IPS Journal of Nutrition and Food Science
IPS J Nutr Food Sci, 2(2): 33-36 (2023)
DOI: https://doi.org/10.54117/ijnfs.v2i2.29

Impact of Pre-Sowing Physical Treatments on The Seed Germination Behaviour of Sorghum (*Sorghum bicolor)*

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Abstract

In the emerging global food scenario, sorghum is a healthy cereal crop used by human beings and has been recognized to have considerable potential to be used in a variety of food products. Germination is a natural processing technique used for the biological activation of grains to improve their nutritional and functional properties. In order to improve the germination of grains, different techniques have been established; therefore, the current study was conducted to analyze the effect of ultrasound and microwave techniques on sorghum grains germination parameters. Overnight steeped sorghum grains were divided into four groups. The first group was not subjected to any treatment. The second group was subjected to ultrasonic treatment, the third group was exposed to microwave treatment, and the fourth group was treated with combined MW & US applications for different intervals. Germinated grains were measured for the shoot length and root length, total weight, germination %, seedling vigor index, and the ratio of root length versus shoot length. It was found that ultrasound-processed treatment US1 (40% amplitude for 5 min) and microwave-processed treatment MW2 (700 W for 5 sec) significantly improved the germination parameters. Pre-sowing physical techniques cause disturbance of the seed coat, which enabled water diffusion into the seeds inducing a higher rate of enzymatic reactions, and the start of the initial development stages consequently resulted in faster and more effective germination.

Keywords: *Sorghum, Germination, Microwave, Sonication, Physical treatment*

Article History

Received: 01 Jul 2023
Accepted: 19 Jul 2023
Published: 22 Jul 2023

Scan QR code to view•

How to cite this paper: Hassan, S. Impact of Pre-Sowing Physical Treatments on The Seed Germination Behaviour of Sorghum (Sorghum bicolor) . *IPS Journal of Nutrition and Food Science*, *2*(2), 33–36. https://doi.org/10.54117/ijnfs.v2i2.29.

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the 5th most important cereal grain crop consumed as a staple food in the world after wheat, rice, corn, and barley (Proietti *et al.*, 2015). It is used as a staple food in 30 different countries for approximately 500 million people living in semi-arid tropical countries (Kumari *et al.*, 2016). Approximately 35% of sorghum is grown for human consumption, and it is also grown for other purposes like fodder, alcohol production, and other industrial productions. The cultivation of sorghum is easier and more economical as compared to other cereal crops because it can withstand the harsh conditions of the environment (Kangama and Rumei, 2005). In Pakistan, the annual production of sorghum is 0.21 M tons with an average yield of 620 kg/ H (Habib *et al.*, 2013). The cultivated area has increased by more than 60%, while still, a limited increase in yield is due to limited resources (Habib *et al.*, 2013; Taylor *et al.*, 2006).

Sorghum is used as a staple food and principal source of energy, protein, vitamins, and minerals by millions of people living in Asia, Africa, and the semi-arid tropics (Maunder, 2002; Raihanatu *et al.*, 2011). But the existence of various anti-nutritional factors creating complexes with many other food components reduces the nutritional value of sorghum grain and also decreases its organoleptic characteristics (Ogbonna *et al.*, 2012). Sprouts developed from the seed after germination contain bioavailable proteins, lipids, vitamins, minerals, and phenolic components (Helal, 2016). Various processing techniques, such as soaking, germination, and cooking, can enhance the nutritional and functional characteristics of seeds (Kajihausa *et al.*, 2014). The germination process separates the anti-nutritional constituents such as phytate or mineral, therefore, making it bio-accessible (Oghbaei and Prakash, 2016). These techniques, individually or in combination, can enhance the nutritional quality of sorghum grains by eliminating the anti-nutritional components (Raihanatu *et al.*, 2011).

