

Advances in Effects of Sound Waves on Plants

Reda H E Hassanien^{1,2}, HOU Tian-zhen^{1,3}, LI Yu-feng¹ and LI Bao-ming¹

¹ College of Water Resources and Civil Engineering, China Agricultural University, Beijing 100083, P.R.China

² Agricultural Engineering Department, Faculty of Agriculture, Cairo University, Cairo 12613, Egypt

³ Key Laboratory of Agricultural Engineering in Structure and Environment, Ministry of Agriculture, Beijing 100083, P.R.China

Abstract

Sound waves technology has been applied to different plants. It has been found that sound waves were at different frequencies, sound pressure levels (SPLs), exposure periods, and distances from the source of sound influence plant growth. Experiments have been conducted in the open field and under greenhouse growing conditions with different levels of audible sound frequencies and sound pressure levels. Sound waves at 1 kHz and 100 dB for 1 h within a distance of 0.20 m could significantly promote the division and cell wall fluidity of callus cells and also significantly enhance the activity of protective enzymes and endogenous hormones. Sound waves stimulation could increase the plant plasma-membrane H⁺-ATPase activity, the contents of soluble sugar, soluble protein, and amylase activity of callus. Moreover, sound waves could increase the content of RNA and the level of transcription. Stress-induced genes could switch on under sound stimulation. Sound waves at 0.1-1 kHz and SPL of (70±5) dB for 3 h from plant acoustic frequency technology (PAFT) generator within a distance ranged from 30 to 60 m every other day significantly increased the yield of sweet pepper, cucumber and tomato by 30.05, 37.1 and 13.2%, respectively. Furthermore, the yield of lettuce, spinach, cotton, rice, and wheat were increased by 19.6, 22.7, 11.4, 5.7, and 17.0%, respectively. Sound waves may also strengthen plant immune systems. It has been proved that spider mite, aphids, gray mold, late blight and virus disease of tomatoes in the greenhouses decreased by 6.0, 8.0, 9.0, 11.0, and 8.0%, respectively, and the sheath blight of rice was reduced by 50%. This paper provides an overview of literature for the effects of sound waves on various growth parameters of plant at different growth stages.

Key words: acoustic technology, sound waves, plants growth

INTRODUCTION

Sound is acoustic energy in the form of an oscillatory concussive pressure wave transmitted through gases, liquids and solids. The lowest frequency classification in the acoustic spectrum is infrasound that has a frequency range less than about 20 Hz. Infrasound has been applied in infrasound diagnosis and therapeutic infrasound. Ultrasound is defined as acoustic waves

at frequencies greater than 20 kHz, which has been widely used in medical practice for at least 50 yr as both diagnostic and therapeutic tools. Ultrasound and infrasound can interact with biological tissues by thermal and mechanical processes (O'Brien 2007; Whittingham *et al.* 2007; Rokhina *et al.* 2009). Audible sound is what human beings hear and has an approximate frequency range between 20 Hz and 20 kHz. Physiological effect of environmental factors such as moisture, light, wind, and temperature on

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Reda H E Hassanien, E-mail: reda.emam@gmail.com; Correspondence LI Bao-ming, Tel: +86-10-62736904, Fax: +86-10-62737570, E-mail: libm@cau.edu.cn

plant stimulus and growth has been well understood. However, little information is available on the effects of audible sound on plants. Audible sound wave technology has recently been applied to plants at various physiological growth stages, e.g., seed germination, callus growth, endogenous hormones, mechanism of photosynthesis, and transcription of certain genes. The sound stimulation could enhance disease resistances and decrease requirements for chemical fertilizers and biocides (Zhang 2012). Plants can produce sound waves at relatively low frequencies of 50-120 Hz spontaneously. Moreover, plants might have a meridian system as in humans and other animals namely, internal frequency. Plants also can absorb and resonate to specific external sound frequencies (Hou *et al.* 1994a, b; Hou and Li 1997a, b). Sound waves can change the cell cycle (Wang *et al.* 1998). Sound waves vibrate the plant leaves and speed up the protoplasmic movement in the cells (Godbole 2013). It has been reported that acoustic biology has become increasingly popular and more attention has been paid to the effects of environmental stresses on the growth and development of plants. However, there is a lot of confusion and contradictions in this area. Some researchers broadcast different styles of music for plants such as rock and roll, jazz, classical, or light music, and extracted different results. Whereas, some other researchers are using different sound frequencies and sound pressure levels to treat plants (Hou and Mooneyham 1999a, b). On the other hand, the mechanisms of sound effect have not been revealed so far. It has also been indicated that some stress-induced genes might be switched on under sound stimulation and the level of transcription increased (Wang *et al.* 2003a). Sound frequency technology stimulates leaf stomata to open; thereby the plant will be able to increase its uptake of spray fertilizer and dew. Sound waves were found to be efficient at getting the herbicide into the plant. Mature weeds can be sprayed with 50% less herbicide and biocide if also treated with sound waves. Therefore, sound waves can decrease the requirements for chemical fertilizer and pesticide (Carlson 2013). Furthermore, the sound and light might interact; both sound energy and light energy could convert and store as chemical energy, which

enhances the photosynthesis system (Meng *et al.* 2012b). However, further experiments addressing this issue must be performed to confirm these results. This paper reviews the effects of sound waves on plants. Plants reviewed include medicinal plants, vegetables and field crops at different growth stages.

