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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Reducing the adverse effects of salt stress on bean seeds exposed to different levels of salt stress by ultrasonic sound waves

Farklı seviyelerde tuz stresine maruz bırakılan fasulye tohumlarında tuz stresinin olumsuz etkilerinin ultrasonik ses dalgalarıyla azaltılması

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ABSTRACT

Irregularities in precipitation due to climate change in recent years cause various adverse conditions in soils. Plants growing in these soils are affected by these adverse conditions and create mechanisms against these adversities. In this study, the resistance to salt stress was investigated as a result of the application of ultrasonic sound waves before sowing to bean seeds under salt stress. Salt stress was created by using NaCl salt at 0, 5, 10 and 15 dS m^{-1} levels. Ultrasonic sound waves were applied at 50 Hz for 20 min in an ultrasonic sound waves device. The study was established in the laboratory of Isparta University of Applied Sciences Faculty of Agriculture, Department of Field Crops with 3 replicates in completely randomized design. In the study, germination (germination rate, average germination time, germination index, vigour index), seedling development (seedling length, root length, dry matter content and salinity tolerance index) were examined in plants grown under stress conditions. When the results were analysed, ultrasonic sound waves had a stimulating effect on non-stressed plants and also increased the salt tolerance of bean plants under 5 and 10 dS m^{-1} salt stress. Except for average germination time and dry matter content, ultrasonic sound wave treatments had positive effects on all traits examined. The results of the study showed that the negative effects of salt stress in beans can be reduced by the application of ultrasonic sound waves.

ÖZET

Son yıllarda meydana gelen iklim değişikliği sebebiyle yağışlarda görülen düzensizlikler topraklarda çeşitli olumsuzlukları ortaya çıkarmaktadır. Bu topraklarda yetişen bitkilerde bu olumsuz koşullardan etkilenmekte ve bu olumsuzluklara karşı mekanizmalar oluşturmaktadır. Bu çalışmada, tuz stresine maruz bırakılmış fasulye tohumlarına ekimden önce ultrasonik ses dalgası uygulamalarının tuz stresine dayanıklılık üzerindeki etkileri araştırılmıştır.Tuz stresi 0, 5, 10 ve 15 dS m^{-1} seviyelerinde, NaCl tuzu kullanılarak oluşturulmuştur. Ultrasonik ses dalgaları ultrasonik ses dalgaları cihazında 50 Hz olarak 20 dk boyunca uygulanmıştır. Çalışmada tesadüf parselleri deneme deseninde 3 tekerrürlü olarak Isparta Uygulamalı Bilimler Üniversitesi Ziraat Fakültesi Tarla Bitkileri bölümü laboratuvarında kurulmuştur. Çalışmada stres koşullarında yetiştirilen bitkilerde çimlenme (çimlenme oranı, ortalama çimlenme süresi, çimlenme indeksi, vigor indeksi), fide gelişimleri (fide uzunluğu, kök uzunluğu, kuru madde oranı ve tuzluluğa tolerans indeksi) incelenmiştir. Sonuçlar incelendiğinde, ultrasonik ses dalgaları stres altında olmayan bitkilerde teşvik edici özelliğe sahip olmuş ayrıca, 5 ve 10 dS m^{-1} tuz stresi altındaki fasulye bitkilerinin ise tuz toleranslarını artırmıştır. Ortalama çimlenme süreci ve kuru madde oranı hariç, incelenen tüm özelliklerde ultrasonik ses dalgası uygulamaları olumlu etkiler ortaya çıkarmıştır. Çalışma sonuçları, fasulyede tuz stresinin olumsuz etkilerinin ultrasonik ses dalgalarının uygulanmasıyla azaltılabileceğini göstermiştir.

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INTRODUCTION

Agriculture is an economic activity that depends on nature. Due to any stress factor encountered in agricultural production, significant yield and quality losses may occur and even production may be interrupted. Abiotic stress factors such as salinity, drought and extreme temperatures are among the main causes of yield losses in crop production in the world (Ilyas et al., 2020). In the world, 6.5% of the total amount of land and 19.5% of irrigated land are affected by salt (FAO, 2022). However, Bouthour et al. (2015) predicted that 50% of arable land will be affected by salinity by 2050.

While salinity shows its direct effect on plants by osmotic and ion stress, its indirect (secondary) effect is shown by structural disorders and synthesis of toxic compounds in the plant as a result of stress. Another reason for the decline in growth and development in plants grown under saline conditions is the inhibition of the uptake and transport of plant nutrients (Cramer & Nowak, 1992). Inhibition of potassium uptake, metabolic toxicity, inhibition of photosynthesis and cell death can be counted among the secondary effects caused by salinity (NaCI) (Hong et al., 2009). Increased salt concentration in the soil solution with salt stress and decrease in water potential cause a decrease in osmotic potential in plant cells. Thus, osmotic stress occurs in the plant root zone (Hussain et al., 2013).

