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Mini Review

Applications of Plant Responses to Sound in Biotechnology and Agriculture

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Abstract

Sound has been identified as a mechanical stimulus that gives rise to various physiological and molecular changes in plants: promote seed germination and plant growth (*in vivo* and *in vitro*), induce stress responses (biotic and abiotic), induce changes in the concentrations of different plant compounds, induce mutations, facilitate genetic transformation, etc. Some of these changes may have important benefits at different scales: research, agriculture, biotechnology or food processing. Here, we summarize the possible applications of sound treatments and the pros and cons of its application.

Keywords: Sound; Ultrasound; Mechano-stimulation; Plant growth

Introduction

In physics, sound is understood to be a vibratory movement that propagates in the form of mechanical waves (audible or not), through a medium (fluid or solid). Therefore, sound involves pressure fluctuations. Sound can be defined as a function of its frequency (Hz) and its amplitude (dB). Sounds audible by humans range from 20Hz to 20kHz. Sounds above 20kHz are known as ultrasound and below 20Hz as infrasound.

When a sound is applied to a plant, what it receives are pressure waves on the surface of its organs, that is, something equivalent to when a plant is touched. Plants can respond to mechanical stimuli (mechano-stimulation) and well-known examples are the responses of carnivorous plants or the leaves of *Mimosa pudica*, but mechanical stimulation is also involved in many other areas such as the regulation of morphogenesis, molecular and physiological changes, generation of Reactive Oxygen Species (ROS), induction of antioxidant enzyme activity, hormonal modulation, altered gene expression, or reorganization of the cytoskeleton [1]. Therefore, it is not surprising that sound, as mechanical stimulation, is also capable of inducing similar responses.

How plants perceive sounds? The general idea is that the mechanisms are similar to those by which plants perceive mechanical stimuli [2]. Mechanical stimuli are perceived at the cytoskeleton-plasma membrane-cell wall interface and cause changes in the permeability, elasticity, and viscosity of cell membranes. That, in turn, would cause changes in ion channel activity (ex, Ca^{2+}), as well as membrane-bound enzyme activity. This, in turn, would initiate ion-mediated signaling responses and signaling events such as increases in protein kinases, peroxidases, antioxidant enzymes, amylase, H^+-ATPase/K^+ channel activities, changes in gene expression [3], protein and metabolite accumulation [4]. Possible changes at the epigenetic level and the accumulation of micro-RNAs have also been reported [5]. Some studies in *Arabidopsis* suggest that trichomes could be especially sensitive to these stimuli [6]. Because of the positive effects it produces in several aspects of plant's life, sound treatments have been exploited significantly in different aspects of biotechnology and agriculture.

Promoting Seed Germination

Priming is a pre-sowing treatment that is widely used to promote germination. Ultrasound treatments have been successfully used as a priming technique in seeds of several species [7]. The effect is dependent on the frequency, intensity and duration of the treatment, and optimal parameters vary with the species. In consequence, the method has to be fine-tuned for each case. Ultrasound treatment makes the seed coat soften, opening many small pores and favouring the absorption of water [8]. Ultrasound treatment also increases amylase activity, starch hydrolysis, the release enzymes from cell walls and increases the presence of free radicals, allowing many biochemical reactions to occur in plant cells. Ultrasound has been shown to have the greatest effect on seed germination but long ultrasound treatments may have a negative effect in germination and induce mutagenesis. Audible sounds do not have this problem and has been demonstrated that is also possible to use them for priming, at least for maize seeds [9].

Impact on Plant Growth

Stimulation of plant growth and development under *in vitro* conditions or agricultural settings by sound treatment has been described in many species [4]. Just one example, the treatment of paddy rice with 400Hz and 106dB produces a significant increase in the shoot height, fresh weight, root system activity, and the cell membrane penetrability [10]. Other described effects are an increase in the grain yield and quality, the numbers of leaves and flowers, the content of chlorophyll, the total length, number, and activity of roots, the height of the plant, the size of the leaves, hypocotyl elongation, etc.

