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Specific Sound Frequency Improves Intrinsic Water Efficiency in Rice Leaf by Imparting Changes in Stomatal Dimensions

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ABSTRACT

Various attempts have been made to increase rice production, including breeding for high-yielding and stress-tolerant varieties, a good crop management system, and increased agricultural input in rice production. Soundwave stimulation has been demonstrated to affect plant growth; thus, this method can be employed in the current rice production methods to improve yield. The study aims to determine the effects of different sound wave qualities on the general growth, physiological, and morphological of rice seedlings. Rice seeds of the MR219 variety were grown under a glasshouse condition in a nested design with five replications and were stimulated with various sound wave frequencies. Various sound wave frequencies, 380, 359, 357, 353, and 350 Hz, were obtained by placing the pot at varying distances (80, 160, 240, 320, and 400 cm, respectively) from the sound source, except control treatment. There were significant effects in some of the parameters: plant height, leaf physiology, and stomatal pore and length when treated with varying sound wave qualities. Plants can be stimulated with 380, 357, and 350 Hz soundwaves frequencies for the best

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photosynthetic experience. In addition, 359 Hz of sound wave stimulation resulted in high water use efficiency, which is beneficial in improving crop performance in drought conditions. Thus, it was demonstrated that the sound wave stimulation method has the potential to enhance rice performance in addition to the regular agronomic practices of rice production in farmers' fields.

Keywords: Leaf physiology, *Oryza sativa* L., photosynthesis, rice growth, rice production, sound wave stimulation, stomatal morphology

INTRODUCTION

Rice (*Oryza sativa* L.) is largely regarded as a vital crop owing to its prominent roles in shaping histories, cultures, diets, and economics for half of humanity (Gomez, 2001). Rice feeds more than three billion people, in addition to one billion people depend on rice cultivation for their livelihood (Skinner, 2012). In 2020, the early report of the total rice production in Malaysia was around 2.3 million tonnes, and 78.4% came from the granary areas (Department of Agriculture [DOA], 2021). Although the government has made many efforts to improve the self-sufficiency level in Malaysia, rice production has only achieved a 63% self-sufficiency level, with the rest being imported to meet the needs of the 33 million people in this country (Department of Statistics Malaysia [DOSM], 2021). Although rice production is gradually increasing, consumption is also increasing, requiring more rice to be imported to meet local demand. Furthermore, the gap between rice production and consumption was projected to widen from 2018 to 2026 (Omar et al., 2019). Therefore, there is an urgent need to increase local rice production to ensure food security instead of relying on imports from neighboring countries to meet domestic consumption.

Various efforts are taken to address the issue of rice shortage, which includes breeding and the introduction of varieties resistant to pests and diseases. These include varieties such as Minghui63-Xa21 and Shanyou63-Xa21, which are resistant to bacterial blight, and Hanyou 2 and Hanyou 3, which have a tolerance to abiotic stresses such as flood responses and drought. (Luo, 2010; Zhai et al., 2001). Other researchers approached the issue from different angles (Stoop et al., 2002), such as the application of various good crop management systems, including a system of rice intensification (SRI) on infertile soils and reduced rate of irrigation while maintaining other agricultural inputs optimally (Kim et al., 2008). Traditional crop production practices involving applying more fertilizers and chemicals to improve performance are environmentally unfriendly. Since 2000, the rice harvested area in Malaysia has remained constant, but rice yield in Malaysia has increased due to the increasing use of nitrogen (N) fertilizers (Herman et al., 2015). Therefore, new eco-friendly approaches need to be developed to ensure sustainability in crop production and be environmentally friendly. Despite the increase of N fertilization, manipulating the photosynthesis of plants is considered a sustainable method to improve crop production. For example, rice variety MR253 showed higher photosynthetic assimilation under the light-saturated condition when treated with 50% N concentration compared to 100% N concentration in a hydroponic system (Herman et al., 2015). This result proved that applying more fertilizer does not necessarily improve crop performance. Similarly, it is believed that sound wave manipulation can be used to enhance photosynthesis at the leaf level, which could potentially improve plant growth and yield rice production.

Interestingly, sound wave has been reported to affect many biological properties in plants (Hassanien et al., 2014). However, whether the effect of sound waves promotes, or limits growth depends on the frequency, intensity, and acting time of the sound wave is not well defined. Stimuli, such as sonic, supersonic, electromagnetic, microgravity, and mechanical vibration, have been previously shown to affect plants. For example, the selectively permeable cell membrane can be injured by environmental factors, such as temperature, salinity, and air pollution. The injury incurred can be thought of as ‘micro-perforations’ that improve the penetrability of the membrane, beneficial in regulating substance movement into or out of the cell. Sound waves with a frequency of 400 Hz can improve the float of the cell membrane and strengthen the mutual function between the membrane’s lipid and protein region (Bochu et al., 2003).

Many studies have demonstrated that music will increase plant growth. Yiyao et al. (2002) reported that plant tissues could be enhanced at a certain range of intensity and frequency of sound waves, but the effect could become the opposite when the sound field is beyond a certain range. Some research also found that the mechanical acceleration wave is needed to promote seed germination (about 70 m/s^2), which is far more than the acceleration of gravity wave (9.8 m/s^2), although this purported value cannot be entirely confirmed due to vibrational acceleration changes that happen continuously in the testing system (Uchida & Yamamoto, 2002). Thus, it is hypothesized that there is a specific sound wave quality, with respect to the varying distances from the sound source, which will be beneficial to improve rice physiologically. In addition, varying sound wave treatments will have differing effects in altering the stomatal and epidermal properties of rice leaves that affect the genetics of rice plants, which could be beneficial in selection for breeding purposes. This study aims to determine the effects of different sound wave qualities on the general growth, physiological, and morphological of rice seedlings.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted in 2018 in a greenhouse of the Institute of Tropical Agriculture and Food Security (ITAFoS), Universiti Putra Malaysia (UPM) (GIS location: 2.98414168 N, 101.7336908 E). Ten rice seeds of MR219 variety were sown in a pot (80 mm [D] × 80 mm [H]) containing paddy field soil (Serdang series), and all the pots were arranged in a nested design with five replications (five pots) per treatment. Treatments were imposed, and growth parameters were assessed within 30–35 days after sowing (DAS). Standard agronomic practices, such as thinning, fertilizing, weeding, and watering, were performed throughout the growing period to maintain good plant growth. Thinning of the seedlings was carried out after 10 days of sowing by leaving only one healthy plant within each pot. Rice plants were fertilized from 14 DAS once a week until 35 DAS with a proprietary blend of hydroponic nutrient solutions from a local garden store.