EFFECTS OF SOUND WAVES ON SEED GERMINATION

Sound waves have been applied to okra and zucchini seeds by using natural sounds of birds and echoes. It was observed that natural sounds had a higher statistical significant effect on number of sprouted okra and zucchini seeds for the main condition and over exposure time ($P < 0.002$) (Creath and Schwartz 2004). Rideau wheat seeds and seedlings exposed to different signal frequencies. The treatment at a sound frequency of 5 kHz and sound pressure level SPL of 92 dB stimulated tiller growth coupled with an increase in plant dry weight and number of roots (Weinberger and Measures 1979). In contrast, Wang *et al.* (2003) investigated the biological effect of sound waves on paddy rice seeds. Results revealed that the germination index, height of stem, relative increase rate of fresh weight ($P < 0.01$), activity of root system, and the penetrability of cell membrane ($P < 0.05$) were significantly increased at sound frequency of 0.4 kHz and SPL of 106 dB. When the sound wave stimulation exceeded 4 kHz or 111 dB, it inhibits the growth of paddy rice seeds. Therefore, sound waves could greatly change the cell cycle of paddy rice cells and speed up its reproduction rate. Sound wave also transfers energy into the cell and drives the sytoplasmic streaming. Sound wave may affect the membrane materials to change the biological function of membrane and enhance cell metabolism. Cell membrane is very sensitive to environmental stimulation and its penetrability influences the plants resistance against harmful materials in a poor environments. Recent evidence illustrated that the young root tips of *Zea mays* were clearly bending towards a continuous sound source and the high bending percentage was measured between 0.2 and 0.3 kHz (Gagliano *et al.* 2012) as shown in Fig. 1.

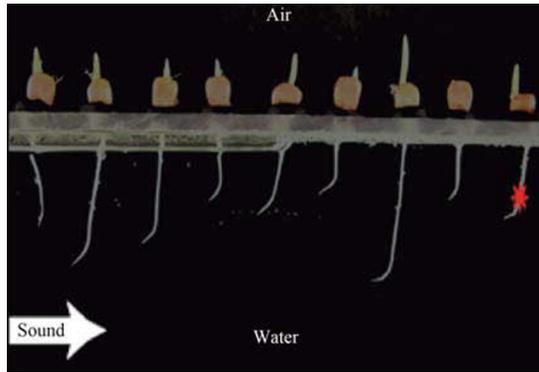


Fig. 1 Behavioural response of young roots of *Zea mays* to a continuous 220 Hz sound coming from left field (white arrow). Root tip clearly bend towards sound source (Gagliano *et al.* 2012).

EFFECTS OF SOUND WAVES ON CALLUS OF MEDICINAL PLANTS

Sound wave stimulation has been applied to certain kinds of medicinal plants in tissue culture especially

on the growth of callus for the last decade in China as shown in Table 1.

Actinidia chinensis callus

Actinidia chinensis (kiwi) is a fruit that has been extensively used in recent decades as a medicinal plant. It is rich in vitamin C, vitamin E and sugars (Arts *et al.* 2000). Sound waves have dual effects on the root development of *Actinidia chinensis* plantlets with a significant differences ($P < 0.05$). Sound waves stimulation increased the root activity, total length and number of roots whereas, the permeability of cell membranes decreased (Yang *et al.* 2004). Furthermore, it was found that adenosine tri-phosphate (ATP) significantly increased at SPL of 100 dB and sound frequency of 1 kHz. ATP is a high-energy molecule used for energy storage by organisms. The increase of ATP content indicates that the anabolism was strengthened in cells. The content of soluble proteins and the activity of SOD

Table 1 Effects of sound waves on the medicinal plants callus

Physiological and biochemical indexes of plants	Optimal sound frequency (kHz), SPL (dB) and exposure period	References
<i>Actinidia chinensis</i> callus		
ATP content, the content of soluble protein	Twice a day, each time 30 min for 20 d at SF of 1 kHz, SPL of 100 dB and at a distance of 0.2 m	Yang <i>et al.</i> (2002, 2003, 2004)
Activity of SOD and activity of indole-3-acetic acid (IAA) oxidase		
Penetrability of cell membrane and cell wall calcium and growth rate	Twice a day each time 30 min for 13 d at SF of 0.8 kHz, SPL of 100 dB and at a distance of 0.2 m	Wang <i>et al.</i> (2002a)
<i>Chrysanthemum</i> callus		
The growth of roots, contents of soluble sugar, protein, and amylase activity	Daily 60 min for 9 days at SF of 1 kHz, SPL of 100 dB and at a distance of 0.2 m	Jia <i>et al.</i> (2003a)
The activity of SOD, content of soluble protein, the absorption rate of calcium and indole-3-acetic acid (IAA) oxidase	Twice a day, each time 30 min for 10 d at SF of 0.8 kHz, SPL of 100 dB and at a distance of 0.2 m	Liu <i>et al.</i> (2002)
The microstructure of plasmalemma for roots and fluidity of lipid	Daily 60 min for 9 d at SF of 1 kHz, SPL of 100 dB and at a distance of 0.2 m	Jia <i>et al.</i> (2003b)
The activity of roots and the content of soluble protein	Daily 60 min for 9 d at SF of 1 kHz, SPL of 100 dB and at a distance of 0.2 m	Jia <i>et al.</i> (2003c)
The activities of SOD, POD, CAT, and the POD isoenzymes	Twice a day, each time 30 min for 9 d at SF of 1 kHz, SPL of 100 dB and at a distance of 0.2 m	Wang <i>et al.</i> (2003a)
PMH ⁻ -ATPase activity, the activity of roots, the content of soluble protein and the permeability of K ⁺ channel	Twice a day, each time 30 min for 15 d at SF of 1 kHz, SPL of 100 dB and at a distance of 0.2 m	Wang <i>et al.</i> (2002b)
The gene expression, POD isoenzymes and the content of RNA and DNA	Daily 60 min for 9 d at SF of 1 kHz, SPL of 100 dB and at a distance of 0.2 m	Zhao <i>et al.</i> (2002a)
Indole-3-acetic acid (IAA) and abscisic acid (ABA)	Twice a day, each time 30 min for 10 d at SF of 1.4 kHz, SPL of 95 dB and at a distance of 0.2 m	Wang <i>et al.</i> (2004)
Indole-3-acetic acid (IAA) and abscisic acid (ABA)	Twice a day, each time 30 min for 10 d at SF of 0.8 kHz, SPL of 100 dB and at a distance of 0.2 m	Wang <i>et al.</i> (2001)
Callus cells of <i>Chrysanthemum</i>		
Callus cells protein kinase and the PMH ⁻ -ATPase activity	Twice a day, 30 min each time for 10 d at SF of 0.8 kHz, SPL of 100 dB and at a distance of 0.2 m	Liu <i>et al.</i> (2001)
	Daily 60 min for 3 d at SF of 1 kHz, SPL of 100 dB and at a distance of 0.2 m	Zhao <i>et al.</i> (2002b)
<i>Dendrobium candidum</i>		
The activity of SOD, CAT, POD, and ascorbate peroxidase (APX)	Daily 60 min for 9 d at SF of 1 kHz, SPL of 100 dB and at a distance of 0.2 m	Li <i>et al.</i> (2008)

