Hydrotropic Root Behavior in Water-Saving Cultivation: A High-Resolution Monitoring Study of Soil Water Dynamics

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Methodology

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Abstract

Background

Subsurface irrigation has been confirmed to have high water use efficiency due to it irrigating only the crop root zone. Hydrotropism allows roots to grow towards higher water content areas for drought avoidance, which has research interests in recent years. However, most hydrotropism studies focused on a single root and were conducted in air or agar systems. The performance of hydrotropism in subsurface irrigation is not clear.

Results

We developed a method to observe and analyze hydrotropism in soil under water-saving cultivation. A wet zone was produced around the whole root system based on using subsurface irrigation method and micro soil water dynamics were observed using high-resolution soil moisture sensors. This method enabled the observation and analysis of plant water absorption activities and the hydrotropic response of the root system. In the analysis, we first applied a high-pass filter and fast Fourier transform to the soil water dynamics data. The results indicated that the plant's biological rhythm of photosynthetic activities can be identified from the soil moisture data. We then observed root growth in response to the dynamics of soil water content in the wet zone. We quantified root distribution inside and outside the wet zone and observed the shape of the root system from the cross-section of the wet zone. The results showed that the root hydrotropic response is not uniform for all roots of an individual plant.

Conclusions

This study verified the feasibility of using high-resolution soil moisture sensors to study root hydrotropic responses in soil during water-saving cultivation. To further evaluate a plant's hydrotropic ability, it is necessary to use statistical analysis and/or a non-deterministic approach. Future studies may also explore developing an automated experimental system and robotic manipulations for getting steady repeatable observation of hydrotropism in water-saving cultivation.

Background

Agriculture represents a major consumer of freshwater, accounting for about 70% of the worldwide total water withdrawal [1,2]. In addition, climate change may result in more frequent occurrences of water shortage and increase the competition for water among urban, industrial, and agricultural demands [3,4]. With increasing water demands from other sectors, irrigation agriculture must increase food production with limited water allocation [1,5]. Crop breeding technologies have been developed for this purpose [6,7]. A major point of crop breeding is to develop ideotypes in irrigation agriculture for more efficient acquisition of water and nutrient in the irrigated area [4,8]. Hydrotropism allows roots to grow actively towards water source for drought avoidance [4,9,10]. Hence, precise analysis of this response in plant and its relation to plant water use efficiency (WUE) is important for breeding of drought avoidance species.

Water-saving irrigation technologies have also been developed to increase WUE, and one such technology is subsurface irrigation [11-15]. This technology uses emitters buried in soil to deliver irrigation water directly to the crop root zone [12,13,15]. Proper management of irrigation water can prevent water logging in topsoil and reduce surface evaporation, run-off, and deep percolation, resulting in improved efficiency of irrigation water use and nutrient uptake [12,13,15]. So far, this technology has shown great performance in field irrigation practice, resulting in higher crop yields and quality with less irrigation water, preventing weed growth, mitigating soil N_2O emissions, and facilitating the use of degraded-quality water [13].

When performing subsurface irrigation, a localized zone of wet soil can be produced. Studies have shown that the geometry of the wet zone under subsurface irrigation can be influenced by irrigation rate, soil hydraulic properties, and root water uptake [14]. In the case of bulk soil, the wet soil particles adhere to each other and form a harder part due to the cohesive force of water molecules. A threshold of penetration pressure exists at the border between wet and dry soil. Therefore, water will not flow into the surrounding dry soil when the penetration pressure is lower than the threshold [16,17]. As water supply continues, the wet zone can be enlarged due to the meniscus phenomenon. Previous studies showed that a spherical wet zone can be stably formed in homogeneous dry soil from a point water source and the size of the wet zone depends on the volume of the supplied water [16,17].

