

Germination of *Lepidium sativum* seeds and regulation of beta-galactosidase enzyme production in response to different abiotic factors

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Abstract

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Seed germination is a complex physiological process influenced by abiotic stress factors, including temperature, salinity, osmotic stress, heavy metals, and irradiance. Our study demonstrates that high temperatures ($\geq 30^{\circ}\text{C}$) and salinity stress (≥ 100 mM NaCl) negatively affect *Lepidium sativum* seed germination, primarily by reducing water availability and altering hormone signaling. Protein synthesis and enzymatic activity, particularly β -galactosidase, are significantly impacted by temperature and salt stress. Osmotic stress due to PEG further inhibits germination by delaying water uptake and starch mobilization. Cadmium chloride exposure results in severe inhibition of germination, likely due to impaired protein metabolism, mineral mobilization, and structural modifications in the cell wall. Conversely, irradiance positively influences seed germination by enhancing gibberellin biosynthesis and β -galactosidase activity, which facilitate cell wall remodeling. The results highlight the critical role of hydrolases in mitigating the effects of abiotic stress, suggesting that β -galactosidase activity serves as a key indicator of germination success under stress conditions. Further research is needed to explore the interactions between stress factors, antioxidant defenses, and hormonal regulation in seed germination under environmental constraints.

Keywords: *Lepidium sativum*; seed germination; β -galactosidase; abiotic stresses; acclimation

Introduction

Garden cress (*Lepidium sativum* Linn.), a member of the Brassicaceae family, thrives in semi-arid regions and is classified as a cool-season annual with an erect herbaceous growth habit. Although all parts of the plant – roots, leaves, and seeds – hold economic value, it is primarily cultivated for its seeds (Behrouzian et al., 2014).

The seeds of *L. sativum* consist of two layers: an outer dead testa and an inner aleurone layer of living endosperm

cells. Germination in this species occurs in two distinct phases: first, the rupturing of the testa, followed by the extension of the lower hypocotyl/radicle (RAD), which penetrates the micropylar endosperm layer (CAP), marking the completion of germination (Morris et al., 2011). This process involves three stages of water uptake: (I) rapid initial imbibition, (II) a plateau phase, and (III) post-germination water uptake leading to embryonic axis elongation. However, in dormant seeds that fail to germinate, phase III does not occur (Bewley, 1997).

During germination, enzymatic activity increases, particularly that of β -galactosidase (BGAL), a glycosidase involved in the degradation of key plant cell wall components, including galactans, arabinogalactans, arabinogalactan proteins, and xyloglucans. Additionally, BGAL plays a role in the hydrolysis of galactolipids and glycoproteins, contributing to cell wall remodeling in various plant processes (Dwevedi and Kayastha, 2010; Le Gall et al., 2015; Tanthanuch et al., 2008).

Plant growth and seed germination are significantly affected by abiotic stresses, particularly water scarcity and increasing soil salinity, both of which pose significant challenges to agricultural productivity (Vinocur and Altman, 2005). Abiotic and biotic stressors – including drought, extreme temperatures, excessive light, pests, and salinity – can disrupt cellular functions. Prolonged or intense stress exposure can overwhelm cellular defense mechanisms, leading to cell damage or death (Van Breusegem and Dat, 2006). While seeds exhibit high resistance to environmental stress, seedlings are the most vulnerable stage in a plant's life cycle (Qu et al., 2008).

Environmental factors, including light, oxygen, and temperature, regulate seed germination and dormancy (Toh et al., 2008). Salinity is a significant constraint on agricultural productivity, as it impairs seed germination and mineral mobility (Munns and Tester, 2008; Tabatabaei, 2014). Temperature fluctuations also play a critical role, as seasonal dormancy and soil seed bank cycling are temperature-dependent (Graeber et al., 2014). While optimal temperatures promote germination and break dormancy, excessively high temperatures reduce viability (Farooq et al., 2004). Furthermore, osmotic stress significantly affects seed germination, as reduced water potential inhibits water uptake and delays germination (Murungu, 2013).

Several studies have demonstrated that β -galactosidase activity increases during seed germination, facilitating water absorption and hydrolyzing food reserves essential for seedling development (Kishore and Kayastha, 2012). However, under abiotic stress conditions such as drought and osmotic stress, galactose accumulation increases, indicating reduced β -galactosidase activity (Dwevedi and Kayastha, 2009).

This study aims to establish a foundation for understanding the seed germination mechanisms of garden cress (*L. sativum*), a plant widely used for medicinal purposes. Specifically, we investigate the regulation of β -galactosidase production under various abiotic stresses and its impact on seed germination. Additionally, we examine the interplay between abiotic stress factors and β -galactosidase regulation to determine optimal enzyme activity levels for applications in food technology.

Materials and Methods

Seed sterilization

For germination, seeds were surface-sterilized by incubation for 15 min in 0.05% SDS dissolved in 70% ethanol, followed by a 1-min rinse in 95% ethanol. After drying, they were spread out on plates for use. The number of germinated seeds was counted after 0, 12, 16, 18, and 24 h of incubation for each specific treatment (Belin et al., 2009).

