Egypt. J. Bot. **Vol. 64,** No. 1, pp. 257-275 (2024)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Physiological and Genetical Responses of *Lepidium sativum* L. Seeds to Ultrasonic Pretreatment under Heat Stress

Amira M. Abd El-Sattar, Eman Tawfik, Eman Z. Ahmed[#]
Botany and Microbiology Department, Faculty of Science, Helwan University, Cairo 11790, Egypt.

MOST seeds prefer temperature below 30°C for germination, but since temperature control in the field is not possible, this study aims to increase *Lepidium sativum* plant tolerance to heat stress by pretreatment with ultrasonic waves. Results revealed that 25°C was the ideal temperature for garden cress seed germination. Heat stress at 30, 35, and 40°C caused a progressive decline in the germination percentage and all growth criteria, and at 40°C no seed germination was observed even after pretreatment with ultrasonic waves. Furthermore, exposure of seeds to ultrasonic vibrations, especially for 20min at temperatures up to 35°C, resulted in a significant increase in germination percentage up to 96% compared to the corresponding control percent 92%, all seedling growth parameters and mineral ion contents at all tested temperatures, in addition to decrease in stress markers. Soluble sugars and proteins also increased, recording 131.64 and 20.65mg/g dry matter respectively compared to corresponding control. The dendrogram and principal component analysis (PCA) plot were designed based on morphological and physiological parameters and showed three groups of variable treatments. A molecular marker RAPD-PCR was applied to estimate the genetic variance resulting from ultrasonic exposure time and heat stress on *Lepidium sativum*. The total polymorphism percentage was 48.61%. In conclusion, ultrasonic waves up to 20min exposure time can be used as seed pretreatment before sowing to help in adaptation to high temperatures.

Keywords: Garden cress, Heat stress, *Lepidium sativum* L., Molecular analysis, Seedling growth, Ultrasonic waves.

Introduction

Agriculture is significantly impacted by climatic conditions, hence efforts to adapt and mitigate agricultural systems should be strengthened. The most harmful increasing abiotic stressors on plant growth and productivity are temperature variations and more research is required to aid plants in adapting to these challenges.

Several plant developmental processes, including seed germination, growth, and development, are adversely affected by heat stress, which lowers plant output (Zhang et al., 2021; Patel et al., 2022). A considerable decrease in yield results from high-temperature conditions as a result of global warming. In the meantime, the world's population is projected to grow quickly, reaching 11 billion people in 2100. To feed the growing population, an increase in global food production of 70% is a difficult undertaking in order to meet the world's food demand and provide food security (Parthasarathi et al., 2022).

According to Jumrani et al. (2022), heat stress caused damage to the structure and operation of the photosynthetic machinery in soybean plants. Taghvaei et al. (2022) found that the germination rate declined to 0.35 and 0.40 seeds per day in both light and dark conditions as the temperature rose from 30 to 40°C. Plants exposed to extreme temperatures experience significant and occasionally fatal negative impacts.

[#]Corresponding author email: em_7891@yahoo.com Tel. 0201145103804
Received 02/04/2023; Accepted 06/10/2023
DOI: 10.21608/ejbo.2023.203597.2299
Edited by: Prof. Dr. Ahmed M. Saleh, Faculty of Science at Yanbu, Taibah University, KSA.

Plants have developed sophisticated heat stress response mechanisms to adapt to such environments. For instance, Liu et al. (2020) found that plants' basic physiological processes such as photosynthesis, respiration, and water metabolism respond to heat stress. Heat stress increases cellular membrane permeability and electrolyte loss, which inhibits cellular activity and lowers thermotolerance (Xalxo et al., 2020). The heat stress response is mediated by a number of epigenetic regulators, including acetyltransferases, methyltransferases, deacetylases, and demethylases (Xu et al., 2017). According to Deng et al. (2018), in order to control gene expression, these epigenetic regulators are attracted to particular histones in chromatin by heat response-associated recruiters (such as TFs, lncRNAs).

One way to increase plant growth and yield is to use physical environmental cues strategically (Trakselyte-Rupsiene et al., 2021). Ultrasound is frequently employed in physical extraction and enhances plant growth. As a result, it has a complicated impact on their biology while not harming plant molecules or cells and instead encourages their biological production (Leon et al., 2018).

After seeds received ultrasound treatment, yield, quality, and antioxidants were positively improved in response to the stress that rice experienced (Rao et al., 2018). Although ultrasonication of seeds did not affect germination rates, it dramatically enhanced the length and weight of the roots and shoots of seedlings that were 7 days old by 23%–68% and 16%–28%, respectively. Starch biosynthesis, IAA biosynthesis, photosynthesis, and the TCA cycle pathways were found to be the most significantly differentially expressed sequences among 107,891 genes (Hidvégi et al., 2022).

When rice seeds were ultrasonically treated (20-40kHz, for 30min.), several growth parameters increased. These include the dry biomass, the net photosynthetic rate in the leaves, grain yield, panicle number, grain number per panicle, and percentage of fissionable grains. his may be due to increased fluidity of cell walls caused by the mobilization of nutrients from endosperm and the larger porosity of barley seeds increased the water diffusion. Superoxide dismutase and peroxidase activity in the leaves has been increased by ultrasonication. Superoxide dismutase activity increased to 84.9%, while peroxidase activity increased by up to 121.5%, depending on the variety and developmental stage (Mo et al., 2020).

Garden cress, or *Lepidium sativum*, is a plant in the Brassicaceae family (Dixit et al., 2020). It is a herbaceous plant that blooms every year, and the seeds are a fantastic source of protein, omega-3 fatty acids, and other nutrients (Adera et al. 2022). The plant was first domesticated in South West Asia and Egypt, but it is currently grown all over the world for its seeds.

Lepidium sativum, has highly significant economic value in its roots, leaves, and seed that this plant has reportedly been used in traditional medicine for a variety of biological functions (Painuli et al., 2022). It is frequently used as an analgesic, hepatoprotective, galactagogue, anti-spasmodic, anti-diarrheal (Balgoon, 2019). Furthermore, the plant is a good source of minerals like calcium, phosphorus, potassium, and zinc as well as proteins, carbs, and dietary fiber, (Alqahtani et al., 2019). The main goal of this study is to investigate the impact of thermal stress on the development of garden cress (*Lepidium sativum*) seedlings and their physiological and genetic performance. The application of ultrasonication is to counteract the detrimental effects of high temperatures on the germination and growth of garden cress seedlings.

Materials and Methods

Experimental design

The study was established at Helwan University's Laboratory of Plant Physiology - Cairo, Egypt. Garden cress seeds were carefully chosen, sterilized for three minutes with a 2.5% sodium hypochlorite solution, and then completely rinsed with distilled water. Ultrasonication at a frequency of 40 kHz (MFUC-80A) at three different time intervals (10, 20 and 30min) and a constant temperature of 25°C. Seeds of each group were germinated at 4 different temperatures 25, 30, 35 and 40 ºC for 7 days. For each treatment, a constant number of seeds (25 seeds) of garden cress seeds were transferred into sterile Petri dish 12cm with Whatman No. 1. filter paper wetted by 10mL distilled water. Four replicates were prepared for each treatment.

Germination percentage and Growth parameters

After seven days, data on germination % and growth traits (seedling vigour index, plumule and radical length (cm), fresh weight of plumule, radicle (mg), plumule to radicle length ratio, and plumule to radical weight (mg)) were collected. When the radicle and plumule were 2mm long, the seed was considered to have germinated. (Chartzoulakis & Klapaki, 2000). In order to record the dry weights of plumule and radicle they were heated in an oven for 48h at 65°C.

Chemical analysis

Total soluble sugars and proteins contents

Anthrone reagent was used to measure the total soluble sugars. (Umbreit, 1959) and total soluble proteins (Lowry et al., 1951). A known weight of germinated seeds was extracted in 5mL of 70% ethanol and completed to a specified volume with distilled water following filtration.

Proline content

The proline concentration was calculated using 0.5g of germinated seeds that were crushed in 10mL of 3% sulfosalicylic acid, after which it was filtered using Whatman filter paper no.2. and was utilized to estimate proline based on (Bates et al., 1973).

Lipid peroxidation

Thiobarbituric acid (TBA) color reaction was used to measure the amount of lipid peroxidation in embryo axes or hypocotyl sections (Hulaev & Oliver, 2006).

Relative permeability of root membranes

The electrolytic conductivity of the fresh root segments was tested 30min after they had been bathed in 25 ml of deionized water. After the root segments were boiled in deionized water in a water bath. The estimated relative permeability was as follows (Zwiazek & Blake, 1991):

$$\text{Relative permeability} = \frac{\text{Electrolytic conductivity of solution at 30 min before heating}}{\text{Electrolytic conductivity of solution after heating}} \times 100$$

Hydrogen peroxide content

In order to measure the endogenous H_2O_2 content. In 5mL of 0.1% TCA, 0.5g of germination-proven seeds were crushed. This procedure according to method described by Loreto & Velikova (2001), 1.5mL of the test solution consisting of 0.5mL of 10mM potassium phosphate buffer (pH 7.0) and 1mL of 1M KI were combined with supernatant. At 390nm, the absorbance of the assay combination was measured.

Mineral ions

Mineral ion content in air-dry leaves for different treatments were estimated at Ecology lab, Faculty of science, Helwan University using Microwave Plasma Atomic Emission Spectroscopy (Agilent Technologies 4210 MP-AES). The operational processes and instrumental settings were modified in accordance with the manufacturer's user handbook.

DNA isolation and RAPD-PCR bioassay

According to Edwards et al. (1991), the total genomic DNA of *Lepidium sativum* L. was isolated using the CTAB method. In this bioassay, six RAPD primers were utilized, however only four of them produced repeatable clear bands "The primers were included in the Table 3. In the Biometra thermocycler, the RAPD-PCR reaction was carried out. The reaction mixture was prepared in a total volume of 25 l, consisting of 12.5 l of Taq master mix (COSMO PCR RED M. Mix, W1020300x), 2 l of genomic DNA, 1 l of each primer (Willowfort), and 9.5 l of double distilled water. The 35 cycles of the reaction programme were broken down into the following steps: 30-sec denaturation at 94oC, 30-sec annealing for each primer at various temperatures as stated in Table 3, and 1min extension at 72°C. The final phase was then extended for a single phase for 10min at 72°C before cooling at 4°C".The amplified PCR product was performed on a ladder gel (New England Biolab, #N3232S) and compared to 1.2% agarose gel.