Since plants absorb water from the roots, a negative pressure difference is generated between the root and the surrounding soil. The formed wet zone may be smaller than the state without plant due to the root water uptake. The water volume of the narrowed region is considered the available water that absorbed by the plant [16,18]. If the volume of available water is controlled through irrigation to balance the moisture flow between the plant and the surrounding soil, the penetration pressure may not exceed the threshold and the wet zone can be maintained at a constant volume. Increasing water supply may increase the gravitational water flow due to the expansion of the wet zone. But since the root zone may also expand due to plant growth and the corresponding increase in water uptake, it can be considered that the plant water uptake dynamically equilibrates with the penetration pressure generated by capillary forces to stabilize the wet zone. In other words, the wet zone changes dynamically because of the influence of the pressure fluctuation caused by the root water uptake that changes the amount of water retained in the wet zone [18]. Thus, how roots respond to the dynamics of the wet zone is important for high WUE irrigation practice.

Roots are essential organs for water and nutrient absorption [3]. Research showed that plants can adapt and regulate water uptake capacity by changing the root system architecture according to changes in their local environment [4,8,19,20]. Studies of the physiological characteristics of the root system can be dated back to 19^{th} century Darwin's study "The power of movements in plants," which stated that the root tips of plants can sense the surrounding soil moisture and modify the direction of root elongation [21]. Root hydrotropism, which allows roots to exploit and intercept localized water resources in soil, may facilitate the utilization of limited water resources [9,10,19,22-24]. In the past decades, two systems have often been used to observe this phenomenon [9,25-29]. In one system, air is placed in an enclosed environment with a concentrated salt solution, and seedlings are mounted on a support, often foam or agar blocks, with the very root tip suspended in air. A water potential gradient can be produced in the root tip between the wet support and the surrounding air [25,26]. Another is the agar-sorbitol system, which places root tips near the border between two growth media with a water potential gradient produced by adding sorbitol to one of the media [26,27]. In both cases, root bending towards higher water potential is considered a hydrotropic phenotype of the test seedling. These systems have been used to identify genes involved in hydrotropism that characterize cellular and molecular events of the response [9,27,30-32]. However, a major issue with these studies is that the ways plants regulate hydrotropism in the laboratory may be different from how they operate in the field condition [10,24,28,33].

To test whether this response happens in natural soil, a few experimental systems have been established. Cole and Mahall [22] tested hydrotropic responses of two coastal dune shrubs under soil conditions. They produced a water potential gradient in soil by injecting water during the seedling growth to create a moisture rich patch laterally next to the growing pot, and roots growing into the patch were considered a hydrotropic response. In the study, they found no compelling evidence for hydrotropic root behavior of the test seedlings. Iwata et al. [23] developed an experimental system to measure hydrotropic response in soil and investigate its role in root system development and crop biomass production. They grew *Arabidopsis* seedlings in rectangular plates covered with soil. A water potential gradient was produced through natural drought by placing a wetter plastic foam on one side of the soil that was half-covered with a lid. Root architecture was scanned by a scanner to study its relationship with moisture distribution. They found that hydrotropism plays an important role in root system development and crop growth. Li et al. [28] developed a sand system to study hydrotropism of *Arabidopsis* and tomato plants. They created water potential gradients in both oblique and vertical directions in soils and found gravity significantly influenced the hydrotropic response in both cases. These systems are efficient to study hydrotropism of primary roots in the early growing stages. But how lateral roots (or fine roots), which form the main root system [3,24,34], respond to water potential gradients has been little studied [9].

Last, a recent study tested the synergic effect of root biomass and hydrotropism on grain yield [35]. The researchers identified hydrotropic phenotypes of maize hybrids using the conventional air system and then performed field trials using hybrids with robust and weak hydrotropic responses. They found a positive interaction between root biomass and hydrotropism in enhancing grain yield [35]. Although the study showed that roots have a positive relationship with water distribution in soil, the root hydrotropic behavior in response to the dynamics of wet zone under subsurface irrigation has not been studied.