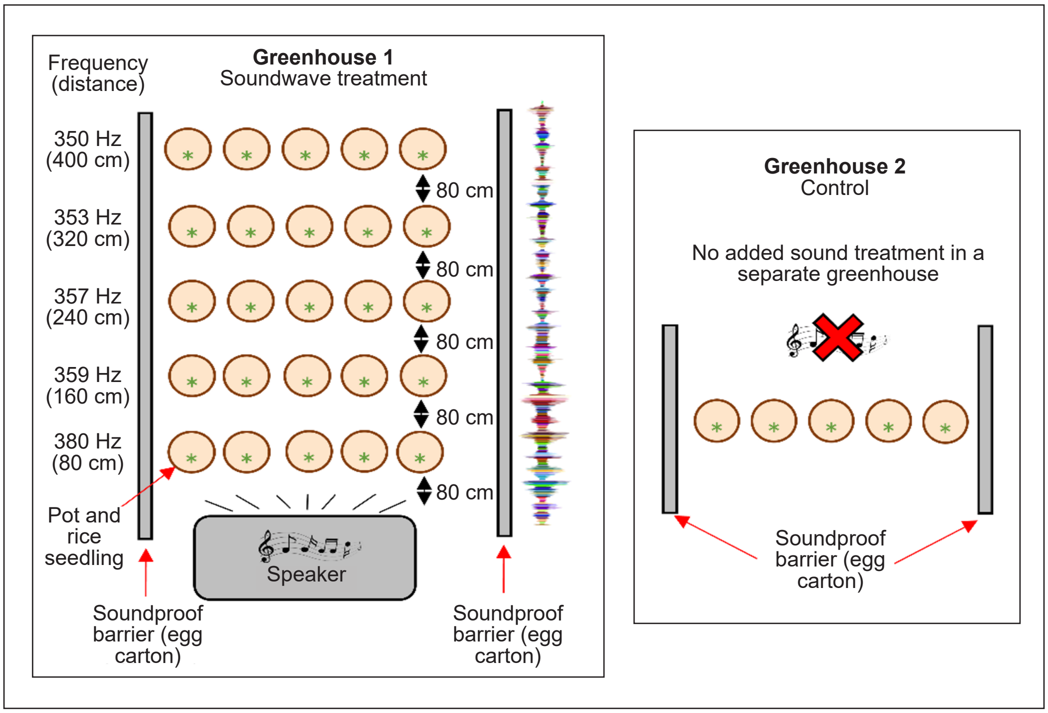
Rice plants were stimulated with different sound qualities in frequency (Hz). Six treatments were imposed on the seeds and seedlings, comprising five sound wave qualities and one control. Different sound qualities, which are 380, 359, 357, 353, and 350 Hz, were obtained by placing the pot at varying distances (80, 160, 240, 320, and 400 cm, respectively) from the sound source with a 600-watt loudspeaker (Aviano Precision, China) with the volume set to 5/10 (Figure 1). The decibel (dB) value, the scale of loudness (intensity of a wave) measured at these distances, is 78, 73, 69, 65, and 60 dB, respectively. The frequencies (Hz) and decibels (dB) were monitored using the FFTWave Android application on mobile phones for sound monitoring. The song used for the sound wave stimulation was a Mozart instrumental song by William McColl. The control treatment received no sound wave stimulation and was in a different greenhouse. The distance between control and treated plants is approximately 28 m (Figures 1a and 1b). The condition between the two greenhouses, especially the sunlight, is the same because the greenhouses are in the same area, and no other building close to this facility can create shade. Sound barrier and absorber using egg cartons were installed to the left and right of the plant rows to reduce the impact of ambient noise. The sound wave stimulation was performed every other day for a month, starting from day 1 of seed sowing for 4 hr in the morning. The duration of the song was 5 min and was repeated during a 4 hr of stimulation period. This whole experiment was conducted only once and was not repeated.

Data Collection

Growth and Appearance. Leaf number was counted manually, and the plant height was measured from the base to the tip of the highest shoot of rice plant using a measuring tape.

Leaf Physiology. Photosynthesis rate (assimilation, A_{400}) and stomatal conductance (g_{sw}) were performed using a portable photosynthesis system (LICOR-6400/XT, USA) to measure the physiological activity of leaves. Gas exchange was measured on the middle part of leaf 5. In the leaf chamber, light intensity, temperature, humidity, and carbon dioxide (CO_2) concentration were set at 1,600 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ PAR, 25^{o}C, 60%, and 400 ppm, respectively. Intrinsic water use efficiency (iWUE) ($\mu mol{\cdot}CO_2{\cdot}mol^{-1}{\cdot}H_2O$) was calculated as the ratio of assimilation

(a)



(b)

Figure 1. (a) A satellite image of two glasshouses used in the experiment and the distance between the experimental locations (about 28 m apart) of sound wave treatments and control treatment (no sound treatment); and (b) the experimental layout in the glasshouse. Rice seedlings treated with sound waves were placed in a different glasshouse (Greenhouse 1) than the non-treated rice seedlings (Greenhouse 2)

rate to stomatal conductance and calculated using the following formula:

$$\mathrm{iWUE} = \frac{\text{Assimilation rate } (\mathrm{A}_{400})}{\text{Stomatal conductance } (\mathrm{g}_{\mathrm{sw}})} \quad [1]$$

Leaf Stomatal Properties. A histology analysis was conducted to determine the properties of leaf stomata. The parameters evaluated were stomatal complex area, stomatal pore area, stomatal densities, percentage stomatal file, stomatal complex length, stomatal complex width, guard cell width, and cell file width. A section of about 1 cm of the fully expanded leaf 5 was cut and placed in Carnoy's fixative that was prepared according to Puchtler et al. (1968) without any modifications. All samples were placed in the Effendorf tubes and bleached with a 15% Clorox® (Malaysia) (sodium hypochlorite) solution for 24 hr until the samples became colorless (to remove the pigments). Then the bleached samples were cut in half, fixed on the slide with a chloralhydrate solution and Arabic gum, and covered with a cover slip.

