



[rexresearch.com](http://rexresearch.com)

## Mi-Jeong JEONG, et al. Frequency Control of Plant Growth

Related – [STERNHEIMER, Joel : Gene Music & Plant Growth](#)

[https://www.researchgate.net/publication/226416083\\_Plant\\_gene\\_responses\\_to\\_frequency-specific\\_sound\\_signals](https://www.researchgate.net/publication/226416083_Plant_gene_responses_to_frequency-specific_sound_signals)

Molecular Breeding 21(2):217-226

DOI: 10.1007/s11032-007-9122-x

### Plant Gene Response to Frequency-Specific Sound Signals

Mi-Jeong Jeong, et al.

#### Abstract

We identified a set of sound-responsive genes in plants using a sound-treated subtractive library and demonstrated sound regulation through mRNA expression analyses. Under both light and dark conditions, sound up-regulated expression of *rbcS* and *ald*. These are also light-responsive genes and these results suggest that sound could represent an alternative to light as a gene regulator. *Ald* mRNA expression increased significantly with treatment at 125 and 250Hz, whereas levels decreased significantly with treatment at 50Hz, indicating a frequency-specific response. To investigate whether the *ald* promoter responds to sound, we generated transgenic rice plants harboring a chimeric gene comprising a fusion of the *ald* promoter and GUS reporter. In three independent transgenic lines treated with 50 or 250Hz for 4h, GUS mRNA expression was up-regulated at 250Hz, but down-regulated at 50Hz. Thus, the sound-responsive mRNA expression pattern observed for the *ald* promoter correlated closely with that of *ald*, suggesting that the 1,506bp *ald* promoter is sound-responsive. Therefore, we propose that in transgenic plants, specific frequencies of sound treatment could be used to regulate the expression of any gene fused to the *ald* promoter.

<http://www.pakbs.org/pjbot/PDFs/46%286%29/11.pdf>

Pak. J. Bot., 46(6): 2015-2020, 2014.

### SOUND FREQUENCIES INDUCE DROUGHT TOLERANCE IN RICE PLANT

MI-JEONG JEONG

[ PDF ]

0.8 kHz enhanced relative water content, stomatal conductance and quantum yield...

KR100795421

### METHOD FOR CONTROL OF GRAY MOLD CAUSED BY BOTRYTIS CINEREA USING A SINGLE FREQUENCY SOUND

Inventor(s): JEONG MI JEONG [KR]; PARK SOO CHUL [KR]; SHIM CHANG KI [KR]; LEE JIN OHK

A method for control of gray mold caused by *Botrytis cinerea* is provided to inhibit growth of *Botrytis cinerea* without specific equipments and technique by treating a plant with a single frequency sound, so that the gray mold is suppressed in an environment-friendly manner. The gray mold caused by *Botrytis cinerea* is controlled by treating a subject plant with a single frequency sound selected from 250-500 Hz generated by a sound generator, wherein the plant is vegetable including tomato, cucumber, strawberry, lettuce, eggplant and red pepper, or flower including lily, gladiolus, rose and carnation.

Gray mold disease control methods using a single sound wave {Method for control of gray mold caused by *Botrytis cinerea* using a single frequency sound}

1 is a photograph showing the survival rate after 24 hours of culture after 1 hour treatment the specific single sound wave then smear the bacteria (*Escherichia coli*) bacteria suspension on solid medium.

2 is then treated with a variety of specific single sound wave two hours of gray mold pathogen (*B. cinerea*), and cultured for 2 days, the treated hyphae a photograph showing a result of comparing the colony growth rate and sporulation of the fungus.

3 is a photograph showing the comparison of the microflora growth and sporulation rate 2 hours after a single specific sound wave 250 Hz, and culturing the mycelia treated for 2 days.

Figure 4 is a picture 2 hours a single 500 Hz and a specific sound waves, showing a comparison of the microflora growth and sporulation ratio and incubated for 2 days, the treated hyphae.

5 is then treated with a single 500 Hz sound wave specified by time and incubated for 5 days the treated hyphae a photograph showing a result of comparing the colony growth rate and sporulation.

Figure 6 is a photograph observed after inoculating gray mold pathogen on Potato agar solid medium after incubation for 1 hour to process a single 500 Hz sound wave particular in the form of 5 days mycelial enlarged at a rate of 400 times optical microscope.

Figure 7 is a photograph observed by expanding the shape of the terminal part of the after culturing gray mold pathogen 1 hour to 500 Hz single specific

sound wave after inoculated on the potato agar solid medium for 5 days after the mycelium in the ratio of the optical microscope 400 times.

Incubation for 8 is then incubated for the treatment time for a single specific sound wave 500 Hz 5 days after inoculation on the new potato agar solid medium stripped off the microflora from the tip of the mycelial three days compared to the colony growth and sporulation rate a photograph of the results.

9 is gray mold spore suspension (1X10<sup>6</sup> spores / mL) to 500 Hz single specific sound wave on the leaves of the inoculated on the leaf of cucumber pathogens treated for 2 hours and then sound the onset area after incubation for 2 days no treatment the picture shown by comparison with the group.

Figure 10 is a photograph showing the area after the onset of incubation the gray mold spore suspension (1X10<sup>6</sup> spores / mL) to 500 Hz sound wave yi single specific inoculated leaves of cucumber pathogens other each time the treatment after two days.

Figure 11 is a photograph showing a detail of the blade in FIG. 10.

12 is for gray mold pathogen spore suspension (1X10<sup>6</sup> spores / mL) to in an easy condition to process by varying the single specific sound wave 500 Hz and then inoculated on the leaf of cucumber, each processing time and induce the onset of 4 il seupsil treatment after showing the result of the appearance of the disease untreated and treated leaf waves.

Figure 13 is a photograph showing a detail of the leaf in Fig.

The present invention relates to a control method of gray mold, more particularly, the growth of the control method and the gray mold pathogen of gray mold comprising the step of treating the single sound wave selected from a frequency of 500 Hz 250 Hz to the target crop It relates to a suppression method. Gray mold pathogen (*Botrytis cinerea*) is strawberries, lettuce, cucumber, eggplant, etc. vegetable crops from Liliium, Gladiolus, such as host range to fungal infections that can cause gray mold on many crops ranging from flowering crops is very large, the conidia and by scattering it can be continued spread, it is the damage on the harvesting of crops by fungal pathogens that cause the bottle in a bar without plant pathogens overwinter in the form of host crops are grown year-round as today. In the case of plant pathogens, to play a big role in the invasion of toxins and enzymes are plants that pathogens are produced, intrusion mechanism of gray mold germs that decompose the cuticle layer of the first gateway to pathogen invasion with such black byeolmunui bottle of cucumber better cuticle enzyme to generate the decomposition by the cuticles are known to invade the plant. And the gray mold disease is endemic ripe fruit or fruit faucets, leaves, etc. petiole, because so rot turned the fruit into dark brown also are falling cause commodity as value and becomes diseased fruits of the harvest is discarded as a gray mold in a commercial farm due to yield losses it should also pass 50% of the yield. Also, because they cause damage during transport during or sell their damages are thought to be much more than the estimate. Therefore, many pesticides for controlling the gray mold are now being marketed. However, the pesticide in the greenhouse during the winter, rather than the infection can result in more in some cases when a certain period of time to promote gwaseup the greenhouse environment, leading to many problems such as environmental pollution. In addition, hot and humid as described above and the amount of light is insufficient House My condition is grayish fungal pathogen and the glass on the growth of such pathogenic bacteria, cucumbers and strawberries, etc. are mainly difficult pesticide in sensitive consumers' response to drug control because the germ the situation then developed the environmentally friendly control methods are required in addition to drug control.

Wateuna There is also an attempt to control using antagonistic microbes as one of the environmentally friendly control methods, a step that requires a lot of effort to put into practice. On the other hand, promote the growth and vitality of the plants, using sound waves and party research has been attempted since 1860 claimed the theory of evolution, Charles Darwin, studies on how to use sound waves to suppress the pest occurs in plant also tried recently to being. And applying the sound wave to the known techniques in plants is a method for promoting the growth of plants by applying a sound wave during the processing of plants in Republic of Korea Patent Publication No. 89-109 in gibberellin solution is disclosed, the Republic of Korea Patent No. 131 133 discloses a frequency Growth promotion of plant by way of music is disclosed below 2000Hz. In addition, a method of Republic of Korea Patent No. 0,325,311 discloses a story-telling sound waves of a frequency of 2 to 20 kHz eumyeokdae the target crop pests suppress generation is disclosed. Method of inhibiting pest generated by using the sound waves as disclosed in the Republic of Korea Patent No. 0,325,311 but is compared with a method using a medicament has the advantage not to induce problems such as environmental pollution and water pollution, destruction of the natural ecology, such as , because the situation is very wide eumyeokdae of the cycle once heard a sonic pest as well as ikchung, and can cause damage to various biological systems in nature scattered around can not be an effective pest control method is difficult to put into practice. How also using experiments wateuna been done a long time effective eumyeokdae and processing time and proper conditions are difficult with the waves to investigate the same for controlling plant pathogens using sound waves to suppress certain microorganisms specifically has not been tried have. Just report parsley and cucumber by targeting such results were obtained are treated with sound waves resulting growth promotion has already known. The present inventors are the treatment of using a sound wave studying the method for inhibiting the growth of certain microorganisms, in particular gray mold pathogen (*Botrytis. Cinerea*), a single sound wave of a particular frequency on crop growth of a specific bacteria is inhibited to identify the occurrence of gray mold is suppressed and completed the present invention.

The object of the present invention is to provide a method of controlling gray mold disease and Growth Suppression of gray mold pathogen (*Botrytis. Cinerea*) comprising the step of treating the particular single sound wave to the target crop.

In order to achieve the above object, the present invention provides a method of inhibiting mycelial growth and spore gray mold pathogen (*Botrytiscinerea*) comprising the step of treating the particular single sound wave. Further to another aspect of the invention, the invention provides a method of controlling gray mold comprising the step of treating the particular single sound wave to the target crop. Hereinafter will be described in more detail the contents of the present invention. The particular single sound wave in the present invention are selected from a single sound wave frequencies 250 to 500 Hz. The single sound wave treatment in the present invention utilizes the acoustic wave generating device. The sound wave generating unit include, but are not necessarily limited to, a computer software program called a sound generator (Sound generator) (Korea Advanced Institute of Science and Technology production) means the computer is built, the single sound processing steps are specifically sound wave generating unit and the growth phase by connecting the speaker installed therein refers to processing the sound waves in single hyphae of fungi or crops. The computer software program of the program is designed to have separated from the granular eumyeokdae unit can process by selecting one of the desired sound waves eumyeokdae. The noise itself extremely off growth phase in order to remove the influence of other sound wave of the sound wave other than those to be processed while the sound wave is treated specially manufactured was named growth phase noise. The target crops in the present invention are gray mold pathogen (*Botrytis cinerea*) to gray mold refers to all crops that may be infected with a disease specifically strawberries, lettuce, cucumber, eggplant, vegetable crops such as peppers, etc. Sahib, gladiolus, rose by says the same floriculture crops. The present inventors, using sound waves to investigate the condition for inhibiting the growth of a particular microorganism *E. coli* (*Escherichia cloi*) to the frequency 50 Hz to process the result of a specific single sound wave selected from 5 kHz, a different result depending on the kind of the processed sound wave It showed. That could be observed that the number of colonies of bacteria decreases considerably compared to the untreated group in the case of one showed the results to reduce the number of colonies, even when treated with 50 or 500 Hz, to process a single sound wave of 125 Hz and 250 Hz .

(See Fig. 1). Frequency of a single sound wave to inhibit the growth of certain microorganisms from the result learned that there is a suitable range. The present invention in one embodiment, gray mold result the bacteria processes the particular single sound wave selected from 5 kHz to 50 Hz frequency, a frequency 250 Hz and 500 Hz when the processing of a single sound wave Growth and sporulation of mycelial rate on the basis of the results of the to obtain a result that is significantly reduced in comparison with the untreated group. However, 50 Hz, When process 125 Hz or 1 KHz acoustic wave, 250 Hz or 500 Hz delayed by not represent a significantly different than when processing the sound wave is different particular single sound wave affects the growth, depending on the type of the target microorganisms are the It could be seen. And transferred to a single sound wave of a frequency of inhibiting the growth derived from the 500 Hz single sound wave is treated hyphae to investigate the effects on sporulation of the next generation microflora of the gray mold

pathogen (*Botrytis cinerea*) on fresh medium of the secondary the result, sound waves processed in the previous generation culture without acoustic treatment to affect the spore formation of the next generation could be observed that the spore formation markedly inhibited (see Fig. 8). Accordingly, the present invention provides a method for inhibiting the growth of gray mold pathogen to undergo a single sound wave from the sound wave of selected frequency 250 Hz to 500 Hz on the target crop. In another embodiment of the present invention to process a single 500 Hz sound wave of the inoculated spores of the gray mold pathogen cucumber leaf it was investigated whether or not the single sound wave suppressing cucumber gray mold disease. As a result, it was confirmed that the preventive effect of 44% to 85% (see Table 1). Accordingly, the present invention is to process a single sound wave from the sound wave of selected frequency 250 Hz to 500 Hz on the target crops provides a method of controlling gray mold. Via the below, embodiments of the present invention will now be described in further detail. These examples are intended for the scope of the invention is only to explain the invention in more detail but not limited to these examples.

by using the effect sound wave of the sound wave for the *Escherichia coli* number of colonies (colony) of *E. coli* (*Escherichiacloii*) specific single sound wave selected from a frequency 50 Hz to 5 kHz to determine the condition for inhibiting the growth of certain microorganisms It was processed. Sonic treatment was treated in a special custom-made noise-free plant growth sangnae order to accurately measure the particular single sound wave treatment effect. This is a self-generated noise about 35-40dB growth phase in the growth environment is designed to be nearly maintenance noiseless conditions are useful to measure the effectiveness of specific sound waves. The suspension of the bacteria in the noise-free growth phase speaker is installed (1x10<sup>8</sup>cfu / mL) is smeared LB solid medium (Luria Bertani: bacto-tryptone 10 g, bacto-yeast extract 5 g, NaCl 10 g and agar to 15 g / L 950 Place the dissolved in distilled water ml to adjust the pH to 7 the addition of 5 N NaOH in about 200  $\mu$ l, and the so-filled 1 liter distilled water and sterilized culture medium solidified by dispensing a chalet after), a sound generator (sound generator, Korea by using the Advanced Institute of Science and Technology), the frequency (50 Hz, 125 Hz, 250 Hz, 500 Hz, 1 kHz) after processing the single sound wave for 2 hours and then transferred to 24 hours incubation in the growth of 37 ° C the solid medium observe the number of colonies of bacteria compared to the number of colonies on the medium are not processed by the sound waves is shown in Figure 1 the results. 1, the sound wave is obtained for the treatment medium, in particular the frequency 125 and the number of colonies resulting from the culture medium significantly reduced the processing of a single sound wave 250 Hz compared to the control group (Fig. 1). <Example 2> gray mold pathogen bar processing conditions of the sound wave to inhibit the growth in accordance with the effect types of the target microbe in the sound waves on the (*Botrytis cinerea*) is estimated to be different, one to inhibit the growth of gray mold pathogen (*Botrytis cinerea*) to explore the frequency of a sound wave, 50 Hz to 5 kHz selected spore-forming bacteria by handling the change of gray mold in one particular sound waves, observed the morphological changes and changes in the next generation of spore-forming pathogens hyphae.

