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PLAUSIBLE MECHANISMS BY WHICH ULTRASONIC WAVES AFFECT SEEDS

ABSTRACT

To study the effect of sonication on the seed germination percentage and rate as well as the cell area of barley (*Hordeum vulgare* L.) a laboratorial experiment was performed as Completely Randomized Design (CRD) with 3 replications. The results indicated that the ultrasonic waves affect the seed germination, germination rate and cell area significantly (at 0.05). The results of the mean comparison tests (LSD, 0.05) showed that the highest germination percentage (100 %), germination rate and cell area (1370.71 μ^2) is achieved through 15 minutes exposure to ultrasonic waves. It is concluded that weakening the seed's cell wall rigidity by sonication results in more and faster water imbibition by the cells and improved germination.

Keywords: cell area, barley, germination, seed dormancy breaking, sonication

INTRODUCTION

Some different mechanisms (called dormancy) are used by plants to postpone germination and protect the seeds until the favorable conditions for seedling are provided. Dormancy breaking and germination stimulation is important for proliferation and early production of important plants. Different methods have been applied to overcome the seed dormancy such as salinity, temperature, humidity (Bradbeer, 1998), light, seed scarification (Jun and Tao, 2004), regulatory hormones (Sozi and Chiesa, 1995) and chemical compounds.

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Recently, ultrasonic waves as a new technic have been used for dormancy breaking. Yaldagard *et al.,* (2008a) reported the usefulness of ultrasonic waves to improve seed germination and plant growth. Ultrasound has been applied to induce faster and greater seed germination of the Norway spruce (Risca *et al.,* 2007), barley (Yaldagard *et al.,* 2008a), orchids (Shin *et al.,* 2011) and other crops (Goussous *et al.,* 2010). Additionally, Power ultrasound as a novel processing method finds wide application in various food processing operations including fruit juice preservation (O'Donnell *et al.,* 2010; Tiwari *et al.,* 2010), enhancing drying rate (Jambrak *et al.,* 2007) and extraction of bioactive compounds (Mason *et al.,* 1996). The physical and mechanical effects such as strong shear forces, particle fragmentation, increased mass and heat transfer, nucleation of seeding induced by ultrasound (Mason *et al.,* 2011) make it very versatile technology for food processing applications.

Barley is the main ingredient for malting and brewing in the manufacture of beer. Germination capacity and the level of α-amylase in barley grains play a significant role in the brewing process of barley. Poor germination capacity of barley reduces malt yield.

There are just a few published articles in the literature which guess the reasons of positive effects of ultrasonic waves on seed germination and other aspects of plant growth. For instance, some recent studies have shown that ultrasound with proper intensity and duration would increase the enzymatic activities or promote the cell growth by stimulating physiological activities of bacterial and callus cells compared to growth without ultrasound (Barton *et al.,* 1996; Liu *et al.,* 2003; Pitt *et al.,* 2003). Different researchers reported that the activity of enzymes increased under mild ultrasound, and in some case high intensities of ultrasound caused a decrease in the activity of alpha-amylase activity (Yaldagard *et al.,* 2008a; Yaldagard *et al.,* 2008b). Nevertheless, there is no research in the literature evaluating the effects of ultrasonic waves on the cells size of seeds and the reasons of improvement of germination by sonication. Thus, this experiment was performed to determine the effects of sonication on the size of the endospermic cells and the germination percentage as well as the germination rate of barley seeds. The reasons of improvement of seed traits were also incerstigated.

MATERIALS AND METHODS

Seed preparation

The barley seeds were provided from Agricultural Research Centre of Karaj (Iran) after which the seeds with the same size were selected (not immature and damaged seeds). They were then soaked for 10 minutes in a solution of 1 part sodium hypochlorite (NaOCl 12.5% w/v) and 2 parts water, and subsequently rinsed by distilled water for three times.

Experiment

The experiment was performed in three replicates in sterilized petri dishes under laboratorial conditions. There were 30 seeds in each replication.

