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Review

Ultrasound in Plant Life and Its Application Perspectives in Horticulture and Agriculture

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Abstract: Acoustic vibrations may induce different changes in plants that perceive them, and plants themselves can also emit acoustic signals. The aim of this review was to cover the past ten years of plant acoustic research and its shortcomings, with a focus on the reflecting, sensing, and emission of ultrasound by plants. Ultrasonication may alter plant growth and development, and an increasing number of studies are being carried out to investigate its effects on both in vitro plant culture and greenhouse or field plant production, as well as on the biochemical and molecular functions of plants. In this paper, we summarized the progress in the use of ultrasound in horticulture and agriculture for enhancing plant growth and development, either in vitro or in vivo, improving yield and crop quality and increasing stress tolerance, as well as for special methodological applications, like sonication-assisted *Agrobacterium*-mediated transformation. Some research gaps, such as the lack of a precise mechanism for plant ultrasound emission, the possible participation of some reactive radicals in ultrasound signaling, the effect of ultrasound on the epigenome, the role of ultrasound in plant-to-plant communication, and whether there is a specific, sound perceiving organ, etc., were also presented. In addition, a predictive vision is described of how ultrasonication of plants and ultrasound detection emitted by plants can be used in the future to develop green and sustainable agricultural and horticultural technologies. Furthermore, based on our current knowledge, a proposal is presented to combine them with machine learning and artificial intelligence for developing novel production technologies.

Keywords: ultrasonication; sound perception; sound emission; sonication-assisted *Agrobacterium*-mediated transformation; sustainability

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1. Introduction

Plants are constantly subjected to rapidly changing environmental conditions due to their sessile nature. They perceive, process, and respond accordingly to these cues, possessing adaptive value in order to improve their fitness [1]. From both biotic and abiotic sources, their surroundings include many naturally present, informative sounds that may warn plants about potential danger or may guide them to resources [2,3]. Sound is a vibration, a mechanical stimulus characterized by mechanical waves of different lengths (frequency, Herz, Hz) and amplitudes (decibels, dB) that travel fast in various media (solid, gas, and liquid) acting as energy and information carriers [4]. There are different types of vibrations according to their medium, namely soil- or air-borne vibrations, where sound transmission takes place through the surrounding soil or air, respectively, or direct vibrations, where the source of the vibration touches the plant (e.g., herbivore chewing) [3].

Generally, the greater the sound amplitude, the greater the mechanical impact on the surrounding medium and thus on the plant, as well [5].

According to our latest knowledge, there is much evidence to support that acoustic vibration (sound or ultrasound) acts on plants leading to growth, developmental, and biochemical and molecular modifications in the perceiving plants [2]. In addition, the latest research has shown that plants are capable of emitting acoustic signs that are species- and stress-specific [6,7]. However, there are research gaps regarding the existence of sound perceiving organs, as well as the exact mechanism of sound or ultrasound production by plants. There has been a lot of debate over whether plants are capable of acoustic communication with each other and other organisms in their environment, whether they use the acoustic signs of the inanimate environment to improve their survival and fitness, and whether they are capable of memorizing previously perceived sound or ultrasound cues by changing their epigenetic state [7–13]. However, in light of the latest studies, this is presumably more than a speculative hypothesis, even if more direct research and evidence related to the acoustic communication of plants are still needed to evaluate and understand this possibility [6,7,13].

This article is an overview of plant acoustic research over the past ten years, focusing on the role of ultrasound in plant life and reviewing the progress and perspectives on the use of ultrasound in horticulture and agriculture.

2. Sound Perception in Plants

Although, according to our recent knowledge, plants lack specialized sensory organs, they have several different apparatuses that detect mechanical stimuli. Considering the magnitude of the mechanical impact, the stronger the impact, the higher the possibility that it will be sensed by the entire cell, rather than the superficial structures, such as the plasma membrane or the cell wall.

Mechanical stresses, for instance, high amplitude sound waves, may cause thigmonasty, which includes morphological changes [14]. These changes occur due to the polymerization/depolymerization of cytoskeletal elements or upon a change in the phosphorylation state of actin filaments that may influence the flexibility of pulvini [15,16]. The cytoskeleton regulates mitosis, meiosis, interaction of organelles, and movements, therefore, sound waves may affect the cell units, their structure, and may cause resonance within the cell. Subtle mechanical stimulus triggers the most sensitive sensory apparatuses, namely, the cell wall–plasma membrane interface and the trichomes. The trichomes may act as a mechanical antenna promoting downstream signaling of sound-dependent responses [17]. Considering that across plant species, trichomes are not ubiquitous, and their density and morphology varies remarkably, they may not be essential for aboveground sound perception but may facilitate stimuli amplification [18]. Roots, on the other hand, may convert belowground mechanical or sound impulses into biochemical signals (e.g., quick and local intracellular increase in Ca^{2+} levels facilitating biochemical responses through modulating different traits). Unfortunately, there are still huge knowledge gaps about the existence of sound perceiving organs due to the lack of research focusing on these topics [19,20].

The plasma membrane, on one hand, is a highly stretchable structure, and, on the other hand, it is filled with mechanosensitive transmembrane, anchored, and intrinsic elements. Upon sound stimuli, these elements may act as transducers of physical signals by stretching, therefore changing the tension of membranes and opening ion channels, facilitating ion fluxes [21]. During stimuli, small fragments of the cell wall may release and become loose (peptides that are non-covalently linked to the wall, such as oligosaccharides), touching the mechanoreceptors on the cell wall–plasma membrane interface causing changes in the chemical and electrical gradient between the apoplast and cellular symplast [22–24]. H^+,

Na^+, and Cl^- ions are transported by the mechanosensitive channel of small conductance-like (MSL, non-selective) channels [25], while K^+ is transported by two pore potassium channels (TPKs) [26]. However, most transmembrane ion channels transport Ca^{2+} ions, namely, mid1-complementing activity (MCA) [27], voltage-independent Ca^{2+} conductance (VICCs) [28], MSL [25], hyperosmolality-gated calcium-permeable (OSCA) [29], defective kernel 1 (DEK1) [30], cyclic nucleotide-gated channels (CNGCs) [31], glutamate-like receptors (GLRs) [32], and PIEZO [33] ion channels. These mechanosensitive ion channels that perceive mechanostimuli are often involved in numerous processes, including the maintenance of the shape and size of epidermal plastids, cell wall integrity (CWI), relief of hypo-osmotic stress during growth and development, stomatal closure, and plant defense [26,34]. Calcium is essential for the expansion and division of cells and is in charge of active H^+-ATPases. Therefore, perturbations in cytosolic Ca^{2+} concentrations and pH shifts may cause specific physiological responses and provoke a downstream signaling cascade. After mechanostimuli (e.g., sound vibrations), through receptor-like kinases (RLKs) or directly, the mechanosensitive channels activate and create a Ca^{2+} wave, which is deciphered via different sensors, such as calmodulins or calmodulin-like proteins (CaMs/CMLs), calcium-dependent protein kinases (CPDKs), calcium and calmodulin-dependent protein kinases (CCaMKs), calcineurin B-like proteins (CBLs) and CBL-interacting protein kinases (CIPKs) [35]. In different cellular components (mitochondria, ER, Golgi apparatus, and vacuoles) with distinctive functions, these Ca^{2+} sensors will translate the acoustic vibrations into physiological, biochemical, and molecular traits [36–38]. In order to restore basal Ca^{2+} levels and prevent any adverse effects to the cellular machinery, multifaceted Ca^{2+} transporters [Ca^{2+} exchangers (CAXs), mitochondrial Ca^{2+} uniporter complex (MCUC), Ca^{2+} ATPases, and P1-ATPases (HMA1)] control the Ca^{2+} influx [2,39].

Subsequently, along with Ca^{2+} and K^+, a reactive oxygen species (ROS) burst occurs, which initiates a period of phytohormone signaling, gene regulation, and protein phosphorylation. The cooperation of these processes is responsible for the maintenance of cellular homeostasis during the transient period following the initial stimuli [40]. The enzymes involved in ROS scavenging (superoxide dismutase, catalase, peroxide dismutase, ascorbate dismutase, and peroxidase) and low molecular weight antioxidants, such as oxidized and reduced glutathione (GSSG and GSH), ascorbic acid (AA), and tocopherol, have shown enhanced activities upon sound or ultrasound vibrations, which are evidence of ROS accumulation after sound treatment [23,41,42]. For the reason that ROS and Ca^{2+} production can be both a cause and an effect [via ROS-induced calcium release (RICR) or Ca^{2+} induced ROS production (CIRP)], they have a reciprocal interaction [43]. Both ROS and Ca^{2+} are signaling molecules that expedite the differential expression of various sound-induced genes; therefore, studies of sound-induced epigenetic changes and chromatin remodeling are undeniably extensive [35,44]. For example, the genes encoding MSL and MCA have an increased expression upon subjecting plants to mechanical stimuli (vibrations) [26,45].

Regulating several adaptive, developmental, and physiological processes (senescence, cell differentiation, flowering, seed germination, and rooting), other than ROS, H_2S and reactive nitrogen species (e.g., NO, a reactive radical) are crucial gaseous signaling molecules in plants [46]. In addition, NO is often connected or interwoven with ROS and Ca^{2+} signaling, for the reason that NO can swiftly react with superoxide radicals creating peroxynitrite, which can oxidize a broad range of biological macromolecules. Furthermore, directly or indirectly, NO is capable of influencing the activity of Ca^{2+} channels. Post-translational modifications (e.g., S-nitrosylation or tyrosine nitration) in target proteins generating confrontational modifications are triggered by NO and H_2S in plants [47,48]. Although H_2S

and NO regulate numerous biochemical and physiological plant processes, unfortunately, there is no information regarding the effect of sound vibrations on their productions [48].

