



OPINION PAPER

Plant acoustics: in the search of a sound mechanism for sound signaling in plants

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Abstract

Being sessile, plants continuously deal with their dynamic and complex surroundings, identifying important cues and reacting with appropriate responses. Consequently, the sensitivity of plants has evolved to perceive a myriad of external stimuli, which ultimately ensures their successful survival. Research over past centuries has established that plants respond to environmental factors such as light, temperature, moisture, and mechanical perturbations (e.g. wind, rain, touch, etc.) by suitably modulating their growth and development. However, sound vibrations (SVs) as a stimulus have only started receiving attention relatively recently. SVs have been shown to increase the yields of several crops and strengthen plant immunity against pathogens. These vibrations can also prime the plants so as to make them more tolerant to impending drought. Plants can recognize the chewing sounds of insect larvae and the buzz of a pollinating bee, and respond accordingly. It is thus plausible that SVs may serve as a long-range stimulus that evokes ecologically relevant signaling mechanisms in plants. Studies have suggested that SVs increase the transcription of certain genes, soluble protein content, and support enhanced growth and development in plants. At the cellular level, SVs can change the secondary structure of plasma membrane proteins, affect microfilament rearrangements, produce Ca^{2+} signatures, cause increases in protein kinases, protective enzymes, peroxidases, antioxidant enzymes, amylase, H^+ -ATPase / K^+ channel activities, and enhance levels of polyamines, soluble sugars and auxin. In this paper, we propose a signaling model to account for the molecular episodes that SVs induce within the cell, and in so doing we uncover a number of interesting questions that need to be addressed by future research in plant acoustics.

Key words: Acoustics, perception, plants, response, signaling, stimulus.

Introduction

To overcome their sessile lifestyle, plants have mechanisms to scrutinize their dynamic surroundings for the copious cues that fine-tune their growth and development. Plants also identify cues that help them acclimatize physiologically towards impending undesirable conditions. The sensitivity of plants has evolved to a level that besides responding to the key physical stimuli of 'light' and 'temperature' they can even perceive mechanical stimuli such as touch, wind, rain, etc. (Heggie and Halliday, 2005; Telewski, 2006). It is important to note that no niche occupied by plants is free of sound,

whether it originates from an organism such as a bee buzzing or a bird chirping, or is produced physically through blowing winds, flowing water, etc. It is thus unsurprising that plants have developed sensitivity towards sound vibrations (SVs) of various ecologically relevant frequencies. A strong example of this is the widespread phenomenon of 'buzz pollination', where pollen from anthers is released only against a particular frequency of SV (buzz) produced by a specific pollinator (De Luca and Vallejo-Marin, 2013). The perception and processing of SVs are ecologically advantageous (Gagliano, 2013b).

Even the unicellular life forms – bacteria and yeast – are responsive towards sound stimulation; SVs trigger colonization in *Escherichia coli* (Gu *et al.*, 2010) and enhance the growth rate of yeast in liquid medium (Aggio *et al.*, 2012). Furthermore, exposure of cells of the microalgae *Picochlorum oklahomensis* to audible sound enhances growth and biomass productivity (Cai *et al.*, 2016). From an evolutionary viewpoint, communication by SV has contributed remarkably in the successful survival of animals (Gagliano *et al.*, 2012a; Gagliano, 2013b): even species without an ear and/or eardrum can perceive sound (Gagliano *et al.*, 2012a). Regardless of the fact that different species have developed substantially different structures for sound perception, processing and response appear to be more-or-less similar. The explanation for this comes from the physical properties of sound waves. In terms of physics, sound waves originate from vibrating objects and are transmitted by the oscillation of the particles in the medium they travel through. These waves can potentially vibrate any object interface with. Even in animals with visible external ears, it is the vibration of the eardrum in particular that is key to the process of hearing (Gagliano *et al.*, 2012a).

Plant membranes are equipped with an enormous number of mechano-sensitive channels that are believed to be responsive to mechanical vibrations (Haswell *et al.*, 2011). SVs can cause an alteration in the tension in biological membranes that could possibly evoke a signaling cascade through activation of these channels (Haswell and Meyerowitz, 2006). Interestingly, a growing body of evidence in the past two decades suggests that SVs of certain frequencies can positively influence processes such as seed germination, root elongation, callus growth, and cell cycling (Gagliano, 2013b; Chowdhury *et al.*, 2014; da Silva and Dobranszki, 2014). In addition, SV treatment has been shown to cause favorable adjustments in plants that prepares them to withstand water scarcity (Jeong *et al.*, 2014). In a critical study, Gagliano *et al.* (2012c) showed an alternative means of communication in plants that had not been reported previously and claimed it to be through SVs. Subsequently, it was shown that young roots of maize seedlings respond to sound treatment by producing SVs (Gagliano *et al.*, 2012a). Although this suggested that plants can emit sound in response to a sound stimulus, clearly further research is warranted to strengthen the idea of communication among plants through SVs. Nevertheless, these reports generated a lot of scientific interest that triggered a series of fruitful discussions/debates in the form of several critical reviews/commentaries (Chamovitz, 2012; Gagliano *et al.*, 2012a, b; Bailey *et al.*, 2013; Gagliano, 2013a, b). These papers are strongly recommended for insights into several issues in plant acoustics, such as (a) whether plants can emit and detect sound stimuli; (b) what could be the benefits and adaptive values of acoustic communication in plants; and (c) is the sound stimuli perceived by plants merely incidental sound or precise signals? These reviews have made two remarkable contributions to plant acoustics. Firstly, two broad areas of research have emerged: particularly, addressing plants' communication with each other through SVs, and tackling the important question of how plants perceive ecologically meaningful acoustic signals. Secondly, attention has

shifted away from the original question of 'whether' plants detect and respond to SVs, to the more direct questions of 'why' and 'how' they do it.

A recent report by Appel and Cocroft (2014) showed that plants became defensive upon exposure to SVs caused by caterpillar's chewing. This demonstrated one of the several possibilities for 'why' perception of sound would be an evolutionarily beneficial adaptive feature in plants. This report proved to be the turning point by establishing the importance of acoustic communication in plants. To substantiate the concept of communication among plants through SVs, Gagliano (2013b) came up with a model for how plants may generate acoustic emissions. However, so far, the discussion on 'how' plants cell perceive acoustic signals has only been superficial.