Statistical analysis

The complete randomized blocks design (CCRBSE. Bas) approach of Snedecor & Cochran (1980) was used to analyses the variance and establish the significance of the data using LSD values at p=0.05. After this investigation, a Duncan's multiple range test was used to compare the various therapies. The community analysis program (1.2) (CAP) was employed on L. sativum in order to estimate the associations between the various variable treatments. PCA covariance was used to aggregate related plant-affecting treatments. Additionally, a dendrogram of linked treatments was generated using the Euclidean complete linkage method. Bands were used to assess the gel electrophoresis test images (1, 0). Bio-Rad Quantity One was used for these computations.

Results

Germination and growth parameters

The collected data revealed that as temperature increased, germination percentage gradually decreased. (Fig. 1A). Germination percentage decreased to 0% at 40 °C compared to 92% for 25°C. However, germination percentage increased after exposure to ultrasonic waves up to 20min generally at temperatures 25, 30, and 35°C.

All morphological characteristics of garden cress, including seedling vigour index, plumule to radicle length ratio, fresh and dried weight of plumule and radicle, and length of plumule and radicle in cm, were negatively impacted by temperatures between 30 and 35°C (Table 1, Fig. 1B) compared to seedling grown at 25 °C. Radicle length increased significantly after 20min of ultrasonic stimulation, rising to 2.1cm from 1.66 cm for the corresponding control.

At high temperatures, both fresh and dry weights of the plumule and radicle greatly reduced. However, ultrasonication for 10 and 20min significantly increased weights.

Physiological parameters

Total soluble sugars, proteins, and proline

Total soluble sugars and proteins showed a significant decrease in response to heat stress up to 35°C. In contrast, ultrasonication for 20min increased total soluble sugars and proteins compared with corresponding controls content (Fig. 1C, D). Proline is a stress marker amino acid that accumulates under different stress types in many plants. It was observed that heat exhaustion caused gradual increase in proline content in germinated seeds, with the highest values recorded at 35°C. On the other hand, ultrasonication significantly decreased proline content compared to corresponding controls at all tested temperatures, particularly at exposure times of 10 minutes. Proline content reduced from 0.71 mg/g for control plants to 0.62 mg/g at sonication for 10min, as can be seen from (Fig. 1E).

Electrolyte Leakage, lipid Peroxidation and hydrogen peroxide

Data presented in Fig. 2A show that electrolyte leakage from root segments of garden cress (*Lepidium sativum*) increased under heat stress. the highest value in relative permeability of root membranes significantly was obtained at 35°C, recording 91.20% in comparison to 21.73% for 25°C seedlings. Additionally, data showed that electrolyte leakage significantly reduced by 10 and 20min of ultrasonic treatments. Ion or electrolyte leakage is generally related to peroxidation of lipids and damage to the cell membrane; it was observed to increase in response to heat or physical stress in a similar trend to that of electrolyte leakage from root segments. The lowest lipid peroxidation levels were recorded at short durations of 10 and 20min of ultrasonic treatments when compared with the corresponding control value. In addition, the highest levels were recorded at 35°C and at sonication time for 30min, as shown in Fig. 2B. H_2O_2 level increased in heat-treated seeds compared to H_2O_2 level in seedlings grown at 25°C, this increase in H_2O_2 during heat stress was associated to oxidative damage in the seeds. Application of ultrasound waves for 10 and 20min reduced H_2O_2 content. However, high heat stress at 35°C and ultrasound duration for 30 minutes increased H_2O_2 content by 9.7% according to Fig. 2C.

Mineral ions

The results concerning the effect of ultrasonic waves in the presence and absence of heat stress on ions accumulation (P, K, Mg, Fe and Na) and K/Na ratio in garden cress seeds are shown in (Table 2). In particular, heat stress significantly decreased P, K, Fe, Mg contents and K/Na ratios and increased Na ions in leaf tissue. In contrast, the application of ultrasound promoted the accumulation of all mineral ions and Na ions.

Molecular analysis

Using six RAPD primers, 22 bands were amplified, 12 of which were monomorphic and 10 polymorphic. (Table 3, Fig. 3). This led to a total polymorphism % of 48.61% in the genetic content of L. sativum in response to the variable stress factors. The Deca-12 primer produced the highest level of polymorphism (66.67%), while the Deca-4 primer had the least variation (33.33%). The results obtained from both morphological and physiological parameters were represented by CAP. The obtained data illustrated the relative relationships of the variable treatments with similar effects. The grouping of treatments was shown in a dendrogram and PCA plot, as in Figs. 4, 5, respectively. The grouping showed three main groups, mainly separated according to the effect of temperature rather than ultrasonic.

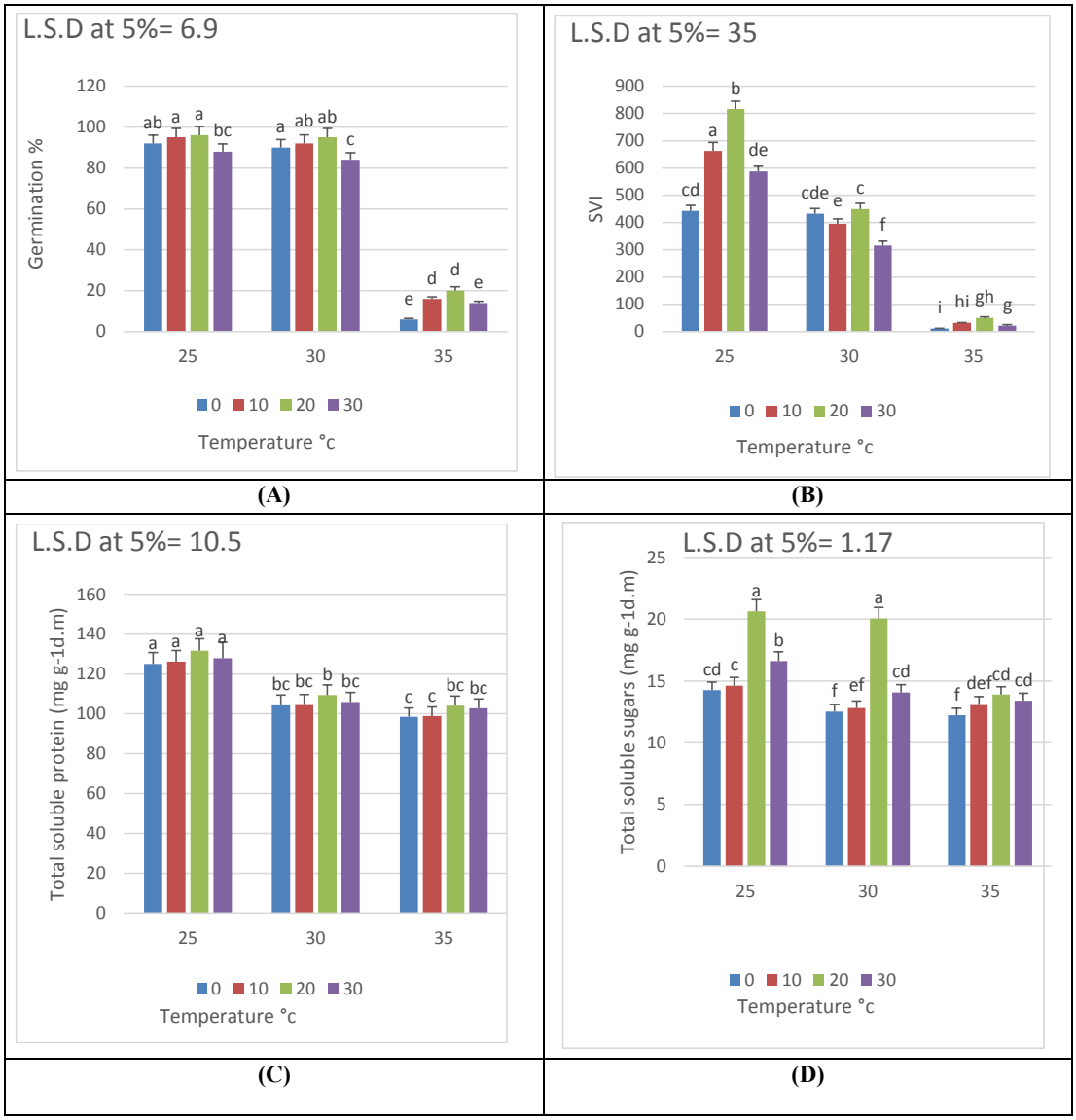

Fig. 1. Effect of ultrasonic exposure time (10, 20 and 30min) and heat stress on (A) germination percentage, (B) SVI, (C) total soluble protein content, (D) total soluble sugars content and (E) Proline content of garden cress at early seedlings stage [Values represent the mean of three replicates. Different letters (a, b, c, d, e, f, g, h and i) indicate statistical differences at 5% probability according to Duncan's test. Error bars are standard errors of the mean]

TABLE 1. Effect of ultrasonic exposure time and heat stress on growth characteristics of garden cress at early seedlings stage. Data shown in the table represent the mean ± standard error, followed by a small letter [Similar letters indicate that means were not different significantly at 5%, probability based on Duncan's test]