increased at 1 kHz and 100 dB. However, those indexes decreased when sound waves stimulation exceed 1 kHz and 100 dB (Yang *et al.* 2002; Yang *et al.* 2003). Superoxide dismutases (SODs) are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen (Raychqudhuri and Deng 2000; Hernandez and Almansa 2002). In higher plants, SODs act as antioxidants and protect cellular components from being oxidized by reactive oxygen species (ROS) (Alscher *et al.* 2002). Reactive oxygen species can form as a result of drought, injury, herbicides, pesticides, ozone, plant metabolic activity, nutrient deficiencies, photoinhibition, temperature above and below ground, toxic metals, and UV or gamma rays.

Chrysanthemum callus

The *Chrysanthemum* was cultivated in China for over 2 000 yr. It has been the national flower of Japan for several hundred years. In America, the *Chrysanthemum* has been hybridized extensively and the flower of *Chrysanthemum* has been used in oriental countries for hundreds of years and it has widely consumed as a medicinal herbal tea (Chen *et al.* 2007). Flos *Chrysanthemum* known in China as Juhua is an important traditional Chinese medicine (TCM) used for “scattering cold”, “cleaning heat and toxin” and “brightening eyes” (Chu *et al.* 2004). Wang *et al.* (2002a) studied the effects of cell wall calcium on the growth of *Chrysanthemum* callus under sound stimulation. There was a significant difference in growth rate between the treated and untreated groups ($P < 0.05$). Thus, the sound stimulation could promote the division and growth of callus cells. Effects of sound stimulation on the metabolism of *Chrysanthemum* roots illustrated that the growth of roots accelerated under certain sound stimulation. The soluble sugar content, protein and amylase activity increased significantly by sound stimulation, but it had no obvious effect on the permeability of membranes (Jia *et al.* 2003c). Sugar is the main product of photosynthesis and the resource of energy for most physiological processes. The content of soluble

protein is close to the growth and division of plant tissue and the accumulating level of soluble protein, which reflects not only the state of necessary substance for division but also the content of enzyme and relative metabolism level. The increase of soluble sugar and protein illustrated that sound stimulation accelerated the anabolism of *Chrysanthemum*. In addition, the increase of amylase activity showed an advancement of sugar decomposition, hence the catabolism changed highly after the sound stimulation. Liu *et al.* (2002) used a sound stimulation generator (SSG) to study the effects of sound field on the growth of *Chrysanthemum* callus by measuring SOD activity, the soluble proteins content, Indole-3-acetic acid (IAA) oxidase activity and the calcium absorption. The sound stimulation generator with sine waves consists of the power supply, amplifier, function generator and speaker. Sound pressure level and frequency have been adjusted by amplifier and function generator, respectively. The callus was put into a sterile room. The distance between the speaker and the callus was approximately 20 cm; a schematic diagram of the device is shown in Fig. 2. Sound waves stimulation can enhance or inhibit the growth of *Chrysanthemum* callus. The activity of superoxide dismutase (SOD), the content of soluble proteins and the absorption rate of calcium in callus were increased with the increase of SPL and frequency. However, these previous indexes were decreased when the SPL and frequency went beyond the limit of 100 dB and 0.8 kHz. The changing tendency of IAA oxidase activity was reversed compared to the above three indexes. The influence of sound waves on the microstructure of plasmalemma for *Chrysanthemum* roots illustrated that the sound stimulation enhanced the fluidity of lipids and sound could also influence the secondary structure of protein not only in cell wall but also in plasmalemma. Sound waves decreased the phase transition temperature. Thus, the decrease of thermodynamic phase transition illustrates the enhancement of the fluidity of the cell wall and membrane, which also enhances the cells to grow and divide faster and easily. The enhancement of cell wall fluidity is one of the mechanisms of the promotion in plant growth by sound waves. Moreover, the electric potential of cell membranes could be changed by the sound field stimulation.

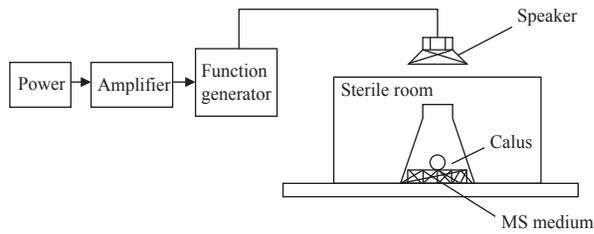


Fig. 2 Schematic diagram of the sound stimulation generator (Liu *et al.* 2002).