Protein kinases, either transmembrane or anchored to the plasma membrane, are also susceptible to mechanical impact, acoustic vibrations, and are involved in the polymerization and acceleration of actin filament growth. Receptor-like kinases [RLKs, including wall-associated kinases (WAKs), FERONIA (FER), THESEUS (THE1), ANXUR1/ANXUR2, and leucine-rich repeat (LRR)], lectin receptor-like kinases (LeRLKs) [23,49,50], and proline-rich extension-like receptor kinases (PERKs) [51] are the best-known mechanosensitive kinases that phosphorylate signaling proteins. Having an extracellular domain that is able to bind cell wall-derived ligands or epitopes, in addition to a cytoplasmic domain (kinase) transmitting those signals, is a unique and distinguishing trait of RLKs. RLKs also regulate the respiratory burst oxidase homolog D (RBOH) activation in the apoplast after sound stimuli, resulting in an increased level of ROS [43]. Receptor-like proteins (RLPs) interacting with cytoplasmic protein kinases are also mechanosensitive proteins, which can transfer information through phosphorylation/dephosphorylation of different signaling proteins or transcription factors (TCH, NAC, XTH, MYB, GA2OX, and HSPS) resulting in differentially expressed responsive genes [52].

Deciphering the molecular components involved in the sound perception of plants is still under debate. The role of these components in plant development will be a matter of intensive research in the future as molecular biology technologies advance. In addition, the effects of sound stimuli are diverse, as the nature of molecular responses is greatly influenced by (ultra)sound patterns, the dynamics of experimental settings, the species in question, and their physiological and environmental conditions. Therefore, it is encouraged to discover and interconnect new elements, taking part in the detection and signaling of acoustic stimuli.

3. The Effect of Ultrasound on the Molecular and Physiological Functioning, Growth, and Development of the Plant

Within a proper range, ultrasonication often has positive effects on plant growth, seed germination, somatic embryogenesis, callus induction, and antioxidant enzyme activity (Table 1). Sonication with sound waves at a low frequency of 60 Hz promoted neo-PLB (protocorm-like body) formation in the Cymbidium Twilight Moon 'Day Light' hybrid [53]. Based on the studies by Farrokhzad and Rezaei [54] and Koochani et al. [55], it appears that ultrasonication at 20–40 kHz enhanced somatic embryogenezis in *V. vinifera* L. (cv. 'Kodori') and *C. sativus* (cv. 'Dastgerd Esfahan') [54,55]. In grapes, proline and tryptophan application with ultrasound waves had the most advantageous effects on callusing frequency, length, width, fresh and dry weight of callus, embryo number per callus, and the percentage of embryos. The treated explants began the flowering stage faster, within two weeks, as opposed to the control, which started two weeks later in the cucumber (Table 1). Ultrasound stimulation can increase the callus formation in apple clonal rootstocks (at 22 kHz, for 60 s) and *Crocus sativus* L. (at 35 kHz, for 5 min), based on the work of Muratova and Papikhin [56] and Firoozi et al. [57]. A significant rise in antioxidant enzyme activity was detected in *Solanum tuberosum* L. after ultrasound stimulation, based on several studies. The concentrations of superoxide dismutase, ascorbate peroxidase, glutathione reductase, and α-tocopherol were increased, contrary to the level of ascorbic acid and endogenous melatonin, which were remarkably decreased [41,42]. Dobránszki et al. [58,59] and Teixeira da Silva et al. [60] examined the expression intensity of differentially expressed genes (DEGs) in potatoes after 20 min of ultrasonication at 35 kHz. Most of the significantly up- and downregulated DEGs participated in biosynthesis, carbohydrate metabolism, catabolism, cellular protein modification, and response to stress. The DEGs encoding universal stress protein, chitinase, catalase, zinc finger proteins, transcription factors (WRKY and MYB), glutathione S-transferase, heat shock proteins, peroxidase, and dehydration-responsive element-binding were detected. Naqvi et al. [61] reported that in case of both a rough (*Salvadora surattense* L.) and a smooth (*S. persica* L.) texture plant, 20 min of sonication combined with 15% sodium hypochlorite treatment proved to be the most appropriate method for controlling bacterial or fungal contamination. The highest number of treated *S. surattense* that survived was about 2.5 times greater compared to the group that had not received ultrasonication treatment (Table 1).

Table 1. Main growth, developmental, biochemical, and molecular effects of ultrasonication of various in vitro explants.

Plant (Species, Cultivar)	Treated Explant	Method of Ultrasonic Treatment	Main Impacts of Ultrasonication	Ref.
Hybrid *Cymbidium* Twilight Moon 'Day Light'	Half-PLBs	Bath sonicator (Iuchi®, Tokyo, Japan) for 0, 1, 5, 10, 20, and 45 min, at 60 Hz and at 25 °C	Total of 5 and 10 min of sonication increased *neo*-PLB formation after 60 days of culture, whereas 1, 20, or 45 min reduced *neo*-PLB formation.	[53]
Grape (*Vitis vinifera* L.) cv. 'Kodori'	Stem internodes	For 0, 60, 120, and 240 s at 20 kHz and 2 W	Ultrasonication for 120 s in combination with 100 µM Trp or Pro gave the best result, including callusing frequency (3.3 and 4.7), length (14.7 and 12.7 mm), width (9 mm each), fresh (6.9 and 6.0 g) and dry (0.28 and 0.23 g) weight of the callus, embryo number/callus (5.5 and 4.0), and the percentage of embryos (81 and 82%), respectively.	[54]
Toothbrush tree (*Salvadora persica* L.) Yellow-fruit nightshade (*Solanum surattense* L.)	Shoot tip and nodal explants	(1) Treatment with 10 and 15% sodium hypochlorite (chemical treatment); (2) sonication for 20 min (physical treatment) (frequency and other details are not specified); and (3) treatment with 10 and 15% sodium hypochlorite under sonication for 20 min (physiochemical treatment)	In *S. surattense*, the highest number of plants survived when treating explants with 15% sodium hypochlorite under 20 min sonication (mean explant number was 2.4 vs. 1.0 without sonication). In *S. persica*, it was 2.8 with 15% sodium hypochlorite, both with and without sonication. This was found as the most proper method for controlling contamination in both rough (*S. surattense*) and smooth (*S. persica*) texture plants.	[61]
Potato (*Solanum tuberosum* L.) cv. 'Desirée'	Nodal explants from 4-week-old in vitro plantlets	Ultrasonication for 20 min at 35 kHz and 70 W (ultrasound is transmitted by air, in homemade ultrasonicator)	After ultrasonication (at 0 and 24 h) the activity of SOD, APS, and GR, as well as the concentration of α-tocopherol, increased. AA concentration significantly decreased. Length and fresh weight of shoots increased by 20 and 24% compared to untreated plants, respectively.	[42]

Table 1. *Cont.*

Plant (Species, Cultivar)	Treated Explant	Method of Ultrasonic Treatment	Main Impacts of Ultrasonication	Ref.
Clonal apple rootstock B9 and clonal rootstock of *Malus siboldy* 14-1	Isosceles triangle-shaped cuts of the lamina with the leafstalk and middle parts of lamina	UZDN-2T ultrasound installation for 60 s, at 22 kHz, power density: 1–1.2; 2–2.6; 3–3.6; 4–6.0; 5–10.0; 6–12.6; and 7–14.9 W/cm^2	Ultrasonication for 60 s activated the callus formation on the leaf disks and increased the frequency of regeneration of adventive shoots in apple clonal rootstocks by 2.5–3.5 times.	[56]
Saffron (*Crocus sativus* L.)	Corms of 1–2 cm in diameter	Bath sonicator (Bandelin electronic®, Berlin, Germany) for 5 min, at 35 kHz and 25 °C, repeated every two weeks for 1 min. Explants were treated with different combinations of PGRs	Ultrasonication with PGRs resulted in significantly increased in vitro callus induction (the highest percentage of callus induction was 100% and a growth of 4.3 g per explant of callus yield).	[57]
Potato (*Solanum tuberosum* L.) cv. 'Desirée'	Single-node segments of 4-week-old in vitro plantlets	Ultrasonication for 20 min, at 35 kHz and at 70 W (ultrasound is transmitted by air, in homemade ultrasonicator)	After ultrasonication (0 h), 29 DEGs were upregulated and 34 DEGs downregulated, after 24 h, 31 and 11, after 48 h, 15 and 64, after 1 week, 16 and 70, and after 4 weeks, 68 and 69 DEGs were up-, and downregulated, respectively. Most of the DEGs play a role in biosynthesis, carbohydrate metabolism, catabolism, cellular protein modification, and response to stress.	[58]
Potato (*Solanum tuberosum* L.) cv. 'Desiree'	Single-node stem segments	Ultrasonicator (Elmasonic X-tra 30 H; Elma Schmidbauer GmbH, Singen, Germany) (ultrasound is transmitted by liquid) for 20 min at 35 kHz, 70 W, and 25 °C	After ultrasonication, 138 (0 h), 72 (24 h), 18 (48 h), 5 (1 week), and 59 (4 weeks) DEGs were upregulated and 6 (0 h), 82 (24 h), 96 (48 h), 172 (1 week), and 107 (4 weeks) DEGs were downregulated. Most of the DEGs coding for universal stress protein, chitinase, catalase, zinc finger proteins, 21 transcription factors, glutathione S-transferase, and 17 heat shock proteins.	[60]
Cucumber (*Cucumis sativus* L.) cv. 'Dastgerd Esfahan'	Hypocotyl pieces form 2-week-old seedlings	Ultrasonic bath (Falc Instruments, Treviglio, Italy) for 0, 5, 10, and 15 min at 40 and 34/722 kHz, 320 Hz	Optimal ultrasonication parameters were 40 kHz and 10 min. The ultrasound treatment accelerated seed germination (in the case of the production of synthetic seeds derived from somatic embryos, it was 76.39 ± 8.17 compared to the control; 55.55 ± 13.37; in the case of the production of synthetic seeds from apical buds it was 86.67 ± 8.43 in comparison with the control: 83.33 ± 8.03). Ultrasonicated explants started the flowering stage faster than, and within two weeks of, the controls.	[55]

Table 1. *Cont.*

Plant (Species, Cultivar)	Treated Explant	Method of Ultrasonic Treatment	Main Impacts of Ultrasonication	Ref.
Potato (*Solanum tuberosum* L.) cv. 'Desirée'	One-node stem segments	(1) Homemade ultrasonicator, ultrasound is transmitted by air; and (2) ultrasound is transmitted by liquid (Elmasonic X-tra 30 H); for 20 min at 35 kHz, 70 W, and at 25 °C	DEGs coding for chitinase, peroxidase, glutathione-S-transferase, transcription factors of ethylene responsive factor, dehydration-responsive element-binding, WRKY, and MYB were significantly expressed in liquid-based ultrasonication, contrary to air-based. Liquid-based US is a stronger abiotic stressor than air-based US.	[59]
Potato (*Solanum tuberosum* L.) cv. 'Desirée'	In vitro single-node segments of stems excised from 4-week-old plantlets	(Elmasonic X-tra 30 H ultrasonicator) for 0, 20, and 30 min, at 35 kHz, 70 W, and at 20 °C	Due to the ultrasound treatment, the three-level antioxidant defense system activated, and the endogenous melatonin level decreased. Melatonin had an important role in the growth and development of plants, and in addition it acted as an antioxidant.	[41]

Abbreviations: AA: ascorbic acid; APS: ascorbate peroxidase; DEGs: differentially expressed genes; GR: glutathione reductase; PLBs: protocorm-like bodies; PGRs: plant growth regulators, Pro: proline; SOD: superoxide dismutase; Trp: tryptophan.

