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SOUND FREQUENCIES INDUCE DROUGHT TOLERANCE IN RICE PLANT

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Abstract

To test the sound's effect on plant and its contribution in drought tolerance, plants were subjected to various sound frequencies for an hour. After 24 h sound treatment, plants were exposed to drought for next five days. During the experiment it was observed that sound initiated physiological changes showing tolerance in plant. Sound frequency with ≥ 0.8 kHz enhanced relative water content, stomatal conductance and quantum yield of PSII (*Fv/Fm* ratio) in drought stress environment. Hydrogen peroxide (H_2O_2) production in sound treated plant was declined compared to control. *Therma*CAM (Infra-red camera) a software which was used to analyze the plant images temperature showed that sound treated plant and leaf had less temperature (heat) compared to control. The physiological mechanism of sound frequencies induce tolerance in rice plants are discussed.

Key words: Frequency, Sound, Drought, Infra Red, Images.

Introduction

Plant is known to absorb sound energy and has been engaged to reduce the noise pollution in inter-city environments (Huisman & Attenborough, 1991; Attenborough, 2002). Unlike wind or waves, sound-energy and its frequency level normally experienced by plants in the environment and do not produce any significant stress on plant. Sound energy passed on to a plant stem can induce bending to its active sound frequencies causing stiffness and elasticity of the stem (Niklas & Moon, 1988). Sound with different frequencies has been exposed to have the greatest effect on plants, specifically on seed germination (Takashi *et al.,* 1992), enzymes activities, soluble sugar, protein contents (Yi *et al.,* 2003) and plant growth (Bhagyalakshmi *et al.,* 1992; Mizoguchi *et al.,* 1996; Braam, 2005; Jeong *et al.,* 2008).

It was known that sound frequencies induced physiological changes differently soon after the sound waves strike on plant. These metabolic changes may be either productive or unproductive. Previously it was found that the treatment of *chrysanthemum* callus with 1.4 kHz sound increases indoleacetic acid levels while decreasing abscisic acid levels (Wang *et al.,* 2004). On the physical nature of sound and hormonal change in plant due to sound frequencies further strengthening the possibility that sound may induce physiological changes causing stress tolerance in plant. Therefore, present study describes the physiological changes induced by sound frequencies in rice plant under stress environment. For this study in particular, those physiological parameters are selected which are being used to examine drought tolerance in various studies (Daves *et al.,* 2000; Chaves *et al.,* 2003; Siddiqui *et al.,* 2008; Kwon *et al.,* 2009; Siddiqui & Khan, 2010; Siddiqui & Khan, 2011).

Material and Methods

Germination and growth: Rice seed Dongjin were collected from the National Center for GM Crops (NCGC), National Academy of Agricultural Science (NAAS), Rural Development Administration (RDA), Korea and washed with distilled water several time before sowing. Seeds were allowed to germinate in 90 mm diameter petri dishes. Four-day old equal size seedlings were transferred to rectangle plastic pot of an area 12 × 4 cm^2. Pots were filled with sandy loam soils (pH 5.8). Ten plants were transferred in each pot and 2 cm^2 distances between the each plant was maintained. Plants were allowed to grow in control environment (EYELA) at 25-28 ± 2°C day / night temperature with 60-80% humidity. Light intensity during the growth varied from 3000-4500 μmole m^{-2} s^{-1}. Three-week old plants were subjected to sound signals with single frequency having different levels such as 0, 0.25, 0.5, 0.8, 1.0, 1.5 kHz. Single frequency with 0 kHz served as control. Plants were irrigated with tap water on alternate days. Drought condition was imposed by stop watering until for next five days reached to 15% soil moisture approximately. Four replicates with ten plants per replicate were used for each treatment and control.

Sound treatment: The single frequency signal was generated using Adobe Audition 3.0 (Adobe System Company). The speaker volume was fixed at 100 dB (Decibel). To avoid experimental error from irrelevant sound, experiments were conducted inside a custom–made noiseless chamber which was provided by Advanced Institute of Science Technology (KAIST) Korea. After one hour sound treatment, plants were kept in growth chamber and allowed to grow. Later, after 24 h of the sound treatment, drought stress was imposed by stop watering for the next 5 days. At 6th day following parameters were examined.