Among the growth and development periods of plants, germination and early seedling development periods are the most sensitive periods to salt stress. For this reason, these periods are considered as critical periods in the examination of the varieties to be grown in terms of salt tolerance or sensitivity. In addition, as a fast, easier and low-cost method, salinity tolerance studies at germination and seedling stages are more preferred than studies to be carried out at later developmental stages (Shelke et al., 2017). Soil salinity causes more detrimental effects on seed germination due to the accumulation of salts mostly in the surface layer (Bybordi & Tabatabaei, 2009). Developing seedlings following germination are initially supported by the mobilisation of seed reserve nutrients (Pandey & Penna, 2017). Rapid germination and vigorous seedling development increase plant salinity resistance and thus play an important role in maintaining plant growth and yield potential (Carpıcı et al., 2009).

Sound is an external factor that has a great influence on the biological index of plants (Yiyao et al., 2002; Yang et al., 2002; Yi et al., 2003) and can promote or suppress growth. Sound is the oscillation of pressure waves transmitted through solids, gases and liquids. As the waves propagate, they carry energy. In addition, sound emits information about the environment and living organisms depend on this information or communicate through wave motion. Humans can hear sounds with frequencies ranging from 20 Hz to 20,000 Hz (Hertz) (Wereski, 2015). Sound waves with frequencies higher than 20 kHz are called ultrasonic sound waves. In ultrasonic sound applications, seeds are exposed to waves between 20 and 100 kHz. Ultrasonic applications are widely used in the food industry in terms of surface sterilisation of the product and sterile material production (Benedito et al., 2002). The combined application of ultrasonic sound waves, temperature and $Ca(OH)_2$ was found to be effective in disinfecting alfalfa seeds contaminated with Salmonella and *E.coli* (Scouten & Beuchat, 2001), and similar results were obtained in broccoli seeds (Kim et al., 2006). Another area of use of ultrasonic sound wave applications is the improvement of germination and elimination of dormancy in seeds. According to the literature studies, it was determined that ultrasonic sound wave applied in different seeds increased germination and emergence rate (Miano et al., 2015; Dönmez, 2018; Rifna et al., 2019). Demirsoy et al. (2020) applied different levels (50% and 100% amplitude) of ultrasonic sound waves at different times (0, 5, 10 and 15 min) to *Solanum torvum* plant. They found that the application of 50% ultrasonic sound wave for 5 min had positive effects on early germination and high germination and emergence rates.

Lahijanian & Nazari (2017) reported that ultrasonic priming creates small porous voids in the seed coat and also weakens the cell wall of seed cotyledons, increasing the absorption and oxygen uptake of seeds. They used a frequency of 42 kHz in the study. Ultrasonic treatment was performed at 0 (dry control and wet control), 10, 20 and 30 minutes and at 30°C. In ultrasonically treated bean seeds, the highest values were obtained from 20 minutes ultrasonic treatment. They stated that it provided a significant increase in seed germination rate (11.30 seeds d^{-1}) and cell area (1467.21 µ2). El-Sattar & Tawfik (2023) applied different doses of NaCl salt and ultrasonic waves for different durations to fenugreek. They found that with increasing NaCl doses, there was a significant decrease in germination percentage and all growth criteria, while the biomass produced by fenugreek increased. They suggested that these were mechanisms developed by the seedlings for salinity tolerance. They determined that the ultrasonic waves they applied had a significant effect on the genetic stability of DNA content, seed germination, early seedling development, and biochemical components under normal and stress conditions. It has been stated by many researchers that ultrasonic sound waves applied in the studies provide positive effects on the output power, but the investigation of the inhibitory effect against stress when applied under abiotic stress conditions specified in the study increases the originality of the study. In the light of this information in this study, the effects of ultrasonic sound waves on germination and early seedling development were examined by applying ultrasonic sound waves for 20 minutes in order to eliminate or minimise the negativities occurring during salinity stress.

MATERIALS and METHODS

This study was carried out in xxxx, Faculty of Agriculture, Field Crops laboratories. 'Nazende' bean variety was used as seed material in the study. Salinity stress was induced by using NaCl salt at 0, 5, 10 and 15 dS m^{-1} levels with electrical conductivity (EC) device (Figure 1). In the study, 25 seeds were used for each treatment and replicate.The control group of the seeds prepared in the study were placed in beakers of pure water (25 ml) at room temperature (25°C) and kept for 20 min (Figure 2). Then the seeds were removed from the water and the seed surface was dried.The device was operated for 30 min with pure water before the seeds were placed.

Figure 1. Electrical conductivity device and prepared solutions (distile water, 5, 10 and 15 dS m^{-1})

Şekil 1. Elektriksel iletkenlik cihazı ve hazırlanmış solüsyonlar (safsu, 5, 10 ve 15 dS m^{-1})

The ultrasonic sound wave treated seeds were placed in balconies and pure water (25 ml) was added. Then, the falcon tubes were placed in the device (vibration frequency 50 Hz and voltage 220 V, Figure 3) together with the stand and ultrasonic sound wave was applied to the seeds at 25°C for 20 min (Figure 4). At the end of this process, the seeds were again removed from the water and the seed surface was dried. In the study, the seeds were established in the incubator at 22°C according to the ISTA rules of paper space (21 x 21 cm) method and continued for 9 days (ISTA, 2010) (Figure 5). During the study, equal amounts of test solutions (0, 5, 10 and 15 dS m-^{1} levels) were applied between each paper when needed.The study was carried out in 3 replicates according to the completely randomized design.