Treatments		Plumule length (cm)	Radicle length (cm)	R/P length ratio	Plumule fresh weight (mg)	Plumule dry weight (mg)	Radicle fresh weight (mg)	Radicle dry weight (mg)
Temperature °C	Sonication time/min							
25°C	0	3.15 ± 0.12 c	1.66 ± 0.08 b	1.89 ± 0.09 de	17 ± 0.77 c	2.3 ± 0.09 b	7.4 ± 0.34 c	0.51 ± 0.02 c
	10	5.06 ± 0.24 ab	1.91 ± 0.09 a	2.64 ± 0.12 b	20.5 ± 0.96 a	2.5 ± 0.11 a	8.15 ± 0.40 a	0.61 ± 0.03 a
	20	6.4 ± 2.13 a	2.1 ± 0.09 a	3.04 ± 0.1 c	20.9 ± 0.94 ab	2.65 ± 0.1	8.96 ± 0.37 b	0.66 ± 0.03 b
	30	5 ± 0.23 b	1.68 ± 0.08 b	2.97 ± 0.13 a	19.7 ± 0.90 b	0.93 ± 0.04	8.11 ± 0.41 b	0. 51 ± 0.02 c
30°C	0	2.8 ± 0.13 cd	2 ± 0.09 a	1.4 ± 0.07 f	12.33 ± 0.56 d	0.706 ± 0.03	3.13 ± 0.14 d	0.216 ± 0.009 d
	10	2.8 ± 0.13 c	1.5 ± 0.07 c	1.8 ± 0.09 de	12.37 ± 0.56 d	0.833 ± 0.04	2.06 ± 0.09 e	0.151 ± 0.007 e
	20	3.03 ± 0.14 c	1.7 ± 0.08 b	1.7 ± 0.08e	12.66 ± 0.58 d	1.266 ± 0.03	2.22 ± 0.10 e	0.166 ± 0.01 e
	30	2.4 ± 0.13 cd	1.36 ± 0.06 d	1.76 ± 0.1 cd	7 ± 0.32 g	1.06 ± 0.05	1.53 ± 0.07 f	0.096 ± 0.03 f
35°C	0	1.3 ± 0.06 e	0.66 ± 0.03 f	1.96 ± 0.09 cde	7.86 ± 0.36 fg	0.543 ± 0.02	1 ± 0.04 g	0.066 ± 0.02 f
	10	1.5 ± 0.07 e	0.5 ± 0.02 g	3 ± 0.13 a	8.69 ± 0.39 ef	0.522 ± 0.02	1 ± 0.03 g	0.065 ± 0.01 f
	20	1.7 ± 0.08 de	0.83 ± 0.04 e	2.04 ± 0.1 cd	9.2 ± 0.42 e	0.931 ± 0.04	1.4 ± 0.07 fg	0.077 ± 0.01 f
	30	1.1 ± 0.05 e	0.43 ± 0.09 g	2.55 ± 0.12 b	5.3 ± 0.24 h	0.811 ± 0.09	1 ± 0.02 g	0.069 ± 0.01 f
L.S.D at 5%		1.1	0.14	0.18	1.2	0.1	0.39	0.03

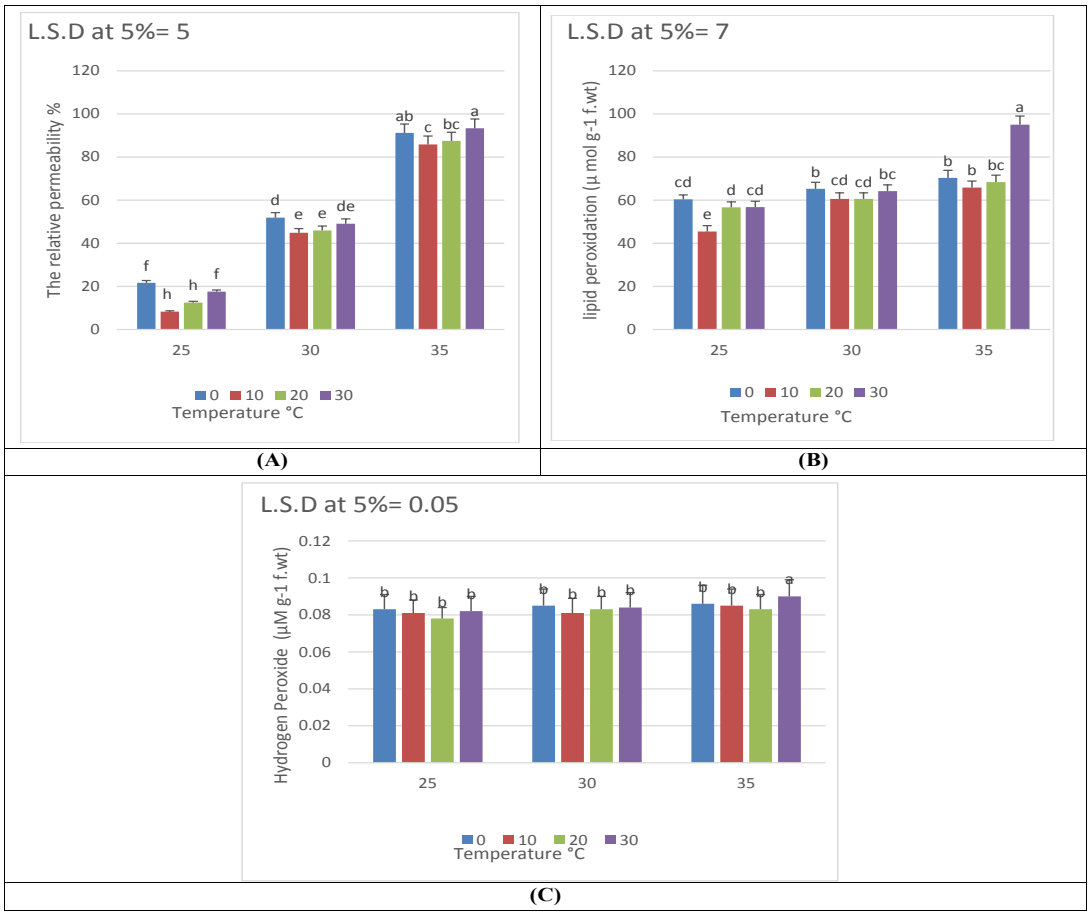

Fig. 2. Effect of ultrasonic exposure time (10, 20 and 30min) and heat stress on (A) The relative permeability, (B) Lipid peroxidation and (C) Hydrogen peroxide content of garden cress at early seedlings stage [Values represent the mean of three replicates. Different letters (a, b, c, d, e, f and g) indicate statistical differences at 5% probability according to Duncan's test. Error bars are standard errors of the mean]

Discussion

The present study data revealed that germination and early seedling growth of garden cress were negatively impacted by an increase in temperature. An imbalance in growth hormones, particularly GA3, which is vital for the germination of seeds, may be the cause of this effect, which may be physiological. It may also have an impact on the production and activity of hydrolytic enzymes, which has an impact on the availability of fundamental nutrients needed for embryonic development. Reactive oxygen species (ROS), which result in lipid peroxidation and ion leakage (Hafez & Fouad, 2020; Tammam et al., 2022; Tourky et al., 2023), are the main cause of structural impact. The negative consequences of heat stress on wheat germination (Mitra & Bhatia, 2008), maize and pearl millet (Ashraf & Hafeez, 2004), sugarcane (*Saccharum officinarum* L.), and other plants have been well reported (Srivastava et al., 2012). Because heat stress significantly impacts plant activities such as seed germination, growth, development, photosynthesis, and reproduction, it has a significant effect on plant growth and the final yield (Hasanuzzaman et al., 2012). Therefore, plants enhance a variety of morphological, physiological, and molecular responses in order to survive under stressful situations. Heat stress causes a decrease in seed germination that results in shorter roots, poor establishment, fewer plants (Toh et al., 2008; Kumar et al., 2011). Heat stress inhibits a number of enzymes involved in starch breakdown and increases abscisic acid production (Essamine et al., 2010). Heat stress also affected seed embryos' potential of producing proteins, which significantly reduced maize seed germination over 37°C (Riley, 1981). At 45°C, the expansion in maize seedlings was entirely halted (Akman, 2009). High temperatures had a negative impact on stand establishment in tomato plants (*Solanum lycopersicum* L.) by causing cell

death and a sharp fall in germination rate (Cheng et al., 2009). Reduced water and nutrient uptake caused by high temperature stress significantly slows plant growth (Huang et al., 2012). Although the impacts of heat stress on roots have received less attention than those on shoots, the majority of earlier research has been on the impact of heat stress on root development and metabolism of carbon (Huang et al., 2008).

TABLE 2. Effect of ultrasonic exposure time and heat stress on mineral ions contents (mg g^{-1}d.m) of garden cress at early seedlings stage [Data shown in the table represent the mean ± standard error, followed by a small letter; similar letters indicate that means were not different significantly at 5%, probability based on Duncan's test]

Treatments		K	Na	K/Na	Mg	P	Fe
Temperature °C	Sonication time / min						
25 °C	0	5.88 ± 0.27 cd	0.928 ± 0.04 f	6.33 ± 0.29 c	1.44 ± 0.06 e	0.59 ± 0.025 cd	0.099 ± 0.01 b
	10	6.25 ± 0.28 bc	0.924 ± 0.05 f	6.76 ± 0.31 b	1.57 ± 0.07 bc	0.64 ± 0.03 ab	0.137 ± 0.03 ab
	20	7.19 ± 0.5 a	0.914 ± 0.04 f	7.86 ± 0.4 a	1.89 ± 0.09 a	0.68 ± 0.04 a	0.154 ± 0.05 ab
	30	6.12 ± 0.27 bc	0.926 ± 0.04 f	6.60 ± 0.30 bc	1.53 ± 0.08 cd	0.61 ± 0.03 bc	0.136 ± 0.03 ab
30 °C	0	5.45 ± 0.20 de	1.99 ± 0.1 d	2.73 ± 0.12 fg	1.40 ± 0.06 de	0.44 ± 0.02 fg	0.073 ± 0.005 b
	10	6.09± 0.27 bc	1.93 ± 0.09 d	3.15 ± 0.2 e	1.46 ± 0.06 cde	0.51 ± 0.02 e	0.108 ± 0.01 b
	20	6.77 ± 0.6 a	1.28 ± 0.05 e	5.28 ± 0.4 d	1.67 ± 0.07 b	0.56 ± 0.03 d	0.115 ± 0.02 a
	30	6.03 ± 0.27 bc	1.97 ± 0.09 d	3.06 ± 0.14 ef	1.45 ± 0.05 cde	0.49 ± 0.02 e	0.094 ± 0.03 b
35 °C	0	5.28 ± 0.20 f	2.78 ± 0.2 a	1.89 ± 0.0 8 h	1.11 ± 0.02 f	0.39 ± 0.02 h	0.055 ± 0.005 b
	10	5.97 ± 0.3 bcd	2.33 ± 0.11 b	2.56 ± 0.12 g	1.19 ± 0.04 f	0.41 ± 0.02 gh	0.063 ± 0.01 b
	20	6.52 ± 0.4 b	2.14 ± 0.10 c	3.04 ± 0.14 ef	1.21 ± 0.05 f	0.48 ± 0.03 ef	0.079 ± 0.01 b
	30	5.31 ± 0.25 f	2.70 ± 0.12 a	1.96 ± 0.09 h	1.15 ± 0.05 f	0.39 ± 0.015 h	0.057 ± 0.005 b
L.S.D at 5%		0.54	0.17	0.42	0.12 f	0.05	0.02

