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Short note

Non-invasive acoustic sensing of tuberous roots of sweet potato (*Ipomoea batatas*) growing belowground

J. Iwase[1], Y. Sato[(*)1], D. Comparini[2, 3], E. Masi [3], S. Mancuso[3], T. Kawano[2, 4]

[1] *Collaboration Center, Kyushu Institute of Technology, Kitakyushu 808-0196, Japan.*
[2] *Faculty and Graduate School of Environmental Engineering, The University of Kitakyushu, Kitakyushu 808-0135, Japan.*
[3] *Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Università degli studi di Firenze, Viale delle Idee, 30, 50019 Sesto Fiorentino (FI), Italy.*
[4] *Universitee Paris Diderot, Sorbonne Paris Cité, Paris Interdisciplinary Energy Research Institute (PIERI), Paris, France.*

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Abstract: The study applies acoustic means for the detection of belowground tuberous roots of sweet potato by developing a novel non-invasive sensing technology based on propagation of frequency-modulated sound through the soil and its detection with acoustic band-pass filtering devices. The presence of tuberous roots hidden in the bed of sandy soil was successfully detected with the proposed acoustic approach, which is still primitive. However, this investigation may induce further studies and developments for belowground sensing and imaging techniques as novel ecological research tools and to support the introduction of vegetables into so-called "plant factories".

1. Introduction

Petty-Clark's law of economics (formally known as Petty's law) (Tsuchiya, 1993) suggests that the proportion of primary industries declines as the economy of a country develops, and in turn, those of secondary and tertiary industries must increase (Kawata, 2011). Today in Japan, agricultural, fishery, and forestry workers make up about 3.51% of the total working population in the nation. In the past six decades, the agricultural force in Japan has been markedly weakened and thinned, partly due to a nation-wide decline in birth rates and a decrease in new employment in the field (http://www.stat.go.jp/data/roudou/longtime/03roudou.htm).

These factors eventually caused highly advanced aging of the agricultural population in Japan. From a macroscopic point of view, many may expect that the decreasing trend in the agricultural working population may continue based on predictions by the Petty-Clark's law (Nakamura, 2009). In contrast, there would be microscopic growth in the working population in specific forms of agriculture. One of the authors of this paper (TK) is currently proposing two models in which agriculture can be converted to higher industries, i.e. as leisure and as a high-tech food production industry (Kawano, 2014).

In the last two decades, prototypic high-tech agricultural farms (so-called "plant factories") aimed at automating the production of fresh produce, chiefly vegetables, under precisely controlled environments have been developed (Ikeda, 2011; Nishida *et al*., 2012; Nishimura *et al*., 2012). Today, such commercially launched "plant factories" cover sprouts, leafy vegetables, and tomatoes (Morimoto, 2005; Shimizu *et al*., 2008). In order to expand the scope of "plant factories" and enable the automated cultivation of a wide range of agriculturally important plant species, the agricultural and horticultural processes which are a successive series of necessary maintenance steps must be translated or re-documented into automatable protocols based on the records of both plant physiological and mechanical data (Kawano *et al*., 2012).

Despite their agricultural importance, twining plants have not been included in the trends of "plant factory" development, possibly due to the belief that handling of growing vines must be achieved manually by experienced specialists (Kawano *et al*., 2012). *Ipomoea* cultivars are examples of such twining crops, whose aerial parts are herbaceous perennial vines and bear alternate heart-shaped green leaves and sympetalous flowers (Sher *et al*., 2001). In addition, root vegetables or crops bearing root products belowground have not been produced in "plant factories" yet, despite the fact that the growth of roots are central

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(*) Corresponding author: sato@lsse.kyutech.ac.jp;
kawanotomi@kitakyu-u.ac.jp

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to the physiology and ecology of higher terrestrial plants including forest trees and agricultural crops.

