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Sonication of seeds increase germination performance of sesame under low temperature stress

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ABSTRACT

A laboratory experiment was conducted to determine the effect of ultrasound (US) exposure time on germination behavior of sesame seeds. All tests were carried out at 20 kHz in a water bath ultrasonic device varying two factors, treatment duration (10, 20 and 30 min) and germination temperature (15, 20 and 25 ºC). Parallel tests were run in which seeds were soaked in water without sonication in order to eliminate the effect of water from US test results. US treatments enhanced seeds water uptake. At mild exposure time it improved sesame seed germination performance and seedling growth at suboptimal temperatures as indicated by higher germination percentage and germination rate. US applying for 20 min had relatively high superoxide dismutase activity; however, had not significant differences with control and US duration for 10 min. The catalase activity was strongly increased by applying the US for a 10 and 20 min. Among the treatments, application of US vibration for 10 and 20 min reduced both of malondialdehyde and H_2O_2 contents, however high US duration (30 min) increased both of the traits. In general, ultrasonic priming technique can be useful for early planting the sesame seeds, and lead to higher yields.

Key words: enzyme activity, germination performance, seedling growth, ultrasound, water uptake

IZVLEČEK

SONIFIKACIJA SEMEN SEZAMA Z ULTRAZVOKOM POVEČA NJIHOVO KALITEV V RAZMERAH HLADNEGA STRESA

V laboratorijskem poskusu smo določali učinke ultrazvoka (US) na kalitev semen sezama. Vsi poskusi so bili izvedenei v ultrazvočni vodni kopeli s frekvenco ultrazvoka 20 kHz , pri čemer smo spreminjali dva dejavnika in sicer trajanje obdelave z ultrazvokom (10, 20 in 30 min) in temperaturo kalitve (15, 20 in 25 ºC). Vzporedno so potekali poskusi, v katerih so bila semena samo namočena v vodi brez ultrazvočne sonifikacije, da bi odpravili učinke vode pri ultrazvočno obdelanih semenih. Obdelava semen z ultrazvokom je v njih povečala privzem vode. Pri srednjih obravnavanjih z ultrazvokom se je izboljšala kalitev in rast kalic pri suboptimalnih temperaturah, kar se je odrazilo kot večji odstotek kalitve in njen hitrejši potek. US obdelava za 20 min je rahlo povečala aktivnost superoksid dizmutase, vendar v primerjavi s kontrolo in obdelavi z US 10 min ni bilo značilnih razlik. Aktivnost katalaze se je pri obdelavah z 10 in 20 min močno povečala. Obdelava z ultrazvokom za 10 in 20 min je zmanjšala vsebnost malondialdehida in H_2O_2, obdelava za 30 min pa je vsebnost obeh parametrov povečala. Na splošno lahko na osnovi te raziskave povzamemo, da je ultrasonična predobdelava semen sezama koristna tehnika za njegovo zgodnjo setev, kar vodi v večje pridelke.

Ključne besede: encimska aktivnost, kalitev, rast kalic, ultrazvok, privzem vode

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1 INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the most important oilseed crops used by humans (Weiss, 2000). It has a high resistance level in drought stress condition, and is therefore appropriate crop for cultivation in dry-land conditions. But in these regions, water availability in the soil can be insufficient to support seed germination and early seedling growth, which are the most sensitive stages for water shortage. In order to conquer for this problem, sesame seeds should be sown earlier in the season, when a lot of water is available, so, canopy closing and growth degree day (GDD) requirement for completing the growing stage is gain earlier and reproductive stage and following seed development and maturity complete early. One of the most important obstacles of this approach is low temperature in the early stages of season that negatively affects seed germination and seedling establishment.

Seed germination and seedling establishment are the most critical stages for survival of plants under unfavorable conditions. One of the most important factors that affect germination and subsequently later stages is low temperature. Seeds can germinate over a wide range of temperatures, but maximum percentage germination is typically reduced at the extremes of the range (Probert, 2000). Germination is divided into three phases: imbibition, activation and post germination growth. The major negative effects of low temperature during germination seem to be associated to the imbibition phase (Hoekstra *et al*., 1992). Arin and Kiyak (2003) reported that difficulty in water uptake under low temperature can influence the later stage of growth and development. For example, emergence percentage decreased under the low temperatures.