Abiotic stress treatment

Thirty seeds of *L. sativum* were incubated in complete darkness in 9 cm Petri dishes on two layers of filter paper moistened with 6 mL of distilled water. Three replicates were created to impose specific abiotic stress as previously designed by Oracz et al. (2012).

Different irradiance treatments

The seeds were incubated in continuous darkness, but various light intensities were established by placing the Petri dishes at different distances from a bank of fluorescent lights. The dishes received a continuous light with different light quantities, measured in terms of photosynthetic photon flux density (PPFD), using a CIRAS-3 portable photosynthesis system (PP Systems, Amstbury, MA, USA). The triplicates received treatments of 5, 50, and 500 μE ($\mu\text{mol photon m}^{-2} \text{s}^{-1}$) (Khan et al., 2003).

Effects of cadmium ions

The seeds were grown in darkness at 25°C with different concentrations of cadmium (Cd) at 5, 20, 50, and 80 mg/L. These concentrations were prepared from a Cadmium standard solution (CdCl_2). At various intervals of germination, the results were taken (Ahmad et al., 2012).

Effects of salinity

Five different sodium chloride concentrations (0, 50, 100, 150, and 200 mM) were used, and the seeds were incubated in darkness at 25°C. Their progress was followed at 12 h, 16 h, 18 h, and 24 h (Rajjou et al., 2006).

Effect of temperature on germination

Seeds were incubated at 20, 25, 27, 30, and 35°C in continuous darkness, and the results were observed at the same time points as in the previous section (Qu et al., 2008).

Osmotic stress treatment

Different water potentials were obtained with different solutions of Polyethylene Glycol (PEG) 8000. PEG solutions were prepared according to Michel (1983) to attain water po-

tentials of 0, -0.05, -0.09, -0.15, -0.22, and -0.31 MPa. Osmotic pressure (OP) was calculated as follows:

$$OP = 1.29 \times C^2 \times T - 140 \times C^2 - 4.0 \times C,$$

where C = PEG concentration; T=Temperature.

Seeds were kept in moist petri dishes with a diameter of 9.0 cm and incubated in a dark incubator maintained at 25°C (Al-Taisan et al., 2010).

Germination Percentage (%)

Seeds were considered germinated when a radicle had emerged from the seed coat. The percentage was then recorded for each specific treatment at 0 h, 12 h, 16 h, 18 h, and 24 h after the start of incubation (Batabyal et al., 2014).

Percentage of germination (%) = (Number of germinated seeds/ Total number of seeds) × 100.

Protein Extraction

After each treatment, the germinated seeds were harvested in liquid nitrogen and stored at -80°C until the subsequent analysis. The seeds were ground into a fine powder using a mortar and pestle and then dissolved in a lysing buffer consisting of 0.22 M mannitol, 0.07M sucrose, 1m M EDTA, 0.3% (wt/vol) PVP and 0.05% (wt/vol) BSA, while maintaining a medium to tissue weight ratio of 2:1. The homogenate was maintained at pH 7.2 with the addition of 1 N KOH. After mixing, the homogenate was centrifuged for 20 min at 10.000 g. The supernatant was further centrifuged at 21.000 g for 75 min, and the resultant soluble fraction was used for enzyme assays. All operations were carried out at 4°C (Sreekala and Lalitha, 1998). The protein content in the supernatant was determined by the Bradford method (Bradford, 1976) using bovine serum albumin (BSA) as a standard.

β-Galactosidase activity

β-Galactosidase activity was determined according to the method described by Kishore and Kayastha (2012). The reaction mixture for activity measurement against Ortho-Nitrophenyl-β-galactoside (ONPG) was prepared in a final volume of 500 µl, consisting of 50 mM glycine-HCl (pH 2.8), 20 mM ONPG substrate, and 20 µl of enzyme extract. The reaction was performed at 37°C for 10 min. The liberated o-nitrophenolate (ONP) was measured spectrophotometrically at 405 nm after stopping the reaction by adding 1.5 mL of sodium tetraborate (20 mM). The specific activity of β-galactosidase is expressed as µmoles of ONP min⁻¹ mg⁻¹ protein.

Statistical analysis

In all experiments, selected seed samples were analyzed, and each assay was performed in triplicate. The results were

expressed as mean±SE. A two-way ANOVA, followed by Tukey's honest significance test, was performed at a 95% confidence level ($P \leq 0.05$) to compare the means of parameters and interactions that were statistically significant. Statistical analysis of the data was carried out using OriginPro (OriginLab Corporation, Northampton, MA, USA, 2023).

Results

Impacts of Abiotic Stresses on Germination Percentage

The effect of temperature on germination percentage (GP) showed no significant change at 20°C compared to the control (25°C). However, at 30°C, GP decreased significantly in a time-dependent manner, reducing by 60%, 50%, 40%, and 30% after 12, 16, 18, and 24 h, respectively, relative to the control. At 35°C, seed germination was inhibited entirely during all incubation periods (12, 16, 18, and 24 hours), indicating thermo-inhibition, as shown in Figure 1A.