Microscopy Analysis. Samples were observed using a LEICA DFC310 FX light microscope (United Kingdom) with a 400× magnification lens. The area of the stomatal complex, guard cell width, stomatal length, interveinal gap, and vein counting were performed using Image J software (version 1.48) (Schneider et al., 2012).

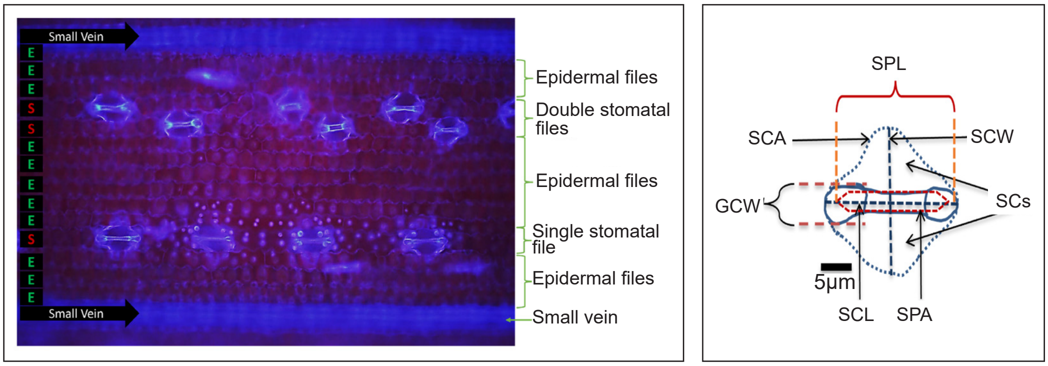
Measurement of Stomatal and Leaf Epidermal Characteristics. For the stomatal complex area, the width and length of the stomata were measured. The stomatal complex in rice consists of a pair of guard cells, and the area of the stomatal complex was calculated based on the measured values of the stomatal complex width and length using the following formula:

$$\frac{(2 \times \mathrm{SPL} \times \mathrm{GCW}) - \mathrm{GCW}^2)}{2} \quad [2]$$

where, SPL = Stomatal pore length; GCW = Guard cell width (Yaapar, 2017)

The guard cell width was determined by drawing the lines from the top to the bottom of the guard cell, and the length of this line was measured as the guard cell width (Figure 2b). The stomatal density (per mm²) was calculated by counting the number of stomatal complexes between two interveinal gap areas. The percentage of stomata files was calculated by counting the number of files with at least one stoma over the total number of cell files. The stomata files were counted between the two parallel veins (Figure 2a).

Statistical Analysis. All data were analyzed in a one-way analysis of variance using SAS 9.4 software using PROC GLM. PROC MIXED was used to analyze data of parameters with missing data and non-constant variance (SAS Institute Inc., 2012). The data were subjected to a normality test to check residuals for normality and constant variance (SAS Institute Inc., 2012). When there were treatment differences, the means were compared and grouped into letter groupings using Fisher's protected least significant difference (LSD) ($P = 0.05$) with n = 5.



(a) (b)

Figure 2. Micrograph images of (a) typical epidermal features and stomatal patterning in a rice leaf (S stomatal files, E epidermal files); and (b) a stomatal complex outline in rice leaves indicating the stomatal complex area (SCA) (surrounded by blue dashed line), guard cell width (GCW), stomatal cell width (SCW), stomatal pore area (SPA) (surrounded by red dashed line), subsidiary cells (SCs), stomatal cell width (SCW), and stomatal pore length (SPL)

RESULTS

The effects of the sound wave in rice plants were evaluated on a few growth and physiological parameters, including plant growth, leaf physiology, and leaf stomatal properties. Each of these parameters corresponded to specific stages of rice growth, allowing a general correlation to be deduced about the vigor and potential yield of the plant. Plant growth parameters include the number of leaves and plant height. Physiological assessments were always performed on the mid-region of the leaf blade of fully expanded leaf 5, including assimilation and stomatal conductance rate. In addition, the assessment of stomata morphologies was performed using samples obtained from leaf 5.

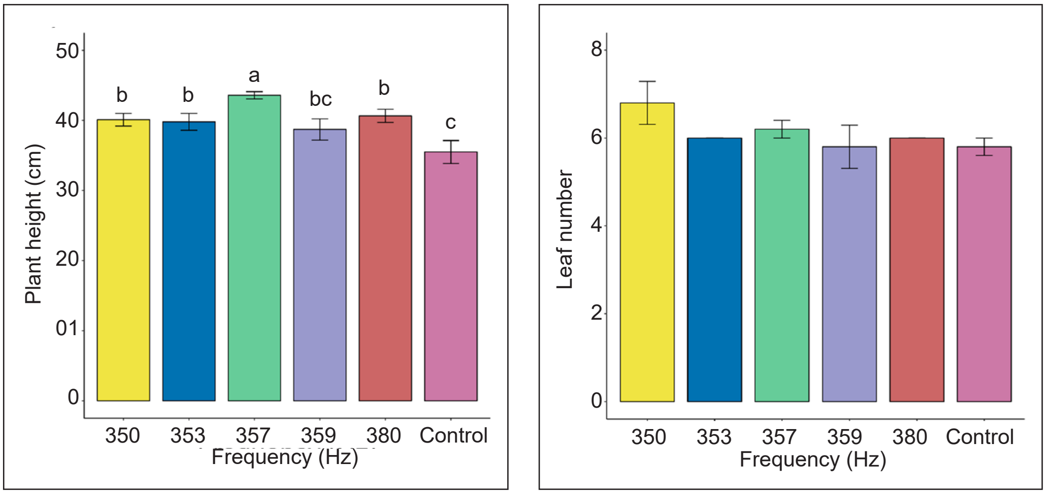
Growth and Appearance

Plant Height. The sound wave treatments significantly affected plant height. The frequency of 357 Hz resulted in the highest average plant height of 43.6 cm, approximately 21% higher than the control (Figure 3a). It was observed that the control and 359 Hz treatment had the lowest plant heights averaging 36 and 39 cm, respectively.