In the light of the above considerations, it appears that simple ignorance of plants being receptive to SVs is the cause of the huge gap in our understanding of acoustic responses in plants. In this paper, we leave this procrastinating debate behind, and instead we bring the focus onto the important question of 'how' SVs are perceived and transduced in plant cells. We present a critical analysis of the cellular changes in response to sound stimulus that have been reported so far with the aim of bridging this knowledge gap. A model is presented showing the probable molecular events that may occur when a plant cell perceives SVs. As well as summarizing the scattered pieces of published information, the proposed model also raises several interesting questions, which, if answered, would connect its isolated component parts together into a working sound-signaling model. This paper forms a fundamental basis for researchers to test several potential hypotheses regarding how SVs are perceived and transduced, and thus will aid scientists to expedite research in this little-explored area of plant biology.

Acoustic responses of plants: the underpinnings

Mankind's proclivity to view nature through the window of our own wisdom has been a reason behind our disbelief that plants can have some senses that are the same as ours. Thus, the Aristotelian view that distinguishes animals from plants based on the capacity to sense has prevailed for so long. However, in the light of the rapid sensory movements exhibited by carnivorous plants, this argument has been shown to hold no truth. With this arises an immediate question – if plants can sense touch, why can't they also sense sound? Studies on plant acoustics commenced back in 1950s with several controversial reports claiming the effect of musical sound on plants (Ekici *et al.*, 2007). Though controversial, these claims attracted increasingly widespread scientific attention towards plant acoustics (Miller, 1983; Collins and Foreman, 2001; Ekici *et al.*, 2007; Jeong *et al.*, 2008; Gagliano, 2013b; Chowdhury *et al.*, 2014; Mescher and De Moraes, 2015). In subsequent studies, to examine the acoustic responses in plants further, scientists adopted the use of natural SVs produced by bird's chirping, cricket's stridulating, bee's buzzing, etc. and obtained compelling results.

For example, such natural SVs were found to accelerate seed germination rates in okra (*Abelmoschus esculentus*) and zucchini (*Cucurbita pepo*) (Creath and Schwartz, 2004). As a further refinement, several researchers have started using SVs of variable single frequencies. Collins and Foreman (2001) applied different frequencies (500, 5000, 6000, 12 000, 14 000 Hz) of SV with the same pressure level (91–94 decibel, dB) to common beans (*Phaseolus vulgaris*) and impatiens (*Impatiens* sp.) and noted frequency-specific responses. Although growth was enhanced in both cases, beans showed maximum growth at 5000 Hz whereas impatiens responded best at 12 000 Hz. Similar results were found in Chinese cabbage and cucumber, where sound stimulation resulted in increased oxygen uptake and levels of polyamines, with Chinese cabbage being more responsive towards natural SVs whereas cucumber responded better to 20 000 Hz (Qin *et al.*, 2003).

It is appropriate to mention here what Chamovitz (2012) proposed: ‘music is not ecologically relevant for plants, but there are sounds that could be advantageous for them to hear’. Certainly, responses of plants to natural SVs that could be potentially stimulating can in no way be replicated by exposing them to SVs of single frequencies artificially. Nevertheless, plants’ sensitivity towards such treatments strongly supports the idea that they do perceive SVs by making adjustments both at the molecular/physiological and morphological levels. Furthermore, as different plant species and tissues respond to different SV frequencies, it appears that this preference to a particular SV frequency could possibly have ecological significance. In the light of this, we highlight here the pressing need for extensive research to address plants’ responses to treatments with different natural SVs recorded at appropriate frequencies (that might enact as a potential stimulus). As well as uncovering the hidden aspects of plant acoustics, this may also reveal evolutionary adaptive features that the responses have produced. We thoroughly discussed the merits of one such study recently conducted by Appel and Cocroft (2014) in a later section of this review.

The growth-enhancing effects of SVs as discussed above accelerated plant acoustics research in a direction more biased towards its utility in biotechnology and agriculture than towards studies examining the ecological significance of plants’ response to natural SV.

Implications of sound treatments in biotechnology and agriculture

Because of the positive effects it produces in several growth parameters of plants, SV treatments have been extensively used in biotechnology and agriculture. In plant tissue culture techniques, SVs have been suggested to increase organogenesis (da Silva and Dobranszki, 2014). Ultrasound (sound above the audible range of 20–20 000 Hz) has been successfully used to enhance the *Agrobacterium*-mediated transformation of several plants, such as *Glycine max*, *Vigna unguiculata*, *Triticum aestivum*, and *Zea mays* (Trick and Finer, 1997). Furthermore, SVs have been shown to stimulate the *in vitro* growth and development of various plant species, such as *Daucus carota*

(Wang *et al.*, 1998), *Oryza sativa* (Liu *et al.*, 2003b), *Aloe arborescens* (Liu *et al.*, 2003a), *Gerbera jamesonii* (Wang *et al.*, 2003b), *Cucurbita pepo* (Ananthkrishnan *et al.*, 2007), *Dendrobium officinale* (Wei *et al.*, 2012), and *Corylus avellana* (Safari *et al.*, 2013). Wang *et al.* (2003a) reported a significant increase in the germination index, shoot height, fresh weight, root system activity, and the cell membrane penetrability of paddy rice treated with SVs of 400 Hz frequency and 106 dB. However, frequencies above this range caused harmful effects, suggesting that SVs only within an optimal range can enhance cells’ physiological activity. Consistent with this, previous studies have shown that audible SVs can be used as a positive growth regulator whereas ultrasound causes negative botanical effects (Miller, 1983; Hassanien *et al.*, 2014). In the case of the medicinal plant *Actinidia chinensis*, SVs increased the total length, number, and activity of roots (Yang *et al.*, 2004). Seeds of *Echinacea angustifolia*, which are highly dormant, showed enhanced germination upon treatment with SVs of 1000 Hz and 100 dB energy (Chuanren *et al.*, 2004). Consequently, Qingdao Physical Agricultural Engineering Research Center in China developed a plant acoustic frequency technology (PAFT) generator that produces SVs at eight variable frequency levels for facilitating agricultural output. Cotton plants exposed to PAFT showed increased height, leaf width, number of boll-bearing branches and bolls, and weight of individual bolls (Hou *et al.*, 2010). Strawberry plants treated with PAFT showed increased photosynthetic characteristics and improved disease resistance without affecting the yield (Qi *et al.*, 2010). Treatment of rice plants with PAFT resulted in the enhancement of both the grain yield and quality: while yield increased by 5.7%, protein content in the grains showed an increase of 8.9% (Hassanien *et al.*, 2014). Application of PAFT resulted in a 17% increase in the yield of wheat together with a rise in the grain’s starch, protein, and fat content by 6.3, 8.5, and 11.6%, respectively (Hassanien *et al.*, 2014). Using the PAFT, numbers of leaves and flowers, content of chlorophyll, and yield were increased in plants including tomato, lettuce, and spinach (Hassanien *et al.*, 2014). SVs have also been implicated in enhancing the postharvest shelf-life of tomato fruits: treatment of tomato fruit with SVs of 1000 Hz delayed ripening as compared to the untreated controls (Kim *et al.*, 2015). The growth-enhancing effect of SVs could potentially reduce the usage of fertilizers by 25% (Hassanien *et al.*, 2014). Plants treated with SVs have been found to exhibit increased immune responses against plant diseases and insect pests. For instance, the spread of sheath blight in rice has been found to be reduced by 50% as a result of SV treatment (Hassanien *et al.*, 2014). SV-mediated acceleration in seed germination as well as plant growth could be due to enhanced levels of growth-promoting phytohormones: one of the means of breaking seed dormancy artificially is through application of growth promoters (i.e. gibberellic acid, cytokinin, and ethylene). In addition, the process of organogenesis, which is promoted by SVs, is under tight regulation by phytohormonal levels (auxin/cytokinin). SV-mediated alterations in phytohormones are discussed later in this paper.

