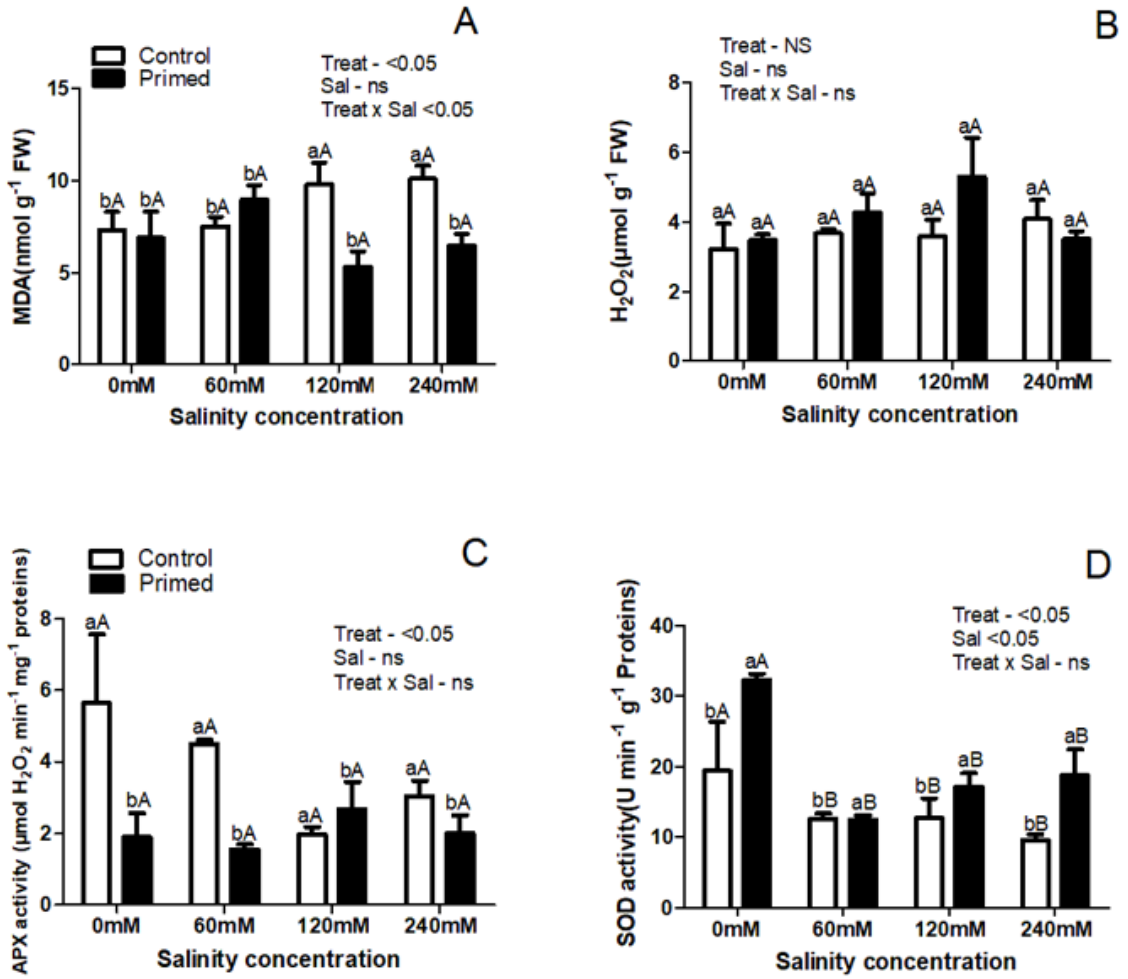


Fig. 5 Estimated levels of lipid peroxidation – MDA (**A**); hydrogen peroxide – H_2O_2 (**B**); ascorbate peroxidase – APX (**C**) and superoxide dismutase – SOD (**D**) of *Helianthus annuus* seedlings. Bars are means ± standard error $(n = 4)$. Capital letters compare differences among salinity concentrations and lower letters compare differences between treatments (control and primed). The same letters or ns (not significant) do not show any significance difference by Tukey's test at 5% significance.

4. DISCUSSIONS

Here, we confirmed the tolerance of sunflower plants (HLADNI et al., 2022) to abiotic stress using salinity. Our results showed that sunflower increased shoot and root mass and length at the highest concentrations, which was an expected response to salinity. However, this happens distinctly with the priming treatment. Priming improved the root length of seedlings when compared to the control at zero salinity, which indicates the importance of this technique as priming improves seedling growth (BAGHERI et al., 2019). At 120mM and 240mM salinity concentrations, seedlings that emerged from primed seeds had greater root lengths than the control. This is a defence mechanism against salinity stress by the seedlings, which involves increasing root growth to increase the water potential of the seedlings and reduce osmotic stress (LIU et al., 2017).