Leaf Number. There was no significant difference in leaf number between any sound wave treatments. However, there is a tendency for the plants that received a frequency of 350 Hz to produce higher leaf numbers compared to other treatments (Figure 3b).

Leaf Physiology. Assimilation rate (A_{400}), stomatal conductance (g_{sw}), and intrinsic water use efficiency (iWUE) were used to assess the performance of leaf 5 after being stimulated with a sound wave of different quality (Hz).

Photosynthesis (A_{400}). A_{400} measurements showed that stimulation with sound waves



(a) (b)

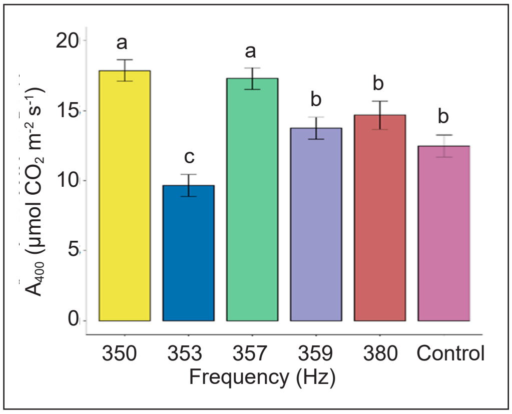
Figure 3. Mean of (a) plant height and (b) leaf number of seedlings grown at different frequencies measured at 80 cm distance interval from the sound wave source. Means with the same letter are not significantly different at $P > 0.05$ using LSD (n = 5). Error bars indicate the standard error of the mean

of 357 and 350 Hz led to the highest rate of photosynthesis (17.3 and 17.9 µmol·CO_2·m^{-2}·s^{-1}, respectively) compared to the other treatments (Figure 4a). Compared to the control, these two treatments increase the assimilation rate by 39 to 43%. Intermediate assimilation rates were observed in plants receiving 380 and 359 Hz sound waves and control treatments (14.67, 17.86, and 12.48 µmol·CO_2·m^{-2}·s^{-1}, respectively). The lowest A_{400} value was observed in plants stimulated with 353 Hz soundwave (9.66 µmol CO_2 m^{-2} s^{-1}).

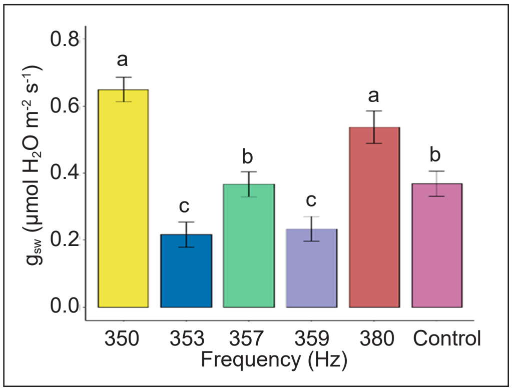
Stomatal Conductance (g_{sw}) and Intrinsic Water Use Efficiency (iWUE). Significantly high g_{sw} values were observed in treatments of 380 and 350 Hz (0.54 and 0.65 µmol·H_2O·m^{-2}·s^{-1}, respectively) compared to the control (0.37 µmol·H_2O·m^{-2}·s^{-1}) (Figure 4b). It is worth noting that the plants stimulated with sound waves of 359 to 353 Hz consistently produced significantly lower g_{sw} (and comparable to the control) compared to the plants stimulated at 380 and 350 Hz. When both A_{400} and g_{sw} were combined as a ratio to assess intrinsic water use efficiency (iWUE), the plants stimulated at 359, 357, and 353 Hz consistently produced significantly high iWUE (60.7, 48.3, and 48.5 µmol CO_2 mol H_2O^{-1}, respectively) compared to plants stimulated at 380 and 350 Hz (Figure 4c).

The iWUE for 359 Hz treatment was the highest among other treatments with an iWUE value of 60.6 µmol·CO_2·mol·H_2O^{-1}, indicating that plants at this distance have high water use efficiency, and it is expected that these plants can tolerate the drought condition well. However, this treatment was not significantly different from plants receiving 357 and 353 Hz with iWUE values of 48.2 and 48.5 µmol·CO_2·mol·H_2O^{-1}, respectively. The control, 380 and 350 Hz showed the lowest iWUE values, namely

37.3, 25.1, and 28.0 μmol·CO_2·mol·H_2O^{-1}, respectively.



(a)



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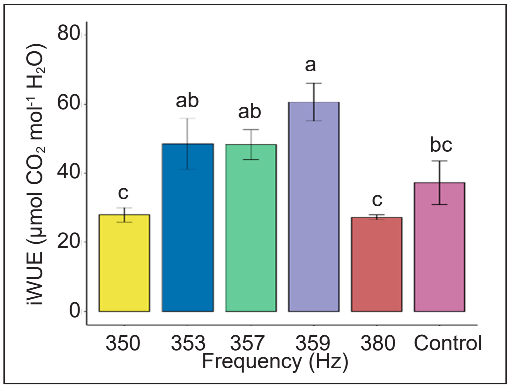


(c)