Collectively, the studies considered above show the potentially large application of SVs in improving crop yield,

defense, nutrient value, etc. Since it is out of the scope of this paper to discuss the detailed benefits gained by SV treatment in biotechnology and agriculture, readers are directed to the dedicated reviews of [da Silva and Dobranszki \(2014\)](#) and [Hassanien *et al.* \(2014\)](#) for this information.

Ecological relevance of sound perception in plants

Whilst studies showing implications of SVs in facilitating agricultural yields and disease resistance have taken a lead in the past two decades, studies deciphering the ecological relevance of such responses have only recently made some advances. Like animals, where the major mode of information exchange is through acoustic communications, recently plants have also been shown to communicate similarly, possibly through sonic vibrations ([Gagliano *et al.*, 2012c](#)). This is further supported by plants sensitivity to detect selective-frequency vibrations and their ability to generate structured, spike-like, acoustic radiations in response ([Gagliano *et al.*, 2012a](#)). Furthermore, plants facilitate several of their vital processes, such as pollination, nitrogen assimilation, etc., through mutualism with animals ([Simon *et al.*, 2011](#); [De Luca and Vallejo-Marin, 2013](#); [Schoner *et al.*, 2015](#)), and such mutualisms have played an important role in shaping ecosystems. Plants and animals communicate in several ways that involve acoustic effects. An example of this is the widely spread phenomenon of ‘buzz pollination’, which is being utilized by approximately 20 000 plant species ([De Luca and Vallejo-Marin, 2013](#)). It has been suggested that buzz pollination evolved to enable the plants to distinguish between pollen thieves and true pollinators. Consistent with this, it has been shown that pollen is released from flowers only upon vibration at the appropriate/selective buzz frequency produced by bees that have coevolved in this process ([Gagliano, 2013a](#)). Importantly, this again highlights the competence of plants to discriminate relevant sonic frequencies from non-relevant ones in an ecological perspective.

There are some other examples where the underlying basis for plant–animal interactions could be the acoustic communication between them. *Marcgravia evenia*, a bat-pollinated Cuban vine, bears a characteristic structure located just above the inflorescence that functions as an echo beacon for attracting bats ([Simon *et al.*, 2011](#)). These are dish-shaped foliage leaves with twisted stalks that bring the leaf blade to an upright position with their upper concave side facing outwards, thus ensuring its effective detection through echolocation. In another example, the pitcher of the carnivorous plant *Nepenthes hemsleyana* functions as an ultrasound reflector and thus enhances their detection by bats to use for roosting. In turn, the plant absorbs nitrogen from the bat droppings, which on average account for ~35% of the total nitrogen assimilated by this plant ([Schoner *et al.*, 2015](#)). Looked at from the animal’s perspective, these structures help the bats to locate plants from which they get food in the form of nectar and pollen, or a safe place to roost. From the plant’s perspective, these bats perform pollination – the most crucial step that ensures continuity of the plant’s genotype – and

help fulfill their nutritional requirement. In layman’s term, plants have evolved these modifications to help ensure their successful pollination and survival. However, the important question that emerges here is what has made the plants evolve and retain these structures – which are so precise as to only perform the function of echo beacons – during the course of evolution? Alternatively, what exactly have the plants taken advantage of in order to attract bats? Exploiting the ultrasound produced by bats in a manner to attract them is not a mere coincidence, rather a possible mechanism employed by the plants. Considering this rigid ecological alliance between these species of plants and bats, which is so tightly and precisely regulated, there is a clear possibility that the ultrasound produced by the bats has facilitated the evolution and/or retention of the receptive plant structures. There is a clear knowledge gap on how the acoustic signals in plant–animal mutualisms have brought about morphological changes in the plants’ structure, which needs exploration in future research.

While the above studies provide an ecological basis for the acoustics of plant–animal interactions, there is a lack of molecular physiological perspective with regards to the responses. We thus highlight the pressing need for studies to elucidate the molecular physiological basis behind these plant–animal mutualisms.

Acoustics of plant herbivory: plants become defensive upon being eaten

Although the studies that show growth-enhancing effects of SV treatments partly bridge the gap in our understanding of SV-mediated morpho-physiological adjustments in plants, they lack an ecological dimension. In a recent study by [Appel and Cocroft \(2014\)](#), *Arabidopsis* rosettes pre-exposed to SVs caused by feeding of the *Pieris rapae* caterpillar were found to exhibit better defense against a subsequent attack by this pathogen. Treated plants accumulated higher levels of defense components such as glucosinolate and anthocyanin compared to untreated plants. This inarguably showed that sensing SVs is indeed ecologically favorable for plants. Most importantly, the authors demonstrated that the plants could specifically recognize the vibrations caused by the caterpillars’ feeding, as vibrations caused by wind or the chirping of leafhoppers failed to elicit a defense response. This adds further support to the contention that perception of SVs is an ecologically adaptive feature in plants and thus they clearly have developed a sophisticated mechanism for perceiving SVs of variable, ecologically relevant frequencies. In addition, this study appropriately addressed the criteria proposed by [Bailey *et al.* \(2013\)](#) that requires assessment of plants’ responses to treatments with recorded sounds (at absolute intensities) that might form potential signals in order to substantiate our understanding of plant acoustics. Rendering the previous scepticism invalid, the study of [Appel and Cocroft \(2014\)](#) thus proved to be the turning point in the field of plant acoustics. It is also important to note here that in several previous studies enhancement in plant defenses upon treatment with SVs at variable single frequencies had also been reported ([Hassanien *et al.*, 2014](#)).