Low temperature during imbibition phase of germination leads to the increase of electrolyte leakage from the seeds, which has been attributed to the disturbance of plasma membrane integrity (Hoekstra *et al*., 1992). Low temperature promotes gel phase formation and increase rigidity, thereby increasing the likelihood of imbibitional injury (Crowe *et al*., 1989). Bochicchio *et al*., (1991) reported that imbibition of seeds is necessary to reorganize fully the structure of cell membrane lipids, but, if during the imbibition process, the temperature is such that membrane lipids are in a gel phase, formation of a continuous bilayer might not be possible, or the bilayer formed might be functionally imperfect. Sharma *et al*., (2011) reported that oxidative stress may be a significant factor in relation to low temperature damage in plants. Reactive Oxygen Species (ROS) play a key role in various events of seed life. In seeds, ROS production has been considered for a long time as being very detrimental, since the works dealing with ROS were mainly focused on seed ageing or seed desiccation, two stressful situations which often lead to oxidative stress. At the opposite, it now appears more and more clearly that ROS would play a key signaling role in the achievement of major events of seed life, such as germination or dormancy release. Many reports have shown that the transition from a quiescent seed to a metabolically active organism (phase II of germination) is associated with ROS generation, suggesting that it is a widespread phenomenon (Bailly, 2004). ROS are involved in endosperm weakening during germination. Cellular antioxidant mechanisms seem to tightly control ROS concentrations, rather than to eliminate them completely, suggesting that some ROS might play normal physiological roles and act as signaling molecules. The reactivation of metabolism following seed imbibition may provide an important source of active oxygen species (AOS). For example, H_2O_2 is produced at the early imbibition period of soybean (Puntarulo *et al*., 1991).

There are several techniques that have positive effect on low temperature tolerance at germination stage. Ultrasound is a novel physical method that involves the application of sound frequencies in the inaudible range (20–100 kHz) to interact with the materials. It was proven that application of ultrasound treatment could change the state of the substances and even accelerate the reactions (Aladjadjiyan, 2007). This technique is unique among existing seed pretreatment methods in that it is simple, cheap, environmentally friendly and multifunctional (Goussous *et al*., 2010). Ultrasound treatment to stimulate germination has been investigated in many seed types including maize, barley, rice and sunflower (Aladjadjiyan, 2002; Florez *et al*., 2007; Yaldagard *et al*., 2008a,

b). Goussous *et al*., (2010) and Yaldagard *et al*., (2008a) showed that US treatments increase seed water uptake, the important stage that was inhibited by low temperatures; so, we use this technique to investigate the beneficial effect of US on seed germination performance in low temperatures. Besides, there has not been any investigation on the effects of US treatment on ROS and antioxidant enzyme content following the ultrasonication. So this research was aimed to determine whether ultrasonic treatment is usable as a seed priming method for sesame.

2 MATERIALS AND METHODS

2.1 Seed materials

Native seed lots of sesame (*Sesamum indicum* 'Oltan') were obtained from Tabriz University, Iran which collected the seed in the same year that the study was undertaken.

2.2 Seed treatment

The ultrasonication experiments were carried out at 20 kHz on the ultrasonic generator (UW2200, Berlin). All tests were performed on samples (50 seeds for each treatment) dispersed in 100 ml of distilled water with direct sonication for 0, 10, 20 and 30 min. To rule out the effect of water in these tests, a control replicate of each test was soaked in a similar volume of water but without sonication. Water circulating in ultrasonic equipment as well as in the water bath was kept at 25 ºC.

2.3 Germination tests

Three replicates of 50 seeds were placed in covered 9-cm Petri dishes containing a single filter paper with 5 mL test solutions. The Petri dishes with seeds were put in sealed plastic bags to avoid moisture loss. Seeds were germinated at constant temperatures (T) of 15, 20 and 25 ºC the dark in an incubator. Germination was scored when the seminal root was 2 mm long.