The seeds of each replicate were exposed to ultrasound waves for different durations independently. The treatments consisted of periods of ultrasonic exposure of 0 minutes (control), 5 minutes, 10 minutes and 15 minutes. The frequency of waves was 42 kHz. For ultrasonic waves exposing, the seeds were soaked in a plastic dish containing distilled water and placed in the ultrasonic apparatus (WiseClean, model WUC-Ao2H, temperature 30°C). After exposing to the waves, the seeds were placed on Wathman No.1 filter papers moistened with 10 ml of distilled water in sterilized petri dishes. Three germination experiments were carried out as the Completely Randomized Design in room temperature (25°C) and darkness. The experiments were performed in January 2016. The statistical analyses were done using SPSS (Statistical Package for Social Sciences) software. The means were compared through the LSD Test at the statistical level of 0.05.

The evaluated features for assessing the effect of ultrasonic waves on the seed were germination percentage, germination rate and area of the endospermic cells.

The germination percentage was calculated via the following formula:

$$G_{\%} = \frac{G}{N} \times 100$$

where;

$G_{\%}$ is percentage of germination, G is the number of germinated seeds and N is the number of total seeds.

The germination rate was investigated for all of the treatments during 5 days with 24-hour intervals. The germinated seeds were counted each 24 hour and the cumulative germination was evaluated in correlation with time (for 5 days).

In order to measure the area of the endospermic cells affected by sonication (and the control) the related tissue (the inner surface of the seed coat) was sampled via a microtome device (model YD-202A). After that, each sample was observed under a light microscope (Leica DM300) equipped with an eye piece micrometer. For each sample (all the replications), the area of 30 cells was randomly measured and the average cell area was calculated.

RESULTS

Germination percentage

The results of variance analysis showed that there are significant differences between the treatments and the control (at the probability level of 0.01) (Table 1). The comparison of means indicated which the highest germination percentage (100%) was achieved through 15 minutes exposure to ultrasonic waves; however, the lowest one (90%) was observed in the control treatment (Table 3).

Table 1

ANOVA table of the seed germination percentages (0.05)

Source	DF	Sum of Square	Mean Square	F
Treatment	3	163.587	54.529	122.613
Error	8	3.558	0.445	

CV: 6.42

Germination rate

Analyses showed significantly different germination rates among treatments. Fig. 1 shows the number of germinated seeds during the period of 5 days for different sonication times. The highest growth rate was related to the treatment 15 minutes and the control had the lowest germination rate.

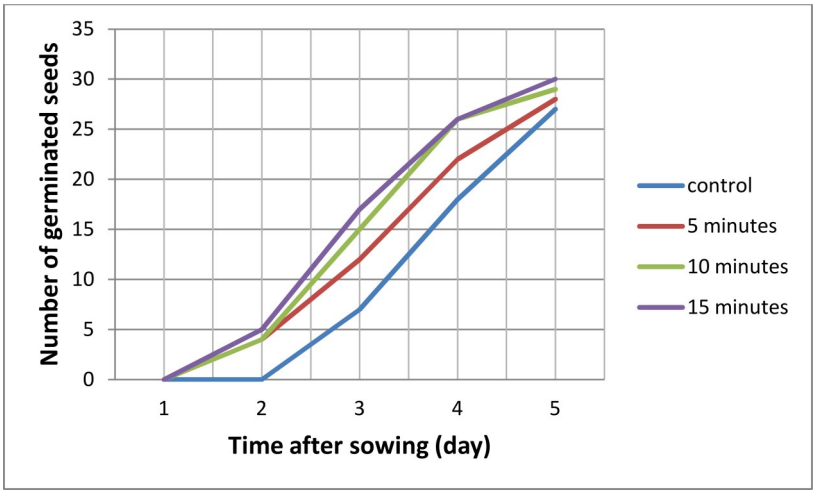

Fig. 1. The number of germinated seeds during 5 days for different sonication times and control.

Cells area

It is obvious from Table 2 that the cells area affected by the sonication was significantly different from the control treatment (at the probability level of 0.01). The results of mean comparison tests linked the highest cell area (1370.71 micron) to the 15 minutes treatment and the lowest cell area (665.04 micron) was related to the control (Table 3).