In the light of all this evidence, we propose that plants may utilize a range of SV frequencies produced by different herbivore feeding and use this as a long-distance signaling mechanism in plant–insect interactions. Most importantly, the study of [Appel and Cocroft \(2014\)](#) also has tremendous potential to reveal many facets associated with the acoustics of plant herbivory. To achieve this, we recommend an expansion of such studies to determine the transcriptomics, proteomics, metabolomics, and hormonal changes associated with the enhanced defense provoked by the SVs of caterpillars' chewing. This would provide important insights into the defense machinery elicited by acoustic signals and the specific factors associated with the signaling and defense responses.

Recently, the establishment of signaling through volatile organic compounds (VOCs) has refuted the view that plants utilize cues but not signals to synchronize with their surroundings. Acoustic signaling through SVs is thus another entry in the list of signals that plants utilize. SVs together with the known systemic resistance triggered by VOC signaling constitute the plants' arsenal for combating pathogen attack effectively. The important question that arises here is why do plants require SV signals when they can counteract the herbivory through VOC signaling? VOC signaling can effectively elicit a successful defense response; however, as a signal, it has both drawbacks as well as advantages. The advantages are: (1) VOC signals are more durable, as chemicals stay in the environment for longer; (2) signals spread in the environment, thus evoking systemic resistance in surrounding plants; and (3) several of these VOCs also attract the carnivorous enemies of the attacking herbivore ([Dicke, 2009](#)). Despite these advantages there are a few major drawbacks of VOC signals: (1) the plant pays a high metabolic cost to generate them; and (2) VOC signals can take a significant time to reach the target plants and they are heavily dependent on the direction of the wind. Is it reasonable that every plant must be bitten first in order to avoid being bitten? When every organism has developed the ability to avoid an unwanted situation through utilizing several means of communication, why should plants be different? Plants would definitely be advantaged by having a quicker and affordable alternative. Acoustic vibrations travel faster over distances and are less affected by the direction of the wind. Furthermore, the metabolic cost of generation of acoustic signals is comparatively low. Recently, the low cost of generating acoustic vibrations by plants has been questioned by researchers ([Bailey et al., 2013](#)); however, generation of acoustic signals through herbivore activity, such as chewing as in the study of [Appel and Cocroft \(2014\)](#), is apparently costless. In the light of these arguments, elicitation of a defense response through acoustic signaling appears relatively fast and cost-effective.

Based on the above discussion, we propose that acoustic signaling is possibly the first line of defense to herbivory, which is then backed up more gradually by VOC signaling, which thus multiplies the signal and/or increases the durability of the stimulus for an overall robust defense response. To further corroborate this view, it would be rewarding to analyze whether chewing SVs themselves are sufficient to induce synthesis of volatile organic compounds. Subsequently,

having deciphered the transcriptomic/proteomic data, the overall acoustic-mediated defense could be compared with VOC-mediated defense in order to highlight the similarities and/or dissimilarities.

Certainly, as next steps, more studies on the acoustics of plant–herbivore interactions are required to reveal how broadly applicable this phenomenon is.

Sound prepares plants against drought

There is a view that plants may utilize drought-induced acoustic signals for communicating with nearby neighbours in order to prepare them for the impending water scarcity ([Gagliano, 2013b](#)). Plants experiencing drought have popping air bubbles in their xylem (through the cavitation process) that produce acoustic emissions when they burst ([Zweifel and Zeugin, 2008](#)). Until recently, this was thought to be the sole mechanism resulting in acoustic emissions by plants. However, with the recent discovery of acoustic emissions by young roots of corn grown hydroponically, it has come to light that drought-induced cavitation is just one of the several ways by which plants produce acoustic vibrations ([Gagliano et al., 2012a](#)). [Jeong et al. \(2008\)](#) treated rice plants with SVs at frequencies overlapping the frequency of those emitted during cavitation (between 50–250 Hz with 65–70 dB) and found a set of genes with altered expression. This was further corroborated by a study showing that SV treatment induced drought responses in rice ([Jeong et al., 2014](#)). The authors found that a prior SV treatment resulted in enhanced relative water content, stomatal conductance, and quantum yield of PSII (F_v/F_m ratio) in the drought-affected plants. There may be several possible reasons for the above response; however, based on the evidence, we strongly suggest that treatment with SVs has priming/hardening effect, which adjusts the physiological conditions within cells such that plants effect drought tolerance more swiftly/effectively than untreated plants. Advent of drought primarily results in reactive oxygen species (ROS) accumulation. Increased ROS evokes a signaling cascade that is linked with sugar-sensing and Ca^{2+} fluxes that trigger acclimatory responses ([Cruz de Carvalho, 2008](#)). Corroboratively, SV treatments have been noted to affect cellular levels of ROS, sugars and Ca^{2+} . We discuss this in detail below.

In a study under more natural conditions by [Falik et al. \(2011\)](#), it was shown that plants experiencing drought can generate a 'drought alarm', which successfully alerts neighboring unstressed plants to close their stomata. Importantly, this response was restricted to plants sharing their rooting volumes, as plants limited to only shoot communication failed to close their stomata. It is thus likely that the drought signal (alarm) is generated by the roots and traveled through the soil for plant-to-plant communication. Although the authors did not assign any modality to such communication, we propose acoustic vibrations as one of the possibilities behind this response. The ability of roots to produce and sense acoustic vibrations ([Gagliano et al., 2012a](#)) corroborates this idea.

Although experimental evidence exists, the question as to whether acoustic communication is utilized by plants as

a drought escape strategy in nature currently remains unanswered, and this has been a major reason behind the scepticism in the belief that such communication exists. Recently, Bailey *et al.* (2013) has argued that the benefits of acoustic communication in drought signaling are hard to envisage given that all plants within the potential range of these signals would in any case experience the same drought stress. We suggest that the authors have perhaps overlooked the pattern of change under natural conditions, which is never sudden but instead is gradual. Although in an agricultural field, which is an artificial system, water status changes more-or-less homogeneously, in a natural landscape it is highly heterogeneous. Additionally, the physiological state will vary from one plant to another, and this is an important factor. Thus, it is plausible that with the gradual advent of water scarcity, plants that fall victim to it first may produce signals to alert others with relatively better conditions and/or physiological state. Nevertheless, the number of studies available for substantiating a wider perspective on acoustics-based drought signaling is seriously limited, highlighting a need for better research in this field.

Impact of sound on plants' cellular processes: sound signaling

To date, the economic concerns of optimizing strategies to enhance crop growth, defense and productivity has gained priority in the field of plant acoustics. However, the important questions of how SVs are perceived and transduced into resulting cellular responses remain unanswered. In one study, da Silva and Dobranszki (2014) attempted to shed some light on this aspect, but remained limited in their conclusions as to how sonication benefits biotechnology. A closer scrutiny of the published studies to date on SV-mediated cellular changes provides the potential to shed light on sound-signaling mechanisms. Hence in this section we provide a broader view summarizing knowledge gained so far, specifically in relation to the molecular changes triggered upon stimulation by SVs. We have brought together these molecular episodes in the form of a signaling model, presented in Fig. 1. As well as connecting the scattered pieces of information into a signaling cascade, the model also raises several important questions, which will help to accelerate research and fill the gaps in our understanding of perception of SVs and associated signal transduction.

