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Review Paper

SOUND WAVE IN PLANT GROWTH REGULATION: A REVIEW OF POTENTIAL BIOTECHNOLOGICAL APPLICATIONS

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ABSTRACT

The sound wave perception of the animal is one of the most important mediums of communication. It contributed significantly in shaping the behavior, ecology and evolution of the organism. This evolutionary conserved communication mechanism in animal is carried out by different specialized organs like ear, antenna and others. However, the sessile organism, plants lack the specialized organ for the perception of sound and still perceive the sound signal. The sound wave perception in plants might also play critical role in shaping ecological and evolutionary implication. The effect of sound waves in the plant might have an advantageous effect which allows it to learn about the surrounding environment using the acoustic energy. The perceptions of sound signal in plants can lead to increase in growth, development and yield potential of plants. Therefore, sound wave treatment in the plant can be used as one of the growth promoter/regulator to increase the yield potential of crops. However, it is yet to identify the molecular receptor that perceives the sound wave in plants. The mechano-sensitive ion channels present in plasma membrane are highly modulated due to sound wave treatment that leads to differential calcium signaling in plants and subsequent regulation of downstream signaling molecules. In this review, author has reported some basic concept of the sound wave research and modulation of molecular responses in plant at cellular and sub-cellular level upon treatment of sound waves.

Key words: Sound wave, Hertz, Decibel, Acoustic energy.

INTRODUCTION

Communication with each other or with one another is ubiquitous in nature and one of the most studied subjects in science. Plants perceive to signaling and respond it according to the perception. The signaling can be of light, electromagnetic, physical, acoustic or chemical one and these signaling plays critical role in communication or the orientation of the organisms. The basic phenomena involve the perception and subsequent processing of signaling/energy embedded in the wave form. Different organisms developed various sensory organs that perceive the wave thus by resulting diversity in sensory organs in organisms. Further, they have developed their sensory organs in such a way that the wave could propagate for subsequent metabolic responses. The plants are sessile in nature and hence they always exposed to different environmental cues all along their life cycle. Different environmental cues are perceived by the plants differently during different time period of their growth and development. Upon perception of the cues, plants develop different physical and chemical signaling mechanism for its orientation, communications and defense programmes (Mohanta *et al.,* 2012). Among different environmental cues perceived by the plants, sound wave perception is one of the most important event (Gagliano *et al.,* 2012; Martens and Michelsen, 1981). Different organism developed different sensory organs with distinct morphological and physiological structure to perceive sound waves signaling. Human and other mammalian organisms have developed an ear pinna which perceives the sound vibration and transmits to the eardrum that converts the acoustic energy to mechanical energy (Groon *et al.,* 2014; Keefe, 2012). Some animals like frogs and birds have no outer ears and still able to perceive the hearing more accurate than humans (Feng *et al.,* 2006). The organisms like fruit flies, mosquitoes and others, the hearing is mediated by Johnstons organ present in the antennae (Göpfert and Robert 2000; Schneider, 1964; Zanini *et al.,* 2014). The reptile organism, snake lacks the outer and inner ear drums and still perceives the vibration wave by the jaw bones (Christensen *et al.,* 2012). These different structural features are responsible for sensing sound/vibrational waves, although there is absence of unique structural features responsible for the perception of sound waves. However, it does not require any specified auditory pathway to perceive and propagate signaling of sound waves. Different organisms generate sound to sense their environment and communication with each other in their close as well as moderately distant proximity. It has reported that plant produces sound waves at relatively low frequencies of 50-120 Hz (Hassanien *et al.,* 2014). However, the ecological and evolutionary importance of generation of sound waves by the plant is yet to be elucidated. However, it can be hypothesized that surrounding sound wave can allow the plant to learn about its surrounding environment. Because the signal of acoustic energy propagate quite rapidly to it's surrounding with minimal fitness cost. However, the generation, emission, and propagation of sound might have significant importance in adaptive mechanism. So, the question arises, what is the site of generation of acoustic energy sound in plants, whether plant perceives external sound waves? If yes, what are the probable receptor sites? What is the threshold intensity and frequency of sound wave perception? Before discussing these things, it is important to understand some of the basic concepts of sound wave for biologists. Therefore, in the review manuscript we presented a few of the basic concepts of sound wave and its application in plants.