Due to the opaque nature of soil-grown roots and the highly varied soil water in time and space, a major difficulty in study root hydrotropism in natural soil is observing and analyzing root growth in response to the dynamics of soil moisture distribution. Modern high-throughput phenotypic technology based on computer vision and machine learning enabled high-resolution measurement of root traits. But this technology requires extracting roots from soil to obtain the high-resolution root images [36,37]. X-ray Computed Tomography (CT) has been widely used to visualize roots in situ. This technology uses a non-destructive technique to visualize the interior of objects in 2-D and 3-D based on the attenuation of an electromagnetic wave [36]. Since the attenuation density of root and soil matrices are similar and highly dependent on soil water content, efforts to visualize root system architecture in soil have focused on the segmentation of roots from the soil pore area [36,38-40]. But so far this technology has limitations in the detection of fine roots and has low contrast in heterogeneous soil and time-consuming user interaction [39].

To test the hydrotropism theory, rigorous experimental design concerning the observation and control of soil water dynamics is important. A major emphasis should be on forming a steep wetting front around the root system, while water supply inside the wet zone should sustain the crop growth. To implement such a study, high-resolution measurement of soil water dynamics around the rooting zone is prerequisite. Previous research has used high-resolution, nondestructive imaging technologies such as x-ray radiation, magnetic resonance techniques, and electrical resistance [41-45]. However, these techniques require tedious calibration of soil parameters to obtain spatial soil moisture distribution [46], and the difficulty in accessing such devices also limits their use [36]. Moreover, most studies of the interaction between soil and root water uptake use single-point soil moisture measurement based on neutron probes or tension meters, which lacks accuracy and representativeness due to the spatially and temporally variant nature of soil-root system [47-49]. As a result, high-resolution measurement of spatiotemporal soil water dynamics has not been achieved.

This paper suggests using a high-resolution soil moisture sensor matrix to measure the micro soil water dynamics in the crop root zone. The system uses minimally sized soil moisture sensors placed in a matrix inside and outside the root zone to obtain precise, real-time signals of the temporal and spatial moisture dynamics around the growing roots. Prior to this study, the authors have used this method to confirm the existence of a wet zone in water-saving cultivation of tomato plants [17,18]. The objective of this paper is to verify the feasibility of using the sensors to observe hydrotropic response in the wet zone by measuring the dynamics of soil water content caused by water absorption of the growing plant.

Results

Soil moisture responses

The soil moisture analysis was based on the same method used in previous works [17,18]. Figure 1 shows the response of soil moisture at each location in a time series from the 88 days after transplanting. The values represent soil moisture at positions close to the root zone and those away from the root zone, which respond to water supply through capillary flow from the point water source. The blue curve at the top is the air temperature inside the growth chamber. The next red line is the volumetric water content (VWC) at the position of the water source where the plant was grown. Water supply was synchronized with the lighting period of the growth chamber. Each peak represents a single water supply event. A fluctuation of water absorption activity can be observed within each lighting period. A fast Fourier transform (FFT) was performed to show the frequency characteristics of plant water absorption. VWC values at the water source varied around 15% to 20% during the growing period (data not shown).

The curves H1, H2, V1, and V2 are, respectively, soil moisture at positions 3 cm and 6 cm horizontally from the water source (H1, H2) and vertically (V1, V2). These values indicated moisture at the boundary zone between wet and dry soil. The curves H3 and V3 show measurements of, respectively, dry soil 12 cm horizontally from the water source horizontally and 17 cm vertically from the water source. The VWC values at these positions were around 5%. These values confirmed that the water supply did not cause excessive water flow into the dry soil. As a result, moisture distribution was in the rooting zone, in the cm order resolution.