The severity of salinity stress and the duration of exposure were also assessed. Treatment with 50 mM NaCl reduced GP after 12 and 16 h of incubation, resulting in 0.7- and 0.6-fold reductions compared to the control, respectively. A notable increase in GP was observed after 24 h, reaching 110% relative to the control. At 100 mM NaCl, GP declined by 80%, 50%, 20%, and 10% after 12, 16, 18, and 24 h of incubation, respectively. The most potent inhibitory effect was observed at 150 mM NaCl, where seed germination was delayed until after 16 h, at which point GP had decreased by 70%. The reduction continued, reaching 50% and 30% after 18 and 24 h, respectively. At 200 mM NaCl, germination was further delayed, resuming only after 18 and 24 hours, with GP reductions of 80% and 60%, respectively, compared to the control. All comparisons of NaCl concentrations and incubation periods were statistically significant, as shown in Figure 1B.

Osmotic pressure was simulated using polyethylene glycol (PEG) to mimic drought stress. At -0.05 MPa, no significant changes in GP were observed. However, at -0.09 MPa, GP decreased to 20% after 12 h and maintained this reduction through 24 h of incubation, compared to the control (0 MPa). At -0.15 MPa, GP dropped to 30% and 10% after 12 and 24 h, respectively. A more pronounced reduction was observed at -0.22 MPa, where GP decreased by 50%, 30%, 30%, and 20% at 12, 16, 18, and 24 h, respectively. At -0.31 MPa, the reduction in GP was significant, reaching 50%, 30%, 30%, and 20% at the respective time points, as shown in Figure 1C.

Cadmium chloride treatments hurt GP, particularly during the early stages of incubation. At a concentration of 5 ppm Cd, GP was reduced by 50%, 40%, and 40% after 12, 16, and 18 h, respectively, compared to the control. More pronounced effects were observed at 20 and 50 ppm,

where GP declined by 60% at 12, 16, and 18 h, further decreasing to 40% after 24 h. At 80 ppm, the reductions were even more severe, with GP decreasing by 70%, 60%, 70%, and 40% after 12, 16, 18, and 24 h, respectively, as shown in Figure 1D.

Light intensity significantly influenced GP. At five μE of light, GP increased by 120% compared to the control (con-

tinuous darkness), maintaining a 110% increase after 12, 16, and 18 h, and reaching 110% at 24 h. At 34 μE , GP was induced further, reaching 120%, 120%, 130%, and 110% at 12, 16, 18, and 24 h, respectively. The highest induction was observed at 500 μE , where GP increased significantly to 140%, 130%, 130%, and 110% after 12, 16, 18, and 24 h, respectively, as shown in Figure 1E.