Relative water content: Six randomly selected leaves were sampled and 4 × 2 cm^2 mid-vein and the edge section of leaves were cut with scissors from each treatment or control. After fresh weight measurement, each sample was placed in a 90 mm air-tight plastic petri plates containing distilled water. After 12-h hydration in dark, the leaf samples were taken out and were dried immediately weighed to obtain fully turgid weight (TW). Leaf samples were then kept in oven at 80°C for 24 h to determine dry weight (DW). Later relative water content were measured using the following formula:

$$\text{RWC } (\%) = [(\text{FW-DW})/(\text{TW-DW})] \times 100$$

where, FW = Sample fresh weight, TW = Sample turgid weight, DW = Sample dry weight.

Osmotic potential: Six randomly selected leaves were taken from each treatment and control. Top-most fully expanded leaves were sampled and 4 × 2 cm^2 of mid-vein and the edge section of leaves were cut and frozen in liquid nitrogen. The frozen leaf tissues were thawed at room temperature prior to the expression of tissue sap. Tissue sap was expressed using a centrifuge MX-305 (Tomy, Tokyo, Japan) at 14,000 rpm for 20 min at 4°C. Osmotic potential of the expressed sap was determined using a vapor pressure osmometer (Wescor, Utah, USA).

Stomatal conductance and dark adapted quantum yield: Stomatal conductance of twenty randomly selected leaves of each treatment and control were examined using a leaf porometer (Model SC-1, Decagon, USA). Measurements of Chlorophyll a fluorescence emission from the twenty randomly selected leaves were monitored with a fluorescence monitoring system (Handy PEA) in the pulse amplitude modulation mode. A leaf, adapted to dark conditions for 30 min. using leaf-clips, was initially exposed to the modulated measuring beam of far-red light (LED source with typical peak at wavelength 735 nm). Original (F_0) and maximum (Fm) fluorescence yields were measured under weak modulated red light (<0.5 $\mu mol\ m^{-2}\ s^{-1}$) with 1.6 s pulses of saturating light (>6.8 $\mu mol\ m^{-2}\ s^{-1}$ PAR). The variable fluorescence yield (Fv) was calculated by the equation of $Fm–F_0$. The ratio of variable to maximum fluorescence (Fv/Fm) was calculated as dark adapted quantum yield of PSII photochemistry was calculated as described by Maxwell and Johnson (2000).

H_2O_2 Quantification: Content of hydrogen peroxide was measured according to the procedure of Velikova *et al*., (2000). Freshly harvested leaf sample (100 mg) were homogenized with 3 ml 0.1% (w/v) trichloroacetic acid (TCA) in ice bath and the homogenate was centrifuged at 12000 g for 15 min. Then 0.5 ml of 10 mM phosphate buffer (pH 7.0) and 1 ml of 1 M Potassium iodide (KI) were added to 0.5 ml of the supernatant. The absorbance of supernatant was read at 390 nm. The amount of H_2O_2 was calculated using the extinction coefficient and expressed as $\mu mole\ g^{-1}$ FW

IR thermal imaging: FLIR-SC-620, FLIR Systems USA were used for the forward looking infra red thermal image sensing in drought stress condition. The system was optimized before experiment up to 30 min. The potted plants of each treated and control were examined. The images were taken at 10 AM morning. All images were captured in a rectangular box of an area about 46 × 30 cm^2. Temperature and relative humidity of inside the box were 24 ± 2°C and 60–70% (RH) respectively. Image-temperature reflectance reports were generated (*Therma* CAM Researcher Pro 2.10).

Statistical analysis: All data from treated and control were subjected to statistical analysis using SPSS 17.0. The values were expressed with mean standard error and t-test. In this study two factor (Sound and Drought) with six sound treatments (0, 0.25, 0.5, 0.8, 1.0, 1.5 kHz) were used. T-test ($p=0.05$) was conducted to compare sound treatments with control and were expressed on bar graphs as an alphabet.