Plasmalemma exists in the outermost part of cells, consisting of membrane lipid and protein. It is the most sensitive and important part for sensing the environmental factors and many substances related to signal transduction exist in plasmalemma (Sun *et al.* 1999; Jia *et al.* 2003a).

Sound waves could greatly change the cell cycle of *Chrysanthemums*. The number of cells in G_0/G_1 decreased while it increased in the S-phase. This indicates that the sound waves accelerated the growth of *Chrysanthemums* (Wang *et al.* 2003b). The growth in plants is defined as the total of cell proliferation in the meristems and the subsequent elongation of cells. Moreover, cell proliferation is usually realized by the process of cell cycle. In normal cells, the cell division cycle is highly regulated and consists of four easily recognized phases: G_1 -phase (preparation for DNA replication), S-phase (DNA replication), G_2 phase (preparation for mitosis), and M-phase (mitosis). S-phase (synthesis phase) is the part of the cell cycle in which DNA is replicated, occurring between G_1 and G_2 phases. Precise and accurate DNA replication is necessary to prevent genetic abnormalities which often lead to cell death or disease (Depamphilis 2003). Exposing *Chrysanthemum* seedlings (*Gerbera jamesonii*) to sound waves elucidated that the activity of roots and the content of soluble protein increased greatly and the PMH^+ -ATPase was sensitive to Ca^{2+} under sound wave stimulation. It was found that the PMH^+ -ATPase could respond to many environmental factors and then regulate the growth and development. In addition, the phosphorylation/dephosphorylation process probably regulates the activity of plasmalemma H^+ -ATPase under sound stimulation. Meanwhile, it was observed that the activities of protective enzymes and

POD isoenzymes increased (Jia *et al.* 2003b; Wang 2003c). Plant plasma membrane H^+ -ATPases are the primary pumps responsible for the establishment of cellular membrane potential in plants and H^+ -ATPase is a major enzyme protein of the plant plasma membrane (PM). PMH^+ -ATPase is a kind of glycoprotein across membranes, and it can play an important role in the processes of growth and development of plants. H^+ -ATPases uses energy from ATP hydrolysis to pump protons from the cytosol to the extracellular space (Elmore and Coaker 2011). The activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and the POD isoenzymes are called protective enzymes or antioxidative defense system against free radicals. The free radicals super oxide radicals O_2^- and hydroxy radicals OH have strong oxidation and can destroy many function molecules in plants. Stimulated *Chrysanthemum* calluses by sound waves illustrated that PMH^+ -ATPase activity of *Chrysanthemum* callus increased apparently and probably Calmodulin-dependent phosphorylation enhances PMH^+ -ATPase activity. Whereas, the effects of sound stimulation on the permeability of K^+ channel of *Chrysanthemum* callus plasma indicated that the opening frequency of the K^+ channel for the stressed group was 50% higher than that of the control group. There was an obvious difference between them ($P < 0.001$) and also the K^+ channel had a close relation with the growth of callus with sound stimulation (Wang *et al.* 2002b; Zhao *et al.* 2002a). Sound wave had no obvious influence on the content of DNA but accelerated the synthesis of RNA and soluble protein. Moreover, some stress-induced genes might be switched on under sound stimulation and the level of transcription increased (Shao *et al.* 2008). The induction effect of sound wave on the dynamic change of endogenous indole-3-acetic acid (IAA) and abscisic acid (ABA) *in vitro* during the differentiation process of *Chrysanthemum* synchronized mature callus has shown that the treated group at a sound frequency (SF) of 1.4 kHz and SPL of 95 dB had significantly ($P < 0.05$) higher IAA levels and lower ABA than that of the control. Subsequently the activation of endogenous IAA and inhibition of ABA are favorable for the callus development and differentiation of mature callus. Sound waves increase capacity of IAA metabolism and inhibit the ABA metabolism in

the mature callus. Therefore, the higher ratio of IAA/ABA in the treated callus implied that a specific gene expression system was associated with endogenous hormone, which was regulated by some signals generated by sound waves stimulation (Wang *et al.* 2004). ABA is a plant hormone, defined as a stress hormone because of its rapid accumulation in response to stresses (Zhang *et al.* 2006; Lovelli *et al.* 2012). IAA is the most important auxin, predominantly produced in cells of the apex and very young leaves of plants. IAA can induce cell elongation and cell division which subsequently affects plant growth and development (Cutler *et al.* 2010; Wilkinson *et al.* 2010). Wang *et al.* (2001) reported that alternative stress of sound waves could change the cell membrane deformability. Moreover, the sound frequency improves the cell membrane deformability. This indicates that cells would change under external force, which made that cells have stronger resistance to the new environment. The effects of alternative stress on Ca^{2+} distribution in *Chrysanthemum* callus cells and the changes of Ca^{2+} distribution in subcellular structures were observed by electron microscopy (EM). There were distinct differences between control and treated groups by sound waves. In the control group cells, Ca^{2+} was concentrated in the vacuole and it was less in other organelles. While in the treated group, Ca^{2+} was concentrated in the vacuole membrane with a linear pattern. Thus, less Ca^{2+} was in the vacuoles and increased notably in cytoplasm, inner lateral of the vacuole membranes and nucleus field. It was also reported that sound stimulation could accelerate the combination of Ca^{2+} and vacuole membrane and the alternative stress could affect the opening and closing Ca^{2+} channel, which causes ion concentration changes on both sides of the membrane and the membrane potential leading the growth of plant tissues. Ca^{2+} calcium ions not only play an important role as a second messenger in plant growth and development but also in the response and adaptation of plants to the various environmental stresses. Different distribution of Ca^{2+} can cause different signal transduction in plants (Liu *et al.* 2001). The effect of sound waves on the PMH^+ -ATPase activity of *Chrysanthemum* callus showed that the PMH^+ -ATPase activity and its phosphorylation profoundly increased. However, a protein kinase inhibitor can effectively inhibit this effect. The

calcium-dependent protein kinase takes part in the effect of sound stimulation on the PMH^+ -ATPase activity of *Chrysanthemum* callus. The Ca^{2+} -dependent protein kinase can transduce the sound signal to the H^+ -ATPase in the plasma membrane. Then, it changes its activity and illustrates the response of cells to the sound stimulation (Zhao *et al.* 2002b). Calcium ion keeps the cell wall membrane and membrane binding protein stable; participates in the regulation of homeostasis and growth (Bush 1995).