2.4 Germination performance measurements

At the end of experiment, Final germination percentage (FGP), germination rates (GR), germination uniformity (GU), seminal root and shoot length were recorded to evaluate germination performance. Daily germination percentage was recorded and subjected to statistical analysis. GR (T_{50}) was defined as days needed to reach 50 % of FGP. GU (T_{10-90}) was defined as days needed for 10 % of FGP to 90% of FGP.

2.5 Seed water uptake

The water uptake of seeds necessary for germination was determined under 15 ºC. For this purpose, three replications of 50 seeds were placed in petri dishes as described for germination experiments, removed at 15 min after initiation of imbibition, drained and blotted with absorbent paper, weighted and placed again into the petri dishes. After 30 min, 1 h and 2 h, the seeds were reweighted as described above. The water uptake was expressed as the seed moisture content at different times.

2.6 Enzyme extractions and assays

Measurements of enzyme activities, lipid peroxidation and H_2O_2 contents were carried out with imbibed but non-germinated seeds and with whole seedlings at 3 days after germination. In order to better discriminate the behavior of the seeds and seedlings, the temperature of 15 °C only has been chosen.

Two grams of peeled seeds or whole seedlings were used for enzymes extraction. The samples were homogenized in 20 ml of 0.1 M potassium phosphate buffer (pH 7.8) centrifuged for 15 min at 16,000×g, at 0 ºC. Extraction was at 4 ºC. The supernatant sample was then stored at -20 ºC prior to assaying. Superoxide dismutase (SOD, EC 1.15.11), catalase (CAT, EC 1.11.1.6) were extracted and assayed according to Bailly *et al*., (1996), and expressed as units per gram FW.

2.7 Evaluation of hydrogen peroxide and malondialdehyde contents

H_2O_2 concentration was measured by the methods of Moloi and Westhuizen (2006). Results are expressed in nmole per gram dry weight. Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) content from 0.2 g (fresh weight) of seeds or seedlings, according to Predieri

et al. (1995). The samples were homogenized in 5 ml sodium phosphate buffer followed by centrifugation for 15 min at 8,000×g. A 0.5 ml aliquot of the supernatant was combined with an equal volume of thiobarbituric acid (TBA) reagent and boiled for 20 min. Absorbance was determined at 532 and 600 nm. MDA concentration was expressed in micromoles per gram dry weight.

2.8 Data analysis

Analysis of variance appropriate to the experimental design was conducted, using SPSS software. Means of each trait were compared according to Duncan multiple range test at $P \leq 0.05$. Excel software was used to draw figures.

3 RESULTS AND DISCUSSION

3.1 Effect of different exposure times on seed water uptake

Before any treatment, seed moisture was 13 %. With increasing the US exposure time, seed moisture increased. At the early times, US treated seeds, particularly US treatment for 30 min, had rapid water uptake compared with control, then water uptake slope (rate of water uptake) was enhanced in control, while reduction was observed in US for 20 and 30 min (Fig. 1). At the end, seed moisture content of control and mild treatment duration (10 and 20 min) were approximately similar. However, at high US duration, seed moisture content was lower than control. One of the possible explanations could be that sonication (for short periods) may create or enlarge fissures in the protective coating surrounding the seed and pericarp and after steeping in the water a significant rise in seedling moisture resulted. US treatment for high duration (30 min) might have led to much cell wall disruption that allowed greater accessibility of cell membranes to the effects of US, thus increasing their permeability and finally leading to post treatment water leakage. Similar results were observed by Goussous *et al*., (2010).

