

SPECIFIC MUSICAL WORK AS A PHYSICAL INDUCER AND GROWTH STIMULATOR IN ROMAINE LETTUCE

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Abstract: For the year 2050, an increase in the population close to 9.7 billion people is estimated, therefore the demand for food will increase and the space for cultivation will decrease. Sound waves have a stimulus and effect on plants, this type of elicitors improves growth conditions, energy metabolism, stress-related gene expression, increases secondary metabolites and disease resistance. The objective of this research is to have a preliminary study to promote musical sound exposure in crops and make their growth more efficient. The results indicated a reduction in the harvest time at 10 weeks compared to the control group that were harvested in 13 weeks, when exposed to 2 specific compositions during their entire cycle. This represented an increase of 28.57% in production.

Keywords: BIO, Agriculture, Yield, Sound waves, Crops, Music.

INTRODUCTION

The usual way of managing agriculture is no longer an option. For the year 2050, a population increase close to 9,700 million people [1] is expected, therefore the demand for food will increase and the space for cultivation will decrease. The challenge is to sustainably improve agricultural productivity to meet demand.

Elicitors are substances from various sources, both inorganic and organic, that can induce physiological effects and changes such as the activation of defensive responses and the accumulation of phytoalexins in the organism to which they are applied [2]. In recent years, the tendency to consume products treated with natural elicitors has increased, since they have great advantages over chemical products because they are less harmful to the ecosystem and because the environmental microflora biodegrades them in situ [3]. Currently, the use of acoustic vibrations has emerged to generate

controlled abiotic stress in plants, this type of elicitors improves growth conditions, energy metabolism, stress-related gene expression, increases secondary metabolites and disease resistance [4].

Living organisms need to perceive their environment and respond to it in order to survive. Plants have been shown to perceive and respond to different types of stimuli, such as light [5], [6], air signals [7] and touch [8]. In recent years, the ability of plants to respond to sound has been reported [9], [10], likewise the sound emissions of documented plants have been documented [11].

The reception of sounds can imply an important selective advantage for plants [12]. Sounds travel fast, are naturally present in the environment, and convey important information about the presence of pollinators, frugivorous herbivores, weather conditions, and essential resources such as water [13]. Specific vibration frequencies, such as those generated by insects, such as the chewing of caterpillars and the buzzing of bees, activate the secondary metabolism of plants and their respective pathways. Thus, studies report that vibrations and sound waves applied to plants improve their physical performance. Commonly, acoustic treatments for plants have used arbitrary frequencies. In jalapeño pepper, a group of signals obtained from water-stressed plants was observed as vibrational patterns using a laser vibrometer to record spectral signals. Acoustic Emission Patterns (AEP), it was suggested that certain vibrations could “emulate” a plant signal through mechanical energy based on the recognition of the vibration pattern. The AEP’s were applied as characteristic vibrations classified as low water stress (LHS), medium water stress (MHS) and high water stress. (HHS) to evaluate its effect on well-watered and healthy plants in two stages of development. In the vegetative stage, gene expression related to

antioxidant and water stress responses was evaluated. The findings are correlated for a better understanding of plant responses to different tones of multifrequency vibration signals with potential for agricultural applications [14].

Likewise, it was reported that flowers increase their amount of nectar when treated with bee sound, which translates into a greater probability of cross-pollination and consequently a higher yield [13].

Therefore, if plants possess even a rudimentary ability to respond to sounds, natural selection is expected to favor these traits, and evolution must lead to improved hearing in plants [15].

Among the proposals to make agricultural productivity more efficient, various experiments have been carried out through sound stimulation to increase and/or improve food production.

In 1960, George F. Smith, stimulating corn in George Gershwin's Rhapsody in Blue for 24 hours, resulted in early shoots and plants with thicker, greener stems. Also affected maize plants weighed 40 g. against 25g. from the control group [12].

Mary Measures and Pearl Weinberg in the mid-1960s exposed high-frequency vibrations to spring and winter grains and seedlings, realizing that depending on the exposure time they responded better to the 5000 Hz frequency, resulting in a double harvest [12].