Sound waves and its source: The sound wave is a mechanical vibration that oscillates at frequency within the audible range of ear (Middlebrooks and Green 1991). The sound vibration creates a pressure variation that interpreted as sound by the ears (Fujimoto 2014). If the pressure variation is highly erratic, it is known as noise. When a sound wave propagates through a medium, they create compression and refraction and the volume of the sound depends upon the pressure differences between the compression and refraction. The lowest sound audible with normal hearing is a zero decibel (0 dB) and a person with good hearing can even able to hear the weaker sound level of minus five (-5) dB (Moore *et al.,* 2014; Takeuchi *et al.,* 2014). The limit of hearing decreases with the increased in the age of the organism (;Bainbridge and Wallhagen, 2014; Kim *et al.,* 2014). The unit of sound is measured in decibel and it's a logarithmic unit. In real life, several sources of sound often occur at the same time and audible as combined with another. When one sound combined with another, the intensity of sound does not become double. For example, addition of 60 dB with 60 dB sounds give rise to only 63 dB that can be calculated by the following formula where SPL meant for sound pressure level.

$$\mathrm{SPL}_{\,(\mathrm{Total})} = 10 \log_{10} \sum_{i=1}^{n} 10^{(\mathrm{SPLi})/10}$$

The sound pressure level fall inversely proportional to the distance 1/r (r = distance) from the sound source (Chen *et al.,* 2011; Johannes *et al.,* 2014). From a spherical wave point source, the sound pressure level (SPL) decreases with doubling of distance by minus six (-6) dB and for cylindrical wave point source, the sound pressure level decreases with doubling of distance by minus three (-3) dB. The sound pressure level in dB without giving distance r to the sound source is useless. Therefore, it is very important to keep this point for any sound wave research in plants or other organisms. The schematic presentation of sound wave treatment in plant is presented in figure 1. The sound propagates through air in the form of longitudinal wave at the speed of 340 m/Sec at 22º C. The speed of the sound largely depends upon different environmental factors like temperature, humidity and speed of the sound increases by 0.6 m/s per degree increase in temperature. If only a single vibration occurs within a certain period of time, it cannot create any wave form and if two or more vibrations occur together within the stipulated time period, they can create waveforms. If the recurrent time period is only a couple of seconds, then the individual vibration will be heard as repeating whooshing sound.

It requires a specified medium for sound to wave to travel and the medium can be solid, liquid (water), or gases. The sound wave cannot travel in absence of medium, i.e. in a vacuum. Although the travel of sound in liquid and air is basically similar, the sound pressure level varied considerably. In solid, the molecules are packed tightly, but in a liquid the molecules are packed with spaces whereas in gases, molecules are packed very loosely. The spaces between two different molecules enable proper transfer of sound waves. Therefore, the movement of a sound wave in a solid is very less due to their compact built up and higher in liquid due to presence ambient spaces between the molecules. The appropriate space between the molecules in liquid enables the sound wave to move four times faster than solid. Plant perceives sound in earth surface and under water ocean surface as well. The sound with the same intensities in water and air per square meter has relative intensities differ by 61.5 dB. The plants present in the earth's surface perceived sound wave from vehicles, jet engine, rain, lighting, industries, and other allied sources, whereas in under water ocean, they received it from a variety of natural sources such as oceanic wave, rain, marine life, military sonars, ships and others. Some sound noise present persistently everywhere in the world and plants continuously comes after it. In soil, the sound moves faster than air due to the perfect molecular arrangement of soil texture and hence it is highly possible that the plant can perceive and propagate underground signaling through their root system far more faster compared to the aerial route. The role of frequency in sound wave application plays a crucial role to have a significant effect on the organisms. The standard measure of the frequency of oscillation of sound wave motion is measured in hertz (Hz). The frequency of sound is 1 Hz when one oscillation occurs per one second and is 1000 Hz when 1000 oscillations occur per one second. The sound wave frequency higher than 20 kilohertz (kHz) is called ultrasound and the sound frequency below 16 Hz are called infrasound. The audible sound frequency of human ranged from 20 Hz to 20 kHz. By keeping the sound intensity constant (sound pressure level) and changing the frequency level (Hz/kHz) frequently and vice versa may not be suitable for the sound wave research. It is important to know the optimum threshold level at what intensity (SPL) and frequency (Hz) level a specific organism responds to a specific kind of sound wave.