Results and Discussion

Sound frequencies enhanced relative water content in plant under drought stress compared to control. However maximum relative water content was found in 1.5 kHz treated plant compared to control (Fig. 1). It was observed that fresh and turgid weights played significant role in leaf water content under drought condition (Hendry & Wallace, 1993; Pilon-Smits *et al.,* 1995; Telewiski, 2006; Rezaei *et al.,* 2006; Siddiqui *et al.,* 2008; Kown *et al.,* 2009). Studies showed that water deficit has exerted a negative effect on relative water content of plant in drought environment (Mekliche *et al.,* 1992). Plant ability to survive in drought depends on its ability to restrict water loss or maintaining high (more than 60%) relative water content (Siddiqui & Khan, 2011). In fact, several workers have reported the existence of a significant positive correlation between drought tolerance and water retention in plant (Kwon *et al.,* 2009; Munns *et al.,* 2010). It was shown that sound caused significant change in cell wall elasticity and flexibility causing considerable effect on cell wall turgidity and thus improving relative water content (Bhagyalakshmi *et al.,* 1992; Mizoguchi *et al.,* 1996; Anthosiewicz *et al.,* 1997; Braam, 2005). Therefore it is presumed that sound enhance relative water content in drought condition could be a reason of drought tolerance in rice.

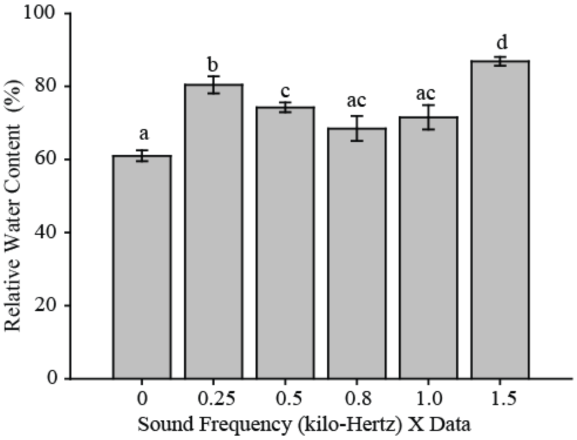

Fig. 1. Effect of sound frequencies on relative water content in drought stress environment. Vertical line on bar graph represent mean standard error (±), similar alphabets stand for non-significant difference at $p<0.05$ (t-test).

Sound frequencies slightly improved osmotic potential and substantially increased dark adapted quantum yield (*Fv/Fm* ratio) in drought stress (Fig. 2). Maximum osmotic potential was recorded in 0.5 and 0.8 kHz frequency treated sample compared to control. Moreover, rest of the treatments displayed more or less similar osmotic potential compared to control. Similarly frequency specific sound improved dark adapted quantum yield (*Fv/Fm* ratio) in treated plant (≤ 0.8 ± 0.045) than in control (< 0.7 ± 0.012).

Sound with different frequencies may induce some metabolic adjustment to synthesize osmotically active organic compound (Jeong *et al.,* 2008). Increases in osmotic potential after sound treatment are the indication of physiological adjustment of the plant in drought stress environment. It was reported osmotic adjustment through attaining osmotic potential in stress environment are related to synthesis of osmotically active osmolytes and polyols (Pilon-Smits *et al.,* 1995; Rezaei *et al.,* 2006; Siddiqui *et al.,* 2008; Kwon *et al.,* 2009; Silva *et al.,* 2010). Further, in stress condition, plants modify the metabolism and synthesize metabolites to adjust homeostasis of plant (Rezaei *et al.,* 2006; Siddiqui *et al.,* 2008).

Sound frequencies improved dark adapted quantum yield (*Fv/Fm* ratio) demonstrating non-significant photosynthesis apparatus damages in drought apart from control (soundless). Quantum yield, photochemical alterations, photo-inhibition or photosynthetic pigments are well known physiological parameters and were found decrease in stress-sensitive plants (Nctondo *et al.,* 2004; Siddiqui, 2007; Silva *et al.,* 2010; Silva *et al.,* 2011). Therefore, maintaining the quantum yield up to 0.8 of the sound treatment may be a reason of enhance tolerance and avoid the drought stress consequences. Further, dark adapted quantum yield (*Fv/Fm*) observation indicated that the photosynthesis processes conserved their normal activities in sound treated plants compared to control. It was reported that frequency-specific sound increase the Aldolase and RUBP carboxylase activity further strengthening the possibility that sound treatment may enhance the photosynthesis in plant (Jeong *et al.,* 2008) In present study, result showed that sound treated plant had less H_2O_2 content production compared to control (Fig. 3). Observation also revealed that H_2O_2 content was gradually declined with sound treatment in drought environment. Reactive oxygen species (ROS) production is linked with normal metabolic processes such as aerobic metabolism (Moller, 2001) and photosynthesis (Asada, 1999). However, ROS production is increased under drought stress during inhibition of $NADP^+$ regeneration, Mehler reaction, and photorespiration. Under drought, ABA accumulation triggers superoxide anion and hydrogen peroxide production through inhibition of CO_2 uptake and alteration in transport electron chain in chloroplasts (Guan & Scandalios, 1998). In addition, H_2O_2 has been detected in ABA response as a mediator of stomatal closure (Desikan *et al.,* 2004) or inhibition of stomatal opening (Yan *et al.,* 2007). The results regarding hydrogen peroxide production in sound treated plant under drought stress condition demonstrating that sound may initiate tolerance in plant.