TABLE 3. Primer Data analysis of RAPD-PCR bioassay with ultrasonic exposure time and heat stress on *Lepidium sativum*

No.	Primer code	Primer's sequence	GC%	Tm	Total number of bands	Total polymorphic bands	Polymorphism %
1	Deca 4	5'-CGTTGGCCCG-3'	80	44	6	2	33.33
2	Deca 11	5'-ATCGGCTGGG-3'	70	39.3	9	4	44.44
3	Deca-12	5′-CTTGCCCACG-3'	70	38.5	3	2	66.67
4	Deca-13	5′-GTGGCAAGCC-3'	70	39	4	2	50
Total					22	10	48.61

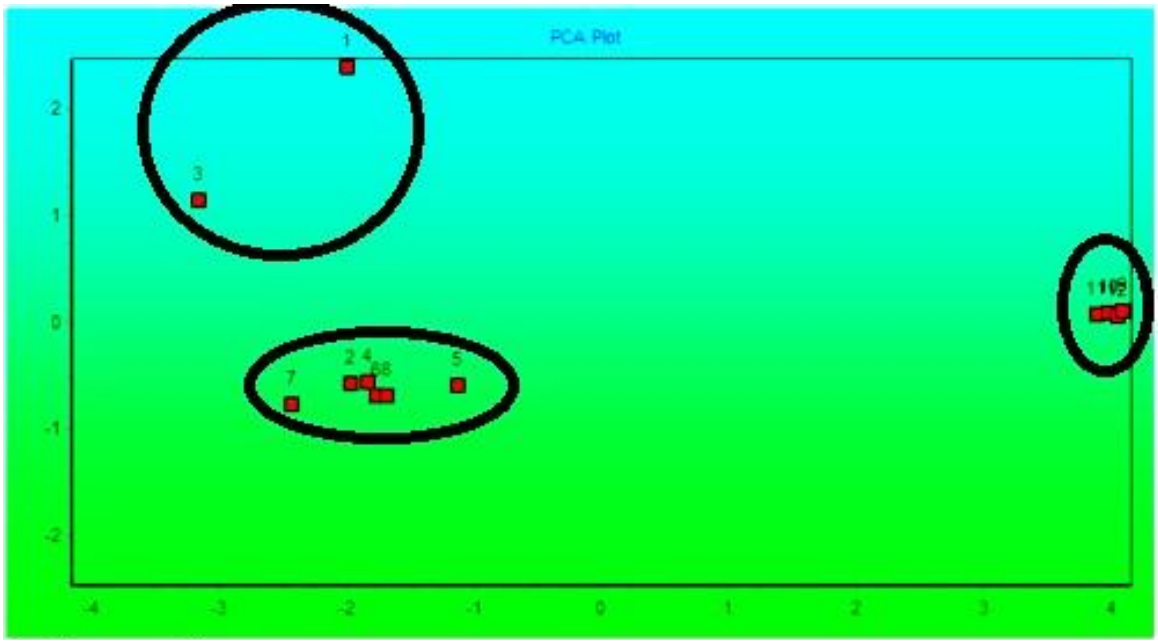

Fig. 3. PCA plot of the variable treatments of *Lepidium sativum* affected by heat and ultrasonic stress, grouping is based on morphological and physiological parameters [(1: 25°C and no sonication, 2: 25°C and 10min sonication; 3: 25°C and 20min sonication; 4: 25°C and 30min sonication; 5: 30°C and no sonication; 6: 30°C and 10min sonication; 7: 30°C and 20min sonication; 8: 25°C and 30min sonication; 9: 35°C and no sonication; 10: 35°C and 10min sonication; 11: 35°C and 20min sonication; 12: 35°C and 30min sonication)]

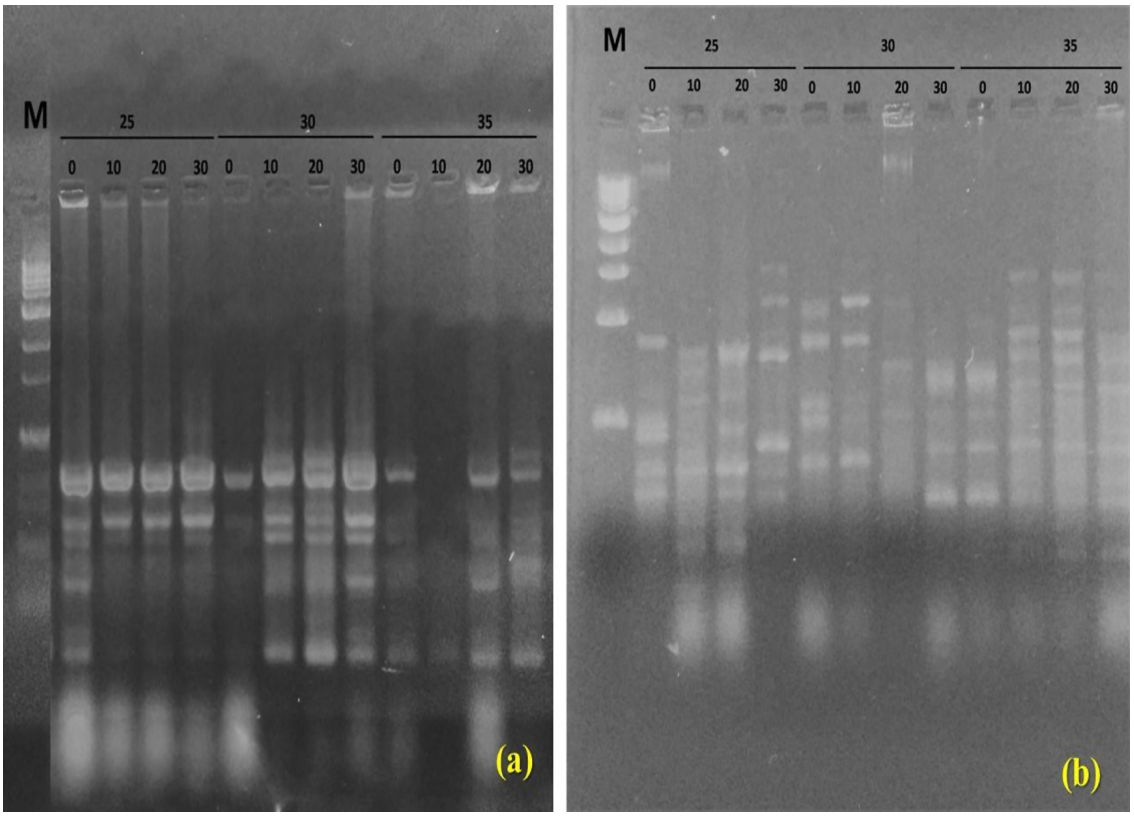

Fig. 4. The agarose gel pattern of *Lepidium sativum* exposed to ultrasonic exposure time and heat stress [a: with Deca-4 primer, b: with Deca-11 primer]

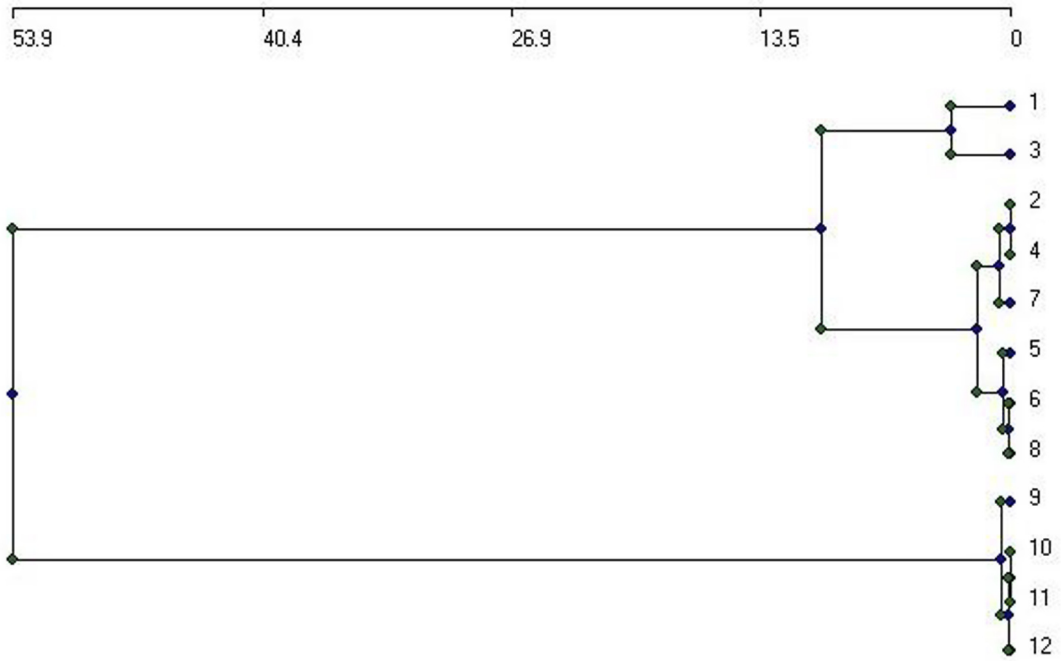

Fig. 5. Dendrogram of the variable treatments of *Lepidium sativum* affected by heat and ultrasonic stress, based on morphological and physiological parameters [(1: 25°C and no sonication, 2: 25°C and 10min sonication; 3: 25°C and 20min sonication; 4: 25°C and 30min sonication; 5: 30°C and no sonication; 6: 30°C and 10min sonication; 7: 30°C and 20min sonication; 8: 25°C and 30min sonication; 9: 35°C and no sonication; 10: 35°C and 10min sonication; 11: 35°C and 20min sonication; 12: 35°C and 30min sonication)]