For in vivo explants, ultrasonication affected germination in many cases (Table 2). Compared to the control, the seed germination percentage was 1.7 times greater in *Atriplex lentiformis* S. Wats, 1.95 times greater in *Zygophyllum eurypterum* L. Boiss and Buhse, and 1.82 times greater in *Cuminum cyminum* L. [62]. Furthermore, 20–59 kHz ultrasonication of the seeds enhanced the germination rate of *Arabidopsis thaliana* L., *Chenopodium album* L. (by 2.8 times), *Triticum aestivum* L. (by 35%), *Trigonella foenum-graecum* L., *Ricinus cummunis* L. (the maximum germination percentage was between 80–100%), and *Zea mays* L. [63–69]. Under stress conditions, ultrasonication also raised the germination parameters in castor bean, fenugreek, and maize (Table 2). The porosity of treated seeds was increased; hence, the seeds could better absorb water and oxygen [68]. The germination rate was intensified by ultrasound stimulation in the beans of *Oryza sativa* L. and *Phaseolus vulgaris* L. (the highest was 77.09%), and in the buds of *Saccharum officinarum* L. [70–72]. Younesian et al. (2017) observed that for sonicated *P. vulgaris* seeds combined with 24-epi-brassinolid foliar application in normal and drought stress conditions, the grain yield increased by about 20% [73]. Similarly, in sugarcane, 2–5 min of ultrasonication could significantly increase the yield [74]. Ultrasonication was advantageous to water absorption in *Glycine max* L. and *O. sativa*, enhancing the hydration of the plants [75,76]. Using an aeroponic system with a fogging period of 15 min and a nutrient solution proved to be the most favorable method in tomatoes because it had a positive effect on the height, root weight and length, fruit weight and length, and yield of the plants [77]. Moreover, a 40.5 and 42 kHz ultrasound treatment had a positive impact on disease resistance in *Solanum lycopersicum* L. and *O. sativa* [78], and it eliminated pathogenic fungi on the seeds of *Triticum aestivum* L. [65], respectively. It was explored in several studies that ultrasonication increased the antioxidant enzyme accumulation in wholegrain brown rice, sugarcane, peppers, soybeans, and red kidney and mung beans [70–72,74,79–81]. In addition to all this, it affected the hormone synthesis pathways, and in *S. officinarum* L., the auxin, abscisic acid, and jasmonic acid content was reduced, and the auxin/abscisic acid ratio was increased, improving germination [71]. In *Z. mays* L., the amount of auxin was enhanced by 5.5%, that of gibberellin by 37.5%, and that of salinic acid by 28.9% [69]. Yu et al. [72] detected that the treatment of red kidney beans resulted in a decrease in fat, starch, and soluble sugar content, while an increase in the total and soluble protein content. After ultrasonication of winter wheat seeds, significantly differentially expressed sequences and differentially methylated regions played a role in starch and indole-3-acetic acid biosynthesis, as well as the photosynthesis and the TCA cycle (tricarboxylic acid cycle) pathways [82]. Ultrasonic waves were beneficial to energy and lipid metabolism and protein hydrolysis in *O. sativa* L. In maize, the quantity of acid protease was enhanced by 96.4%, α-amylase by 73.8%, and β-amylase by 49.1% (Table 2). Furthermore, it was noticed that secondary metabolite production was also stimulated by the ultrasound treatment in different plants [66,72,76,81].

Table 2. Main growth, developmental, biochemical, and molecular effects of ultrasonication in various in vivo explants.

Plant (Species, Cultivar)	Treated Explant	Method of Ultrasonic Treatment	Main Impacts of Ultrasonication	Ref.
Big saltbrush (*Atriplex lentiformis* S. Wats), Cumin (*Cuminum cyminum* L.), *Zygophyllum eurypterum* L. Boiss. and Buhse	Seeds	Ultrasonication (Elma, e 30 h; Elmasonic, Singen, Germany) for 0, 1, 3, 5, 7, and 9 min at 42 kHz	Ultrasonication for 5 min had the highest effect on the germination percentage in *A. lentiformis* (68.00%) and *Z. eurypterum* (73%) seeds; furthermore, 7 min of ultrasonication was the most effective for gemination of *C. cyminum* (80%) seeds, in comparison with the controls (40%, 37.5%, and 44%, respectively).	[62]
Arabidopsis thaliana L. (Col-0 ecotype)	Seeds (fresh, artificially deteriorated, naturally aged)	Ultrasonic bath (USC-1400, Unique, São Paulo, Brazil) for 30 s to 64 min at 45 kHz and 0.028 W m^{-3} volumetric power, at 24 °C	Ultrasonication for 30 s significantly increased germination in artificially deteriorated and naturally aged seeds. More pores are present on the coat of treated seeds (vs. control), which can be the reason for the increased germination rate.	[63]
Red bean (*Phaseolus vulgaris* L.)	Seeds	Ultrasonic bath (digital ultrasonic, Model 4820-CD(Song Young International. Co., Taiwan.) for 3 min at 24 kHz, at 32 °C	24-epi-brassinolid foliar application combined with ultrasonication increased grain yield by about 20%.	[73]
Soybean (*Glycine max* L.) Merr	Seeds	Airborne acoustic ultrasound generator for 30 min at 25 kHz combined with using plasma activated water (PAW)	Airborne ultrasonication can increase the hydration of plants and the ability of seeds to absorb water. When using plasma treated water, faster growth and taller plants were detected.	[75]
Tomato (*Solanum lycopersicum* L. cvs. 'Momotaro', 'Moneymaker' Cabbage (*Brassica oleracea* L. var. capitata cv. 'Shikidori' Rice (*Oryza sativa* L. cvs. 'Aichi-asahi', 'Kinuhikari'	1- or 2-week-old plants	Aerial ultrasound oscillator (was developed) (1) Ultrasound at frequency of 40.5 kHz, ca. 100 dB for 2 weeks (24 h per day). The oscillator was set over the plants at a distance of 70 cm; (2) ultrasound at frequencies of 19.8 and 28.9 kHz	Ultrasound of 40.5 kHz increased disease resistance in tomatoes and rice and reduced the incidence of *Fusarium* wilt and blast diseases, respectively.	[78]

Table 2. *Cont.*

Plant (Species, Cultivar)	Treated Explant	Method of Ultrasonic Treatment	Main Impacts of Ultrasonication	Ref.
Tomato (*Solanum lycopersicum* L.) *Lycopersicon esculentum* (Mill.)	Whole plants	Fogging period for 10, 15, and 20 min at the ultrasonic frequency of 50 and 107 kHz, and 2.1 MHz, using an aeroponic system	Frequency of 50 kHz and a 15 min fogging period of the nutrient solution were the most advantageous parameters, which had significant effect on the plant height, root weight and length, fruit weight, fruit length, and plant yield.	[77]
Wholegrain brown rice (*Oryza sativa* L.) subsp. *Japonica*	Grains	Ultrasonic irradiator (Tianhua Co., Jining, China) for 5, 10, 15, and 30 min at 400 W (28 kHz, 17.83 W cm^{-2})	Ultrasonication for 5 min caused the highest germination rate. The starch content in pretreated grains decreased, while the reducing sugar content increased. Ultrasonication increased the accumulation of antioxidants, GABA (with 48.9% and 56.9%, in naturally germinated grains and HIU-stimulated grains, respectively), and Pro (17.6 mg/100 g in naturally germinated and 19.5 mg/100 g in HIU-treated samples). Nutritional index of free amino acids together with the in vitro bioaccessibility of Ca and Fe (from 202.1 mg/kg to 259.6 mg/kg) were also raised significantly.	[70]
Common lambsquarters (*Chenopodium album* L.)	Seeds	Ultrasound bath (Bandelin DT 255 H model with internal dimensions of 325 mm × 175 mm × 305 mm and volume of 5.5 L) for 0, 5, 10, 15, and 30 min at 35 kHz and 230 W	Ultrasound treatment improved the germination. The highest percentage of germination was 83.3% after 15 min ultrasonication, compared to the control (29.8%).	[64]
Winter wheat (*Triticum aestivum* L.) variety Zebrets	Seeds	Ultrasonic bath (CARRERA—SINUS, Model: 2501) (Supply voltage: 230 V; 50 Hz; 50 W) for 3, 9, and 21 min at 42 kHz	Ultrasonication of contaminated seeds for 3 min showed a significant rise in the germination (by 35%) and the germinating energy of seeds (by 46%). At a frequency of 42 kHz with a duration of more than 3 min ultrasonication can destroy pathogenic fungi on seeds.	[65]
Fenugreek (*Trigonella foenum-graecum* L.)	Seeds	Ultrasonic bath (FALC Instruments, Treviglio, Italy) for 0.5 and 10 min at 40 and 59 kHz and 300 W, at 25 ± 0.5 °C	Ultrasonication for 5 min improved germination-related properties. It enhanced the effect of nicotinic acid promoting defense responses and the production of secondary metabolites, including trigonelline biosynthesis.	[66]
Winter wheat (*Triticum aestivum* L.) cv. 'SE15'	Seeds	Ultrasonication for 5 min, at 30 kHz and 70 W	Ultrasonication of seeds increased the length and weight of roots (by 23–68%) and shoots (16–28%) of 7-day-old seedlings significantly. DEGs play a role in starch biosynthesis, indole-3-acetic acid biosynthesis, photosynthesis, and TCA cycle pathways (these were affected by changes in DNA-methylation, as well). It resulted in DNA hypomethylation, which altered the accessibility of some genes for transcription.	[82]