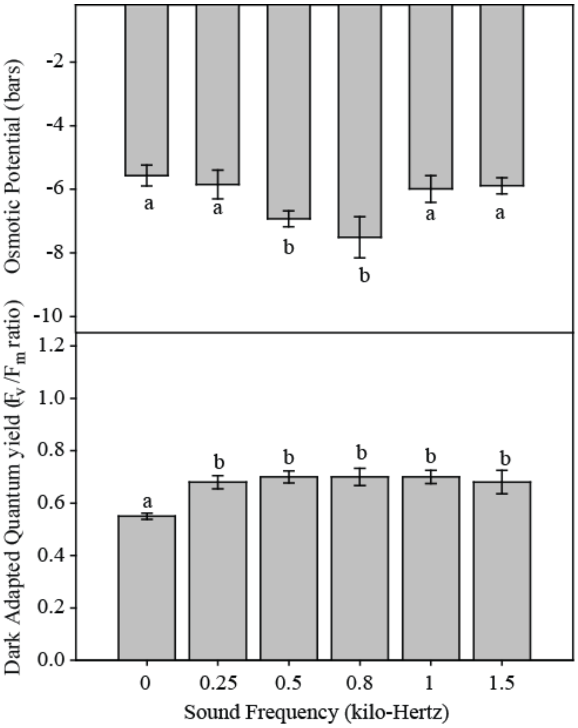

Fig. 2. Effect of sound frequencies on osmotic potential and quantum yield of photosystem II (*F_v/F_m ratio*) in drought stress environment. Vertical line on bar graph represent mean standard error (±), similar alphabet stand for non-significant difference at $p<0.05$ (t-test).

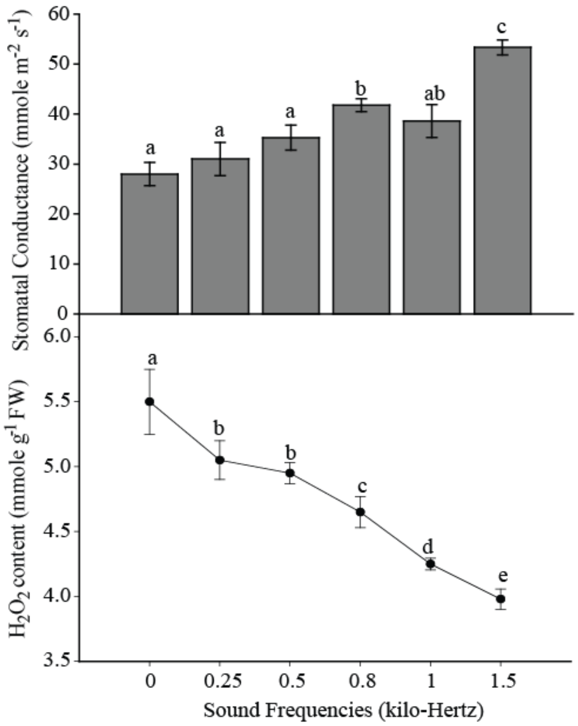

Fig. 3. Effect of sound frequencies on stomatal conductance and hydrogen peroxide production in drought stress environment. Vertical line on bar graph represent mean standard error (±), similar alphabet stand for non-significant difference at $p<0.05$ (t-test).