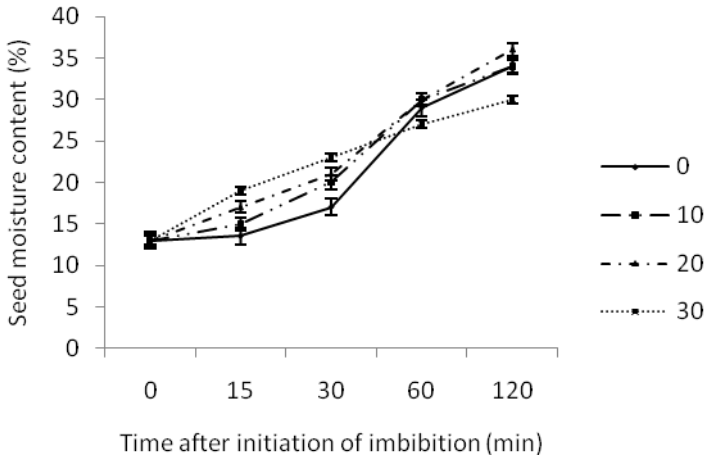

Figure 1: Seed moisture content of sesame seeds soaked in water in ultrasonic device (US) for 0 - 120 min

3.2 Effect of different us exposure times and temperatures on germination performance

Germination percentage (GP) of sesame seeds increased with increasing the treatment duration, reaching the limit (80 %) at 20 min. this GP value was approximately 12 % above that of the control seeds. However, at long duration (30 min), GP significantly decreased (approximately 18 %). Similar result was reported by Goussous *et al*., (2010) that showed that application of mild US duration improved germination percentage of wheat seeds. The effects of ultrasonic treatment on seed germination depend on frequency of

ultrasonic wave and exposure time as well as on plant species (Aladjadjiyan, 2012). It is noticeable from Table 1 that significant differences also existed in GP among incubation temperatures. Seeds of sesame germinated better at 25 ºC. It is also observed that, at suboptimal temperature (15 ºC), 20 min US treatment, enhanced GP by 22 %, but at optimal temperatures improvement of GP by 20 min US was only 2 % above control. Therefore, US treatment had more pronounced effects on germination at low temperatures than at optimum ones. US duration treatment significantly affected germination rate (GR) and uniformity (GU) (Table 1). With increasing the US duration, GR increased. The highest GR (0.27) was obtained at 30 min, reaching 28 % above control. GU decreased by US treatments, however, difference between control and 10 min US was not significant. In general, US priming technique improved sesame seed germination performance at 15 ºC as indicated by higher GP and GR (Table 1), indicating an improved of chilling tolerance.

Germination performance improvement by US application had been reported by Goussous *et al*. (2010) on wheat, Yaldagard *et al*. (2008) on barley and Aladjadjiyan (2002) on carrot. It is demonstrated that US exerts its major effects by inducing mechanical effects (acoustic cavitation) and disruption of plants cell walls, thereby increasing water uptake, important phase of germination that are negatively affected by low temperatures. The extra absorbed water reacts freely and readily with the cell embryo, so, metabolic processes such as gibberellic acid release and activation of enzymes expedited (Yaldagard *et al*. 2008a), and then will significantly reduce the mean germination time and will increase the rate and yield of germination. At long period of US (30 min) decreased germination performance. It is possible that US treatment for 30 min was too extreme to be tolerated by the small and fragile seeds of sesame leading to lysis of cells.

In this study non-sonicated seeds only exposed to water were used as control. Hydration of seeds by soaking in water for various periods without allowing seminal root emergence is one method of priming, which generally improves germination performance (Ashraf and Foolad 2005). Therefore, sonication priming can be used instead of usual priming ways (hydropriming).

Table 1: Germination percentage, rate and uniformity of sesame seeds exposed to different duration of ultrasound (US) treatment and incubated at three temperatures; 15, 20 or 25 ºC

US duration	Temperature (ºC)	Germination		
		Percentage (%)	Rate (1/day)	Uniformity (day)
0	15	62^{f}	0.19^{j}	4.73^{a}
	20	70^{def}	0.21^{h}	4.07^{bc}
	25	82^{ab}	0.23^{f}	3.69^{cd}
10	15	72^{cdf}	0.20^{i}	4.32^{b}
	20	74^{bcd}	0.23^{f}	3.8^{cd}
	25	84^{a}	0.24^{e}	3.77^{cd}
20	15	76^{abcd}	0.25^{d}	3.67^{cd}
	20	80^{abc}	0.26^{c}	3.5^{d}
	25	84^{a}	0.22^{g}	3.37^{d}
30	15	51^{g}	0.31^{a}	3.74^{cd}
	20	62^{f}	0.29^{b}	3.72^{cd}
	25	64^{ef}	0.22^{g}	3.52^{d}

Different letters in each column indicate significant difference at $P \leq 0.05$.