The application of new and emerging novel technologies is one of the main challenges to the improvement of food quality. Seed pre-treatments, including physical and chemical treatments, are widely used to enhance plant performance. To stimulate plant growth, as a substitute for chemical methods, physical methods appeal more consideration of agricultural producers. These physical methods more efficiently improve food quality without damaging its safety (Aladjadjiyan, 2011). Various physical treatments, for example, microwaves and electric irradiations, are popular techniques to improve seed enactment (de Sousa Araújo *et al.*, 2016). These physical technologies affect the biochemical and physiological process of growing seeds without harmfully affecting the environment; therefore, they can enhance plant performance. Chemical technologies are also widely used, but they can create a burden for their disposal in the environmental cycle (Sharma *et al.*, 2015).

For facilitating the sowing process, protecting seeds, and improving their growth and development in severe environmental situations, now different techniques have been established. Recently, ultrasound has gained the attention of experts. The process of cavitation contains a series of phenomena, including the creation, development, and collapse of micro-bubbles produced in a liquid when ultrasound waves travel through the medium (López-Ribera and Vicient, 2017). The promotion of cell growth can be achieved by the use of ultrasound waves in proper intensity and duration, which stimulate seed physiological activities or increase its enzymatic activities (Chowdhury *et al.*, 2014). Similarly, microwave treatment is also effective, as high-frequency microwaves have positive effects on plant growth, germination, and enzymatic activity (Soran *et al*., 2016). The main objective of this study was to assess the germination potential of sorghum grains after the application of ultrasonic and microwave techniques by using their different treatment levels. Different growth parameters were analyzed.

Material and Methods

Raw material procurement

Sorghum grains were purchased from a local grain market in Faisalabad (Pakistan) and cleaned to remove stones, dust, glumes, stalks, light materials, and broken and undersized grains. Sorted grains were kept in polyethylene bags to avoid moisture uptake and contamination before use.

Grains treatment

Grains were soaked in 5% sodium hypochlorite solution for surface sterilization, followed by washing until they reached neutral pH (Hamidi *et al*., 2013). Afterward, grains were soaked in excess water for 22 h at room temperature. The steeping water was drained off, and the grains were washed twice and divided into four portions.

Processing of grains

Sorghum grains were grouped into four portions, each having 100-gram grains. The first portion was not subjected to any technique which served as a control group (C). The second portion was further divided into four treatments, and grains were subjected to ultrasonic treatments using two ultrasonic intensities (US_1: 40%; US_2: 60%) with two-time durations (t_{US1}: 5 min; t_{US2}: 10 min) at constant power (750 W), and frequency (20 kHz) (Machikowa *et al*., 2013). The third portion was also divided into four treatments and exposed to microwave treatments at two power levels (MW_1: 450 W; MW_2: 700 W) for two-time durations (t_{MW1}: 15 sec; t_{MW2}: 30 sec) at the constant microwave frequency (2450 MHz) (Aladjadjiyan, 2010). Similarly, the fourth portion was also divided into four treatments and exposed to both microwave (MW_1, MW_2) and ultrasonic (US_1, US_2) treatments at two different time levels (t_{US}, t_{MW}). The detailed treatment plan is presented in Table 1.

Table 1. The treatment plan for processing of sorghum grains through different techniques

Ultrasonic group (US)			Microwave group (MW)			Combined group (UM)		
Treatment	Time (min)	Amplitude (%)	Treatment	Time (sec)	Power (watt)	Treatment	Time US (min): MW (sec)	amplitude (%): power (watt)
Control (C)								
US_1	5	40	MW_1	15	450	UM_1	5:15	40:450
US_2	5	60	MW_2	15	700	UM_2	5:15	60:700
US_3	10	40	MW_3	30	450	UM_3	10:30	40:450
US_4	10	60	MW_4	30	700	UM_4	10:30	60:700

Note: The selected treatment conditions for ultrasound (US) and microwave (MW) processing were based on some preliminary trials.

Germination process

All the untreated (control) and treated grains were subjected to germination through the method reported by Nour *et al.* (2016) with little modification. About 100 seeds from each treatment were germinated separately to analyze different growth parameters. Grains placed on moist paper towels in germination trays were covered with another sheet of paper towel. The trays were shifted into an incubator at 25±2 °C for 48 hrs to conduct germination. Trays were watered two or three times daily to stimulate the germination activity. The grains were considered to be germinated when both the root and shoot had emerged up to ≥ 0.5 cm (Geressu and Gezaghegne, 2008).