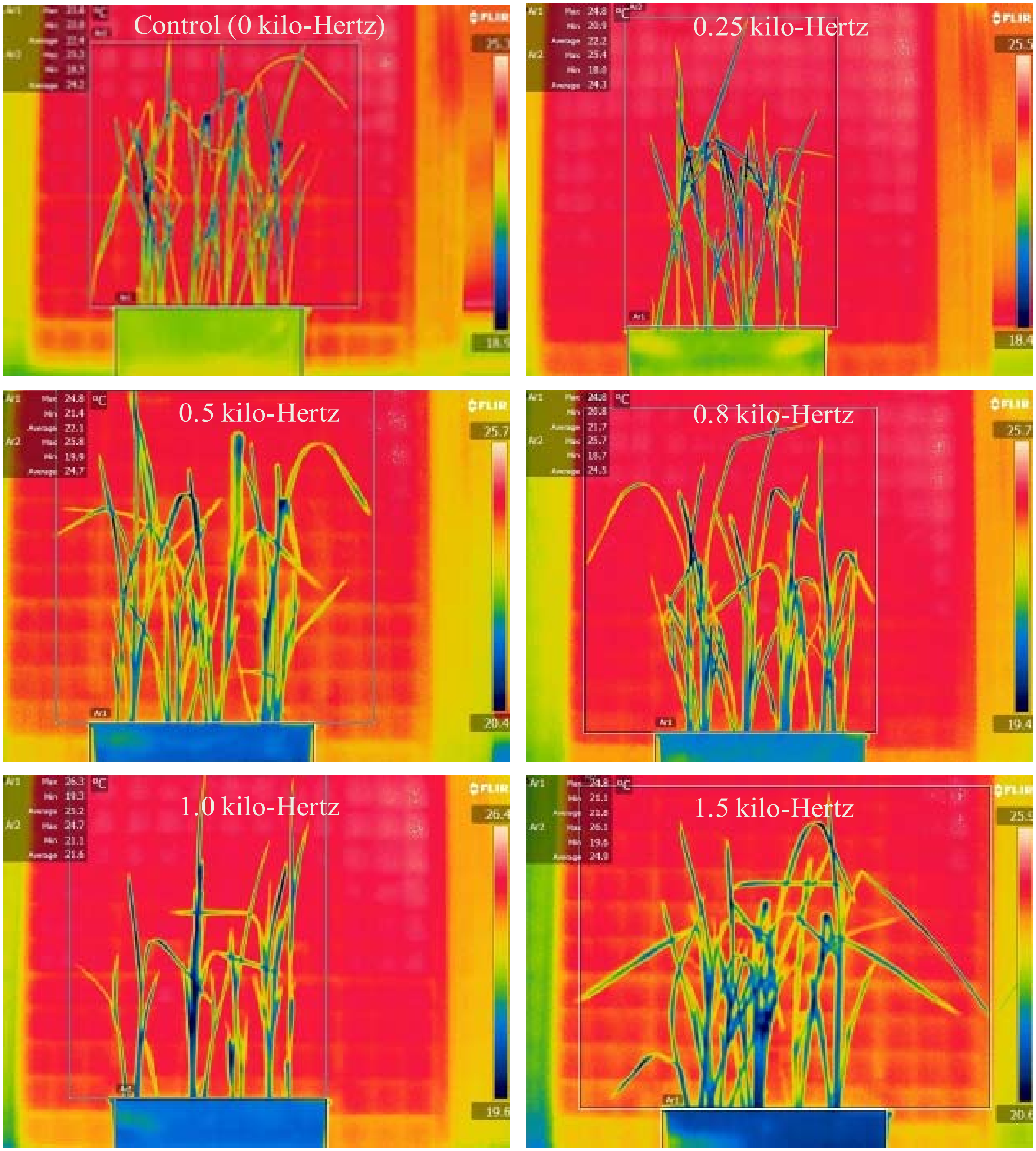

Fig. 4. Effect of sound frequencies on plant temperature/heat in drought stress environment. Note Infra-Red thermal images were taken by FLIR SC-620 camera. Reflected temperature reading was pasted at the top of each.

It was observed that sound frequencies triggered the stomatal conductance in stress environment compared to control (Fig. 3). Stomatal conductance increased linearly with the increased with sound frequencies in drought stress environment. However, 1.5 kHz treated plant showed maximum stomatal conductance compared to other treatment.