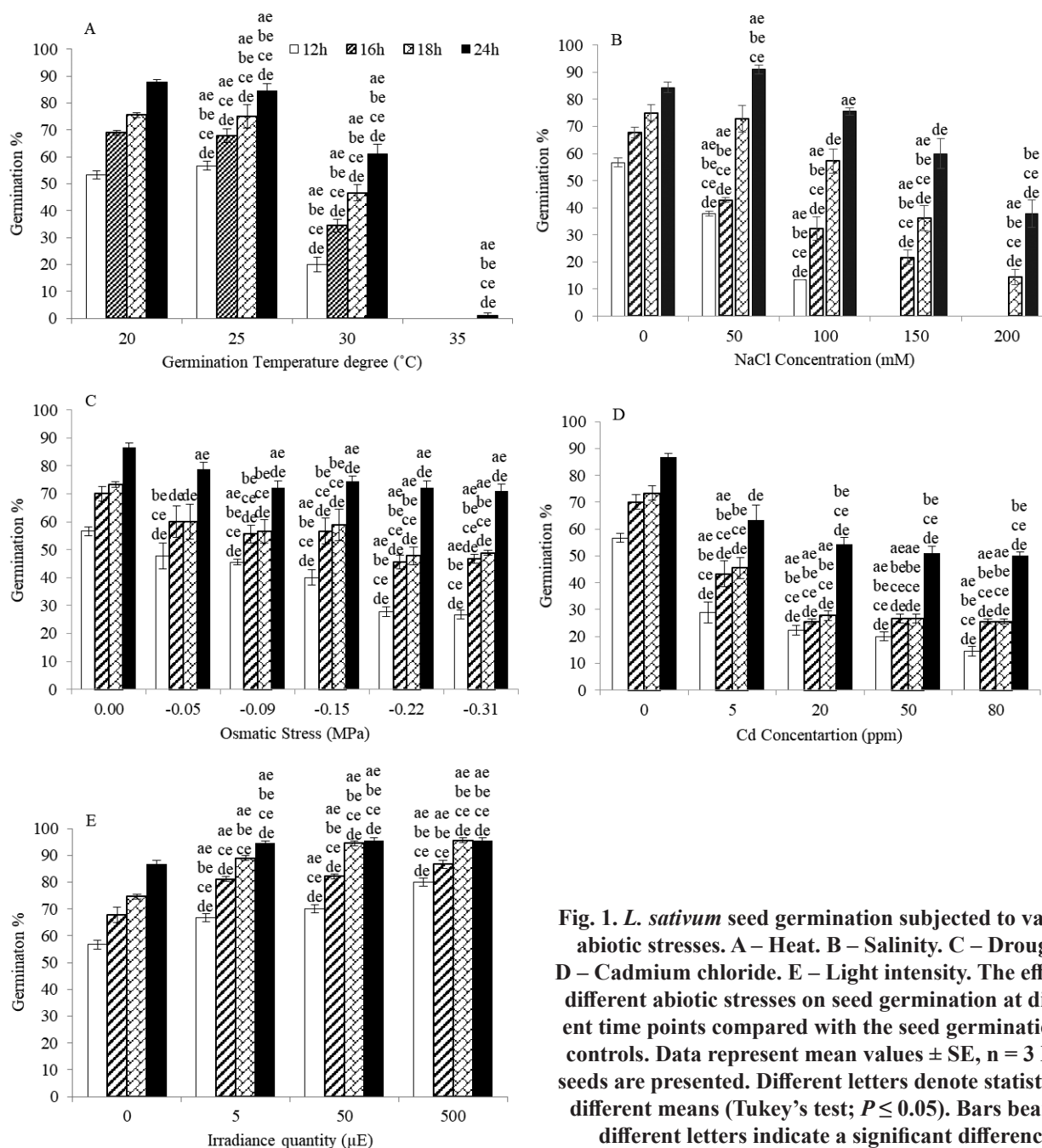


Fig. 1. *L. sativum* seed germination subjected to various abiotic stresses. A – Heat. B – Salinity. C – Drought. D – Cadmium chloride. E – Light intensity. The effect of different abiotic stresses on seed germination at different time points compared with the seed germination in controls. Data represent mean values $\pm$ SE, $n = 3 \times 30$ seeds are presented. Different letters denote statistically different means (Tukey's test; $P \leq 0.05$). Bars bearing different letters indicate a significant difference

Protein content

Temperature had a slight, non-significant reductive effect on protein content after 12 and 18 h of incubation at 20°C and 30°C compared to the control (25°C). However, at 35°C, protein content was consistently reduced across all germina-

tion periods, indicating a temperature-dependent decline, as shown in Figure 2A.

Protein content decreased significantly in response to NaCl concentration in a time-sensitive manner, particularly at early time points. At 50 mM NaCl, protein content was re-