Application of Sound Waves to Plants: Different environmental factors like temperature, light, wind, etc. brings different physiological effects in plant growth, development and stress responses (Eller *et al.,* 2014; Hunt and Jaffe, 1980). In past two decades, importance was given to understand the mechanism of plant-to-plant communication which was basically based on chemical communication. Researches in these fields are well documented, but the role of the sound wave in plant growth and development is in its infantile stage. The application of sound wave technology to plants has been applied since long days and recently gained considerable attention. It has been found that the sound waves at different sound pressure levels (SPLs), frequencies, distances from the sound source and exposure periods influences plant growths and development (Hassanien *et al.,* 2014). Different researcher reported that sound wave treatment induces seed germination, and in numbers of lateral roots (Creath and Schwartz 2004). Behavioral response of *Zea mays* root to sound wave treatment (220 Hz) was demonstrated by Gagliano *et al.,* in 2012 (Gagliano *et al.,* 2012). They found, root tip immersed in water were bent towards the water-borne source of the sound. It also increases the plant dry weight (Weinberger and Measures 1979), chlorophyll content, photosynthetic rate (Fan *et al.,* 2010a; Meng *et al.,* 2012) by activating photosystem II, increases stomata opening (Pujiwati 2014), increases IAA and GA level and reduces ABA levels (Bochu *et al.,* 2004; Hassanien *et al.,* 2014; Xiaocheng *et al.,* 2003), increases soluble sugar and protein content (Yi *et al.,* 2003), speed up protoplasmic movements of cells (Hassanien *et al.,* 2014), and changes in cell cycle (Bochu *et al.,* 1998). Sound treatment also enhances disease resistance and hence decreases the requirements for chemical fertilizers and biocides (Zhang, 2012). Carlson (2013) reported that matured weed can be sprayed with 50% less herbicide and biocide if treated with sound wave (Carlson, 2013). The disease resistance activity of tomato plant was enhanced considerably by the application of sound waves (Table 1). The susceptibility of late blight, gray mold, aphids, spider mites and virus diseases decreased by 11.0%, 8.0%, 8.0%, 6.0% and 9.0% respectively (Table 1) (Hou *et al.,* 2009).The sheath blight of rice was reduced by 50% when it was exposed to PAFT (plant acoustic frequency technology) generator (Hou *et al.,* 2010; Yu *et al.,* 2013). The results from three year's experiments on rice and sound technology showed that sound wave treatment can reduce the application of chemical fertilizers by about 25% (Hassanien *et al.* 2014). Treatment of sound wave accompanying with the spraying of micronutrient promoted the growth of tomatoes (fresh weight) (59.5%, $p<0.01$), accelerated tomato ripeness and increased the yield (13.9%, $p<0.001$) (Hou and Mooneyham, 1999). Treating spinach with agri-wave technology led to stimulated growth rate and increased yield (22.7%) (Hou and Mooneyham, 1999). This also led to the increased in sugar (37.5%), vitamin A (35.6%), B (40%), and C (41.7%) contents. The yield potential of rice pot experiments was increased by 25% when it was exposed to PAFT (plant acoustic frequency technology) generator. Similarly the yield potential of wheat also increased by 17% (Hassanien *et al.,* 2014). Sound wave treatment significantly enhanced the yield of sweet pepper, tomato, and cucumber. When experiments were conducted with fungi, it has found that sound wave mediated growth of the fungal mycelium was increased by 15%, and accelerates fruiting of edible mushroom (Jiang *et al.,* 2011). Mushroom treated with sound waves increased the yield potential by 8.0 to 15.8% and fruit size by 2.4-43.3% respectively (Table 1) (Jiang *et al.,* 2011).

The sound wave can enhance the fluidity of lipids and influence the secondary structure of proteins in the plasmalemma by changing the α-helix and β-sheet conformation (Yi *et al.,* 2003). The sound wave can decrease the phase transition temperature and hence decrease the thermodynamic phase transition. This leads to increase the fluidity of the cell wall and plasma membrane and enhances the cell to grow and divide faster. This increase in the rate of cell division can be one of the major mechanisms for promotion of plant growth by sound waves. At sound frequency of 0.4 kHz and sound pressure level (SPLs) of 90 dB, the structure of plasma membrane protein changes significantly (Zhao *et al.,* 2002). It causes an increase in α-helix and decrease in β-turn structures. This shows that the secondary structure of protein is very sensitive to the stimulation of sound waves. These changes in secondary structure of membrane protein may be responsible for the increase in the fluidity of plasma membrane.

