**Freie Universität Berlin**

# Master thesis

# Adaptive value of sound vibrations emitted by plants using the example of maize

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# Content

# List of figures

# Abstract

Plant bioacoustics is a widely unresearched field. In this study, sound vibrations of maize seedlings were recorded by a laser vibrometer. The sound vibrations of the primary root were further investigated under different states of the plant. The tip of the primary root emitted the strongest sound vibrations. The vibrations consisted of single impulses as well as of periods of impulses of varying amplitudes up to 1.2 µm, which could last over 40 seconds. The dominant frequencies could vary within milliseconds, and ranged from less than 1 kHz to the maximum setting of 100 kHz. The vibration patterns differed under different conditions of the plant. In single impulses of cut roots, within the ultrasound range the peak frequency of 58 kHz dominated, which was higher than the peak frequency of single impulses of 54 kHz in drought-stressed roots. Varying light conditions did not result in significant change of vibration patterns. The vibrations were not transmitted through the plant, indicating that they do not have a direct informative value within the plant. However, they can propagate through the air as sound and can be perceived by other organisms.

Keywords: plant bioacoustics, acoustic emission, ultrasound, vibration, maize, drought stress.

Deutsch:

Plant bioacoustics ist ein weitgehend unerforschtes Feld. In dieser Studie wurden die Schallvibrationen der Primärwurzel von Mais-Setzlingen anhand eines Laser-Vibrometers aufgenommen. Die Vibrationen bestanden sowohl aus einzelnen Impulsen als auch aus Perioden von aneinandergereihten Impulsen mit variierenden Amplituden bis zu 1.2 µm, die über 40 Sekunden andauern konnten. Die dominanten Frequenzen konnten innerhalb von Millisekunden variieren, und traten in einer Bandbreite von weniger als 1 kHz bis hin zur maximalen Einstellung von 100 kHz auf. Die Vibrationsmuster unterschieden sich bei verschiedenen Zuständen der Pflanze. Einzeln auftretende Impulse von verletzten Wurzeln enthielten dominante Frequenzen von 58 kHz, die höher waren als die einzelnen Impulse von 54 kHz bei Wassermangel. Variationen der Lichtintensität ergaben keine signifikant unterschiedlichen Vibrationsmuster. Die Vibrationen wurden nicht auf andere Organe innerhalb der Pflanze übertragen, was darauf hinweist, dass sie keinen direkten informativen Wert für die Pflanze selbst haben. Jedoch können sich die Vibrationen als Schall fortsetzen und von anderen Organismen wahrgenommen werden.

Schlagwörter: Plant bioacoustics, akustische Emission, Ultraschall, Vibration, Mais, Trockenstress.

# 1 Introduction

Plant bioacoustics is a widely unresearched field. Plants, from trees to herbaceous plants, emit sound vibrations[1]. Most known are the sound vibrations emitted in relation to water scarcity[2][3][4]. Several studies showed an increase of acoustic emission events with an increase of water stress[5][6][7]. Further on, a study by Qiu et al. on tomato plants showed an increase of acoustic emissions with decreasing soil humidity whereas at the stage of severe water stress, the acoustic emission events decreased again. Laschimke at al. found out that plants emit sounds incessantly not only during transpiration, but also during re-hydration. Zweifel et al.[8] recorded the loudest acoustic emissions in tress before sunrise, when the amount of water in trees is the highest. Khait et al. succeeded in recordeding ultrasonic sound vibrations from a distance, emitted by tomato plants 10 cm away, with a focus on an ultrasonic frequency range of 20 – 150 kHz. The plants emitted sounds over the whole frequency range, with different amplitude peaks. The sound of drought-stressed plants had a mean peak (the frequency with maximum energy) of around 50 kHz.

However, the emissions of sound vibrations are not only related to the water status of the plant. A study on cutted leaves of tomato and tobacco plants have shown that the emission of sound vibrations have characteristic patterns and frequencies which differ from the sound vibrations emitted under water stress. Cutted plants emitted sound of a mean peak frequency of 57 kHz.

In general, there are diverse oscillatory processes in plants, from circumnutation[9], periodic nutrient uptake[10] to periodic ion fluxes during cell growth[11]. However, these frequencies

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[1] Ritman, K. T. et al. (1988).

[2] Johnes et al. (1986).

[3] Salleo et al. (1992).

[4] Matthiews et al. (1992).

[5] Borghetti et al. (1989).

[6] Lo Gullo et al. (1991).

[7] Kikuta et al. (2008).

[8] Zweifel et al. (2008).

[9] The shoot tip exhibit circular movements caused by repeatign cycles of different growth speed od the cells around the shoot tip. Also the root shows circumnutation during the vertical growth which happens in the oscillation range of 1 h.

[10] Nutrient uptake as well as rhythmical character causing oscillations in the frequency range of several minutes (Shabala et al. (1997)) to several hours (Khalitonashivili et al. (1997)). Rhythmic fluctuation can also be caused by stress, e.g. salt-stress. (Macduff et al. (1996)).

[11] Tissue growth in the elongation zone of the root apex is caused by periodic ion fluxes.

starting at several minutes per period are far too low from reaching sound range.

Regarding oscillations in the sound range, the mechanism behind as well as their adaptive value is still unknown, though some hypotheses exists. The prevalent hypothesis describes the formation of gas bubbles in the water columns due to water scarcity, known as cavitation, as the the origin of acoustic emissions. Cavitation causes a rapid relaxation of the tension in the column that produces a sound vibration[12]. Indeed the acoustic emissions rise in quantity with increasing water scarce conditions. Most studies have been conducted with tree species, the acoustic emission of which are comparatively louder (36 dB) and larger in quantity than herbaceous species[13]. The measurement range in these studies is between 20 and 300 kHz. The reason is that below 20 kHz the ambient noise is stronger, whereas in the high ultrasound ranges including MHz range the amount of acoustic emissions are so high that it is technically difficult to properly count and store the signals[14].

However, not all acoustic emissions can be explained by cavitation only. In another study[15], high acoustic activity was recorded both during a period of high water loss by transpiration as well as during re-hydration. In fact, only a minor amount of the recorded acoustic emissions could be attributed to cavitation. When cavitation happens, the end of the broken water column violently retracts along the vessel tube, which causes a characteristic fast fading sound vibration. However, most of the acoustic emissions showed lasting oscillations of acoustic origin, which cannot be attributed to cavitation. It is argued that these bubbles are capable to store and release energy due to their high elasticity. It is assumed that this energy transports water through the xylem in peristaltic waves. This hypothesis points to the cell walls of the xylem as a sound origin, where the regrouping of countless tiny gas bubbles causes sound vibrations[16]. The authors state that the water is actually not lifted up not by the negative pressure caused by transpiration, but by these countless tiny gas bubbles.

Can cell metabolism cause vibrations of the plant surface as well? Ion fluxes in the cell membrane cause a change in the transmembrane potential, which lead to vibrations until a

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[12] Qiu et al. (2002).

[13] Mayr (2016).

[14] Laschimke et al. (2006).

[15] Laschimke et al. (2006).

[16] Laschimke et al. (2006).

new equilibrium is established. These oscillations are supposed to generate acoustic emissions in the ultrasound range[17].

Further on there are assumptions that microtubules, tubular proteins as part of the cyrosceleton of eucaryotic cells, can generate high-frequency mechanical vibrations,[18] with a maximum frequency of 100 MHz to 10 GHz depending on the model. Most models calculate the lowest frequency around 5 MHz[19]. These potential sound vibrations are not addressed in this thesis; the maximum frequency setting was 350 kHz.

Can cell metabolism also generate vibrations of lower frequencies? In studies with bacteria, vibration patterns have been measured, which increased when bacteria were in glucose liquid, but when put in an antibiotic liquid, decreased to almost 0 within 7 minutes. These vibrations which were measured up to around 100 Hz were most likely caused by the cells, prodiving strong evidence for vibrations of lower frequencies linked to the cell metabolism[20].

However, a comprehensive characterisation of the sound vibrations, the differences between species or within the same species, as well as sound emissions under different states of the plant are yet to be identified.

Regarding the effect of sound vibrations on plants, being a rather new research area, there is already a solid basis of studies. On the level of physiological activity, sound vibrations can stimulate germination, growth and yield by enhancing the physiological activity of cells[21], in a certain frequency range and amplitude. Regarding the root system, sound vibrations can increase its total number, length and activity[22]. Further on, a treatment with sound vibrations can lead to a better immunity against pathogens. It can also result in a better drought tolerance; in a study on rice plants, a treatment of 1.5 kHz single frequency resulted in a maximum water content of the plant[23], which increased its drought tolerance[24].

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[17] Perel'man et al. (2006).

[18] Thackston et al. (2019).

[19] Pokolny et al. (1997).

[20] Johnson et al. (2017).

[21] Examples on the effect on cellular level: Sound vibrations can change the secondary structure of plasma membrane proteins, affect microfilament rearrangements, produce Ca 2+ signatures, cause increases in protein kinases, protective enzymes, peroxidases, antioxidant enzymes, amylase, H + -ATPase / K + channel activities, and enhance levels of polyamines, soluble sugars and auxin. See Mishra et al. (2016).

[22] Weinberger et al. (1979).

[23] Jeong et al. (2014).

[24] Jeong et al. (2014).

Thus, it is suggested that sound vibrations can have a priming effect on plants[25].

There are remarking examples of plant response to specific ecological sound vibrations: *Arabidopsis* recognises when only chewing sound of caterpillars are played to the plant, and respond with initiating a defense reaction[26]. The sound vibrations of a buzzing bee caused the flower of *Oenothera drummondii* to produce sweeter nectar within three minutes, increasing the chances of pollination[27].

Regarding the root physiology, the different zones of functions and cellular activities of the root apex are described shortly. The root apex is the place where cell division and elongation happen in a precise way so that new tissue is formed in a regular pattern[28]. Further on it has different functions of coordinating plant behaviour. Starting from the tip there is the cell division zone (0 – 1.5 mm from the tip), the transition zone (1.5 – 3 mm from the tip), the cell elongation zone (3 – 7 mm from the tip) and the mature zone[29]. The cell division zone contains actively dividing cells of undifferentiated type. It is protected by the root cap. The root cap protects the root apex as it grows through the soil. In the transition zone, the cells are undergoing a preparatory phase for rapid elongation[30][31]. They are neither dividing nor elongating rapidly, instead they focus on integrating sensory informations which they receive from the root cap. Based on the received sensory informations it instructs the motoric responses of cells in the overlying elongation zone[32]. In this zone, electric activity peaks and cells are highly active[33].The „root-brain-hypothesis“ formulated by Charles and Francis Darwin states that the root apex behaves as a brain-like organ, which coordinates informations and direct movements of the plant. They documented that the root apex „*acts like the brain of [...] the lower animals; the brain being seated within the anterior end of the body, receiving impressions from the sense-organs, and directing the several movements*”[34]. This „brain“ is located in the transition zone of the root apex. In the elongation zone, the cells then elongate fastly (quadruplicate in just two hours in Arabidopsis), and slow down towards the mature zone. In the mature zone, the

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[25] Mishra et al. (2016).
[26] Appel et al. (2014).
[27] Veits et al. (2018).
[28] Torrey, J. et al. (1977).
[29] Baluška (2013c).
[30] Baluška et al. (1996).
[31] Sivaguru, M. et al. (1998).
[32] Baluška et al. (2013a).
[33] Baluska et al. (2013a).
[34] Darwin (1880).

cells elongate slightly further to reach their mature lengths. Root hairs also fully develop in this zone[35].

Plants perform complex information processing; they do this with the use of action potentials and of synaptic modes of cell-to-cell communication[36]. How the integration of sensory informations among different plant organs and areas works are still unknown – discovering the underlying signalling mechanisms is the target of the emerging field of Plant Neurobiology[37]. Plants make decisions and can learn from experiences – such behavior is researched in the field of plant intelligence. Regarding their sound emissions, it is still unknown if a plant can regulate these and if they have any adaptive value.

In this thesis, firstly comprehensive measurements and analyses of pea and maize seedlings were conducted in a pilot study (experiment 1). In the pilot study it was found that beside the shoot apex, the root apex was the most active area in emitting sound vibrations. Among both plants, the maize root emitted the strongest vibrations. Based on these results, a series of follow-up experiments are conducted with maize seedlings focusing on the sound vibrations of the primary root (experiment 2). In this experiment 2, the correlations of the sound vibrations to environmental conditions (i.e. humidity, light) and the reactions to the cut-off of the root tip as well as the leaf will be investigated (experiments A - F).

The differing cellular activities and functions of the root apex zones might cause differing vibration patterns. In experiment A, the vibration patterns of the cell division zone and the elongation zone will be investigated and compared.

When measuring vibrations of one spot it is interesting to understand if these vibrations are actually generated in that spot or are just transmitted to there from another part of the plant. Especially for strong vibrations this would be relevant to understand.

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[35] Baluška et al. (2006a).

[36] Baluška et al. (2006b).

[37] Calvo (2016); in fact, plant lack the foundations of a nervous system, the neurons. However, they have neurotransmitters (e.g. dopamin, glutamate, serotonine), and GABA which can act as a signalling molecule. 'Neuroid conduction', the propagation of electrical events in the cell membranes, takes place in plants as well.

Regarding possible adaptive values of vibrations for the plant, an insight into to what extent the vibrations are carried within the plant would give further indications whether these vibrations have an informative value or functions for other parts or processes within the plant. This will be investigated in experiment B.

An injury causes strong reaction processes in the plant. It will be investigated if the sound vibrations change when the leaf (experiment C) gets cut-off. In experiment D, the root will be decapitated at the transition zone, and the reaction is measured in the elongation zone which is located above the transition zone. Already Charles Darwin and his son Francis Darwin have done development studies on maize roots including the racapping of the root cap, and formulating the „root-brain-hypothesis“. However, these findings remained a hypothesis because later experiments could not confirm that the root apex acts like a sensory organ controlling root tropism. But as turned out, in those experiments the root cap has just been removed badly. Later studies could prove the hypothesis[38]. If solely the root cap is removed without wounding the cell division zone, the root is able to regenerate it within 30 – 40 hours[39]. However, the gravitropic behaviour is lost and the roots grow straightly[40][41], which happens even in a faster time. However, in this experiment D the cut-off removed the underlying cell division zone as well, and reactions are examined.

Regarding light-related behaviour of the plant it is interesting to note that not only aboveground parts of the plant are able to sense light, even the roots have different photoreceptors[42]. Light can penetrate the soil surface and thus reaches the root system as well. The photoreceptors which can sense light at wavelengths from the short-wave UV-B to the long-wave far-red regions are located in the root apex region. Light sensed by the roots of maize seedlings influence the gravitropic bending of the roots[43]. The different types of photoreceptors are specifically located in different root zones. Since blue light cannot penetrate deep beneath the soil, receptors for blue light are mostly located in the roots close to the soil surface. When these shallow roots get exposed to light, these

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[38] Baluska (2013a).
[39] Barlow et al. (1981).
[40] Burbach et al. (2012).
[41] Baluska (2009).
[42] Mo et al. (2015).
[43] Schaefer et al. (1911).

photoreceptors mediate different measures to protect the plant; for maize*,* the roots grow away from the light (negativ phototropic bending), another effect known from the well-studied *Arabidopsis* is that lateral root growth is suppressed. This increases the drought tolerance of the plant[44]. Thus, root phototropism may serve in the optimization of the orientation of the entire root system[45]. In experiment E, the vibration patterns upon illumination will be analysed.

Finally it will be investigated how the vibrations of the root change with increasing drought.

Research question: Do the sound vibrations of maize have adaptive value?