<1-1> Flora, using the 6 mm bore Cork (Cork bore, diameter 6mm) of gray mold pathogen within the growing noise of the sound effect of the speakers are installed on a gray mold spore forming bacteria potato extract medium (Potato Dextrose Agar, inoculated on PDA), and by using the sound generator (sound generator, Korea Advanced Institute of Science and Technology), the frequency (50 Hz, 125 Hz, 250 Hz, 500 Hz, 1 kHz) processes the single sound wave for 2 hours and then 25 ° C incubated for two days moved on, and then compared the growth of mycelial growth and sporulation rate of the gray mold pathogen. As a result, when processing the sound waves to obtain a result that is significantly reduced in comparison with the untreated group, especially a frequency 250 Hz and 500 Hz sound wave single treatment of growth and sporulation rate of hyphae (Fig. 2). Therefore, the most effective single sound wave to inhibit the growth of gray mold pathogen was found to be of a single sound wave selected from a frequency of the frequency 250 Hz to 500 Hz. In addition, the inhibition of spore formation by the single sound wave repeatability, spore formation is significantly reduced by the same process three times in the same manner as above the 250 Hz and 500 Hz single sound wave in order to check whether a persistent 3 the results and 4 shown, respectively. As shown in Fig. 3 and 4, are compared to the untreated group inhibit both sporulation if the single sound wave is treated it can be seen that the that the repeatability sporulation inhibitory effect of gray mold pathogen by the single sound wave treatment. In order to compare the spore formation inhibition effect of the acoustic treatment time, of observing the microflora of 500 Hz 0.5 times the single sound wave, 1 hour, 2 hours, 4 hours, 8 hours treated gray mold pathogen After incubation for 5 days result, the processing time and was independent of the sporulation effectively suppressed in all of the processing time, the most effective treatment time of which was found to be 2 hours (Fig. 5). In conclusion, it was found that a single treatment of the sound waves to be repeated, continues to inhibit the formation of gray mold spores, germs be an effective method of controlling gray mold.

<1-2> using the effective microflora 6 mm bore Cork (Cork bore, diameter 6mm) of gray mold germ of sound waves on the morphology of the mycelium of gray mold pathogen in potato extract agar medium (Potato Dextrose Agar, PDA) inoculation, and it was observed to expand the culture, and then morphological characteristics of fungal mycelium after 1 hour a single sound wave of 500 Hz moved on the growth of 25 ° C for 5 days with 400-fold magnification under an optical microscope the results 6 and shown in Fig. As it is shown in Figure 6, in the untreated group were able to observe the form of the hyphae typical of the single sound processing microflora was observed in most of the mycelia are dissolved. And 7, the sound waves are processed in a single colony was found that mycelium terminal region is changed to the abnormal form can be observed that the ratio between the diaphragm and the diaphragm or or melting phenomenon. By the result of the above, processing of a single sound wave of the present invention was found to be also able to suppress not the mycelium of gray mold pathogen development properly and ensure that abnormal deformation in the mycelial growth and sporulation of the next generation. <1-3> single sound wave of the next frequency to suppress the generation of the gray mold pathogen growth effect of the sound wave for the formation of spores (nextgeneration) of the gray mold pathogen in order to determine the effect on the sporulation of the next generation, 500 Hz of the sound waves over time (0.5, 1, 2, 4, 8 hours), processed and incubated for 5 days the colony 6 mm derived from the sound wave of the the colony and untreated derived from mycelia treated hyphae corkscrew bores (Cork bore, shown in diameter 6mm) new potato agar extract, respectively inoculated to the medium, and additional incubator of 25 ° C without sonic treatment using (after 3 ilgan cultured in incubator) a sporulation rate in the results are 8 compares each it was. As shown in Figure 8, in the case of a single colony derived from the mycelial sound wave treatment of the present invention it could be seen that the spore formation is significantly suppressed in the next generation. However, the effect was clearly visible by sonic treatment time.

From the above results, the processing of a single sound wave of the present invention could, as well as spore formation of gray mold pathogen suppresses also sporulation of the next generation to prevent secondary infection caused by spores scattering seen that effective control methods of gray mold.

<Example 3> The gray mold control effect processing by the single sound wave of the sound wave 3 weeks to, a spore suspension (1x10<sup>6</sup> spore / mL) of the gray mold pathogen in order to determine whether effective control methods of gray mold of the actual target crops the sound waves of 500 Hz was treated by time (0.5, 1, 2, 4, 8 hours) was inoculated on the leaf of cucumber and smear with a sound generator (sound generator, Korea Advanced Institute of Science and Technology) on the noise-free growth sangnae. And a sound wave over a cucumber treatment temperature is 23-25 ° C, humidity maintained at 70% in humidity in fact induces onset were cultured in a dark state were examined daily disease progress. Pathogens second day gray mold this was confirmed from the occurrence of after inoculation, the area of outbreak was significantly lower in the treated cucumber leaves a single sound wave is compared to the untreated group (Figure 9). The sound waves of 500 Hz over time to after a treatment with (0.5, 1, 2, 4, 8 hours), leading to the onset in the same manner as the leaf of cucumber to 2 days and the untreated group to identify an appropriate sonic treatment time comparison is shown in Fig. 10 and 11 by. The resulting sound wave is treated cucumber leaves was significantly inhibited the incidence of gray mold, there were no significant processing time in accordance with the sound waves. In addition, observation of the fungus 4 days leaf that after inoculation to ensure that the maintenance, the present invention of a continuous gray mold control effect by a single sound wave treatment, onset could see that a significant progress, the same as the results of 2 days aspects as compared to the untreated group was observed that the onset is significantly inhibited (see Fig. 12 and 13). Lesion with respect to the case of processing the same way as when processing the sound waves of 500 Hz to cucumber leaves by time (0.5, 1, 2, 4, 8 hours), and sound waves of 250 Hz and the on cucumber leaves at the same time as the to obtain the area ratio by calculating a control is obtained from the lesion area ratio shown in Table 1 below.

As a result, the commercial processing of a single sound wave was confirmed that the antifungal activity of about 44- 85%. Through this, the frequency 250 Hz to 500 Hz in the processing of a single sound wave selected is was found to be an effective method of controlling gray mold of the actual target crops. \* Lesion area ratio = (area of disease occurrence / total ipmyeonjeok) x100, \* = The control (untreated lesion area-treated lesion area / untreated) X 100

As described above, processing of a single sound wave selected from a frequency of 250 Hz to 500 Hz on the target crop is an effective method of controlling

gray mold and disease, Growth Suppression of gray mold pathogen. In particular the Gray Control method of fungal disease treatment method is very simple and is processed without the need for special equipment or techniques available environmentally friendly control methods, the growth of gray mold pathogen, as well as inhibition

**Related :**

[https://www.researchgate.net/publication/282286677\\_Sound\\_waves\\_delay\\_tomato\\_fruit\\_ripening\\_by\\_negatively\\_regulating\\_ethylene\\_biosynthesis\\_and\\_sig](https://www.researchgate.net/publication/282286677_Sound_waves_delay_tomato_fruit_ripening_by_negatively_regulating_ethylene_biosynthesis_and_sig)  
**Postharvest Biology and Technology** 12/2015; 110:43-50.  
**DOI: 10.1016/j.postharvbio.2015.07.015**

**Sound waves delay tomato fruit ripening by negatively regulating ethylene biosynthesis and signaling genes**

**J.-Y. Kim, et L.**

**ABSTRACT**

Regulation of tomato fruit ripening may help extend fruit shelf life and prevent losses due to spoilage. Here, tomato fruit were investigated whether sound treatment could delay their ripening. Harvested fruit were treated with low-frequency sound waves (1. kHz) for 6. h, and then monitored various characteristics of the fruit over 14-days at 23. ±. 1. °C. Seven days after the treatment, 85% of the treated fruit were green, versus fewer than 50% of the non-treated fruit. Most of the tomato fruit had transitioned to the red ripening stage by 14 days after treatment. Ethylene production and respiration rate were lower in the sound-treated than non-treated tomatoes. Furthermore, changes in surface color and flesh firmness were delayed in the treated fruit. To investigate how sound wave treatment effects on fruit ripening, the expression of ethylene-related genes was analyzed by quantitative real-time RT-PCR analysis. The expression level of several ethylene biosynthetic (ACS2, ACS4, ACO1, E4 and E8) and ripening-regulated (. RIN, TAGL1, HB-1, NOR, CNR) genes was influenced by sound wave treatment. These results indicated that sound wave treatment delays tomato fruit ripening by altering the expression of important genes in the ethylene biosynthesis and ethylene signaling pathways.

<http://www.ThePowerOfSound.com/Gardening>

The following information is in reference to Chapter 17, page 210 of **The Power Of Sound.**

**Symp Vibe**

**Music in the Garden**

“Measuring Effects of Music, Noise, and Healing Energy Using a Seed Germination Bioassay. “ The objective of this 2004 study was to measure biologic effects of music, noise, and healing energy without human preferences or placebo effects using seed germination as an objective biomarker. A series of five experiments were performed utilizing okra and zucchini seeds germinated in acoustically shielded, thermally insulated, dark, humid growth chambers. Conditions compared were an untreated control, musical sound, pink noise, and healing energy. Healing energy was administered for 15-20 minutes every 12 hours with the intention that the treated seeds would germinate faster than the untreated seeds. The objective marker was the number of seeds sprouted out of groups of 25 seeds counted at 12-hour intervals over a 72-hour growing period. Temperature and relative humidity were monitored every 15 minutes inside the seed germination containers. A total of 14 trials were run testing a total of 4600 seeds.

Results: Musical sound had a highly statistically significant effect on the number of seeds sprouted compared to the untreated control over all five experiments for the main condition and over time. This effect was independent of temperature, seed type, position in room, specific petri dish, and person doing the scoring. Musical sound had a significant effect compared to noise and an untreated control as a function of time while there was no significant difference between seeds exposed to noise and an untreated control. Healing energy also had a significant effect compared to an untreated control and over time with a magnitude of effect comparable to that of musical sound. Conclusion: This study suggests that sound vibrations (music and noise) as well as biofields (bioelectromagnetic and healing intention) both directly affect living biologic systems, and that a seed germination bioassay has the sensitivity to enable detection of effects caused by various applied energetic conditions.<sup>67</sup>

International fascination grows about the effect of music and sound on agriculture. To wit, “Music Can Help Plants Grow Faster” appeared in the <sup>[1]</sup>Times of India, August 30, 2007. <sup>[2]</sup>“Vegetable Growing Technique Not Music to Everyone’s Ears” was the title of the China Daily in September 2007. Both articles referred to a Korean research study entitled “Plant Genes Switched on by Sound Waves,” published in the August 2007 edition of New Scientist (London),

“...Mi-Jeong Jeong of the National Institute of Agricultural Biotechnology in Suwon, South Korea, and colleagues claim to have identified two genes in rice that respond to sound waves. The findings follow a host of similar, but unsubstantiated, claims that plants respond to sound. If the researchers are correct, they say their discovery could enable farmers to switch specific crop genes on and off, such as ones for flowering, by blasting sound into the fields.”

**US7600343**

**Method of stimulating plant growth**

Inventor(s): SCHULTHEISS REINER, et al.

The method of stimulating a plant substance is disclosed. The method has the steps of activating a pressure pulse or an acoustic shock wave generator or source to emit pressure pulse or acoustic shock waves; and subjecting the plant substance to the pressure pulse or acoustic shock waves stimulating said plant substance wherein the substance is positioned within a path of the emitted shock waves. In one embodiment the emitted pressure pulse or shock waves are divergent or near planar. In another embodiment the emitted shock waves are convergent having a geometric focal volume of point at a distance of at least X from the source, the method further comprising positioning the substance at a distance less than the distance X from the source. The substance is a plant tissue having cells. The tissue can be a seed, zygotic embryo or somatic embryogenic culture of somatic embryos of plants.; The plant may be a vegetable, tree, shrub or tuber. The tissue may be a part of the root system, a part of the stem system or a part of the leaf system. The method of stimulating includes activating the cells within the treated tissue thereby releasing growth factor proteins or other chemical compositions promoting growth and accelerating germination or plant growth.