In soil- or sand-based conventional agricultural fields, a variety of root vegetables are produced. Such vegetables having highly developed roots include beet (*Beta vulgaris* ssp. *vulgaris*), turnip known as *Kabu* in Japan (*Brassica rapa* L.), carrot (*Daucus carota* L.), radish and *Daikon*-Japanese white radish (*Raphanus sativus* L.), Chinese yam known as *Nagaimo* in Japan (*Dioscorea oppositifolia* L.), and burdock known as *Gobō* in Japan (*Arctium lappa* L.). Among the root vegetables, sweet potato (*Ipomoea batatas* L.), cassava (*Manihot esculenta* Crantz), and yacón or so-called Andes potato (*Smallanthus sonchifolius*) are typical examples of vegetables bearing starchy tuberous roots. In addition to the above true root vegetables, there are groups of undersoil vegetables that develop root-like organs as storage of starch, sugars, and nutrition. Economically important crops with undersoil organs such as corms of taro (*Colocasia esculenta*), tubers of potato (*Solanum tuberosum*), and rhizomes of lotus (*Nelumbo nucifera*), ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), and ginseng (*Panax ginseng*), are examples of vegetables with specialized stems. Furthermore, vegetables producing bulbs can be included in the undersoil vegetables, mostly belonging to *Allium* such as garlic (*Allium sativum* L.), onion, and shallot (*Allium cepa* L.).

Today, soils or sands are hardly manipulated in sensor-equipped "plant factories" while hydroponic cultures are mostly favored. In order to introduce such root vegetables in "plant factories", it is necessary to monitor the belowground properties and processes where plant roots take place, mainly spatial occupation by the growing root system and changes in water, salts, and other elements that can influence productivity and functioning of the soil ecosystem. However, dynamic growth and development of a root system are poorly understood since roots growing belowground are invisible to conventional optical means.

Before facing the challenges to growing under-soil vegetables in the controlled environment inside "plant factories", appropriate non-invasive sensing technologies allowing real-time monitoring of belowground plant growth must be developed and applied. Due to the light absorbing nature of the soils, optical measurements are difficult to apply. Three-dimensional computed tomography with X-ray scanning technology might be a candidate technology if plants are grown in laboratory-scaled small pots (We have developed non-invasive scanning protocols for garlic bulbs, data not shown). However, X-ray scanning cannot be employed on-site in plant producing facilities.

In this study, we attempted to detect the presence of tuberous roots which were hidden in the sandy bed. For this purpose, a novel instrumental set-up was designed for modulation and demodulation of sound signals passed through soil or sand with and without growing plant tissues.

Sweet potato, known as Satsuma-imo in Japanese, is a dicotyledonous crop that belongs to the family of *Convolvulaceae*. Due to its starchy and sweet-tasting tuberous roots, this plant is often considered both a starch producing crop and a fiber-rich root vegetable (Purseglove, 1968; Woolfe, 1992).

2. Materials and Methods

Acoustic sensing system

Frequency-modulated sound signals propagated through sandy soil containing plant tissue samples or not were monitored using a pair of acoustic probes, a flat sound radiator, and sensing microphones (Fig. 1A, B). In order to eliminate the background sound noise, a signal from the reference (ground) microphone was used. To generate the modulated sound signal at the frequency of interest, a function generator (DFG-6020 20MHz DDS, EZ Digital Co. Ltd., Gwangju, Korea) was used (Fig. 1C). Signals received by the sensing microphone were amplified by a AT-MA2 microphone amplifier (Audio-Technica, Tokyo, Japan) and band-filtered to demodulate using an acoustic *band-pass filter* (Multi-function filter 3611 NF Corporation, Yokohama, Japan), and the amplitude of the sound at specific frequency captured through the system was analyzed using an oscilloscope (TBS 1064, Tektronix Inc., OR, USA).