Sound vibration, membrane and mechano-sensitive channels

Stimulus perception at the cell's surface is a prerequisite for a successful signal transduction event that culminates in plant response(s). Signals are sensed by different receptors present mostly on the plasma membrane (Alexandersson *et al.*, 2004). Hitherto, most of the receptors are identified to be proteins; however, another way of eliciting a transduction event is through altering the membrane potential, i.e. the voltage across the plasma membrane (Trewavas and Malho, 1997).

To facilitate this, ions are fluxed in or out of the cell through pumps or channels situated in the plasma membrane (Pandey *et al.*, 2007). For a plant cell to perceive SVs there has to be a membrane-associated receptor(s) or alternatively a membrane potential-based mechanism. Recent advancements in this field have proposed the existence of a mechano-sensory network in plants, similar to what operates in animal cells. Like gravity, wind, tidal currents, and rain, SVs are also pressure waves that have a mechanical influence (Telewski, 2006). For perception of a mechanical stimulus in plants, Telewski (2006) suggested two mechano-receptor models: (a) a plasmodesmata-based cytoskeleton-plasma membrane-cell wall (CPMCW) network, and (b) stretch-activated ion channels. Interestingly, the latter has been shown to be operational in bacterial cells under mechanical stimulation. It is reported that membrane tension results in extension of the pores of mechano-sensitive channels, like the mechano-sensitive channels of small conductance (MscS) and MscS-like (MSL) channels present in the bacterial cell membrane (Okada *et al.*, 2002). It is important to note that MSL proteins are widely distributed and are found in yeast and plants besides bacteria (Haswell and Meyerowitz, 2006). Furthermore, reports suggest that treatment with SVs brings about microfilament rearrangement, eventually resulting in increased tension or decreased cell membrane deformability in plants (Liu *et al.*, 2001). Additionally, through circular dichroism spectral analysis, SVs were observed to trigger modifications in the secondary structure of the cell membrane-associated proteins: an increase in the α -helix and decrease in the β -turn within the secondary structure of membrane proteins (Zhao *et al.*, 2002b). Of course, this also necessitates a cell wall associated modification. Consistent with this, Johnson *et al.* (1998) noted strong induction of TCH4, a xyloglucan endotransglucosylase/hydrolase (XTH) that modifies the cell wall (Campbell and Braam, 1998), upon exposure to SVs (50 Hz, 30 min). We here propose that SVs may result in activation of the MSL channels in plants, thereby evoking a membrane potential-based signaling cascade. Direct evidence to this, however, is lacking and warrants elucidation by further research (Fig. 1). Interestingly, it has been shown that expression of Arabidopsis MSL3 can complement the phenotype of bacterial cells defective in MS-ion-channel activity (Haswell and Meyerowitz, 2006). This corroborates that MSLs may have a similar function in plants, where they activate upon disturbances in the membrane state and results in suitable responses. Between the two classes of aforementioned putative plant mechano-sensors, stretch-activated channels appear to play a primary role in sound signaling. Two types of stretch-activated channels have been identified in plants to date: MSLs, the non-selective channels, and Mid1-complementing activity (MCA) proteins, which are specifically Ca^{2+} channels (Leblanc-Fournier *et al.*, 2014). Interestingly, expression of Arabidopsis MCA1 has been found to enhance the mechano-sensitive channel activity in the *Xenopus laevis* oocyte plasma membrane (Furuichi *et al.*, 2012), suggesting that plants' Ca^{2+} -specific channels (MCA) can also respond to mechanical stimulus as well as MSLs. Notably, several studies have suggested efflux/influx of Ca^{2+} upon stimulation by SVs, which directly relates to the