**RELATED APPLICATIONS**

[0001] This application is a continuation in part of U.S. patent application Ser. No. 11/122154 filed on May 4, 2005 entitled "Pressure Pulse/Shock Wave Therapy Methods and an Apparatus for Conducting the Therapeutic Methods" and U.S. patent application Ser. No. 11/071152 filed on Mar. 4, 2005 entitled "Pressure Pulse/Shock Wave Apparatus for Generating Waves Having Nearly Plane or Divergent Characteristics" and also claims benefit of priority to U.S. Provisional Patent Application Ser. No. 60/701,277 filed Jul. 21, 2005, U.S. Provisional Patent Application Ser. No. 60/621,028 filed Oct. 22, 2004 and of U.S. Provisional Patent Application Ser. No. 60/642,149 filed Jan. 10, 2005, the disclosures of which are incorporated herein by reference in their entirety.

## FIELD OF THE INVENTION

[0002] The present invention relates to a method for stimulating plant growth generally and more particularly to a method to accelerate embryonic stimulation and germination of seeds or somatic embryogenesis formation of plant clones.

## BACKGROUND OF THE INVENTION

[0003] Plants are cellular tissue laden organisms having a genetic code for each plant within each species and plant variety. Within these genetic codes are variations or alterations which affect growth rates, yield and disease resistance.

[0004] Plants provide a large source of food, clothing, building supplies, paper products and medicines not to mention landscaping and beauty.

[0005] Rainforests contain no less than 60% of all higher plant species known on earth and they provide all that is needed for human survival, including remedies for disease. Their highly complex molecular structures often surpass the imagination of the chemical scientist and cannot easily be reproduced in the laboratory. More than 25% of all prescription drugs in Organisation for Economic Cooperation and Development (OECD) countries (contrasted with 60% in Eastern Europe) prove to consist of unmodified or slightly altered higher plant products. Natural drugs and medicinal plants, along with other non-timber forest products, already yield an important economic value. These few examples should make one realize how much modern drug delivery depends on sustainability and how vulnerable it is to the exhaustion of natural resources.

[0006] Plants are the source of many of our most important pharmaceuticals. Despite this, we know little about optimizing the production of these valuable secondary products in whole plants or cell and tissue cultures. Cultural practices to optimize pharmaceutical production in field or greenhouse grown plants have not been rigorously determined or have been of little benefit in increasing levels of the desired compounds. Considerable effort has been made to generate plant-derived pharmaceuticals economically in plant cell or tissue culture, with relatively few successes. As a result there is an apparent need to naturally stimulate growth and reproduction of these valuable plant species. The secret or key to continuing growth of such genetically complex plants will most likely occur by stimulation of growth factors within the plant's own tissues.

[0007] Commercially plants and plant products generate many hundreds of billions of dollars of commercial activity per year.

[0008] World demand for plant products is increasing very rapidly. The world demand for paper in 1997 was expected to increase by 50% by the year 2010. This places a huge demand on the timber industry which is concurrently seeing a surge in world demand for lumber products.

[0009] Trees like all of our plant products are renewable and in order to keep pace with increasing demands, faster growing and maturing trees are needed to avoid rapid deforestation on a worldwide basis.

[0010] Plants generally are taken somewhat for granted particularly in their role of influencing climate changes. Singularly no other species has a more positive role in affecting the global environment.

[0011] US patent publication 2005/0125161 A1 entitled "Differentially-Expressed Conifer cDNAs, and Their Use In Improving Somatic Embryogenesis" assigned to Institute of Paper Science and Technology provides a useful insight into current trends in coniferous trees and discloses a relational database of cDNA molecules, including those corresponding to Loblolly Pine Major Intrinsic Protein (MIP), which are differentially expressed during plant embryogenesis. The invention further related to the use of DNA arrays for evaluating gene expression in somatic and zygotic embryos. The invention encompassed related nucleic acids, proteins, antigens, and antibodies derived from these cDNAs as well as the use of such molecules for the staging, characterization, and manipulation of plant embryogenesis, in particular conifer embryogenesis. The cDNAs and related nucleic acids, proteins, antigens, and antibodies derived from these cDNAs are useful in the design, selection, and cultivation of improved crops, specifically including coniferous trees, which provide raw materials for paper and wood products.

[0012] Similarly, in US 2003/0074697 A1 entitled "Cotton Plants with Improved Cotton Fiber Characteristics and Methods for Producing Cotton Fibers From These Cotton Plants", the inventors extensively studied the mechanisms of fiber elongation and formation in cotton plants from the viewpoints of molecular biology to improve the characteristics of cotton fibers. As a result, they found that this purpose can be attained by introducing a gene coding for endoxyloglucan transferase, which is deeply associated with the cell elongation and greatly expressed in the cotton fibers and ovule tissues at the cotton fiber elongation stage, or a gene coding for catalase or peroxidase, which is a hydrogen peroxide eliminating enzyme, into cotton plants and over-expressing these genes in the cotton fiber cells.

[0013] The result is a finer cotton fiber with a resultant higher yield. In this patent these benefits are achieved in an early stage by detection of a positive hybridization signal only from cDNA probe prepared from the ovules on the fifth day of flowering.

[0014] In US 2005/0044592 entitled "Plant Growth Modulation" teaches the use of one or more genes, encoding a protein of the elongator complex to modulate plant growth wherein there results an over expression of the DRL-1 gene to stimulate growth of leaves and roots, the subject matter of this publication being incorporated herein by reference in its entirety.

[0015] As in the other patents, stimulation occurs at the embryonic or early stage of plant development while the resultant growth modulation can occur throughout the life of the plant.

[0016] To better understand the fundamental aspects of the present invention the complexities of plants generally should be appreciated. In the background of US 2005/0044592 a summary of plant development is recited which reports findings of a variety of plant scientists which is repeated below.

[0017] Plants develop mainly post-germination from an embryo with a rudimentary body plan. The embryonic apical-basal axis is delineated by apical meristems that determine the future growth direction of the organism. The embryonic radial axis determines the identity and arrangement of tissues in concentric layers. During development pattern formation, growth and differentiation are overlapping rather than consecutive events. These processes are reiterated throughout the life cycle upon formation of every new organ. Axis formation is the basis for pattern formation within the whole plant body, an organ or even a tissue.

[0018] In Arabidopsis, leaves initiate post-germination at specific positions at the periphery of the shoot apical meristem according to a radial pattern imposed by the plant hormone auxin (Reinhardt et al., 2000). The repression of the homeobox gene SHOOT MERISTEMLESS and the activation of the myb gene ASYMMETRIC (AS) are crucial for leaf initiation (Long et al., 1996; Byrne et al., 2000). AS imposes a dorsal-ventral asymmetry upon the radial symmetry of

the leaf primordium (Byrne et al., 2000). Dorsal identity in the leaf blade is promoted by the PHABULOSA and PHAVOLUTA transcription factors (TF) (McConnell et al., 2001) and ventral identity by the YABBY and KANADI TFs (Siegfried et al., 1999; Sawa et al., 1999; Kerstetter et al., 2001). Four tissues are specified along the dorsi-ventral axis: the upper epidermis and palissade parenchyma with dorsal identity, the spongy parenchyma and the lower epidermis with ventral identity.

[0019] In the primary root the radial axis of the radicle (embryonic root) is reinforced by positional information that originates from the top to the bottom, i.e. from mature cells to initial cells (van den Berg et al., 1995) and polar auxin transport (Sabatini et al., 1999). Tissues are arranged in concentric layers: the epidermis, the cortex, the endodermis, the pericycle and the vascular bundle. SCARECROW and SHORT ROOT are important genes for cortex specification (Scherer et al., 1995; Di Laurenzio et al., 1996), TORNAO 1 & 2 are important for epidermis specification (Cnops et al., 2000). Pattern formation in the primary root epidermal cell layer results in root hair cell files alternating with non-hair cell files which are formed at the anticlinal wall of two underlying cortex cells (Dolan et al., 1993, 1994). The gaseous hormone ethylene and auxin positively regulate root hair cell identity (Masucci et al., 1996). TRANSPARENT TESTA GLABRA1 and CAPRICE are positive regulators of root hair cell identity; GLABRA2 is a negative regulator (Di-Cristina et al., 1996; Wada et al., 1997; Walker et al., 1999).

[0020] The shoot apical meristem is essential for the formation of the vegetative plant body. Regulated cell division activity and changes in the orientation of cell plates precede the initiation of leaf primordia. Growth of leaf primordia occurs mainly along the length (proximo-distal axis) and width (centro-lateral axis) direction and is restricted along the thickness (dorsi-ventral axis) direction because of pattern formation in tissue layers. Early growth processes in leaves occur mainly by anticlinal cell divisions leading to the sheet-like structure of the blade. These growth processes are coupled with dorsi-ventral pattern formation (Siegfried et al., 1999; McConnell et al., 2001; Eshed et al., 2001). Late growth occurs by cell expansion processes (Tsuge et al., 1996; Kim et al., 1998). Pattern formation in lateral growth results in the distinction between lamina and petiole (van der Graaff et al., 2000). Restriction of growth determines the final shape and size of the leaf organ. At flower induction, the SAM changes identity to an inflorescence meristem of which the structure and activity resembles that of the SAM except it produces floral meristems as lateral organs instead of leaf primordia. The onset of cell division in plants and animals is controlled at the G1/S transition of the cell cycle by the retinoblastoma protein that in a hypo-phosphorylated state binds and inactivates the general transcription factors E2F. Upon a mitogenic signal, such as sucrose or cytokinin activated cyclin D/CDK complexes hyper-phosphorylate retinoblastoma and derepress E2F. By preventing cell cycle entry into S-phase, retinoblastoma plays a role in cell differentiation as well (de Jager and Murray, 1999). The cross-talk between cell cycle progression and developmental programs is a new and exciting area of research and the first reports have been published (Gaudin et al., 2000; De Veylder et al., 2001). Regulation of gene expression at the transcriptional level is an important and universal mechanism of controlling developmental programs. Classes of specific TFs recognize upstream promoter boxes in specific sets of genes. Through direct or indirect interaction with the general TFs the RNA polymerase II (RNAPII) transcription initiation complex is either activated or repressed. The specific TFs are activated by environmental or developmental stimuli that are transduced from the cell plasma membrane into the nucleus. Evidence in yeast and humans is accumulating that the control of expression of sets of genes is also mediated by the process of transcription elongation. The RNAPII transcription elongation complex forms the unfolded structure of transcribing nucleosomes (Walia et al., 1998). The elongation reaction is stimulated by a large variety of factors of which some prevent pausing or stalling of the RNAPII complex and others model the chromatin for transcription. The degree of chromatin condensation is modulated by histone acetyltransferases and deacetylases (Walia et al., 1998; Wittschieben et al., 1999). Elongating RNAPII holoenzyme co-purified with a multisubunit complex, Elongator, whose stable interaction is dependent on the hyperphosphorylated state of the RNAPII carboxy-terminal domain (Otero et al., 1999). The elongator complex consists of two subcomplexes: one consists of ELP1 (Otero et al., 1999), ELP2, a WD40 repeat protein (Fellows et al., 2000) and ELP3, a histone acetyltransferase (Wittschieben et al., 1999), the other one of ELP4, ELP5, and ELP6 (Krogan and Greenblatt, 2001; Winkler et al., 2001). Most components of Elongator are well conserved from yeast to man (Hawkes et al., 2001). Phenotypes of *elpA* mutants in yeast were slow growth adaptation, slow gene activation and temperature sensitivity and demonstrated that the ELP genes play a role in the activation of inducible genes in the adaptation to new growth conditions (Wittschieben et al., 1999; Otero et al., 1999; Fellows et al., 2000; Krogan and Greenblatt, 2001; Winkler et al., 2001). Mutations in man in one of the Elongator components cause familial dysautonomia, a well-known disorder (Hawkes et al., 2001). We identified the DEFORMED ROOT AND LEAF1 (DRL1) gene, a homolog of the yeast TOT4/KT112 gene (Butler et al., 1994; Frohloff et al., 2001). TOT genes were identified in search of mutants resistant to the *Kluyveromyces lactis* toxin zymocin. TOT1, TOT2, and TOT3 are isoallelic to ELP1, ELP2 and ELP3 and hence TOT equals elongator. TOT4/KT112 encodes a protein that associated with the elongator complex (Frohloff et al., 2001). The *tot4* mutant displays similar phenotypes as deficient elongator mutants, in addition to slow growth, G1 cell cycle delay and hypersensitivity to Calcofluor White and caffeine. The inventors in US 2005/0044592 demonstrated that in higher plants DRL1 is important for pattern formation and growth processes.

[0021] The above related findings demonstrate that plants undergo a systemic response via a form of cross talk or cellular communication. This finding is consistent with a similar cellular communication found in mammals. In each organism be it a plant or mammal, cellular stimulation can result in a release of proteins and other chemical compositions relating to growth factors.

[0022] In attempts to activate such growth stimulations U.S. Pat. No. 5,819,467 entitled "Method of Stimulating Plant Growth" a conductive helical coil was spaced around a stem of a growing plant to stimulate growth by inducing an electromotive force or EMF.

[0023] Similarly in Canadian patent application CA 2 375 695 entitled "An Invention to Enhance Plant Growth and Germination" proposed growth and germination of some species of plants may be enhanced by exposure to a static magnetic field wherein permanent magnets were placed in a bank or array near the plants.

[0024] The present invention also has the object of stimulating plant growth and accelerating seed germination which is summarized as follows.

## SUMMARY OF THE INVENTION

[0025] The method of stimulating a plant substance is disclosed. The method has the steps of activating a pressure pulse or an acoustic shock wave generator or source to emit pressure pulse or acoustic shock waves; and subjecting the plant substance to the pressure pulse or acoustic shock waves stimulating said plant substance wherein the substance is positioned within a path of the emitted shock waves. In one embodiment the emitted pressure pulse or shock waves are divergent or near planar. In another embodiment the emitted shock waves are convergent having a geometric focal volume of point at a distance of at least X from the source, the method further comprising positioning the substance at a distance less than the distance X from the source. The substance is a plant tissue having cells. The tissue can be a seed, zygotic embryo or somatic embryogenic culture of somatic embryos of plants. The plant may be a vegetable, tree, shrub or tuber. The tissue may be a part of the root system, a part of the stem system or a part of the leaf system. The method of stimulating includes activating the cells within the treated tissue thereby releasing growth factor proteins or other chemical compositions promoting growth and accelerating germination or plant growth. Definitions

[0026] "Altered expression of a gene" means that in the genetically modified plant an amount of messenger RNA is produced that is significantly different from an untransformed control plant, grown under the same conditions.