In the case of *in vitro* cultures, sounds have been shown to stimulate the growth and development in various plant species [11]. For example, potato *in vitro* node segments with a single leaf were exposed to ultrasound (35kHz, 70W, for 20min) and the treatment accelerated shoot growth, but inhibited the development and growth of roots [12].

Impact on Stress Responses

Another major benefit obtained by sound treatment is the increased plant resistance to abiotic stresses and the plant immunity against plant diseases and pests. For example, *Arabidopsis* plants pre-exposed with sound were found to be more tolerant to *Botrytis cinerea* infection [13] and more resistant to drought [3]. Sound treatments induced in *Arabidopsis* a significant up-regulation of abiotic stress-responsive genes [3] and the accumulation of proteins related with stress responses [14]. Similar responses to abiotic stress have been shown in other species like potato, rice, wheat and *Dendrobium candidum*.

The effect of increased resistance to pathogens is especially interesting because it opens up the possibility of carrying out preventive treatments without the need to use chemicals. Increased immune responses against fungi after sound treatment has been observed in *Arabidopsis* [13] and rice [11]. On the other hand, *Arabidopsis* plants pre-exposed to the sounds caused by feeding of the *Pieris rapae* caterpillar were found to exhibit better defence against a subsequent attack and treated plants accumulated higher levels of defence components such as glucosinolate and anthocyanin compared to untreated plants [15].

Changes in Composition

Sound waves can induce changes in a large number of plant compounds such as hormones, alkaloids, flavonoids, phenols, terpenes, quinones, etc. We have already mentioned that sounds induce changes in different hormones associated with stress and in the activity of enzymes, antioxidants, gene expression and protein accumulation. Some of these components may have an interest in human nutrition and sound processing opens several possibilities regarding the enhancement of healthy aspects of plant-based food products [16].

The application of ultrasounds is an emerging technology for different aspects of food processing and, in special, for the processing of fruits and vegetables. For example, sound treatments increase the postharvest shelf-life of tomato fruits inducing a delay in ripening as compared to the untreated controls [17]. Many other examples can be found in three recent reviews [18-20].

Mutagenesis and Genetic Transformation

Induced mutagenesis provides a possibility to enrich genetic variability in crops. Irradiation and treatment with chemical mutagens are the two major methods used to induce mutations in plants, but the use of ultrasound treatments is also possible [21].

Sonication of tissue during infection with *Agrobacterium* mediated genetic transformation has been reported to enhance the efficiency of the process by producing small and uniform fissures and channels throughout the plant tissue, allowing the access of *Agrobacterium*. Sonication has been shown to augment transformation efficiency, particularly in species recalcitrant to transformation. For example, sonication of 60kHz during 40s of *Leptadenia pyrotechnica* explants, before the *Agrobacterium* infection, produced about 4-times more stable transformations than the control [22].

Conclusion

That plants are capable of responding to different types of sound in different ways is already evident and in some cases with obvious concrete applications such as *in vitro* cultivation, food processing or seed germination. In other cases, such as promoting growth and production or inducing resistance to stress or pathogens, its practical application is not, at the moment, so evident. On one hand, sound stimulation could decrease requirements for chemical fertilizers and pesticides. However, there are also some problems. First, this technology would not be a zero cost to the environment. First, it requires machines and electrical consumption. Second, it causes noise pollution that can disturb human communities, but also animals and it can alter the behavior of animals either positively or negatively for the crop. For example, it could ward off birds that feed on pest insects. On the other hand, it could alter the plant species present in the farmlands. For example, sounds can affect pollination and seed dispersal, altering the composition of the plant community. In any case, before its application on a large scale, it would be necessary to carry out many studies and fine-tune the doses and the characteristics of the sound treatments to optimize them and make them ecological friendly and cheaper. A better understanding of the molecular mechanisms behind plant sound perception is also imperative. Other technical challenges are facing this technology. The sound pressure level falls inversely proportional to the distance from the sound source, which makes difficult to apply the same sound treatment in large fields. In addition, the leaves of plants absorb sound which may somehow contribute to sound attenuation. These problems could be solved, for example, by using a robot-guided mobile sound sources. In any case, on a small scale, indoors and with high-value products, it seems promising techniques for improving crop yield, defence, nutrient value, etc.

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