Data processing of soil water dynamics

To analyze plant physiological water absorption activities, we extracted the frequency content in the soil moisture data using signal processing techniques. Since the plant absorbs water only from the wet zone, the short-period fluctuations at the water source should be caused by plant water absorption activities. So, it is possible to use signal processing techniques to determine how often these activities happen. In Fig.1, there were two obvious periodical responses at the water source that were caused by water supply events. One is the longer period that synchronized with the growth chamber's daily setting; the other is at the top of the curve that was caused by water supply events at 2 h intervals during the lighting period each day. We needed to remove these two frequencies using a high-pass filter. The selection of the filter type and the determination of cut-off frequency were based on trial and error using the MATLAB R2018a software. In this study, we used the Butterworth high-pass filter. The sampling frequency of the experimental data was 0.00334 Hz. The passband cutoff frequency (Wp), stopband starting frequency (Ws), ripples in passband (Rp), and minimum actuation in stopband (Rs) of the Butterworth filter were 0.00018 Hz, 0.00015 Hz, 3 db, and 20 db respectively.

Figure 2(a) shows the filtered waveform of soil water dynamics in time domain at the water source. It should be noted that short-period fluctuations can be seen within each lighting period. This indicates that the waveform reflects the state of photosynthetic activity. The waveform shows that the plant water absorption is not evenly distributed in the time series and the activity of water absorption fluctuates at different frequencies.

Next, to find the natural frequency of the waveform, we applied fast Fourier transform (FFT) to the filtered moisture data. Figure 2(b) shows the amplitude frequency after FFT of the filtered data. Distinct frequencies around 0.00022 Hz, 0.00034 Hz, and 0.00045 Hz (76 min, 49 min, and 37 min into the period) can be identified from the waveform. Since theses frequencies were not identified at other locations of the soil (data not shown), and the period was around the multiple of the water supply interval (120 min), these frequencies can be considered to be caused by plant water absorption activities, and the biological rhythm of plant photosynthesis can be identified from the soil moisture data.

Metric potential gradient

The soil moisture values were transferred to water potential through a water retention curve of the experimental soil (Fig.3). The water potential value at the water source varied from -0.22 MPa (15%) to

-0.1 MPa (20%). In this experiment, accumulated soil water content in the wet zone fluctuated around -0.22 MPa (pF 3.4), which was the wilting point and can be treated as a threshold for water supply. The plant recovered from wilting by absorbing water after each water supply event when water potential peaked around –0.1 MPa. The plant cannot absorb water from the dry soil where the water potential is much lower than the wilting point. Therefore, the wet soil area for plant growth and the dry soil area below the wilting point were clearly separated in the same soil. As shown in Fig.3, the results indicate that a stable soil water potential gradient close to 0.02 MPa/mm was produced near the wet/dry boundary.

Root distribution at the wet/dry soil boundary during crop growth

To observe root growth in response to soil water dynamics in the wet zone, we disturbed Pot 1 several times during the experiment and examined root distribution at the wet/dry boundary (Fig. 4). In this study, Pot 2 severed as a static reference for which observation was conducted only once at the end of the experiment. Changes in growing stages of shooting, flowering, and fruit maturing of both Pot 1 and Pot 2 were observed, which represent changes in plant physiological status that may affect the structure of the root system. As shown in Fig. 4, we observed many root tips distributed at the boundary, but not obviously penetrating into the dry soil.

The gravimetric water content on the wet and dry sides and at the boundary area was determined using the conventional oven dry method. The moisture level of the dry soil was maintained around 10% during the growing period. The fluctuation of soil moisture in the wet soil may be caused by water supply and plant root water uptake. An average of 12% moisture difference can be seen between the dry and wet soil.

The relationship between plant height and water supply for Pot 1 is shown in Fig.5. During the experiment, the plant increased its height in response to the volume of water supply in the wet zone. A linear relationship between the plant height and the cumulative water supply can be observed. The plant absorbed water from the wet zone where the roots mostly distributed. Because no water loss from the root zone was observed, it can be assumed that almost all water supplied to the wet zone were absorbed by the plant.