Minor research questions:

Experiment 1 (Pilot study)

A. What are the characteristics of the vibrations in time scale and frequency scale?
B: Can regular vibration patterns be found?
C: Are the vibration patterns specific to the measured organ?

Experiment 2

A: What are the characteristics of the vibrations of the cell division zone and the elongation zone of the root apex?
B: Are the vibrations measured at one point actually created there, or could the vibrations be transmitted from another organ, e.g. the shoot or the root?
C: Do leaf vibrations change when the tip of the leaf gets cut-off?
D: How does the root react to the cut-off of the root tip in terms of vibrations?
E: How does the root react to illumination in terms of vibrations?
F: How does the root react to an increasingly dry condition in terms of vibrations?

# 2 Methods

Measuring instrument:

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[44] Galen et al., (2007).
[45] Kutschera et al. (2012).

First, an ultrasound microphone with a frequency range of 2 kHz – 200 kHz (Avisoft CM16) and approximate sensitivity of 500 mV/Pa has been tried out but over w few minutes no sound coudl be recorded, so it was considered to be not sensitive enough for the small seedlings. In consequence, an optical measurement by a laser Doppler vibrometer was chosen, which enables an undistorted and fine measurement of vibrations; this device measures the distance between the device and the measuring object with a laser beam and visualise the vibrations, thus the change in distance, in the form of amplitudes and frequencies. The device with the highest resolution was chosen (20 mm/s velocity) and successfully tested in the pilot study. For better reflectivity of the laser beam, the measured parts of the seedlings were wrapped in aluminium foil. For the measurement, the seed part was placed on a grid of thin wooden sticks. No temperature change was recorded behind the aluminium foil throughout 15 minutes of laser radiation. It is the first time that the roots of maize seedlings are comprehensively measured by a laser vibrometer[46]. Not the vibration of air, but the actual vibration of the surface is measured. These include the measurements of vibrations which are either too low in amplitude or too low in intensity for moving the air and thus become sound. A clear threshold, which of the recorded vibrations actually propagate as sound is not possible on the basis of the recorded scale of amplitude and frequency. However, this is further discussed in the chapter 3.3 which presents additional findings.

Location:

Since there is hardly any comprehensive research about sound vibrations of plants yet, it is of particular importance to make sure that the recorded vibrations are actually vibrations emitted by the plants, and not ambient noise. For this, the room ideally has to be soundproof so that no ambient noise can enter. The most anechoic room found is a special underground room at Technische Universität Berlin[47]. Above 125 Hz, most frequencies are filtered. Test measurements confirmed some frequencies below 125 Hz. To filter the frequencies below 125 Hz, the device was set to cut-off all frequencies below that. As a further measure to prevent possible interference of ambient noise, the measurements were conducted out of working hours of surrounding places. One reason that the existing studies focused on frequencies from 20 kHz on is because the

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[46] An article by Gagliano et al. (2012) depicts the recording by a laser vibrometer, however, the source of the figure is not available, see chapter Discussion.

[47] http://fd.tu-berlin.de/einrichtungen/reflexionsarmer-raum/ (viewed 08.10.2021)

background noise is weaker in the ultrasound range. The research presented in this thesis was undertaken in an experimental setting in a room close to soundproof, therefore lower frequencies could be well recorded too.

Data analysis:

Since the program from the company of the vibrometer was not sophisticated enough for the data anylsis for this thesis, a Python script was developed in cooperation with physicists[48]. In the frequency analysis done by fast Frourier transform (FFT), a frequency range is considered as dominant when its maximum amplitude is at least 1.5 times higher than the maximum amplitude of the rest of the frequency range. In this way they could be distinguished from general amplitude fluctuations over the range.

Germination and growth condition:

The germination and growth took place in the same space where the measurements were conducted, to ensure same environmental conditions for the seedlings[49]. Seedlings of maize (Golden Bantam, Zea mays L. subsp. mays, from Demeter) were germinated in humid filter rolls wrapped in aluminium foil. The seeds were first soaked in water for 24 hours before being placed in the filter rolls. Each roll contained five seeds and the seed rolls were placed vertically in a cup which contained 20 ml of water (replenished daily). In the pilot study, an additional species was used for comparison: pea (*Pisum sativum,* from Demeter). Upon germination, the seedlings were transferred into aeroponic devices with a constant temperature and humidity condition[50]. Artificial illumination by cold white LED light (6500 K daylight, 470 lm, Paulmann) was now delivered on a 8-h light:16-h dark photoperiod (09:00–17:00 light period).

## 2.1 Methods of experiment 1

The measuring device is a Laser Doppler Vibrometer, Polytec OFV 5000 with the measurement head OFV 505, which can measure frequencies up to 350 kHz. Its finest

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[48] See chapter „Acknowledgement“.

[49] An upbringing in a common growth chamber would include a ventilatory system with constant noise, the later transfer into the silent measurement room would mean a big change of environmental conditions.

[50] Simultaneously measured by Hygro Button data-logger.

setting is 20 mm/s which is enough to record the vibrations of young seedlings. Controls were measured prior and in between the measurement series to properly detect possible ambient noise. The vibrations were recorded in time scale, measuring the velocity. In the data analysis the speed data (in [μm/s]) was converted into position data (in [m]) to have the actual displacement of the plant, meaning the distance and thus the amplitude in which the plant vibrated. For the frequency analyses, which were conducted on the basis of the time scale data, it was useful to have short measurements, because a FFT shows only the average amplitudes of the frequencies in the measurement. So the shorter the measurement, the more accurate the variation of the frequency amplitudes can be analysed[51]. Thus the usual duration of each measurement was < 1 second. However, some longer measurements of several seconds as well as 100 seconds have been conducted as well.

Hundreds of seedlings have been grown and more than 600 measurements were taken. Four parts of the plant were measured: seed, stem, top of stem, and root. The stem was measured at 2 cm from the shoot tip. The top of the stem was measured within the top few millimeters. The first leaf of older seedlings was measured as well. The root was divided into four parts: upper, middle, lower part, and the root tip (lowest few millimeters).

The control should ideally be a non-living object with the same plasticity as a plant, so that it reflects the environmental vibrations in the same manner as the plant. Different objects which come close to that has been tried out, e.g. thin wooden stick or foam. In the end a piece of aluminium foil was chosen which has the same laser reflectivity and the same weight. The pattern of vibration reflection of the control was tested and the foil was adjusted so that both plant and control reflected ambient noise in a similar amplitude.

## 2.2 Methods of experiment 2

Longer measurements in experiment 1 revealed that the vibrations show distinctive patterns over this longer time range. So experiment 2 focusses on longer measurements (up to 100 sec.). With the meanwhile developed Python script it became possible to

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[51] In discussion with the technical expert of the vibrometer company, such a short measurement time was thought to be most useful for the characterisation and analysis of the vibrations.

properly analyse longer measurements which were recorded with the finest setting.

Two laser Doppler vibrometer were used to enable parallel measurements (Polytec VibroGo and IVS 500, for single measurements until 100 kHz, and parallel measurements until 25 kHz). It was tested if the two devices indeed display the vibrations in the same amplitude and frequencies, by producing certain noises; this could be confirmed. First, parallel measurements were conducted with the control. In this way, ambient noise could be precisely distinguished, and the characteristics of plant vibrations could be identified and results from the pilot study could be confirmed, and further parallel measurements of different parts of the plant could be conducted. With the available technology, a parallel measurement was possible with frequencies up to 25 kHz. However, single measurements were possible up to 100 kHz. So measurements of frequencies over 25 kHz are single measurements.

The number of seedlings were reduced to 5 in each of the experiments (A – F) in order to keep the research in the frame of this thesis.

For the characterisation as well as comparison of the vibrations, the mean absolute value of displacement and the maximum amplitude of each measurement is used. The average amplitude is not used because the plant vibrates in a very wide range of frequencies so the average amplitude turns out to be extremely small (in the scale of 0.0001 nm), the same counts when only a part of the frequency range is selected, e.g. the first 20 kHz. So instead, the loudness of the vibration was measured by using the average absolute value of displacement. Displacement is the distance which the plant is moved in space during vibration, creating a vibration pattern in time scale. The average of all values of the sound waves are the average absolute values of displacement, further on in this thesis abbreviated as „average value“[52].

### 2.2.1 Experiment A: Comparison of different zones of the root apex

In this experiment, four-days-old seedlings were used. Measured points were the cell division zone (1 mm from tip of the root cap), and the cell elongation zone (3 – 7 mm from

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[52] This method was discussed and confirmed by Dr. Boné from Max Planck Institute of Astronomy.

the tip). A parallel measurement of the transition zone would have been too close for the two laser beams.

### 2.2.2 Experiment B: Transmission of vibrations within the plant

The roots were between 7 to 10.5 cm long, the shoots between 3.5 to 7 cm. The shortest distance between two measured points was 7 cm (5 cm root, 2 cm shoot). The duration of measurement was 1.5 minutes. This enables to depict the dynamics of the root vibrations and thus investigate if this dynamic is shown in the shoot as well.

A parallel measurement of two spots on the plant was conducted. As one measuring point the root tip was chosen, one of the most strongly vibrating parts of the Maize seedling. As the second point, a central part of the shoot was chosen, which is another organ and which does not generate strong vibrations in the amplitude range of the root. So it is suitable to investigate if the vibrations of the root tip are transmitted to here.

The measurement points were the middle part of the shoot which was 1 – 2 cm from the shoot tip, and 2 – 3 mm from the root tip which is the highly active transition zone. The shoot was between 5 and 8 cm long and the root between 2 and 5 cm long. The shortest distance between the measurement points was 6 cm (5 cm root and 1 cm of shoot).

The duration of measurement was 1.5 minutes x 4. This enables to depict the dynamics of the root vibrations and thus investigate if this dynamic is shown in the shoot as well.

The criteria to confirm a transmission of vibration through the plant is that stronger vibrations of the root are visible in the vibration patterns of the shoot. The stronger vibrations have to occur at the same time, the pattern might be a bit different due to the differences in the tissue. If there are dominant frequencies of the root vibration, they would be visible in the shoot measurement as well, because vibrations do not change in frequencies when transmitted.

In the pilot study as well as in experiment 2A, patterns of plant vibrations were identified (as a difference to ambient noise (control)). The maximum amplitude of the control was mostly in the range of 0.5 to 1.5 nm, the highest amplitude of ambient noise reached 10 nm. In order to distinguish ambient noise in this analysis, vibrations only with a higher amplitude than 20 nm will be considered.

### 2.2.3 Experiment C: Reaction upon injury: Leaf

Plant and control were measured parallelly. The leaf was wrapped in aluminium foil except the tip of the leaf. 5 mm of this tip was cut-off, and the change of the vibration patterns were measured 2 – 3 mm from that part. The first measurement was taken 10-20 seconds after the cut. The measurement was divided into several measurements of 90 seconds each, with a 30 seconds break in between[53].

### 2.2.4 Experiment D: Reaction upon injury: root tip

The measurement range was up to 100 kHz. Five days old Maize seedlings were used. The first two centimeters of the root is wrapped in aluminium foil so that the radiation of the laser beam does not fall on any part of the root. The root apex is cut at the transition zone (1.5 – 3 mm from the root tip). The position of the laser point stays the same throughout the measurement series. After the cut-off, five subsequent measurements of 100 sec. each were conducted, so 8 min. 20 sec. in total, with 20 sec. break in before the next measurement starts (which was needed to prepare for the next measurement). This experiment consists of two parts (D1 and D2). In experiment D1, beside the LED light which simulates daylight condition, an additional light was switched on one hour before the first measurement for practical purpose. In order to exclude possible influences which the extra light might have caused on the vibration behaviour fo the root, a follow-up experiment was conducted without extra light (experiment D2).

### 2.2.5 Experiment E: Reaction upon illumination

For illumination, an LED bulb with white light (Paulmann 230 V, 470 lm, daylight 6500 K)

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[53] The maximum measurement time depends on the chosen sampling rate.

was used, which comes close to daylight condition. The light reached the plant with 7 lux. This time, the roots was were 1 – 2 cm long. Due to the short size of the radicle, it was not necessary to wrap the root, instead a tiny piece of aluminium foil was attached and could hold by the moisture on the root surface. The root cap and the transition zone were not covered by aluminium foil. The measurement point was 5 mm from the tip in the elonation zone. Six measurements were conducted with alternating light conditions (off and on), each for 90 seconds (experiment E1). The first measurement was conducted in darkness.

A follow-up experiment (experiment E2) was conducted with continuous illumination over 15 min (a series of 15 measurements of 40 sec. each, with a 20 sec. break in between) with 3 – 4 cm long roots. Only the root cap was not covered in aluminium foil.

### 2.2.6 Experiment F: Reaction to increasing drought

Two groups of seedlings which are in different states of humidity were used in this experiment. One group is freshly rolled out of the filter paper, and its roots were 0.7 – 1.7 cm long. The other group of seedlings are a few days older. Their roots were 2.9 – 4.4 cm long, and have already been growing under aeroponic conditions for three days. They have experienced a humidity level below 70% for a few times, so the roots are more susceptible to drought. The reactions to a humidity level below the critical humidity level of 70% are examined (humidity level of 62.9%).

Measurement point is the elongation zone, 5 mm from the tip of the root. The frequency range was 100kHz. For each seedling, four measurements a 100 sec. were taken, with a break of 30 min. between the measurements so that the effect of water scarcity becomes clearly visible. For the comparison between the seedlings, the average value of displacement and the maximum amplitude of each measurement are used.

# 3 Results

## 3.1 Results of experiment 1

Both pea and maize vibrations have occasional periods of stronger vibrations which do not occur in the control. These periods of stronger vibrations, which are at least twice the maximum amplitude of the basic vibrations, occur irregularly. A longer measurement over minutes revealed that these patterns are not a continuous phenomenon; typically the patterns decline after several seconds (figure 1c).

Within the same species, the occurence of periods of stronger vibrations varies between the organs of the same age, but their time and frequency patterns are similar to each other and difficult to distinguish. Different species differ in vibration patterns.

Comparisons of maize seedlings of different dryness revealed that the seedlings in condition of increased dryness emitted stronger vibrations. The difference in humidity was the only aspect which differed between these two measurements. This result is consistent with the results of other studies where acoustic emissions get stronger with increasing dryness[54]. Experiment F will investigate this topic.

### 3.1.1 Time scale

Pea vibrations:

In the range of milliseconds, pea sound vibrations consists of irregularly occuring single impulses with varying amplitudes (figure 1a), which last for around 0.1 sec.

In the range of seconds, periods of stronger vibrations occur. Stronger vibrations are defined as those in which the maximum amplitude is at least twice the maximum amplitude of the basic vibrations. Vibrations are considered as stronger if the maximum amplitude is at least twice of the maximum amplitude of the basic vibrations. These periods consist of regular impulses in a row, occuring each 0.1 – 0.12 sec. The overall period consists of increasing and then decreasing amplitudes (figure 1b).

The highest amplitudes were measured in the shoot tip in the last repetition of a measurement series, with an amplitude of 1.1 µm. The strongest vibration in the root tip

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[54] Qiu et al. (2002).

reached 800 nm.

In longer time scale, these regular patterns vary in amplitude and duration (figure 1c).