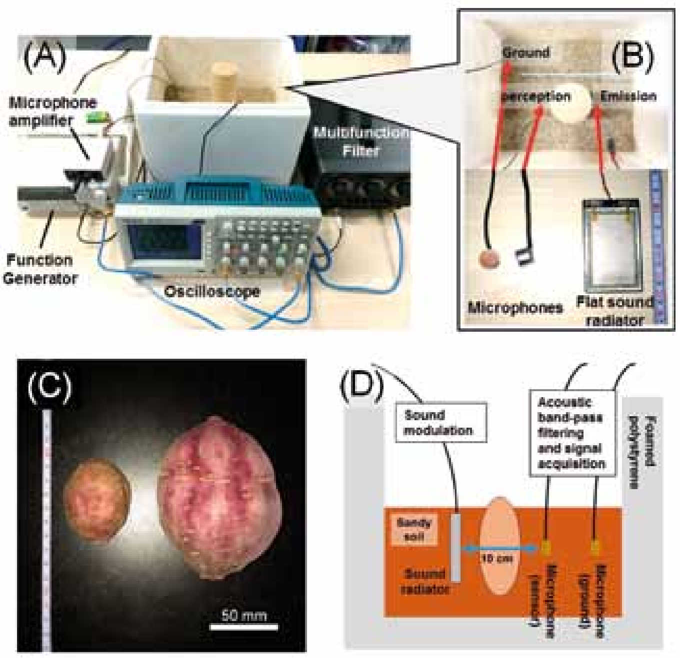

Fig. 1 - Equipment for the acoustic sensing of belowground plant tissues. (A) Experimental configuration. Modulation and demodulation of acoustic signal propagated in a sandy soil bed were preliminarily tuned with and without wooden stick. (B) A pair of acoustic probes designed for emitting and receiving the modulated and band-pass filtered sound signals in the soil or sand. Left, sensing and reference microphones. Right, flat-shaped sound radiator with directional sound propagation. (C) Sweet potato tuberous roots used for demonstration. (D) Experimental design.

Experimental procedure

An experimental set-up (Fig. 1) inside the sand-filled container of polystylene foam with or without model tissues embeded in the sand, was used for acoustic measurements. Band-modulated sound signals were emitted and detected at a constant distance of 100 mm, by a pair of acoustic probes, namely, a directional sound radiator and receivers (microphones), both positioned in the bed of dry (*ca*. 1% of water content) or wet (*ca*. 10% of water content) sands.

The given sound frequency was in the range between 100 and 20,000 Hz. Changes in the height of voltage signal between the peaks and valleys of the recorded sound waves were recorded (Fig. 2A) and expressed as the extent of signal intensity, reflecting the perception of band-pass filtered sound after propagation of band-modulated sound. Mean values of the height of voltage signal obtained at each frequency (n=5) were used to obtain a series of regression lines with the least-square method. Data were recorded in the absence (blank control) and presence of tubers.

Plant materials

Since *Ipomoea* plants naturally grow in sandy fields, and sand-culture is often employed for cultivation of sweet potatoes (Leonard *et al*., 1948; Spence and Humphries, 1971), commercially available river-sand mixture was chosen as tuber root supporting medium. Assuming there is the necessity to monitor tuberous roots inside a plant growth container, a planter made of foamed polystyrene filled with dry or wet river-sand mixture was employed as a model platform for acoustic sensing demonstrations.

Inside the container, two types of sandy soil (volume: 4L; area: 220 mm x 262 mm, depth: 100 mm) with known water content (dry at *ca*. 1 or wet at *ca*. 10% w/w) were tested. Portions or intact sweet potato tuberous roots of different sizes were detected and compared with the absence of any vegetable tissue (blank control). In particular, two intact tuberous roots with a central swelled portion (45 and 82 mm in diameter) (Fig. 1C) were obtained from a local market and used as model vegetable tissues. As a model for sampling tissues with smaller mass, a cut plug (cylindrical section of 20 mm in diameter) of tuberous root was prepared and used for acoustic measurements.