Sound with high frequency enhances the stomatal conductance in drought stress environment as compared to sound untreated sample. Stomatal response after sound treatment in drought stress condition is indicating unique approach to combat drought consequences. It was observed that drought-tolerant plants exploit the fitness by lessening leaf size and stomatal conductance to water vapor in response to water scarcity (Nativ *et al.,* 1999; Ares *et al*., 2000). Plants might adapt physiologically to drought conditions by reducing stomatal conductance to water vapor and thereby increasing their water-use efficiency. In drought condition, drought tolerant plant may adapt two different strategies 1) long lived annuals and perennials, decreasing leaf size and/ or stomatal conductance (Geber & Dawson, 1997; Querejeta *et al.,* 2003). In contrast, shorter lived annuals may maximize fitness by increasing stomatal conductance (low WUE) in order to increase net rates of carbon gain to avoid drought stress. This strategy may allow them to grow rapidly, flower early, and increase yield prior to the onset of substantial soil drying (Geber & Dawson, 1997; McKay *et al.,* 2003). When drought is experienced at later developmental stages, selection should favor decreased stomatal conductance (high WUE) and smaller leaves, whereas when plants experience drought at early developmental stages, increased stomatal conductance (low WUE) should be selected for and leaf size may be of no adaptive value. In other words, sound treatment may favor a tolerance strategy of surviving drought and delaying reproduction during a longer growing season but may favor a strategy of rapid growth and reproduction to avoid stress during a short growing season.

For drought tolerant plant, water status and stomatal regulation are important physiological attributes and were effectively maintained in drought stress environment and optimizes the growth. Therefore it may be suggested that frequency-specific sound may trigger certain gene and these gene may involved in controlling the stomatal conductance under drought stress environment. There are several reports that show genes such as calmodulin and ABA were highly expressed in plant after mechanical stress (Braam & Davis, 1990; Antosiewicz *et al.,* 1997; Dutta & Robinson, 2004; Braam, 2005). Further, Jeong *et al.,* (2008) identified a set of sound responsive genes in plant using sound treated subtractive library and demonstrated through mRNA expression analysis. The dual hormonal role of ABA in controlling the stomata regulation are well established in stress environment (Hartung *et al.,* 1998; Daves *et al.,* 2000; Jones, 2004; Buckley, 2005; Siddiqui *et al.,* 2008; Silva *et al.,* 2011).

We have used a thermal camera (FLIR SC-620, FLIR-System, USA). Forward Looking Infra Red thermal (FLIR) images of treated and control plant was conducted (Fig. 4). IR images showed significant variation in temperature in treated and control plants (Table 1). Average leaf temperature was recorded lesser in 0.5, 1.0 and 1.5 kHz frequency-specific-sound treated plant compared to control. Less temperature in sound treated plant representing the less heat produced under drought condition. However, in drought sensitive plant more heat or temperature was expecting in drought condition due to the scarcity of the water. In case of water deficiency either salt or drought, an immediate plant response is a reduction in transpiration to reduce water loss, increasing leaf temperature (Munns *et al*., 2010). Further, leaf temperature is considered to be an immediate indicator showing plant water status and it may be related to stomatal conductance and relative water content (Idso *et al.,* 1981; Manzoni *et al.,* 2011; Zia *et al.,* 2012).

Table 1. Average plant and pot temperature of drought treated and untreated plant. Reading was an average of three values analyzed by *Therma* CAM researcher pro 2.10 FLIR software.

Sound treatment kHz	Pots temperature (˚C)	Average plant temperature (˚C)	Average leaf temperature (˚C)
Control (0)	22.4^a	23.8^a	24.2^a
0.25	22.2^a	24.8^b	24.3^a
0.5	22.1^a	24.8^b	24.7^b
0.8	21.7^b	24.8^b	24.5^b
1	21.6^b	26.3^c	25.2^c
1.5	21.5^b	24.8^b	24.9^c

Note: Similar alphabets represent non-significant difference at $p<0.05$

Conclusion

Present study is concluded that frequency specific sound induced substantial improvement in leaf water status and dark adapted quantum yield. Further sound initiates effective stomatal regulation and maintain the appropriate water status when plants were subjected to stress condition. This physiological evidence indicates that sound enhances drought tolerance in plant however; detail molecular and physiological arguments are needed.

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