Table 2. *Cont.*

Plant (Species, Cultivar)	Treated Explant	Method of Ultrasonic Treatment	Main Impacts of Ultrasonication	Ref.
Sugarcane (*Saccharum officinarum* L.) varieties ROC22, LC05–136, YT93–159	Buds	Plant seed production increase processor (JD-1 L, Guangzhou Golden Rice Agricultural Science & Technology Co., Ltd., Guangzhou, China). Ultrasonication for 1, 2, and 5 min at 20–40 kHz	Ultrasonic treatment for 2–5 min increased the length, diameter, and germination rate of buds and enhanced the antioxidant enzyme activities. It reduced the contents of auxin, abscisic acid, and jasmonic acid, and increased the ratio of auxin/abscisic acid. In ROC22 and YT93–159, 2 and 5 min of ultrasonication, respectively, increased the bud length by about 90%. In YT93–159 and LC05–136, 5 min of ultrasonication, enhanced the bud diameter and germination rate by about 30%. The gene expression of gibberellin synthesis was upregulated, while that of abscisic acid synthesis was downregulated.	[71]
Pepper (*Capsicum annuum* L.) cv. 'Çetinel'	Seeds	Ultrasonic bath (Elma-Elmasonic S, Singen, Germany) for 0, 15, and 30 min at 40 Hz with magnetic field treatment (0, 0.3, 0.9, and 1.1 Tesla) for 5 min	Magnetic field treatment with ultrasonication decreased the malondialdehyde (19% and 35%, respectively) and hydrogen peroxide (52% and 58%, respectively) content, and increased the catalase enzyme activity. The endogenous melatonin content increased, too, giving tolerance against drought stress.	[79]
Glutinous rice (*Oryza sativa* L.)	Germinated brown glutinous rice	Ultrasound device for 5 min pulse on and 25 min pulse off, with an ultrasonic generator (28 kHz), a power switch (30, 40, 50 W), temperature switch, and an automatic timing unit (330 mm × 150 mm × 330 mm ($L \times H \times W$)) Ultrasonication combined with 2% $CaCl_2$ stress.	Higher contents of gamma-aminobutyric acid (3.29-fold), pyruvic acid (7.63-fold), glycerol (4.88-fold), glutamate (2.02-fold), and glucose (1.32-fold) were accessed due to the 30 W ultrasound treatment and 2.0% $CaCl_2$ stress at 9 h pre-germination.	[76]
Castor bean (*Ricinus communis* L.)	Seeds	Ultrasonic bath (KS-150EI, Ningbo Haishu Kesheng Ultrasonic Equipment Co., Ltd., Ningbo, China) bath capacity of 4 L (300 × 150 × 100 mm) for 0, 3, 6, 9, 12, 15, 20, 25, 30, 35, 40, 50, and 60 min at 40 kHz and 150 W and at 15, 20, 25, 30, and 35, 40 °C	Ultrasound treatment for 12 min at 30 °C increased germination parameters in both aged and non-aged seeds especially under drought and salt stresses. Under normal conditions, GP_{max} was between 80 and 100% for, at most, 6 days of accelerated aging without, and, at most, 10 days with ultrasonication. When decreasing water potential by 1.2 MPa (using NaCl), GP_{max} remained above 40% for, at most, 2 days of accelerated aging without, and, at most, 6 days with ultrasonication.	[67]

Table 2. *Cont.*

Plant (Species, Cultivar)	Treated Explant	Method of Ultrasonic Treatment	Main Impacts of Ultrasonication	Ref.
Fenugreek (*Trigonella foenum-graecum* L.)	Germinated seeds	Bath ultrasonication (Digital ultrasonic cleaner, MFUC-80A, Biobase Meihua Trading Co., Ltd., Shangdong, China) for 10 and 20 min at 40 kHz and 25 °C	Ultrasonication for 10 and 20 min had a significant effect on seed germination, early seedling development, and biochemical components under normal and salinity stress, also increasing the porosity of seeds, enhancing their ability to absorb water and oxygen.	[68]
Soybean (*Glycine max* L.)	Seeds	(1) Ultrasonication (Ultrasonic Homogenizer, JY92-IIN, Shanghai Drawell Scientific Instrument Co., Ltd., Shanghai, China) for 30 min at 400 W and 20–25 kHz at 25 °C; (2) 250 µM spermidine treatment	qRT-PCR analysis demonstrated that most isoflavone reductase genes, (primarily GmIFR9/17 and GmIFR36) were remarkably upregulated in soybean cotyledon, followed by hypocotyl and root tissues under spermidine and ultrasonication treatments.	[80]
Red kidney bean (*Phaseolus vulgaris* L.)	Beans	Water bath high-intensity ultrasonication for 10 min at 20 kHz and 250, 350, and 450 W, combined with H_2O_2 treatment, at 25 °C	Ultrasonication for 10 min at 350 W resulted in the highest germination rate (77.1%), increased total and soluble protein and ash content, and while reducing the fat, starch, and soluble sugar content, increased the accumulation of phenolic and flavonoid compounds, AA, and GABA, antioxidative capacity, improved amino acid composition, and protein digestibility.	[72]
Mung bean (*Vigna radiata* L.) variety Ming Mung Bean	Seeds	Ultrasonic generator (KQ-250E, Kunshan Ultrasonic Instrument Co., Kunshan, China) for 3–15 min at 40 kHz and 240–360 W (1) Traditional germination (2) Ultrasonic pretreatment and germination (3) Ultrasonic-Ca^{2+} pretreatment and germination	Ultrasound and ultrasound-Ca^{2+} pretreatments significantly increased the polyphenol content and enhanced the antioxidant capacity during germination. Ultrasound pretreatment stimulated flavonoid biosynthesis, while ultrasound-Ca^{2+} pretreatment promoted the tyrosine synthesis pathway.	[81]
Maize (*Zea mays* L.) *ZD958*	Seeds	Ultrasonic processor (5ZCG-T6; Guangzhou Jindao Agricultural Technology Co., Ltd., Guangzhou, China) for 10–60 s at 20–40 kHz and at 20–25 °C	Ultrasonication for 40 s increased the acid protease (by 96.4%), α-amylase (73.8%), and β-amylase (49.1%) content. Most of the DEGs play a role in ribosome, proteasome, and pyruvate metabolism, sesquiterpenoid, triterpenoid, and phenylpropanoid biosynthesis, and oxidative phosphorylation. The amount of auxin (by 5.5%), gibberellin (37.3%), and SA (28.9%), and different TFs, were enhanced. Seed germination and growth under salt, drought, and waterlogging stresses were improved.	[69]

Table 2. *Cont.*

Plant (Species, Cultivar)	Treated Explant	Method of Ultrasonic Treatment	Main Impacts of Ultrasonication	Ref.
Potato (*Solanum tuberosum* L.) cvs. Denar, Lord, Owajca, Vineta, Satina, Tajfun, Syrena, Zagloba	Tubers	Ultrasonication in aquatic environment in an ultrasonic bathtube devicet for 10 min (at 18 °C); 200 W; and 50 Hz	Both total and marketable tuber yields of ultrasonically pre-treated plants were increased (5.5%) but a cultivar-dependent way.	[83]
Sugarcane (*Saccharum officinarum* L.) varieties ROC22, LC05–136, YT93–159	Buds	Ultrasonic treatment machine (developed by Guangzhou Jindao Agricultural Technology Co., Ltd.). Ultrasonication for 1, 2, and 5 min at 20–40 kHz, dry treatment	Optimal ultrasonication time was 2–5 min. Cane yield increased by 2.2–22.1% and sugar yield by 0.3–21.2% in 2021, while in 2022, by 1.2–12.6% and 0.38–8.4%, respectively. It was beneficial to photosynthesis, root systems, and caused a more rapid growth.	[74]

Abbreviations: AA: ascorbic acid; GABA: gamma-aminobutyric acid; Pro: proline; TCA: tricarboxylic acid cycle; GP_{max}: maximum germination percentage; DEGs: differentially expressed genes; SA: salicylic acid; TF: transcription factor.

According to recent in vitro and in vivo studies (Tables 1 and 2), the main processes of sensing and transduction mechanisms of ultrasound stimuli, which may lead to various growth and developmental alterations in plants, are summarized in Figure 1.

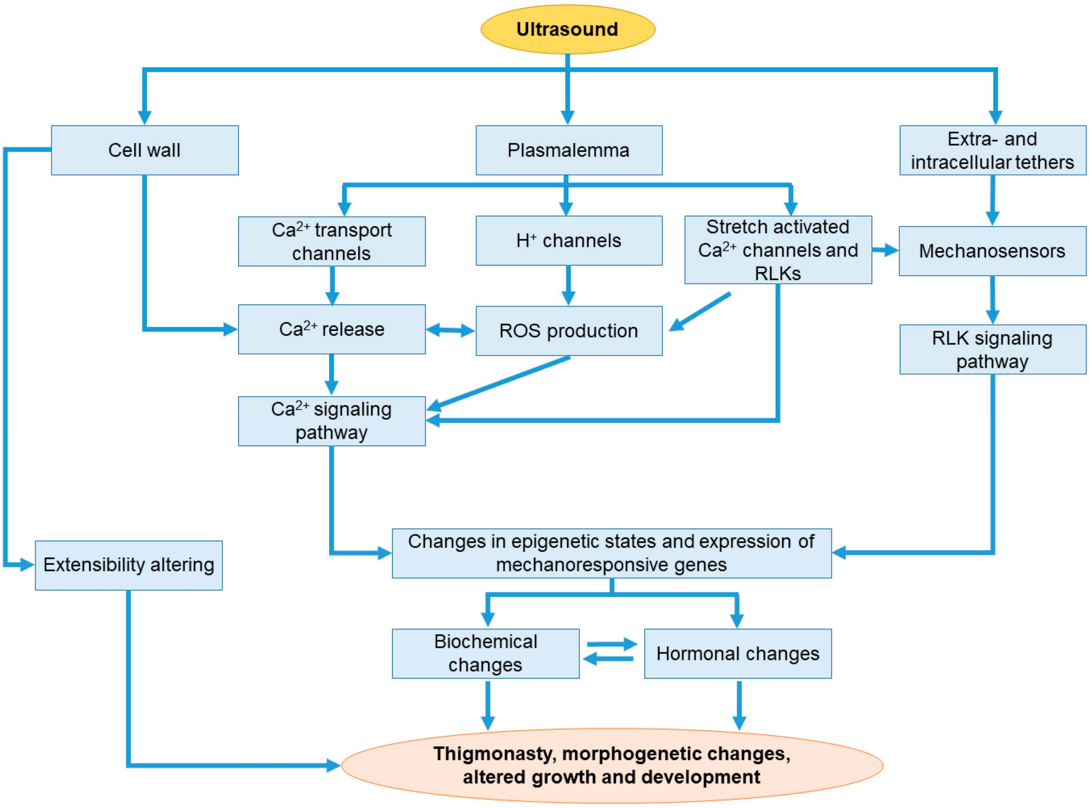

Figure 1. Summary of main processes involved in sensing and transduction of ultrasound cues and how they affect molecular, biochemical, and physiological functioning of plants (abbreviations: ROS—reactive oxygen species; RLK—receptor-like kinase).