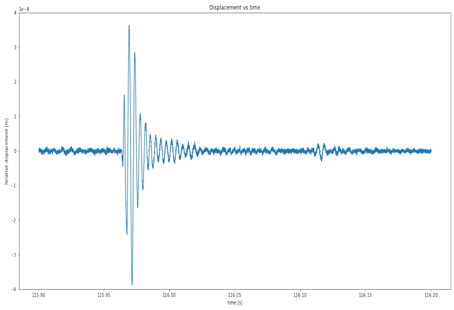

**Figure 1a. A single impulse of pea root.** Recorded until 12 kHz. Duration: 0.1 sec. Maximum amplitude:. 39 nm

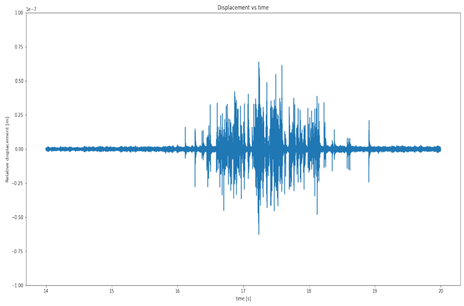

**Figure 1b. A period of stronger vibrations of a pea root.** Duration: 2 sec. Maximum amplitude: 70 nm.

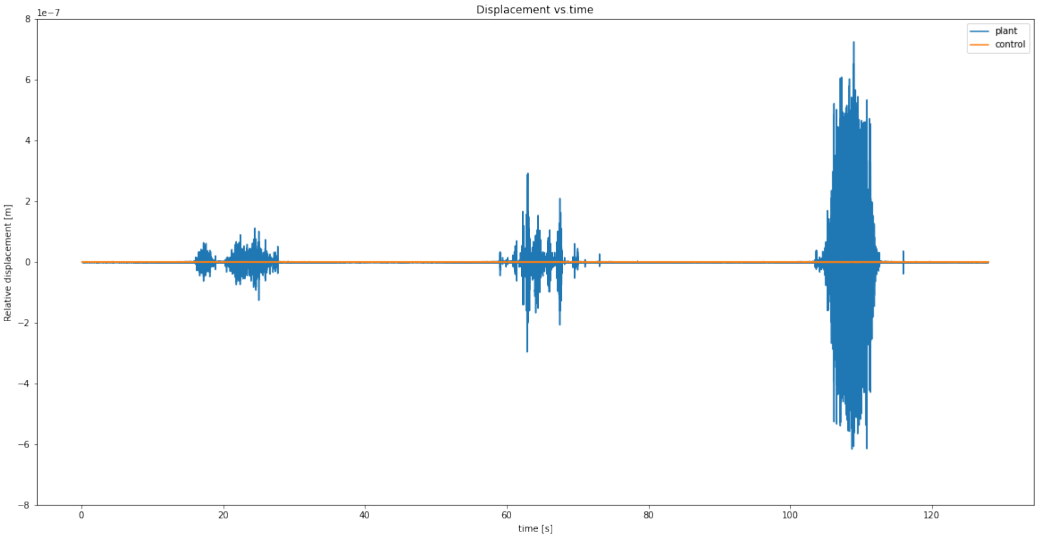

**Figure 1c. A long-term measurement of a pea root.** The vibrations of the mature zone of a pea root (blue) and the parallely measured control (orange) during a 130 sec. measurement.

Maize vibrations:

Maize sound vibrations also contain periods of stronger vibrations. The highest was found in the root tip, reaching 1.2 μm. Same as for pea, a typical pattern is a series of regular stronger vibrations. In 2/3 of these periods, the impulses occurred every 0.1 – 0.14 sec. In the roots, this pattern was most pronounced. These stronger vibrations usually increase, reach a peak and then decrease again, like as in the pea. Single impulses as in figure 1a did not occur. A single impulse within a period of stronger vibrations is depicted in figure 2.

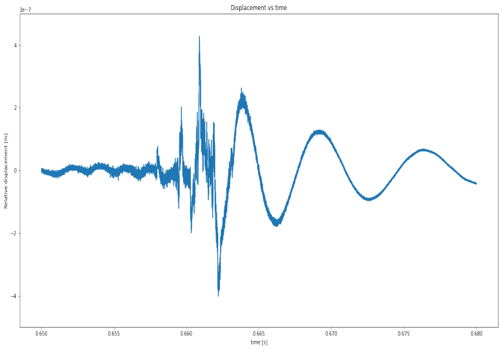

**Figure 2. Strongest impulse of a maize root.**
Duration: 0.1 sec. Maximum amplitude: 450 nm.

### 3.1.2 Frequency scale

Within the frequency curve, dominant frequencies occur. They are highly variable over time, even within one organ of an individual. They can even change within milliseconds. Analyses of root, shoot, leaf and seed vibrations could not result in finding a lasting frequency pattern. Most of the time the dominant frequencies are located within 1 kHz, but those of stronger vibrations even reaches tens of kHz-ranges (figure 4a).

With increasing frequency, the impulses are overlapped by the noise component of the electronic/measuring device. This is seen by a continuous increase in amplitude with increasing frequency from around 10 – 20 kHz.
The height of this curve depends on how strong the plant vibrations are, but the gradient of the curve is generally the same. Thus, with this measuring technique, the emitted frequencies in the higher ranges remain unclear.

Frequencies of pea vibrations:

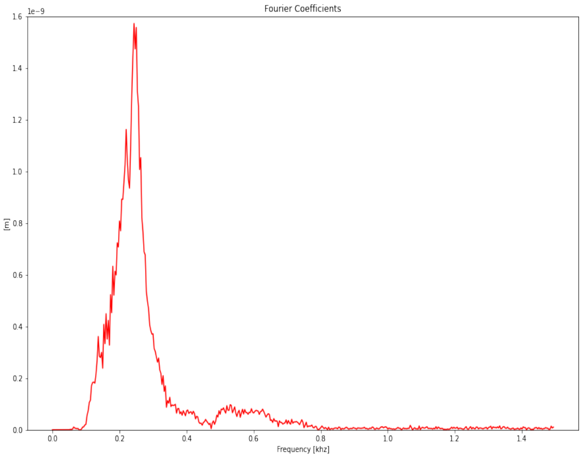

**Figure 3a. Frequency curve of the impulse depicted in fig. 1a.** Beside the highest peak around 220 Hz it contains dominant frequencies around 500 – 800 Hz. Further analysis of the Pisum root revealed a frequency peak at 400 Hz.

Frequencies of maize vibrations:

Ultrasound vibrations only occurred in the highest impulses, and can vary within milliseconds. For example in the highest impulse depicted in figure 2, the highest frequency peaks are found in the ultrasound range at 36 kHz and 22 kHz. However, in the second impulse which followed 0.38 sec. later, the previously dominant frequency of 36 kHz is not dominant anymore (figure 4b). Previous as well as subsequent stronger vibration patterns in that measurement do not contain distinctive dominant frequencies.

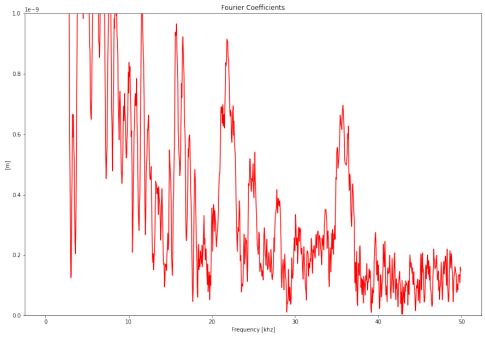

**Figure 4a. Frequency curve of the impulse depicted in fig. 2.** The highest frequency peak is around 36 kHz. Scale of the y-axis: 1 nm.

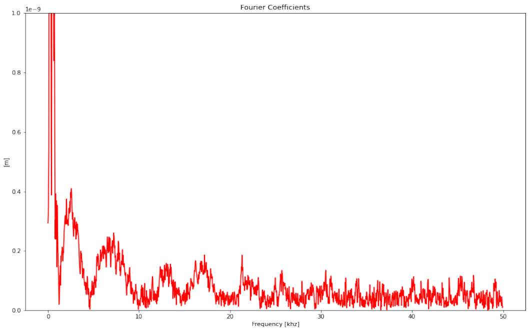

**Figure 4b. Frequency curve of the second highest impulse.** It followed 0.1 sec. later and contains dominant frequencies up around 20 kHz. The previously dominant frequency of 36 kHz is not dominant anymore. Scale of the y-axis: 1 nm.

It can be generalised that dominant frequencies can change within milliseconds. Actually, many months of analysing the change of frequencies in detail revealed a surprising variability.

How do the vibrations differ among the plant organs? In both pea and maize, the vibrations of the root tip as well as the shoot tip showed stronger vibrations than the stem and the seed. The vibrations were strongest in the root tip of maize. Vibrations of other organs (i.e. seed) are lower in amplitude.

Further on it was analysed if any correlation of the patterns to environmental conditions (i.e. humidity) can be found. Thus, two measurement series of maize stems with 6 hours time difference after the last watering were conducted. The comparison between them revealed that the stems in the later measurement under increased drought conditions emitted stronger vibrations as well as higher amplitudes of frequencies over the whole frequency range. These initial findings were further investigated in experiment 2.

As a general correlation, higher vibration amplitudes, meaning stronger vibrations, contain a wider range of dominant frequencies.

Based on the findings in the pilot study, the sound vibrations are categorised into three categories: Vibrations with amplitudes less than 20 nm (category A), vibrations with an amplitude range of 20 – 100 nm (category B) and vibrations with amplitudes over 100 nm.

## 3.2 Results of experiment 2

### 3.2.1 Experiment A: Comparison of different zones of the root apex

In the cell elongation zone, more measurements with diffuse impulses were found which were more difficult to distinguish from the basic vibrations in comparison with the cell division zone. Irregular higher impulses occurred, for around 0.1 sec. (figure 5a). The amplitude of the highest impulse reached 9 nm, which is 22.5 times higher than those of the average rest of the vibrations in that measurement.

As for the frequencies, it could be generalised that the cell elongation zone has a dominant frequency range until around 500 Hz, followed by distinctive patterns of dominant frequency peaks occurring up to 2.3 kHz (0.003 nm[55]). The short measurements in this experiment could confirm the finding of the pilot study that frequencies change within milliseconds, as shown in figure 5c – e. During the impulse, the highest frequency peak was at 1.7 kHz (0.09nm). Right after the impulse, only a small peak was left (0.035 nm). The simultaneously measured control (figure 5f) does not contain any dominant frequencies. As an example, of two measurements with only 5 seconds in between, the analysis of the impulse of the first measurement (impulse up to 7 nm) showed that it consists of dominant frequencies at 0.7 kHz (0.02 nm), 1.05 kHz (0.006 nm), and 1.3 kHz (0.004 nm). They can only be found within this impulse; other parts do not contain clearly dominant frequencies. A repeated measurement of the same point contains a further impulse (9.5 nm), the analysis of which revealed dominant frequencies at 1.1 kHz (0.06 nm), 1.7 kHz (0.1 nm), 2.2 kHz (0.02 nm), which again exist only in the impulse. This example shows well that on the one side, the measured impulses contain certain dominant frequencies which only exist in the respective impulse. On the other side the impulses, consists of different frequencies. Dominant frequencies vary as well among different individuals of the same age and same length of the root. After the impulse, other dominant frequencies can appear, as a frequency peak of 3.3 kHz appeared after the impulse in the first measurement.

Though dominant frequencies change over time, all measurements contain dominant frequencies in the range of 600 to 700 Hz. In sum, peaks were found at 610, 660 and 700 Hz, among which 660 Hz was only found in impulses. Within an individual seedling under constant environmental conditions, the dominant frequency pattern of the cell elongation zone changes over time – even within milliseconds. The patterns vary between individuals of the same age as well.

As for the cell division zone, impulses occur in close succession, with interruptions (figure 5b). The highest measured amplitude is 7.5 nm. Compared to the cell elongation zone, the range of the lower level dominant frequencies varies more; it reached up to 1 kHz. However, the frequency peaks found in the cell elongation zone could be found in most of

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[55] These amplitudes are relative to the whole measurement duration. So a selection of a more narrow section would show higher amplitudes. However, they can be used as references for comparisons between measurements of the same duration.

the measurements of the cell division zone as well. The impulse in the cell elongation zone has a 7.5 times higher average amplitude than the rest. The amplitude of the highest point is 22.5 times higher than of the average rest.

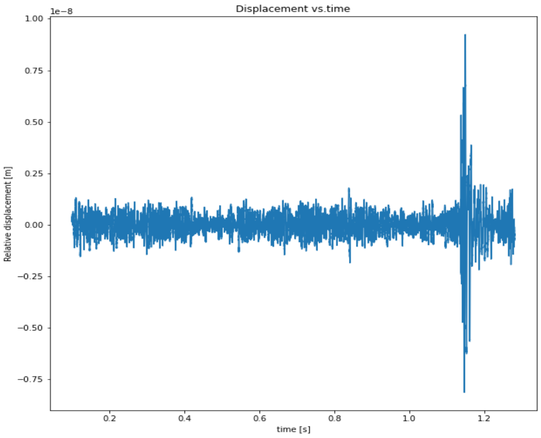


**Figure 5a. Cell elongation zone.** The impulse reaches up to 9.5 nm.

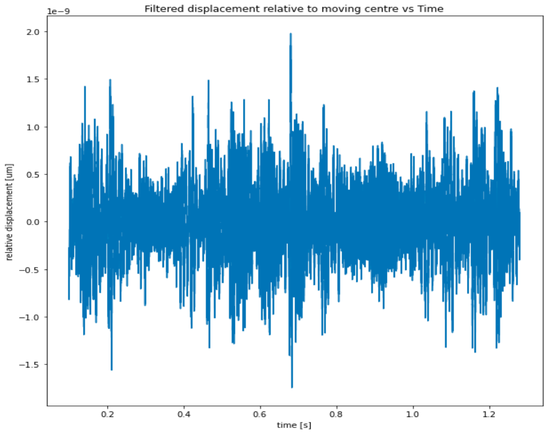


**Figure 5b. Cell division zone.** The amplitudes reach 2 nm.

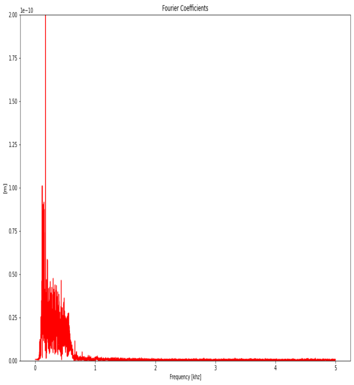


**Figure 5c. Frequency curve of vibrations depicted in fig. 5a, *before* the impulse (until 1.1 sec.).** Scale of y-axis: 20 nm, axis: 5 kHz.

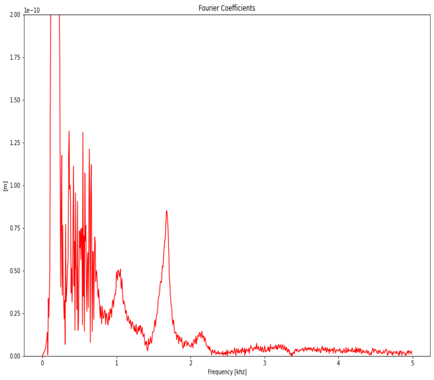


**Figure 5d. Frequency curve of vibrations depicted in fig. 5a, *during* the impulse (1.1 – 1.25 sec.).** Scale of y-axis: 20 nm, x-axis: 5 kHz.

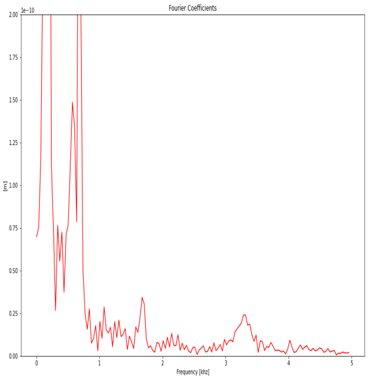


**Figure 5e. Frequency curve of vibrations depicted in fig. 5a, *after* the impulse (from 1.25 sec.).** Scale x- of y-axis: 20 nm, x-axis: 5 kHz.