Figure 2. Soaking of bean seeds in distile water (control group)
Şekil 2. Fasulye tohumlarının saf suda bekletilmesi

Figure 3. Ultrasonic sound wave application device
Şekil 3. Ultrasonik ses dalgası uygulama cihazı

Figure 4. Ultrasonic sound wave application to bean seeds
Şekil 4. Fasulye tohumlarına ultrasonik ses dalgası uygulaması

Figure 5. Image of the first and last day of the trial according to the paper in between method

Şekil 5. Kağıt arası yöntemine göre denemenin ilk ve son gününe ait görüntü

20-0: Ultrasonic sound wave applied and (0 dS m-1) control group; 20-5: Ultrasonic sound wave applied and 5 dS m-1 salt applied group; 20-10: Ultrasonic sound wave applied and 10 dS m-1 salt applied group; 20-15: Ultrasonic sound wave applied and 15 dS m-1 salt applied group; 0-0: Control group without ultrasonic sound wave; 0-5: Control group and 5 dS m-1 salt applied group; 0-10: Control group and 10 dS m-1 salt applied group; ; 0-15: Control group and 15 dS m-1 salt applied group

20-0: Ultrasonik ses dalgası uygulanmış ve (0 dS m-1) kontrol grubu; 20-5: Ultrasonik ses dalgası uygulanmış ve 5 dS m-1 tuz uygulanan grup; 20-10: Ultrasonik ses dalgası uygulanmış ve 10 dS m-1 tuz uygulanan grup; 20-15: Ultrasonik ses dalgası uygulanmış ve 15 dS m-1 tuz uygulanan grup; 0-0: Ultrasonik ses dalgası uygulanmamış kontrol grubu; 0-5: kontrol grubu ve 5 dS m-1 tuz uygulanan grup; ; 0-10: kontrol grubu ve 10 dS m-1 tuz uygulanan grup; ; 0-15: kontrol grubu ve 15 dS m-1 tuz uygulanan grup

The seeds with 2 mm radicle in the seeds checked every day were called germinated seeds and germinated seeds were counted every day. Germination rate (%) was calculated as the ratio of the number of germinated seeds to the total number of seeds at the end of the period specified in ISTA rules (9 days) (Karaman & Kaya, 2017). Germination index was obtained by dividing the proportion of germinated seeds per day by the number of counting days (Wang et al., 2004).

Germination index = ∑ (Proportion of germinated seeds per day)/(Counting day) **Eq.(1)**

Mean germination time (MGT) was obtained by dividing the sum of the product of the number of germinated seeds and the number of germination days by the total number of germinated seeds (Kaya et al., 2005; Karaman & Kaya, 2017).

$MGT = \sum(fx)/\sum f$ **Eq.(2)**

f: Number of germinated seeds; x: Germination day

For germination index and average germination time, germinated seeds were counted every day and the day when germination was fixed was considered as the last counting day. At the end of the 9th day of the study, shoot and root lengths were determined by measuring with a millimeter ruler on 10 seedlings randomly selected from the replicates in each treatment. Vigor index (VI; germination strength) is obtained by summing the seedling and root lengths and multiplying by the germination rate (Sivritepe, 2011).

VI = Germination rate x (rootlet length + seedling length) **Eq.(3)**

After the seedlings and roots were measured, their wet weights were determined. Shoot and root wet weights were weighed and then dried in an oven at 65°C until constant weight was reached. The dried seedlings were weighed on a precision balance and their dry weights were determined. Finally, dry matter rate was calculated by dividing the dry weight by the wet weight and multiplying the result by 100 (Temel and Tan, 2020). Salt tolerance index (STI) was calculated according to the formula:

STI = (Total wet weight at Sx / Total wet weight at S0) x 100 **Eq.(4)**
Sx: Salt concentration, S0: Control (Kuşvuran et al., 2015)

Evaluation of data
The data were subjected to analysis of variance with the MINITAB statistical package programme according to the completely randomized design and means were compared to Tukey test at 0.05 level.

RESULTS and DISCUSSIONS

The results of analysis of variance for all traits examined as a result of ultrasonic sound wave applications for the inhibition of abiotic stress of bean seed treated with different salt doses are given in Table 1. When Table 1 was examined, the differences between ultrasonic sound wave applications, salt doses and ultrasonic sound wave applications x salt doses interactions were found to be statistically significant (at 0.01 and 0.05 levels) in all traits except average germination time and dry matter content. Only salt doses were found statistically significant ($p<0.01$) in the mean germination time and salt doses and ultrasonic sound waves application were found statistically significant ($p<0.01$) in the dry matter ratio. The averages and difference groupings for germination traits are given in Table 2, and the averages and difference groupings for seedling traits are given in Table 3.