3.3 Seed germination trends

Control seeds were germinated well when incubation temperature was 25 ºC, but US treatment seeds were not significantly affected by temperatures at early stages of germination. Higher temperature caused rapid germination in US and control seeds (Fig. 2). The optimum temperature for sesame seed germination is 25 ºC (Bennet, 2011). The temperature below the optimum, decreased germination rate (Bradford, 2002). Langham (2007) reported that with soil temperatures around 25°C, the seed imbibes enough moisture and encourage a rapid germination. At all of the temperatures, high US duration (30 min) had lowest GP, but cumulative GP slop (i.e. GR) was high. At low temperature (15 ºC), GP curve for control seeds was lower than 10 and 20 min US, however, with increasing the temperature, GP curve for control seeds was approached to 10 and 20 min US duration curves. At 25 ºC, control and mild US duration treatments (10 and 20 min) had approximately similar curves (Fig. 2). Therefore, US priming technique for appropriate duration were more beneficial at suboptimal temperature than optimal ones. And it can improve germination performance of sesame seeds under chilling stress.

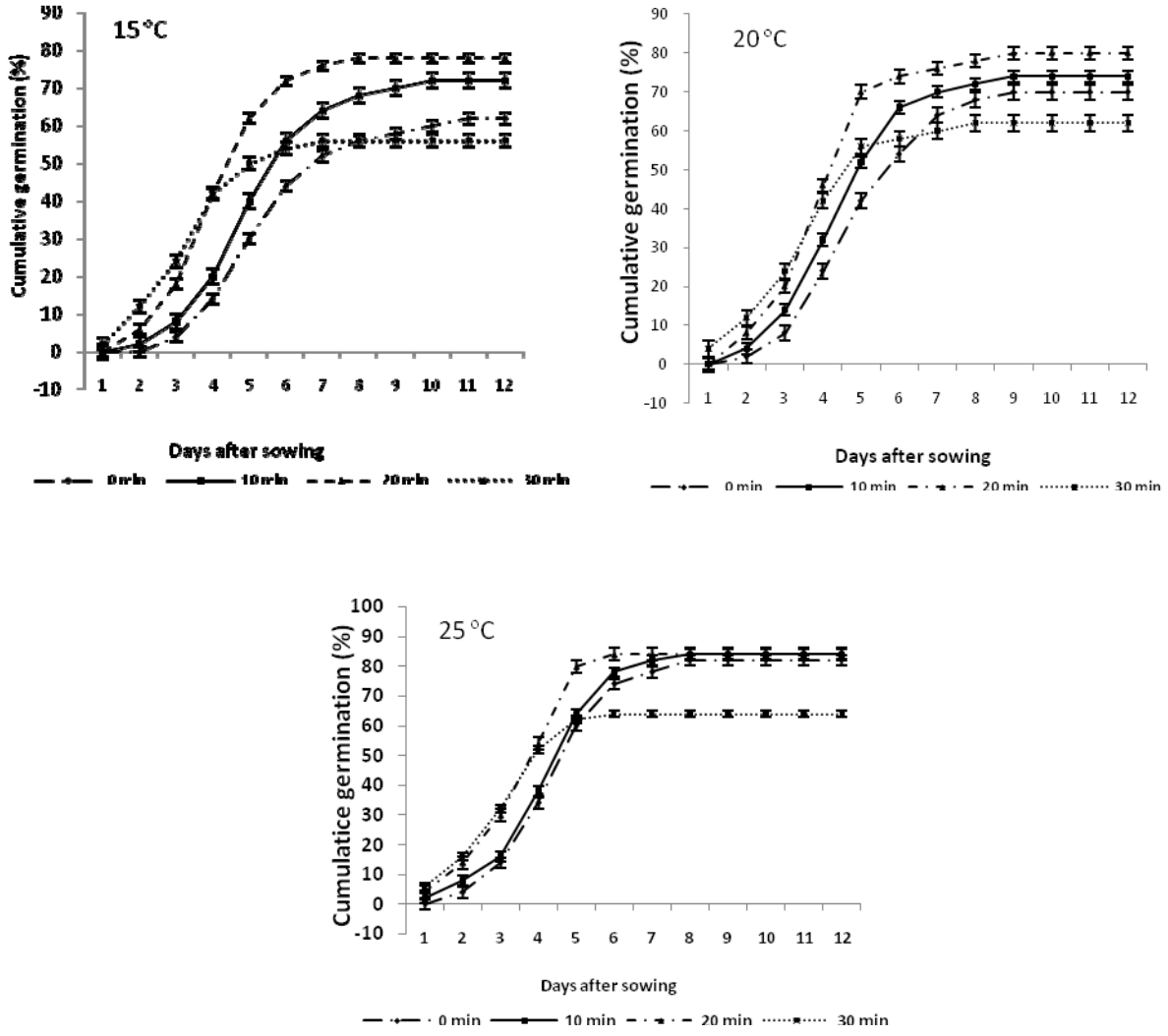

Figure 2: Cumulative seed germination of US treatment and control seeds under different temperatures (15, 20 and 25 ºC)

3.4 Effect of us and temperature treatments on seedling growth