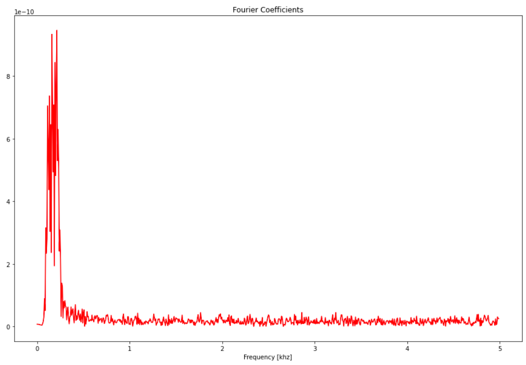


**Figure 5f. The frequency curve of the control during the impulse of the root (sec.1.1 – 1.25).** For comparison, the control does not contain dominant frequencies.

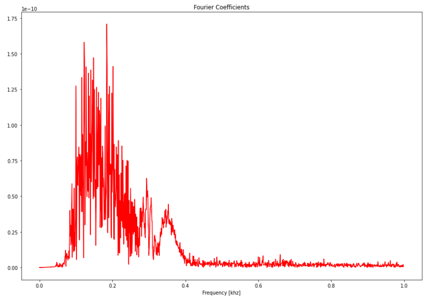


**Figure 5g. Frequency curve of the cell division zone depicted in figure 5b.** It consists of a frequency peak around 360 Hz, which is missing in the control (figure 5h).

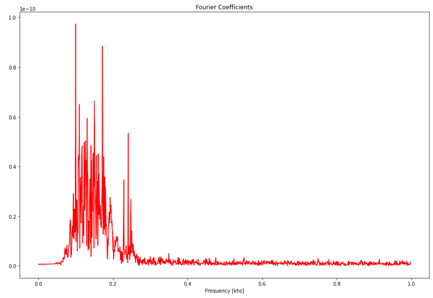


**Figure 5h. Frequency curve of the control of the vibrations depicted in figure 5b.**

Both measured root zones contain a range of dominant frequencies, which reach up to 500 Hz in the cell elongation zone and up to 1 kHz in the cell division zone. Further on in both plant root regions impulses occur, which have an amplitude of 4 - 10 nm, compared to the basic vibrations of 0.5 – 3 nm. The impulses contain frequencies, which are missing in the rest of the measurement.

On the basis of the given data, vibration patterns of the plant root show great variation in time and among individuals of the same age. The analysis shows that these are indeed plant vibrations. However, for determining possible characteristic patterns, a wider data basis is necessary. The same counts for the described differences between the cell elongation zone and the cell division zone.

### 3.2.2 Experiment B: Transmission of vibrations within the plant

In all measurements, the root tip vibrations were not visible in the shoot. Both root tip and shoot vibrations have their own vibration patterns, which do not correlate with one another. Even the strongest vibrations of the root tip could not be recognized in the vibrations of the shoot (see figure 6a). As an addition, the strongest shoot vibrations could not be detected in the root tip vibrations. This indicates that the vibrations are not transmitted throughout the plant.

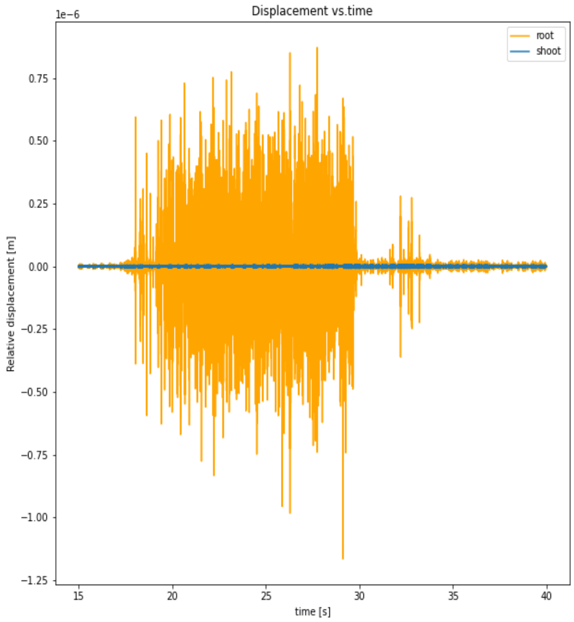


**Figure 6a. Strongest vibrations of a root tip (orange).** They reached up to 1.2 µm, with a duration around 15 sec. The shoot vibrations (blue) are not affected by the stronger root vibrations.

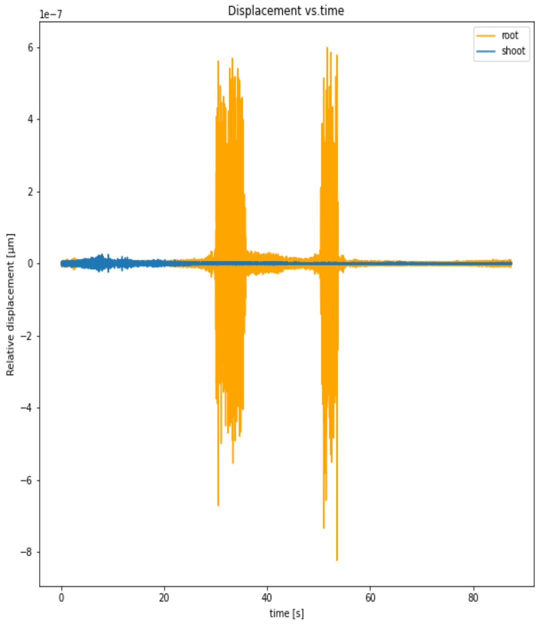


**Figure 6b. Another, more prevalent pattern of strong vibrations of the root tip.** They reached up to 820 nm and lasted up to 10 sec. Both vibrations do not affect each other.

As a further measure to work out the differences between the two vibrations, their frequencies were analysed. If the vibrations are transmitted throughout the plant, the vibrations of spot 1 must contain the frequency pattern of spot 2, because frequencies do not change when transmitted. The analysis of the period of strong root vibrations shows that the root vibrates stronger in the whole frequency range, with dominant frequencies up to 4 kHz, which are missing in the shoot vibrations. This confirms the findings from the vibration patterns.

However, these findings still leave the possibility that vibrations are transmitted within the same organ (figure 6c). Thus, a follow-up experiment was conducted in which different parts of the same root were measured. Results show that the vibration patterns are not transmitted even within the same organ. However, on a lower amplitude level, there are short time impulses, which are seen in both measurements (6c - e). If vibration patterns are seen in both measurements, the possibility of ambient noise needs to be considered as well. An analysis of a lower amplitude range (<10 nm) reveals that occasional impulses from the upper part of the root begin 0.3 sec. earlier than the vibrations in the root apex. This indicates that these are indeed plant vibrations. In 4 of 5 cases during this measurement, these root vibrations occurred prior to strong vibration patterns of the shoot.

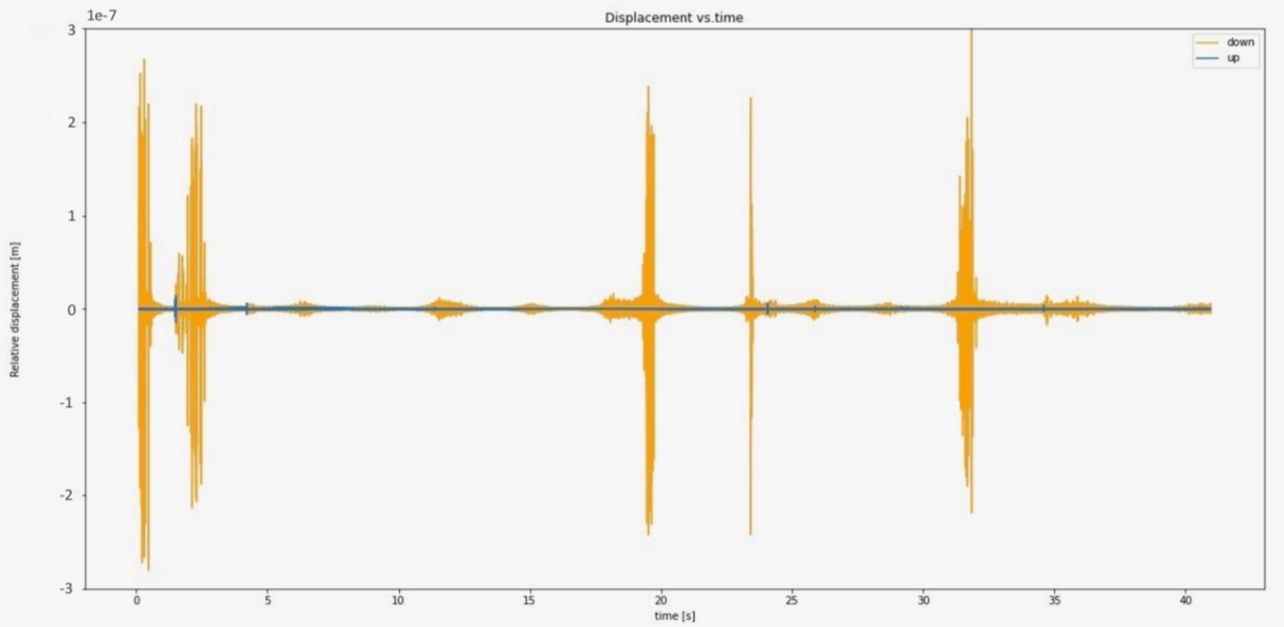


**Figure 6c. Parallel measurements of the root apex (orange) and the mature zone of the root (blue) in a frequency range of 800 Hz – 25 kHz.** The root length was 7.5 cm, the measuring points were 0.5 cm from tip for the apex and 4.5 cm from tip for the mature zone. Scale of y-axis: 300 nm.

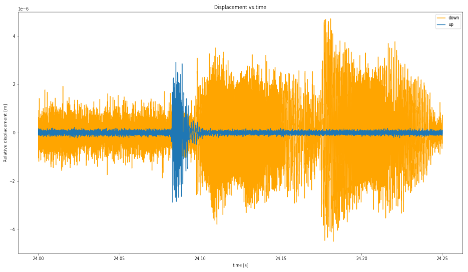


**Figure 6d. Enlargement of an impulse of the mature zone of the root.** It reaches up to 50 nm.

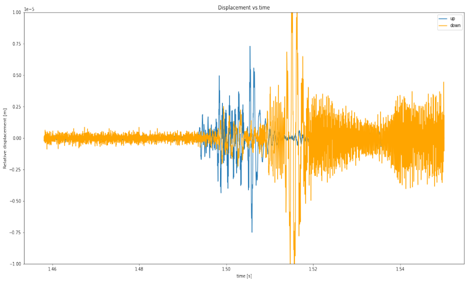


**Figure 6e. A further enlargement of root vibrations.** They reach up to 150 nm.

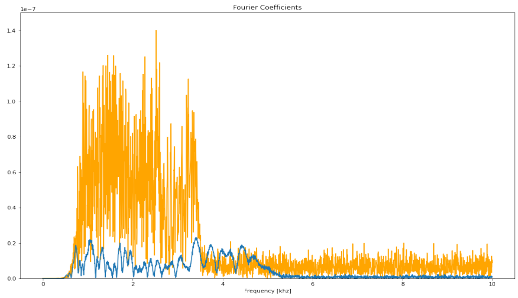


**Figure 6f. Frequency curve of vibrations depicted in figure 6d.**

The frequency analysis of the impulse of the mature zone shows that although it has lower amplitudes, it contains dominating frequencies up to 5 kHz, whereas the root tip has dominant frequencies up to 3.5 kHz. This is contrary to most findings where stronger vibrations also contain higher frequency ranges.

### 3.2.3 Experiment C: Reaction upon injury: Leaf

After the leaf was cut, two of five seedlings showed periods of stronger vibrations of up to seven times higher amplitude (700 nm). In further two seedlings, twofold higher amplitudes were recorded. In one case no difference was seen.

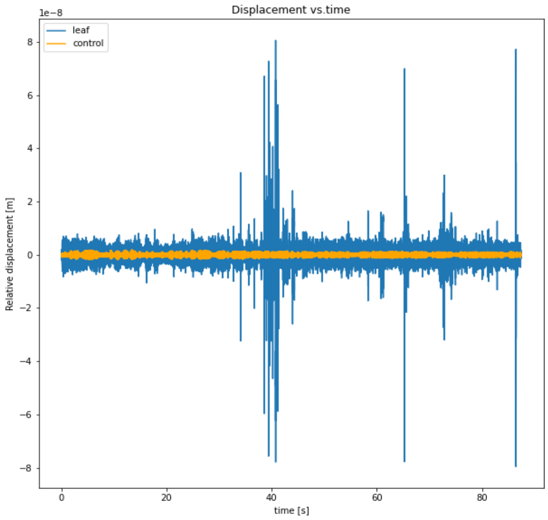


**Figure 7a. Vibrations of leaf (blue) and control (orange) before the cut.** They reached up to 80 nm during a measurement time of 90 seconds.

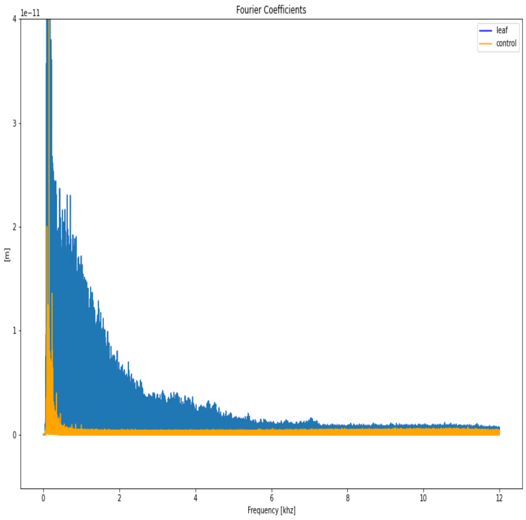


**Figure 7b. The frequency curve of vibrations depicted in figure 7a.** It reveals dominant frequencies up to 7.5 kHz.

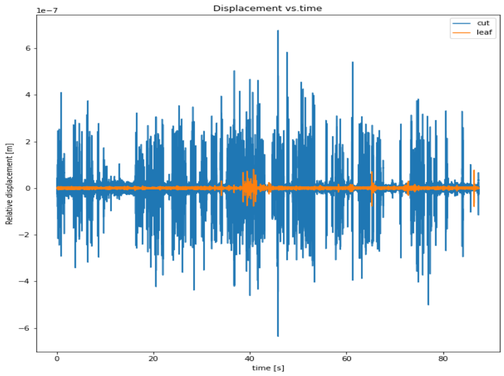


**Figure 7c. The leaf vibrations *before* the cut (depicted in figure 7a, this time in orange), and *after* the cut (blue).** Duration after cut: 6 min.

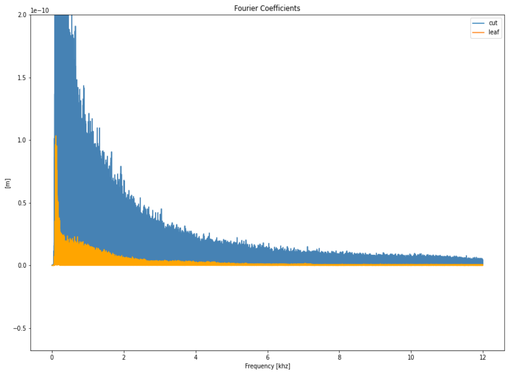


**Figure 7d. Frequency curve of vibrations *before* (orange) and *after* the cut (blue).** The vibrations after the cut vibrate stronger in the whole recorded frequency range.