Germination properties
The highest germination rate value in terms of salt doses was obtained from 0 dS m^{-1} dose (99.67%), while the lowest germination rate was obtained from 15 dS m^{-1} (45.0%) salt dose application (Table 2). Increasing salt doses caused a significant decrease in germination rate. The germination rate of ultrasonic sound waves application was significantly higher than the control. When the interaction of ultrasonic sound wave application applied as salt stress inhibitor was examined, the germination rate varied between 34% and 100%. The germination rate of the control application (no ultrasonic sound wave application) (100%) and ultrasonic sound wave application (99.3%) of 0 dS m^{-1} dose had the highest values, while the germination rate of bean seeds without ultrasonic sound wave application at 15 dS m^{-1} dose had the lowest value (34.0%). Germination index of bean seeds decreased with the increase in salt doses. Compared to the control, germination index decreased by 69.7% with the highest salt dose application. Germination index increased by 33.1% with ultrasonic sound wave application. Interaction of salt doses and ultrasonic sound wave application showed that the highest values of germination index were found in the control application (no ultrasonic sound wave application) (10.79) and ultrasonic sound wave application

(10.69) at 0 dS m^{-1} dose and the lowest values were found in the control application at 15 dS m^{-1} (1.88) and 10 dS m^{-1} (2.57) doses.The mean germination time decreased with increasing salt doses.The longest germination time was realised at O dS m^{-1} and 5 dS m^{-1} doses. The change in germination time with ultrasonic sound applications did not show a statistical difference. This is because increasing salt doses inhibit germination. However, in the formula, germination time is calculated by multiplying the number of germinated seeds by the number of days to germination, divided by the total number of germinated seeds (Karaman & Kaya, 2017). The reason the study found a lower average germination time at higher doses is thought to be due to germination being completed early at higher concentrations and the suppression of re-germination due to salt concentrations. Vigour index was significantly higher (1063.46) with ultrasonic sound treatments. Vigour index averages (306.69-1326.31) decreased in parallel with the increase in salt doses. The highest vigour index was obtained with ultrasonic sound wave application at a dose of 5 dS m^{-1}, and the lowest was determined in the control without ultrasonic sound wave at a dose of 15 dS m^{-1}.

Table 1. Results of analysis of variance for ultrasonic sound wave applications for the inhibition of abiotic stress of bean seed

Çizelge 1. Fasulye tohumlarının abiyotik stresinin inhibisyonu için ultrasonik ses dalgası uygulamalarına ilişkin varyans analizi sonuçları

		Germination rate		**Germiantion Index**		**Mean Germination Time**	
Sources of variation	**SD**	**Adj MS**	**F Value**	**Adj MS**	**F Value**	**Adj MS**	**F Value**
Ultrasonic sound waves (A)	**1**	1472.67	77.17**	46.76	101.39**	1.04	0.77 ns
Salt doses (B)	**3**	3612.56	189.30**	66.45	144.09**	7.54	5.59**
AxB	**3**	314.56	16.48**	8.62	18.70**	1.61	1.20 ns
Error	**16**	19.08		0.46		1.34	
		Vigor Index		**Shoot length**		**Root length**	
	SD	**Adj MS**	**F Value**	**Adj MS**	**F Value**	**Adj MS**	**F Value**
Ultrasonic sound waves (A)		790113	327.74**	7.43	87.79**	20.57	79.48**
Salt doses (B)		1365640	566.47**	7.15	84.48**	30.11	116.37**
AxB		86501	35.88**	0.36	4.33*	2.83	10.96**
Error		2411		0.08		0.25	
		Dry matter rate		**Salt tolerance index**			
	SD	**Adj MS**	**F Value**	**Adj MS**	**F Value**		
Ultrasonic sound waves (A)		273.50	57.16**	11353.8	305.93**		
Salt doses (B)		129.30	27.02**	2213.1	59.63**		
AxB		12.58	2.63 ns	3663.8	98.72**		
Error		4.78		37.1			

**:Significant at 0.01 level; *:significant at 0.05 level; ns: not significant).

Germination rate decreased with increasing salt doses. However, ultrasonic sound waves applications increased germination rate compared to the control. Ultrasonic sound wave applications increased the germination rate in parallel with the increase in salt doses, especially except the control application. This increase was 11% at 5 dS m^{-1}, 67% at 10 dS m^{-1} and 65% at 15 dS m^{-1}. In the light of the data obtained in this study, the application of ultrasonic sound waves improved the salt stress on germination and seedling characteristics. Ultrasonic sound waves create microscopic cracks in the seed coat. These cracks increase water uptake and facilitate faster water flow. The extra water absorbed by the seed accelerates mass transfer by allowing it to react freely and easily with the cell embryo. This releases gibberellic acid and accelerates metabolic activity in the cells of the aleurone layer. Ultrasonic sound waves interfere with the structure of the cell membrane, helping to mobilize nutrients in the endosperm (Wang et al. 2020; El-Sattar and Tawfik, 2023). Salty conditions create oxidative stress in the plant, increasing the accumulation of reactive oxygen species (ROS). The application of ultrasonic waves increases the activity of antioxidant enzymes (such as SOD, CAT, and POD) in the seed. These enzymes protect cell membranes by neutralizing ROS and increasing germination rates. According to the literature studies, seed germination is one of the most important stages affecting seedling formation and yield of any crop under abiotic stress conditions. Indeed, salt stress has an inhibitory effect on seed germination and seedling development (Dutta & Bera 2014). The accumulation of sodium and chloride ions on seeds under salinity creates an external osmotic potential that restricts adequate water uptake. This phenomenon leads to poor activation of hydrolytic enzymes and seed germination is reduced in saline environments (Khajeh-Hosseini et al., 2003). Furthermore, seed dormancy is also increased under salinity stress, which may be responsible for delayed and reduced seed germination of salt-sensitive crops (Murillo-Amador et al., 2002). In legumes, especially beans, seed germination was significantly delayed and decreased under salinity conditions. When the literatures were examined, it was determined that chitosan had a mitigating effect on sorghum (Mulaudzi et al., 2022) and soybean (Mehmood et al., 2020) under salt stress. Karaman (2023) investigated the effects of salicylic acid, $CaCl_2$ and GA_3 applications on chickpea genotypes exposed to different levels of salt stress. As a result of the study, it was stated that GA_3 application at low levels reduced salt stress. As a result of ultrasonic sound waves application, it has been determined that it promotes germination in many seeds such as corn, barley, rice and sunflower (Aladjadjiyan, 2002; Florez et al., 2007; Yaldagard et al., 2008).