Observation of root distribution at the end of the experiment

The hydrotropism theory assumes that all roots will grow towards the point water source. In addition, since the wet zone was controlled by the water-saving cultivation method, it can be assumed that the root zone will be inside the wet zone. To confirm these two assumptions, the wet zone was taken out from the cultivated soil at the end of the experiment for observation and analysis of root distribution. Figure 6 shows the roots of Pot 2, which were not disturbed during the experiment as those of Pot 1. As shown in the figure, roots of Pot 2 obviously grew out of the wet/dry soil border into the dry soil for several centimeters. Also, we observed several roots left at the bottom when we removed the wet zone. This may be caused by the gravitropic effect being stronger than the hydrotropic response.

We then cut the roots that grew out of the border along the edge of the border (approximately more than 1 cm) and recorded the numbers of the roots. We also measured root length in the dry soil and obtained the dry weight of roots inside and outside the wet soil. Table 1 shows these and other results including root dry biomass and root weight density inside and outside the wet zone, root number and root length outside the wet zone, the total dry mater, yield, and WUE (Table 1). The percentages of root dry weight outside the wet soil were 2.63% and 5.61%, the total root numbers outside the wet zone were 82 and 138, and the WUE were 6.96 g/L and 5.30 g/L for Pot 1 and Pot 2, respectively. The mean root length outside the wet zone were 2.1 cm for both plants. As these results showed, root distribution characteristics at the dry/wet soil border and their relationships with yield and WUE can be precisely measured and analyzed.

Table 1 Root distribution inside and outside the wet zone, plant yield, and water use efficiency.

	Pot 1	Pot 2
Root dry biomass inside (g)	1.48	1.01
Root dry biomass outside (g)	0.04	0.06
Total root dry biomass (g)	1.52	1.07
Percentage of root outside (%)	2.63	5.61
Root weight density inside (g/L)	3.72	1.93
Root weight density outside (g/L)	0.05	0.07
Ratio of density outside to inside (%)	1.34	3.63
Total root number outside	82	138
Total root length outside (cm)	172.2	290.4
Mean root length outside (cm)	2.1	2.1
Total dry mater (g)	6.12	4.71
Number of fruits	2	1
Yield (g)	36.06	32.14
Water use efficiency (g/L)	6.96	5.30

Root skeleton from cross-section of the wet zone

The soil moisture dynamics show that the plant absorbed water mainly from the region close to the water source. To observe the root architecture inside the wet zone, we sliced the wet zone with a razor blade to observe the root skeleton from the cross-section as shown in Fig.7. The hole at the center of the soil shows where the water supply tube had been placed. It can be found that some of the roots orientated towards the inside of the wet zone, while some roots grew outwards. A vertex root architecture can be observed at the cross-section. It appears that the roots grew downward and around obstacles (the water supply tube) and then bent back towards the inside of the wet zone where the moisture content is higher. Although some roots overcame the effect of gravity and grew towards water, the hydrotropic response was not a universal response for all the roots observed in this study.

Our observation confirmed that hydrotropism allows roots to grow towards water source. Hydrotropism has been confirmed in the laboratory germination experiments. In this study, it was confirmed in an actual crop cultivation practice. Our observation also confirmed that the root system is a limited zone caused by hydrotropism. This makes it possible to control the root area and thus establish new cultivation techniques for crop growth control and/or increased planting density. This result also indicated that it is possible to control root development by applying a moisture gradient [50].

Discussion

This study observed and analyzed root hydrotropic behavior in soil under water-saving condition. A major focus of the study is to produce a wet zone during crop growth from where plants can absorb water. During the experiment, the total size of the wet zone did not much change because the accumulated water content has been maintained at a constant level in the wet zone. But the soil water content retained in the wet zone fluctuated due to plant root uptake. We measured the soil water dynamics around the wet zone using a high-resolution soil moisture sensor matrix. The results showed that the roots absorbed water only from the wet zone. Also, we analyzed plant water absorption activities by applying a high-pass filter and fast Fourier transform to the soil moisture data. The results showed that plant water absorption oscillated at different frequencies in accordance with water supply events. The oscillation of photosynthetic activities has been demonstrated in previous studies, which is regulated by stomata and depend on environmental and soil water conditions [51,52]. In this study, we identified the exact frequencies of water absorption activity.