The average amplitude of the measurement before the cut is 1.52 nm, and 9.42 nm five minutes after the cut; the amplitudes after the cut are 6.2 times higher than the amplitudes before the cut, with the highest amplitudes reaching seven times higher than the one before the cut.

The stronger vibrations after the cut do not occur continuously; in one measurement there were no stronger vibrations for the whole measurement duration of 90 seconds. There is no regularity among the measured points of how long the higher amplitudes continue in close succession (as seen in figure 7c). They consist of varying durations and amplitudes. However, the occasional higher amplitudes continued to occur for the whole duration of the measurement series of 23.5 minutes. The highest amplitude after the cut reached 680 nm, which was 17.4 times higher than the highest amplitude before the cut.

As an additional note, a parallel measurement with the root tip during stronger vibrations of the leaf showed a much lower amplitude of root vibrations (< 50 nm).

### 3.2.4 Experiment D: Reaction upon injury: root tip

Experiment D1: After the cut-off of the root cap, all seedlings shows a more dynamic vibration behaviour of the root. Periods of strong vibrations of the category C (> 500nm) occurred after the cut-off in four of five seedlings (see figure 8a), occurring up to six times in one measurement series.

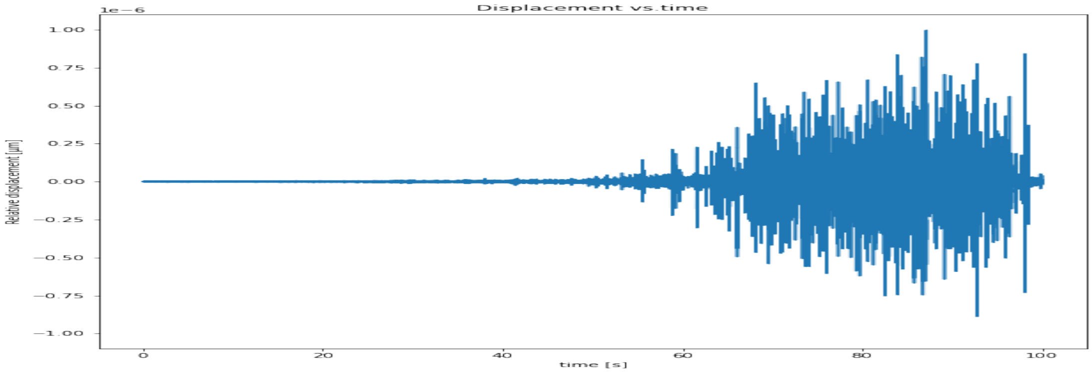


**Figure 8a. Experiment A: Example of a period of strong vibrations of a root after the cut-off.** The strong vibrations started 4 min. 14 sec. after the cut-off.

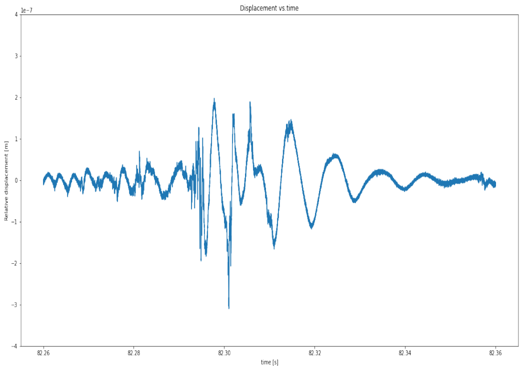


**Figure 8b. A single impulse from the period of stronger vibrations depicted in figure 8a.**

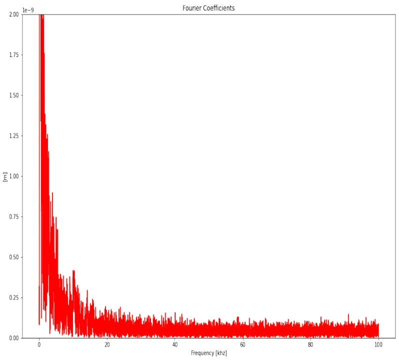


**Figure 8c. Frequency curve of the depicted in figure 8b.**

The frequency analysis of a single impulse shows dominant frequencies around 10 kHz. They were not fround in other impulses, once again confirming the character of fast changing dominant frequencies.

Typically, the pattern of category A (20 – 100 nm) over several seconds occurred after the cut-off; this was the case in four of five seedlings.

Regarding the basic vibrations, in all seedlings they rose to a higher average amplitude in the first measurement after the cut. However, there is no continuous rise of the amplitudes of the basic vibrations. The same counts for the occasionally occuring periods of strong vibrations throughout the minutes of measurement after the cut-off; a period of strong vibration can be followed by minutes without any stronger vibrations.

Experiment D2: Measurements in experiment D2 confirms the results of the previous experiment D1 regarding the patterns. Four of five seedlings reached vibrations of category A, but only one seedling reached vibrations of category B (four times). The period of strongest vibrations were measured 3 min. 17 sec. after the cut and lasted until the end of the measurement 43 sec. later (figure 8d). The average amplitude during this period (sec. 57 – 100) was 31.4 times higher than the average vibrations amplitude before (sec. 0 – 57).

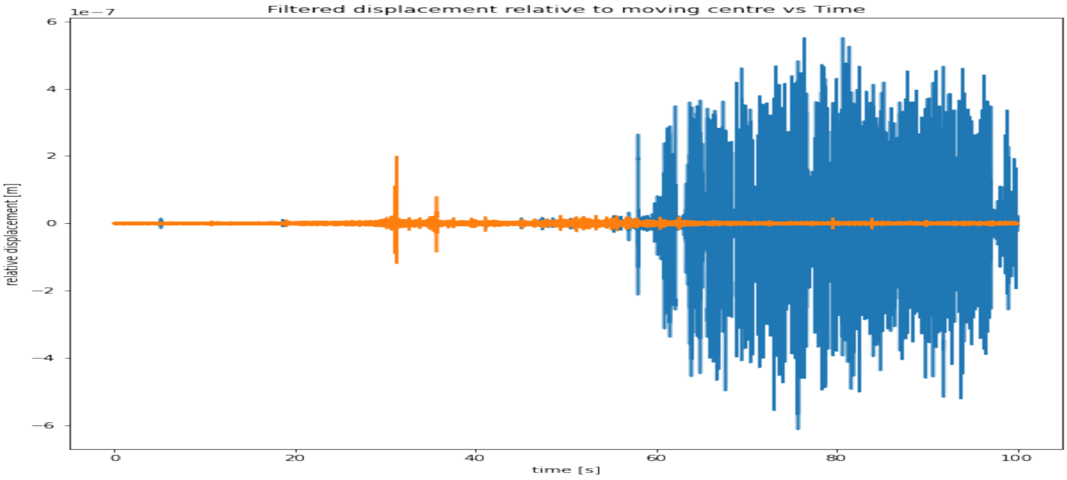
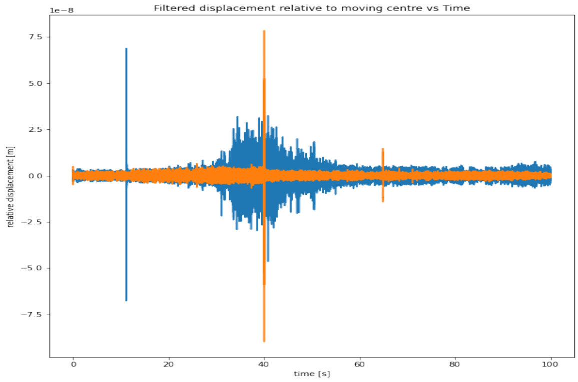


**Figure 8d. Vibrations before (orange) and after the cut-off (blue).**

Besides the amount of periods of strong vibrations of category C, also the amplitudes reached were higher in experiment D2 (up to 1.2 µm).

As a common pattern in both experiments, all seedlings emitted stronger vibrations of category A (until 100 nm, single vibrations exceeding this amplitude can occur). In four of five seedlings, these periods lasted longer than 10 seconds (up to around 30 seconds) (see figure 8e and 8f).



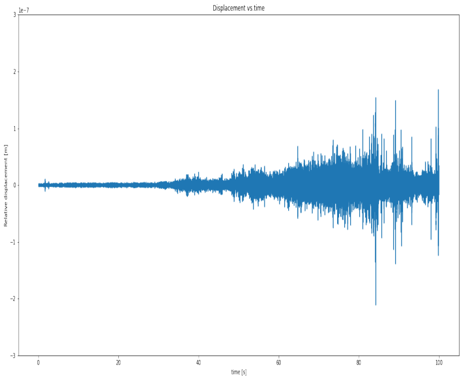
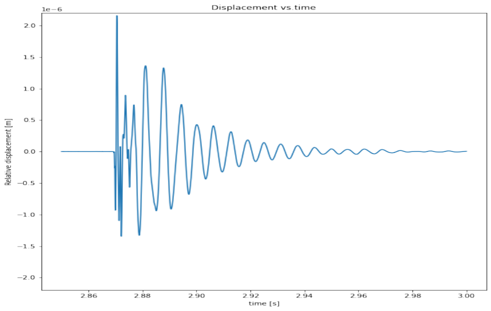


**Figure 8e. Vibration period of category A (20 –100 nm) in D1 (before the cut (orange) and after the cut (blue).**

**Figure 8f. Vibration period of category A (20 – 100 nm) in D2.**

Further on it could be generalised that regarding the strong vibrations, the seedlings differ in the duration when the first period of strong vibrations appear and in their amplitudes.

However, a striking finding is that single, strong impulses in higher ultrasound range occurred. The strongest impulse reached an amplitude up to 2.2 µm (9 min. after the cut-off in exp. D2),  with a frequency peak at 58 kHz (figure 8i).



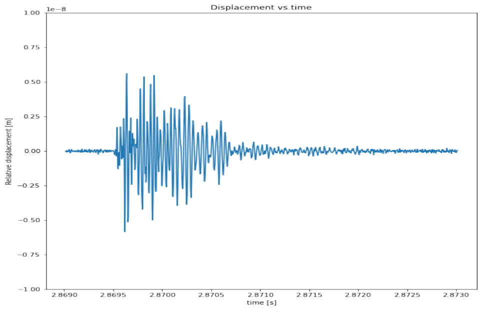


**Figure 8g. An single impulse, occurring apart from periods of stronger vibrations.**

**Figure 8h. Same impulse as depicted in figure 8g, in ultrasonic range.**

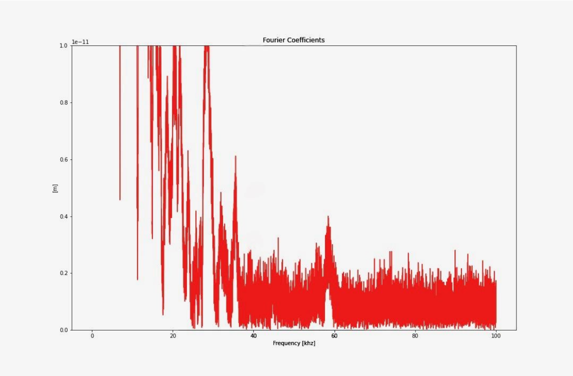


**Figure 8i. Frequencies of the impulse depicted in figure 8g showing a peak at 58 kHz.**

### 3.2.5 Experiment E: Reaction upon illumination

Experiment E1 with alternating light conditions: In three of five seedlings, the root vibrated stronger when the light was switched on. However, in one of the three cases, the value is not much higher than when the light was off (figure 9 maize 3). This difference between the different light states (0.352 nm) is smaller than the differences within the same light state of the same plant (0.042 nm), so the difference is considered as not significant. A correlation of the average value or maximum amplitude to a light state was considered as not significant if the value difference between the light states was smaller than the differences within the same light state of the same plant.

Further on, in three of five seedlings the maximum amplitude reached in each measurement was higher when the light was actually off. Investigations included an analysis of the maximum amplitude and the average value as well as their relation to each other, a chategorization of the higher amplitudes according to their range of amplitude as well as an analysis of their distribution among the two conditions; however none of these approaches revealed any further correlations. In addition, the frequency analysis showed no particular dominant frequencies. Thus, in three of five seedlings, the average value of vibrations were stronger when the light was on, but further correlations of the illumination with the vibration patterns were not found for any of the seedlings.

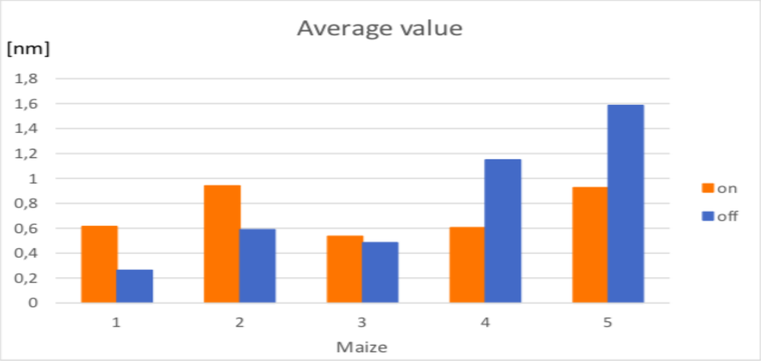


**Figure 9. The average value of vibrations when the light was on (red) and off (blue).**

However, the roots did occasionally emit stronger vibrations during the measurement series. Vibrations with high amplitudes of category B (>100nm) occurred, they were found in both light states and in sum in most measurements periods. Two vibration periods with the highest amplitudes of category C (>500nm) actually occurred when light was off (second and third time).

Since the first experiment (E1) showed conflicting results, a follow-up experiment with continuous illumination (E2) was conducted. This time, a bending behaviour could be recorded. After an initial bending towards the laser (min. 2 – 4), the root moved away from the beam. The speed was highest between min. 4 and 8 (figure 10). However, a particular frequency pattern could not be attributed to the bending behaviour. Vibrations of all measurements, with one exception, remained below 20 nm[56], so they were not adduced for comparison.

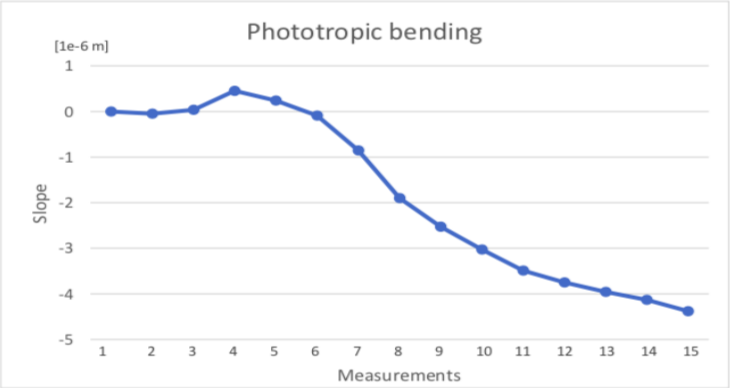


**Figure 10. Example of phototropic reactions of the root.**
The gradient to which extent the root moved from the beginning until the end of a measurement is depicted as a slope.

### 3.2.6 Experiment F: Reaction to increasing drought

Over the time, the vibrations of both seedling groups contain periods of stronger vibrations in amplitude ranges of category B (>100 nm) and C (>500 nm). Both the new and the adapted seedlings have in common that from 60 min. on, the average values are in a similar amplitude range (0.15 – 0.88 nm). After 90 minutes, the average value of both dropped below the level at the beginning of the drought condition (minute 0). However, there is a striking difference between two groups.