Table 2. Effect of ultrasonic sound waves on different levels of salt application on germination characteristics of beans

Çizelge 2. Ultrasonik ses dalgalarının farklı seviyelerde tuz uygulamasının fasulyenin çimlenme özellikleri üzerine etkisi

	Germination rate (GR;%)			Germiantion Index (GI)		
Salt doses	**Control**	**Ultrasonic sound wave application**	**Mean**	**Control**	**Ultrasonic sound wave application**	**Mean**
0 (control)	100.0 a	99.33 a	***99.67 A***	10.79 a	10.69 a	***10.74 A***
5 dS m^{-1}	84.00bc	93. 00 ab	***88.50 B***	7.30 c	10.06 ab	***8.68 B***
10 dS m^{-1}	48.00 d	80.33 c	***64.17 C***	2.57 e	8.34 bc	***5.46 C***
15 dS m^{-1}	34.00 e	56.00 d	***45.00 D***	1.88 e	4.63 d	***3.26 D***
Mean	**66.50 B**	**82.17 A**		**5.64 B**	**8.43 A**	

	Mean Germination Time (MGT; day)			Vigor Index (VI)		
Salt doses	**Control**	**Ultrasonic sound wave application**	**Mean**	**Control**	**Ultrasonic sound wave application**	**Mean**
0 (control)	7.70	7.41	***7.80 A***	1270.0 b	1382.61 ab	***1326.3 A***
5 dS m^{-1}	7.96	7.63	***7.56 A***	979. 76 c	1455.39 a	***1217.6 B***
10 dS m^{-1}	5.52	7.41	***6.46 AB***	356.67 d	998.31 c	***677.5 C***
15 dS m^{-1}	5.15	5.56	***5.35 B***	195.87 e	417.52 d	***306.7 D***
Mean	**6.58**	**7.00**		**700.57 B**	**1063.46 A**	

Seedling properties

Shoot length increased by 28.5% with ultrasonic sound wave application. In parallel with the increase in salt dose, shoot length decreased from 5.55 cm in the control treatment to 3.06 cm in the 15 dS m^{-1} salt treatment. In terms of salt dose x ultrasonic sound wave interaction, seedling length varied between 2.73 cm and 5.96 cm. The highest values were obtained at 0 dS m^{-1} and 5 dS m^{-1} salt doses with ultrasonic sound waves and the control without ultrasonic sound waves, and there was no statistical difference between the seedling lengths of the plants in these treatments. The lowest seedling length was determined at 15 dS m^{-1} salt dose (2.73 cm and 3.40 cm). Although there was a numerical difference in seedling length at this dose, this difference was statistically insignificant (Table 3).

The highest (6.62 cm) and the lowest (3.58 cm) root length values were determined at 5 dS m^{-1} and 15 dS m^{-1}, respectivelty. Increasing salt doses caused a significant decrease in root length. In parallel with shoot length, root length also increased with ultrasonic sound wave applications. The highest root length (10.02 cm) was obtained at 5 dS m^{-1} salt dose with ultrasonic sound wave applications, while the lowest root length (3.10 cm) was obtained at 15 dS m^{-1} in control treatment. There was no statistical difference between 15 dS m^{-1} salt dose control treatment, ultrasonic sound wave treatment and 10 dS m^{-1} control treatment (Table 3). The dry matter rate increased with the increase in salt doses. Dry matter ratio was highest in 15 dS m^{-1} salt dose treatment with 34.65% and lowest in 0 dS m^{-1} control treatment with 24.97%. In the study, dry matter content decreased by 21% with ultrasonic sound wave application compared to the control. Salinity tolerance index values were determined according to the control. The highest average salt tolerance index values were determined at 5 dS m^{-1} dose with 102.15, followed by 10 dS m^{-1} dose with 90.40, 15 dS m^{-1} dose with 73.08 and 0 dS m^{-1} dose with 58.46. Salinity tolerance index significantly increased with the application of ultrasonic sound waves. Salt tolerance index varied between 66.51 and 116.91 with the application of ultrasonic sound waves in salt stress of bean seeds.