Growth parameters

After germination, all germinated grains, including control and treated groups, were collected for analysis of growth parameters, including shoot length (SL) and root length (RL) in cm, total weight in gram (g), germination % (GP) of grains, seedling vigor index (SVI), and RL/SL ratio (Aladjadjiyan, 2010; Abdul-Baki and Anderson, 1973).

GP was calculated according to the following formula:

$$GP = (N_g/N_t) \times 100$$

Where N_g refers to the total number of germinated grains, N_t is the total number of grains used.

SVI was calculated by the following formula:

SVI = seedling length (cm) x germination percentage (%)

Where, Seedling length = root length + shoot length

Statistical analysis

The obtained data were subjected to statistical analysis to determine the significance level. Data are given as mean values along with their standard deviation. Data were analyzed at a 5% significance level, using analysis of variance (ANOVA) (Minitab software version 17) and least significant difference (LSD) post hoc test (Statistical Software SPSS 21).

Results and Discussion

Effect of ultrasound processing

It is understandable from the results that the ultrasonic treatment significantly affected the germination frequency (G) along with root length (RL), shoot length (SL), total weight, and seedling vigor index (SVI), while root length/shoot length ratio (RL/SL) was not significantly ($p > 0.05$) changed. The means for germination parameters in ultrasonic-treated seeds are presented in Table 2. The results showed that seed germination percentage increased at 40% (US_1: 94.00±3.00 %) & 60% (US_2: 85.00±1.00 %) of total ultrasonic amplitude for 5 minutes as compared to untreated control sprouts (78.00±2.00 %). However, increasing exposure time by up to 10 minutes decreased seed germination. The stimulating efficiency of ultrasonic treatment on sorghum grains was observed at the amplitude level of 40% of the output power with an exposure time of 5 minutes (US_1). The variation in duration for ultrasonic amplitude has also resulted in variations in the root and shoot lengths and the total weight of sprouts. From the data, the mean root length of the treated seedlings ranged 1.40±0.60 to 2.80±0.10 cm, compared to 2.40±0.30 cm of the control treatment. A similar result was detected in shoot lengths of the processed seedlings, which ranged from 1.10±0.20 to 2.20±0.30 cm compared to 1.90±0.10 cm of the control. The total weight range for treated seeds was 1.51±0.06 to 2.56±0.04 g, while that of control was 2.42±0.20 g. Treatment US_1 at the amplitude of 40% for the duration of 5 minutes produced maximum RL (2.80±0.10 cm), SL (2.20±0.30 cm), and TW (2.56±0.04 g). The size of the seedlings and weight were decreased at 40% (US_3) and 60% (US_4) amplitude for 10 min. Similarly, treatments US_1 & US_2 gave high values of RL/SL ratios of 1.29±0.22 & 1.92±0.24, respectively. It is clear from the results that the use of ultrasonic amplitude increased the SVI % more than the control (335.53±21.02 %). The duration of ultrasound exposure to seeds resulted in SVI % variations. The maximum SVI (US_1: 470.40±33.80 %) of seedlings was observed at 5 minutes of ultrasonic exposure with 40% amplitude. While the amplitude of 40% (US_3) and 60% (US_4) for 10 min decreased the SVI. Prolonged treatments of ultrasonic at higher amplitude for longer exposure time possibly imposed injury on the embryo and decreased the germination parameters, as shown in Table 2. Long-time duration of sonication (10 min) declined germination performance. It's possible sonication treatment of 10 min might be too high to be endured by small and fragile sorghum grains resulting in a cell lysis process and alternations of metabolic reactions, including endogenous hormonal balance (Chen *et al.*, 2013).