As for the new seedlings, after they got exposed to a dry condition for the first time, they

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[56] Vibration patterns in this small range were not considered because they are difficult to distinguish from basic vibrations (see methods).

show a more dynamic vibration behaviour, so that the average values of vibrations among the seedlings differ more (between 0.1 and 4 nm) compared to the adapted seedlings 0.17 – 0.64 nm); the difference between the seedlings of this group is highest at the beginning (exemplary vibrations see figure 1), and the vibrations of the new seedlings have a higher average value than the adapted seedlings (4.02 times higher, figure 11a). After 30 min., the average value of all new seedlings has dropped; they are found in a relatively small range (0.07 – 0.76 nm, figure 11a yellow curve, figure 11c). For four of the five seedlings, this is also the smallest average value over the 90 minutes. The same development can be found for the maximum amplitude of each measurement, which drops from a range of 2.8 – 500 nm (min. 0) to a range of 6.3 – 32 nm (figure 11c)[57]. However, after the initial drop down of the average value, the vibrations begin to rise again during the next one hour (min. 30 – min. 90); from min. 30 to min. 60, the average value increased 2.5-fold compared to the previous value (maximum amplitude increased 6.1-fold), and from min. 60 to min. 90 it increased further 4.2-fold (maximum amplitude 46.3-fold) (figure 11c).

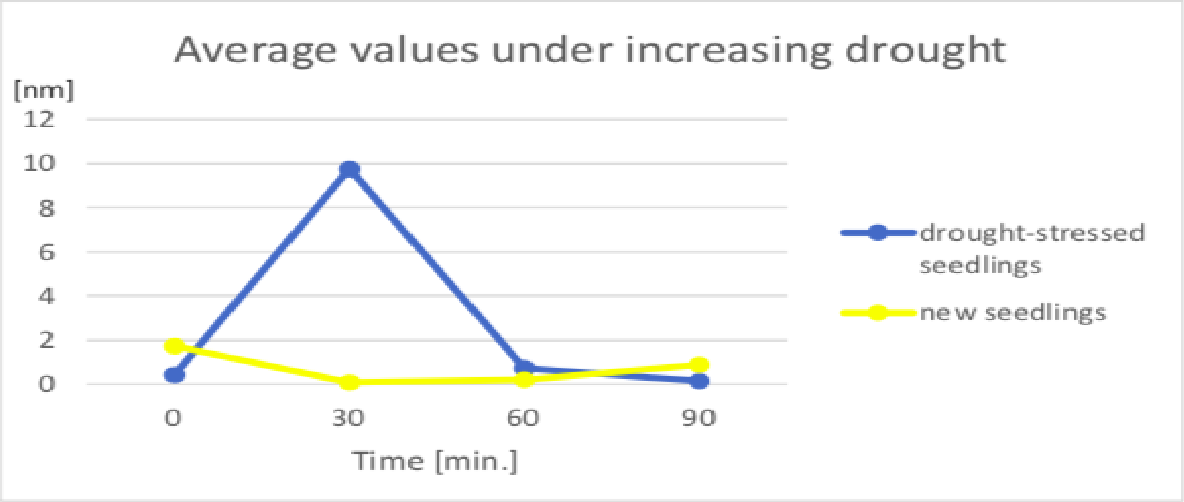

**Figure 11a. Average values of the vibrations of both groups.**

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[57] As a note, the range of maximum amplitudes at a certain time is usually high, because the periods of stronger vibrations occur at different times for each seedling.

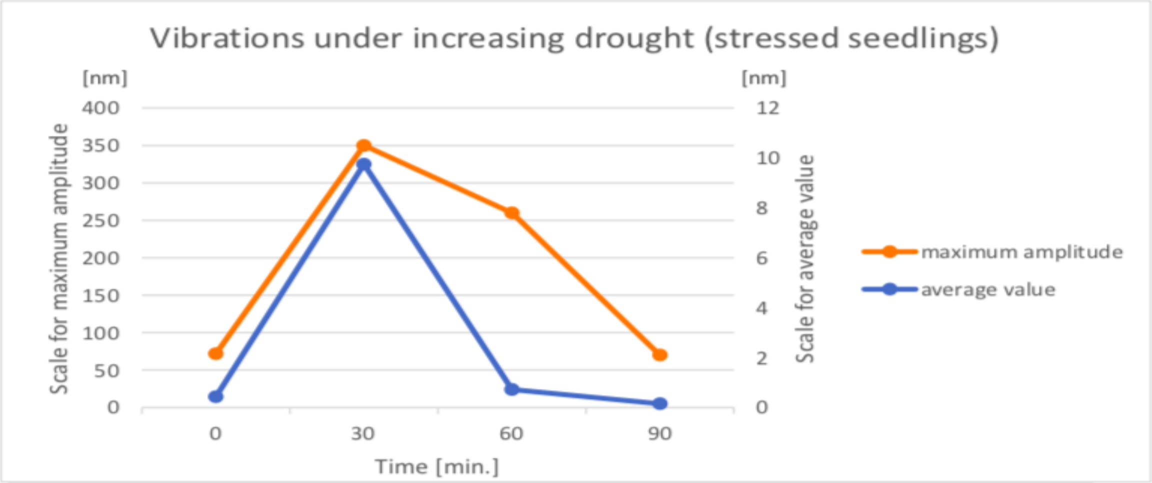

**Figure 11b. The mean average value as well as the mean maximum amplitude of all drought-stressed seedlings over the time of 90 min.**

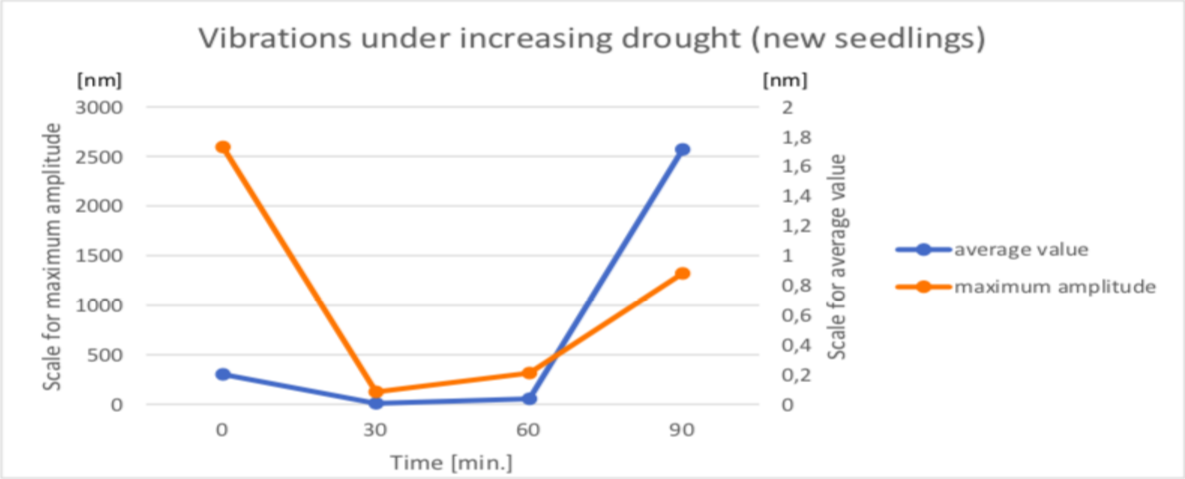

**Figure 11c. Vibration behavior of new seedlings.**

As for the seedlings, which have been exposed to aeroponic conditions for a few days and have already experienced drier conditions, their reaction takes another path. After 30 min., their mean vibration level as well as the amplitude of the strongest vibrations have increased to a maximum (22.7-fold, figure 11b). Afterwards, over the next hour (min. 30 – 90) they drop down to a level lower than at the beginning of the dry condition (min. 0).

A further, striking finding of this experiment is the emission of strong impulses in the higher ultrasound range with the highest mean frequency peak at 54 kHz (figure 12a – e). Further frequencies in the untrasound range which are not continuously dominant were found at 37 kHz, 75 kHz, and the range of 20 – 30 kHz. The highest impulse reached an amplitude of 5.8 µm (figure 12a).

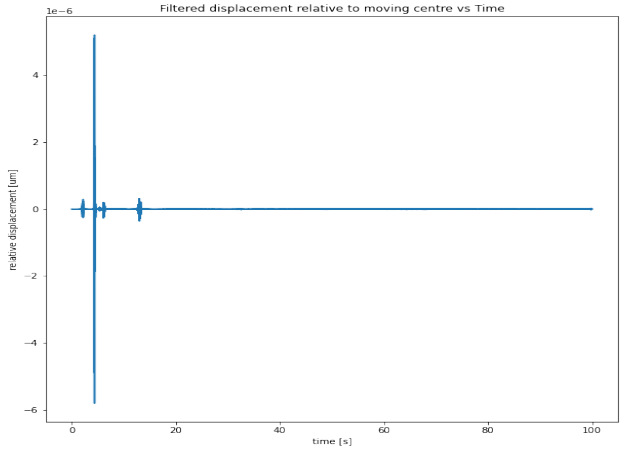

**Figure 12a. High impulse of a new seedling.** It reached 5.8 µm. The highest impulse has frequency peaks in the ultrasound range as well.

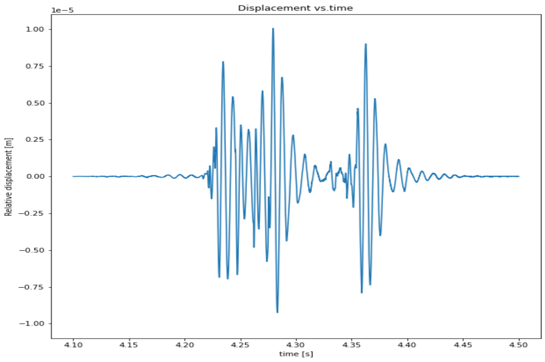

**Figure 12b. The first impulse depicted in figure 12a.**

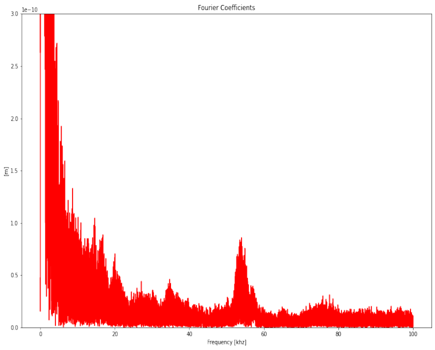

**Figure 12c. Frequency curve of the impulse depicted in figure 12b.**

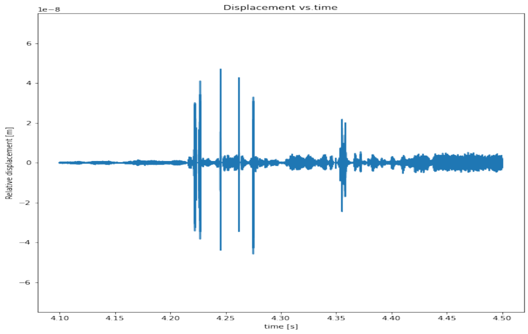

**Figure 12d. The whole impulse depicted in figure 12b, in the ultrasonic frequency range.**

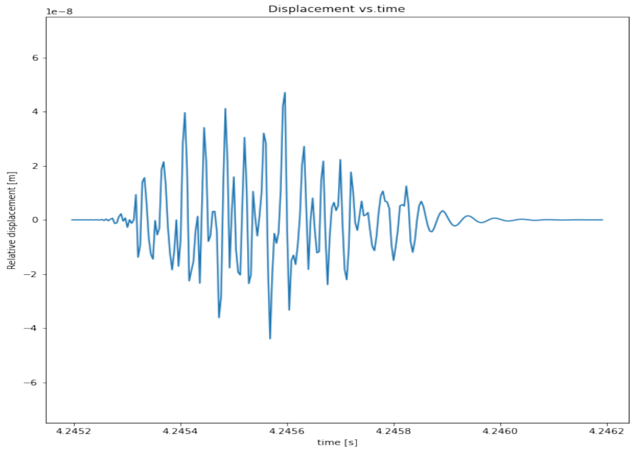

**Figure 12e. Enlargement of a single „click“.** Time scale is 1 ms, with a peak frequency at 56 kHz. It further contains frequency peaks at 22 kHz.

Between these single „clicks“, the frequencies of 80 kHz dominate, with higher amplitudes up to the recorded maximum frequency of 100 kHz.

## 3.3 Additional findings

Characterization of shoot vibrations:

During the experiment 2B, where root tip and shoot was measured in parallel, strong vibrations of the shoot occurred as well. A closer look on the strong vibrations of the shoot makes clear that the impulses of the shoot are not transmitted to the root tip either (figure 13).

Characterisation of the vibration patterns of the shoot (1 – 2 cm from shoot tip) and the root tip (2 – 3 mm from root tip): In these measurements over six minutes, the shoot has slightly more periods of strong vibrations (category C (>100nm) shoot = 11, root = 8), but root vibrations reach a much higher average of the maximum amplitudes of each measurement (1.7 times; shoot = 562.73 nm, root = 967.5 nm). The strongest vibrations of the shoot reached 850 nm, of the root reached 1.2 µm.

Though the patterns of shoot and root do not correlate to each other, the types of patterns can be similar (figure 13a – c, e). In the following, besides comparing both vibration patterns, a closer analysis of the shoot vibrations are conducted. The analysis of shoot vibrations confirms again the fast changes of dominant frequencies. In this example, the frequencies until 1.3 kHz dominate the central part of the strong vibration period (figure 13d), 2 seconds later frequencies around 1.7 kHz vibrate stronger. In sections of strong shoot vibrations where dominant frequencies were visible, they ranged from 1.3 to 2 kHz. Stronger vibrations contain higher frequencies than the basic vibrations. However, a rise of the vibration amplitude did not correlate to a rise of the dominant frequency range.

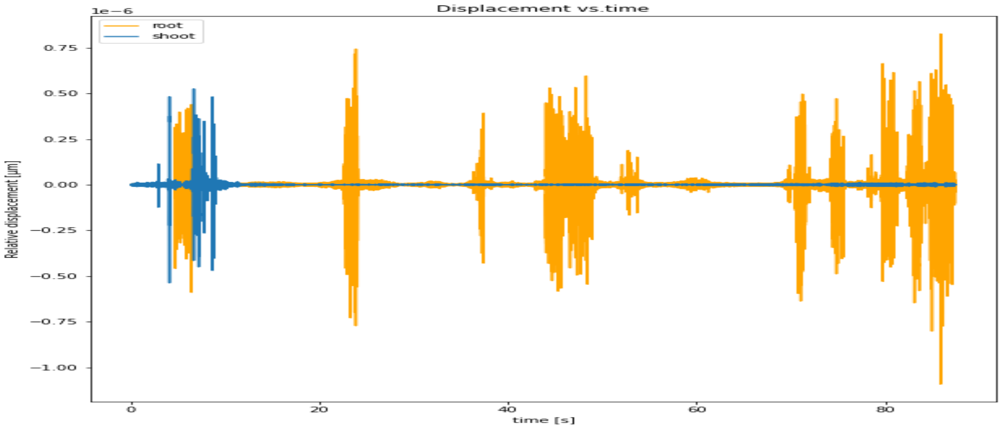

**Figure 13a. Vibrations of similar patterns of shoot (blue) and root tip (orange).** They reached around 600 nm with a duration of several seconds. They both vibrated in the whole frequency range.