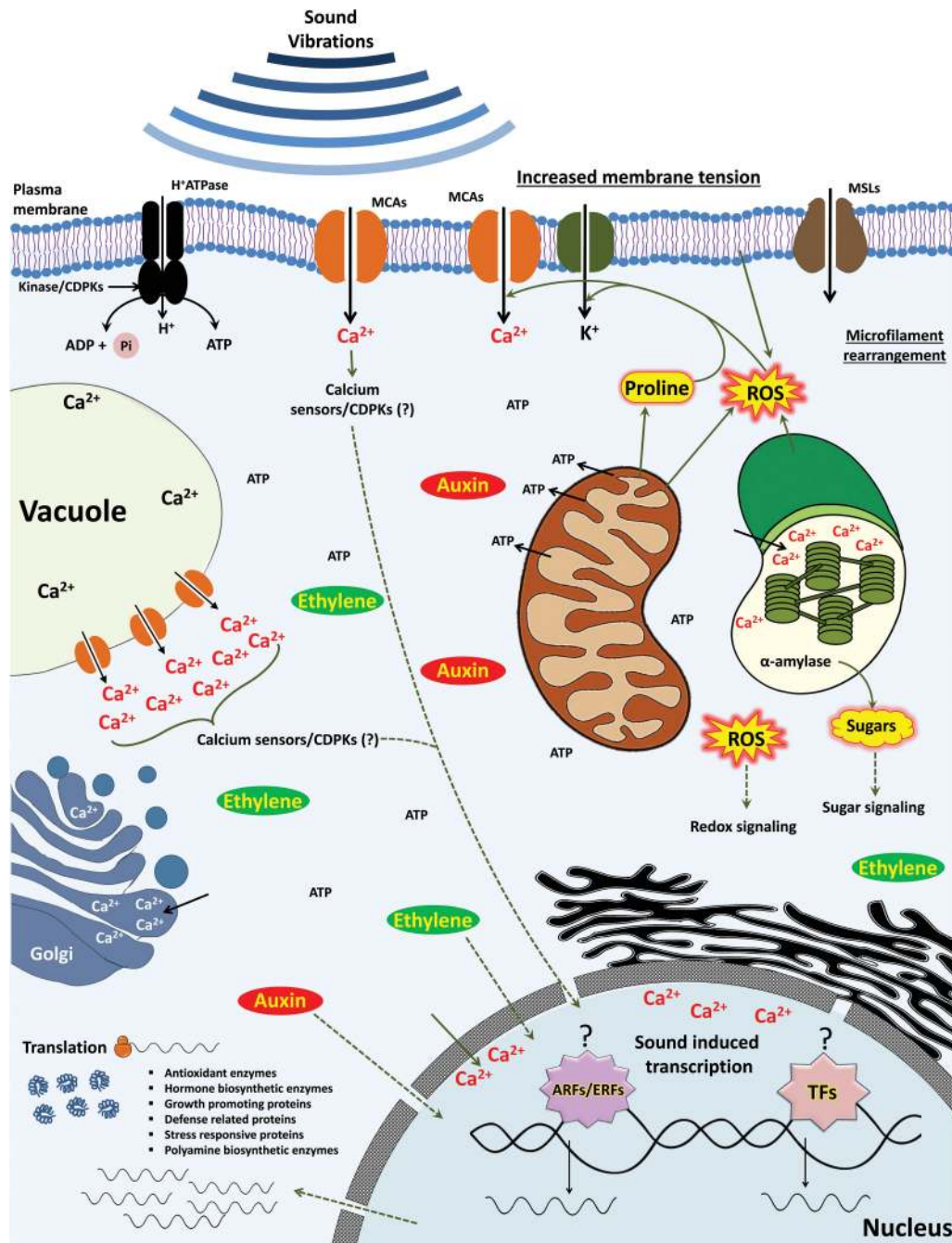


Fig. 1. A model summarizing the molecular events triggered within a plant cell upon stimulation by sound vibrations (SVs). SVs result in cell wall modification and microfilament rearrangement in plant cells. The primary site of activity is the plasma membrane (PM), which upon disturbance through SVs evokes a signaling cascade that eventually culminates into a response. The most promising molecular candidate acting as a second messenger of SVs is the calcium ion, Ca^{2+} . MSLs and MCA are the two possible PM-localized channels facilitating the SV-mediated efflux/influx of Ca^{2+} . This results in generation of Ca^{2+} transients critical for sound signaling. Ca^{2+} is sensed possibly through various Ca^{2+} sensors and/or CDPKs, which pass the message through phosphorylation/de-phosphorylation to different signaling proteins or to transcription factors, eventually resulting into gene expression. Proline and ROS, which are increasingly synthesized in SV-treated cells, together facilitate further activation of Ca^{2+} channels along with K^+ channels. α -Amylase activity and, correspondingly, sugar levels increase in cells treated with SVs. Together, Ca^{2+} , ROS and sugars may evoke separate signaling cascades resulting in increased gene expression. Auxin and ethylene that are implicated in the SV-mediated response may directly target ARFs and ERFs, respectively, to mediate gene regulation. Several classes of genes/proteins that are differentially expressed/activated upon stimulation of the plant cell by SVs are shown. The energy required in the above processes is derived from ATP, which is increasingly synthesized in sound-sensitized cells. Solid lines in the model are the steps supported by published studies whereas dotted lines represent the hypothesis we present, and which thus need to be analyzed in future plant acoustic research. MCA, Mid1-complementing activity; MSL, MscS-like; CDPK, calcium-dependent protein kinase; ROS, reactive oxygen species; ARFs, auxin response factors; ERFs, ethylene response factors; TFs, transcription factors.

possible activity of the MCA channels under such conditions (Fig. 1). Additionally, a report has suggested an increase in fluidity of the membrane upon stimulation by SVs (Yi *et al.*,

2003a). This hints at the possible involvement of lipid signaling in transduction of the SV stimulus, which proceeds downstream through cross-talk with Ca^{2+} signaling (Mishra and

Grover, 2014). Although there are ample reports suggesting a possible Ca^{2+} -based mechanism for SV signaling, it is too early to assign lipid signaling as the initiation of this response.

Cellular calcium

Intracellular Ca^{2+} constitutes one of the most important players in the plants' signal transduction pathways. The calcium ion, the so-called second messenger, diffuses readily to convey the information from an extracellular source to a cellular target protein (enzyme) (Sanders *et al.*, 2002). Generally, cytoplasmic Ca^{2+} concentration remains several folds lower than its concentration in the cell wall. Upon receiving a stimulus, the cell's cytoplasmic Ca^{2+} concentration briefly increases (Ca^{2+} transients), which facilitates a signaling cascade and the generation of responses (Hepler, 2005). Importantly, Ca^{2+} transients, which are unique depending upon the stimulus (White and Broadley, 2003), have been noted in cells treated with SVs; in *Chrysanthemum* callus cells, SV treatment brings about major redistribution of Ca^{2+} (Liu *et al.*, 2001). Upon treatment with SVs, vacuolar Ca^{2+} starts accumulating in the vacuolar membrane together with an increase in cytoplasmic Ca^{2+} concentration (Liu *et al.*, 2001). Further, the concentration of Ca^{2+} has also been noted to increase in the nucleus, the Golgi complex and in chloroplasts (Liu *et al.*, 2001). A direct connection between Ca^{2+} transients and the observed accelerated growth phenotype in *Chrysanthemum* callus was established through pharmacological studies. It was noted that addition of EGTA (a Ca^{2+} chelator that specifically works at the cell wall level by blocking the movement of Ca^{2+}) to medium lacking in Ca^{2+} negatively impacted the SV-mediated acceleration in *Chrysanthemum* callus growth (Wang *et al.*, 2002). This was further corroborated by a similar response in the phenotype when a specific Ca^{2+} channel blocker, Verapamil, was added to the medium (Wang *et al.*, 2002). This strongly suggests that SVs generate Ca^{2+} signatures by transportation of cell wall-associated Ca^{2+} to the cytoplasm through activity of Ca^{2+} channels, thereby eliciting a signal cascade (Fig. 1). In order to shed more light on this, we highlight the need to employ different fluorescence resonance energy transfer (FRET)-based genetically encoded Ca^{2+} -sensors to study the SV-stimulated generation of Ca^{2+} spikes in a temporal and spatial manner.

In light of the above discussion, the cell wall–cell membrane continuum appears to be critical in sensing the SVs and the subsequent generation of a Ca^{2+} signature. Thus, depending on the frequency of the SVs the impact would vary, affecting Ca^{2+} channels and generating specific Ca^{2+} signatures. We propose that different Ca^{2+} signatures could form the underlying basis for plants to distinguish between different acoustic signals to generate different responses.