Because soil water content dynamically changed in the wet zone due to plant water absorption activities, it is necessary to observe how roots grew in response. In this study, the water potential gradient produced by the water supply was about 0.02 MPa/mm at the front of the irrigation water. This gradient is sufficient to induce hydrotropic response [53]. According to hydrotropism, roots modify their growth direction towards higher moisture. In this study, roots stopped growth in the dry soil and most root biomass was distributed inside the wet zone (Table 1). On the other hand, some roots grew out of the wet/dry soil border for several centimeters (2 cm in average), which suggests a weaker hydrotropic response of these roots. Gravitropism, which induces roots to grow to deeper soil, also plays an important role in root formation as described in previous studies [4,9,28]. Accordingly, in this study, roots growing in dry soil at the bottom of the wet zone were observed. In plant physiology, elongation of roots is caused by turgor pressure in the growing tissue, and growing tissues show lower turgor when grown in a medium with low water potential. Therefore, the limited supply of water in the dry soil and turgor pressure in the root-tip cells may be insufficient to drive cell elongation [4,19], which explains why the roots stopped growing when they penetrated into the dry soil.

To analyze root distribution, we quantified the roots inside and outside the wet zone. In this study, we cut the roots along the edge of the wet/dry border of the wet zone to measure dry biomass distribution outside the wet zone. Because this part of the roots did not grow towards higher water content, it can be considered that these roots grew against the hydrotropic response. In this study, the root dry biomass in the dry soil were 2.63% and 5.61%, respectively, for the two plant samples. According to hydrotropism, each root serves as a water sensor which can perceive water potential gradients and modify their growth direction towards higher moisture [27]. It can be assumed that plants with fewer roots distributed in the dry soil can perceive wet zones more precisely, which is preferable in subsurface irrigation to make more efficient use of the irrigation water in the wet zone [8,54,55]. Our study provides a method to quantify root distribution that is against hydrotropic responses. Our data are based on limited samples. To provide more robust evidence on the effect of hydrotropism, more observation samples and statistical analyses or non-deterministic principles are needed.

In our study, the plant absorbed water only from the wet zone produced by the subsurface irrigation method. According to the soil moisture dynamics measured by the high-resolution moisture sensor matrix, the plant absorbed water mainly from the region close to the water source. We observed that the root skeleton showed a vertex shape with some roots bent back towards the water source for water acquisition (Fig.7). It can be assumed that hydrotropism acted on these roots inside the wet zone. On the other hand, since some roots grew out of the wet zone, it can be assumed that the hydrotropic response is not uniform among roots within an individual crop. To evaluate the plant hydrotropic ability, more samples are needed to perform statistical analyses.

Root hydrotropic responses in soil have been reported before in actual crop cultivation practice, but the study was conducted on seedling roots by maintaining a sharp border between two soil layers over a period of one to two weeks [9]. Because the water potential gradient in soil varies considerably, observing hydrotropism of the whole root system throughout plant reproductive stages is difficult. This study used a soil moisture sensor matrix method to measure the soil water dynamics in crop rooting zone. The characteristics of soil water content can be obtained inside and outside the wet zone. The use of in-situ measurement for soil moisture profiling along soil depth has been reported before, but the resolution used in previous studies is insufficient when studying plant physiological activities because bulky sensors used in multi-points measurement may disturb soil structure and result in measurement errors [48,49]. In this study, we increased the observation resolution to the centimeter order using small soil moisture sensors, and a sharp water potential gradient was maintained at the wet/dry boundary throughout the growing period. As a result, the response of plant physiological activities to soil water content can be precisely observed and analyzed.