The frequency of the ultrasonic wave, the length of exposure, and the type of plant all affect how well seeds germinate after receiving ultrasonic treatment. Garden cress seeds were briefly ultrasonically treated to increase germination rates and early seedling growth (Aladjadjiyan, 2012). In low temperatures, ultrasonic technology improved sesame seed germination and seedling growth with a brief exposure time (Shekari et al., 2015). One probable explanation is that sonication enlarges the cracks in the protective layer around the seed and pericarp, which causes the seedlings to retain significantly more moisture after being steeped in water. By creating microscopic cracks on the seed coat, which also makes the cotyledon cell walls less stiff, ultrasonic waves may speed up the germination of common bean seeds. This will cause the seeds to absorb more water more quickly, which will lead to cell enlargement, accelerated starch hydrolysis, faster germination as well as other physiological changes (Karagöz & Dursun, 2021; Lahijanian & Nazari, 2017). Many researchers point out that exposing ultrasonically treated seeds to ultrasonic hydration leads to an increase in enzymatic activity, especially alpha amylase (Miano et al., 2016). Ultrasonic waves have a big impact on plants by causing mechanical effects (acoustic cavitations) and disrupting their cell walls, which increases water uptake. This was also reported by Goussous et al. (2010) on wheat, Yaldagard et al. (2008) on barley, and Aladjadjiyan (2002) on carrot. According to Zheng et al. (2008), ultrasound treatment can affect the development of some plant organs, with a low dose of ultrasound stimulating division of cells, a medium dose of ultrasound inhibiting division of cells, and a large dose of ultrasound causing cell demise when ultrasonically processing seeds. It was also shown that mild ultrasonic processing, which stimulates the plant root cell to divide rapidly, improves plant development, and encourages the plant to strike roots, increases the germination rate of spinach and cabbage seeds. According to Aladjadjiyan (2012), the length of a lentil seedling increased with the duration of its exposure to ultrasonic waves in an approximately linear fashion. In the case of fenugreek, a similar finding was made previously (Kapoor & Pande, 2015; Ratnakar & Rai, 2013) in spinach (Keshavarzi et al., 2011), in oat (Chauhan et al., 2016), and in common lambsquarters (Babaei-Ghaghelestany et al., 2020). Stress induced by high temperatures decreases water content, membrane

permeability, and nutrient uptake, which adversely affects germination and considerably delays plant growth. However, increased acoustic cavitation induced by ultrasound and increased seed coat porosity improved water absorption and oxygen availability.

The creation of micro-pores and micro-cracks caused by sonication means that the seeds are more permeable to water and oxygen entry (Miano et al., 2016).

Under heat stress conditions, soluble sugar and protein content in seedlings showed a significant decline when compared to control seedlings. Specific cells and tissues experience programmed cell death at severe heat stress as a result of protein denaturation. A slow death is seen at moderate heat stress for an extended period of time (Hasanuzzaman et al., 2010). Heat stress lowers nutrient absorption per unit root surface because it depletes labile carbon (non-structural carbohydrates). Additionally, heat stress reduces the quantity of protein used for food intake by limiting the movement of sugars from shoots to roots and harming plant roots (Huang et al., 2008). As a result of the proteins' decreased activity, nutritional absorption is lowered. Similar results were shown earlier (Bochu et al., 2003) on rice, where physical stress caused by ultrasonic wave resulted in a substantial increase in total soluble sugars and proteins. According to Yi (2003), Chrysanthemum's levels of soluble carbohydrates, protein, and amylase activity rose when exposed to ultrasound waves. This shows that sound activation may enhance Chrysanthemum growth and root metabolism. Plants regularly use osmoregulation, cell turgor maintenance, mitigating ROS-induced damage using defense mechanisms, as well as the impact of ultrasonic therapies on soluble protein accumulation (Anjum et al., 2011). This positive response to ultrasonication could be explained by an increase in hydrolytic enzymes amylase and protease activity under stress conditions. The increased organic solutes present in cells especially those resulting from hydrolysis of storage food in seeds, such as soluble sugars and soluble proteins, lower the cellular water potential as a mechanism of osmoregulation which helps in more water uptake without any harmful effect on seed metabolism.

As a response to thermal stress, proline also accumulates in many plants. Proline, an amino acid also known as a stress marker, increases in plant tissue under stress to improve plant tolerance as plants acquire various small molecules in the presence of heat to help them survive. These molecules of low molecular weight are named osmolytes (Sakamoto & Murata, 2002). Plants acquire a range of osmolytes, including sugars, proline, ammonium, and sulphur compounds, when the temperature is high (Sairam & Tyagi, 2004), whereas ultrasonication for brief, moderate durations of time reduced the proline concentration. In reaction to stress, proline carries out a variety of functions including osmotic correction (Voetberg & Sharp, 1991), protection of macromolecules, carbon and nitrogen regulation of cytosolic acidity (Van Rensburg et al., 1993), mitigating lipid membrane oxidation (Okuma et al., 2004), and acting as an osmo-protectant (Kishor et al., 2005; Hoque et al., 2008). Proline boosts the activity of antioxidant system enzymes, enhancing Nicotiana tabacum's resistance to salt stress (Hoque et al., 2008), whereas proline content was decreased through brief, moderate periods of ultrasonication. Biopolymers are hydrated by proline buildup, which enables them to persist as a viable energy source during times of growth inhibition (Kala & Godara, 2011). On numerous plant species, similar outcomes have been attained, (Alqurainy, 2007): on bean and pea, (Chen et al., 2008), and on common bean (Ahmad et al., 2009).

Asada (2006) showed that heat also contributes to the accumulation of dangerous reactive oxygen species, which are the main contributors to the development of oxidative stress in plants. The majority of ROS are formed in photosystem I and photosystem II reaction centers. Yet, the peroxisomes and mitochondria of plants are also involved in the formation of ROS. (Soliman et al., 2011). Even relatively brief heat stress can produce ROS, the two main components of which are believed to be hydrogen peroxide and superoxide (Apel & Hirt, 2004). On the other hand, applying ultrasonic vibration for 10 and 20min reduced the amount of H_2O_2, although a long ultrasonication period (30min) combined with a temperature of 35°C increased the amount of H_2O_2. These findings concur with those of (Shekari, 2015; Pan et al., 2020). Ma et al. (2022) reported during the subsequent storage period, sweet potato slices exposed to ultrasonic treatment for 10min dramatically reduced H_2O_2 concentration, SOD and CAT levels rose in ultrasonic-treated slices as a result, inhibiting the buildup of H_2O_2. While

macromolecule aggregation was prevented, molecular weight was decreased, solubility was enhanced, and ultrasonic hastened the breakdown of H_2O_2, the primary structure did not alter noticeably. This was also reported by Alfalahi et al. (2022) on soybean, who examined antioxidant gene expression in the seedling tissues and indicated a significant stimulatory effect of ultrasonication on catalase and superoxide dismutase antioxidant gene expression.

The relevant data showed that physical stress and heat could have an impact on lipid peroxidation and electrolyte leakage. Furthermore, under heat stress, the amount of Thiobarbituric acid reactive substances (TBARS) increased, which was connected to secondary oxidative damage (Stepien & Klobus, 2005). Membrane permeability is significantly impacted by heat stress. The capacity of the cell membrane to function under heat stress is necessary for the activities of respiration and photosynthesis (Blum, 1988). This can be taken into consideration that even the treatment must be used in accurate doses so as not to be included as a new stress on plant. For that, ultrasonic waves at low doses only activated the antioxidant system in the plant, which decreased or controlled ROS production, protecting cell phospholipid membranes from peroxidation, which is indicated by the decrease in ion leakage also.

Under heat stress, the amount of TBARS increased, which was connected to secondary oxidative damage (Stepien & Klobus, 2005). Membrane permeability is significantly impacted by heat stress. The capacity of the cell membrane to function under heat stress is necessary for the activities of respiration and photosynthesis (Blum, 1988). Molecules under heat stress move more quickly and with more kinetic energy against plant membranes, breaking chemical bonds in the process. Membranes become more porous and fluid as a result of heat stress because it dehydrates proteins and raises the content of unsaturated fatty acids (Savchenko et al., 2002). The structure (tertiary and quaternary) of membrane proteins is altered by heat stress, which also makes membranes more permeable and causes electrolyte leakage, negatively affecting membrane biological functions and integrity. In a variety of agricultural plants, including cowpea, cotton, sorghum, and barley, reduced membrane thermostability due to an increase in electrolyte leakage has been observed (Wahid & Shabbir, 2005; Wahid, 2007). The amount of electrolyte that leaks out of plants depends on their tissues, organs and developmental stages. For illustration, older leaves in the maize experienced more severe heat-related plasma-lemma damage than younger leaves (Karim et al., 1999). The amount of saturated fat in sugarcane increased at higher temperatures because increased leaf temperature reduced the heat tolerance of the plants (Wahid, 2007). Heat stress causes malondialdehyde levels in plant leaves to drastically increase (Hurkman et al., 2009; Mohammed & Tarpley, 2010). Early growth stages of the wheat plantlets that had been exposed to heat stress had much higher MDA, and later growth stages had even more MDA. Heat stress also reduces the activities of enzyme antioxidants (Miller et al., 2009). According to Chen et al. (2013), ultrasonic therapy can help plants get rid of ROS by raising the activity of antioxidant enzymes. When seeds are subjected to ultrasonic waves, the plants' physiological processes change (Tyagi et al., 2014; Chemat et al., 2017). Ultrasonic vibration has an impact on microstructures, cellular enzyme activity, and plant metabolism (Yusaf, 2015). However, compared to untreated seeds, seeds exposed to ultrasonic waves are able to absorb more energy from their surroundings as they grow and develop. This is because ultrasound increases the activity of the enzymes superoxide dismutase and peroxidase and reduces malondialdehyde levels (Liu et al., 2018). Taken into consideration the results to present. Depending on the species and tissues examined, heat stress has been shown to affect the nutritional profiles of plants in a variety of ways. (Sehgal et al., 2018; Soares et al., 2019). Huang et al. (2012) found that high- temperature stress limits nutrient and water absorption, which greatly slows plant growth. Heat stress also alters the way sources and sinks interact with plants, modulating plant nutrient concentrations and reducing the amount of hormones released from roots (Huang et al., 2008). By lowering root metabolic rate, protein levels or activities involved in nutrient uptake, and root mass, abrupt, high heat events can injure roots and decrease nutrition uptake, claims Giri (2013). Roots are typically more heat-sensitive than shoots, hence heat stress often has an impact on them. As a result of global warming and rising heat waves, an 11-day heat treatment during anthesis resulted in a substantial shift in the amounts of 15 different elements in quinoa seeds compared to control seeds (Tovar et al., 2022). Because low-energy ultrasound may easily permeate through

cell membranes and walls, it can alter cellular metabolisms or encourage nutrition intake. Pb content was lower in the ultrasonic-treated rice plants compared to the control, according to Rao et al. (2018). This finding raises the possibility that in Pb-polluted soils, treating rice seeds with ultrasonic waves could increase rice growth and decrease brown rice Pb accumulation.