Seedling length and dry weight were significantly affected by priming technique. The size of the seedlings was larger in treatments where seeds were primed by 20 min or 10 min, and smaller in control and 30 min US treatment. At all temperatures, seedling size of mild US treated seeds (20 min), was higher than control, but high US duration at low incubation temperatures produce more slender seedlings (compared to control) (Fig. 3). At higher temperatures, 30 min US treatment of seeds for 30 min decreased seedling's growth. Their size is lower than in controls. According to literature low temperature promotes gel phase formation and increase rigidity of membrane, so, it seems that, in this situation, membrane may be able to tolerate mechanical changes that caused by US duration for 30 min. At higher temperatures, however, membrane rigidity is eliminated (Nijsse *et al*., 2004), which presumably decrease tolerance to high duration US treatment. In a research conducted by Fateh *et al*. (2012) on fennel, they showed that US treatment had adverse effect on seedling growth. Aladjadjiyan (2012) reported that seedling length of lentil had approximately linear relationship with US exposure time, and with increasing the US time, these traits increased.

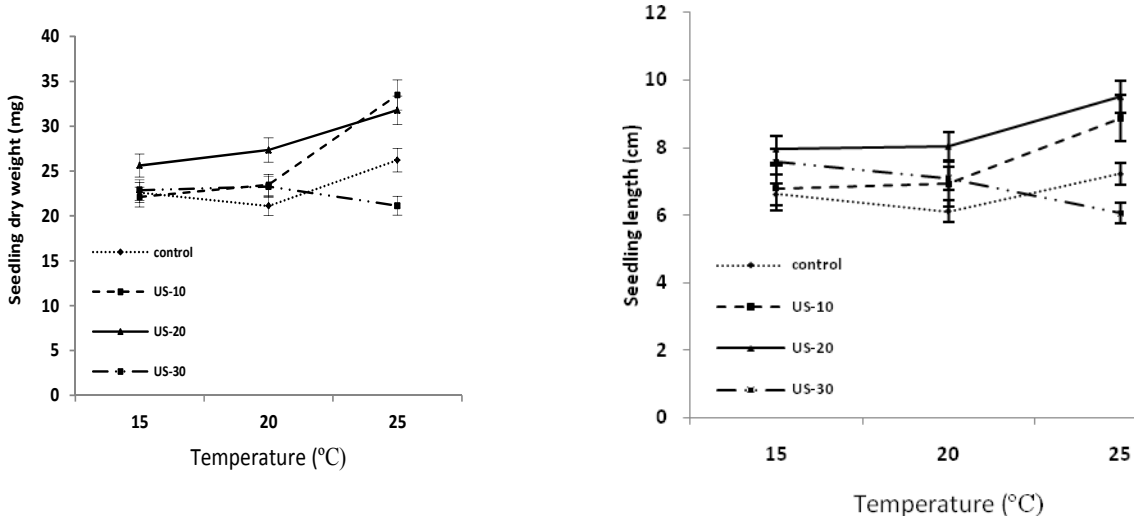

Figure 3: Effect of US treatments on seedling dry weight and length at different temperatures