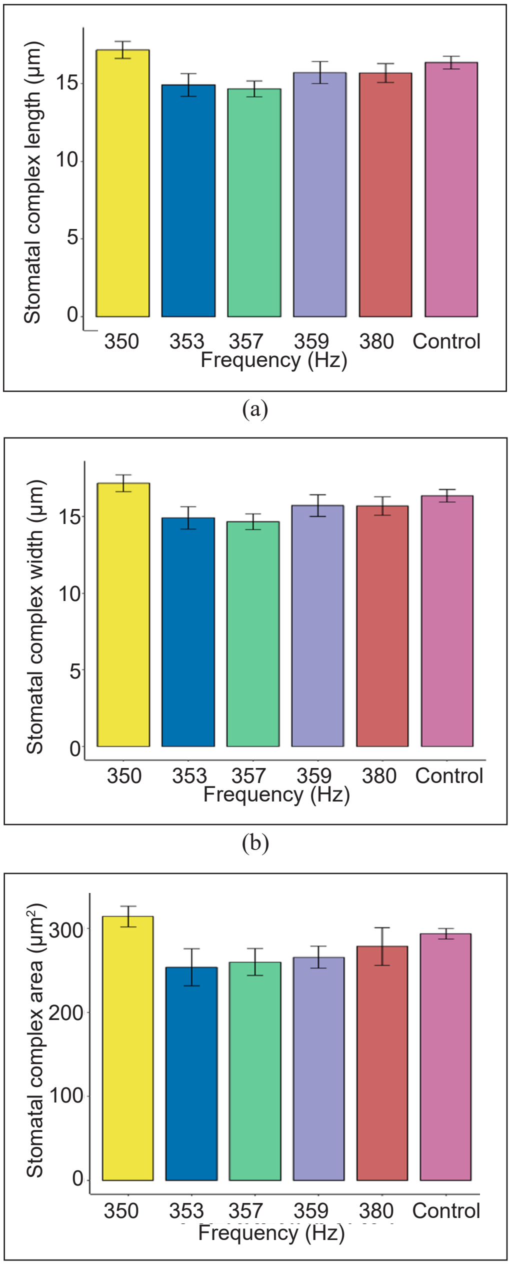
Figure 4. Mean of (a) assimilation rate (A_{400}); (b) stomatal conductance (g_{sw}); and (c) intrinsic water use efficiency (iWUE) of seedlings grown at different frequencies measured at 80 cm distance interval from the sound wave source. Means with the same letter are not significantly different at $P > 0.05$ using LSD (n = 5). Error bars indicate the standard error of the mean

Leaf Stomatal Properties

Effects of Sound Wave on Stomatal and Epidermal Area. Stomata are small epidermal pores on leaves that regulate the movement of CO_2 and water in and out of the plants, respectively. Therefore, the attribute of stomata is crucial to ensure plants have a balanced gas exchange where they can maintain enough CO_2 for carbon fixation while minimizing water loss. The parameters quantified in this experiment included stomatal complex area (SCA), stomatal pore area (SPA), stomatal density (SD), percentage stomatal file (PSF), stomatal complex length (SCL), stomatal pore length (SPL), stomatal cell width (SCW), guard cell width (GCW), and cell file width (CFW) (Figure 2).

Stomatal Complex (Area, Length, and Width). Different frequencies of sound wave treatment have no significant effect on the SCL, SCW, and SCA (Figures 5a-5c). However, there is a tendency for the SCA to increase for plants stimulated with a 350 Hz sound wave (314.2 µm²) compared to the control treatment (293.6 µm²) (Figure 5c).

Stomatal Pore (Length and Area) and Guard Cell Width. It was observed that sound wave treatment had a significant impact in altering the length (SPL) and area of the stomatal pore (SPA) but not the width of the guard cell (GCW) (Figures 6a-6c). Significantly greater pore length was

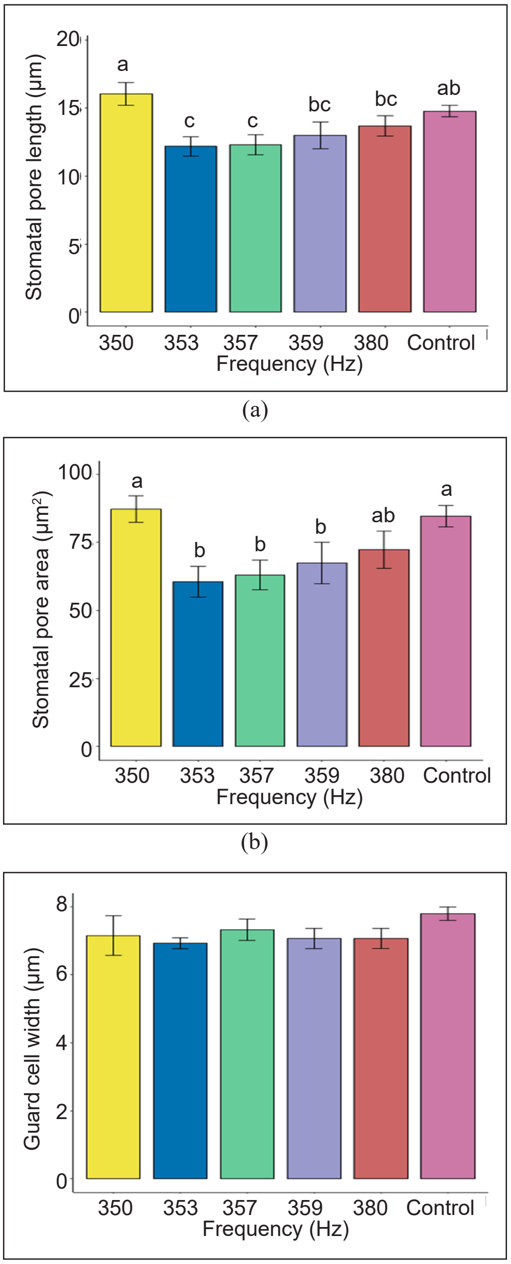


(a)

(b)

(c)

Figure 5. Mean of (a) stomatal complex length (SCL); (b) stomatal complex width (SCW); and (c) stomatal complex area (SCA) of seedlings grown at different frequencies measured at 80 cm distance interval from the sound wave source. Means with the same letter are not significantly different at $P > 0.05$ using LSD (n = 5). Error bars indicate the standard error of the mean



(a)

(b)