Table 3. Effect of ultrasonic sound waves on different levels of salt application on seedling characteristics of beans
Çizelge 3. Ultrasonik ses dalgalarının farklı seviyelerde tuz uygulamasının fasulyenin fide özellikleri üzerine etkisi

	Shoot Length (SL; cm)			Root Length (RL; cm)		
Salt doses	**Control**	**Ultrasonic sound wave application**	**Mean**	**Control**	**Ultrasonic sound wave application**	**Mean**
0 (control)	5.15 abc	5.96 a	***5.55 A***	7.55 b	7.96 b	***7.76 B***
5 dS m^{-1}	4.45 c	5.63 ab	***5.04 B***	7.21 b	10.02 a	***8.62 A***
10 dS m^{-1}	3.26 d	5.04 bc	***4.15 C***	4.17 c	7.40 b	***5.79 C***
15 dS m^{-1}	2.73 d	3.40 d	***3.06 D***	3.10 c	4.07 c	***3.58 D***
Mean	**3.90 B**	**5.01 A**		**5.51 B**	**7.36 A**	

	Dry Matter Rate (DMR; %)			Salinity tolerance Index (STI)		
Salt doses	**Control**	**Ultrasonic sound wave application**	**Mean**	**Control**	**Ultrasonic sound wave application**	**Mean**
0 (control)	27.05	22.88	***24.97 C***	0.0 e	116.91 a	***58.46 D***
5 dS m^{-1}	27.40	23.08	***25.24 C***	94.51 bc	109.79 ab	***102.15 A***
10 dS m^{-1}	35.21	25.89	***30.55 B***	76.07 d	104.73 ab	***90.40 B***
15 dS m^{-1}	39.25	30.05	***34.65 A***	66.51 d	79.65 cd	***73.08 C***
Mean	**32.23 A**	**25.48 B**		**59.27 B**	**102.77 A**	

In the study, the highest tolerance index was obtained in the application of ultrasonic sound wave at 0 dS m^{-1} salt dose and the lowest was obtained in the untreated control dose. At control dose, there was no statistical difference between the ultrasonic sound wave applications at 5 and 10 dS m^{-1} doses. These results show that the hypothesis stated in the study, that is, the application of ultrasonic sound waves to the seed before sowing in salt stress has an inhibitory effect on salt stress in the seed, is supported. Shekari et al. (2015) reported that ultrasonic sound waves create bubbles in the water, which are necessary for seed germination. This creates mechanical pressure on the seed, increasing cell wall fluidity and creating micropores and microcracks in the cell wall. They reported that the application of ultrasonic sound waves increased germination characteristics and increased root and shoot lengths of seedlings, while also stimulating enzyme activity and the initiation of other biological processes. In this study, ROS accumulation increased in bean seedlings under salt stress. Consequently, antioxidant enzymes were activated, and the seedlings had higher salt tolerance indices (116%) than the control groups. As shown in Table 3, the application of ultrasonic sound waves at control, 5, and 10 dS m^{-1} doses resulted in increases of 16.91%, 16.17%, and 37.68%, respectively. Many researchers have investigated the effect of ultrasonic priming on the germination rate of different plants. Generally, ultrasonic treatment has a positive effect on germination rate. For example, the germination rate in pea, chickpea, wheat, watermelon and mung bean increased by 93%, 20%, 36%, 16% and 25%, respectively (Chiu and Sung 2014, Goussous et al. 2010, Miano et al. 2015).

Ultrasonic sound waves increase the porosity of the seed, enhancing its ability to absorb water and oxygen. In addition, ultrasonic sound waves accelerate its passage in aleurone cells by allowing extra absorption, water reacts easily with the cell embryo, releasing gibberellic acid and accelerating metabolic activities in the embryo (Wang et al., 2020; El-Sattar & Tawfik, 2023). For this reason, it is estimated that the seeds encountering stress as a result of ultrasonic sound wave applications to the seeds are less affected by this situation. In the study, dry matter content increased with increasing salt concentration, while decreases were observed at all doses of ultrasonic sound waves. As seen in Figure 5, germination began at the 15 dS m^{-1} dose, but seedling growth was slower than at the other doses. Therefore, it is believed that the dry matter content was higher during this initial growth period because the substances contained within the seed were inactive. Furthermore, the application of ultrasonic sound waves increases water uptake and oxygen availability by increasing porosity in the seed, which is thought to reduce the effects of salt stress.

Rapid and uniform germination had a positive effect on root and shoot length in seedlings (Ramteke et al. 2015, Liu et al. 2016). Yang et al. (2003) observed increases in ATP synthesis, cell resistance, and superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) enzyme levels with ultrasonic sound waves. They even found that increased enzyme levels in leaves, roots, and shoots were associated with a decrease in malondialdehyde levels. SOD and CAT enzymes play an important role in the conversion of superoxide (O_2^-) radical ions into O_2 (oxygen) and H_2O_2 (hydrogen peroxide) molecules. Therefore, an increase in the level of these enzymes serves as an important source for the reduction of stress metabolites (Dikilitaş et al., 2018)

Today, climate and soil are very important for the sustainability of agriculture. These conditions vary every year due to climate crises. Despite these conditions, the world population also needs to be fed healthily. Cultivation should be done in order to feed human beings. For this reason, alternative ways should be sought against abiotic stress conditions that arise due to the change in climatic conditions for cultivation. The important issue among alternative ways is the applicability of the determined method and its transferability to practice.