4. Ultrasound Emission by Plants

According to the latest studies of the last decade, as presented in the previous sections, we now have quite a lot of information about the growth–developmental and biochemical responses of plants induced by ultrasound. Additionally, we begin to explore the gene transcription and DNA methylation changes that are behind these responses. The question was rightly raised that if plants are able to sense acoustic signals, like ultrasound, are they capable of emitting them?

It has been proven for a long time that plants can produce both audible and ultrasonic acoustic signals, mainly if they are drought-stressed. This had been attributed to cavitation and embolism caused by the transpiration pull allowing air to enter the xylem, leading to vibrations in the vessel wall [84–87]. Audible acoustic emission was first detected by Milburn and Johnson [84] in *Ricinus* during leaf rehydration. The investigations of Ritman and Milburn [85] showed that larger vessels produced acoustic signals with lower frequencies, while smaller ones emitted signals, rather, in the ultrasonic frequency range. They assumed that ultrasonic acoustic emission, at least partly, is generated by other processes than cavitation, i.e., the acoustic signal emissions by plants may occur independently from cavitation, as later described by others [88–91]. Recently Khait et al. [6] proved that the overlap between cavitation and the emitted and recorded ultrasound was only partial. Plants are able to produce a wide range of acoustic emissions at specific frequencies, for varying durations and with different intensities in response to different environmental stress conditions. It means that naming transpiration and cavitation as the sole cause of plant ultrasound emission is an infinite simplification, even if they can modify the emitted acoustic signals [7,92,93]. However, the exact mode of sound and ultrasound production by plants is not yet clear.

The specificity of the ultrasound emission is determined by the type of environmental stress and by plant species [6,7]. The information-carrying property of the emitted ultrasonic sound was investigated by Khait et al. [6], both in an acoustic chamber and under greenhouse conditions. Tomato (*Solanum lycopersicum* L. cv. Hawai 7981) and tobacco (*Nicotiana tabacum* L. cv. Samsun NN) plants were stressed either by drought or by cutting. With the development of machine learning algorithms, they were able to distinguish the emitted ultrasounds in a range of 20–150 kHz. They concluded that the emitted ultrasound (20–100 kHz) was specific for the type of the stress and thereby it might play an information-carrying role. The number of sounds emitted differed depending on species and stress conditions. It was, after cutting, 25.2 per hour and 15.2 per hour while, in response to drought stress, 35.4 per hour and 11.0 per hour, for tomato and tobacco, respectively, compared to the control plants where it was under 1.00 per hour. Both the ultrasound intensity and the mean peak frequency were different in both species and in response to both stresses when measured from a 10 cm distance from plants. The watering status and the transpiration rate of the plants studied in the greenhouse and the emitted sound signal pattern (number of sounds per hour) were in strong correlation with each other in tomatoes. The more water-deficient the plant was, the more sounds it emitted, and after watering, the sound emissions decreased. The physiological state of the plant could be surveyed with high precision (99.7%) by measuring the emitted ultrasound. The authors assumed that insects or mammals are able to detect the plant ultrasound emissions from a 3–5 m distance. In addition, they successfully detected the ultrasound emission in other herbaceous plants (wheat, corn, cactus, and henbit) or even in grapevines, but not from woody species, like almonds or the woody part of grapevines. Furthermore, in the case of tomatoes, another type of specific ultrasound emission was detected when tomatoes were infected with tobacco mosaic virus (TMV).

5. The Plant's Benefit from Reflecting, Sensing, and Emitting Ultrasound

In nature, there are some nice examples of mutual benefits and increased fitness in bats and plants based on the ultrasonic reflection of plants. Some plant species (e.g., *Mucuna holtonii, Marcgravia evenia,* and *Marcgravia evenia*), which are pollinated by bats, develop a leaf or inflorescence structure. They have properties to reflect the ultrasound emitted by bats in such a way that they attract bats by helping their echolocation and, therefore, their feeding, and at the same time, bats increase the success of plant pollination (up to 50%) [94,95]. Pitcher plants (*Nepenthes* sp.) living a carnivorous lifestyle attract bats with their special structure that reflects the bats' ultrasonic sound. The plants provide roost for the bats in the pitchers, while about one-third of all nitrogen in plant foliage comes from bat droppings [96].

The latest research results presented in the previous sections indicate that plants are capable of sensing and emitting different frequencies of ultrasound. Considering that the emitted ultrasound is informative [6], there is a chance that neighboring plants and other organisms can gain information from them about environmental stresses or threats. Similarly, the detection of ultrasound vibrations using different properties from their environment and from other living beings can also carry information for plants. It can affect their growth, development, and even stress tolerance. Therefore, it can be assumed that the airborne ultrasound emissions and sensing of plants can be a way of biocommunication [6,10,13,97].

The use of ultrasound, or sound in general, for plants to communicate with each other and with other creatures living in the environment is a cheap and fast way for them to obtain information about their environment (ultrasound emitted by stressed plants, sound of water, chewing sound of herbivores, buzzle of pollinators, etc.) and to share information with those living in their environment (other plants, animals, and fungi) [92]. It allows plants to quickly learn about threats appearing in the near environment and to wait for them in preparation. Being in preparation means the formation of a primed state, when the metabolic, hormonal, and biochemical pathways of the plants are modified, and even certain epigenetic modifications occur [2,13,98]. Furthermore, sound can induce tolerance to other stresses, like drought, as was described in *Arabidopsis* and rice [63,99]. From the other side, sound or ultrasound can help to map environmental possibilities, such as water sources, or the appearance of symbiotic organisms in the near environment, and the plant responds to them with modified growth (in direction or extent) [2,3]. Plants are able to distinguish between sounds of different frequencies (audible and ultrasound) in their environment, and act accordingly. As an example, beach evening-primrose (*Oenothera drummondii* Hook.) attracts pollinators by producing sweeter nectar after sensing the sound emitted by its pollinator (1 kHz), but does not react to ultrasound (35 kHz) emitted by bats [100], which facilitates the success of the pollination.

Therefore, "hearing" and "talking", i.e., sensing and emitting sound waves, and the ability to discriminate between sounds of different frequencies or patterns are ecologically beneficial traits even for plants, even in the lack of specialized organs. In addition, they are an adaptation of importance in plant life [2]. In terms of energy and transmission, the use of acoustic signals is more favorable in some cases than the synthesis and spread of chemical substances [92,98].

6. Perspectives on Ultrasonication and Ultrasound Detection in Horticulture and Crop Production

Taking into account the research results of the last decade, Tables 1 and 2 summarize what changes in growth, development, and molecular function occurred in plants, when different plant parts, whether it was in vitro or ex vitro, or if the intact plants were exposed to ultrasound (Tables 1 and 2). The methodological and technological achievements and perspectives that ultrasound application has achieved and, based on our current knowledge, is expected to enable plant biotechnology, agriculture, and horticulture practices are summarized in this chapter.

6.1. Use of Ultrasonication in Plant Tissue Culture for Enhancing In Vitro Growth and Development

Ultrasonication is of methodological importance under in vitro conditions. It was able to control contamination (both bacterial and fungal) during in vitro culture establishment, enhance callus development, somatic embryogenesis, and modify shoot or root growth and biomass in various plant species (Table 1). Thereby, ultrasonication in various in vitro explants can be incorporated into plant micropropagation and other plant tissue culture techniques, both to enhance their efficiency and to trigger developmental changes in vitro.

6.2. Sonication-Assisted Agrobacterium-Mediated Transformation (SAAT)—A Methodological Application of Plant Ultrasonication in Gene Transformation

The method of sonication-assisted *Agrobacterium*-mediated transformation (SAAT) was first described by Trick and Finer in 1997 [101]. This technique involves exposing the target plant tissue to the ultrasound treatment, which causes microwounds on the tissue in the presence of *Agrobacterium*. SAAT enhanced the transient GUS (β-glucuronidase) expression in soybeans, and, according to Zhang et al. [102,103], it was significantly higher (41.4% and 54.2%, respectively) compared to the control (9% and 11%, respectively). The highest frequency of *Pinus elliottii* Ehgelm. zygotic embryos with transient GUS expression were noticed, too, after ultrasonication using *Agrobacterium* [104]. In *Dierama erectum* Hillard, embryonic shoot apical meristems proved to be the best target tissue for transformation, with an efficiency in GUS gene expression of about 67%, compared to about 40% measured for calluses (Table 3) [105]. *Agrobacterium*-mediated gene transfer with ultrasonication enhanced the transformation efficiency in many species. In *Coffea canephora* Pierre ex A.Froehner, a transformation efficiency of 0.5–4% was measured, based on the number of putatively transformed hygromycin-resistant somatic embryos [106]. In *Beta vulgaris*, the highest transformation efficiency of 54% was observed when using diploid sugar beet explants, compared to efficiencies between 10 and 36% yielded by haploid explants, depending on the treatment [107]. In *Catharanthus roseus* (L.) G. Don, 10 min of ultrasonication increased it to 6%, compared to 3.5% measured using the conventional method [108]. In *Paspalum vaginatum* Sw., a high transient transformation efficiency of about 95% was observed with 5 min of ultrasonication, significantly exceeding the value of about 75% measured without it [109]. In *Passiflora cincinnata* Mast., transformation efficiencies up to 21.36% were observed using ultrasonication, in contrast to 13.63% in the case of the control [110]. In *Hevea brasiliensis* Müll.Arg., transformation efficiency was improved by ultrasonication at 40 kHz, mostly when using a treatment of 50 s and *Agrobacterium* at 0.45 OD_{600} [111]. In *Malus x domestica*, Borkh., wounding by scratching or 10 min of ultrasonication improved transformation efficiency to 9.3 roots per rooted explant, compared to 4.4 measured with no wounding (Table 3) [112]. Ultrasonication also improved transformation frequency in some cases. It was increased from the control value of 17% to 35% when using ultrasonication in *Dendrobium catenatum* [113]. It was increased by 2.2-fold to 60% using ultrasonication compared to manual wounding in *Oroxylum indicum* (L.) Benth. ex Kurz [114]. In *Verbascum nigrum* L., the infection frequency was 83%, detecting the first neoplastic roots at leaf wound sites two weeks after the transformation (Table 3).