It is reported that sound can positively influence drought resistance in rice:

Oryza sativa [16] plants were subjected to various sound frequencies for an hour. After 24 h sound treatment, plants were exposed to drought for next five days. During the experiment it was observed that sound initiated physiological changes showing tolerance in plant. Sound frequency with ≥ 0.8 kHz enhanced relative water content, stomatal conductance and quantum yield of

PSII (Fv/Fm ratio, also various investigations report various effects of sounds on plants such as; like arabidopsis [17] wind, rain, touch and vibration, *Actinidia chinensis* callus [18], *Phaseolus vulgaris* bean [19], *Fragaria* strawberry [20], *Tagetes erecta* L., *Catharanthus roseus* L., *Trachyspermum ammi* L., *Dendranthema grandiflorum* L., *Hibiscus rosa-sinensis* L., *Epipremnum aureum* L., *Duranta repens* L., *Ocimum sanctum* L [21], carrots *daucus carota* [22], rice *Oryza sativa* [23], okra *Abelmoschus esculentus* and zucchini *Cucurbita pepo* [24].

The objective of this work was to create a specific composition from frequencies that romaine lettuce emits in the soil during its growth to make the development period more efficient through exposure to the sound material created.

MATERIALS AND METHODS

The study was carried out at the Amazcala Campus of the Autonomous University of Querétaro (UAQ). The investigation was carried out in two general stages: 1) Data collection and sound composition, and 2) Sound composition exposure to lettuce.

In both stages we worked with romaine lettuce plants platinum Geneva seeds, sown in soil under greenhouse conditions. An 8 m x 9 m macro tunnel with a diffuse plastic roof with 30% shading of 700 gauges and white anti-aphid mesh walls of 10 x 16 threads per square centimeter was used for a period of 2 years.

MATERIAL FOR EXPERIMENTATION

Culture material (1st stage):

- Substrate: 33.3% Peatmoss (1 kg), 33.3% grit (1 kg) and 33.3% Tezontle (1 kg) total 3kg
- Romaine lettuce seed, Geneva seed platinum

- Irrigation system: drip by high-density polyethylene tape with drippers every 20cm, caliber 8000 with a flow of 2 l/hour.
- 12 pots 8.5 inches in diameter
- 12 pots 5.5 inches in diameter

Equipment for the creation of the musical work:

- Computer
- Cubase 5 program
- Audio interface
- Sound Force 10 Program

Equipment for recording the underground vibration phenomenon of the plant (Fig. 1):

- 1 pc CPU 1214C (14 DI 24V DC; 10 O 24V DC; 2 AL) PS 24V DC, Siemens 6ES7214-1AG40-0XB0
- 1 PM1207 Power Supply, 120/230 V AC, output: 24 V DC, Siemens 6EP1332
- 1 pc Analog Input 4 AI; 13-bit, Siemens 6ES7231-4HD32-0XB0
- 1 pc Analog Input 8 AI; 13-bit, Siemens 6ES7231-4HD32-0XB0
- 1 pc Memory Card, 24Mb, Siemens 6ES7954-8LF03-0AA0
- 1 pc Phenolic Plate 10 x 10 cm, Steren MC100
- 2 pcs Transfer sheets for printed circuit boards, Steren PNP-010
- 2 pcs Base for integrated circuit and 14 pins, Steren IC14
- 10 pcs. Ceramic disc capacitor, from 0.001UF (Micro Farads) to 500Volts, Steren C.001-500 S
- 10 pcs Radial Electrolytic Capacitor, 100UF (Micro Farads) at 25 volts, Steren E100-25R
- 4 pcs TTL Integrated Circuit of 6 inverters

4 pcs SN74LS04N

- 30 m, tinned cable for connections, red color, gauge 22AWG C22R-100
- 30 m tinned cable for connections, black color, gauge 22 AWG C22N-100
- 1 piece Simantic Step 7 Basic 15 (package) Siemens 6ES7822-0AA05-0YA5
- 1 PLC S7-1200 CPU 1214 DC/DC/DC Siemens brand, model 6ED1052-1CC01-0BA6

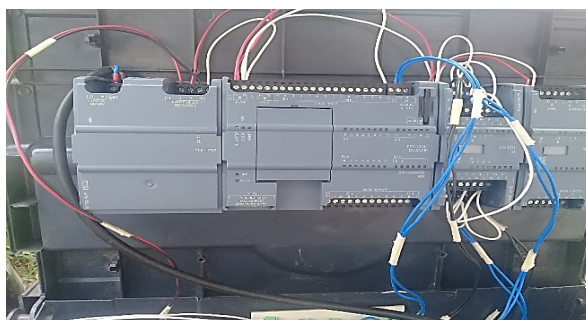


Figure 1. Measurement equipment. General operation of the equipment: PLC. Analog input module, Smith Trigger.

Amplifying equipment (Fig. 2).