[0027] "Coding sequence" is a nucleotide sequence, which is transcribed into mRNA and/or translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to mRNA, cDNA, recombinant nucleotide sequences or genomic DNA, while introns may be present as well under certain circumstances.

[0028] A "curved emitter" is an emitter having a curved reflecting (or focusing) or emitting surface and includes, but is not limited to, emitters having

ellipsoidal, parabolic, quasi parabolic (general paraboloid) or spherical reflector/reflecting or emitting elements. Curved emitters having a curved reflecting or focusing element generally produce waves having focused wave fronts, while curved emitters having a curved emitting surfaces generally produce wave having divergent wave fronts.

[0029] "Divergent waves" in the context of the present invention are all waves which are not focused and are not plane or nearly plane. Divergent waves also include waves which only seem to have a focus or source from which the waves are transmitted. The wave fronts of divergent waves have divergent characteristics. Divergent waves can be created in many different ways, for example: A focused wave will become divergent once it has passed through the focal point. Spherical waves are also included in this definition of divergent waves and have wave fronts with divergent characteristics.

[0030] "Embryo" a discrete mass of cells with a well defined structure that is capable of growing into a whole plant.

[0031] "Extracorporeal" occurring or based outside the living body or plant structure.

[0032] "Functional fragment of a gene" refers to a fragment of a gene that can be used in a functional way. Typical functional fragments are the promoter region and the coding sequence. However, the term refers also to parts of the coding sequence that encode for a functional fragment of the protein, i.e. a domain of the protein that is functional on its own.

[0033] "Functional fragment of the protein," as used herein, refers to a fragment of the protein that, on its own or as part of a fusion protein still retains the possibility to modulate plant growth. Typical functional fragments are fragments essential for the protein-protein interaction in the elongator complex. Specifically for DRL1, functional fragments are the conserved domains from AA 56 to 94, from AA 138 to 159 (including a GTPase G4 consensus motif) and from AA 245 to 265, the ATP/GTP binding domain from AA 8 to 15, and the Calmodulin-binding domain, comprising AA 258 to 272, preferentially comprising AA 249 to 276, more preferentially comprising the C-terminal 100 AA. A preferred embodiment is a functional fragment comprising SEQ ID NO:16, preferably consisting essentially of SEQ ID NO:16, even more preferably consisting of SEQ ID NO:16.

[0034] "Gene," as used herein, refers both to the promoter region of the gene as well as the coding sequence. It refers both to the genomic sequence (including possible introns) as well as to the cDNA derived from the spliced messenger operably linked to a promoter sequence.

[0035] A "generalized paraboloid" according to the present invention is also a three-dimensional bowl. In two dimensions (in Cartesian coordinates, x and y) the formula  $y < n > = 2px$  [with n being  $< > 2$ , but being greater than about 1.2 and smaller than 2, or greater than 2 but smaller than about 2.8]. In a generalized paraboloid, the characteristics of the wave fronts created by electrodes located within the generalized paraboloid may be corrected by the selection of  $(p(-z, +z))$ , with z being a measure for the burn down of an electrode, and n, so that phenomena including, but not limited to, burn down of the tip of an electrode  $(-z, +z)$  and/or disturbances caused by diffraction at the aperture of the paraboloid are compensated for.

[0036] "Expression of a gene," as used herein, refers to the transcription of the gene into messenger RNA.

[0037] "Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A promoter sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the promoter sequence.

[0038] "Overexpression of a gene" means that more messenger RNA is produced in the genetically modified plant than in an untransformed control plant, grown under the same condition.

[0039] "Ovule" The body which, after fertilization, becomes the seed.

[0040] A "paraboloid" according to the present invention is a three-dimensional reflecting bowl. In two dimensions (in Cartesian coordinates, x and y) the formula  $y < 2 > = 2px$ , wherein p/2 is the distance of the focal point of the paraboloid from its apex, defines the paraboloid. Rotation of the two-dimensional figure defined by this formula around its longitudinal axis generates a defacto paraboloid.

[0041] "Plane waves" are sometimes also called flat or even waves. Their wave fronts have plane characteristics (also called even or parallel characteristics). The amplitude in a wave front is constant and the "curvature" is flat (that is why these waves are sometimes called flat waves). Plane waves do not have a focus to which their fronts move (focused) or from which the fronts are emitted (divergent). "Nearly plane waves" also do not have a focus to which their fronts move (focused) or from which the fronts are emitted (divergent). The amplitude of their wave fronts (having "nearly plane" characteristics) is approximating the constancy of plain waves. "Nearly plane" waves can be emitted by generators having pressure pulse/shock wave generating elements with flat emitters or curved emitters. Curved emitters may comprise a generalized paraboloid that allows waves having nearly plane characteristics to be emitted.

[0042] A "pressure pulse" according to the present invention is an acoustic pulse which includes several cycles of positive and negative pressure. The amplitude of the positive part of such a cycle should be above about 0.1 MPa and its time duration is from below a microsecond to about a second. Rise times of the positive part of the first pressure cycle may be in the range of nano-seconds (ns) up to some milli-seconds (ms). Very fast pressure pulses are called shock waves. Shock waves used in medical applications do have amplitudes above 0.1 MPa and rise times of the amplitude are below 100 ns. The duration of a shock wave is typically below 1-3 micro-seconds ( $[mu]s$ ) for the positive part of a cycle and typically above some micro-seconds for the negative part of a cycle.

[0043] "Promoter of a gene" as used herein, refers to a functional DNA sequence unit that, when operably linked to a coding sequence and possibly placed in the appropriate inducing conditions, is sufficient to promote transcription of the coding sequence.

[0044] "A protein of the elongator complex," as used herein, means that the protein belongs to the multisubunit complex Elongator, as known to the person skilled in the art or to a protein associating with the complex. Preferentially, the protein has structural and/or functional homology with one of the proteins ELP1, ELP2, ELP3, ELP4, ELP5, ELP6 or TOT4/KT112 as described in *Saccharomyces cerevisiae*.

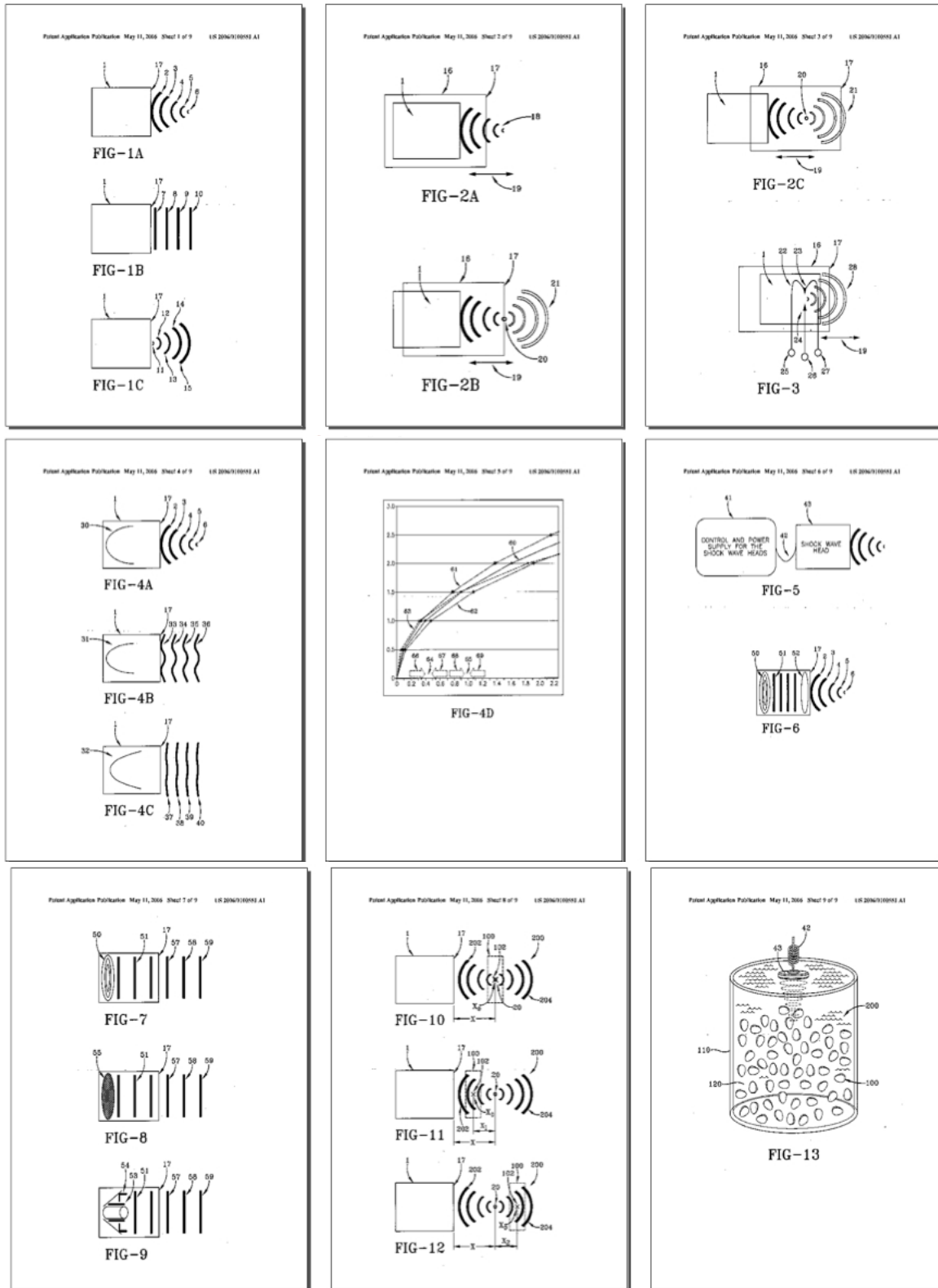
[0045] "seed" The ripened ovule, consisting of the embryo and its proper coats.

[0046] "Somatic Embryogenesis" a type of plant tissue culture where a piece of a donor plant is excised, cultured and induced to form multiple embryos.

[0047] Waves/wave fronts described as being "focused" or "having focusing characteristics" means in the context of the present invention that the respective waves or wave fronts are traveling and increase their amplitude in direction of the focal point. Per definition the energy of the wave will be at a maximum in the focal point or, if there is a focal shift in this point, the energy is at a maximum near the geometrical focal point. Both the maximum energy and the maximal pressure amplitude may be used to define the focal point.

[0048] "Zygotic Embryogenesis" is a sexual or asexual reproductive process that forms new plants. The process of embryogenesis may occur naturally in the plant as a result of sexual fertilization or asexual processes, these embryos are called zygotic embryos and develop into seeds, which germinate giving rise to seedlings.

BRIEF DESCRIPTION OF THE DRAWINGS



[0049] The invention will be described by way of example and with reference to the accompanying drawings in which:

[0050] FIG. 1a is a simplified depiction of a pressure pulse/shock wave (PP/SW) generator with focusing wave characteristics.

[0051] FIG. 1b is a simplified depiction of a pressure pulse/shock wave generator with plane wave characteristics.

[0052] FIG. 1c is a simplified depiction of a pressure pulse/shock wave generator with divergent wave characteristics.

[0053] FIG. 2a is a simplified depiction of a pressure pulse/shock wave generator having an adjustable exit window along the pressure wave path. The exit window is shown in a focusing position.



[0054] FIG. 2b is a simplified depiction of a pressure pulse/shock wave generator having an exit window along the pressure wave path. The exit window as shown is positioned at the highest energy divergent position.

[0055] FIG. 2c is a simplified depiction of a pressure pulse/shock wave generator having an exit window along the pressure wave path. The exit window is shown at a low energy divergent position.

[0056] FIG. 3 is a simplified depiction of an electro-hydraulic pressure pulse/shock wave generator having no reflector or focusing element. Thus, the waves of the generator did not pass through a focusing element prior to exiting it.

[0057] FIG. 4a is a simplified depiction of a pressure pulse/shock wave generator having a focusing element in the form of an ellipsoid. The waves generated are focused.

[0058] FIG. 4b is a simplified depiction of a pressure pulse/shock wave generator having a parabolic reflector element and generating waves that are disturbed plane.

[0059] FIG. 4c is a simplified depiction of a pressure pulse/shock wave generator having a quasi parabolic reflector element (generalized paraboloid) and generating waves that are nearly plane/have nearly plane characteristics.

[0060] FIG. 4d is a simplified depiction of a generalized paraboloid with better focusing characteristic than a paraboloid in which  $n=2$ . The electrode usage is shown. The generalized paraboloid, which is an interpolation (optimization) between two optimized paraboloids for a new electrode and for a used (burned down) electrode is also shown.

[0061] FIG. 5 is a simplified depiction of a pressure pulse/shock wave generator being connected to a control/power supply unit.

[0062] FIG. 6 is a simplified depiction of a pressure pulse/shock wave generator comprising a flat EMSE (electromagnetic shock wave emitter) coil system to generate nearly plane waves as well as an acoustic lens. Convergent wave fronts are leaving the housing via an exit window.

[0063] FIG. 7 is a simplified depiction of a pressure pulse/shock wave generator having a flat EMSE coil system to generate nearly plane waves. The generator has no reflecting or focusing element. As a result, the pressure pulse/shock waves are leaving the housing via the exit window unfocused having nearly plane wave characteristics.

[0064] FIG. 8 is a simplified depiction of a pressure pulse/shock wave generator having a flat piezoceramic plate equipped with a single or numerous individual piezoceramic elements to generate plane waves without a reflecting or focusing element. As a result, the pressure pulse/shock waves are leaving the housing via the exit window unfocused having nearly plane wave characteristics.

[0065] FIG. 9 is a simplified depiction of a pressure pulse/shock wave generator having a cylindrical EMSE system and a triangular shaped reflecting element to generate plane waves. As a result, the pressure pulse/shock waves are leaving the housing via the exit window unfocused having nearly plane wave characteristics.