Table 3. Effects of ultrasonication-assisted *Agrobacterium*-mediated transformation.

Plant (Species, Cultivar)	Treated Explants	Method of Ultrasonic Treatment	Main Impacts of Ultrasonication	Ref.
Slash pine (*Pinus elliottii* Engelm.)	Mature zygotic embryos	Bath sonicator (Model PC5, L&R Manufacturing Co, Keamy, NJ, USA) for 15, 30, 45, 60, 75, or 90 s at 55 kHz, using *Agrobacterium* at 0.3, 0.6, 0.9, 1.2, 1.5, or 1.8 $OD_{600\ nm}$	The highest frequency of embryos with GUS transient expression was obtained after ultrasonication for 50 s and infection with *A. tumefaciens* OD_{600} = 0.9.	[104]
Coffea canephora L.	Somatic embryos	Ultrasonication for 30 s at 20 kHz and 80% amplitude (Bandelin Sonoplus Ultrasonicator, Bandelin electronic GmbH & Co. KG, Berlin, Germany)	The transformation efficiency (0.5–4%) was recorded by observing the number of putatively transformed hygromycin-resistant somatic embryos.	[106]
Sugar beet (*Beta vulgaris* L.)	Petiole and midrib explants from two-week-old shoots	Ultrasonication for 15–240 s using *Agrobacterium rhizogenes* at 0.05 and 0.5 OD_{600} (frequency and other sound-related details not specified)	Ultrasonication for 15 s with *Agrobacterium* OD_{600} = 0.5 were the most effective conditions for transformation. The highest transformation efficiency was 54%.	[107]
Soybean (*Glycine max* L.) Merrill	Cotyledonary node	Bath sonicator (KH2200B, Kunshan Hechuang, Kunshan Ultrasonic Instruments Co., Ltd., Kunshan, China) for 0 and 3 min at 40 kHz, using *Agrobacterium* at 0.8 OD_{600}	Transient GUS expression was significantly higher (41.4%) than in not ultrasonicated (9.0 ± %) samples. Ultrasonication created micro-wounds for *Agrobacterium* infection and increased transformation efficiency by disrupting the synthesis of isoflavones.	[103]
Dark mullein (*Verbascum nigrum* L.)	Hairy root cultures	Sonication for 45 s at 35 kHz (UCI-50Raypa® R. Espinar S.L., Barcelona, Spain), using *Agrobacterium rhizogenes* at 0.8 OD_{600}	Two weeks after the transformation, first neoplastic roots were detected at the wound sites of *V. nigrum* leaves and the infection frequency was 83%.	[115]
Soybean (*Glycine max* L.) Merrill	Cotyledonary-node explants	Bath sonicator (KH2200B, Kunshan Hechuang, Kunshan Ultrasonic Instruments Co., Ltd., Kunshan, China) for 0 and 15 s at 40 kHz	As a result of ultrasonication, the transient GUS expression was (54.2%), significantly higher than that of not the sonicated (11%).	[102]
Catharanthus roseus L.	Hypocotyls	Bath sonicator (Imeco Ultrasonics, IMECO Ultrasonic Cleaning Mashine Manufacturer, Maharashtra, India) for 10 min, using *Agrobacterium* at 0.8 OD_{600} (frequency and other sound-related details not specified)	The transformation efficiency was 6.0%, compared to 3.5% with the conventional method.	[108]
Dierama erectum Hilliard	Embryonic shoot apical meristems, hypocotyls, and organogenic callus	Ultrasonication (Julabo Labotechnik GmbH, Seelbach, West Germany) for 0, 10, 20, 30, 40, 50, and 60 s at 35 kHz, using *Agrobacterium* at 0, 0.2 0.4, 0.8, 1.6, and 2 OD_{600}	The optimal duration of ultrasonication was 30 s, with *Agrobacterium* at 1.6 OD_{600}. The embryonic shoot apical meristems proved to be the best target tissue for transformation, yielding a GUS gene expression efficiency of about 67%, compared to about 40% yielded by calluses.	[105]

Table 3. *Cont.*

Plant (Species, Cultivar)	Treated Explants	Method of Ultrasonic Treatment	Main Impacts of Ultrasonication	Ref.
Dendrobium catenatum L.	Primary protocorms generated from seeds	Sonication (Scientz, Ningbo, China) for 1, 2, 3, 4, and 5 min at 300 W and 40 kHz, using *Agrobacterium* at 0.4, 0.6, and 0.8 OD_{600} Three surfactants (Tween® 20, Triton™ X-100, and Silwet®-77) (Solarbio) at either 0.001% (*v*/*v*) or 0.01% (*v*/*v*) concentration were added to the *A. tumefaciens* suspension	The most advantageous condition for ultrasonication was 300 W at 40 kHz for 3 min, and the optimal OD_{600} was 0.6. The combination of the ultrasound treatment for 1 min and 0.001% (*v*/*v*) Triton™ X 100 was the optimal pretreatment to reach the highest transformation frequency (35%, compared to 17%).	[113]
Seashore paspalum (*Paspalum vaginatum* O. Swart) cv. 'Sea Spray'	Embryogenic callus from multiple explants	Ultrasonication for 5 and 10 min at 40 kHz, using *Agrobacterium* at 0.1, 0.6, and 1.2 OD_{600}	A high transient transformation efficiency (about 95%) was observed when using *Agrobacterium* concentration of OD_{600} = 0.6 with 5 min of ultrasonication (compared 75% without ultrasonication).	[109]
Passion fruit (*Passiflora cincinnata* Mast.)	Somatic embryos at the cotyledonary stage	Bransonic Ultrasonic Cleaner (B1210E-Mt, Branson Ultrasonics Corp., Brookfield, WI, USA) for 15 and 30 s at 47 kHz and 80 W, using *Agrobacterium* at 0.5 OD_{600}	The highest transformation efficiency (21.4%) was observed by ultrasonicating SEs for 30 s, in contrast to 13.6% in the control.	[110]
Hevea rubber tree (*Hevea brasiliensis* Müll.Arg.)	Mature cotyledonary somatic embryos	Ultrasonication for 10, 30, 50, and 70 s at 40 kHz, using *Agrobacterium* at 0.45, 0.6, and 0.75 OD_{600}	The best transformation efficiency was observed by using *Agrobacterium* at a concentration of OD_{600} = 0.45, and 50 s of ultrasonication.	[111]
Oroxylum indicum L.	Embryonic axis, cotyledon, leaf, and callus	Ultrasonication (Citizen, Mumbai, India) for 10–100 s at 50 MHz and 80% amplitude, using *Rhizobium rhizogenes*	Ultrasonication increased transformation frequency by 2.2-fold (60%) compared to manual wounding. Ultrasonication with 15 mM $CaCl_2$ caused the best transformation efficiency (84%).	[114]
Apple (*Malus domestica* Borkh.) genotype 'M26'	Whole leaves	Bath sonication (Bandelin Sonorex Super 10 P, type DK 102 P, Bandelin electronic GmbH & Co. KG, Berlin, Germany) for 5, 10 min, at 35 kHz and 60 W L^{-1}, using *Rhizobium rhizogenes* at 0.5 OD_{600}	Wounding by 10 min of ultrasonication improved transformation efficiency. The 4.4 roots/explant measured with no wounding was increased to 9.3 with ultrasonication.	[112]

Abbreviations: GUS: β-glucuronidase.

The SAAT method can be enhanced with vacuum infiltration. This combined method was applied successfully by Stevens and Pijut [116] and Palla and Pijut [117] in the *Fraxinus profunda* (Bush) Bush and *Fraxinus americana* L., respectively (Table 4). In *Prunus serotine* Erhart, a transformation rate of 6.7% was measured using 60 and 90 s of ultrasonication and 21.7% using 15 min of vacuum infiltration, while the combination of the two treatments yielded poorer results [118]. However, this can be considered as an exception in the articles included in this review, as typically, the combination of the two treatments yielded the best results in similar cases (Table 4). Using such a combined treatment, Mayavan et al. [119] measured a maximum transformation efficiency of 29.6% in *Saccharum officinarum* L. Kapildev et al. [120] found an increase in transformation efficiency to 46.2% in *Vigna mungo* (L.) Hepper, from 25.6% measured for the control. Rani et al. [121] found that sonication increased the transformation efficiency to 10.8% in *Carthamus tinctorius* L. when combined with vacuum infiltration, while it was only 6.8% without it. Karthik et al. [122] achieved 33.6% using both treatments, and 19.6% without sonication, in *Arachis hypogaea* L. Vasudevan et al. [123] measured 17.3%, compared to 7.6% using ultrasonication only and 1.3% without treatment, in *Citrullus lanatus* (Thunb.) Matsum. and Nakai. Karthik et al. [124] measured 37%, compared to 18.6% in the case of the control, in *Momordica charantia* L. Vasudevan et al. [125] achieved a transformation efficiency of 17.33%, compared to 8.00% in the untreated case, in *Citrullus lanatus*. Zhang et al. [126] applied a combined treatment that yielded 18.6%, compared to about 6.7% for a simple 30 min of soaking, in *Puccinellia tenuiflora* (Griseb.) Scribn. and Merr. Song et al. [127] measured values up to 4.45%, contrary to control values up to 0.8%, in *Vaccinium myrtillus*. Saravanan et al. [128] achieved 38.0%, in contrast to 6.6% measured in untreated explants, in *Glycine max* L. Chandrasekaran et al. [129] found that a combination of the treatments yielded a transformation efficiency of 12.33%, compared to 6.33% yielded by the control, in *Pisum sativum* L. (Table 4). In *Gossypium hirsutum* L., the maximum transformation frequency observed was 28.66% when using ultrasonication combined with vacuum infiltration [130]. In *Fraxinus mandshurica* Rupr., Qi et al. [131] found that the maximum number of regenerated shoots was yielded by the combined treatment, increasing the number of adventitious buds per hypocotyl from 33.23 to 77.67 (Table 4). In *Glycine max*, ultrasonication combined with vacuum infiltration led to the highest total expression of GUS, according to King et al. [132]. In *Withania somnifera* (L.) Dunal, this combination enhanced transformation efficiency, resulting in a GUS foci frequency of 84% at the regeneration sites (Table 4) [133]. Sonication combined with vacuum infiltration also proved to be the most advantageous method in the case of *Tricosanthes cucumerina* L., studied by Subramanyam et al. [134] and in *Abelmoschus esculentus* (L.). Moench, studied by Manickavasagam et al. [135], where 39.3% and 54% of infected seeds were germinated, respectively (Table 4).