Dendrobium candidum Wall. ex Lindl

Dendrobium candidum Wall. ex Lindl, known as Shihu, is a precious herbal plant in Chinese traditional medicine (Bao *et al.* 2001). It has been used in Chinese medicine therapy because it possesses the functions of clearing heat, benefiting the eyes and its immunomodulatory effects (Wang *et al.* 2011). The aerial parts of *Dendrobium* plants including *D. candidum* are often collected, cut into pieces, and then dried for medicinal usages (Zha *et al.* 2009). The effect of sound waves stress on *Dendrobium candidum* Wall. ex Lindl has shown that the activities of antioxidative enzymes were enhanced in different organs of *D. candidum*, as leaves, stems and roots. The different organs of *D. candidum* might produce accumulations of active oxygen species (AOS) under initial treatment of sound wave stress (Li *et al.* 2008). It was clear that later AOS might start to reduce due to the enhancement of antioxidant enzymes activities after sound waves treatments. The increased regulation of these enzyme activities would help to reduce the buildup of AOS and protect plant cells from oxidative damage. Active oxygen species have dual actions during plant stress responses (Dat *et al.* 2000) and proposed as a central component of plant adaptation to both biotic and abiotic stresses (Kim *et al.* 1998).

EFFECT OF PLANT ACOUSTIC FREQUENCY TECHNOLOGY (PAFT) ON VEGETABLES

The plant acoustic frequency technology (PAFT) generator manufactured by the Qingdao Physical

Table 2 Operation method of plant acoustic frequency technology generator

Operating condition	Wave band
Air temperature 10-20°C	1
Irrigation and air temperature 10-25°C	2
Air temperature 20-25°C	3
Air temperature 25-28°C	4
Air temperature 28-30°C	5
Irrigation and air temperature 25-30°C	6
Irrigation and air temperature 30-35°C	7
Air temperature 35-40°C	8

Agricultural Engineering Research Center in China, has eight variable frequency levels from 0.06 to 2 kHz and sound pressure levels (SPLs) from 50 to 120 dB for a distance about 50-100 m. PAFT produces an intermittent pulse of sound waves frequencies. As shown in Table 2, the wave band can be adjusted according to the plant meridian system, air temperature and relative humidity. The new generation of PAFT generator uses solar energy to operate and the control system for the sound generator uses a remote control. The effective coverage area for the PAFT ranges from 2 500 to 15 000 m²

The effect of PAFT generator on plant characteristics and the changes of chlorophyll fluorescence of cucumbers and strawberries were studied and showed that the numbers of flowers and fruits, as well as the content of chlorophyll, the net photosynthetic rate, the photochemical efficiency of PS II (F_v/F_m) and non-photochemical quenching were significantly ($P<0.05$) increased after 42 d of treatments in greenhouses. The sound pressure level was monitored with a precision sound pressure level meter. Therefore, the acoustic frequency treatment could improve the activity of PS II reaction centers; enhance the electron transport and the photochemical efficiency of PS II (Fan *et al.* 2010; Zhou *et al.* 2010; Meng *et al.* 2011, 2012a). Moreover, the acoustic frequency significantly stimulated the producing of endogenous hormones, such as, IAA, GA and ZR and also increased its contents in six kinds of vegetables including cucumber, tomato, muskmelon, cowpea, and eggplant (Huang and Jiang 2011; Zhu *et al.* 2011; Meng *et al.* 2012b).

The treated strawberries with the PAFT were grown stronger than the control group and their leaves were deeper green. They also were shifted 1 wk earlier in blossoming and bearing fruit. Photosynthetic rate had

significantly increased ($P<0.05$). The strawberries resistance against disease and insects were enhanced with a little effect on the yield (Qi *et al.* 2010).

Hou *et al.* (1999a) investigated the effect of agri-wave technology on the plant meridian system to improve the yield and quality of plants. Agri-wave technology is to broadcast intermittent pulses of sound waves by using the PAFT and spraying a compound of microelement fertilizer on the leaves once every other week. The results illustrated that the agri-wave technology significantly promoted the growth of tomatoes (fresh weight of the branch, stems and leaves), which were significantly (59.5%, $P<0.001$) higher than the untreated group. It also accelerated the tomato ripeness, increased the yield (13.9%, $P<0.001$) and improved its quality. Treating spinach with agri-wave technology stimulated the growth rate and increased the yield of spinach. The yield of the treated spinach increased by 22.7 and 22.2% and the sugar content of the treated spinach was increased by 37.5%, vitamins A, C and B were increased 35.6, 41.7 and 40.00%, respectively. In greenhouse testing, the average weight of three species of lettuce treated by agri-wave technology was 44.1% greater than that of the control group (Hou *et al.* 1999b). It has also been reported that applying PAFT to protect vegetable production in greenhouses increased the yield of the vegetables (Fig. 3-A). The yields of treated sweet pepper, cucumber and tomato were significantly higher than that of the control group. In addition, it improved crop quality and enhanced disease resistance. The spider mites, aphids, gray mold, late blight, and virus disease of tomatoes in greenhouses decreased by 6.0, 8.0, 9.0, 11.0, and 8.0%, respectively (Hou *et al.* 2009; Cai 2012; Jiang and Huang 2012).