3.5 Enzyme activities

SOD activity did not significantly change during germination phase. So this enzyme activity was similar from imbibition phase to seedling growth. Whereas, about CAT activity; after imbibition, CAT activity had increased by 29 % and the activity of this enzyme was higher at seedling stage than imbibition time (Fig. 4). It can be concluded that SOD activity is not correlated with seed germination, and it may be mainly involved in preserving the viability of seeds and protecting them from reactive oxygen species formed during storage. CAT activity changes during germination are demonstrating that this enzyme in addition to had role in storage time; it had a detoxifying role at germination stage too. These results are in agreement with certain finding of Yeh *et al*. (2005), which showed that activity of antioxidant enzymes such as CAT and SOD is closely related with germination and storage longevity.

Among the treatments, 20 min US treatment induced relatively high SOD activity. There was, however, not significant difference between this treatment and shorter US treatment (10 min) and the control. High duration of US (30 min) decreased SOD activity. At all of the treatments, SOD activity changes were not significant between imbibed seeds and seedlings (Fig. 4). The CAT activity was strongly increased by applying the US for a moderate time (10 and 20 min), thus

enhancing the antioxidant defence of the cells. Our data also showed that US treatment for a long time (30 min) reduced CAT activity in the both of imbibed seeds and seedlings. Chen *et al*. (2013) reported that seeds exposed to ultrasonic vibration showed high CAT and SOD activities, so, improved resistance to cadmium and lead in wheat seedling. Stimulation of CAT activity by seed priming was also reported by Bailly *et al*. (2002).

The catalase intervenes in the respiration of plants, which caused degradation of the endosperm and cotyledons reserve substances and synthesis of some necessary substances for nutrition and embryo growth. Therefore, it seems that stimulation of germination process and seedling growth by ultrasonic vibration may be attributed to CAT activity. Bailly *et al*. (2002) showed that improvement of germination performance by priming was clearly associated with higher CAT activity.

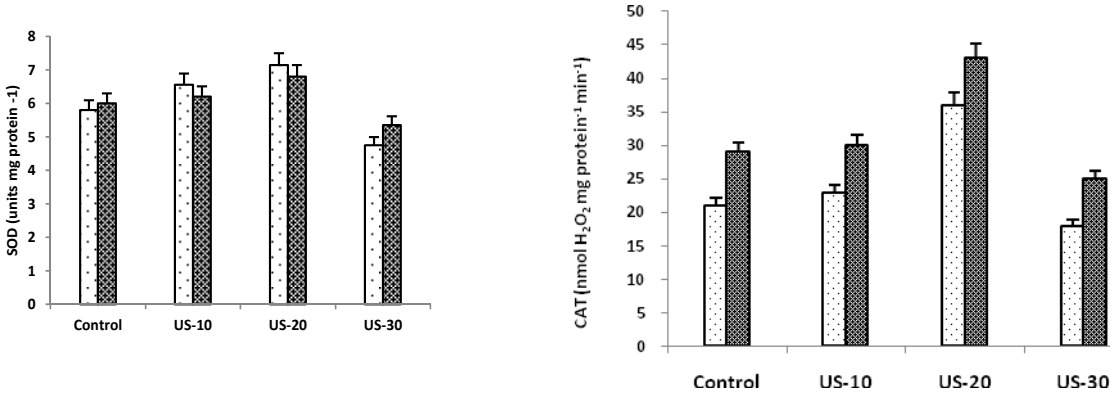

Figure 4: Effects of US treatments on SOD and CAT activities in imbibed seeds and seedlings