Table 4. Effects of combined application of ultrasonication-assisted *Agrobacterium*-mediated transformation with vacuum infiltration (VI).

Plant (Species, Cultivar)	Treated Explants	Method of Ultrasonic Treatment	Main Impacts of Ultrasonication	Ref.
Black cherry (*Prunus serotina* Ehrh.) genotype BC3	Leaves	Ultrasonication (Branson Ultrasonics, Brookfield, USA) for 0, 30, 60, and 90 s at 40 kHz, and VI for 0, 5, 10, and 15 min at 63.5 cm Hg, using *Agrobacterium* at 0.5, 1.0, 1.5, and 1.8 OD_{600}	Optimal parameters of treatment were 15 min of VI without ultrasonication with a transformation efficiency of 21.7%. In contrast, 60 and 90 s of ultrasonication yielded 6.7%.	[118]
Pumpkin ash (*Fraxinus profunda* (Bush) Bush	Mature hypocotyls from 3- to-7-day-old embryos	Sonication for 90 s and VI for 10 min at 62.5 cm Hg with *Agrobacterium* at 0.6–1 OD_{600} (frequency and other sound-related details not specified)	Pumpkin ash hypocotyls were successfully transformed with *Agrobacterium* by sonication combined with VI.	[116]
Soybean (*Glycine max* L.) cv. 'Williams 82'	Entire seedling	Ultrasonication (Sharpertek Ultrasonic cleaner, Model# SH180-6L; Sharpertek USA, Auburn Hills, MI, USA) for 0, 20, 30, and 40 s at 40 kHz with VI for 0, 5, and 15 min and 3 × 5 min at 483 mm of Hg, using *Agrobacterium* at 0.6, 0.8 OD_{600}	Ultrasonication for 30 s, combined with three 5-min VI, led to the highest total expression of GUS.	[132]

Table 4. *Cont.*

Plant (Species, Cultivar)	Treated Explants	Method of Ultrasonic Treatment	Main Impacts of Ultrasonication	Ref.
White ash (*Fraxinus americana* L.)	Hypocotyls from mature embryos	Sonication for 90 s, with VI at 62.5 cm of Hg for 10 min with *Agrobacterium* at 0.4–0.6 OD_{600} (frequency and other sound-related details not specified)	Sonication for 90 s with 10 min of VI treatment was first reported to successfully regenerate transgenic plants.	[117]
Ashwagandha (*Withania somnifera* L.) cv. 'Dunal'	Six-day-old nodal explants	Ultrasonication for 5, 10, 15, 20, and 25 s at 30 kHz and VI for 5, 10, 15, and 20 min, using *Agrobacterium* at 0.1, 0.2, 0.5, and 1.0 OD_{600}	Ultrasonication for 10 s, combined with 10 min VIand *Agrobacterium* at 0.2 OD_{600}, enhanced transformation efficiency. GUS foci frequency was 84%.	[133]
Snake gourd var. AGMMB2 (*Tricosanthes cucumerina* L.)	Seeds (decoated, 12 h old)	Bath sonicator (model 1510 Branson, Branson Ultrasonics, Kanagawa, Japan) for 0, 10, 20, 30, 40, 50, and 60 min at 40 kHz with VI for 0, 1, 2, 3, 4, and 5 min at 750 mm of Hg, using *Agrobacterium* at 1.0 OD_{600}	Sonication for 30 min, combined with 3 min of VI, proved to be the most advantageous method; 39.3% of infected seeds were germinated.	[134]
Sugarcane (*Saccharum officinarum* L.) hybrid varieties Co 62175, Co 6304, Co 8021, Co 86032, Co 6907	Nodal cuttings from 6-month-old plants	Bath sonicator (model 2510 Branson, Branson Ultrasonics, Kanagawa, Japan). Sonication for 0, 2, 4, 6, 8, and 10 min at 40 kHz, in combination with VI for 0, 1, 2, 3, and 4 min at 0, 50, 100, 250, 500, and 750 mm of Hg	Sonication for 6 min, followed by 2-min VI at 500, were found to be the optimum to achieve the maximum transformation efficiency of 29.6% (with var. Co 62175).	[119]
Okra (*Abelmoschus esculentus* L.) Moench, var. Arka Anamika	Seeds	Bath sonicator (model 1510 Branson, Branson Ultrasonics, Kanagawa, Japan) for 0, 10, 20, 30, 40, and 50 min at 40 kHz, in combination with VI for 0, 1, 2, 3, 4, and 5 min at 750 mm of Hg with *Agrobacterium* at 0.6 OD_{600}	The transformation efficiency of okra was significantly improved by 30 min of sonication and 3 min of VI; 54% of infected seeds were germinated.	[135]
Black gram (*Vigna mungo* L.) cv. 'T9'	Cotyledon with embryo axis	Sonication (Branson Ultrasonics, Brookfield, WI, USA) for 0, 1, 2, 3, 4, 5, and 6 min and VI for 0, 1, 2, 3, 4, 5, and 6 min at 100 mm of Hg, using *Agrobacterium* at 0.8 OD_{600} (frequency and other sound-related details not specified)	Sonication for 3 min and 2-min of VI enhanced transformation efficiency of (46.2%), when compared to the control (25.6%).	[120]
Safflower (*Carthamus tinctorius* L.)	Seeds (decoated, 3 days old)	Bath sonicator for 10, 20, 30, 40, 50, and 60 s and VI for 5, 10, and 15 min at 250, 500, 750, and 1000 mm of Hg, using *Agrobacterium* at 0.5 OD_{600}	A combination of sonication (30 s) and VI (750 mm of Hg for 10 min) increased the transformation efficiency to 10.8% from 6.8%.	[121]
Peanut (*Arachis hypogaea* L.) cv. 'CO7'	One-half of the seeds (cotyledon with full embryo)	Bath sonicator (1510 Branson, Branson Ultrasonics, Kanagawa, Japan) for 0, 2, 4, 6, 8, and 10 min at 40 kHz, combined with VI for 0, 1, 2, 3, 4, and 5 min at 750 mm of Hg, using *Agrobacterium* at 0.8 OD_{600}	Sonication for 6 min, with 3 min VI of seed explants, resulted in the highest transformation efficiency of 33.6%. (without sonication 19.6%)	[122]

Table 4. *Cont.*

Plant (Species, Cultivar)	Treated Explants	Method of Ultrasonic Treatment	Main Impacts of Ultrasonication	Ref.
Watermelon (*Citrullus lanatus* Thunb.) cv. 'Arka manik'	Cotyledonary node explants from 7-day-old in vitro grown plants	Bath sonicator (Model 2510 Branson, Branson Ultrasonics, Kanagawa, Japan) for 10–50 s at 40 kHz with VI for 1–5 min at 100 mmHg	Optimal duration of ultrasonication (30 s) and VI (2 min) increased transformation efficiency to 17.3% from 7.6% and 1.3%, with and without ultrasonication.	[123]
Bitter gourd (*Momordica charantia* L.)	Seeds	Bath sonication (1510 Branson model, Branson Ultrasonics, Kanagawa, Japan) for 0, 5, 10, 15, 20, and 25 min at 40 kHz, combined with VI for 0, 2, 4, 6, 8, and 10 min at 750 mm of Hg, using *Agrobacterium* at 0.8 OD_{600}	The highest transformation efficiency (37%) was recognized by using ultrasonication for 15 min with VI for 6 min, compared to 18.6% in case of the control.	[124]
Watermelon (*Citrullus lanatus* Thunb.) cv. 'Arka manik', 'Sugar baby', 'Arka muthu', 'IIHR-14'	Embryonic axis attached with single cotyledon	Bath sonication for 1–5 min with VI for 0–5 min at 100 mm of Hg (frequency and other sound-related details not specified)	Optimal durations were 3 min for sonication and 2 min for VI, which enhanced the transformation efficiency to 17.33%, compared to 8.00% in the untreated case.	[125]
Alkaligrass (*Puccinellia tenuiflora* Griesb.) Scribn. And Merr.	8-week-old embryogenic calluses	Explants were incubated with *Agrobacterium* suspension (0.3–0.4 OD_{600}) (1) for 30 min; (2) for 10 min, then under ultrasonication for 10 min, and after that, another incubation for 10 min; (3) under vacuum for 10 min, and next, under ultrasonication for 10 min, following vacuum incubation (frequency and other sound-related details not specified)	The highest transformation efficiency (18.6%) resulted from vacuum for 10 min, ultrasonication for 10 min, and then vacuum for 10 min, compared to about 6.7% for simple 30 min of soaking.	[126]
Cotton (*Gossypium hirsutum* L.) cv. 'SVPR-2'	Seeds	Ultrasonication (Model 2510 Branson, Branson Ultrasonics, Kanagawa, Japan) for 20, 40, 60, 80, and 100 s at 60 kHz, combined with VI for 30, 60, 90, 120, and 150 s at 250, 500, and 750 mmHg, using *Agrobacterium* at 0.4, 0.6, 0.8, 1.0, and 1.2 OD_{600}	Optimal parameters of transformation were 60 s of ultrasonication, combined with 90 s of VI at 500 mmHg, using *Agrobacterium* at 0.1–1.0 OD_{600}, which resulted in 28.66% maximum transformation frequency.	[130]
Manchurian ash (*Fraxinus mandshurica* Rupr.)	Hypocotyls	Explants were (1) immersed in *Agrobacterium* suspension for 15 min; and (2) sonicated for 90 s and then vacuum-infiltrated at 0.8 MPa for 10 min, using *Agrobacterium* at 0.6 OD_{600} (frequency and other sound-related details not specified)	Sonication for 90 s and 10 min vacuum treatment resulted in the maximum number of regenerated shoots. The number of adventitious buds per hypocotyl was doubled from 33.2 to 77.7.	[131]
Bilberry (*Vaccinium myrtillus* L.)	Calluses	Sonication (Qsonica Part No. Q700, Shanghai, China) for 2 s at 20 kHz, combined with VI for 10 min	Sonication with VI significantly increased the transformation efficiency (up to 4.45% from 0.8%).	[127]