[0066] FIG. 10 is a simplified depiction of a pressure pulse/shock wave (PP/SW) generator with focusing wave characteristics shown focused with the focal point or geometrical focal volume being on a substance, the focus being targeted on the location X0.

[0067] FIG. 11 is a simplified depiction of a pressure pulse/shock wave (PP/SW) generator with the focusing wave characteristics shown wherein the focus is located a distance X, from the location X0 of a substance wherein the converging waves impinge the substance.

[0068] FIG. 12 is a simplified depiction of a pressure pulse/shock wave (PP/SW) generator with focusing wave characteristics shown wherein the focus is located a distance X2 from the location X0 wherein the emitted divergent waves impinge the substance.

[0069] FIG. 13 shows shock waves being transmitted through a container or vat having a plurality of plant tissues to be treated.

## DETAILED DESCRIPTION OF THE INVENTION

[0070] The present invention relates to the use of various pressure pulse wave patterns or acoustic shock wave patterns as illustrated in FIGS. 1-12 for stimulating plant growth. Each illustrated wave pattern will be discussed later in the description; however, the use of each has particularly interesting beneficial features that are a remarkably valuable new tool in the effort to accelerate plant growth and production.

[0071] The present invention employs the use of pressure pulses or shock waves to stimulate a cellular response stimulating a tissue growth process that activates the tissue to initiate a systemic growth process.

[0072] In the pressure pulse or shock wave method of treating a plant tissue, a zygotic embryo or seed or somatic embryos of the plant or cultures of such embryos are positioned in a convenient orientation to permit the source of the emitted waves to most directly send the waves to the target site to initiate pressure pulse or shock wave stimulation of the target area or zone with minimal, preferably with little or no obstructing features in the path of the emitting source or lens. Assuming the treatment region is accessible through an open access region then the shock wave head 43 can be inserted and placed directly on or adjacent to the treatment region 200. Assuming the target area or site is within a projected area of the wave transmission, a single transmission dosage of wave energy may be used. The transmission dosage can be from a few seconds to 20 minutes or more dependent on the condition. Preferably the waves are generated from an unfocused or focused source. The unfocused waves can be divergent, planar or near planar and having a low pressure amplitude and density in the range of  $0.00001 \text{ mJ/mm}^2$  to  $1.0 \text{ mJ/mm}^2$  or less, most typically below  $0.2 \text{ mJ/mm}^2$ . The focused source preferably can use a diffusing lens or have a far-sight focus to minimize if not eliminate having the localized focus point within the tissue. Preferably the focused shock waves are used at a similarly effective low energy transmission or alternatively can be at higher energy but wherein the tissue target site is disposed pre-convergence inward of the geometric focal point of the emitted wave transmission. In treating some hard to penetrate regions, the pressure pulse more preferably is a high energy target focused wave pattern which can effectively penetrate through outer structures prior to being dampened while still exposing the plant to activating pressure pulses or shock waves. This emitted energy preferably stimulates the plant cells without rupturing cellular membranes. The surrounding healthy cells in the region treated are activated initiating a growth mechanism response stimulating new growth. In the case of embryonic tissues, the cells are activated stimulating accelerated germination when planted in a nutrient rich environment such as soil.

[0073] These shock wave energy transmissions are effective in stimulating a cellular response and can be accomplished without creating the cavitation bubbles in the tissue of the target site when employed in other than site targeted high energy focused transmissions. This effectively insures the tissue or plant does not have to experience the sensation of cellular membrane rupturing so common in the higher energy focused wave forms having a focal point at or within the targeted treatment site.

[0074] This method permits the lens or cover of the emitting shock wave source to impinge on the plant or tissue directly or through a transmission enhancing gel, water or fluid medium during the pressure pulse or shock wave treatment. The treated area can withstand a far greater number of shock waves based on the selected energy level being emitted. For example at very low energy levels the stimulation exposure can be provided over prolonged periods as much as 20 minutes if so desired. At higher energy levels the treatment duration can be shortened to less than a minute, less than a second if so desired. The limiting factor in the selected treatment dosage is avoidance or minimization of surrounding cell rupturing and other kinds of damage to the surrounding cells or tissue while still providing a stimulating cell activation or a cellular release or activation of proteins or functional fragments of the protein or other chemical composition that modulates growth factors.

[0075] The underlying principle of these pressure pulse or shock wave therapy methods is to enrich the treatment area directly and to stimulate the plant's own natural growth capability. This is accomplished by deploying shock waves to stimulate cells in the surrounding tissue to activate a variety of responses. The acoustic shock waves transmit or trigger what appears to be a cellular communication throughout the entire anatomical structure, this activates a generalized cellular response at the treatment site, in particular, but more interestingly a systemic response in areas more removed from the wave form pattern. This is believed to be one of the reasons molecular stimulation can be conducted at threshold energies heretofore believed to be well below those commonly accepted as required. Accordingly not only can the energy intensity be reduced in some cases, but also the number of applied shock wave impulses can be lowered from several thousand to as few as one or more pulses and still yield a beneficial stimulating response. The key is to provide at least a sufficient amount of energy to activate growth reactions. The treatment is particularly beneficial in early stages of plant growth, but also can be employed with appropriate transmission medias to treat infected or damaged mature plants such as infected trees which when subjected to shock waves activates a cellular defense response to an intrusion of for example parasitic diseases.

[0076] Ideally the present invention is best suited for large scale farming and nursery operations where seedlings are harvested in large quantities.

[0077] As shown in FIG. 13 the treated plant tissue can be seeds, zygotic embryos, or somatic embryogenesis cells placed in a nutrient rich environment or culture medium which easily allows the transmitted waves to pass through each seed or cluster of embryogenic cells to trigger the growth protein modulation. Thereafter the treated plant tissues can be planted in soil or nutrient medium to initiate root generation and full germination.

[0078] In practice treated bean seeds were planted along with untreated control seeds. The treated seeds sprouted on average two days before the control seeds. This finding is consistent with the findings of a Canadian patent 2,376,695 which used an array of magnets to produce a magnetic field in proximity to the planted seeds. The distinction and benefit of the present invention is the treatment is applied one time to a mass quantity of seeds prior to planting. The cellular stimulation having been triggered no further stimulation was required. The vegetative foliage of the treated bean plants was superior in growth and appearance as well evidencing a pronounced long-term benefit.

[0079] Additional shelf life testing needs to be conducted to see if the effect of shock waves is transitory. That is how long treated seeds, embryos or seedlings can be held in storage until planting and still see the beneficial accelerated germination and improved quality of plant structure.

[0080] As further shown in FIG. 13 the pressure pulse or shock wave head 43 can be immersed in a nutrient rich fluid medium or culture 120 of zygotic embryos, seeds or somatic embryos or embryonic tissues 100. The treated tissue 100 can be one or more such embryo or seeds 100, preferably many more. As shown a large container or vat 110 is shown holding many thousands of such plant tissues 100. The shock wave head 43 is connected via cabling 42 base to a wave generator or source (not illustrated). After treating the plant tissue or seeds 100 the treated embryonic plants can be potted or planted to initiate the germination process. As can be appreciated such a process is also ideally suited for hydroponic planting processes as well. The treated plant tissues can form trees, bushes, tubers, cotton, or vegetables like soybean, corn, peanuts, beans, melons, citrus fruit trees, avocados or any other plants including grasses. The plants may be of flowering varieties or seed producing varieties such as walnut, pecan and other tree born nut producing plants. The resultant treated plant tissue may be of a plant variety which is used in manufacture of medicines or other pharmaceutical drugs. The treatment may be directed to the root system and stimulation thereof or the leaf system or stem. The treated tissue may be at a graft site or may be plant tissue of one or more zygotic embryos or one or more somatic embryos which is micro-propagated from somatic embryo in vitro from minute pieces of tissue or individual cells such as in cloning.

[0081] Assuming the treated seeds need not be potted or planted immediately then the above method could have an important role in large scale seed production. Otherwise the beneficial attributes may be better suited for nurseries and large scale planting operations wherein improved plant growth rates are financially rewarding.

[0082] Nevertheless the use of such pressure pulses and acoustic shock waves can be very beneficial to plant production in terms of accelerated growth.

[0083] FIG. 1a is a simplified depiction of the a pressure pulse/shock wave (PP/SW) generator, such as a shock wave head, showing focusing characteristics of transmitted acoustic pressure pulses. Numeral 1 indicates the position of a generalized pressure pulse generator, which generates the pressure pulse and, via a focusing element, focuses it outside the housing to treat plants or embryos of plants. The affected plant tissue is generally located in or near the focal point which is located in or near position 6. At position 17 a water cushion or any other kind of exit window for the acoustical energy is located.

[0084] FIG. 1b is a simplified depiction of a pressure pulse/shock wave generator, such as a shock wave head, with plane wave characteristics. Numeral 1 indicates the position of a pressure pulse generator according to the present invention, which generates a pressure pulse which is leaving the housing at the position 17, which may be a water cushion or any other kind of exit window. Somewhat even (also referred to herein as "disturbed") wave characteristics can be generated, in case a paraboloid is used as a reflecting element, with a point source (e.g. electrode) that is located in the focal point of the paraboloid. The waves will be transmitted into the plant tissue via a coupling media such as, e.g., ultrasound gel or oil or a nutrient rich fluid and their amplitudes will be attenuated with increasing distance from the exit window 17.

[0085] FIG. 1c is a simplified depiction of a pressure pulse shock wave generator (shock wave head) with divergent wave characteristics. The divergent wave fronts may be leaving the exit window 17 at point 11 where the amplitude of the wave front is very high. This point 17 could be regarded as the source point for the pressure pulses. In FIG. 1c the pressure pulse source may be a point source, that is, the pressure pulse may be generated by an electrical discharge of an electrode under water between electrode tips. However, the pressure pulse may also be generated, for example, by an explosion, referred to as a ballistic pressure pulse. The divergent characteristics of the wave front may be a consequence of the mechanical setup shown in FIG. 2b.

[0086] FIG. 2a is a simplified depiction of a pressure pulse/shock wave generator (shock wave head) according to the present invention having an adjustable or exchangeable (collectively referred to herein as "movable") housing around the pressure wave path. The apparatus is shown in a focusing position. FIG. 2a is similar to FIG. 1a but depicts an outer housing (16) in which the acoustical pathway (pressure wave path) is located. In a preferred embodiment, this pathway is defined by especially treated water (for example, temperature controlled, conductivity and gas content adjusted water) and is within a water cushion or within a housing having a permeable membrane, which is acoustically favorable for the transmission of the acoustical pulses. In certain embodiments, a complete outer housing (16) around the pressure pulse/shock wave generator (1) may be adjusted by moving this housing (16) in relation to, e.g., the focusing element in the generator. However, as the person skilled in the art will appreciate, this is only one of many embodiments of the present invention. While the figure shows that the exit window (17) may be adjusted by a movement of the complete housing (16) relative to the focusing element, it is clear that a similar, if not the same, effect can be achieved by only moving the exit window, or, in the case of a water cushion, by filling more water in the volume between the focusing element and the cushion. FIG. 2a shows the situation in which the arrangement transmits focused pressure pulses.

[0087] FIG. 2b is a simplified depiction of the pressure pulse/shock wave generator (shock wave head) having an adjustable or exchangeable housing around

the pressure wave path with the exit window 17 being in the highest energy divergent position. The configuration shown in FIG. 2b can, for example, be generated by moving the housing (16) including the exit window (17), or only the exit window (17) of a water cushion, towards the right (as shown in the Figure) to the second focus  $f_2$  (20) of the acoustic waves. In a preferred embodiment, the energy at the exit window will be maximal. Behind the focal point, the waves may be moving with divergent characteristics (21).

[0088] FIG. 2c is a simplified depiction of the pressure pulse/shock wave generator (shock wave head) having an adjustable or exchangeable housing around the pressure wave path in a low energy divergent position. The adjustable housing or water cushion is moved or expanded much beyond  $f_2$  position (20) so that highly divergent wave fronts with low energy density values are leaving the exit window (17) and may be coupled to a plant tissue. Thus, an appropriate adjustment can change the energy density of a wave front without changing its characteristic.

[0089] This apparatus may, in certain embodiments, be adjusted/modified/or the complete shock wave head or part of it may be exchanged so that the desired and/or optimal acoustic profile such as one having wave fronts with focused, planar, nearly plane, convergent or divergent characteristics can be chosen.

[0090] A change of the wave front characteristics may, for example, be achieved by changing the distance of the exit acoustic window relative to the reflector, by changing the reflector geometry, by introducing certain lenses or by removing elements such as lenses that modify the waves produced by a pressure pulse/shock wave generating element. Exemplary pressure pulse/shock wave sources that can, for example, be exchanged for each other to allow an apparatus to generate waves having different wave front characteristics are described in detail below.

[0091] In certain embodiments, the change of the distance of the exit acoustic window can be accomplished by a sliding movement. However, in other embodiments of the present invention, in particular, if mechanical complex arrangements, the movement can be an exchange of mechanical elements.

[0092] In one embodiment, mechanical elements that are exchanged to achieve a change in wave front characteristics include the primary pressure pulse generating element, the focusing element, the reflecting element, the housing and the membrane. In another embodiment, the mechanical elements further include a closed fluid volume within the housing in which the pressure pulse is formed and transmitted through the exit window.

[0093] In one embodiment, the apparatus of the present invention is used in combination therapy. Here, the characteristics of waves emitted by the apparatus are switched from, for example, focused to divergent or from divergent with lower energy density to divergent with higher energy density. Thus, effects of a pressure pulse treatment can be optimized by using waves having different characteristics and/or energy densities, respectively.

[0094] While the above described universal toolbox of the present invention provides versatility, the person skilled in the art will appreciate that apparatuses that only produce waves having, for example, nearly plane characteristics, are less mechanically demanding and fulfill the requirements of many users.