When the results obtained in this proposed project are evaluated, it is determined that ultrasonic sound waves application is very effective in terms of germination and seedling development in case of salt stress compared to the control. For this reason, it was determined that the application of ultrasonic sound waves for 20 minutes before sowing the seeds in the presence of any salt stress increases both germination, seedling development and salt resistance. As a result, considering global warming and significant climate changes that lead to salinity and low productivity it is recommended to apply ultrasonic sound waves to the seeds before sowing in salinity stress.

STATEMENT OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR'S CONTRIBUTIONS

The authors declare that they have contributed equally to the study.

STATEMENT OF ETHICS CONSENT

This article does not contain any studies involving human or animal subjects, so ethical approval is not required.

REFERENCES

Aladjadjiyan, A. (2002). Study of the infuence of magnetic feld on some biological characteristics of *Zea mays*. *Journal of Central European Agriculture*, *3* (2), 89-94.

Benedito, J., Carcel, J.A., Gonzalez, R., & Mulet, A. (2002). Application of low intensity ultrasonics to cheese manufacturing processes. *Ultrasonics*, *40*, 19-23.

Bouthour, D., Kalai, T., Chaffei, H.C., Gouia, H., & Corpas, F.J. (2015). Differential response of NADP-dehydrogenases and carbon metabolism in leaves and roots of two durum wheat (*Triticum durum* Desf.) cultivars (Karim and Azizi) with different sensitivities to salt stress. *Journal of Plant Physiology*, *179*, 56-63.

Bybordi, A., & Tabatabaei, J. (2009). Effect of salinity stress on germination and seedling properties in canola cultivars (*Brassica napus* L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 37* (2), 71-76.

Carpıcı, E.B., Celik, N., & Bayram, G. (2009). Effects of salt stress on germination of some maize (*Zea mays* L.) cultivars. *African Journal of Biotechnology*, *8* (19), 4918-4922.

Chiu, K.Y., & Sung, J.M. (2014). Use of ultrasonication to enhance pea seed germination and microbial quality of pea sprouts. *International Journal of Food Science & Technology*, *49* (7), 1699-1706.

Cramer, G.R., & Nowak, R.S. (1992). Supplemental manganese improves the relative growth, net assimilation and photosynthetic rates of salt-stressed barley. *Physiologia Plantarum, 84* (4), 600-605.

Demirsoy, M., Aydın, M., & Gürbüz, B. (2020). Farklı ultrasonik ses dalgası uygulamalarının *Solanum torvum* tohumlarındaki çimlenme ve çıkış değerleri üzerine etkisi. *Bilecik Şeyh Edebali Üniversitesi Fen Bilimleri Dergisi, 7* (1), 354-362.

Dikilitaş, M., Balak, M.V., Şimşek, E., & Dikilitaş, S.K. (2018). Ses dalgaları ile hücrelerde geri dönülmez DNA hasarları oluşturmak mümkün müdür?. *Harran Tarım ve Gıda Bilimleri Dergisi*, *22* (4), 560-571.

Dönmez, F. (2018). Ultrasonik ses dalgası uygulamalarının ıspanak tohumlarında çimlenme ve çıkış üzerine etkileri. (Publication No. 521849) [Yüksek Lisans Tezi, Süleyman Demirel Üniversitesi].

Dutta, P., & Bera, A.K. (2014). Effect of NaCl salinity on seed germination and seedling growth of mungbean cultivars. *Legume Research-An International Journal*, *37* (2), 161-164.

El-Sattar, A.M.A., & Tawfik, E. (2023). Effects of ultrasonic waves on seedling growth, biochemical constituents, genetic stability of fenugreek (*Trigonella foenum-graecum*) under salinity stress. *Vegetos*, *36* (4), 1427-1436.

FAO (2022). The Future of Food and Agriculture: Trends and Challenges. Available online: http://www.fao.org/3/a-I6583e.Pdf (Accessed date: 30 September 2022).

Florez, M., Carbonell, V., & Martínez, E. (2007). Exposure of maize seeds to stationary magnetic felds: Efects of germination and early growth. *Environmental and Experimental Botany, 59*, 68-75. https://doi.org/10.1016/j.envexpbot.2005.10.006

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* (2), 231-242.

Hussain, S., Khaliq, A., Matloob, A., Wahid, M.A., & Afzal, I. (2013). Germination and growth response of three wheat cultivars to NaCl salinity. *Soil and Environment*, *32* (1), 36-43.

Ilyas, N., Mazhar, R., Yasmin, H., Khan, W., Iqbal, S., Enshasy, H.E., & Dailin, D.J. (2020). Rhizobacteria isolated from saline soil induce systemic tolerance in wheat (*Triticum aestivum* L.) against salinity stress. *Agronomy*, *10* (7), 989-1008.

ISTA. (2010). International Rules for Seed Testing. https://www.seedtest.org/en/international-rules-forseed-testing- content---1--1083.html

Karaman, R. (2023). Reaction of chickpea genotypes to salinity-inhibiting applications at different salt stress levels. *Gesunde Pflanzen*, *75* (5), 1823-1831.

Karaman, R., & Kaya, M. (2017). Mercimeğe (*Lens esculanta* Moench) uygulanan farklı klor tuzu ve dozlarının kimi ilk gelişme özelliklerine etkisi. *Journal of Agricultural Sciences*, *23* (1), 10-21.