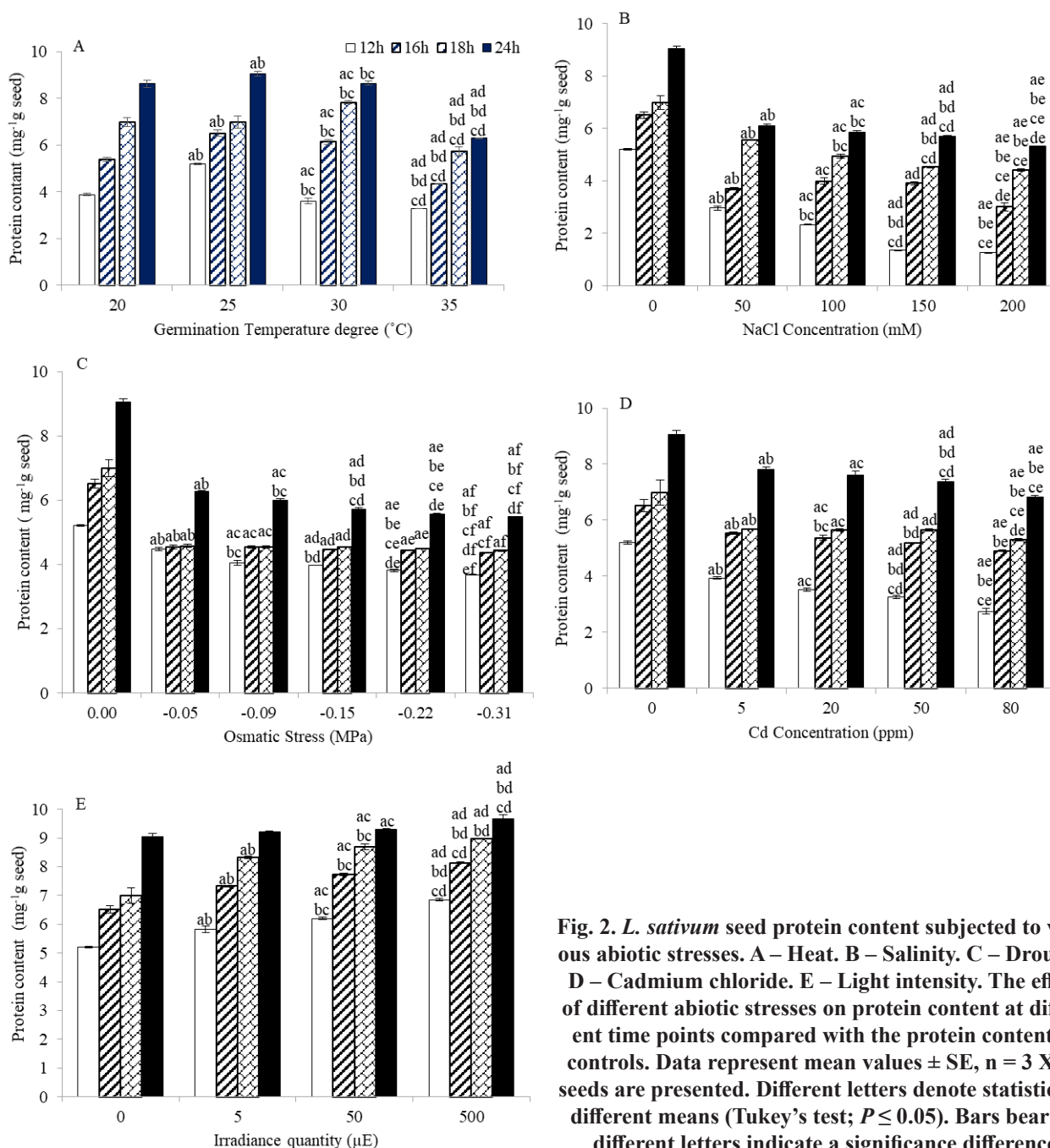


Fig. 2. *L. sativum* seed protein content subjected to various abiotic stresses. A – Heat. B – Salinity. C – Drought. D – Cadmium chloride. E – Light intensity. The effect of different abiotic stresses on protein content at different time points compared with the protein content in controls. Data represent mean values $\pm$ SE, $n = 3 \times 30$ seeds are presented. Different letters denote statistically different means (Tukey's test; $P \leq 0.05$). Bars bearing different letters indicate a significance difference

duced by 40%, 40%, 20%, and 30% after 12, 16, 18, and 24 h of incubation, respectively. At 100 mM NaCl, reductions of 60%, 40%, 30%, and 40% were observed over the same time points. A more pronounced effect was observed at 150 mM NaCl, where protein content declined by 70%, 40%, 40%, and 40%, respectively. In contrast, at 200 mM NaCl, reductions reached 80%, 50%, 40%, and 50%, respectively, as shown in Figure 2B.

Osmotic pressure significantly affected protein content in most conditions tested. At -0.05 MPa, reductions of 10% and 30% were observed after 12 and 18 h, respectively, compared to the control. At -0.09 MPa, protein content decreased uniformly by 30% across all time points. Similar trends were observed at -0.15 MPa, with reductions of 20%, 30%, 40%, and 40% after 12, 16, 18, and 24 h, respectively. The effects at -0.22 MPa and -0.31 MPa were nearly identical to those at -0.15 MPa, showing reductions of 30%, 30%, 40%, and 40% at the respective time points, as shown in Figure 2C.

Cadmium chloride significantly reduced protein content across all incubation periods. At a concentration of 5 ppm Cd, protein content declined by 20%, 10%, and 10% after 12, 16, and 24 h, respectively, compared to the control. At 20 ppm, reductions of 30%, 20%, 20%, and 20% were observed simultaneously. At 50 ppm, protein content dropped by 40% after 12 h but showed a 20% reduction at 16, 18, and 24 h. The most severe reduction occurred at 80 ppm Cd, where protein content decreased by 50%, 20%, 20%, and 20% over the incubation periods, as shown in Figure 2D.

Protein content was significantly induced under all tested irradiances. At five μ E, protein content increased by 110%, 110%, and 120% after 12, 16, and 18 h, respectively, compared to the control. At 50 μ E, induction reached 120% at 12, 16, and 18 hours, while at 24 h, it was slightly lower at 102%. The highest induction was observed at 500 μ E, with protein content reaching 130% after 12, 16, and 18 h and 110% after 24 h, as shown in Figure 2E.

β -galactosidase specific activity

The reduction in β -galactosidase specific activity was observed across all incubation periods (12, 16, 18, and 24 h) at 20°C, 30°C, and 35°C compared to the control (25°C). At 20°C, activity was reduced to 70%, 60%, 50%, and 60%, respectively. At 30°C, reductions of 70%, 60%, and 50% were recorded. The most pronounced decrease occurred at 35°C, where β -galactosidase activity dropped to 94%, 92%, and 91% at the respective time points, as shown in Figure 3A.

Salinity had a concentration-dependent effect on β -galactosidase activity, particularly at early time points. A significant reduction was first observed at 100 mM NaCl after 16 h of incubation, where activity decreased by 30% compared to

the control. At 150 mM NaCl, β -galactosidase activity was significantly reduced after 12 and 16 h, reaching 92% and 50% of the control level, respectively. At the highest concentration (200 mM NaCl), reductions of 92%, 90%, 40%, and 50% were observed after 12, 16, 18, and 24 h, respectively, as shown in Figure 3B.

A significant reduction in β -galactosidase specific activity was observed under osmotic pressures of -0.22 MPa and -0.31 MPa. The reduction was most evident at 12 h of incubation, where activity decreased by 40% in both conditions compared to the control, as shown in Figure 3C.

Cadmium exposure had a strong inhibitory effect on β -galactosidase activity. At a concentration of 5 ppm Cd, activity was significantly reduced by 30% after 12 and 16 h of incubation. At 20 ppm Cd, reductions of 40% were observed at 12, 16, and 18 h. When Cd concentration increased to 50 ppm, activity declined by 50%, 40%, and 40% at 12, 16, and 18 h, respectively. The most substantial reduction was observed at 80 ppm Cd, where β -galactosidase activity decreased by 70%, 70%, and 40% at the respective time points, as shown in Figure 3D.