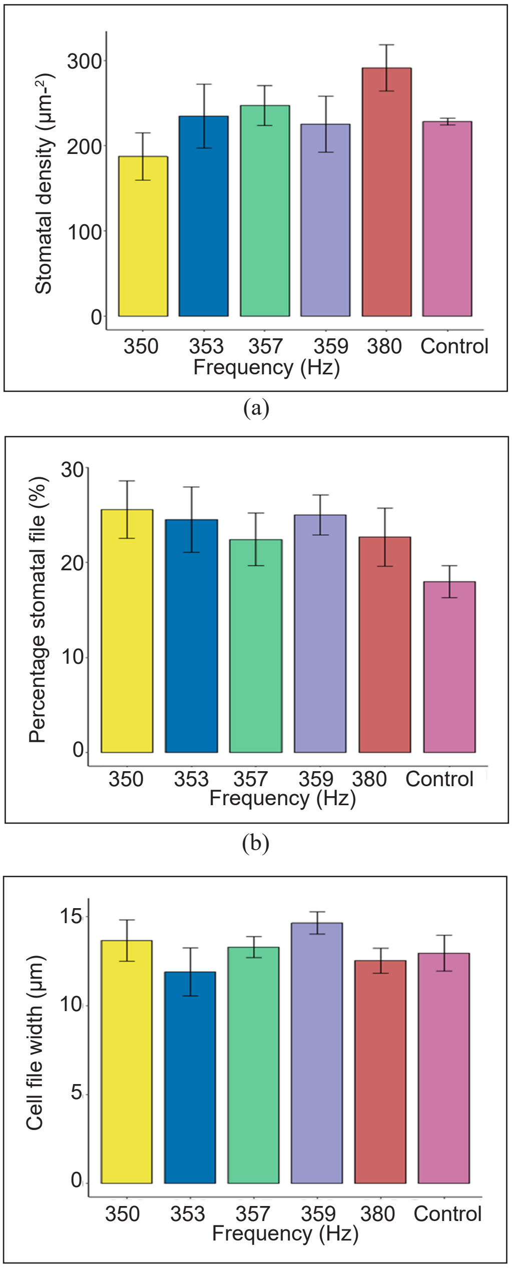
(c)

Figure 6. Mean of (a) stomata pore length (SPL); (b) stomatal pore area (SPA); and (c) guard cell width (GCW) of seedlings grown at different frequencies measured at 80 cm distance intervals from the sound wave source. Means with the same letter are not significantly different at $P > 0.05$ using LSD (n = 5). Error bars indicate the standard error of the mean

observed in 350 Hz stimulated plants and in the control treatment with mean values of 16.1 and 14.8 µm, respectively. It also showed that lower frequency has a significant effect in increasing the pore length. The shortest pore length was observed in plants receiving 353 Hz (12.19 µm) sound wave, but not significantly different from the 357, 359, and 380 Hz sound wave treatments with mean pore lengths of 12.3, 12.9, and 13.7 µm, respectively (Figure 6a). The 353 Hz sound wave stimulation produced the smallest pore length, and alikeuction was approximately 8 and 27% compared to control and 350 Hz treatments, respectively.

The largest SPA was observed in control, 380, and 350 Hz with a mean of 72.3, 87.23, and 84.63 mm^2, respectively. The smallest pore area was observed in plants that received 353 Hz, but this treatment is not significantly different from plants that received 380, 359, and 357 Hz. Sound wave treatments significantly reduced the pore area by 20–28% compared to the control. The GCW was not significantly affected by different sound wave treatments; however, the control treatment tends to have wider guard cells compared to other treatments.

Stomatal Density, Percentage Stomatal File, and Cell File Width. There was no significant difference in stomatal density (SD), percent stomatal file (PSF), and cell file width (CFW) of the rice plant when exposed to different sound wave treatments (Figures 7a-7c). The mean SD ranged from 187 to 291 per mm^2, with plants stimulated with 380 Hz tending to have a higher SD



(c)

Figure 7. Mean of (a) stomatal density (SD), (b) percentage stomatal file (PSF), and (c) cell file width (CFW) of seedlings grown at different frequencies measured at 80 cm distance interval from the sound wave source. Means with the same letter are not significantly different at $P > 0.05$ using LSD (n = 5). Error bars indicate the standard error of the mean

and those stimulated with 350 Hz tending to have a lower SD. For PSF, the mean was between 18 and 26%, and there is a tendency for plants stimulated with 350 Hz to increase PSF, and the control treatment had a lower PSF as compared to other treatments. For CFW, the mean ranged from 11.9 to 14.65 µm, with plants exposed to 359 and 353 Hz sound waves tending to have higher and lower CFW, respectively.

Correlation Between Physiological and Morphological Attributes

The correlation analysis suggested that many of the stomatal morphological parameters have intermediate to strong correlations among each other, as compared to the correlation within growth/physiological parameters, as well as the correlation between growth/physiological and morphological parameters (Figure 8). The above results suggested that most stomatal characteristics are independent of leaf growth/physiological characteristics, except for the SPA and SPL, which had significant intermediate positive relationships with g_{sw}, a physiological parameter. Between the leaf physiological attributes, A_{400} shows a significant intermediate positive correlation with stomatal conductance. In addition, iWUE shows a significantly high positive correlation with stomatal conductance. On the other hand, within the leaf morphological attributes, intermediate positive correlations were observed between SCA and SCW, SCL and GCW, SCL and SCW, SPL and SCW, and SPA and GCW. Strong positive correlations were observed between SPL and SCA, SPA and SCA, SPA and SCW, SPA and SCA, and SPL.

DISCUSSION

This experiment studied the effect of sound wave qualities on three different components of crop performance in rice: plant growth, leaf physiology, and stomata morphology. In the first part of this study, the effects of sound waves on general plant growth and leaf physiological parameters were evaluated. The second part of the study evaluated the effects of sound waves on changing the morphological properties of the stomata and epidermis of rice leaves. The combined results from plants' physiological and morphological traits provide insight into how these components affect rice seedling growth.