- 1 breadboard
- 2 integrated SN74HC14N
- 10 ceramic capacitors of 100pF
- 10 resistors of 10K Ohms

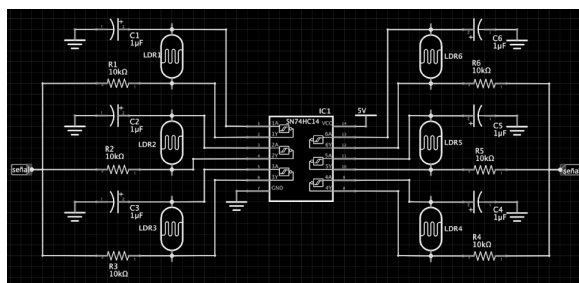


Figure 2. Integral circuit, amplifier.

The function of the integrated circuit is the amplification of the noise that is in the ground, the data is stored in the PLC.

SAMPLE GROUP RECORD

A sample group of 12 plants (Fig. 3) was cultivated for three months in order to obtain measurements of the frequencies emitted by the root during its growth.

Once the lettuces germinated, the installation of the measuring equipment and data collection were carried out., carried out by means of an integral amplification circuit inserting two cables directly to the substrate to each of the individuals, which were connected to the PLC to store the information in volts, generating 1 data every minute 24/7 for 13 weeks.



Figure 3. Lettuces in a greenhouse with measuring equipment installed.

By week 9, the lettuces had already reached the size to be transplanted into an 8.5” pot and drip irrigation was installed, thus continuing the data recording.

An average temperature of 19.45°C and an average humidity of 72% were reported.

The amount of irrigation per day in the different phenological stages per week:

- Week 1 to 4: 2.5 l/m²
- Week 5 and 6: 3 l/m²
- Week 7 and 8: 3.75 l/m²
- Week 9: 3.75 l/m²
- Week 10 to 13: 3.25 l/m²

DATA ANALYSIS AND SELECTION OF FREQUENCIES FOR THE CREATION OF THE SOUND-MUSICAL WORK

The data collected with a 5 volt signal was entered into the PLC through the analog channels, these were emptied into an Excel sheet in a CSV file. The files were separated by plant, yielding a total of 10,212 records per week. Then a database was generated in the program: TIA Portal where all Excel data was saved. To change the database to audio, an audio interface was connected to the analog channel of the PLC, reproducing all the data from the TIA Portal program to the Sound Force 10 program. This way, the collected data was changed to audio format to analyze the recorded frequencies.

Each audio was analyzed by means of the Cubase 5 program, through the following process:

1. A new project was created
2. An audio of the transformed material lasting 10 minutes was inserted.
3. Once the audio was clean, a selection of 2:30 minutes was made (equivalent to one day of recording).
4. The Q-clone stereo equalizer was used to analyze the frequencies through the pick hold (Fig.4)
5. The maximum and minimum frequency points were located
6. The data was emptied into an Excel table.
7. Of the total data, the frequencies of greatest repetition were selected



Figure 4. Pick Hold sample for frequency analysis.

Steps 1 to 8 were repeated for each of the 31 seeded individuals.

Two compositions were made for the treatment since it was taken into account that individuals are living beings and as such require different elements depending on their stage of growth.

For the first composition, 34 frequency records were taken, ranging from 60 to 13,586 Hz (Table 1):

60	70	91	176	191	285	294
320	356	537	541	544	588	592
600	626	1146	1175	1286	2305	2320
2563	4342	4373	4404	4699	4733	4768
4803	11175	11341	12710	2876	13586	

Table 1. Frequencies (Hz) Composition 1.

With a duration of 5 minutes (300 seconds). The duration of each frequency was corresponding in seconds to the repetition time in the data analysis. For example, during the 300 seconds, 516 repetitions of all the frequencies analyzed were recorded and the frequency 13,586 Hz was repeated 50 times, its duration within the composition corresponding to 29 seconds.

In the 2nd composition, 94 frequencies were used from 50 to 13586 Hz (Table 2).

With a duration of 5 minutes, in the same way as in composition 1.

The compositional criteria start from the Pythagorean proportions applied to the musical intervals, appearing more frequently and adjusted as close to octaves (2:1), fifths (3:2) and thirds (5:4). (Maggiolo, s.f.).