Light exposure significantly enhanced β -galactosidase specific activity at most incubation times. At five μ E, activity was induced to 120%, 160%, and 160% of the control after 12, 16, and 18 h, respectively. At 50 μ E, induction reached 120%, 160%, and 170% of the baseline over the same time points. The highest induction was observed at 500 μ E, where β -galactosidase activity increased to 120%, 170%, and 170% compared to the control, as shown in Figure 3E.

Discussion

Temperature

Temperature plays a crucial role in various physiological processes in seeds. First, temperature and moisture content determine the rate of seed deterioration. Second, temperature influences dormancy loss in dry seeds and modulates dormancy patterns in wet seeds. Third, in non-dormant seeds, temperature regulates the germination rate (Roberts, 1988). Mukhopadhyay (2010) reported a significant decline in germination rate when the incubation temperature exceeded 30°C, indicating thermal inhibition of germination. The pattern of germination decline in this study aligns with Mukhopadhyay's findings. Thermoinhibition at elevated temperatures is linked to the expression of the *9-cis-epoxy-carotenoid dioxygenase (NCED)* gene (Argyris et al., 2008, 2011). In general, genes associated with abscisic acid (ABA) biosynthesis are more highly expressed under conditions that inhibit germination. Specifically, *NCED4*, a key gene in the ABA biosynthetic pathway, is upregulated in response to

high temperatures. Under such conditions, gibberellin (GA) synthesis is suppressed, while GA promotes ABA catabolism and raises the upper temperature threshold for seed germination (Gonai et al., 2004).

The ability of seeds to germinate under extreme temperatures may be related to the sensitivity of the embryo to temperature (Riley, 1981). However, the rate of protein

synthesis in seeds exposed to high or low temperatures is reduced compared to those incubated at 25°C. In our experiments, this decline in protein synthesis correlates with temperature sensitivity, suggesting that protein synthesis in *L. sativum* seeds is particularly temperature-dependent (Riley, 1981). Following germination, hydrolase activity, including that of β -galactosidase, contributes to endosperm cell wall