The high-resolution measurement system developed in this study requires considerable time and labor for system installation and data collection, which restricts its use to obtain replicable data. Also, offline measurement using a data logger may risk inadequacy or missing data due to the possibility of disconnection of cables during data collection. Moreover, to perform more detailed analyses of roots' directional growth, non-destructive methods such as X-ray Computed Tomography (CT) will be necessary to capture continuous images of the roots' growth. In the future, multidimensional information processing techniques such as probability distribution or non-deterministic modelling will also be necessary for high-throughput measurement of spatiotemporal, multidimensional data of roots and soil moisture dynamics.

Conclusions

In this study, we observed root hydrotropic behavior under water-saving condition by using an original observation method, which is to produce a zone of wet soil around the whole root system based on subsurface irrigation and then observe the micro soil water dynamics using a high-resolution soil moisture sensor matrix. Doing so enabled the observation and analysis of plant water absorption activities as well as the hydrotropic responses of the root system. In the study, we applied a high-pass filter and fast Fourier transform to the soil water dynamics data. The results indicated that the plant biological rhythm of photosynthetic activities can be identified from the soil moisture data. We then observed root growth in response to the dynamics of soil water content in the wet zone. We quantified root distribution inside and outside the wet zone and observed the shape of the root system from the cross-section of the wet zone. The results showed that root hydrotropic response is not uniform for all roots of an individual plant. Our observation results verified the feasibility of using the high-resolution soil moisture sensor matrix to study plant hydrotropic behavior during water-saving cultivation. Future studies may also explore developing an automated experimental system and robotic manipulations for getting steady repeatable observation of hydrotropism in water-saving cultivation.

Materials And Methods

Experimental system description

The experimental system has been described in our previous studies [17,18]. This study focused on producing a localized zone of water retention surrounding the growing roots that can sustain a small growth of the plant. We used high-resolution soil moisture sensors to observe micro soil water dynamics near the rooting zone. The objective of using this method is to create a stable and steep water potential gradient around the growing roots. The experimental system consisted of cultivars (two tomato samples, Anemo, Japanese variety), environmental control components (Fig.8 parts (5)~(8)), and rooting zone moisture measurement sensors (Fig.8 part (1)). The tomatoes were grown in cylindrical pots that are 25 cm in diameter and height (Pot 1 and Pot 2 in Fig.8). An industrially regulated soil, burnt red clay, was used, which had a homogeneous composition and granularity with no organic fiber elements. The soil was dried in oven at 110°C for 24 h and then passed through a 1 mm sieve. Soil was placed into pots with a slight compaction. The packed dry bulk density was 0.75 g/cm^3. Sample tomatoes were transplanted into the pots at 30 days after sowing. Prior to transplantation, roots were carefully cleaned of the original sowing soil.

Water source was made by a round fibrous cloth (A-1, Toyobo, Tokyo, Japan), which was equipped at the tip of a plastic pipette to directly supply water into the rooting zone (Fig.8 part (3)). Infiltration of water through the fibrous cloth can produce uniform water globes by capillary water flow [17,18]. The size of the water globes depends on the amount of water supply and crop water absorption. The water supply was set at 10 ml per event, 4-7 events a day. A capacitance-type soil moisture sensor matrix (EC-5, Meter) was placed at the water source, 3 cm, 6 cm, 12.5 cm horizontally and 3 cm, 6 cm, 17 cm vertically from the water source (Fig. 8 part (1)). The data was automatically recorded by a data logger (EM5b, meter) at a sampling interval of 5 min. Irrigation timing was manually determined by observing the crop growth and soil moisture condition. Nutrients were supplied by adding liquid fertilizer of 5:5:5 (Hyponex, Osaka, Japan) to the irrigation water at a ratio of 1:500.