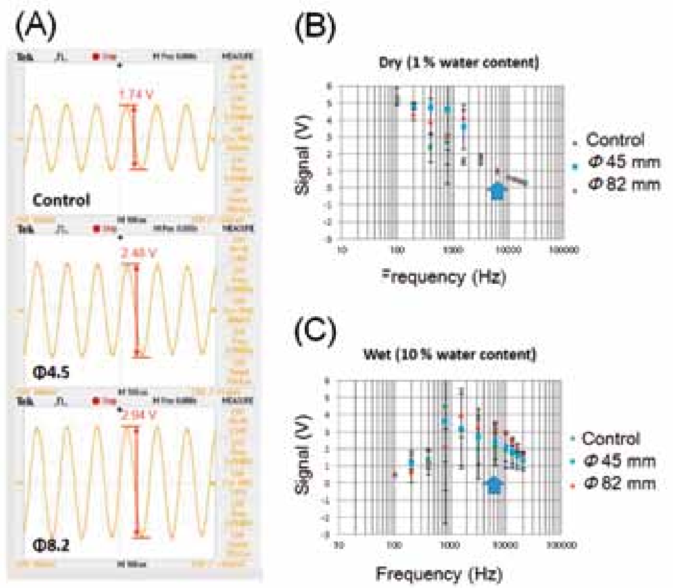

Fig. 2 - Detection of sound signals propagated in the sand layer with and without sweet potato tuberous toots. (A) Typical raw records of signal modulated at *ca*. 6.4 kHz (6.386-6.413 kHz). Changes in signal intensity (voltage changes between the peaks and valleys of the waves) were monitored. Relationship between the frequency and yield of sound signal in the beds of dry sand (B; water content, *ca*. 1%) and wet sand (C; water content, *ca*. 10%) were compared. Arrows in (B) and (C) indicate signal yields at 6.4 kHz. Error bars, S.D. (n= 5).

3. Results and Discussion

Sound signal detection

The intensity of sound signals propagated through sand differed noticeably depending on the sound frequency (Fig. 2). Fractuations of signal intensities were shown to be higher in the relatively lower range of frequency examined (100-1,600 Hz) in both sand samples. Compared to the dry sand, the wet sand likely enhanced the yield of sound signals in the presence of sweet potato tissues, especially at the higher range of frequency examined (3,200-20,000 Hz). Figure 2A reports typical raw records of sound signals showing a size-dependent enhancement at 6.4 kHz in the wet condition. Thus, data from the wet sand bed was used for further analysis to elucidate the effect of tissue size on signal acquisition.

Sound signal relationships

Relationships between sound signal frequency and yield in the beds of dry and wet sands were compared by obtaining a series of regression lines with the least-square method (Fig. 3A). Correlation coefficients (r^2) for four regressions were between 0.8825 and 0.9991. To evaluate tissue size-dependent changes in the yield of sound signals in the wet sand bed, two indices (i.e. index of subtraction and index of ratio) were proposed and the processed data were used to obtain the linear regressions and were compared with other linear regressions obtained for raw signal data (Fig. 3B, C). With the index of subtraction (sample-blank) (Fig. 3B), correlation coefficients (r^2) for three regressions were between 0.7148 and 0.9623. With the index of ratio (sample / blank) (Fig. 3C), correlation coefficients (r^2) for three regressions were between 0.7829 and 0.8885. The regressions obtained for the index of subtraction highlighted the relationship between the signal yield and tissue size increases since the coefficients for x in the linear regressions increased with size: $2 \cdot 10^5 x$ for the ø20 mm sample, $3 \cdot 10^5 x$ for the ø45 mm sample, and $5 \cdot 10^5 x$ for the ø82 mm sample (Fig. 3B).

Further studies

During frequency-modulated sensing of plant tissues, the impact of acoustic signals on plant growth must be minimized, as recently demonstrated by Gagliano *et al.* (2012 a, b) who suggested that the growing tips of plant roots could be attracted by acoustic signals with specific spectra raging between 200 and 400 Hz, if plant roots were continuously exposed to the sound stimulus. One possible way to avoid such influence during acoustic sensing is to use signals in a frequency range higher or lower than the plant activating range of sound (<200 Hz, >400 Hz), or to use sound only for short periods when required. Since the range of frequency recommended for monitoring belowground plant tissues was shown to be between 3,200 and 20,000 Hz (Fig. 3), the impact of applied sound on plant growth could be avoided.