EFFECT OF PLANT ACOUSTIC FREQUENCY TECHNOLOGY (PAFT) ON FIELD CROPS

Cotton plants were treated by using PAFT (Fig. 3-B). Results revealed that the height of the treated samples, the width of the 4th expanded leaf from terminal one, boll-bearing branches, number of bolls, and single boll weight increased by 1.7, 5.2, 1.1, 9.2, and 3.3%,

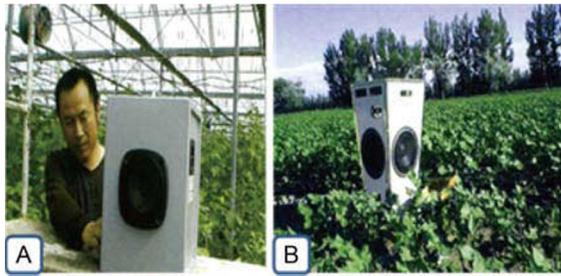


Fig. 3 Treatments of plant acoustic frequency technology generator cucumber in the greenhouse (Hou *et al.* 2009, A) and cotton in the open field (Hou *et al.* 2010a, B).

respectively. Meanwhile, the yield of treated cotton significantly increased in average of 12.7%. It has also reported that the effect of PAFT with four speakers, on cotton plants depends on the distance from the sound source in different directions. The minimum yield increase was 5.2% at a distance from PAFT about 30 m and at SPL of 75-110 dB and the maximum yield was 18.6% at a distance ranged from 30 to 60 m with a SPL of 70-75 dB, when the distance exceeded 150 m there was no effect (Hou *et al.* 2010a).

The yield of rice in pots experiment and in the open field increased in average by 25.0 and 5.7%, respectively and the yield of wheat increased in average by 17.0% when exposed to PAFT generator. Meanwhile, sound waves also improved the rice yield quality. For instance, head rice rate and protein content of rice increased by 5.9 and 8.9%, respectively; starch, protein and fat content of wheat increased by 6.3, 8.5 and 11.6%, respectively. As shown in Table 3, the PAFT has been applied to different plants during a different growth stages. Thus, applying this technology could effectively strengthen plant immune systems against plant diseases and insect pests. The sheath blight of rice was reduced by 50%. In addition, the results of 3 yr experiments on rice and sound technology showed that acoustic technology could reduce the amount of fertilizer by about 25.0% (Hou *et al.* 2010b; Yu *et al.* 2013).

EFFECTS OF SOUND WAVES ON DIFFERENT PLANTS

Jeong *et al.* (2008) identified a set of sound-responsive

genes in plants using a sound-treated subtractive library and demonstrated sound regulation through mRNA expression analyses. The experiments were conducted under light and dark conditions and results showed that sound could represent an alternative to light as a gene regulator. Ald mRNA expression significantly increased with treatment at 0.125 and 0.250 kHz, but it significantly decreased at 0.050 kHz. Moreover, they proposed that in transgenic plants, specific frequencies of sound treatment could be used to regulate the expression of any gene fused to the Ald promoter.

Effects of sound waves on the structure of the protein in tobacco cells revealed that the change of plasma membrane protein structure is closely related to the SPL and frequency. At a SF of 0.4 kHz and SPL of 90 dB, the sound waves made significant changes on the membrane protein structure, caused an increase in α -helix and a decrease in β -turn. This proves that the secondary structure of membrane protein is highly sensitive to the stimulation of sound waves and the change of the secondary structure of membrane protein may lead to the fluidity increase of the plasma membrane. The sound stimulation significantly decreased the phase transition temperature and the speed of cells growth (Sun and Xi 1999; Zhao *et al.* 2002c). It was mentioned that sound stimulation at a SF of 1 kHz and SPL of 100 dB promoted the soluble protein and sugar in cytoplasm, which indicates higher metabolism level and a vigorous state of cell division in a certain sound stimulation administrated to *D. morifolium* callus (Zhao *et al.* 2003). Sound waves increased the mycelium growth by about 15%, accelerated fruiting, achieved earlier maturity and extended the picking period by about 3-8 d. The audio treatment also increased the yield of edible mushrooms by 8.0-15.8% and also increased the fruit size by 2.4-43.3%, respectively. Moreover, the growth and propagation of *Chlorella pyrenoidosa* were significantly improved by sound waves at a sound frequency of 0.4 kHz (Jiang *et al.* 2011, 2012). Martens and Michelsen (1981) investigated the vibration of leaves of four plant species in a sound field using a laser Doppler vibrometer system. All leaves behave as linear mechanical systems when driven by sound and noise at SPL of 100 dB at reference pressure of 20 μ Pa. The vibration velocities of