A study conducted by Shekari *et al.* (2015) showed an increase in the seedling length of sesame seeds when sonicated for 10 min, and seedling length decreased on prolonged exposure for 30 min. According to work described by Aladjadjiyan (2012), the seedling length of lentils had a direct relationship with US exposure time. It is confirmed that ultrasound applies its major effects by causing mechanical changes (acoustic cavitation) and disturbance of cell walls. The ultrasonic application could fragment the seed shell causing larger porosity on the surface of barley grains by the captivation of ultrasound (shock waves). Thus, increasing water uptake and water retention capacity in dry grains result in better hydration. The extra absorbed water reacts freely and readily with the cell embryo, so metabolic processes in the form of gibberellic acid release and activation of enzymes expedited and increased the rate of enzyme-catalyzed hydrolysis reactions within the seeds (Chen *et al.*, 2013).

Effect of microwave processing

According to the presented data of means regarding germination parameters given in Table 3, it could observe that both exposure time and microwave power influenced germination frequency. For the untreated control treatment, the percentage of germination frequency was 78.00±2.00 %. Microwave seed treatment for 15 sec time at different power levels, 450 W (MW_1) & 700 W (MW_2), resulted in a significant increase of this parameter as 92.00±1.25% and 95.00±3.00%, respectively. Germination capacity decreased significantly for MW_3 (72.00±0.90%) & MW_4 (67.00±1.50%) when the same power levels were used for 30 sec. In general, microwave seed treatment (MW_4) for time 30 sec at 700 W negatively affected this parameter. Samples exposed to radiation for 15 sec (MW_1 & MW_2) demonstrate the biggest stem and root length, while higher exposure times of 30 sec (MW_3 & MW_4) again lead to a decrease of these parameters. For exposure of 15 sec at 700 W (MW_2), SL was 2.06±0.45 cm, and RL was 2.90±0.50 cm longer than the control. The samples exposed for 30 sec (MW_4) gave shorter SL (1.20±0.60 cm) and RL (1.60±0.20 cm). It can be noticed that at higher radiation amplitude and longer exposure times, the positive effect of microwave stimulation is weaker. Similarly, the total weight for the samples treated with 450 W (MW_1) at 15 sec was 2.53±0.50 g, and for those treated with 700W (MW_2) was 2.55±0.30 g, so higher than the control (2.42±0.20 g). At the same time, RL/SL ratio was higher only in treatment MW_1 (1.50±0.05) as compared to the control (1.27±0.22). It is concluded that for 700 W, the exposure at 15 sec (MW_2) is more effective in later stages of development than the exposure at 30 sec. The presented results regarding the vigor index (SVI) showed output powers of 450 W & 700 W (MW_1 & MW_2) as stimulation of the growth (SVI: 414.41±51.62 % & 473.73±105.10 %, respectively) at 15 sec. The exposure time of 15 sec is a noticeable growth stimulation compared with controls. Microwave radiation power of 700 W for 30 sec (MW_4: 188.40±57.80 %) showed a decrease in SVI than control (335.53±21.02 %). Results can be described as an effect of absorbed energy. Radiations with high output power for long exposure time resulted in more energy absorption by the object. The stimulated germination might be due to the disruption of the seed coat through microwave treatment, which enabled water diffusion into the seeds inducing a higher rate of enzymatic reactions and the start of the development, consequently resulting in fast and effective germination (Iuliana *et al.*, 2013a).

The results obtained in this investigation correspond well with investigation of Naeem *et al.* (2013), the effect of microwave radiations on okra and corn seed germination and plant growth were studied. Seeds were exposed to microwave radiation (2450 MHz) for 0 (control), 1, 2, and 3 sec and showed a decrease in germination percentage of 80%, 40%, and 25%, respectively, as compared to control (100%). Barley seeds exposed to microwave treatment at 400 W and 720 W with a frequency of 2.45 GHz for 0, 10, and 20 sec showed the best results for the treatment with output microwaves power of 400W for the 20 sec (Iuliana *et al.*, 2013b). The influence of microwave irradiation (400 & 720 W) treatment on the development of barley seeds with a frequency of 2.45 GHz for the exposure time 0, 30, 60, and 90 sec was studied. Seeds exposed to irradiation for a shorter period of 30 sec and lower microwave power 400 W showed higher germination capacity (Iuliana *et al.*, 2013a).