[0095] As the person skilled in the art will also appreciate that embodiments shown in the drawings are independent of the generation principle and thus are valid for not only electro-hydraulic shock wave generation but also for, but not limited to, PP/SW generation based on electromagnetic, piezoceramic and ballistic principles. The pressure pulse generators may, in certain embodiments, be equipped with a water cushion that houses water which defines the path of pressure pulse waves that is, through which those waves are transmitted. In a preferred embodiment, a plant tissue is coupled via a nutrient rich fluid, ultrasound gel or oil to the acoustic exit window (17), which can, for example, be an acoustic transparent membrane, a water cushion, a plastic plate or a metal plate.

[0096] FIG. 3 is a simplified depiction of the pressure pulse/shock wave apparatus having no focusing reflector or other focusing element. The generated waves emanate from the apparatus without coming into contact with any focusing elements. FIG. 3 shows, as an example, an electrode as a pressure pulse generating element producing divergent waves (28) behind the ignition point defined by a spark between the tips of the electrode (23, 24).

[0097] FIG. 4a is a simplified depiction of the pressure pulse/shock wave generator (shock wave head) having as focusing element an ellipsoid (30). Thus, the generated waves are focused at (6).

[0098] FIG. 4b is a simplified depiction of the pressure pulse/shock wave generator (shock wave head) having as a focusing element a paraboloid ( $y^2 = 2px$ ). Thus, the characteristics of the wave fronts generated behind the exit window (33, 34, 35, and 36) are disturbed plane ("parallel"), the disturbance resulting from phenomena ranging from electrode burn down, spark ignition spatial variation to diffraction effects. However, other phenomena might contribute to the disturbance.

[0099] FIG. 4c is a simplified depiction of the pressure pulse/shock wave generator (shock wave head) having as a focusing element a generalized paraboloid ( $y^n = 2px$ , with  $1.2 < n < 2.8$  and  $n > 2$ ). Thus, the characteristics of the wave fronts generated behind the exit window (37, 38, 39, and 40) are, compared to the wave fronts generated by a paraboloid ( $y^2 = 2px$ ), less disturbed, that is, nearly plane (or nearly parallel or nearly even (37, 38, 39, 40)). Thus, conformational adjustments of a regular paraboloid ( $y^2 = 2px$ ) to produce a generalized paraboloid can compensate for disturbances from, e.g., electrode burn down. Thus, in a generalized paraboloid, the characteristics of the wave front may be nearly plane due to its ability to compensate for phenomena including, but not limited to, burn down of the tips of the electrode and/or for disturbances caused by diffraction at the aperture of the paraboloid. For example, in a regular paraboloid ( $y^2 = 2px$ ) with  $p=1.25$ , introduction of a new electrode may result in  $p$  being about 1.05. If an electrode is used that adjusts itself to maintain the distance between the electrode tips ("adjustable electrode") and assuming that the electrodes burn down is 4 mm ( $z=4$  mm),  $p$  will increase to about 1.45. To compensate for this burn down, and here the change of  $p$ , and to generate nearly plane wave fronts over the life span of an electrode, a generalized paraboloid having, for example  $n=1.66$  or  $n=2.5$  may be used. An adjustable electrode is, for example, disclosed in U.S. Pat. No. 6,217,531.

[0100] FIG. 4d shows sectional views of a number of paraboloids. Numeral 62 indicates a paraboloid of the shape  $y^2 = 2px$  with  $p=0.9$  as indicated by numeral 64 at the  $x$  axis which specifies the  $p/2$  value (focal point of the paraboloid). Two electrode tips of a new electrode 66 (inner tip) and 67 (outer tip) are also shown in the Figure. If the electrodes are fired and the tips are burning down the position of the tips change, for example, to position 68 and 69 when using an electrode which adjusts its position to compensate for the tip burn down. In order to generate pressure pulse/shock waves having nearly plane characteristics, the paraboloid has to be corrected in its  $p$  value. The  $p$  value for the burned down electrode is indicated by 65 as  $p/2=1$ . This value, which constitutes a slight exaggeration, was chosen to allow for an easier interpretation of the Figure. The corresponding paraboloid has the shape indicated by 61, which is wider than paraboloid 62 because the value of  $p$  is increased. An average paraboloid is indicated by numeral 60 in which  $p=1.25$  cm. A generalized paraboloid is indicated by dashed line 63 and constitutes a paraboloid having a shape between paraboloids 61 and 62. This particular generalized paraboloid was generated by choosing a value of  $n > 2$  and a  $p$  value of about 1.55 cm. The generalized paraboloid compensates for different  $p$  values that result from the electrode burn down and/or adjustment of the electrode tips.

[0101] FIG. 5 is a simplified depiction of a set-up of the pressure pulse/shock wave generator (43) (shock wave head) and a control and power supply unit (41) for the shock wave head (43) connected via electrical cables (42) which may also include water hoses that can be used in the context of the present invention. However, as the person skilled in the art will appreciate, other set-ups are possible and within the scope of the present invention.

[0102] FIG. 6 is a simplified depiction of the pressure pulse/shock wave generator (shock wave head) having an electromagnetic flat coil 50 as the generating element. Because of the plane surface of the accelerated metal membrane of this pressure pulse/shock wave generating element, it emits nearly plane waves which are indicated by lines 51. In shock wave heads, an acoustic lens 52 is generally used to focus these waves. The shape of the lens might vary according

to the sound velocity of the material it is made of. At the exit window 17 the focused waves emanate from the housing and converge towards focal point 6.

[0103] FIG. 7 is a simplified depiction of the pressure pulse/shock wave generator (shock wave head) having an electromagnetic flat coil 50 as the generating element. Because of the plane surface of the accelerated metal membrane of this generating element, it emits nearly plane waves which are indicated by lines 51. No focusing lens or reflecting lens is used to modify the characteristics of the wave fronts of these waves, thus nearly plane waves having nearly plane characteristics are leaving the housing at exit window 17.

[0104] FIG. 8 is a simplified depiction of the pressure pulse/shock wave generator (shock wave head) having an piezoceramic flat surface with piezo crystals 55 as the generating element. Because of the plane surface of this generating element, it emits nearly plane waves which are indicated by lines 51. No focusing lens or reflecting lens is used to modify the characteristics of the wave fronts of these waves, thus nearly plane waves are leaving the housing at exit window 17. Emitting surfaces having other shapes might be used, in particular curved emitting surfaces such as those shown in FIGS. 4a to 4c as well as spherical surfaces. To generate waves having nearly plane or divergent characteristics, additional reflecting elements or lenses might be used. The crystals might, alternatively, be stimulated via an electronic control circuit at different times, so that waves having plane or divergent wave characteristics can be formed even without additional reflecting elements or lenses.

[0105] FIG. 9 is a simplified depiction of the pressure pulse/shock wave generator (shock wave head) comprising a cylindrical electromagnet as a generating element 53 and a first reflector having a triangular shape to generate nearly plane waves 54 and 51. Other shapes of the reflector or additional lenses might be used to generate divergent waves as well.

[0106] With reference to FIGS. 10, 11 and 12 a schematic view of a shock wave generator or source 1 is shown emitting a shock wave front 200 from an exit window 17. The shock wave front 200 has converging waves 202 extending to a focal point or focal geometric volume 20 at a location spaced a distance X from the generator or source 1. Thereafter the wave front 200 passes from the focal point or geometric volume 20 in a diverging wave pattern as has been discussed in the various other FIGS. 1-9 generally.

[0107] With particular reference to FIG. 10 a plant tissue 100 is shown generally centered on the focal point or volume 20 at a location X0 within the tissue 100. In this orientation the emitted waves are focused and thus are emitting a high intensity acoustic energy at the location X0. This location X0 can be anywhere within or on the tissue 100.

[0108] With reference to FIG. 11, the plant tissue 100 is shifted a distance X toward the generator or source 1. The tissue 100 at location X0 being positioned a distance X-X1 from the source 1. This insures the tissue 100 is impinged by converging waves 202 but removed from the focal point 20. When the tissue 100 is impacted, this bombardment of converging waves 202 stimulates the cells activating the desired response as previously discussed.

[0109] With reference to FIG. 12, the tissue 100 is shown shifted or located in the diverging wave portion 204 of the wave front 200. As shown X0 is now at a distance X2 from the focal point or geometric volume 20 located at a distance X from the source 1. Accordingly X0 is located a distance X+X2 from the source 1. As in FIG. 10 this region of diverging waves 204 can be used to stimulate the tissue 100 which when the tissue is a cellular tissue stimulates the cells to produce the desired growth effect or response.

[0110] As shown in FIGS. 1-12 the use of these various acoustic shock wave forms can be used separately or in combination to achieve the desired effect of stimulating growth.

[0111] Furthermore such acoustic shock wave forms can be used in combination with chemical treatments, gene therapy or cloning or plant grafting or cross pollination methods and when so combined the stimulated cells will more rapidly grow increasing productivity and potentially improving yields.

[0112] The present invention provides an apparatus for an effective treatment of plant tissues, which benefit from high or low energy pressure pulse/shock waves having focused or unfocused, nearly plane, convergent or even divergent characteristics. With an unfocused wave having nearly plane, plane, convergent wave characteristic or even divergent wave characteristics, the energy density of the wave may be or may be adjusted to be so low that side effects including cellular membrane damage do not exist at all.

[0113] In certain embodiments, the apparatus of the present invention is able to produce waves having energy density values that are below 0.1 mJ/mm<sup>2</sup> or even as low as 0.000 001 mJ/mm<sup>2</sup>. In a preferred embodiment, those low end values range between 0.1-0.001 mJ/mm<sup>2</sup>. With these low energy densities, side effects are reduced and the dose application is much more uniform. Additionally, the possibility of harming surface tissue is reduced when using an apparatus of the present invention that generates unfocused waves having planar, nearly plane, convergent or divergent characteristics and larger transmission areas compared to apparatuses using a focused shock wave source that need to be moved around to cover the treated area. The apparatus of the present invention also may allow the user to make more precise energy density adjustments than an apparatus generating only focused shock waves, which is generally limited in terms of lowering the energy output.

[0114] The treatment of the above mentioned plant tissue or body of a plant is believed to be a first time use of acoustic shock wave therapy. None of the work done to date has treated the above mentioned plant treatments with convergent, divergent, planar or near-planar acoustic unfocused shock waves of low energy or high energy focused shock waves in a transmission path from the emitting source lens or cover to the target site.

[0115] It will be appreciated that the apparatuses and processes of the present invention can have a variety of embodiments, only a few of which are disclosed herein. It will be apparent to the artisan that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

[0116] The use of acoustic shock waves to plant tissue stimulates a cellular response of the treated tissues as well as a cellular response in any surrounding tissue. This response activates otherwise dormant cells to increase the plant's growth mechanisms, allowing the cells to rapidly replicate to initiate the growth process.

[0117] A further benefit of the use of acoustic shock waves is there are no known adverse indications when combined with the use of other nutrients. In fact the activation of the cells exposed to shock wave treatments only enhances cellular absorption of such nutrients making them faster acting than when compared to non stimulated cells. As a result, it is envisioned that the use of one or more nutrients prior to, during or after subjecting the plant tissue to acoustic shock waves will be complimentary to the treatment or pre-conditioning treatment. It is further appreciated that certain uses of pesticides can be altered or modified to lower risk or adverse side effects when combined with a treatment involving acoustic shock waves as described above.

[0118] Another aspect of the present invention is the use of acoustic shock waves can be combined with organic food farming. The treatment does not require genetic alteration or manipulation to accelerate the otherwise natural growth of plant tissue as such the use of acoustic shock waves is compatible with organic farming practices as well as the new fields of genetic engineering.

US8701340

**Methods and apparatus for improving plant growth**

Inventor : ADAMS ARTHUR HENRY

**FIELD**

[0002] These teachings involve equipment and methods for horticultural and agricultural productivity enhancement.

**BACKGROUND**

[0003] The field of cultivating plants has spurred technological advances from the plow, to artificial irrigation, to hybridization and now to advances in the application of DNA research. In the area of subtle influences that alter a plants environment, some have experimented with "talking to their plants" and playing Mozart for them. While neither of those techniques has found widespread use, there is a growing body of serious research regarding the effects of sound and vibrations on plant growth. Like all living organisms, plants have highly complex sensory networks for monitoring their surroundings, and are known to modify their growth and development to suit their environment. For example, plants exposed to a variety of mechanical perturbations, such as wind or touch, undergo physiological and developmental changes that enhance resistance to subsequent mechanical stress. Developmental changes in response to mechano-stimulation are collectively known as thigmomorphogenesis.

[0004] The short paper "Biochemical and physiological changes in plants as a result of different sonic exposures" by Yu-Chuan Qin, Won-Chu Lee, Young-Cheol Choi and Tae-Wan Kim that was published in Elsevier's Ultrasonics journal (41 (2003) 407-41) investigates the biochemical mechanisms that might be involved in some of these phenomena. Chinese cabbage and cucumbers at two growth stages were the researchers' subjects. For each plant type three groups were constituted. Besides a control group that was not subject to any artificial acoustic treatment, one group was exposed to steady ultrasonic (US) waves of 20 k Hz, while the other was exposed to so-called "green music" (GM) consisting of a combination of classical music and natural sounds including bird songs. Both O<sub>2</sub> intake and polyamines content were measured. In brief, they found Chinese cabbage reacting more positively to the GM and the cucumbers to the US. However, for each quantity measured, either one or the other or both of the sonically exposed plants had greater readings than those of the control plants. That paper's charts of the polyamines content measurements are reproduced as FIGS. 1A and 1B. The caption of the Chinese cabbage growth graph in that paper is:

"Polyamine content (nmol/gFW) of Chinese cabbage seedlings: (A) 15 d and (B) mature plant (70 d) as a result of different acoustic exposures. Error bars represent the standard deviations of the means of polyamine contents." And the caption in that paper of the cucumber data is: "Polyamine content (nmol/gFW) of cucumber seedlings: (A) 15 d and (B) mature plant (70 d) as a result of different acoustic treatments. Error bars represent the standard deviations of the means of polyamine contents."