For composition 1, the first thing that was taken into account were the frequencies with the highest number of repetitions during

50	60	70	80	82	90	91	92	100	103
111	113	114	124	125	133	157	165	178	180
181	200	285	287	289	291	294	295	320	323
325	327	332	334	537	541	544	588	592	596
600	630	1146	1153	1160	1168	1175	1269	1286	1294
1302	1310	1319	1328	1338	2291	2305	2320	2334	2563
2582	2600	2619	2637	2654	2672	4279	4311	4342	4373
4404	4699	4733	4768	4803	4838	5052	5712	7569	7680
8320	10252	10326	10400	10474	10687	10966	11036	11105	11258
11341	12710	12876	13586						

Table 2. Frequencies (Hz) Composition 2.

weeks 1 to 3: 13.586 Hz, 12.876 Hz and 12,710 Hz, these three frequencies were placed within 5 minutes as described below:

- A second of these frequencies was placed at the beginning, at the end, in the middle and then in halves until ending with the total seconds of each frequency, having a slight offset to avoid their splicing. Figure 5 visually shows the placement of the high frequencies (Fig. 5).



Figure 5. Distribution of treble frequencies in the composition 1. On the left side it shows the frequency that is being used, and each colored line indicates where that frequency is within 5 minutes and its duration that goes from 1 second to 5 seconds, with the thickness of the line we can see that throughout the piece its duration is similar except at the end that lasts more seconds.

Subsequently, the following frequencies were placed in chords, maintaining mostly the Pythagorean proportions to form them, mixing low frequencies with high frequencies and creating motifs that would be repeated during the 5 minutes.

Likewise, the criterion of placing the closest frequencies as joint degrees was also used to soften the drastic changes from low to high frequencies in composition 1 (fig. 6).



Figure 6. Placement of the remaining frequencies. On the left side it shows the frequency that is being used, and each colored line indicates where that frequency is within 5 minutes and its duration that goes from 1 second to 5 seconds.s.

For composition 2, the same criteria was applied as in composition 1 for the frequencies with the longest exposure time from week 4 to

13, being in this case the frequencies 13,586 Hz and 12,710 Hz. To maintain the same order as in the Composition 1 (Fig. 7) the same criterion was applied with the frequency 12.876 Hz despite the fact that it did not occur as many times.



Figure 7. Distribution of treble frequencies in composition 2. On the left side it shows the frequency that is being used, and each colored line indicates where that frequency is within 5 minutes and its duration that goes from 1 second to 5 seconds.

However, the accommodation of the other frequencies was different since there was a greater number of data than composition 1. In this case, the frequencies were used to cover all 5 minutes, without splicing frequencies, making the appearance of a new frequency maintaining the Pythagorean proportions for the most part, for example: 11,258Hz (F9) 4,768Hz (D8) 111Hz (A2), having that order of appearance for 1 second each, resembles the arpeggio of a Dm chord (D-F-A) using different heights. Following this example, all frequencies were used during the 300 seconds, always taking into account the relationship of seconds with the number of repetitions in the frequency analysis (Fig. 8).



Figure 8. Frequency distribution composition 2. On the left side, the frequency being used is displayed, and each colored line indicates where that frequency is within 5 minutes and its duration ranging from 1 second to 5 seconds.

STAGE 2: TREATMENT APPLICATION

Materials:

Crop:

- Substrate: 33.3% peatmoss, 33.3% grit and 33.3% tezontle
- Romaine lettuce seed, Geneva seed platinum
- Irrigation system: drip by high-density polyethylene tape with drippers every 20cm, caliber 8000 with a flow of 2 l/hour.

Sound application equipment:

- Koss horn model BTS1
- Alarm Clock Xtreme application version 6.1.2
- Cell Phone Motorola xt 914 /Tablet7 Samsung Galaxy A6
- Extension of 20 meters.
- Wooden box
- Wooden tables
- Volteck TEM-8 Timer

This stage began on December 14, 2018, on the same date that the planting of the lettuce group began where the registration and storage of. It was done this way to have the same climatic conditions. There was a sample group of 12 plants which were not subjected to acoustic treatment.

Lettuces were germinated in a 38-space germination tray, with Peatmoss. The transplant was done in soil for an area of 3.5m³ (3.5 m long x 1 m wide).

After a bibliographic analysis in which the documented experiments report different schedules and reproduction times ranging from 5 minutes up to 48 hours of treatment with hours such as 5:30 am, 12:00 pm, likewise in some antecedents the time or hours of

treatment are not mentioned, the following treatment times were decided:

1. Composition 1: 4.5 weeks, 5 minutes every 3 hours making a total of 25 minutes of treatment
2. Composition 1: 2.5 weeks. 10 minutes of treatment every 3 hours making a total of 50 minutes after transplantation.
3. Composition 2: 4 weeks 15 minutes of treatment every 3 hours making a total of 75 minutes
4. Reproduction was chosen to be every 3 hours from 6:00 a.m. to 6:00 p.m. in order to stimulate the individual during its photosynthesis process. Applying each treatment at 6:00 a.m., 9:00 a.m., 12:00 p.m., 3:00 p.m. and 6:00 p.m.
5. Between the stage changes from seedling to transplant, it was decided to maintain composition 1 for 6.5 weeks in order not to have additional variables to the change of habitat and the increase in exposure time from 5 to 10 minutes.