Effect of combined processing

It is indicated through results that combined treatment application significantly affected the germination % along with root length, shoot length, total weight, and seed vigor index, while the root length/shoot length ratio was not significantly ($p > 0.05$) changed. Sorghum grains were treated with both ultrasonic and microwave applications to check that their effect on germination was either synergistic or antagonistic. According to means values (Table 4), germination frequency showed a maximum value of 75.00±2.50 % at UM_2 (US: 60 % for 5 min & MW: 700 W for 15 sec) higher than UM_1, UM_3 and UM_4 but lower as compared to control (78.00±2.00 %). Similarly, as compared to other treatments (UM_1, UM_3 & UM_4), UM_2 showed high values of other growth parameters like SVI (224.66±7.50 %), RL (1.50±0.10 cm), SL (1.50±0.10 cm), TW (2.16±0.06 g) and RL/SL (1.00±0.05) but all these values were lower than control. The combined application of ultrasound and microwave negatively affected the germination and growth parameters because all the values were significantly decreased as compared to the control. In combination, both techniques caused more intense effects, which may negatively affect metabolic processes and cause alterations in endogenous hormonal balance, thus negatively reducing germination. Microwave heating and ultrasonic waves are among the most simple, inexpensive, and valuable tools. Besides saving energy, these green techniques promote faster and more selective transformations (Cravotto and Cintas, 2007).

In a study, *Melia dubia* seeds were treated with a combined application of microbial consortia and microwave energy. Seeds were exposed to microwave energy (2450 MHz) for 7.5 minutes, followed by seed pelletization with selected microbial consortia (Ravi *et al.*, 2012). It was recorded the highest germination percentage of 68% over control as compared to 5 min, 10 min, 2.5 min & 20 min of microwave energy. Even though many methods, like stratification with sandpaper, acid, and hot water treatments, help in cracking the seed coat and allowing imbibition, the methods did not work with the study material. Many authors reported that the low seed germination in hard seed-coated and physiologically dormant seeds could be overcome by various physical and chemical treatments. The effect of priming with gibberellic acid and chilling stratification on improving germination and growth of Eastern black walnut seeds was studied. The highest percentage of seed germination (69.27 %) was recorded with the combined treatment of two months of chilling and gibberellic acid (400 ppm) (Parvin *et al.*, 2015).

Table 2. Germination parameters for ultrasound-processed sorghum sprouts

Treatments	G (%)	RL (cm)	SL (cm)	RL/SL	TW (g)	SVI (%)
C	78.00±2.00^{abcd}	2.40±0.30^{ab}	1.90±0.10^{abc}	1.27±0.22^{ns}	2.42±0.20^{abcd}	335.53±21.02^{abcd}
US_1	94.00±3.00^{abcd}	2.80±0.10^{abc}	2.20±0.30^{abc}	1.29±0.22^{ns}	2.56±0.04^{abcd}	470.40±33.80^{abcd}
US_2	85.00±1.00^{abcd}	1.90±0.40^{a}	1.20±0.50^{ab}	1.92±0.24^{ns}	2.09±0.02^{abcd}	263.43±5.40^{abcd}
US_3	64.00±2.00^{abcd}	1.50±0.20^{ab}	1.30±0.10^{ab}	1.17±0.24^{ns}	1.56±0.01^{abc}	179.33±12.00^{abcd}
US_4	47.00±1.50^{abcd}	1.40±0.60^{ab}	1.10±0.20^{ab}	1.23±0.32^{ns}	1.51±0.06^{abc}	116.70±33.85^{abcd}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) are indicated by various letters. ns: non-significant; a: one treatment is significantly different from one other treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from the other three treatments; abcd: one treatment significantly different from the other four treatments.