Khajeh-Hosseini, M., Powell, A.A., & Bingham, I.J. (2003). The interaction between salinity stress and seed vigour during germination of soyabean seeds. *Seed Science and Technology, 31* (3), 715-725.

Kim, H.J., Feng, H., Kushad, M.M. & Fan, X. (2006). Effects of ultrasound, irradiation, and acidic electrolyzed water on germination of alfalfa and broccoli seeds and *Escherichia coli* O:157:H7. *Journal of Food Science, 71* (6), 168-173.

Kuşvuran, A., Nazlı, R.I., & Kuşvuran, Ş. (2015). The effects of salinity on seed germination in perennial ryegrass (*Lolium perenne* L.) varieties. *Türk Tarım ve Doğa Bilimleri Dergisi, 2* (1), 78-84.

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

Liu, J., Wang, Q., Dura, K., Liu, X., Cui, J., & Gui, J. (2016) Effects of ultrasonication on increased germination and improved seedling growth of aged grass seeds of tall fescue and Russian wildrye. *Scientific Reports, 6* (1), 22403

Mehmood, A., Mubarak, N.M., Khalid, M., Walvekar, R., Abdullah, E.C., Siddiqui, M.T.H., ... & Mazari, S. (2020). Graphene based nanomaterials for strain sensor application—a review. *Journal of Environmental Chemical Engineering*, *8* (3), 103743.

Miano, A., Forti, V., Abud, H., Gomes-Junior, F., Cicero, S., & Augusto, P. (2015). Effect of ultrasound technology on barley seed germination and vigour. *Seed Science and Technology, 43* (2), 297-302.

Mulaudzi, T., Nkuna, M., Sias, G., Doumbia, I. Z., Njomo, N., & Iwuoha, E. (2022). Antioxidant capacity of chitosan on sorghum plants under salinity stress. *Agriculture, 12* (10), 1544.

Murillo-Amador, B., López-Aguilar, R., Kaya, C., Larrinaga-Mayoral, J., & Flores-Hernández, A. (2002). Comparative effects of NaCl and polyethylene glycol on germination, emergence and seedling growth of cowpea. *Journal of Agronomy and Crop Science, 188* (4), 235-247.

Pandey, M., & Penna, S. (2017). Time course of physiological, biochemical, and gene expression changes under short-term salt stress in *Brassica juncea* L. *The Crop Journal, 5* (3), 219-230.

Ramteke, M., Ghune, N., & Trivedi, V. (2015). Simulated binary jumping gene: A step towards enhancing the performance of real-coded genetic algorithm. *Information Sciences, 325*, 429-454.

Rifna, E., Ramanan, K.R., & Mahendran, R. (2019). Emerging technology applications for improving seed germination. *Trends in Food Science & Technology, 86*, 95-108.

Scouten, A.J. & Beuchat, L.R. (2001). Combined effects of chemical heat and alfalfa seeds. *Journal of Applied Microbiology 92*, 668-674.

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

Shelke, H.D., Lokhande, A.C., Kim, J.H., & Lokhande, C.D. (2017). Photoelectrochemical (PEC) studies on Cu_2SnS_3 (CTS) thin films deposited by chemical bath deposition method. *Journal of Colloid And Interface Science, 506*, 144-153.

Sivritepe, H.Ö. (2011). Tohum canlılığının değerlendirilmesi. *Alatarım, 10* (2), 94-105.

Temel, S., & Tan, M. (2020). Kuru koşullarda yetiştirilen farklı kinoa çeşitlerinin kaba yem kalite özellikleri açısından değerlendirilmesi. *Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi, 6* (2), 347-354.

Wang, J., Wang, J., Vanga, S.K., & Raghavan, V. (2020). High-intensity ultrasound processing of kiwifruit juice: efects on the microstructure, pectin, carbohydrates and rheological properties. *Food Chemical, 313,* 126121. https://doi.org/10.1016/j.foodchem.2019.126121.

Wang, Y.R., Yu, L., Nan, Z.B., & Liu, Y.L. (2004). Vigor tests used to rank seed lot quality and predict field emergence in four forage species. *Crop science, 44* (2), 535-541.

Wereski, M. (2015). The threshold of hearing. *The STEAM Journal*, *2* (1), 21-24.

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

Yang, X.C., Wang, B.C., & Duan, C.R. (2003). Effects of sound stimulation on energy metabolism of *Actinidia chinensis* callus. *Colloids and Surfaces B: Biointerfaces, 30,* 67-72.

Yang, X., Wang, B., Liu, Y., Duan, C. & Dai, C. (2002). Biological effects of *Actinidia chinensis* callus on mechanical vibration. *Colloid Surface B*, *25*, 197-203.

Yi, J., Bochu, W., Xiujuan, W., Chuanren, D., & Xiaocheng, Y. (2003). Effect of sound stimulation on roots growth and plasmalemma H+ -ATPase activity of chrysanthemum (*Gerbera jamesonii*). *Colloids and Surfaces B: Biointerfaces, 27*, 65-69.

Yiyao, L., Wang, B., Xuefeng, L., Chuanren, D., & Sakanishi, A. (2002). Effects of sound field on the growth of *Chrysanthemum callus*. *Colloids and Surfaces B: Biointerfaces, 24,* 321-326.