Table 2

ANOVA table of the cells' area (0.05)

Source	DF	Sum of Squares	Mean Square	F
Treatment	3	957.587	125.651	131.327
Error	8	7.621	0.957	

CV:9.28

Table 3

Mean comparison results of the germination percentages and the cells' area (LSD Test, 0.05)

Sonication time [min]	Germination percentage [%]	Cell area [micron]
0	*90 d*	665.04 d
5	*93.33 c*	883.96 c
10	*96.66 b*	1183.71 b
15	*100 a*	1370.71 a

The means with the different letter in the columns are significantly different

DISCUSSION

Germination percentage and rate

The results of this study indicate that sonication could significantly improve germination percentage and rate. In agreement with our results, Yaldagard *et al.,* (2008a) reported that the sonication positively affects the germination percentage and rate; very high exposure times however resulted in the lower germination percentage and speed (Yaldagard *et al.,* 2008a). Other studies show that ultrasound has been applied to induce faster and greater seed germination of the Norway spruce (Risca *et al.,* 2007), orchids (Shin *et al.,* 2011) and other crops (Goussous *et al.,* 2010). There are a few definite proofs showing the reasons for the positive effects of sonication on seed germination and germination acceleration. For example, It has been assumed that sonication may increase the pores sizes of the seed shell for better hydration by enlarging the cells (Yaldagard *et al.,* 2008a); however, this has not been documented through experimental analyses. They also mentioned the cell wall fluidity as a possible result of ultrasonic stimulation as a cause for better germination. The cavitation during sonication is another possible reason that induces micro-fissures on the grain surface and may improve imbibition of moisture for enhanced germination rate (O'Donnell *et al.,* 2010). These reasons all were just the assumptions cited in the literature.

Cells area

According to the results of this study ultrasonic waves enhanced the cells size significantly compared to the control treatment. Obviously, a plant cell is enlarged when the central vacuole is filled with water. Water to be transfer into the central vacuole needs to pass the cell wall and plasma membrane. Thus, it could be concluded that when the seeds are exposed to sonication the cell walls undergo some changes by which their rigidity is lost and water is better absorbed be the seeds. It has been shown that ultrasound causes cavitation in aqueous solutions, which is an effective factor in damaging the cell wall of the micro-organisms (Elliott *et al.,* 1995). When a bubble collapses, a strong shear rate is generated in the environment that breaks the chemical bounds in the cells' wall and membranes' (Dubbs, 1996). Even though it is not clear if sonication causes cavitation on the plant cell wall, micro-cracks and micro-voids are formed on the bacterial cell wall (Tabatabaie and Mortazavi, 2008). It has also been reported that the cell volume and size increase when exposed to the sonication (Tabatabaie and Mortazavi, 2008). Now, it can be said that water is the key of increase in the seed germination percentage and rate. Water deficit is one of the most serious problems for germination, a crucial phase of plant life (Gill *et al.*, 2003). The sequence of events leading to the emergence of the radicle through the seed coat is governed by water uptake from the external medium (Kaur *et al.*, 1998). Water availability plays a significant role in enzymatic reactions, in solubility and transportation of metabolites, and as a reagent in the hydrolytic breakdown of proteins, lipids and carbohydrates in the storage tissues of germinating seeds (Bewley and Black, 1994). The activity of some enzymes such as α-amylase in *Cicer arietinum* cotyledons (Gupta *et al.*, 1993; Kaur *et al.*, 1998; 2000) or α- and β-amylase in *Medicago sativa* germinating seeds (Zeid and Shedeed, 2006) is reduced by water stress. In conclusion, water imbibition changes the physiological activities of the seeds resulting in the germination enhancement and rate. It could be concluded that ultrasonic waves weaken the cell wall rigidity as well as forming micro-voids on the seeds cell wall. This leads to more water imbibition followed by increase in the α-amylase activity. To support this, Yaldagard et al (2008b) showed that the α-amylase activity of barley seeds significantly increased by sonication. α-amylase is an enzyme by which the starch is hydrolyzed. Increase in the α-amylase activity followed by more starch hydrolysis can support faster and higher nutrients transfer to the embryo resulting in germination.

CONCLUSIONS

This experiment analyzed the effects of sonication on germination percentage and rate as well as the seed cells' area. Overall, ultrasonic waves enhance the germination percentage and rate of the seeds through weakening the cells' wall rigidity resulting in more and faster water imbibition by the cells. This leads to enlargement of the seed cells followed by faster release of α-amylase which accelerates hydrolysis of the starch and germination occurs.

Economic benefits

There are rich backgrounds in terms of the positive effects of ultrasonic waves on seed germination and plant growth. But so far, in seed sciences, unfortunately sonication is only used for experimental purposes. In fact, sonication devices in the market are very small and do not work for the industrial aims. The authors would like to strongly advise the seed companies and mechanical engineers to design an ultrasonic bath in industrial volume. This will definitely yield economic profits and bring many gains for farmers.

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