Table 3. Germination parameters for microwave-processed sorghum sprouts

Treatments	G (%)	RL (cm)	SL (cm)	RL/SL	TW (g)	SVI (%)
C	78.00±2.00^{abcd}	2.40±0.30^{abcd}	1.90±0.10^{ns}	1.27±0.22^{ns}	2.42±0.20^{ns}	335.53±21.02^{ab}
MW_1	92.00±1.25^{abcd}	2.70±0.30^{abc}	1.80±0.20^{ns}	1.50±0.05^{ns}	2.53±0.50^{ns}	414.41±51.62^{ab}
MW_2	95.00±3.00^{abcd}	2.90±0.50^{abc}	2.06±0.45^{a}	1.41±0.07^{ns}	2.55±0.30^{ns}	473.73±105.10^{abc}
MW_3	72.00±0.90^{abcd}	2.00±0.10^{abcd}	1.50±0.40^{ns}	1.41±0.26^{ns}	2.38±0.01^{ns}	251.82±18.45^{ab}
MW_4	67.00±1.50^{abcd}	1.60±0.20^{abcd}	1.20±0.60^{a}	1.55±0.19^{ns}	2.38±0.53^{ns}	188.40±57.80^{abc}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) are indicated by various letters. ns: non-significant; a: one treatment is significantly different from one other treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from the other three treatments; abcd: one treatment significantly different from the other four treatments.

Table 4. Germination parameters for combined application processed sorghum sprouts

Treatments	G (%)	RL (cm)	SL (cm)	RL/SL	TW (g)	SVI (%)
C	78.00±2.00^{abc}	2.40±0.30^{abcd}	1.90±0.10^{abc}	1.27±0.22^{ns}	2.42±0.20^{abcd}	335.53±21.02^{abcd}
UM_1	63.00±0.70^{abcd}	1.30±0.40^{a}	1.20±0.30^{a}	1.07±0.06^{ns}	1.57±0.40^{abcd}	157.17±42.35^{abc}
UM_2	75.00±2.50^{abc}	1.50±0.10^{a}	1.50±0.10^{ns}	1.00±0.05^{ns}	2.16±0.06^{abcd}	224.66±7.50^{abcd}
UM_3	52.00±1.00^{abcd}	1.10±0.30^{a}	1.10±0.60^{a}	1.14±0.40^{ns}	1.42±0.01^{abcd}	113.80±44.60^{ab}
UM_4	41.00±0.50^{abcd}	0.90±0.50^{a}	1.00±0.40^{a}	1.17±0.24^{ns}	1.33±0.03^{abcd}	77.86±3.15^{abc}

Mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) are indicated by various letters. ns: non-significant; a: one treatment is significantly different from one other treatment; ab: one treatment is significantly different from other two treatments; abc: one treatment is significantly different from the other three treatments; abcd: one treatment significantly different from the other four treatments.

Conclusion

Physical stimulating technologies are an innovation in the research area of seed invigoration. Novel technologies, such as sonication and microwave, could be easily adapted to stimulate germination. Microwave and ultrasonic processing of sorghum grains resulted in significantly improved germination percentage and growth parameters. The results showed that seed germination percentage was maximum at 40% (US_1: 94.00±3.00 %) amplitude for 5 minutes. The same treatment (US_1) produced maximum RL (2.80±0.10 cm), SL (2.20±0.30 cm), TW (2.56±0.04 g), RL/SL ratio (1.29±0.22), and SVI % (470.40±33.80 %). Ultrasound treatment can enhance enzymatic and physiological activities if applied at an appropriate amplitude level and duration. Microwave seed treatment for a time of 15 sec at a power level of 700 watts (MW_2) resulted in a significant increase of germination percentage 95.00±3.00 % as compared to the control. Treatment MW_2 showed the highest results of other growth parameters such as RL: 2.90±0.50 cm, SL: 2.06±0.45 cm, TW: 2.55±0.30 g, SVI: 473.73±105.10 %). Microwave electromagnetic treatment caused disturbance of the seed coat, which enabled water diffusion into the seeds inducing a higher rate of enzymatic reactions, and the start of the initial development stages consequently resulted in faster and more effective germination. However, more research is needed to determine the effect of processing through novel technologies as an important tool in terms of value addition for cereals, especially on the nutritional and viscoelastic properties of sorghum.

Acknowledgment

The author thanks the Higher Education Commission of Pakistan for financial support.

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