Signaling Events: From the above discussion, it is apparent that the sound wave has significant impact in plant growth, development as and disease resistance as well. The changes in plant growth, development and enhanced disease resistances are undoubtedly related with different sophisticated signaling cascades. The expectation in differential changes at the transcriptome and proteome level is highly apparent. The activity of superoxide dismutases (SOD) and catalases (CAT), peroxidases (POD) enzyme in *Actinidia chinensis* (kiwi) was increased by the application of sound wave at 1 kHz and 100 dB (Table 2) (Xiujuan *et al.,* 2003; Yang *et al.,* 2002; Yiyao *et al.,* 2002). The stimulation of SOD was decreased when stimulation exceeded 1 kHz. The reactive oxygen species (ROS) resulted due to different biotic and abiotic stresses and SOD enzymes act upon it as an anti-

Table 1. Application of sound waves to crop plant and their change in phenotype..

Plant Materials	Sound Frequency (kHz) and Sound Pressure Level (dB)	Exposure Time Period	Sound Source Distance	Physiochemical Changes	References
Tomato	PAFT generator, 0.08-2 kHz, 100 dB	3 hours in once every other day from 7.00-10.00 AM		Increased yield of by 13.2%	(Hassanien *et al.,* 2014)
Sweet pepper	PAFT generator, 0.08-2 kHz	3 hours in once every other day from 7.00-10.00 AM	5-50 m	Increased yield by 30.05%	(Hassanien *et al.,* 2014)
Cucumber	PAFT generator, 0.08-2 kHz	3 hours in once every other day from 7.00-10.00 AM	5-50 m	Increased yield by 37.1%	(Hassanien *et al.,* 2014)
Rice	400 Hz, 106 dB	30 min twice a day for 2 days		Increased seed germination rate, stem height, fresh weight, rooting ability, activity of root system and penetrability of cell membrane	(Bochu *et al.,* 2003)
Rice	0.3 to 6 kHz, 80 dB	3 hours everyday		Increased in rice growth, yield and quality	(Hassanien *et al.,* 2014)
Cucumber	PAFT generator, 0.08-2 kHz	3 hours in once every other day from 7.00-10.00 AM	5-50 m	Increased in numbers of flowers, fruits and chlorophyll content	(Fan *et al.,* 2010b)
Cotton				Increased in shelf life of fruit and disease resistance	(Hou *et al.,* 2010)
Cow pea	0.340-3.3 kHz, 40 db-80 dB	Everyday 240 min, from 8.30-11.30 AM and from 2.00 to 5.00 PM		Increased in growth and yield	(Huang and Jiang, 2011)
Egg plant	0.340-3.3 kHz, 40 db-80 dB	Everyday 240 min, from 8.30-11.30 AM and from 2.00 to 5.00 PM		Increased in growth and yield	(Jiang *et al.,* 2011)
Muskmelon	0.340-3.3 kHz, 40 db-80 dB	Everyday 240 min, from 8.30-11.30 AM and from 2.00 to 5.00 PM		Increased in growth and yield	(Hassanien *et al.,* 2014)
Strawberry	PAFT generator, 0.08 to 2 kHz, 100 dB	3 hours every day from 7.00 to 10 AM	5-50 m	Increased in numbers of leaves, flower and quality of yield	(Qi *et al.,* 2010)
Wheat	0.4 kHz, 104 dB	3 hours, once in every other day from 7.00 AM to 10.00 AM	0.2 m	Seed germination, stem height, increased activity of root system	(Weinberger and Measures, 1979)
Mushroom	0.340-3.3 kHz, 40 db-80 dB	Everyday 240 min, from 8.30-11.30 AM and from 2.00 to 5.00 PM		Increase yield by 8.0-15.8% and fruit size by 2.4 to 43.3%	(Jiang *et al.,* 2011)
Tomato disease				Decrease diseases by 6.0, 8.0, 9.0, 11.0, and 8.0% caused by spider mite, aphides, gray molds, late blight and viruses respectively	(Hassanien *et al.,* 2014)

Table 2. Effect of sound waves in plant and their changes at the molecular level.