The increase in shoot dry matter was significant among the salinity concentrations in the shoots. According to (KARLOVA et al., 2021), it is possible that it is a more effective way by plants to tolerate stress by increasing their mass to absorb more water and nutrient, but it could also be a way to loss of water by transpiration. Therefore, we evaluated leaf area. The leaf area results indicated that there was a significant difference between the treatments and salinity concentrations. The leaf area was relatively low in both treatments at all levels of salinity, except at 240 mM salinity, where seedlings that emerged from control seeds had a relatively higher leaf area, which may be due to an increase in NaCl concentration in the leaves, which could induce premature senescence (SADDIQ et al., 2021).

Chlorophyll a, chlorophyll b, and carotenoids results showed no significant differences between the treatments and salinity concentrations. The concentrations of chlorophyll b and carotenoids were similar in both treatments at all salinity concentrations. However, chlorophyll a content increased with increasing salinity in both treatments. This indicates that the seedlings used more chlorophyll a as a defence mechanism to maintain photosynthesis by absorbing light and protecting them from oxidative damage caused by reactive oxygen species (YANG, B. et al., 2019).

The priming treatment also increased the levels of reducing and total soluble sugars. Seedlings that emerged from primed seeds had higher concentrations of reducing sugars than the control at all concentrations of salinity. It has been reported that priming helps to improve

seedling growth (SADDIQ et al., 2019) by increasing the effect of reducing sugars in seedlings, including nutrient and central signalling, plant growth and development, stress tolerance responses, and disease resistance (CIERESZKO, 2018). During the priming procedure, the drying step could increase soluble sugars in general, improving seed desiccation tolerance, which is maintained in seedlings and probably makes them more salt tolerant. Because salinity causes osmotic stress responses, increasing soluble sugars can help prevent stress damage (AFZAL; CHAUDHARY; SINGH, 2021).

Priming also reduced oxidative damage caused by lipid peroxidation (MDA). Seedlings that emerged from primed seeds had lower concentrations of MDA than the control at salinity concentrations of 120mM and 240mM. This shows that priming improves tolerance to salinity stress by limiting the formation of reactive oxygen species (RAHIMI, 2013). Even though the hydrogen peroxide content showed no differences between seedlings from primed or non-primed seeds, lipid peroxidation was under control in seedlings from the priming treatment. Therefore, we can assume that priming could improve the antioxidant system, which probably did not occur at the highest salt concentrations in the control treatment.

To control the generation of reactive oxygen species, seedlings from the priming treatment increased SOD activity at the highest salt concentrations compared to the control. This indicates that priming helps increase plant tolerance to salinity stress by boosting the activity of enzymatic antioxidants (SADDIQ et al., 2019). This also indicates that the superoxide anion is the major ROS produced by salt treatments in sunflower seedlings. However, seedlings from primed seeds were more suitable for increasing SOD activity, which in turn corroborates the observations of lipid peroxidation, in which primed seeds presented lower levels of lipid peroxidation. In addition to SOD activity, priming also increased APX activity compared to the control, at 120mM of salt. We can suppose that the higher activity of APX is to control the formation of MDA, but it was not enough to control MDA formation at 240mM salinity concentration in this study.

The proline content was reduced at high salinity concentrations, and for proteins, the concentrations were relatively the same at all salinity concentrations in both treatments, but higher than that of proline. These results showed that the seedlings used more molecular mechanisms through stress-responsive proteins than the biochemical method of using proline

to tolerate salinity stress (ABID et al., 2018; TANI et al., 2019). However, despite being an important compatible osmolyte for salt stress tolerance, mainly due to priming treatment, the proline content did not increase with salinity.

These results suggest that for the sunflower hybrid used here, the most important strategy accessed during the priming procedure, which was retained by the seedlings, was related to the enzymatic antioxidant system and soluble sugars, instead of proline accumulation. It can be concluded that the priming technique is useful for increasing salinity tolerance in sunflower seedlings by the recruitment or permanency of some osmolytes, such as soluble sugars, and is capable of increasing antioxidant capacity by enhancing SOD and APX activity under high salinity.

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FINAL CONSIDERATIONS

Ultimately, it can be determined that primed seeds exhibited better phenotypic performance compared to control seeds. However, the results showed that both treatments were able to tolerate oxidative stress levels, indicating that short-term storage duration did not impact the seeds' physiological characteristics.

Based on these results, it can be deduced that the phenotypic traits of the seedlings were not impacted by salinity. The sunflower seedlings exhibited an ability to withstand the physiological effects of salinity, particularly at higher concentrations. Furthermore, seedlings that emerged from primed seeds displayed greater tolerance to the salinity effect compared to seedlings that emerged from control seeds.

Through this research, we have successfully narrowed the gap in the use of halopriming technique in maintaining the quality of sunflower seeds during short-term storage while also improving their tolerance to salinity stress.

More research may allow for the halopriming technique to be used to enhance the rate of sunflower seed germination after storage, as demonstrated in this study, and to determine if sunflower tolerance to salinity can be developed beyond the seedling stage. Farmers and other stakeholders may leverage the halopriming technique to optimize sunflower production under challenging conditions.