Only a few markers and accessions were employed in previous *L. sativum* research studies, so they may not have accurately represented the genetic diversity and variability present in the genetic materials (Tawfik, 2021). Any crop plant's genetic resources can be evaluated for variability, which provides a good basis for a deliberate breeding program focused at genetic advancement. Many molecular markers have been used to conduct comprehensive research on the genetic diversity of crop plants. There have only been a few marker-based genetic studies on *L. sativum* previously published. Using 10 RAPD primers on 15 different *L. sativum* genotypes, Sharma et al. (2015) conducted a polymorphism and diversity analysis and found 86.27% polymorphism. Bansal et al. (2012) used 32 RAPD primers to detect 82.59% of polymorphism across 18 genotypes. ISSRs were used by Kaur et al. (2015) to assess the genetic diversity of 15 genotypes of *L. sativum*, and Kumar et al. (2012) combined RAPD and ISSR markers to do the same (Nadeem et al., 2018; Kumar & Yadav, 2019).

Conclusions

Short-term exposure to ultrasonic waves can improve different heat tolerance mechanisms in plant starting from germination stage. It is recommended to use inexpensive and eco-friendly methods like ultrasonic waves for short periods as a pretreatment of seeds to improve germination percent and plant growth. The exposure of plants to these stress factors (ultrasonic and heat) affects the genetic content of this plant. The morphological and physiological behavior of the plant was affected by this genetic difference. This might be due to mutation in some essential genes and so, the function of the expressed proteins will be altered. The results of this study would offer valuable insights for managing crop productivity and producing heat resistant crops.

Competing interests The authors report no conflicts of interest regarding this work.

Authors' contributions: All the authors participated in practical work, writing and revising manuscript. Dr Amira + Dr Eman physiology part practical + Writing + revision. Dr Eman Tawfik Genetics practical + Writing + revision.

Ethics approval: Not applicable

References

Adera, F., Yusuf, Z., Desta, M. (2022) Physicochemical properties and biological activities of garden cress (*Lepidium sativum* L.) seed and leaf oil extracts. *Canadian Journal of Infectious Diseases and Medical Microbiology*, **2022**, Article ID 2947836. doi: 10.1155/2022/2947836.

Ahmad, P., Jaleel, C.A., Azooz,, M.M., Nabi, G. (2009) Generation of ROS and non-enzymatic antioxidants during abiotic stress in plants. *Botany Reseach International,* **2**(1), 11-20.

Akman, Z. (2009) Comparison of high temperature tolerance in maize, rice and sorghum seeds, by plant growth regulators. *Journal of Animal and Veterinary Advances,* 8, 358-361

Aladjadjiyan, A. (2002) Study of the influence of magnetic field on some biological characteristics of *Zea mays* L. *Journal of Central European Agriculture,* **3**, 89-94

Aladjadjiyan, A. (2012) Physical factors for plant growth food quality. In: "*Food Production, Approaches, Challenges and Tasks*". Intech. DOI:10.5772/32039.

Alfalahi, A.O., Alobaidy, B.S., Almarie, A.A., Dhanoon, O.M., Qasem, J.R., Almehemdi, A.F., Najda, A. (2022) Ultrasonic treatment enhances germination and affects antioxidant gene expression in soybean (*Glycine max* L. Merr). *Agronomy*, **12**(10), 2446. https://doi.org/10.3390/agronomy12102446

Alqurainy, F. (2007) Responses of bean and pea to vitamin C under salinity stress. *Research Journal of Agriculture and Biological Sciences*, **3**(6), 714-722

Anjum, S.A., Wang, L.C., Farooq, M., Hussain, M., Xue, L., Zou, C.M. (2011) Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. *Journal of Agronomy and Crop Science,* **197**(3), 177-185.

Apel, K., Hirt, H. (2004) Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology,* **55**, 373-399

Alqahtani, F.Y., Aleanizy, F.S., Mahmoud, A.Z., Farshori, N.N., Alfaraj, R., Al-sheddi, E.S., Alsarra, I.A. (2019) Chemical composition and antimicrobial, antioxidant, and anti-inflammatory activities of *Lepidium sativum* seed oil. *Saudi Journal of Biological Sciences,* **26**, 1089–92

Asada, K. (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology*, **141**(2), 391-396

Ashraf, M., Hafeez, M. (2004) Thermo-tolerance of pearl millet and maize at early growth stages: growth and nutrient relations. *Biologia Plantarum,* **48**(1), 81-86.

Babaei-Ghaghelestany, A., Alebrahim, M.T., MacGregor, D.R., Khatami, S.A., Farzaneh, R.H.N. (2020) Evaluation of ultrasound technology to break seed dormancy of common lambsquarters (*Chenopodium album*). *Food Science & Nutrition,* **8**, 2662-2669 DOI: 10.1002/fsn3.1547

Balgoon, M.J. (2019) Assessment of the protective effect of lepidium sativum against aluminium induced liver and kidney effects in albino rats. *BioMed Research International,* **2019**, 4516730. doi: 10.1155/2019/4516730

Bansal, D., Bhasin, P., Yadav, O., Punia, A. (2012) Assessment of genetic diversity in *Lepidium sativum* (Chandrasur) a medicinal herb used in folklore remedies in India using RAPD. *Journal of Genetic Engineering and Biotechnology*, **10**, 39-45

Bates, L.S., Waldren, R.P., Teare, I.D. (1973) Rapid determination of free proline for water-stress studies. *Plant Soil,* **39**(1), 205-207

Blum, A. (1988) "*Plant Breeding for Stress Environments*". Boca Raton (FL): CRC Press Inc. https://doi.org/10.1201/9781351075718

Bochu, W., Xin, C., Zhen, W., Qizhong, F., Hao, Z., Liang, R. (2003) Biological effect of sound field stimulation on paddy rice seeds. *Colloid Surfaces B,* **32**, 29-34

Chartzoulakis, K., Klapaki, G. (2000) Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. *Scientia Horticulturae (Amsterdam),* **86**(3), 247-260

Chauhan, A., Rajput, N., Kumar, D., Kumar, A., Chaudhry, A.K. (2016) Effect of different salt concentration on seed germination and seedling growth of different varieties of oat (*Avena sativa* L.). *International Journal of Information Research and Review*, **3**(7), 2627-2632

Chemat, F., Rombaut, N., Sicaire, A., Meullemiestre, A., Fabiano-Tixier, A., Abert-Vian, M. (2017) Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics Sonochemistry*, **34**, 540-560.

Chen, J.B., Jing, R.L., Mao, X.G., Chang, X.P., Wang, S.M. (2008) A response of PvP5CS2 gene to abiotic stresses in common bean. *Acta Agronomica Sinica,* **34**, 1121-1127

Chen, Y.P., Liu, Q., Yue, X.Z., Meng, Z.W., Liang, J. (2013) Ultrasonic vibration seeds showed improved resistance to cadmium and lead in wheat seedling. *Environmental Science and Pollution Research,* **20**(7), 4807-4816.

Cheng, L., Zou, Y., Ding, S., Zhang, J., Yu, X., Cao, J., Lu, G. (2009) Polyamine accumulation in transgenic tomato enhances the tolerance to high temperature stress. *Journal of Integrative Plant Biology,* **51**(5), 489-499

Deng, X., Qiu, Q., He, K., Cao, X. (2018) The seekers: How epigenetic modifying enzymes find their hidden genomic targets in Arabidopsis. *Current Opinion in Plant Biology,* **45**, 75–81.

Dixit, Jr. Iii V., Kumar, I., Palandurkar, K., Giri, R., Giri, K. (2020) *Lepidium sativum*: Bone healer in traditional medicine, an experimental validation study in rats. *Journal of Family Medicine and Primary Care*, **9**(2), 812-818.

Edwards, K., Johnstone, C., Thompson, C. (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Research,* **19**, 1349. doi: 10.1093/nar/19.6.1349

Essamine, J., Ammar, S., Bouzid, S. (2010) Impact of heat stress on germination and growth in higher plants: physiological, biochemical and molecular

repercussion and mechanisms of defense. *Journal of Biological Sciences,* **10**, 565-572

Giri, A. (2013) Effect of acute heat stress on nutrient uptake by plant roots. *Master's Thesis*. University of Toledo; Toledo, OH, USA

Goussous, S.J., Samarah, N.H., Alqudah, A.M., Othman, M.O. (2010) Enhancing seed germination of four crop species using an ultrasonic technique. *Experimental Agriculture,* **46**, 231-242.

Hafez, R., Fouad, A. (2020) Mitigation of genotoxic and cytotoxic effects of silver nanoparticles on onion root tips using some antioxidant scavengers. *Egyptian Journal of Botany,* **60**(1), 133-145.

Hasanuzzaman, M., Hossain, M.A., Fujita, M. (2010) Physiological and biochemical mechanisms of nitric oxide induced abiotic stress tolerance in plants. *American Journal of Plant Physiology*, **5**, 295-324.