Since ultrasound energy can increase the permeability and selectivity of cell membranes, thus promoting cell wall growth (Qi et al., 2010), it has been hypothesized that overall plant growth may benefit from enhanced permeability and selectivity of the cell membrane. However, sound wave qualities have a certain value that promotes or hinders plant growth. This study identified two soundwave frequencies, namely 357 and 350 Hz, which promote plant performance by significantly increasing several parameters, namely assimilation rate, stomatal conductance, and plant height. The above findings suggested that an increase or decrease in these parameters might correspond to the nature of the wave propagation, which has a peak and trough. The two peaks could correspond

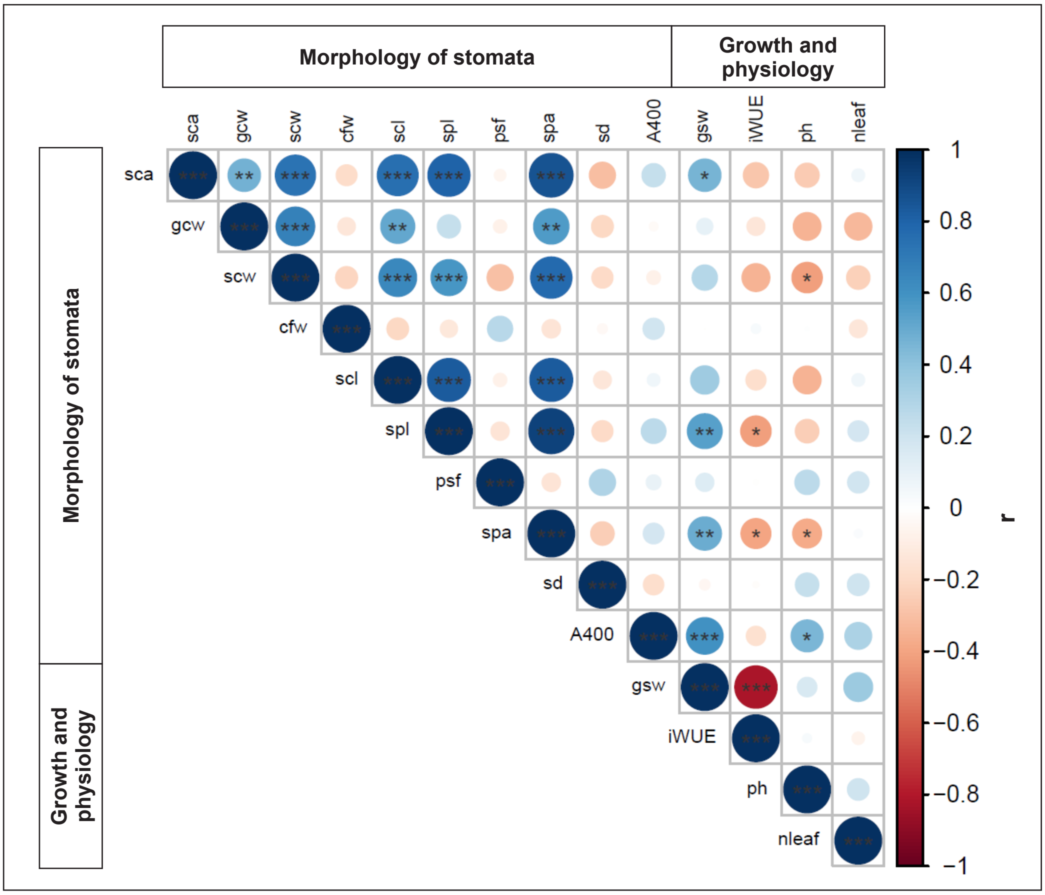


Figure 8. Pearson correlation coefficients between plant leaf characteristics, leaf physiology, and stomatal morphology of rice (MR219) grown at Ladang 15, UPM, in 2017

Note. *, **, *** Statistically significant at $P < 0.05$, 0.01, and 0.001, respectively; r = Correlation coefficient; sca = Stomata complex area; gcw = Guard cell width; scw = Stomata complex width; cfw = Cell file width; scl = Stomata complex width; spl = Stomata pore length; psf = Percentage of stomatal file; spa = Stomata pore area; sd = Stomatal density; A400 = Assimilation rate (CO_2 400 ppm); g_{sw}= Stomatal conductance; iWUE = Intrinsic water use efficiency; ph = Plant height at day 28; nleaf = Number of leaves at day 28

Non-star circle means not statistically significant at $P > 0.05$. Blue and red circles indicate positive and negative correlations, respectively. The size and color intensity of the circle indicates the strength of the correlation between parameters. Large circles mean a strong correlation, and small circle means a weak correlation

to the frequency of 357 and 350 Hz or 240 and 400 cm from the sound source (high values), while the trough could correspond to frequencies of 359 and 353 Hz or 160 and 320 cm from the sound source (low values). Although insignificant, other parameters also show a similar trend in sound wave propagation at slightly different distances. The above speculation needs to be proven, but a plausible explanation is that music can enhance the uptake of nutrients from the soil, resulting in better plant metabolism and increased growth and performance (Chowdhury et al., 2014). Sound waves also can affect stomatal movement, so this may have an important impact on leaf gas exchange capacity (Cai et al., 2014).

Physiological data also revealed interesting findings which could be used to support the earlier results in general plant growth. It was previously reported that the frequencies of 357 Hz significantly improved plant height compared to plants that did not receive sound wave stimulation. The above finding is consistent with the photosynthesis measurements where the plants stimulated with similar sound wave quality also showed significantly higher assimilation rates compared to the control (Table 1).

The morphological study of stomatal and epidermal properties of rice leaf in this study included many dimensional categories of the stomata, including area, frequency, length, and width. The results showed that different sound wave qualities had minimal effects on stomatal and epidermal properties, suggesting that stomatal and epidermal properties are not very responsive to different sound wave qualities. It has been reported that stomata size is inversely related to stomata density (Büssis et al., 2006; Doheny-Adams et al., 2012), and the increase in stomata density is compensated by a decrease in stomata size (Büssis et al., 2006). It implies that plants with high stomata density will have small stomata and vice versa. It has been shown that mutants with low stomata density and large size have reduced transpiration rates when

Table 1
Summary of mean comparison when compared to control treatment only

When compared to the control			Frequency (Hz) [Decibel (dB)]				
			350 (60)	353 (65)	357 (69)	359 (73)	380 (78)
Growth and physiology	Growth	Leaf number (nleaf)					
		Plant height (ph)	⬆	⬆	⬆		⬆
	Leaf physiology	Assimilation rate (A_{400})	⬆	⬇	⬆		
		Stomatal conductance (g_{sw})	⬆	⬇		⬇	⬆
		Water use efficiency (iWUE)				⬆	
Morphology (stomata)	Area, width, and length	Stomatal complex length (SCL)				⬇	⬇
		Stomatal complex width (SCW)					
		Stomatal complex area (SCA)					
		Stomatal pore length (SPL)				⬇	⬇
		Stomatal pore area (SPA)		⬇	⬇	⬇	

Note. ⬆ Increase; ⬇ Decrease
The green and red arrows indicate a significant increase or decrease, respectively. The mean comparison was performed using least significant difference (LSD) at a significant level of $P < 0.05$

grown in different CO_2 concentrations (200, 450, and 1,000 ppm) and water regimes (70 and 30%) (Doheny-Adams et al., 2012), suggesting that plants with these traits may be beneficial in drought conditions where water availability is limited.