Table 4. *Cont.*

Plant (Species, Cultivar)	Treated Explants	Method of Ultrasonic Treatment	Main Impacts of Ultrasonication	Ref.
Soybean (*Glycine max* L.) Merrill cv. 'JS335'	The apical meristem of modified half-seed	Sonication for 0, 1, 10, 20, and 30 min with VI for 0, 1, 10, 20, and 30 min, using *Agrobacterium* at 1.0 OD_{600} (frequency and other sound-related details not specified)	Sonication for 10 min, combined with 10 min VI, resulted in the highest transformation efficiency of 38.0%, compared to the control of 6.6%.	[128]
Pea (*Pisum sativum* L.) cv. 'Ageta 6'	Cotyledonary nodes	Sonication (Branson, MO, USA) for 10–50 s at 40 kHz with VI for 1–6 min at 750 mm Hg, using *Agrobacterium*	The highest transformation efficiency was 12.3%, resulting from 30 s sonication combined with 3 min VI, compared to 6.3% yielded by the control.	[129]

Abbreviation: GUS: β-glucuronidase; VI: vacuum infiltration.

In some cases, the frequency and other details related to ultrasonication were not specified in the articles reviewed. This is indicated in the cases affected in Tables 1, 3 and 4.

6.3. Advances and Perspectives on Ultrasound Applications in Agricultural and Horticultural Technologies

Current application possibilities of plant ultrasonication in various agricultural and horticultural practices in fields or in greenhouses are based on mechano-priming, and include amending growth and development, improving yield and crop quality, and increasing stress defense and tolerance (Tables 1 and 2). The mechano-priming effect of ultrasound is based on the fact that it also acts as an abiotic stressor for plants [59] and induces stress-defense responses [41,42]. Ultrasonication affects plant gene transcription and DNA-methylation, as well, and its impact is memorized epigenetically in plants for at least a short period of time [58,60,82]. In addition, acoustic stimuli can prime plants for other abiotic and biotic stresses [46].

Priming effects of ultrasound have been widely utilized for seeds to increase germination rate, to enhance the germination capacity of aged seeds, and to intensify the early seedling growth and development (Table 2). The number of pores in the seed coat increased in response to seed ultrasonication, which allowed them for enhanced water uptake and higher germination percentage [75,76]. Furthermore, ultrasonic treatment of winter wheat seeds caused changes in DNA methylation and gene transcription in young seedlings, which enabled their increased growth [82]. Based on the latter study, the plants were able to epigenetically memorize, for at least a short period of time, the ultrasound exposure.

However, beyond seed treatment, ultrasound has much wider application possibilities in agriculture and horticulture. A roadmap for its commercial utilization is summarized in Figure 2. Considering that plants respond to various stresses by emitting specific ultrasound signals and patterns [6,7], their monitoring allows us a real-time assessment of the current physiological state of the plant, such as nutrient or water deficiency, or whether the plant is suffering from any other biotic or abiotic stress [9,39,97]. State-of-the-art technologies, like machine learning (ML) and artificial intelligence (AI), can be effectively used to process and interpret data on emitted ultrasound [7,9]. This enables immediate intervention and targeted stress mitigation and management. Furthermore, deciphering the information-carrying nature of ultrasound emitted and perceived by plants provides an opportunity to formulate predictions for plants about future challenges. This may include developing specific ultrasound treatments to improve plant health, enhance or modify plant growth and development, or alerting the plant to potential danger by using a specific ultrasound pattern [6,7,13]. Targeted treatment using ultrasound is an eco-friendly tool for mechano-priming in sustainable agriculture [13,78]. In addition, training a plant with various types of ultrasonic waves may enable the development of stress or multi-stress resilience in various crops.

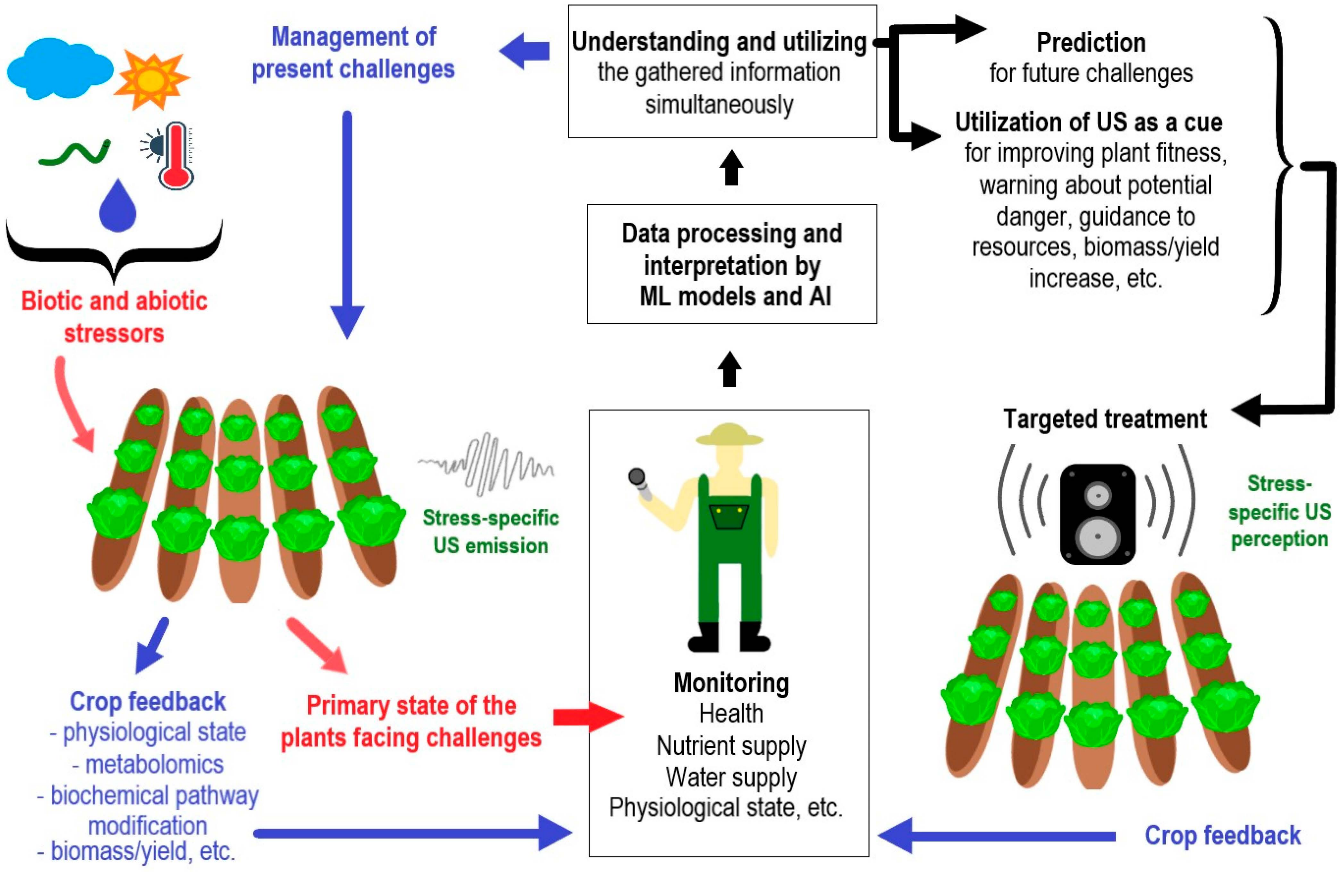

Figure 2. Possible perspectives on applying ultrasound in sustainable agri-and horticulture. Ultrasound emitted by plants can be applied for monitoring the physiological and health state of plants (red arrows). Data processing and interpretation can be done using machine learning (ML) models and artificial intelligence (AI). As a result of data processing, the gathered information and continuous crop feedback can be used for managing present challenges (blue arrows), and simultaneously, for the targeted ultrasound treatment of plants (black arrows).

7. Concluding Remarks

As we realized that ultrasound act on plants, including their growth and development, transcriptomics, and even epigenetics, and that plants themselves can emit ultrasound, the question arises, logically, if emitted ultrasound can be a way of communication between plants or between the plant and its environment (including animals and microbes) [11–13]. Can the plant use ultrasound to warn of environmental stress events or to signal environmental resources? In addition, whether and how can ultrasound emitted by plants affect life forms in their environment, or at least in their nearby environment? In the absence of enough direct evidence currently available, the questions cannot be answered clearly with either yes or no. However, taking into account nature's cost–benefit principle and based on the latest studies [6,7,13], with the stress-specificity of the emitted ultrasound, it is theoretically and potentially possible [6,7,13]. Thereby, the acoustic emission is not merely, as said by Gagliano et al. [92], "an incidental mechanical by-product attributable to cavitation alone" but may have adaptive value. Undoubtedly, however, in practice, more and detailed direct experimental evidence is still necessary to understand its role in environmental adaptation and biocommunication.

What seems to be clear, especially based on the experimental evidence from the last decade reviewed in this article, ultrasonication is able to modify or enhance plant growth and development in several ways (Tables 1 and 2). Thereby, ultrasound is well suited for increasing the efficiency in certain in vitro laboratory methods, e.g., gene transformation mediated by *Agrobacterium* (Tables 3 and 4), or for incorporating it into certain greenhouse or green in-field agricultural technologies (Tables 1 and 2; Figure 2). Moreover, in the future, by measuring the sound or ultrasound emitted by plants, rapid and reliable monitoring or diagnostic systems can be developed in horti- and agriculture (Figure 2). With these, it will be possible to measure and track the current physiological or health status and nutrient or water supply in plants [7,97].

We consider it important to mention the biggest limitation of the studies on ultrasonication of plants. This includes the often incomplete data on the properties of the ultrasound used for treating plants or plant parts, e.g., the volume (dB), the power (W), or the frequency (kHz) of the ultrasound (see the notes in Tables 1 and 2).

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