Kinases, gene-regulation, and enzymes

Another important player in plants' signal transduction pathway is protein kinases (Sheen, 1996). These enzymes phosphorylate and alter the function of various target proteins, for example transcription factors, and thus have a direct

control over the regulation of gene expression (Choi *et al.*, 2005). Evidence suggests that cells treated with SVs exhibit enhanced protein kinase activity (Zhao *et al.*, 2002c). Thus, we propose that phosphorylation of signaling proteins and/or transcription factors triggered by SV-mediated activation of protein kinases may eventually lead to up-regulation of responsive genes (Fig. 1). Consistent with this, several genes are differentially expressed upon stimulation by SVs (Wang *et al.*, 2005; Jeong *et al.*, 2008; Shao *et al.*, 2008; Safari *et al.*, 2013). It has been reported that SVs accelerate mRNA synthesis (Wang *et al.*, 2003b; Zhao *et al.*, 2003). In a preliminary study, Wang *et al.* (2003b) noted that stimulation by SVs resulted in differential expression of six cDNAs with sizes ranging between 200–600 bp. Jeong *et al.* (2008) performed mRNA expression analysis and found a set of SV-responsive genes in the SV-treated subtractive library. They reported that under both dark and light conditions genes encoding the Rubisco small subunit (*RBCS*) and aldolase (*ALD*) were invariably up-regulated. Further, transgenic plants containing the β -glucuronidase (*GUS*) reporter gene downstream to the *ALD* promoter were found to express *GUS* upon treatment with SVs (250 Hz for 4h), strongly suggesting that the *ALD* promoter is SV-responsive. Shao *et al.* (2008) reported both up- and down-regulation of certain genes upon treatment of *Chrysanthemum* with SVs. Safari *et al.* (2013) found enhancement in expression of the genes encoding catalase (*CAT*) and phenylalanine ammonialyase (*PAL*) in *Corylus avellana* cells upon treatment with low-intensity ultrasound. Through use of microarrays, we have investigated global transcriptomic changes in Arabidopsis upon treatment with SVs (500 Hz, 80 dB) and recorded up-regulation of some kinases (e.g. *MPK11*) and transcription factors (*MYB77*, *DREB26*, and *RAV1*) (Gene Expression Omnibus microarray data accession no. GSE68944; unpublished data).

Interestingly, the regulation of gene expression upon SV stimulation is also observed at the level of proteins. Kwon *et al.* (2012) performed proteomic analysis of leaf tissues harvested from Arabidopsis plants treated with SVs of 500 Hz and got exciting results. Employing two-dimensional gel electrophoresis (2-DE), they found 38 differentially regulated spots in the treated protein samples. Further, using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and MALDI-TOF/TOF MS the differentially regulated proteins were identified to be functionally involved in photosynthesis, stress and defense, nitrogen metabolism, and carbohydrate metabolism. Although this study corroborates differential gene expression upon SV treatment, a detailed transcriptomic analysis entailing global gene expression changes is lacking. Such a study would certainly reveal the possible targets of protein kinases, such as signaling proteins, transcription factors, etc.

One of the direct targets of kinases under stimulation by SVs is H^+ -ATPases; it has been reported that *Chrysanthemum* callus cells treated for 1h with SVs of 100 dB and 1000 Hz showed significantly increased activity of H^+ -ATPases (Zhao *et al.*, 2002a). Evidence that activation of H^+ -ATPases is mediated by its phosphorylation through kinase activity has come from pharmacological studies. It was noted that inhibition of

protein kinase by the inhibitor staurosporine strongly inhibited H⁺-ATPases activity (Jia *et al.*, 2003). Furthermore, the component functioning upstream to the protein kinases in the activation of H⁺-ATPases was found to be Ca²⁺. Notably, factors such as Ca²⁺ concentration, blockage of Ca²⁺ passage through Verapamil, and inhibition of the Ca²⁺ carrier (A23187) affect the activity of H⁺-ATPases; specifically, chelating the Ca²⁺ decreases the activity of H⁺-ATPases (Zhao *et al.*, 2002a). Thus, it appears that the Ca²⁺ transient leads to activation of Ca²⁺-dependent protein kinases, thereby resulting in enhanced phosphorylation of H⁺-ATPases leading to its increased activity (Fig. 1). Importantly, H⁺-ATPases create an electrochemical gradient in the plasma membrane, which plays an essential role for plant responses to environmental stimuli. Thus, we highlight here that the phosphorylation-dephosphorylation events seem to be critical in the signal transduction pathway of the SV stimulus.

Along with protein kinases, enzymes involved in scavenging of ROS have also been reported to show enhanced activities in response to SVs (Li *et al.*, 2008; Safari *et al.*, 2013). Protective enzymes, namely superoxide dismutase (SOD), catalase (CAT), peroxide dismutase (POD), and ascorbate dismutase (APX) along with peroxidase (PRX) show increased activity upon stimulation through SVs. This suggests that upon SV stimulation the amount of ROS in the cell transiently increases. Direct evidence for ROS accumulation is the initial increase in malondialdehyde (MDA), a by-product of lipid peroxidation by ROS, after SV treatment, which gradually decreases with increasing antioxidant activities (Li *et al.*, 2008). Importantly, ROS are considered as a general stress signal and have been implicated in signaling (Corpas *et al.*, 2015). An increase in ROS and associated signaling is the primary response to drought (Cruz de Carvalho, 2008). Thus, enhanced level of ROS upon SV stimulation goes hand-in-hand with our earlier explanation for the enhanced drought tolerance observed in rice plants stimulated through SVs (Jeong *et al.*, 2014). Furthermore, ROS can also regulate the activity of membrane ion-channels, specifically the one regulating the Ca²⁺ and K⁺ ion influx/efflux (Pottosin *et al.*, 2014). Corroboratively, evidence suggests that SV stimulation increases the activity of K⁺ ion channels through enhancing their frequency of opening (Zhao *et al.*, 2002a). Importantly, K⁺ ion channels are directly involved in stomatal closure and opening, which is critical during drought stress. Thus, enhancement in K⁺ ion channel activity together with ROS and antioxidant enzymes forms a strong basis for increased competency of plants to withstand impending drought. Recently, it was found that the activity of Ca²⁺ and K⁺ ion channels is regulated by a synergistic effect of ROS and polyamines (PAs) (Pottosin *et al.*, 2014). Importantly, an elevation in the level of PAs in plants treated with SVs has been reported (Qin *et al.*, 2003). Thus, SV-mediated increases in the levels of ROS and PAs possibly facilitate the Ca²⁺ influx, which strengthens Ca²⁺-mediated elicitation of signaling events in response to SVs (Fig. 1).