In rice leaves, one of the most important properties in photosynthesis is the guard cell width, which controls the gas and water movement in and out of the leaf. In this study, most of the stomatal properties, namely stomatal complex length, stomatal complex width, stomatal complex area, guard cell width, stomatal density, percentage stomatal file, and cell file width, did not respond significantly to sound waves stimulation. However, significant responses were observed in stomatal pore length and area, indicating that stomatal cells have been stimulated by the sound wave, which could have resulted in the differential gene expression for these traits. It was reported that RNA and soluble protein content increased in chrysanthemum cell culture stimulated with sound waves suggesting an alteration in gene expression (Xiujuan et al., 2003). In addition, SPA (Figure 6b), which trend was also similar to the stomatal complex area, suggests a significant correlation between these two parameters (Table 1).

Sound vibration is a mechanical stimulus that can cause thigmomorphogenetic responses in plants (Telewski, 2006). Therefore, to a certain degree, soundwave treatments could alter the stomatal pore size. It was documented that stomatal features, including stomatal pore size, influenced stomatal conductance (Fanourakis et al., 2015). This study observed an intermediate positive correlation between conductance and stomatal pore length and area, suggesting the tendency of stomatal pore length and area to affect stomatal conductance (Table 1).

In addition, it was found that certain high frequencies tend to reduce the length and area of the stomata, while those of plants stimulated at 350 Hz (60 dB) were unaffected compared to the control treatment. The assimilation rate for plants stimulated with 350 Hz (60 dB) is the greatest compared to other treatments, including the control (Figure 4a). The current finding contradicts those of Hou et al. (2009), who reported that when using four speakers as sound wave treatment at a different planting distance in cotton, the minimum yield was obtained in plants grown at a relatively far distance (30 m) with a sound wave intensity range of 75–110 db. Evidence from another study showed that net photosynthesis measured weekly in strawberry plants treated with sound waves of 100 dB and a frequency of 40–2,000 Hz was not significant compared to the control (no sound wave), except during the fourth sound stimulation. Although insignificant, there were tendencies to improve net photosynthesis when treated with sound waves, potentially leading to higher yield. Similarly, the sound wave treatment tends to produce a higher fruit number compared to the control (Meng et al., 2012)—different ranges of sound intensity measured in decibels used for multiple frequencies in sound wave studies.

As previously mentioned, the intensity of a wave is the energy carried by the wave per unit of time per unit area at that point. On the other hand, frequency measures sound quality or the number of sound waves per second. At a given frequency, the intensity can vary depending on how much energy the sound wave carries. Therefore, when treating the plants with sound waves, the researcher can adjust the intensity of a specific frequency of interest.

Additionally, this study found that the stomata density of plants treated at higher frequencies (Figure 7a) tended to have smaller pore sizes (length and area) (Figures 6a and 5b). Although the effect between frequencies in stomata density was insignificant, the response pattern for stomata density and pore size (area and length) showed a similar trend to the results reported in a study that manipulated the genetics of stomata density in *Arabidopsis*. It was also implied that the plants with small pore sizes and high stomatal density showed significantly higher water use efficiency and were likely to adapt well to drought environments (Franks et al., 2015). In another study, rice plants with reduced stomatal density exhibited the ability to conserve water and tolerate drought while maintaining rice yield (Caine et al., 2019).

It was observed that a certain frequency of sound waves significantly changes the membrane protein's structure and affects the cell membrane's permeability and fluidity (Zhao et al., 2002). In addition, it was observed that the activity of plasmalemma H^+ ATPase (proton pumps), which regulates biochemical and physiological processes in plant growth, increases by 19.8% in plants treated with sound waves (Yi et al., 2003). It is believed that vibrations induced by sound waves can enhance the permeability of membranes and be useful in regulating the movement of substances in or out of the cell, thereby enhancing plant growth. Bochu et al. (2003) showed that sound waves with a frequency of 400 Hz could improve the buoyancy of the cell membrane and strengthen the mutual function between lipid and protein regions of the membrane.

It is worth mentioning that having a high photosynthetic rate alone, although sensible, means that resulting in relatively more vigorous plants will not always be helpful if the plant is experiencing water scarcity. There are two key findings in this study. First, if water is never an issue, rice plants can be stimulated with 380, 357, and 350 Hz soundwaves frequencies to achieve the best photosynthetic experience. Second, suppose water efficiency is the aim. In that case, stimulating the rice with a 359 Hz sound wave is the answer because the relatively high amount of carbon is assimilated for a one-unit amount of water lost. Nevertheless, it is worth noting that music comes with various sound qualities; thus, if the same physiological improvement is achieved, similar sound quality in terms of hertz and decibel should be applied regardless of the music type.

CONCLUSION

The current study aimed to determine the effects of sound wave quality on rice plant characteristics, including height, stomatal properties, and physiological performances. These findings suggest that, in general, sound waves between the frequencies of 350 and 370 Hz could promote plant growth performance in terms of increased plant height. The proportionate carbon assimilation and stomatal conductance rates could be the reason for the growth increase, particularly at 350 Hz frequency. It was also shown that the pore dimensions of the stomata were alterable at frequencies higher than 350 Hz. The distinct significant effects of the sound wave in certain parameter classes suggest its useful potential as a stimulus like many other abiotic factors, such as light and temperature. The ability of sound wave stimulus to increase yield and achieve better water use efficiency indicates alternative options are always available in improving seedlings' establishment in rice, thus potentially leading to an enhanced overall rice yield and production. Also, since rice production is water intensive, reducing the water supply in the rice can adversely affect yield. Therefore, sound wave stimulation at a specific frequency, which can change the properties of stomata, resulting in the plant using water more efficiently, allows plants to produce a higher yield with limited water availability.

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