3.6 Lipid peroxidation and H_2O_2 content

Lipid peroxidation (degradation) was evaluated by determination of seed malondialdehyde content. MDA is one of the final products of the peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage (Sharma *et al*., 2011). MDA contents of seeds and seedlings are summarized in Fig. 5. In all of the treatments, MDA contents were higher in imbibed seeds than in seedlings. Despite an active metabolism associated with seedling growth, there was no increase in lipid peroxidation. Our data showed that hydrogen peroxide content was not significantly changed during seedling development following the imbibition, despite of producing by mobilization of stored reserves by β-oxidation in glyoxysomes, a type of peroxisome (Bewley and Black, 1994). Indeed, peroxisomes are also the site of localization of catalase, which eliminates H_2O_2. Therefore, production of H_2O_2 by converting the lipid reserves into sugars during the first stages of seedling development can be reduced by catalase activity that increased in this period.

Among the treatments, application of US vibration for 10 and 20 min reduced both of MDA and H_2O_2 contents, however high US duration (30 min) increased both of the traits. It is notable that MDA and H_2O_2 enhancement in imbibed seeds is higher than seedlings (Fig. 5). Imbibed control and US treated seeds for 10 min, and further growth of seedlings generated by these seeds, did not lead to significant changes in H_2O_2 content. It seems that increase in MDA and H_2O_2 content by US priming may be attributed to increase in antioxidant enzymes activity such as catalase that could be able to eliminate these detoxifying agents.

The results obtained underline the likely involvement of CAT in germination and early growth of seedlings of sesame thus suggesting that control of H_2O_2 homeostasis is an important event in expression of seed vigor. However, H_2O_2, together with other ROS, may have many cellular

targets, such as proteins and DNA (Sharma *et al*., 2011). Alternatively, CAT also plays a key role in H_2O_2 removal during fatty acid β-oxidation in glyoxysomes (Olsen and Harada, 1995). Therefore, high CAT activity could be associated with better mobilization of lipid reserves and faster seedling development.

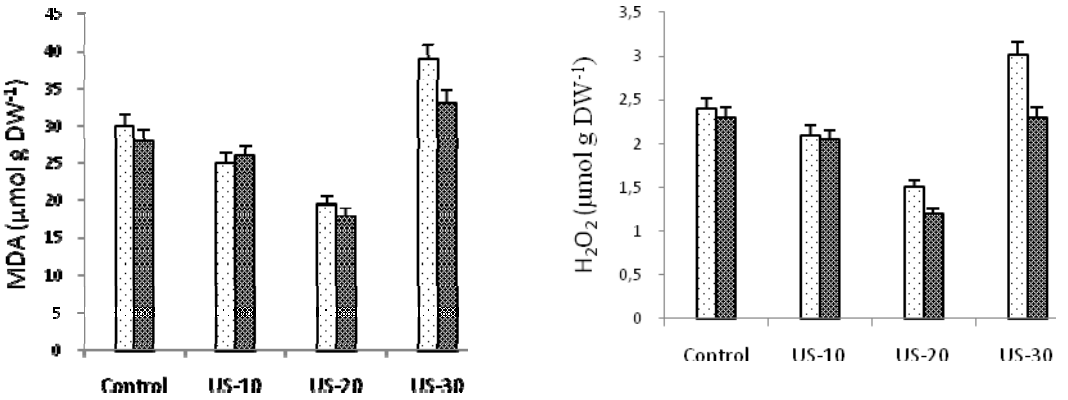

Figure 5: Effects of US treatments on MDA and H_2O_2 contents in imbibed seeds ⬚ and seedlings ▩

4 CONCLUSION

Results of this research indicated that US treatment effectively enhanced germination performance and seedling growth of sesame seeds especially at suboptimal temperatures. This increase in germination performance could have a positive impact on the success of early field establishment and canopy closer leading to better plant establishments and influence weed managements. So, this technique can be useful for alleviating the adverse effect of suboptimal temperature on seed germination, therefore, it can be very important in earlier canopy closing and finally improve yield.

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