Phytohormones and SV-mediated plant growth

The most common plant response noted upon SV stimulation is the enhancement in its growth. Phytohormones regulate

growth responses in plants, which result from rapid cell division/elongation. Thus, an alteration in phytohormone levels and cell cycle favoring rapid cell division upon SV treatment is a prerequisite. By using a flow cytometry approach, Wang *et al.* (2003b) showed that SVs indeed accelerate cell division by changing the cell cycle of *Chrysanthemum*; there is a decrease in the number of cells in the G0/G1 phase with a corresponding increase in the ones in the S phase. This strongly suggests that SVs can accelerate growth through increasing cell division. This was further corroborated by studies suggesting alterations in phytohormone levels upon SV stimulation. Interestingly, SVs have been shown to significantly increase accumulation of endogenous phytohormones, such as, indole-3-acetic acid (IAA), gibberellin (GA) and cytokinin (zeatin riboside, ZR) in plants such as cucumber, tomato, muskmelon, cowpea, and eggplant (Wei *et al.*, 2012; Hassanien *et al.*, 2014). However, in *Chrysanthemum* callus cells treated with SVs (1400 Hz, 95 dB), an increase in the levels of IAA occurred with a parallel decrease in the stress phytohormone abscisic acid (ABA) (Wang *et al.*, 2004). Importantly, whereas the phytohormones IAA, GA, and ZR facilitate cell division/elongation and overall plant growth, ABA is a negative regulator with growth-inhibitory activity. It thus appears that SV-mediated growth enhancement is under tight regulation of growth-promoting phytohormones. In addition, SV-mediated accumulation of GA supports our proposed contention that this could be the reason for enhanced germination in dormant seeds. Besides phytohormones, increased levels of soluble sugar and protein concentration within SV-treated cells (Yi *et al.*, 2003b; Zhao *et al.*, 2003) represents high metabolic activity and a state of vigorous cell division, and the energy required in the process comes from ATP, which is synthesized more upon SV treatment (Yang *et al.*, 2003). Thus, SV treatment mimics environmental stimuli that bring positive changes in the molecular-physiological status of the plants, thus promoting growth.

Appel and Cocroft (2014) suggested the possible involvement of ethylene signaling in elicitation of defense responses. Previously, ethylene signaling was shown to modulate the SV-dependent enhancement in Arabidopsis seed germination (Uchida and Yamamoto, 2002). In contrast, SVs have been shown to delay tomato fruit ripening through inhibiting ethylene biosynthesis and signaling genes (Kim *et al.*, 2015). In line with this, we propose that SV as a stimulus interacts with developmental cues and enacts in contrasting ways in a tissue-specific manner. In addition, it is noteworthy that plants' responses to mechanical vibrations produced by touch are also regulated in part by ethylene signaling (Braam, 2005; Chehab *et al.*, 2009). This emphasizes a possible molecular cross-talk between the two mechanical stimuli, sound and touch. The molecular cross-talk in the signaling of sound and touch stimuli is discussed in detail the next section.

Mechano-sensing of sound and touch: possibility of a cross-talk

As previously discussed, SVs being pressure waves like touch, have a mechanical impact on the cell's membrane. Due to

the mechanical nature of SVs and touch stimuli, overlapping signal transduction molecules could well form the basis for cross-talk between the two sensory systems. Further, considering the turbulent environment that surrounds immobile plants, an overlap in the mechano-stimuli of touch and sound is very likely to occur. A close examination of the molecular episodes elicited by the two stimuli reveals many similarities that endorse this argument. Being mechanical in nature, both stimuli elicit signaling events across the plasma membrane through possible excitation of mechano-sensitive channels (Haswell and Meyerowitz, 2006). It is important to note that, as in case of sound, mechanical stimuli such as touch and wind also generate Ca^{2+} transients in plant cells (Braam, 2005). Notably, Arabidopsis genes that are up-regulated in response to touch encode for proteins involved in cell wall modification, calcium binding, kinases, transcription factors, and defense (Braam, 2005). These are the proteins that are involved in a calcium-dependent signaling cascade that seemingly are functional in case of SV signaling as well. This possibility is further strengthened by SV- (50 Hz, 90 dB) mediated up-regulation of some touch-responsive genes (e.g. *TCH2*, *TCH3*, and *TCH4*) in Arabidopsis (Johnson *et al.*, 1998). Our microarray study (see above) also revealed up-regulation of several touch-stimulated genes (e.g. *CML38*, *MYB77*, *DREB26*, *HSPRO2*, and *RAV1*) by SV in Arabidopsis (Gene Expression Omnibus microarray data accession no. GSE68944; unpublished data). Like SV stimulation, mechano-stimulation by touch also results in ROS production (Monshausen *et al.*, 2009). Mechano-stimulation results in lateral root induction, a phenotype that has been attributed to auxin accumulation in the roots (Monshausen and Gilroy, 2009). As auxin also accumulates after SV treatment, this further highlights the interconnected responses between touch and sound stimuli. Like sound, touch also results in accumulation of PRXs (Saidi *et al.*, 2009).

Although all these similarities highlight the molecular cross-talk between the two signaling events, we strongly emphasize that SVs as a stimulus is ecologically distinct from touch and the responses of plant to sound are tailored accordingly. For instance, most of mechanical stimuli, such as wind, lead to stunting and an increase in secondary growth, whereas SV, in general, promotes growth.

Conclusions and future perspective

The perception and processing of vibrations in the form of sound waves are very advantageous from an ecological perspective, and thus it is unjustified to exclude plants from this exciting field of study. From the recent discoveries that have been made it is amply clear that plants perceive the SV stimulus, which is appreciably different from other mechanical stimuli. Our attempt to critically assess SV-mediated cellular adjustments in this paper has resulted in a model defining the sound-signaling pathway (Fig. 1). There appear many similarities in the sound- and touch-signaling pathways, and thus, the field of plant acoustics can benefit from the information available in signaling of thigmoresponses. However,

the molecular components involved in the signaling of the SV stimulus in plants are still debated. Deciphering the precise role(s) of phytohormones in SV-mediated regulation of plant development and growth will be a matter of extensive research. ROS and sugars are versatile molecules also implicated in signaling and thus their probable role(s) in the SV-mediated response need to be thoroughly investigated. With the advancement of molecular biology technologies, there is certainly a need to make available the whole genome transcriptomic maps to identify all the genes specifically affected by the SV stimulus. This will highlight the similarities and/or dissimilarities among the acoustic and mechano-perceptions and help to decipher acoustic signaling in plants. Several specific knowledge gaps have been highlighted in the text and Fig. 1 of this paper. Most importantly, we urge for more studies on the response of plants exposed to natural SVs that may provide a beneficial stimulus, recorded at the correct and appropriate intensities.

Finally, more focused attention is needed to unravel the hidden facets in this under-studied field of plant biology. The time has come to move on from the debate about whether plants can sense and communicate SVs, which has constrained us so far in our understanding of plant communication: scientists should now set themselves the task of revealing the fascinating details that are currently hidden in the field of plant acoustics. We should be enthusiastic about this new emerging field of plant research that holds the promise to provide us with a new dimension to look at plant as a perceiving organism: much smarter and more sensitive to various environmental stimuli than we might think.

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