[0006] Studies have also focused on specific frequencies' effects, for example "Plant gene responses to frequency-specific sound signals", Mi-Jeong Jeong, Chang-Ki Shim, Jin-Ohk Lee, Hawk-Bin Kwon, Yang-Han Kim, Seong-Kon Lee, Myeong-Ok Byun and Soo-Chul Park. (Mol Breeding (2008) 21:217-226) published Springer's Molecular Breeding journal. They demonstrated sound affecting plant growth through mRNA expression analyses.

[0007] Others have looked at the issue of the effect of vibration on plant growth. One relevant article is: "Growth Promotion by Vibration at 50 Hz in Rice and Cucumber Seedlings", Hideyuki Takahashi, Hiroshi Suge and Tadashi Kato. (Plant CellPhysiol. 32(5): 729-732 (1991)). They looked at the effect of 50 Hz vibration and mention that a motivation of their study was the issue that motors and other mechanical apparatus in a green house might produce sounds with unintended and unexpected effects on plants.

[0008] FIG. 3 shows a reproduction of that paper's "FIG. 1." Its caption is:

"Germination of rice and cucumber seeds as affected by vibration at 50 Hz Data is shown as the percentage of germinated seeds in a time-course study. Top (A), rice seeds under submerged conditions; middle (B), rice seeds on filter paper; bottom (C), cucumber seeds on filter paper. Open (O) and closed circles indicate the control and the vibrated seeds, respectively. One hundred seeds were used for each treatment."

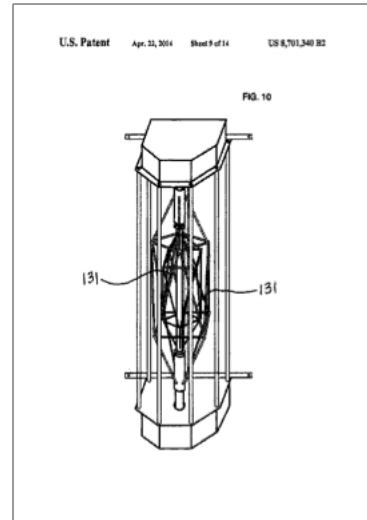
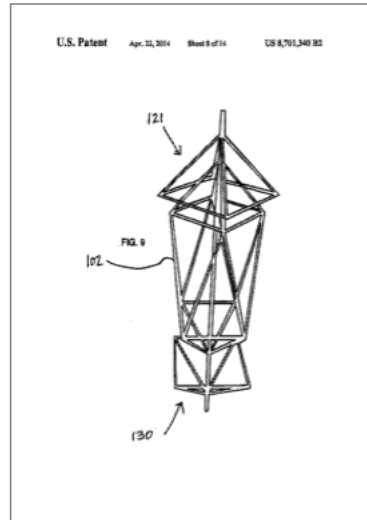
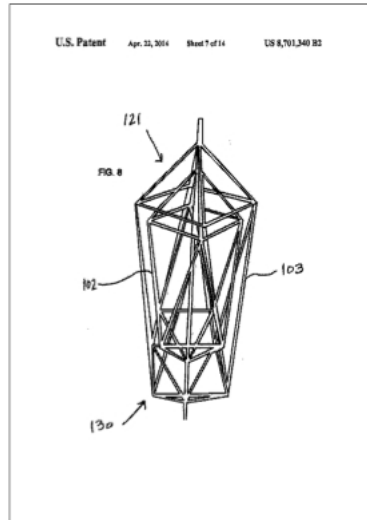
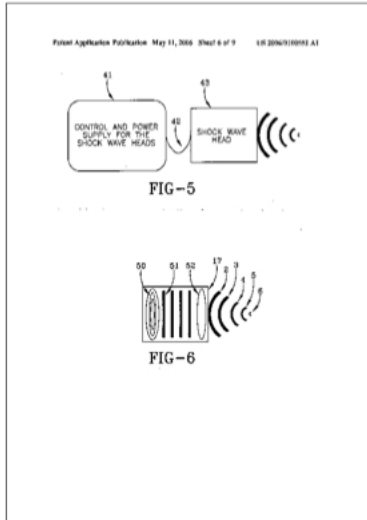
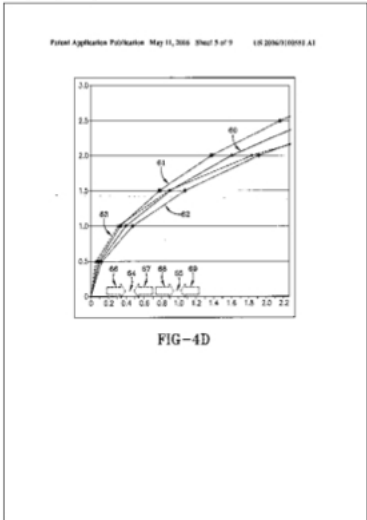
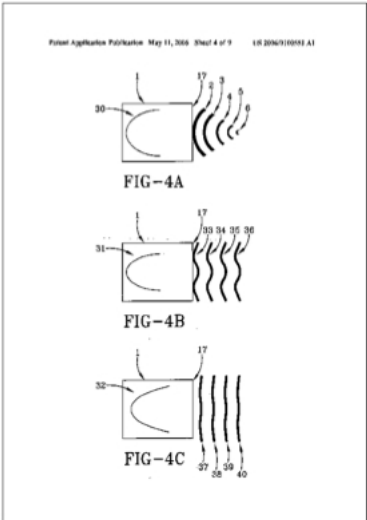
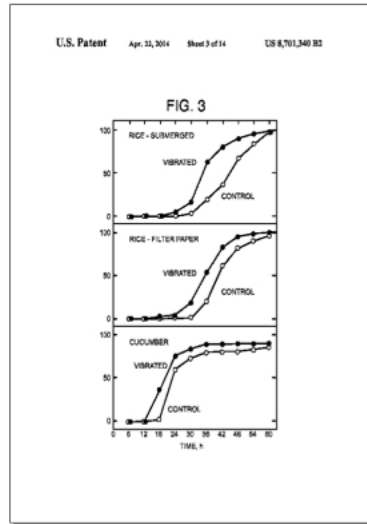
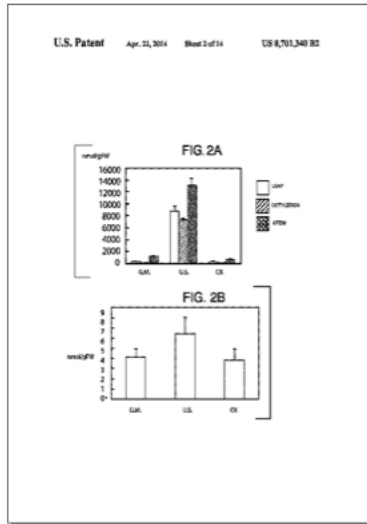
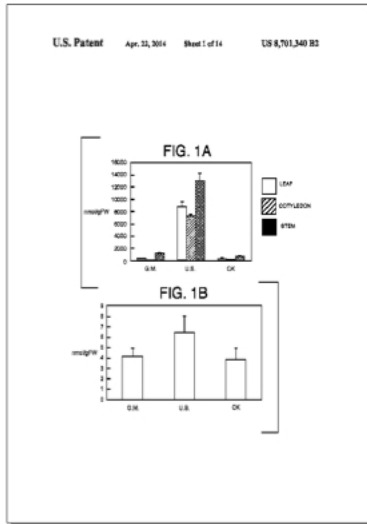
[0010] U.S. Pat. No. 7,600,343 dated Oct. 13, 2009 by Reiner Schultheiss, et al, discusses the effect of shock waves on plant growth.

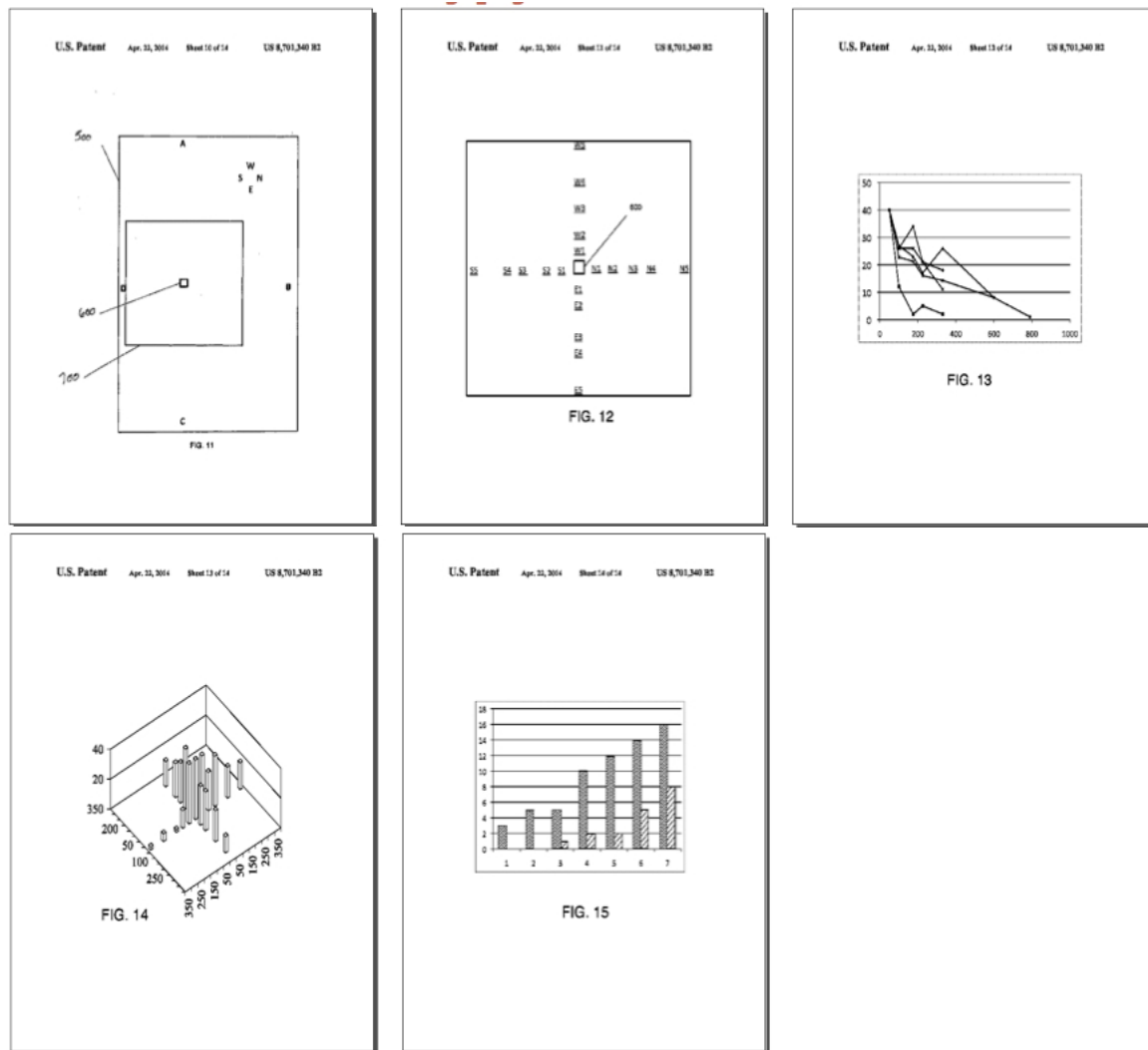
[0011] However, previous attempts to improve plant growth along the lines of the research above have not made it into routine, large-scale, commercial use. Systems and methods are needed which can improve plant growth in ways compatible with our current environmental imperatives that are also inexpensive to deploy and maintain. Preferably, solutions would avoid chemical fertilizers and chemical pesticides and be simple to deploy in both the developed world and the developing world.

**SUMMARY**

[0012] System and methods consistent with these teachings involve two counter-rotating geometric space frame structures that may be thought of as concentric. When energized and operated proximate to growing plants, the growth rate of those plants can be enhanced. Experimental results have shown its operation to be associated with effective increase in plant growth.

**BRIEF DESCRIPTION OF DRAWINGS**





[0013] FIGS. 1A and 1B together constitute a re-drawing of FIG. 1 of the paper of Yu-Chuan Qin, et al;

[0014] FIGS. 2A and 2B together constitute a re-drawing of FIG. 2 of the paper of Yu-Chuan Qin, et al;

[0015] FIG. 3 is a re-drawing of FIG. 1 of the paper of Hideyuki Takahashi, et al;

[0016] FIG. 4 shows a perspective view of the first example embodiment;

[0017] FIG. 5 shows a perspective view of upper and lower pyramids of the inner armature of the unit of FIG. 4 in isolation;

[0018] FIG. 6 shows a plan view of the armature components of FIG. 5;

[0019] FIG. 7 shows a perspective view of the inner armature from the perspective view as in FIG. 5;

[0020] FIG. 8 shows the apparatus of FIG. 7 and shows an identical but larger outer armature;

[0021] FIG. 9 shows the inner armature of FIG. 7 with the larger pyramids of the outer armature arranged above and below it;

[0022] FIG. 10 shows a unit of the second embodiment version;

[0023] FIG. 11 shows a schematic diagram of a tomato field in which experiments were performed noting location of the test area;

[0024] FIG. 12 is the test area of FIG. 11 expanded to show the physical location of test plants and equipment in the test area;

[0025] FIG. 13 is a graph showing the number of tomatoes as a function of distance from the unit;

[0026] FIG. 14 is the same data as seen in FIG. 13 displayed 2-dimensionally as relative to its position in the field;

[0027] FIG. 15 shows tomato growth using an indoor unit;

## DETAILED DESCRIPTION

### [0028] Introduction

[0029] The global population is estimated to reach 9 billion people by 2050. There is an increasing loss of arable land caused by desertification and decreasing water supplies caused by melting glaciers and erratic precipitation patterns. It may be difficult to feed the world's population in the future.

Equipment and methods to enhance plant growth are therefore of high global importance.