Plant Materials	Sound Frequency (kHz) and Sound Pressure Level (dB)	Exposure Time Period	Distance from Sound Source	Physiochemical Changes	References
Chrysanthemum callus	1 kHz, 100 dB	60 min daily for 3 days	0.2 m	Enhanced activities of PMH^+-ATPase	(Zhao *et al.,* 2002)
Chrysanthemum callus	1 kHz, 100 dB	30 min twice a day for 9 days	0.2 m	Enhanced activities of SOD, POD, CAT	(Xiujuan *et al.,* 2003)
Chrysanthemum callus	0.8 kHz, 100 dB	30 min twice a day for 10 days	0.2 m	Enhanced SOD, soluble protein, absorption rate of Ca^{2+}ion and Iindole-3-acric acid oxidase	(Yiyao *et al.,* 2002)
Chrysanthemum plant	1 kHz, 100 dB	Daily 60 min for 9 days	0.2 m	Changes in gene expression, POD isoenzymes and DNA and RNA content	(Hongbo *et al.,* 2008)
Chrysanthemum callus	1.4 kHz, 95 dB	30 min twice a day for 10 days	0.2 m	Higher Indole-3-acetic acid and lower abscisic acid content	(Bochu *et al.,* 2004)
Chrysanthemum callus	1 kHz, 100 dB	Daily 60 min for 6 days	0.2 m	Enhanced plasma membrane H^+-ATPase activity	(Zhao *et al.,* 2002)
Chrysanthemum callus	1 kHz, 60 dB	Daily 60 min for 9 days	0.2 m	Changes in microstructure of plasmalemma	(Yi *et al.,* 2003)
Actinidia chinensis callus	1 kHz, 100 dB	30 min twice a day for 20 days	0.2 m	Increased in ATP and soluble sugar content	(Xiaocheng *et al.,* 2003)
Dendrobium candidum	1 kHz, 100 dB	Daily 60 min for 9 days	0.2 m	Enhanced activities of SOD, CAT, and POD	(Li *et al.,* 2008)
Dendranthema morifolium	1 kHz, 100 dB	30 min every day for 15 days	0.2 m	Increased in soluble protein and sugar content in cytoplast	
Cucumber and cabbage	Green music, 20 kHz, 75 dB	3 hours every day for 15 days		Increased in levels of polyamines (PAs), increased uptake of oxygen	(Qin *et al.,* 2003)
Nicotiana	0.4 kHz, 90 dB	60 min every day	0.2 m	Changes in plasma membrane of tobacco cells and fluidity of the cell membrane, increase in α-helix and decrease in β-turns	(Zhao *et al.,* 2002)

oxidant thus protects cellular component from oxidation and cell death (Mohanta *et al.,* 2012). The SOD activity of *Chrysanthemum* callus was increased with the increase in SPL and sound frequency.

The plasma membrane bound H^+-ATPase activity was enhanced in *Chrysanthemum* callus due to sound wave treatment in calcium dependent protein kinase (CPK) mediated phosphorylation event. The H^+-ATPases were the glycol-proteins responsible for the establishment of cellular membrane potential (Elmore and Coaker, 2011; Levin *et al.,* 2002). They utilize energy from ATP hydrolysis to pump protons from the cytosol to the extracellular space. The opening and permeability of potassium ion channel (K^+) was higher in sound wave treated group compared to the control *Chrysanthemum* callus. The sound wave stress also led to differential Ca^{2+}distribution in *Chrysanthemum* callus. The Ca^{2+} ion was concentrated to the vacuolar membrane in a linear pattern. The alternative sound stress might be responsible for the opening of Ca^{2+} channels that led to the changes in membrane potential (Liu *et al.,* 2001). The Ca^{2+} ions act as an important second messenger and helps the plants for its growth, development and disease resistance activities (Kanchiswamy *et al.,* 2013). Besides this, Ca^{2+}ion acts in diverse signaling event during different biotic and abiotic stresses. The Ca^{2+} mediated calcium dependent protein kinases can transduce the sound signal to H^+-ATPase in the plasma membrane.