Hasanuzzaman, M., Nahar, K., Alam, M.M., Fujita, M. (2012) Exogenous nitric oxide alleviates high temperature induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by modulating the antioxidant defense and glyoxalase system. *Australian Journal of Crop Science*, **6**, 1314-1323

Hidvégi, N., Gulyás, A., Dobránszki, J. (2022) Ultrasound as a hypomethylating agent, remodels DNA methylation and alters mRNA transcription in winter wheat (*Triticum aestivum* L.) seedlings. *Physiologia Plantarum*, **174**(5), e13777. https://doi.org/10.1111/ppl.13777

Hoque, M.A., Banu, M.N.A., Nakamura, Y., Shimoishi, Y., Murata, Y. (2008) Proline and glycinebetaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells. *Journal of Plant Physiology,* **165**(8), 813-824

Huang, B., Rachmilevitch, S., Xu, J. (2012) Root carbon and protein metabolism associated with heat tolerance. *Journal of Experimental Botany*, **63**(9), 3455-3465.

Huang, D., Wu, W., Abrams, S.R., Cutler, A.J. (2008) The relationship of drought-related gene expression in Arabidopsis thaliana to hormonal and environmental factors. *Journal of Experimental Botany*, **59**(11), 2991-3007

Hulaev, V., Oliver, D.J. (2006) Metabolic and proteomic markers for oxidative stress. New tools for reactive oxygen species research. *Plant Physiology,* **141**, 367–372.

Hurkman, W.J., Vensel, W.H., Tanaka, C.K., Whitehand, L., Altenbach, S.B. (2009) Effect of high temperature on albumin and globulin accumulation in the endosperm proteome of the developing wheat grain. *Journal of Cereal Science*, **49**(1), 12-23.

Jumrani, K., Bhatia, V.S., Kataria, S., Alamri, S.A., Siddiqui, M.H., Rastogi, A. (2022) Inoculation with arbuscular mycorrhizal fungi alleviates the adverse effects of high temperature in soybean. *Plants (Basel),* **11**(17), 2210. doi: 10.3390/plants11172210

Kala, S., Godara, A.K. (2011) Effect of moisture stress on leaf total proteins, proline and free amino acid content in commercial cultivars of *Ziziphus mauritiana*. *Journal of Scientific Research,* **55**, 65-69

Kapoor, N., Pande, V. (2015) Effect of salt stress on growth parameters, moisture content, relative water content and photosynthetic pigments of fenugreek variety RMt-1. *Journal of Plant Sciences*, **10**(6), 210-221.

Karagöz, F.B., Dursun, A. (2021) Ultra-sonic sound applications used in seed viability, seedling growth and plant development of ornamentals. *Journal of the Institute of Science and Technology,* **11**, 3416-3428.

Karim, M.A., Fracheboud, Y., Stamp, P. (1999) Photosynthetic activity of developing leaves of Zea mays is less affected by heat stress than that of developed leaves. *Physiologia Plantarum,* **105**(4), 685-693.

Kaur, A., Kumar, R., Rani, S., Grewal, A. (2015) Genetic diversity analysis of *Lepidium sativum* (Chandrasur) using inter simple sequence repeat (ISSR) markers. *Journal of Forestry Research,* **26**, 107-114.

Keshavarzi, M.H.B., Rafsanjani, M.S.O., Moussavinik, S.M., Abdin, M.Z. (2011) Effect of salt (NaCl) stress on germination and early seedling growth of Spinach (*Spinacia oleracea* L.). *Annals of Biological Research,* **2**(4), 490-497

Kishor, P.B.K., Sangam, S., Amrutha, R.N., Laxmi, P.S., Naidu, K.R. (2005) Regulation of proline

biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. *Current Science*, **88**(3), 424-438.

Kumar, S., Kaur, R., Kaur, N., Bhandhari, K., Kaushal, N., Gupta, K., Bains, T.S., Nayyar, H. (2011) Heat-stress induced inhibition in growth and chlorosis in mungbean (*Phaseolus aureus Roxb*.) is partly mitigated by ascorbic acid application and is related to reduction in oxidative stress. *Acta Physiologiae Plantarum,* **33**(6), 2091-2101

Kumar, S., Goyal, R., Sheorayan, A., Kajla, S., Yadav, O., Mangal, M. (2012) Assessment of genetic diversity in *Lepidium sativum* using RAPD and ISSR markers. *Annals of Applied Biology,* **28**, 93-97

Kumar, V., Yadav, H.K. (2019) Assessment of genetic diversity in Lepidium sativum L. using inter simple sequence repeat (ISSR) marker. *Physiology and Molecular Biology of Plants,* **25**(2), 399-406.

Lahijanian, S., Nazari, M. (2017) Increasing germination speed of common bean (*Phaseolus vulgaris*) seeds by ultrasound treatments. *Seed Technology*, **38**(1), 49-55

Leon, A.D., Perera, R., Nittayacharn, P., Cooley, M., Exner, A.A. (2018) Ultrasound contrast agents and delivery systems in cancer detection and therapy. *Advances in Cancer Research*, **139**, 57-84.

Liu, J., Zhang, R., Xu, X., Fowler, J.C., Miller, T.E.X., Dong, T. (2020) Effect of summer warming on growth, photosynthesis and water status in female and male *Populus cathayana*: Implications for sex-specific drought and heat tolerances. *Tree Physiology*, **40**, 1178–1191.

Liu, X., Chen, Z., Liu, Q., Gao, Y.N., Zhou, W.N., Cui, X.W., Wang, Q.Z. (2018) Effects of ultrasound on the germination and seedling growth of three aged forage seeds. *The Journal of Applied Ecology,* **29**(6), 1857-1866.

Loreto, F., Velikova, V. (2001) Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiology,* **127**, 1781–1787.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry,* **193**(1), 265-75.

Ma, X., Dong, L., He, Y., Chen, S. (2022) Effects of ultrasound-assisted H_2O_2 on the solubilization and antioxidant activity of yeast β-glucan. *Ultrasonics Sonochemistry*, **90**, 106210. https://doi.org/10.1016/j.ultsonch.2022.106210

Miano, A.C., Pereira, J., Castanha, N., Matta Jr., M.D., Augusto, P.E.D. (2016) Enhancing mung bean hydration using the ultrasound technology: description of mechanisms and impact on its germination and main components. *Scientific Reports,* **6**(1), 38996. Doi:10.1038/srep38996

Miller, G., Schlauch, K., Tam, R., Cortes, D., Torres, M.A., Shulaev, V., Dangl, J.L., Mittler, R. (2009) The plant NADPH oxidase RbohD mediates rapid, systemic signaling in response to diverse stimuli. *Science Signaling,* **2**, 36-45.

Mitra, R., Bhatia, C.R. (2008) Bioenergetic cost of heat tolerance in wheat crop. *Current Science,* **94**, 1049-1053

Mo, Z., Liu, Q., Xie, W., Ashraf, U., Abrar, M., Pan, S., Duan, M., Tian, H., Wang, S., Tang, X. (2020) Ultrasonic seed treatment and Cu application modulate photosynthesis, grain quality, and Cu concentrations in aromatic rice. *Photosynthetica*, **58**(3), 682– 691.

Mohammed, A.R., Tarpley, L. (2010) Effects of high night temperature and spikelet position on yield-related parameters of rice (*Oryza sativa* L.) plants. *European Journal of Agronomy*, **33**(2), 117-123

Nadeem, M., Nawaz, M.A., Shahid, M.Q., Dog˘an, Y., Comertpay, G., Yıldız, M., O¨ zkan, H. (2018) DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment,* **32**, 261-285.

Okuma, E., Murakami, Y., Shimoishi, Y., Tada, M., Murata, Y. (2004) Effects of exogenous application of proline and betaine on the growth of tobacco cultured cells under saline conditions. *Soil Science and Plant Nutrition,* **50**(8), 1301-1305

Painuli, S., Quispe, C., Herrera-Bravo, J., Semwal, P., Martorell. M., Almarhoon, Z.M., et al. (2022) Nutraceutical profiling, bioactive composition,

and biological applications of *Lepidium sativum* L. *Oxidative Medicine and Cellular Longevity,* **19**, 2910411. Doi: 10.1155/2022/2910411

Pan, Y., Chen, L., Pang, L., Chen, X., Jiaa, X., Li, X. (2020) Ultrasound treatment inhibits browning and improves antioxidant capacity of fresh-cut sweet potato during cold storage. *The Royal Society of Chemistry,* 9193-9202. DOI: 10.1039/c9ra06418drsc.li/rsc-advances

Parthasarathi, T., Firdous, S., David, Mariya E., Lesharadevi, K., Djanaguiraman, M. (2022) "*Effects of High Temperature on Crops*". Intec Open. Doi: 10.5772/intechopen.105945

Patel, K., Bidalia, A., Tripathi, I., Gupta, Y., Priyanka, A.P., Rao, K.S. (2022) Effect of heat stress on wild type and A7a knockout mutant Arabidopsis thaliana plants. *Vegetos*, **35**, 168-178.

Rao, G., Ashraf, U., Huang, S., Cheng, S., Abrar, M., Mo, Z., Pan, S., Tang, X. (2018) Ultrasonic seed treatment improved physiological and yield traits of rice under lead toxicity. *Environmental Science and Pollution Research,* **25**, 33637-33644

Ratnakar, A., Rai, A. (2013) Effect of sodium chloride salinity on seed germination and early seedling growth of *Trigonella foenum-graecum* L. Var. Peb. *Octa Journal of Environmental Research*, **1**(4), 304-309

Riley, G. (1981) Effects of high temperature on protein synthesis during germination of maize (*Zea mays* L.). *Planta*, **151**, 75-80

Sairam, R.K., Tyagi, A. (2004) Physiology and molecular biology of salinity stress tolerance in plants. *Current Science,* **86**(3), 407-421.

Sakamoto, A., Murata, N. (2002) The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. *Plant, Cell & Environment,* **25**(2), 163-171.

Savchenko, G.E., Klyuchareva, E.A., Abramchik, L.M., Serdyuchenko, E.V. (2002) Effect of periodic heat shock on the membrane system of etioplasts. *Russian Journal of Plant Physiology*, **49**(3), 349-35.