Table 3 Application of sound waves on vegetables and different field crops

Plant	Growth indexes	Optimal sound frequency (kHz), SPL level (dB) and exposure period	References
Tomato, lettuce, spinach	The number of flowers, fruits and the content of chlorophyll Number of leaves, flowers and yield quality Disease resistances	The PAFT generator at SF ranged from 0.08-2 kHz and SPL 100 dB, once every other day, 180 min each time, from 7:00 a.m. to 10:00 a.m.	Hou and Mooneyham (1999a) Meng <i>et al.</i> (2011) Meng <i>et al.</i> (2012a)
Wheat	Tiller growth, plant dry weight and number of roots Seeds germination, height of stem, activity of root system, the penetrability of cell membrane and mRNA expression analyses	Acoustical instrument at SF of 5 kHz and SPL of 92 Twice a day 30 min each time for 2 d at SF of 0.4 kHz, SPL of 104 dB and at a distance of 0.2 m The PAFT generator at SF ranged from 0.08-2 kHz and SPL ranged from 50 to 100 dB, once every other day, 180 min each time, from 7:00 to 10:00	Weinberger <i>et al.</i> (1979) Wang <i>et al.</i> (2003)
Rice	Rice growth, yield and quality	Acoustical instrument at SF ranged from 0.3 to 6 kHz and 80 dB every day 180 min	Hou <i>et al.</i> (2010b) Yu <i>et al.</i> (2013)
Cucumber, sweet pepper	The number of flowers, fruits and the content of chlorophyll	The PAFT generator at SF ranged from 0.08 to 2 kHz and SPL 100 dB, once every other day, 180 min each time,	Fan <i>et al.</i> (2010) Zhou <i>et al.</i> (2010)
Strawberry	Number of leaves, flowers and yield quality	from 7:00 to 10:00. The distance ranged from 5 to 50 m	Qi <i>et al.</i> (2010)
Cotton	The shelf life for fruit and disease resistances		Hou and Mooneyham, (1999b) Hou <i>et al.</i> (2009) Hou <i>et al.</i> (2010a) Meng <i>et al.</i> (2012b)
Cucumber and cabbage	The level of polyamines (PA _s), the vitamin C and uptake of oxygen O ₂	Exposed to “green music” for 180 min daily and 20 kHz continuous wave ultrasound at SPL of 75 dBA	Qin <i>et al.</i> (2003)
Tobacco	The plasma membrane of tobacco cell and the fluidity of the cell membrane	Daily 60 min at SF of 0.4 kHz, SPL of 90 dB and at a distance of 0.2 m	Zhao <i>et al.</i> (2002c)
<i>Dendranthema morifolium</i>	The soluble protein and sugar in cytoplasm	Twice a day each time 30 min for 15 d at SF of 1 kHz, SPL of 100 dB and at a distance of 0.2 m	Sun <i>et al.</i> (1999)
<i>Edible mushroom</i> , cowpea, eggplant, muskmelon	The growth, yield and nutrient component	Range at SF of 0.340-3.3 kHz, SPL of 40 dB-80 dB every day 240 min from 8:30 to 11:30 a.m., and from 2:00 to 5:00 p.m.	Jiang <i>et al.</i> (2011), Zhu <i>et al.</i> (2011), Huang and Jiang (2011)
Different crops	Plant height, number of leaves and flowers The yield, earlier maturity, long shelf life and disease resistances	Dan Carlson company at SF of 3-5 kHz once every other day for 180 min	Carlson (2013)

the leaves at frequencies of 0.5-5 kHz were varied between 10^{-5} and 3×10^{-4} m s⁻¹, while the vibration velocity of air particles was 0.005 m s⁻¹ at 100 dB SPL. The vibration velocities of the leaves are thus 1-3 orders of magnitude smaller than that of the air particles. This means that only a part of the sound waves energy reached the leaf caused the leaf to vibrate and the other part of sound waves energy was reflected and diffracted around the leaf as if the leaf did not move at all. Sound waves might be absorbed by the leaf tissue and converted into heat (Martens *et al.* 1982). Although the amount of sound waves energy absorbed in this way by a single leaf is very small, this mechanism may somehow contribute to sound attenuation by plants and plant communities. Dan Carlson invented sonic bloom which involves a unique combination of sound and fo-

liar spray of seaweeds and found that in the frequency ranging from 3 to 5 kHz caused the stomata to open and increased the uptake of ‘free’ nutrients available in the atmosphere, including nitrogen and moisture in the form of humidity in the morning dew. Meanwhile, the sound attracts more birds than usual along with a large increase in the number of butterflies. Mosquitoes and other pests are thus consumed plus it increases resistance to pests and diseases by strengthening the plant’s immune system (Carlson 2013). Liu *et al.* (2012) reported that sound waves significantly effected on the behavior of drosophila. Drosophilas perform the avoidance and suppressed feeding in response to the acoustic radiation and the rate of avoidance to the acoustic radiation was 22.48%. It was also reported that exposing *Tetracus citri* Chen (Chinese citrus fly)

to a resonating acoustic at frequency of 0.055 kHz and sound pressure level of 120 dB for 30 min everyday in the flask had significant impact on the lifespan and increased the mortality rate of *Tetracus citri* Chen by 42.8% (Chen *et al.* 2013). The different musical elements had positive effects on root growth and mitotic divisions in onion root tip cells (Ekici *et al.* 2007). It has been demonstrated that audible sound affect on microbial cell metabolism when exposing yeast cells to music, high and low frequency sonic vibration and silence in liquid culture. All sonic stimuli tests not only increased the growth rate of the yeast cells by 12% but also reduced biomass production by 14%. Moreover, different metabolic pathways were affected by various sound frequencies (Aggio *et al.* 2012). Wilson and Dunton (2009) reported that seagrass have revealed acoustic phenomena and the effective sound speed in the plant-filled resonator was strongly dependent on plant biomass when exposed to a low frequency of 0.5-2.5 kHz.