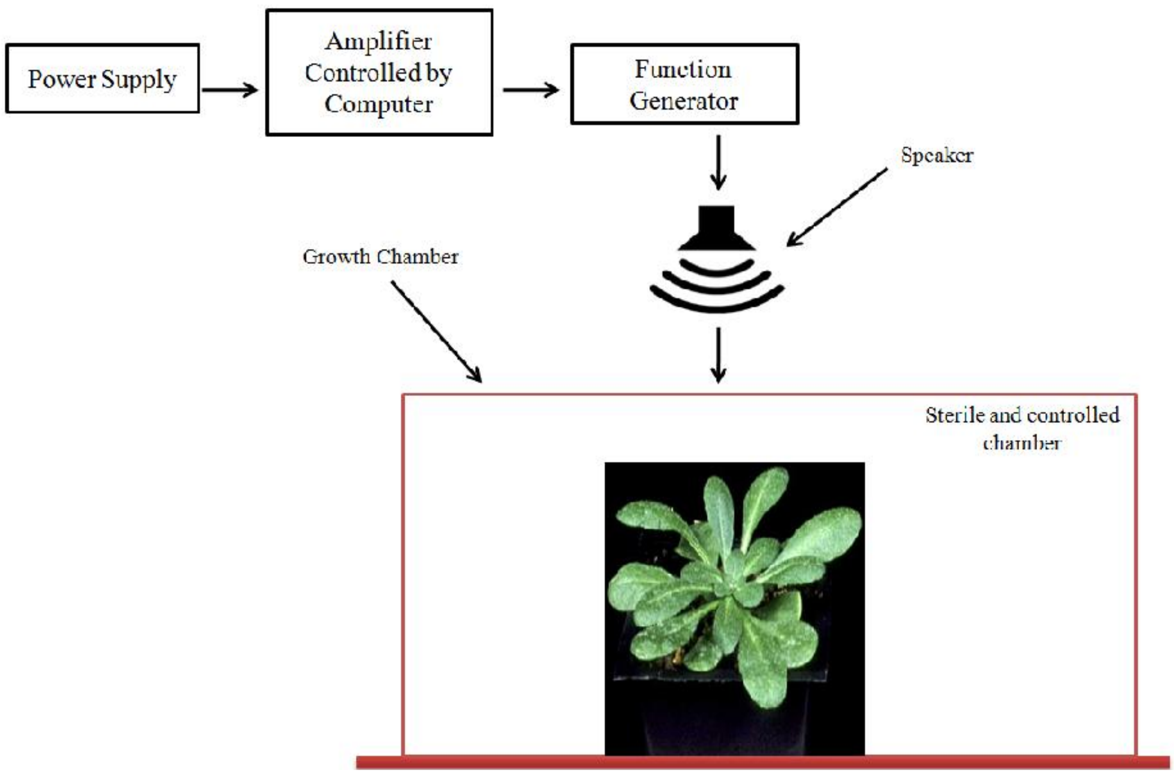

Figure 1. Schematic presentation of sound generator and treatment chamber require for sound treatment in plants. Sound pressure level decreases with increase in distances. So, proper care should be taken regarding origin of sound source (speaker) and its distance during sound treatment to plants.

Challenges of Sound Wave Research: The sound treatment is very difficult to apply in the field level because it might result in noise pollution for human and other organisms. Different species perceives sound levels differently and hence sound wave (frequency and intensity) beneficial for one organism may be harmful for growth and development of another organism. So, it is better advised to perform and restrict sound wave treatment to closed environments.

Conclusion and Future Perspectives: The sound wave perception in plants is a new field of research and need to be investigated thoroughly. The sound wave researches in crop plants like wheat, rice, tomato, cucumber and other plants are reported by different research groups and found that sound wave act as a growth regulator. Therefore, sound wave treatment can be used as a potential biotechnological application in plant growth promotion. Although sound wave treatment in crop plant gained significant attention, researches in model plant *Arabidopsis thaliana* are very scanty. Application of high throughput transcriptomics, proteomics and metabolomics study will be very helpful to find out the potential sound receptor gene. Elucidation of sound receptor gene will be very helpful to elucidate the sound responsive and its regulatory pathways for its better application in plant growth promotion.

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