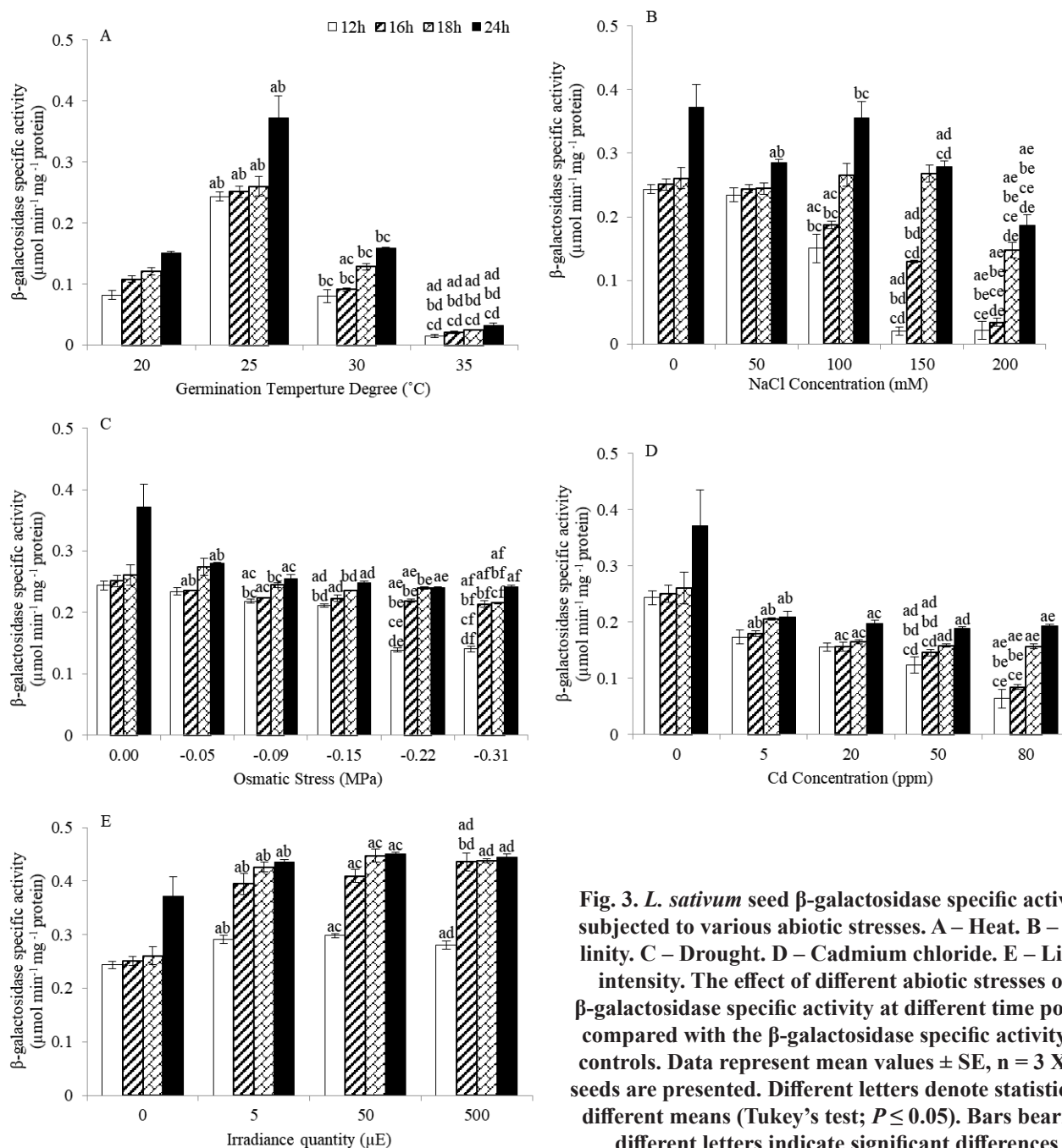


Fig. 3. *L. sativum* seed β -galactosidase specific activity subjected to various abiotic stresses. A – Heat. B – Salinity. C – Drought. D – Cadmium chloride. E – Light intensity. The effect of different abiotic stresses on β -galactosidase specific activity at different time points compared with the β -galactosidase specific activity in controls. Data represent mean values $\pm$ SE, $n = 3 \times 30$ seeds are presented. Different letters denote statistically different means (Tukey's test; $P \leq 0.05$). Bars bearing different letters indicate significant differences

degradation, facilitating cotyledon expansion by reducing mechanical resistance. This enzymatic process also enhances the diffusion of degradation products into the cotyledons (Suda et al., 2003). Endosperm cap weakening is promoted by GA and inhibited by ABA, as demonstrated by studies on hormone signaling networks (Morris et al., 2011). The *DELAY OF GERMINATION1 (DOG1)* gene regulates delayed germination by suppressing GA-responsive genes involved in cell wall remodeling in a temperature-dependent manner. This regulation is primarily influenced by the temperature at which seeds are synthesized (Graeber et al., 2014).

Salinity

Our experiment demonstrated that salinity negatively impacted germination, with both concentration and duration of exposure playing critical roles. Similar findings were reported by Habibi and Abdoli (2013), who observed that salinity reduced seed viability and germination percentage. Specifically, they found that exposure to 150 mM NaCl decreased the germination rate from 95.3% to 89.7%. The adverse effects of salinity on seed germination may be attributed to direct ion toxicity, resulting from increased anion and cation concentrations (Panuccio et al., 2014). Additionally, salinity reduces water availability and disrupts the mobilization of stored reserves, potentially affecting the structural integrity of proteins (Demir and Mavi, 2008; Kim et al., 2006). It also influences endogenous hormone levels by increasing inhibitory hormones, such as abscisic acid (ABA), while reducing stimulatory hormones, like cytokinins (Çavusoglu and Kabar, 2010; Kim et al., 2006).

Salt stress induces physiological and biochemical changes, including inhibition of protein synthesis, disruption of reserve hydrolysis product translocation from the cotyledons to the embryo, and delayed enzyme solubilization and activation (Gomes-Filho et al., 1996). Our findings align with these observations, as protein content significantly decreased in response to NaCl concentration in a time-dependent manner. These changes in protein synthesis under salt stress may be attributed to variations in mRNA transcription, transport, stability, or translation efficiency (Dell'Aquila and Spada, 1993).

Furthermore, salinity affected the specific activity of β -galactosidase during seed germination, with this effect being concentration-dependent, particularly at early time points. Notably, β -galactosidase activity increased following exposure to 100 mM NaCl. Over time, prolonged incubation led to a further increase in enzyme activity, likely due to enhanced enzyme solubilization as seeds absorbed water. However, salinity delays the water uptake required for enzyme solubilization and activation, suggesting that salt stress

inhibits the timely activation of β -galactosidase (Enéas-Filho et al., 1995).

Osmotic stress

Higher concentrations of osmotic stress result in lower germination percentages (Bialecka and Kępczyński, 2010; Demir and Mavi, 2008; Rasaei et al., 2013; Schellenberg et al., 2013). In this study, the inhibition of germination in polyethylene glycol (PEG)-treated seeds was attributed solely to osmotic effects resulting from the reduced water potential of the solutions (Zhang et al., 2010). Similarly, Almansouri et al. (2001) reported that PEG negatively affects starch remobilization kinetics by reducing α -amylase activity. Additionally, osmotic stress enhances the expression of *LOS6/ABAI*, a gene that encodes zeaxanthin epoxidase, which plays a crucial role in ABA biosynthesis. This suggests that ABA is crucial for gene regulation under osmotic stress conditions (Xiong et al., 2002).

Low water potential caused by osmotic stress slows water uptake, prolongs the water uptake plateau, and subsequently delays or prevents germination. PEG-induced drought stress results in a gradual decrease in water potential, which correlates with reduced germination and inhibition of storage reserve degradation (Khademi et al., 1991). Under drought stress, key physiological processes such as cell wall expansion, protein synthesis, and enzymatic activity are inhibited. In particular, the de novo synthesis of proteolytic enzymes, which are responsible for breaking down storage proteins, is essential for germination, highlighting the importance of both soluble and insoluble protein degradation in seed germination (Khademi et al., 1991).