CHALLENGES FOR ACOUSTIC FREQUENCY TECHNOLOGY APPLICATION

There are a number of challenges which are facing this technology. First, the PAFT causes noise pollution for both animals and human communities especially labors on the open field farms and in greenhouses. Therefore, to manage this problem sound waves application should be short early in the morning at 5:00-9:00 a.m. in the huge farms more than two square hectares. In addition, the leaves of plants absorb sound which may somehow contribute to sound attenuation by plants and plant communities, since the number of leaves of one full grown tree equals about 2×10^5 leaves. However, this noise might be beneficial for some plants. Francis *et al.* (2012) found that noise pollution (around 95 dB) in natural gas wells area indirectly increased artificial flower pollination by hummingbirds, but altered the community of animals that prey upon and disperse *Pinus edulis* seeds, potentially explaining reduced *P. edulis* seedling recruitment in noisy areas because black-chinned hummingbirds are more likely to visit and nest in noisy areas. Second, the sound pressure

level falls inversely proportional to the distance $1/r$ from the sound source. That is the $1/r$ law or distance law from its point of source as shown in eqs. (1) and (2). Since the intensity (I) is power (P) divided by area (A), the bigger the area, the lower the intensity:

$$I=P/A=P/(4\pi r^2) \quad (1)$$

For a spherical wave of a point source, the sound pressure level (SPL) decreases with doubling of distance by (-) 6 dB so SPL decreases with the ratio $1/r$ to the distance. Whereas, for a cylindrical wave of a line source the sound pressure level (SPL) decreases with doubling of distance only by (-) 3 dB. Thus, sound pressure levels decreases with the ratio $1/\sqrt{r}$ to the distance. Moreover, the other sounds such as bird's songs, jets, cars, loud music, explosions and people activities could change the certain sound pressure level. The sound pressure level L_p in dB without the given distance r to the sound source is useless:

$$L_2=L_1-20 \text{ Log } [r_2/r_1] \quad (2)$$

Where, L_1 , sound pressure level at reference distance r_1 dB SPL; L_2 , sound pressure level at another distance r_2 dB SPL; r_1 , reference distance r_1 (m); r_2 , another distance r_2 (m).

The decrease of sound pressure levels (SPL) could manage by increasing the interaction area between the plant acoustic frequency technology generators. Third, there is a lot of confusion and contradictions about this technology in terms of frequencies and exposure periods. Furthermore, the interactions between the environmental factors and sound waves could effect on the plant growth and each plant has a different response to sound frequency. Therefore, the future work should be set under a completely environmental control systems (air temperature, relative humidity, CO₂, lighting, water, soil and nutrition) to avoid any other environmental effects. Meanwhile, using the nutrient solution of foliar spray after sound waves treatment could achieve good results. Hence, some practical researches are needed to perform on the open fields at a large scale to quantify the effects of sound waves on plants and to find out some evidences for benefits and advantages of sound technology.

CONCLUSION

The world population increase presents a challenge

to scientists and researchers to investigate the possibilities for utilizing new and green technologies to increase the production of food. Using sound waves technology can enhance the plant immune system thereby; avoid many problems associated with the environmental pollution and the economic costs of chemical fertilizers and herbicide. The aim of this review was to present the effects of audible sound waves on some plants in different growth stages. The previous studies have shown that sound waves at different kinds of acoustics, sound pressure levels and frequencies for a certain exposure time affect on plants growth. Results of previous studies revealed that sound wave stimulation increases the growth of callus and accelerates the anabolism of callus cells at a frequency of 1 KHz and sound pressure level (SPL) of 100 dB (0.01 W m^{-2}) for 60 min once or twice a day as continuous sine waves for 9-12 d. Sound waves enhance the division and growth of callus cell significantly ($P < 0.05$), increase the activities of protective enzymes, and enhance the fluidity of lipids. Furthermore, sound waves could influence the secondary structure of protein not only in cell wall but also in plasmalemma. However, the optimal sound frequency stimulation will change according to the exposure time and period of application. Different plant species have various responses to sound stimulation at different growth stages. The optimal sound stimulation for seed germination of sound waves was at SPL of 100 dB and frequencies of 0.4-0.8 kHz every day for one hour. Using plant acoustic frequency technology (PAFT) to treat plants for 3 h d^{-1} , in the morning once every other day significantly increases the yield for different crops (6-30%). Viewing of the sound stimulation mechanism from the physicists platform, plants might have a meridian system as in humans and other animals. The frequencies of external sound stimulation along with the plant spontaneous sound frequency are in line and then the resonance occurs. Moreover, when the sound waves energy reached the leaf; part of sound energy vibrates the leaf and the other part of sound waves energy reflects and diffracts around the leaf and that part of sound waves energy effects on the insects around plants. Viewing of the sound stimulation mechanism from the biologists platform, sound

stimulation caused leaf stomata to open so the plant will be able to increase its uptake of spray fertilizer and dew, especially in the morning. The sound stimulation is also very efficient at getting the herbicides into the plant. Mature weeds can be sprayed with 50% less herbicide and biocide. Therefore, sound stimulation decreases the requirements for chemical fertilizer and pesticide (15-25%) as well as decreasing the plants diseases and improving the plant immune systems. Moreover, absorption efficiency of light energy markedly increased by sound waves, which is resulted in more electron transport between original quinine receptors on the recipient side of PS II, more light energy used for photochemical reaction and finally less superfluous excitation energy (Aspinell and Paleg 1981; Meng *et al.* 2012b). Sound waves stimulate the endogenous hormones. Thus, acoustic frequency technology promotes plant growth, increases production and improves yield quality.

FUTURE TRENDS OF ACOUSTIC TECHNOLOGY APPLICATIONS

Results of the previous studies showed that different species of plants have a various responses to sound waves stimulation in different growth stages. Thus, further research is needed to confirm these results.

Using sound waves for desirable plants could stimulate them to grow while undesirable plants (weeds for instance) could be inhibited, which has been done with electromagnetic energy, in this case sound waves, pulsed to the right set of frequencies thus affecting the plant at an energetic and sub molecular level.

Improve and spread this technology to promote the plant production worldwide and integrate the multi-physical agricultural technologies, such as electric, magnetic, optical, thermal, and nuclear and emerge into a single practical technology.

The mechanism of how sound affects the cell cycle and growth of plants needs further research and more scientific studies for unambiguous understanding.

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