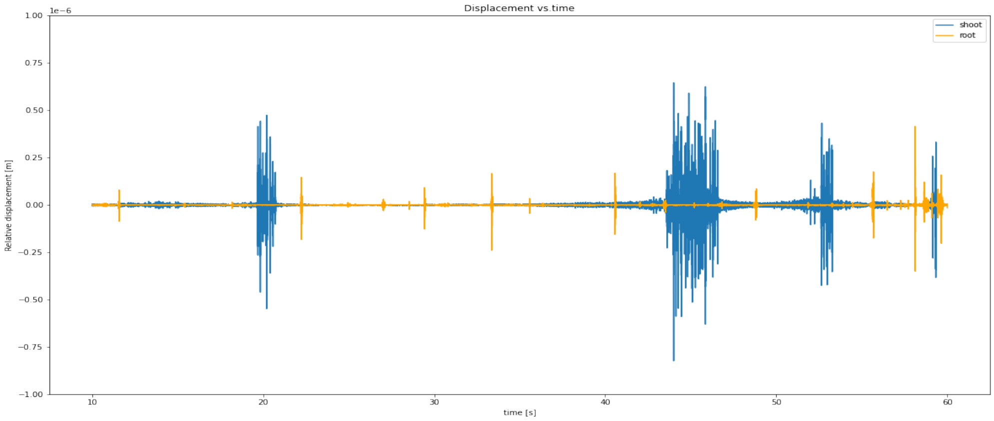

**Figure 13b. Further example of the shoot vibrations pattern.** They are in a similar amplitude range (500 - 850 nm), and last for up to 10 sec. The independence from root tip vibrations are clearly visible.

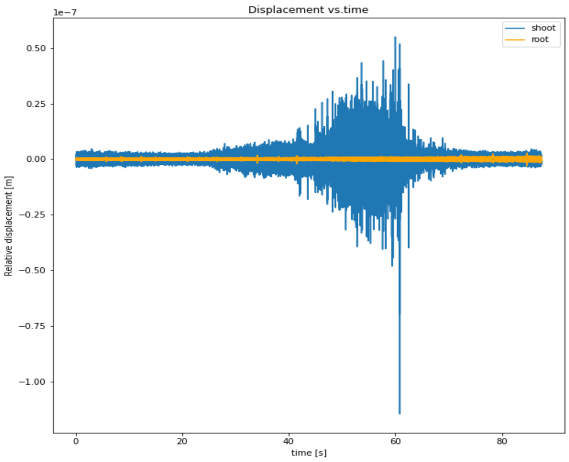

**Figure 13c. Shoot vibration patterns.** They occur on a lower amplitude level (category A (20 – 100 nm)). These vibrations last for around 10 – 30 sec.

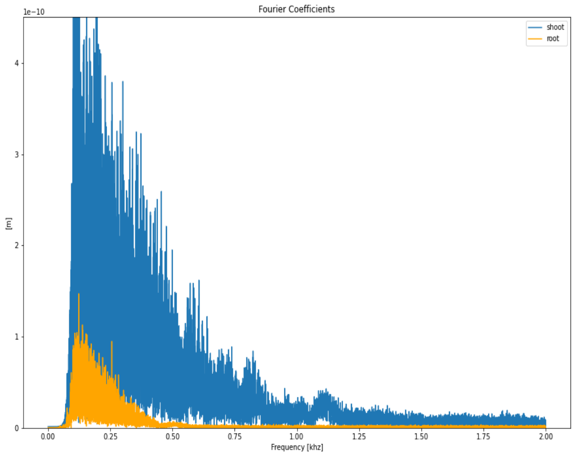

**Figure 13d. Example of frequencies of the part of a stronger vibration period of 10 sec. of the shoot (blue).** It is compared to the root tip (orange).

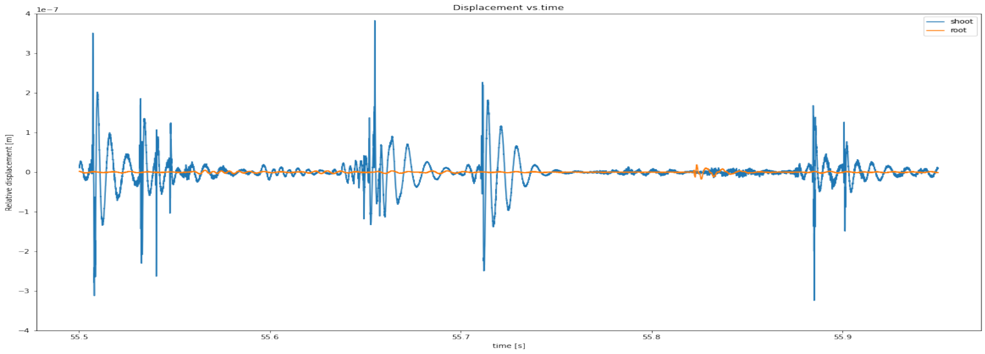

**Figure 13e. Single impulses of periods of strong vibrations of the shoot (blue).** They reached up to 400 nm. They are not transmitted to the root tip.

Lasting displacements of strong vibrations:

Strong vibrations, regardless of the duration, cause a lasting displacement of the seedling in space (figure 14). Depending on the duration of these vibrations the root might be placed back to its previous position, or it could keep growing at its new position. A strong vibration of 1.1 µm amplitude (with several peak frequencies up to 75 kHz, lasting for 0.1 sec.) caused a lasting displacement of around 1.8 µm. It is the first time that plant vibrations could be recorded in such a precise way. All vibrations of category C caused a lasting displacement of the root. Whether this phenomenon has any adaptive value is yet to be researched.

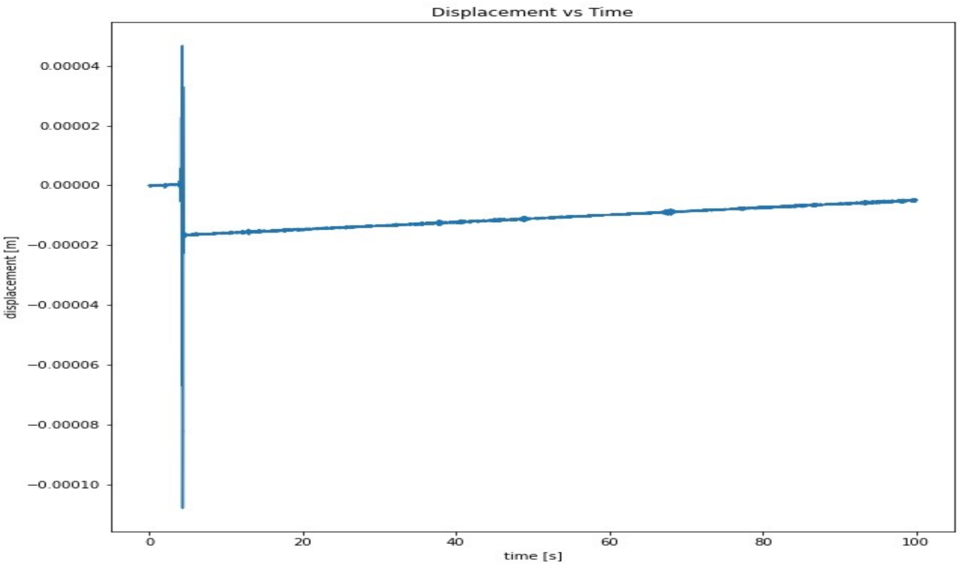

**Figure 14. Depiction of a strong impulse which caused a lasting displacement of the root.** Note: The slight slope of the vibrations over time is technically related.

Characterisation of the maximum amplitudes:

As the graph (figure 15) shows, there is a tendency ($R^2$ = 0.13) that the maximum amplitude increases with the increase of the average value of the vibrations. It means, the higher the average value of vibrations, the higher the maximum amplitude of single impulses or periods of impulses reaches (with a correlation of $R^2$ = 0.13).

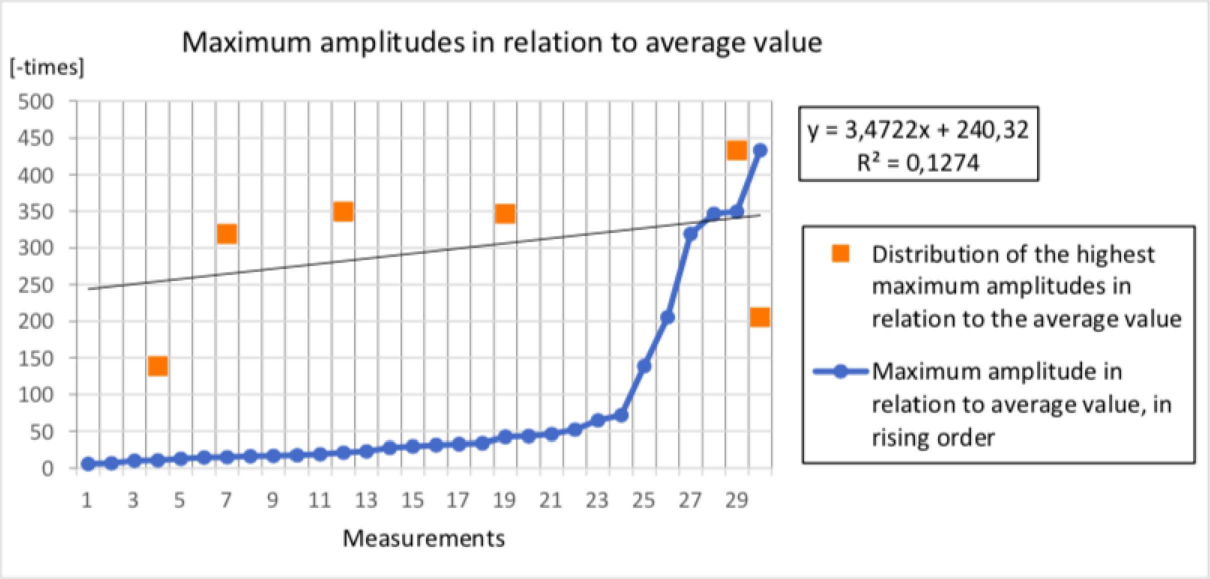

**Figure 15. Relation of maximum amplitude to the average value of vibrations.** The sum of all 30 measurements, arranged in the increasing order of the relation of the maximum amplitude to the average value of the vibrations for each of the 30 measurements (blue). The distribution of the highest values - when

maximum amplitude is >100 times higher than the average value - are shown in orange, with the tendency (black line).

Propagation of the measured vibrations in air:

When looking at the adaptive value of vibrations which are transmitted as sound to the environment, it is essential to find out how far the sound vibrations reach. Based on that, it can be said if an organism in a certain distance can perceive the sound. However, an exact calculation of how far the sound waves travel is not possible because the surface from where the sound is being emitted is very complex in case of a living organism as the plant, but it is possible to conduct a simplified calculation for a crude estimation. For this, the method of Huygen's Principle is applied, with the following results:[58]

Example A (one of the highest measured single impulses): A single impulse of the amplitude 1 µm, emitted by the root tip with a radius of 0.5 mm, vibrates with a frequency of 57 kHz. At a distance of 10 cm from the root, the sound waves of 57 kHz still have an amplitude of 285 nm.

Example B: A period of strong vibrations with an amplitude of 500 nm, emitted by the root tip with a radius of 0.5 mm vibrates with a frequency of 1 kHz. At a distance of 10 cm from the root, the sound waves of 100 nm only have an amplitude of 2.5 nm.

Considering the excluded factors the real amplitude would be even lower. However, it gives an idea about how the sound waves would propagate through air.

# 4 Discussion

Discussion of the methods:

This experiment revealed that even in measurements where no distinctive impulses are seen and the measurement is in a similar amplitude range as the control, plant vibrations could still be determined with the help of the frequency analysis.

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[58] For explanations, see attachment.

Since vibrations are measured directly at the surface of the plant, vibrations which do not expand as sound are likely to be included. This enables an insight into further vibration processes in the plant which happen simultaneously to the sound emissions.

Due to the sensitivity of the seedlings, the reaction they showed could also be the influenced by other factors; e.g. sometimes it was not easy to place them vertically on the grid so their change of position in space might have caused reactions in form of different sound vibrations. The advantage of using a laser vibrometer is that the device does not touch the plant directly, as it is the case in most of the studies on plant acoustics. However, it is necessary to protect the plant from direct laser light, and an attachment of aluminium foil is necessary for ensuring a good reflection of the signal power. The wrapping of the root into aluminium foil might already have caused a stress reaction of the root, which could have influenced the vibration behaviour, for example after the cut-off of the root tip, but also before that; this could explain some stronger vibrations which occurred already before the cut-off in two seedlings in experiment D1 and in one seedling in experiment D2. However, the vibrations were still weaker than after the cut-off.

Due to the necessary breaks inbetween the measurements, it is possible that periods of strong vibrations of less than 20 sec. occurred which was not recorded. So this has to be taken into account when looking at the mentioned numbers of periods of strong vibrations after the cut-off. But still a comparison within the seedlings is possible, because due to the irregular occurance of these periods, the probability that one period was missed during a measurement break is the same among the seedlings.

Discussion of the results:

In general, this study revealed that not only old grown trees or a few weeks old plants are able to emit sound vibrations, but also plants which are only a few days old. Regarding possible mechanisms of sound production, this implies that the production of sound vibrations, especially of ultarsound vibrations, does not depend on an advanced developmental stage of the physiology.

This study also revealed that beside the so far studied species, maize (Poaceae) and pea (Fabaceae) are able to emit sound vibrations as well.

It can be generalised that sound vibrations of maize as well as of pea consists of a close succession of single impulses, each lasting for around 0.1 sec. The dominant frequencies can vary within milliseconds. In the strongest impulses of maize roots, ultrasonic frequency peaks up to 36 kHz occurred. Lower frequencies dominate around 220 – 240 Hz. As tested in another study[59], this is actually the frequency range to which the roots of maize seedlings reacted with a positive bending of their root tip towards the origin of the sound. Possible correlations are yet to be researched. Further on, the clarity of the stronger vibrations vary between the organs. The patterns of stronger vibrations over time vary between the species. The strongest vibrations were measured in maize root tips (1.2 µm).

The different clarities of the impulses in different organs could be related to different tissue structures from where the vibrations originate. The same could be assumed for the root tip of maize which showed more pronounced vibrations, since the cells in the root apex of pea and maize have a different arrangements[60].

Typically, around 5 to 10 sec. prior to and after a period of strong vibrations, impulses of category A (20 – 100 nm) occur.

For the first time, plant sound vibrations which are in the range below ultrasound (20 kHz) could be identified and described. The method of a parallel measurement with the control provided strong evidence that plants emit sound vibrations in the lower ranges from 220 Hz on.

However, also ultrasound frequencies could be successfully recorded and related to different reactions of the plant. Frequency peaks in the ultrasound range occurred up to 75 kHz. Further peaks in this range occurred around 20-22 kHz. Single impulses can occur repeatedly, several times in a 90 sec. measurement. However, most of the impulses do not contain frequencies in ultrasound range – they consist of frequencies in the lower range, mostly below 2 kHz, but can go up to over 10 kHz. The highest ultrasound frequencies occurred in single impulses not accompanied by a period of strong vibrations. However, it was not always the case that impulses with the same amplitude as the one which contains

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[59] Gagliano et al. (2012).

[60] Barlow et al. (1982).

ultrasound, contain frequencies in ultrasound range as well.

Regarding correlations between different types of vibrations, single impulses can occur solely without stronger vibrations happening before, during or after the impulse. However, during periods of stronger vibrations, they occur in higher amounts. This indicates that periods of stronger vibrations might stimulate or initiate these impulses. Since the impulses contain different frequency peaks it is possible that they are of different origins.