Both salinity and drought in the growing medium hinder seed germination by limiting water uptake. According to Mehra et al. (2003), PEG molecules do not permeate seeds; once the water potential of the seed and its environment reaches equilibrium, water absorption ceases. Water stress induced by PEG can also lead to the degradation and inactivation of essential hydrolases, further impairing germination (Pratap and Sharma, 2010).

Cadmium chloride

Cadmium has been shown to negatively impact GP, exhibiting an inhibitory effect consistent with previous reports (Ahmad et al., 2012; Pavel et al., 2013). Similar studies have demonstrated that Cd(II) significantly reduces germination at concentrations above 30 ppm, although at lower levels, cadmium can serve as a micronutrient for plants (Pavel et al., 2013). The delayed germination observed in cadmium-exposed seeds may be attributed to the inhibition of both storage protein catabolism and plant protein anabolism, ul-

timately reducing seed germination (Gianazza et al., 2007). Furthermore, cadmium disrupts mineral and carbohydrate mobilization during germination by inhibiting the activities of α -amylase and invertase, which in turn affects membrane integrity (Sfaxi-Bousbih et al., 2010).

A decline in protein abundance during germination under cadmium stress may result from decreased protein synthesis, increased protein degradation, or interactions with cadmium-induced peptides such as $(\gamma\text{-Glu-Cys})_n\text{-Gly}$, $(\gamma\text{-Glu-Cys})_n$, and $(\gamma\text{-Glu-Cys})_n\text{-Glu}$. This ultimately reduces free peptide availability and inhibits protein synthesis (Rauser, 1995; Gubrelay et al., 2013).

Cadmium stress also reinforces cell junctions, leading to enhanced tissue cohesion and Cd sequestration within plant cells (Douchiche et al., 2010). Instead of degrading cell wall polysaccharides through enzymatic activity (e.g., cellulase and β -galactosidase), plants respond by increasing the levels of cell wall components such as pectin and hemicellulose. These polysaccharides play a crucial role in binding heavy metals, preventing cellular damage, and irreversibly inhibiting growth (Le Gall et al., 2015).

Irradiance

Light, in terms of both irradiance levels and spectral composition, plays a crucial role in enhancing seed germination by increasing the germination rate (Lone et al., 2014; Gross, 1985; Ologundudu et al., 2013). This effect is mediated by pigments and phytochromes, which function as photoreceptors. The presence of at least five phytochrome genes suggests that different members of this family contribute to distinct aspects of photoregulation during germination (Shinomura, 1997). In *Arabidopsis thaliana*, phytochrome PhyA and PhyB are the primary active forms that promote seed germination (Shinomura et al., 1994). Light facilitates germination by stimulating the biosynthesis of active GAs and upregulating GA 3 β -hydroxylase gene expression via phytochrome PhyB (Ogawa et al., 2003; Yamaguchi et al., 1998).

Gross (1985) further explored the effect of irradiance levels and spectral composition on seed germination, while Bellaloui et al. (2012) noted that shading reduced protein content during germination due to lower nitrogen assimilation. Additionally, lipolytic and proteolytic enzymes play a role in softening the seed coat, thereby reducing seed hardness. Enzymes involved in key metabolic pathways, particularly those associated with the tricarboxylic acid cycle, were found in dry seeds and remained stable or accumulated further during early germination under light exposure (Angelovici et al., 2011; Weitbrecht et al., 2011). β -Galactosidase, a potential cell wall remodeling enzyme, facilitates the

release of monosaccharides from pectin and hemicellulose, providing an energy and carbon source for developing cells (Lee et al., 2007). Moreover, light-induced GA biosynthesis triggers pectin modification by activating pectin hydrolases, such as pectate lyase, polygalacturonase, and β -galactosidase, while downregulating the repressors of pectin esterases (Munteanu et al., 2014).

Conclusion

Seed germination is a complex physiological process influenced by abiotic stress, which can have either positive or negative effects depending on its type, duration, and severity. Key abiotic stressors such as temperature, soil salinity, drought, and heavy metal contamination regulate germination through hydrolase activity, particularly β -galactosidase production and function. Our study found that elevated temperatures (30–35°C) and high soil salinity (≥ 100 mM NaCl) significantly inhibited *L. sativum* seed germination, especially during the early exposure phase (12 h). Similarly, water deficit negatively impacted germination, while increased light intensity had a positive effect, as reflected in enhanced β -galactosidase activity. Exposure to cadmium chloride, simulating heavy metal soil contamination, exhibited extreme toxicity at all stages of germination, further underscoring the critical role of hydrolases, including β -galactosidase.

Overall, our findings highlight the significant impact of abiotic stress on seed germination through the regulation of β -galactosidase activity. However, further research is required to explore the contributions of other factors, such as antioxidant enzyme activity, non-enzymatic antioxidants, and plant hormones, in mitigating stress-induced germination inhibition.

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Author Contributions

W.A. performed the research. K.A. designed the project, supervised its execution, and discussed the results, subsequently writing the article. T.H., Y.H., and E.R. contributed to writing the manuscript and did the editing of the manuscript. Percentage participation is equal for all co-authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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