### [0030] Structure

[0031] In a first example embodiment seen in FIG. 4, a machine includes a platform 100 supporting a vertical axle 101. Rotatably coupled to the axle are an inner armature 102 and an outer armature 103. Each of these armatures is supported by respective bearings 104 104 and are arranged to separately, freely rotate about the axle. A source of motive force in this example, are two DC motors 105 and are mechanically connected by belt drives 106 to each armature to provide for their respective rotation in opposite directions. In this first example device, the outer armature 103 revolves clock-wise with the inner armature 102 going counter-clockwise. The relative rotation of the armatures is not set to a fixed relationship by mutual gearing, for example. The motors are capable of being adjusted to cause each armature to rotate in a range of 400 to 500 rotations per minute. In this example the upper surface of the top supports a solar cell panel 402 that provides the energy to operate the motors. Those skilled in the art will be familiar with the specific current requirements of various motors that may be used and the energy storage than may be required. In this example the electricity from the solar panels is used to charge batteries. The motors, under the control of a timer and speed controllers, is then driven from the batteries.

### [0032] Armatures

[0033] The inner and outer armatures of this version are each a geometric frame constructed from stainless steel rods. Aluminum rods may also be used. The armatures are of the same geometric configuration with the difference being that the outer armature 103 is a scaled up instance of the configuration of the inner armature 102. Therefore the inner armature will be initially described in isolation. This also allows for clearer drawings than those showing the entire machine.

### [0034] Inner Armature

[0035] Its height is the dimension that would be from its top to its bottom when configured on the axle on the platform in a usage configuration. In this first version the inner armature's 102 overall height, is about 28.5 inches. The upper-most and lower-most elements are rods of a diameter of about  $\frac{3}{8}$ ", centered within the armature body that fits over the axle. The armature can be thought of as a space frame with its top and bottom portions being symmetrically arranged rectangular, right pyramid space frames. To allow this description to be more meaningful, the lengths of the space frame rods will be expressed in relation to the length (L) of the rods that make up the upper and lower pyramids. For this inner pyramid L is 9". As seen in FIG. 5, the upper, inner pyramid 121 has a base 122 that is formed by four rods of length L 128 arranged to represent the sides of a square. From each corner 223 224 225 226 of that base is a rod 129 representing a vertex of the pyramid. The rods' other ends all meet near an apex 126. In this case the vertices rods' are also of length L. These identical lengths of base and vertex segments result in a shape with faces that are at about 52 degrees to the plane of the base.

[0036] The upper pyramid, 121 as mentioned, is complimented by an identical lower pyramid 130 that is of an identical space frame configuration. However, in constituting the armature, the lower pyramid has its apex 136 pointing downward. Its position is symmetric relative to the upper pyramid with the exception of being rotated by 45 degrees about a line connecting the two apex points 126 136. This rotational offset is better seen in FIG. 6 that shows a top plan view of the pyramids of FIG. 5.

[0037] As seen in FIG. 7 as well as other figures, the two pyramids are spaced apart by connecting rods 131 that interconnect the corners of the upper pyramid's base 122 with corners of the lower pyramid's base 133. Each corner is connected to the two nearest corners of the opposing pyramid's base. For example, a particular corner of the upper pyramid 224 has one attached connecting rod 131 whose other end is attached to corner 235 of the lower pyramid 130. A second connecting rod is attached to the same upper pyramid corner 224 and its other end is attached to the lower pyramid at a second corner 234. The six remaining connecting rods are similarly attached to the pyramids creating the symmetric geometric shape of the inner armature. Since the pyramids are rotationally offset, these eight equal length rods take a form of the letter "V". In this example, the length of those rods are all of length  $1.8*L$ . The consequence to these relative rod dimensions is that the height of the pyramids is  $0.707*L$  while the distance between bases is  $0.95*L$ . The total length from apex to apex is therefore  $(2*1.8+0.95)*L$  or  $3.1*L$ . As mentioned, the pyramids' respective vertices meet near the apex. In fact, they terminate at the upper and lower support tubes 127 137 respectively. These support tubes are centered on the armature's apex-to-apex centerline.

[0038] FIG. 8 shows an inner armature surrounded by an outer armature, both on a common axle through their support tubes. In this version, the outer armature has rods making up its pyramids that are length 11 inches. Bearings support the armatures for rotating on the axle. They provide for independent driving of each armature in their respective rotations. The total height of the dual armature assembly is about 36 inches from apex to apex.

### [0039] Assembly Method

[0040] The various rods and pipes that make up most parts of the armatures are welded together in this first example. Since one is completely inside of the other, the outer armature is welded together after being assembled around the inner armature. FIG. 9 shows a partially assembled unit. The outer upper and lower pyramids are in place surrounding the inner armature. The next step would be to weld the outer armature's connecting rods to the appropriate corner locations on the two large pyramids.

### [0041] Variations

[0042] While a solar powered unit may be ideal for field use, motors running on AC mains power might be more suitable for use in a green house or in an indoor hydroponic application. Rather than having a motor for each armature, it is known to those skilled in the art to use a single motor with gearing or other mechanical coupling to have one motive source turn the armatures in opposite directions. The entire unit may be scaled up or scaled down, keeping the proportions constant.

### Second Example Embodiment

[0043] A similar geometric shape to the first example unit is present in the second example embodiment. However, the second embodiment is intended for indoor use. The armatures rods are each one half the total length of the corresponding structure in the first example. This version is shown in FIG. 10. Rather than a platform and pipes to support it, this unit is in a self-contained cabinet.

### [0044] Operation

[0045] For outdoor operation the embodiment of example one can be supported from the ground by 2-inch galvanized pipes approximately one foot into the ground that support the platform near its four corners. The unit is placed in a field in proximity to the crops to be effected. The solar panel is connected to a battery that, in turn, is connected to a timer and to speed controllers for the two motors. The timers are recommended to be set to operate the apparatus three to five times during daylight hours at equally spaced intervals for equal durations. An example operation is operating for five minutes, three times a day, at intervals that split the daylight hours into four segments. Since the sunlight available is variable, those skilled in the art will recognize the function of the battery in providing a steady source of energy to rotate the armatures at a predetermined rate for a predetermined duration. The rate for each armature respectively can be between 400 and 500 revolutions per minute.



**[0046] Alternate Modes of Operation**

[0047] A smaller, indoor unit is bolted to the floor the plants are resting upon. Alternatively it is bolted to a wall that is, in turn, abutting and secured to that floor. An indoor unit would most likely be powered from AC, as mentioned.

[0048] AC could also power an outdoor unit. Other modes of powering could be a wind turbine substituting for the solar panels. Another could involve deriving power from the flow of water in an irrigation system.

**[0049] Experiments to-Date****[0050] Outdoors Experiment**

[0051] Two primary experiments have been performed. A large-scale outdoor trial was performed at Eclipse Farms in the City of Oxnard, Calif. The crop grown there is Roma tomatoes. A unit 600 constructed as the first example embodiment was installed and operated as discussed in the operation section above in a 35-acre rectangular field 500. Within the field a square of 10 acres 700 (660 feet per side) had a unit placed at its center. Plant locations were marked off along lines due North, East, South and West from the center location to the perimeter of the square. The unit was activated on Jul. 20, 2009 and controlled by the inventor during the testing.

[0052] In FIG. 12 the location of five plants per line were marked as plants to be measured in the experiment. Starting from the unit (in each respective compass point direction) the distances from the center were 50 feet, 100 feet, 175 feet, 225 and 330 feet. In addition there were four other plants marked for testing. These four plants were along the same four lines at the four points at which respective line intersects the acre plot perimeter. In FIG. 11 these points are designated A, B, C and D. The 20 plant locations within the square are designated in FIG. 12. The five "West" plant locations are designated W1, W2, W3, W4 and W5. The "East" plant locations are designated E1, E2, E3, E4, and E5 and so on for the North and the South.

[0053] The twenty-four marked test plants were measured for over fourteen weeks. Those measurements included counting the tomatoes each week. In addition, in the first few weeks, both the number of flowers and the number of tomatoes were counted twice a week.

**[0054] Results**

[0055] The number of tomatoes in the twenty locations on the E-W and N-S lines within the 10 acres is shown in FIG. 13 as a line graph. That figure shows the N 301, S 302, E 303 and W 304 tomato counts as a function of feet from the unit. Also seen is a curve representing the average 305. This graph demonstrates a fall off of the effect of the unit with distance which is consistent with many physical phenomena. The same data is displayed in a three-dimensional format in FIG. 14. The X and Y positions represent the plants' location relative to the unit. The Z heights represent the number of tomatoes on the plant at that location when counted at the end of the experiment.

[0056] It can be seen that in all directions there is a general falling off of tomato count as distance from the unit increases.

[0057] The other four tomatoes were not at constant distances from the unit since the overall field is rectangular. A and C are at 460 feet, B is at 270 feet, D is at 20 feet. Those data points are also included in the graph of FIG. 13.

**[0058] Indoors Experiment**

[0059] A second set of experiments were performed indoors. In fact it was on a balcony of an apartment in Marina Del Rey, Calif. These experiments were performed during 2008. The tests used different plants including Celosia, Tomatoes, and Pepper plants. The plants were sourced in twos from a nearby Home Depot. Plants of similar height and girth were chosen. If one was arguable slightly larger than the other, that one was made the control plant.

[0060] The procedure was that the potted plants were placed a few inches apart on a table on the balcony. Lab calibrated beakers were obtained and used to measure water and nutrients that were applied equally to both plants. Every week the plants positions were changed to account for any difference in sunlight.

[0061] Each day the test plant was taken to another room inside where a Biowave unit was bolted to the wall. The motors of this machine were set a yard away from the machine itself (to be further from the plant) so that any magnetic field from the motors would not impact on the plants. Also the motors were screened with expanded metal and grounded to further reduce any EM radiation. This was measured with a magnetometer.

[0062] The test plant was placed beside the machine for 15 minutes a day and then returned to the balcony. In one test run the test plant had 15 blossoms, while the control plant had 7 blossoms. This experiment was repeated at least 18 times during 2008 with both with of the other plants. All of the tests showed the test plants with greater growth than the control plants.

[0063] Four of the 18 tests were with organic tomatoes. In all these cases the test plant to control plant tomato-count ratio ranged from 4 times to 2. The results of one particular test run are shown in FIG. 15. The more densely hatched columns represent the number of tomatoes on the test plant. The other columns represent the control plant. Both are plotted at one week intervals over seven weeks. Additionally, in at least one documented test run, the test plant also had significantly less insect damage than the control plant.

[0064] A tomato from one of the experiment's test plants was analyzed for its Brix content by Silliker Labs of Cypress Calif. The USDA average Brix rating for an organic tomato is 4.9%. The tests performed by this independent lab showed the test tomatoes to have a Brix rating of 10.5%.

**[0065] Theory of Operation**

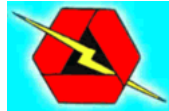
[0066] No particular theory of operation is presented or known. Various known mechanisms may be involved including acoustic waves transmitted through the air or vibrations transmitted through the ground. Gravity is another possible communication medium. The papers mentioned in the background section, "Biochemical and physiological changes in plants as a result of different sonic exposures" by Yu-Chuan Qin, Won-Chu Lee, Young-Cheol Choi and Tae-Wan Kim and "Growth Promotion by Vibration at 50 Hz in Rice and Cucumber Seedlings", by Hideyuki Takahashi, Hiroshi Suge and Tadashi Kato. (Plant CellPhysiol. 32(5): 729-732 (1991)). These and other publications evidence serious researchers studying possibly not-yet-understood factors that can affect plant health and growth.

[0067] Publications that teach subtle influences on plant growth include, "Plant gene responses to frequency-specific sound signals" Mi-Jeong Jeong, Chang-Ki Shim, Jin-Ohk Lee, Hawk-Bin Kwon, Yang-Han Kim, Seong-Kon Lee, Myeong-Ok Byun and Soo-Chul Park., "Growth of the Cellular Slime Mold, Dictyostelium discoideum, Is Gravity Dependent" Yukishige Kawasaki\*, Takeshi Kiryul, Kenji Usui1, and Hiroshi Mizutani, Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194, Japan. Another paper showing an effect of music and even less tangible inputs causing differences in plant growth is "Measuring Effects of Music, Noise, and Healing Energy Using a Seed Germination Bioassay" From the journal of alternative and complementary medicine Volume 10, number 1, 2004, pp. 113-122 Katherine Creath, Ph.D. (Optical Science), Ph.D. (Music), 1-3 And Gary E. Schwartz, Ph.D.1, 3.

[0068] Still other scholarly papers that may be relevant are from Plant and Cell Physiology, 2002, Vol. 43, No. 6 647-651. "Effects of Mechanical Vibration on Seed Germination of Arabidopsis thaliana" (L.) Heynh. Ayuho Uchida<sup>1, 3</sup> and Kotaro T. Yamamoto<sup>1, 2, 4</sup> and 1. J Gravit Physiol. 1996 April; 3(1):69-74. Also, "Gravity related features of plant growth behavior studied with rotating machines". Brown A H. Collaborators: Brown A H. University of Pennsylvania (Philadelphia), USA.

[0069] Those skilled in the art will recognize that these and other teachings suggest that occurrences proximate to plants may have effects on their growth even though the mechanisms may not be understood. They represent serious researcher's efforts to understand subtle influences on plant germination and growth.

[0070] These seven papers mentioned above are hereby incorporated by reference in their entirety. In particular, FIGS. 2A and 2B are reproductions of charts appearing in the paper "Biochemical and physiological changes in plants as a result of different sonic exposures" mentioned above. FIG. 2A shows polyamine content (nmol/gFW) of cucumber seedlings. FIG. 2B shows a mature plant with a different acoustic treatment. Error bars represent the standard deviations of the means of polyamine contents. It can be seen from these figures that the polyamine uptake is greatest in the plants exposed to ultrasound.



**Your Support Maintains this Service --**

**BUY**

**The Rex Research Civilization Kit**

**... It's Your Best Bet & Investment in Sustainable Humanity on Earth ...  
Ensure & Enhance Your Survival & Genome Transmission ...  
Everything @ rexresearch.com on a Thumb Drive or Download !**

**[ORDER PAGE](#)**

---