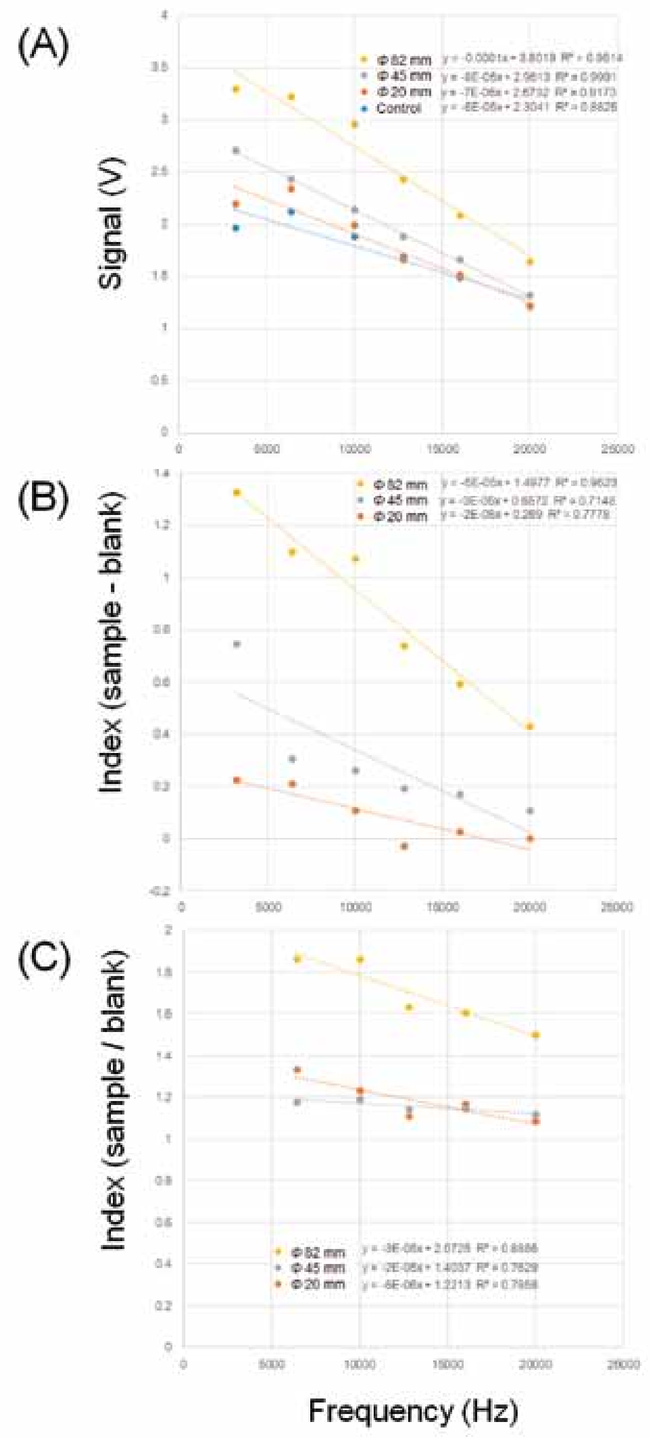

Fig. 3 - Signals for belowground tuberous roots differed in size based on the acoustic recording at the higher range of frequency (between 3,200 and 20,000 Hz). Data recorded in the absence (blank control) and presence of tuberous roots of sweet potato (20, 45 and 85 mm in diameter) were plotted. Mean values obtained at each frequency were used (n = 5). (A) Plots and linear regression of raw signal. (B) Evaluation of the signal with the subtraction index (sample-blank). (C) Evaluation of the signal with the ratio index (sample/blank).

In the present work we have developed a novel, non-invasive sensing technology for the detection of belowground plant tissues based on sound propagation in the soil. We employed tuberous root tissue of sweet potato as model material to test the instrumental set-up which was specifically designed for modulation and demodulation of sound signals through soil or sand with and without plant tissues.

It is well known that growth of tuberous roots of sweet potato is highly sensitive to changes in temperature (Spence and Humphries, 1971), moisture (Spence and Humphries, 1971; Eguchi *et al.*, 2012), nutrient (Leonard *et al.*, 1948), phytohormone levels (Spence and Humphries, 1971), and oxygen supplies (Eguchi and Yoshida, 2011) in the supporting sand. However, to date, underground growth of sweet potatoes' tuberous root was rarely studied with non-invasive real-time monitoring approaches. Further studies are still needed, although there are several technical problems to be overcome, chiefly, sensitivity and resolution of the sensing units.

Ecologists, ecohydrologists, and biogeochemists need detailed insights into plant properties and processes where plant roots reside, including changes in water, salts, and other elements that can influence ecosystem productivity and functioning (Jayawickreme *et al.*, 2014). However, limitations in data and some confusion over terminology, together with a strong dependence on a small set of conceptual frameworks, have limited the exploration of root function in terrestrial models (Smithwick *et al.*, 2014). Relying on traditional mechanical sampling and observation techniques can be costly, time consuming, and poorly feasible, especially if the spatial scales involved are large (Jayawickreme *et al.*, 2014). Therefore, non-invasive real-time monitoring may largely benefit both the ecological research community and the field of "plant factory" research and development.

Although the present study succeeded in only the primary test case, we hope it will stimulate further studies and development by scientists and engineers.

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