Sehgal, A., Sita, K., Siddique, K.H.M., Kumar, R., Bhogireddy. S., Varshney, R.K., et al. (2018) Drought or/and heat-stress effects on seed filling in food crops: Impacts on functional biochemistry, seed yields, and nutritional quality. *Frontiers in Plant Science*, **9**,1705. 10.3389/fpls.2018.01705

Sharma, J., Joshi, A., Dodiya, N., Jain, P., Rajoriya, S., Rajamani, G., Jain, D. (2015) Assessment of genetic diversity in *Lepidium sativum* L. (Chandrasur) genotypes using morphological and molecular markers. *Journal of Cell & Tissue Research,* **15**, 4783-4790.

Shekari, F., Mustafavi, S., Abbasi, A. (2015) Sonication of seeds increase germination performance of sesame under low temperature stress. *Acta Agriculturae Slovenica*, **105**(2), 203-212.

Snedecor, G.W., Cochran, W.G. (1980) "*Statistical Methods*", 7th ed., Ames, IA: The Iowa State University Press

Soares, J.C., Santos, C.S., Carvalho, S.M.P., Pintado, M.M., Vasconcelos, M.W. (2019) Preserving the nutritional quality of crop plants under a changing climate: Importance and strategies. *Plant and Soil,* **443**(1-2), 1-26.

Soliman, W.S., Fujimori, M., Tase, K., Sugiyama, S.I. (2011) Oxidative stress and physiological damage under prolonged heat stress in C3 grass *Lolium perenne*. *Grassland Science*, **57**(2), 101-106

Srivastava, S., Pathak, A.D., Gupta, P.S., Shrivastava, A.K., Srivastava, A.K. (2012) Hydrogen peroxide-scavenging enzymes impart tolerance to high temperature induced oxidative stress in sugarcane. *Journal of Environmental Biology,* **33**(3), 657-661

Stepien, P., Klobus, G. (2005) Antioxidant defense in the leaves of C3 and C4 plants under salinity stress. *Physiologia Plantarum,* **125**(1), 31-40

Taghvaei, M., Nasrolahizadehi, A., Mastinu, A. (2022) Effect of light, temperature, salinity, and halopriming on seed germination and seedling growth of *Hibiscus sabdariffa* under salinity stress. *Agronomy,* **12**, 2491. https://doi.org/10.3390/agronomy12102491

Tammam, A., El-Aggan, W., Badry, H., Abou-Shanab, R., El-Sawy, S. (2022) Influence of local isolates of arbuscular mycorrhizal fungi on growth and physiological performance in *Zea mays* grown in phosphorus-deficient calcareous soil. *Egyptian Journal of Botany,* **62**(1), 131-148.

Tawfik, E. (2021) Different cultivation media conditions affecting behavior and genetic stability of *Lepidium sativum* L. *Natural Resources for Human Health,* **1**(2), 85-90.

Toh, S., Imamura, A., Watanabe, A., Nakabayashi, K., Okamoto, M., Jikumaru, Y., Hanada, A., Aso, Y., Ishiyama, K., Tamura, N. (2008) High temperature-induced abscisic acid biosynthesis and its role in the inhibition of gibberellin action in Arabidopsis seeds. *Plant Physiology,* **146**(3), 1368-1385

Tourky, S., Abo-Hamed, S., Saleh, H., Shukry, W. (2023) Evaluation of the role of cobalt nutrition in the oxidative machinery of drought-stressed rice (*Oryza sativa* L.) plants at the reproductive stage. *Egyptian Journal of Botany*, **63**(1), 175-191.

Tovar, J.C., Berry, J.C., Quillatupa, C., Castillo, S.E., Acosta-Gamboa, L., Fahlgren, N., Gehan, M.A. (2022) Heat stress changes mineral nutrient concentrations in *Chenopodium quinoa* seed. *Plant Direct*, 1-13. https://doi.org/10.1002/pld3.384

Trakselyte-Rupsiene, K., Juodeikiene, G., Cernauskas, D., Bartkiene, E., Klupsaite, D., Zadeike, D. (2021) Integration of ultrasound into the development of plant-based protein hydrolysate and its bio-stimulatory effect for growth of wheat grain seedlings *in vivo*. *Plants*, **10**(7), 1319. https://doi.org/10.3390/plants10071319

Tyagi, V.K., Lo, S., Appels, L., Dewil, R. (2014) Ultrasonic treatment of waste sludge: a review on mechanisms and applications. *Critical Reviews in Environmental Science and Technology*, **44**(11), 1220-1288.

Umbreit, W.W. (1959) "*Manometric Techniques*". Minnieap. Burgess Publ. Co.

Van Rensburg, L., Krüger, G.H.J., Krüger, H. (1993) Proline accumulation as drought-tolerance selection criterion: its relationship to membrane integrity and chloroplast ultrastructure in *Nicotiana tabacum* L. *Journal of Plant Physiology*, **141**(2), 188-194

Voetberg, G.S., Sharp, R.E. (1991) Growth of the maize primary root at low water potentials. III. Role of increased proline deposition in osmotic adjustment. *Plant Physiology*, **96**, 1125-1130

Wahid, A. (2007) Physiological implications of metabolites biosynthesis in net assimilation and heat stress tolerance of sugarcane (*Saccharum officinarum*) sprouts. *Journal of Plant Research,* **120**(2), 219-228.

Wahid, A., Shabbir, A. (2005) Induction of heat stress tolerance in barley seedling by pre-sowing seed treatment with glycinebetaine. *Plant Growth Regulation,* **46**(2), 133-141.

Xalxo, R., Yadu, B., Chandra, J., Chandrakar, V., Keshavkant, S. (2020) Alteration in carbohydrate metabolism modulates thermotolerance of plant under heat stress. In: "*Heat Stress Tolerance in Plants: Physiological, Molecular and Genetic Perspectives*", Wani, S.H., Kumar, V. (Eds.), pp. 77–115. 1st ed. John Wiley and Sons Ltd.; Hoboken, NJ, USA.

Xu, Y., Zhang, S., Lin, S., Guo Y, Deng, W., Zhang, Y., Xue, Y. (2017) WERAM: A database of writers, erasers and readers of histone acetylation and methylation in eukaryotes. *Nucleic Acids Research*, **45**, D264–D270

Yaldagard, M., Mortazavi, S.A., Tabatabaie, F. (2008) Influence of ultrasonic stimulation on the germination of barley seed and its alpha-amylase activity. *African Journal of Biotechnology*, **7**, 2465-2471

Yi, J., Bochu, W., Xiujuan, W., Daohong, W., Chuanren, D., Toyama, Y., Sakanishi, A. (2003) Effect of sound wave on the metabolism of chrysanthemum roots. *Colloids and Surfaces B*, **29**, 115-118

Yusaf, T. (2015) Evaluating the effect of heat transfer on cell disruption in ultrasound processes. *Annals of Microbiology,* **65**(3), 1447–1456.

Zhang, H., Zhu, J., Gong, Z., Zhu, J. (2021) Abiotic stress responses in plants. *Nature Reviews Genetics*, **23**, 104-119

Zheng, W.X., Wu, S.J., Yang, Y. (2008) Ultrasound application in agriculture and prospect. *Agricultural Outlook*, **1**, 115-119

Zwiazek, J.J., Blake, T.J. (1991) Early detection of membrane injury in black spruce (*Picea mariana*). *Canadian Journal of Forest Research*, **21**(3), 401-404

الاستجابات الفسيولوجية والوراثية لبذور حب الرشاد للمعالجة بالموجات فوق الصوتية تحت الإجهاد الحراري

اميرة محمد عبد الستار، إيمان توفيق، إيمان زكريا احمد
قسم النبات والميكروبيولوجى- كلية العلوم – جامعة حلوان – القاهرة – مصر.

تفضل معظم البذور درجة حرارة أقل من 30 درجة مئوية للإنبات، ولكن السيطرة علي درجة الحرارة حقلياً غير ممكنة، تهدف هذه الدراسة إلى تحفيز مقاومة نبات حب الرشاد للإجهاد الحراري عن طريق المعالجة المسبقة بالموجات فوق الصوتية وكشفت النتائج ذلك كانت درجة الحرارة 25 درجة مئوية هي درجة الحرارة المثالية لإنبات بذور حب الرشاد. الإجهاد الحراري عند 30، 35 و 40 درجة مئوية تسبب في انخفاض تدريجي في نسبة الإنبات وجميع قياسات النمو و عند 40 درجة مئوية لم يلاحظ أي إنبات البذور حتى بعد المعالجة بالموجات فوق الصوتية. علاوة على ذلك، تعريض البذور للذبذبات فوق الصوتية، خاصة لمدة 20 دقيقة عند درجات الحرارة حتى 35 درجة مئوية، أدى إلى زيادة معنوية في نسبة الإنبات تصل إلى 96% مقارنة إلى البذور الغير معاملة 92%، بالإضافة إلى زيادة كل مظاهر نمو البادرات ومحتوى النبات من العناصر عند جميع درجات الحرارة المختبرة، بالإضافة إلى انخفاض علامات الإجهاد. كما ارتفعت السكريات القابلة للذوبان والبروتينات أيضاً حيث سجلت 131.64 و20.65 ملجم/جم مادة جافة على التوالي مقارنة بالبذور الغير معاملة. كان تحليل dendrogram والمكونات الرئيسية (PCA) التى صممت على أساس المعايير المورفولوجية والفسيولوجية وأظهرت ثلاث مجموعات من العلاجات المختلفة. تم تطبيق المعلم الوراثي RAPD-PCR لتقدير التباين في المحتوى الجيني الناتج عن وقت التعرض بالموجات فوق الصوتية والإجهاد الحراري على حب الرشاد. وكانت نسبة تعدد الأشكال الناتجة من اختلاف حزم الحمض النووي نتيجة لاستخدام بادئات مختلفه هى 48.61%. لذلك نوصى بتعريض البذور إلى الموجات فوق الصوتية لمدة 20 دقيقة كمعالجة مسبقة للبذور قبل الزراعة للمساعدة في التكيف مع درجات الحرارة المرتفعة.