The only article stating results of the recordings of maize roots by a laser vibrometer is by Gagliano et al. (2012), which shows a figure of the measurement of the sound vibrations of young maize roots with a laser Doppler vibrometer. However, it is a figure extracted from a phD, which is not available; neither it was possible to get any further informations about the figure from the authors nor the phD has been published, against the statement in the article. Necessary informations are missing, such as the property of the measurement chamber or the growth environment, so it is unknown under which condition the plants were measured (in air or in water, the light condition during measurement etc.) which all affects the vibrations of the plant, as could be shown in this Master thesis. The cut-off frequency is not mentioned either. Also an important point of consideration is that only the velocity of the vibration is shown, which is the speed in which the root was vibrating, but not the displacement in space (so the differing positions), which is what actually matters. The speed can be very similar to the actual displancement, but can also be very different (single impulses seen in the position data are not necessarily reflected in the speed data). So speaking of „loud [...] and frequent clicks“ is not correct because this cannot be concluded from the velocity. For this Master thesis, the data, which were first recorded as speed data, were converted so that it displays the relative displacement of the vibrations, which is the amplitude of the sound vibrations. So a direct comparison with the results in this Master thesis is not possible. However, because of the lack of research in this field, it still makes sense to do a limited comparison with considering the abovementioned limitations. The figure (figure 16) shows a similar pattern of vibrations as could be measured in the frame of this thesis. Frequent „clicks“ are visible within a durantion of 1 sec. It is assumed that the vibrations in the figure of Pagano have been recorded in the ultrasonic range. In this Master thesis, some amplitudes also reached a velocity of 2 cm/sec. So they could actually be plant vibrations, but further informations are needed for verification.

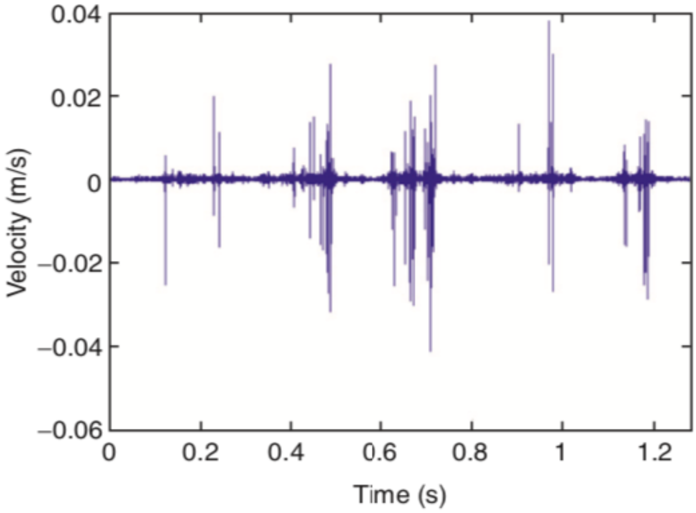

**Figure 16. Maize root vibrations depicted in speed data, by Gagliano et al. (2012).**

Comparing the results of cut and drought-stressed plants, there are clear differences between the vibrations of drought-stressed and cut roots. Regarding the single vibration impulses (those which are not accompanied by a period of lower amplitude vibrations), under dry condition the amplitude was in the range of 5 – 5.8 µm. The pattern of the impulse in the ultrasound range differed from each other. The mean peak frequency was 54 kHz, with further temporarily dominant frequencies. Whereas single impulses of cut roots only reached an amplitude of 2.2 µm, but with a higher peak frequency of 58 kHz. Further peak frequencies were at 28 kHz and 36 kHz, which were more dominant than 58 kHz. This result is coherent with the study on tomato and tobacco plants[61], where a peak frequency in a similar range is found (around 54 – 58 kHz). A further coherence is that cut plants emitted a higher peak frequency (tomato: 57.3±0.7 kHz, tobacco: 57.8±0.7 kHz) than drought-stressed plants (tomato: 49.6±0.4 kHz, tobacco: 54.8±1.1 kHz).

When other vibration patterns are considered as well, further differences can be found. In both conditions, vibrations of category B (>100 nm) and C (>500 nm) occur, which consist of lower frequencies up to 12 kHz. However, the periods of strong vibrations are more pronounced in cut roots. In cut roots, the vibration period last up to 40 sec. and reach up to 1.2 µm, whereas in drought-stressed roots  the period is significantly shorter (up to 5 sec.) and lower in amplitude (up to 620 nm).

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[61] Khait et al. (2019).

Further on, the root react to the cut-off of the leaf as well as the root tip with different patterns. The vibration patterns after the cut-off of the root-tip can differ greatly from those after the cut-off of the leaf tip. Whereas the periods of strongest vibrations of leaves can continue throughout the measurement duration of 90 sec., they are less dense and their amplitudes mostly remain in category B (20 – 100 nm). Considering physiological aspects, it is known that when certain parts of the plant are removed, the plant can actually rebuild it. This is also the case when the root cap is removed. Without the root cap the root can penetrate the soil less[62], which prevents it from reaching further nutritions and water resources. When the root is decapped, the plant initiates an increase in the cell growth and division rate of the cells lying beneath the exposed cell division zone, i.e. a group of cells called the quescient center[63], which do not divide as fast as cells of the rest of this zone[64]. The regeneration happens very fast in only 30 – 40 h. However, in this experiment not only the root cap but also the cell division zone has been removed. Under this circumstance the root might not be able to rebuild its root cap. Instead, there are distinctive cellular activities going on as a reaction to the decapping, e.g. dramatic oscillation in Ca2+ flux[65]. These wound-induced reaction processes might be reflected in the patterns of strong vibrations. Regardless of the possible physiological mechanisms behind the vibrations, results of drought-stressed plants as well as of cut plants regarding the frequencies in this study are supported by recent findings by Khait et al[66]. It would be useful in future studies to remove solely the root cap and compare how the different physiological reactions are expressed in vibration patterns.

When a root gets illuminated, reactions on the physiological level begins immediately[67]. That is why in experiment E1 it was expected that illumination of the roots causes stronger vibrations or distinctive vibration patterns during this period, since the roots are known to show photophobic behaviour. However, this did not happen. The light intensity of 7 lux is enough to cause photophobic reactions of the roots. The roots were fresh and had not been exposed to aeroponic conditions before, so there was no influences of possible water stress. Bending of the roots was assumed to cause vibrations due to the elongation and

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[62] Iijima (2008).
[63] Barlow (1974).
[64] Iijima (2008).
[65] Kochian et al. (1992).
[66] Khait et al. (2019).
[67] Baluska et al. (2012).

growth of cells. The bending behaviour of the root was also analysed and there was no continuous bending. So this result is in coherence with the results that no distinctive vibrations were measured during the periods of illumination. In a study by Baluska et al. (2012), rapid bending of the roots occurred after around 20 min. of illumination with white light[68]. However, in the range of nm as in this experiment, the bending can already be measured earlier. The different results in E1 and E2 indicate that the bending behaviour might have been disturbed by the frequent change of light state. The fastest bending behaviour is accomplished by cells which are transferred from the transition zone to the elongation zone, where they elongate and thus cause the bending of the root tip[69]. However, it might take some minutes until this effect can be measured. This might explain why in an illumination duration of 1.5 minutes, no effect was seen. Another reason could be the age of the seedlings. In only a few days old roots, some physiological developments are not accomplished yet[70], which could effect the phototropic behavior. This might explain why no bending behavior was recorded in the first group. In the second group, which had 2 – 3 cm longer roots, phototropic bending could be recorded, although not all seedlings showed phototropic bending.

To have a look at the processes on the physiological level regarding the effect of illumination, it is known from *Arabidopsis* that the root of a seedling releases massive amounts of so-called reactive oxygen species (ROS), already a few seconds after illumination[71]. ROS act as stressed-induced signals, which regulate, among other processes, root development and growth[72]. This initiates the negativ phototropic bending of the root. The release of ROS happens only seconds after illumination, and spread throughout the plant[73]. The measurement started several seconds after the illumination[74], so ROS might have already begun to be released. If the release of ROS result in vibrations of the root surface within the recorded frequency range of 125 Hz – 25 kHz, it should be visible in the recordings during illumination. However, none of the seedlings showed a correlation of the amplitude level with the light condition.

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[68] Baluska et al. (2012).
[69] Baluška et al. (2012).
[70] Hochholdinger et al. (2004).
[71] Yokawa et al. (2015).
[72] Baluska et al. (2012).
[73] Baluska et al. (2012), Yokawa et al. (2015).
[74] There was a technical distance between the light switch and the computer, so the measurement could start a few seconds after illumination.

Further on, the analysis of stronger vibration patterns indicate that it is most likely that the periods of stronger vibrations are rather related to another stimulus or stimuli. One factor could be the change of environment from the humid filter rolls to a surrounding of humidity less than the required 70%. Another factor could be the wrapping in aluminium foil, which might have caused a stress reaction. However, each seedling reacted differently, and varied in time and duration of the stronger vibrations as well as in the maximum amplitude reached.

The maximum amplitudes reached in this experiment E were higher when the light was off. Also the reactions upon the cut-off of the root tip which occurred when the light was off resulted in stronger vibrations. This could indicate that illumination might be related to the lower amplitudes of stronger vibrations during this state. Further research with longer durations of illuminations would be useful, including different states of the seedlings.

The observation in the pilot study that the seedlings in condition of increased drought emitted stronger vibrations, could later be confirmed. The seedlings in experiment 2 which have been growing in aeroponics for a few days, with experiencing critical humidity lewels, are assumed to be more vulnerable to drought than the new seedlings, which still have some moisture adhering to the roots. So after the drought-stressed seedlings get exposed to a critical humidity level again, they generate stronger vibrations as a reaction to the drought condition, which reaches their peak at min. 30. After that, the roots begin to dry which is reflected in the decreasing level of vibrations. As the study by Khait et al. revealed, the pattern of increasing and then decreasing level of sound vibrations is the same also for tomato and tobacco plants[75]. However, their roots grew in soil, where the humidity level decreases much slower, so the process of increase and decrease of the sound vibrations even takes several days.

Studies done on tomato plants by Qiu et al.[76] show that acoustic emissions do not always correspond to the water condition of the plant; the acoustic emissions increased under moderate water stress, but decreased under severe water stress conditions. This result was confirmed by Khait et al.[77] It was assumed that under mild or in the absence of water stress conditions, there is often a time lag between the transpiration and water supply from

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[75] Khait et al. (2019).
[76] Qiu et al. (2002).
[77] Khait et al. (2019).

the root as long as the transpiration rate increases, which leads to cavitation. Whereas under moderate or severe water stress con- ditions, AE do not increase with the increase of the transpiration rate, because the root system cannot absorb enough water to replenish the conduits with cavitation.

For the new seedlings which are freshly placed out of their filter rolls, with moisture still adhering to the roots, they are assumed to have a better capacity to deal with drought. However, as being freshly rolled out of the filter paper, they immediately react to the huge change of surrounding conditions with periods of stronger vibrations seen at the beginning of the measurements. This might be a reaction to the stress which the seedlings experienced when they are suddenly placed out of their zone of very high humidity to the drier surrounding. 30 min. later, they arrange to the new conditions, and the vibration level drops, which could be close to the level before their initial reaction after they have been displaced. Afterwards, their level of vibrations rises continuously, as a reaction to the increasing drought. However, this process takes much longer than for the drought-stressed plants since they had a far better humidity condition at the beginning of the measurements. It is assumed that coherent with the findings by Qiu et al. and Khait et al., the moisture at the root of the seedlings provide enough humidity to be absorbed by the roots. When this moisture is transpirated and evaporated, the sound vibrations of the new seelings begin to rise, as the figures showed. Further measurements over a longer period of time would be useful to further investigate these results and interpretations.

The existing studies which measured the sound vibrations of plants used microphones, so the unit was in dB, or the amount of acoustic emissions were counted. So a comparison of amplitudes recorded in these experiments is not possible. It was tried out to develop a formula to estimate the measured sound vibrations of maize seedlings in dB, but the calculated results were not realistic. However, based on the frequency analyses the acoustic emissions measured in dB are considered equivalent to at least the vibrations of category C (>500nm) in this study. Parallel measurements in future studies by both laser vibrometer and microphone would enable a better attribution of the different types of vibrations.

The vibrations are not transmitted to other organs of the plant, indicating that they are not attributed to an informative value in this regard. However, regarding the same organ (root), there are impulses which occurred 0.3 sec. prior to stronger vibrations of the other part, so there might be another physiological mechanism correlating to both parts which causes these vibrations. Despite the high variability of the frequencies, the reults of cut and drought-stressed plants indicate that there are sound vibrations with a characteristic pattern in time as well as in frequency scale. These vibrations can propagate through air. As the study by Khait et al. shows, the ultrasound of several weeks old tomato and tobacco plants could be recorded in 10 cm distance, and calculations in this study gave an estimation about the propagation of the recorded sound vibrations. However, also closer distances in the range of mm or even less can be considered regarding possible adaptive values. Since the root emits the strongest vibrations, organisms which live underground, including funghi which penetrate the roots of plants, might be able to perceive and react to these vibrations. It would be interesting to play back the recorded sound vibrations to organisms, and also to the plant itself and investigate possible reactions. In fact, artificial sound vibrations in both higher and lower ranges can initiate processes in the metabolism of plants[78][79]. In further research, it would be interesting to compare the effect of these sounds with that of plant-generated sounds.

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[78] Rodrigo-Moreno et al. (2017).

[79] Gosh et al. (2016).

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# 7 Attachment

Derivation of the formula about sound propagation in air:

For the calculation of how fast the sound wave attenuates in air, minor aspects such as temperature, reflection of the vibration, and energy which is lost for vibrating the air, are not considered. Considering that one spot of the plant is vibrating, the sound waves expand spherically.

Abbreviations:
A: Amplitude of the frequency of the plant.
A2: Amplitude which reaches the surface of an object.
ω: 2 pi* Frequency („angular frequency“)
sqrt: Square root √
r0: Radius 0: Half of the thickness of the tissue which is vibrating, in this case the root.
r: Distance between the plants and a object.

The plant is vibrating with a certain amplitude and frequency, and they have a certain (kinetic) energy associated to it (E).

$$\langle E \rangle = 1/2\ m \langle v^2 \rangle$$

The velocity of the plant is changing (vibrating back and forth), so there is a varying energy value, but constant average energy. Sound is propagating this energy to the other plant. However, we are interested in the average amplitude, because this is the value which the plant receives in average. Calculation of the energy which reaches plant 2:

$$\langle E(r) \rangle = \langle E0 \rangle (r0/r)^2 \text{ (spherical wavefront)}$$

Position on the surface (which is measured), associated to a certain frequency:
$x=A*\sin(\omega*t)$

Velocity of the plant associated with certain frequency (derivaion of the first formula):
v=A*w*cos(ω*t)

Average of the velocity^2
<v^2> = 1/2 A^2*ω^2

This average of velocity will be used for the average energy of the plant surface.
<E0> = 1/2 m <v^2>

Energy which reaches plant 2:
<E(r)> = 1/2 m <v2^2>

v2: verlocity of plant 2:
<v2^2> = 1/2 A2^2*ω^2

If solved for A2:
A2= A(ω)*r0/r

Example A: Single impulse of the amplitude 1 μm, emitted by the root with a radius of 0.5 mm (r0) vibrates with a frequency of 57 kHz (A(ω). The distance to an object (A2) is 100 mm.
A2 = 1 * 0.5/100
A2 = 0.285
The waves of 57 kHz reach the object in 10 cm distance with an amplitude of 285 nm.

## Selbständigkeitserklärung

Hiermit erkläre ich, dass die vorliegende Arbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt wurden, alle Stellen der Arbeit, die wortwörtlich oder sinngemäß aus anderen Quellen übernommen wurden, als solche kenntlich gemacht wurden und die Arbeit in gleicher oder ähnlicher Form noch keiner Prüfungsbehörde vorgelegen hat.

Berlin, den 05.10.2021