Distribution of the lettuce in the greenhouse (Fig. 9), around the lettuce, wood was placed to make use of the sound reflection effect.

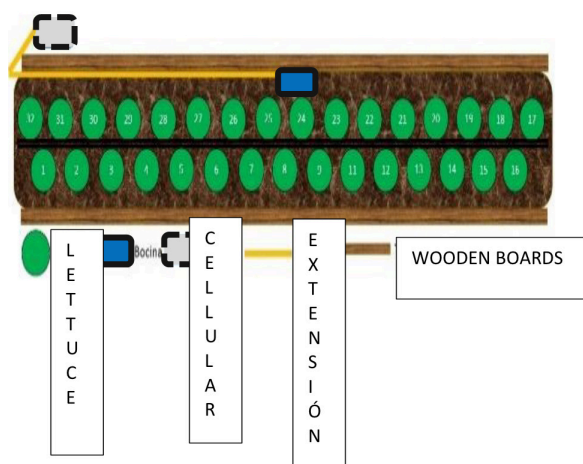


Figure 9. Distribution of lettuce in the greenhouse.

The lettuces were harvested on March 8, 2019, turning 11 weeks old (Fig. 10).



Figure 10. Lettuce in week 11 ready to harvest.

For the measurement of lettuce growth, in the first instance a leaf of each lettuce was marked to have the measurement of the same leaf throughout the cycle, however this leaf in 90% of the lettuces was not the highest, it is for this reason, it was decided to measure the maximum height of the lettuce instead of the marked leaf with a flexometer, taking centimeters as the unit of measurement.

The horn was always kept in the central part of the crop, in the seedling stage lettuces 26, 27 and 28 received the treatment directly and after transplanting the horn remained in front of lettuces 23, 24 and 25.

Irrigation was the same as with the sample group.

The average temperature was 19.50 °C and an average humidity of 65%.

DISCUSSION AND RESULTS

The harvest time of romaine lettuce according to SAGARPA is 14 weeks (López López, Magaña Lira, & Vázquez Romero, 2014). The results were compared with literature and with a control group of 12

individuals, under the same conditions and within the same greenhouse, which were harvested at week 13. Individuals with acoustic treatment were harvested at week 11, however from After week 10, the harvest could be carried out since the growth in the following 2 weeks was minimal and 77.4% of the lettuces were already in the physiological stage of harvest.

Our results showed an increase in production of 28.57%, which represents a reduction to 10 weeks in the harvest time of romaine lettuce with acoustic treatment.

To obtain the percentage of growth, the measurements in centimeters that were carried out every 3rd day during the entire phenological cycle of the lettuce were used, measuring its length. The week with the highest percentage of average growth was week 6 with 334.99%, this being one week after the transplant, having the composition 1 for 10 minutes every 3 hours in a range from 6:00 a.m. to 6:00 p.m. (Table 3).

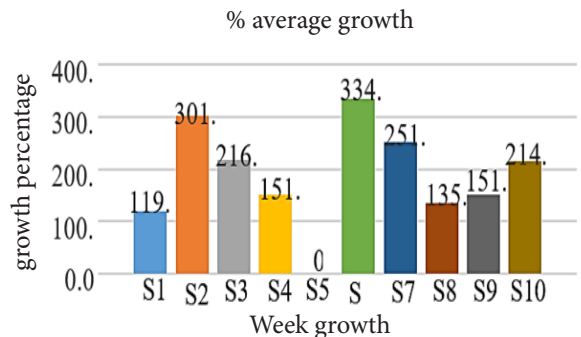


Table 3. Weekly growth percentage.

Although individual 32 sprouted 15 days later, it was harvested the same week as the other plants.

CONCLUSION

When analyzing the results of the experiment, it was determined that an acoustic elicitor helps for the production of romaine lettuce and optimize its productivity, however,

it is necessary to carry out a greater number of repetitions to see if the increase in decibels in the treatments affects positively or negatively. the productivity. Likewise, carry out various tests, leaving only one composition per group and thus have at least 4 different experimental groups: Composition 1, Composition 2, White noise and no sound (control). Standardizing treatment.

Similarly, before the application of this technique to agriculture, it is expected to have a greater number of tests directly in the field to determine its effectiveness for its replicability in different parts of the Mexican Republic and to be able to make this technique one more option for cultivation to solve food sovereignty.

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