The experimental system was placed in a climate-controlled growth chamber (KCLP-1500LED-NCS, NK-system, Japan) 151.2 cm in height, 120 cm in width, and 80 cm in length. In the experiment, two environmental conditions were manually controlled each day. The chamber was illuminated with fluorescent lamps under condition 1 (8:00~22:00), and the lamps were turned off under condition 2 (22:00~8:00). Air temperature and maximal humidity were set at 30°C and 60% under condition 1 and 22°C and 60% under condition 2. The microclimate inside the growth chamber was measured using an air temperature-humidity sensor (HMP-155, Vaisala, Vantaa, Helsinki, Finland), a solar radiation sensor (LI-190, Li-Cor, Lincoln, NE, USA), and a CO_2 sensor (GMT-222, Vaisala). The experiment was conducted from September 28th, 2016 to January 23rd, 2017, which covered the period from crop transplantation to fruit bearing. The mean values of the measured environmental parameters through the experiment are shown in Table 2.

Table 2 Mean value of the experimental parameters inside the growth chamber

	Time	Temperature (°C)	Humidity (%)	Quantum （umol/m^2/s）	CO_2 (ppm)
Control condition 1 (Light on)	8:00～22:00	30	36	191	412
Control condition 2 (Light off)	22:00～8:00	22	60	0.5	416

Root observation and analysis

To observe whether hydrotropic growth modification happen in soil, we first observed root distribution at the wet/dry soil border by removing a small amount of dry soil next to the border. The moist soil particles adhere to each other and form a harder part that can be distinguished from the dry soil, which is softer. Therefore, the border can be clearly recognized and the root distribution at the border can be visually observed. Reference soil moisture was confirmed using the oven dry method by collecting three soil samples at wet, dry, and boundary areas. A photo of the observation area at the wet/dry soil border was captured. After each observation, prepared dry soil was used to recover the soil. This visual observation was conducted for Pot 1 at different growth stages as determined by numbers of branches, flowering, and fruit bearing. Pot 2 severed as a static reference for which observation was conducted only once at the end of the experiment. We also observed the growth height and daily water supply volume of both pots for growth analysis. These were non-penetrative observations.

To analyze whether hydrotropism plays a dominant role under water-saving conditions, we quantified root distribution outside and inside the wet zone. The observation method used during and after the growing period is shown in Fig.9. When cultivation ended, we stopped water supply for 5 days to balance the water flow in the soil-root system. The soil moisture in the wet zone decreased to the level of moisture at the boundary area. The decreasing of soil moisture did not destroy the shape of the wet zone. Then we carefully dug out the wet zone from the cultivated soil to analyze the root distribution on the dry and moist sides. We cut the roots that grew out of the border (approximately more than 1 cm) using a scissor along the line of the border, and then recorded the numbers (Fig.9). We then measured the length of the cut part of roots that distributed outside the wet zone. For Pot 1, the wet zone was sliced using a razor blade at the shoot base to observe the root architecture from the cross-section of the wet zone. The roots both inside and outside the wet zone were carefully sieved and washed to separate them from the soil and then dried in the oven at 80°C for 24 h to measure the dry weight. In this study, we calculated the percentage of root dry weight outside the wet zone and the root weight density inside and outside the wet zone. The root weight density inside the wet zone was calculated using the dry weight inside divided by the volume of the wet zone (which was assumed to have a spherical shape). The root weight density outside was determined using the dry weight outside divided by the volume of the dry layer where the roots exist.

Abbreviations

FFT: Fast Fourier transform; WUE: Water use efficiency; VWC: Volumetric water content.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files]. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

QL designed the experiment and conducted the research, analyzed the data, and drafted this paper. TS designed the experiment, provided the concept, and edited the manuscript. SS supervised the research project, provided the concept, and revised the manuscript. ML supervised the study, provided the funding, and revised the manuscript. All authors read and approved the final manuscript.

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Figures

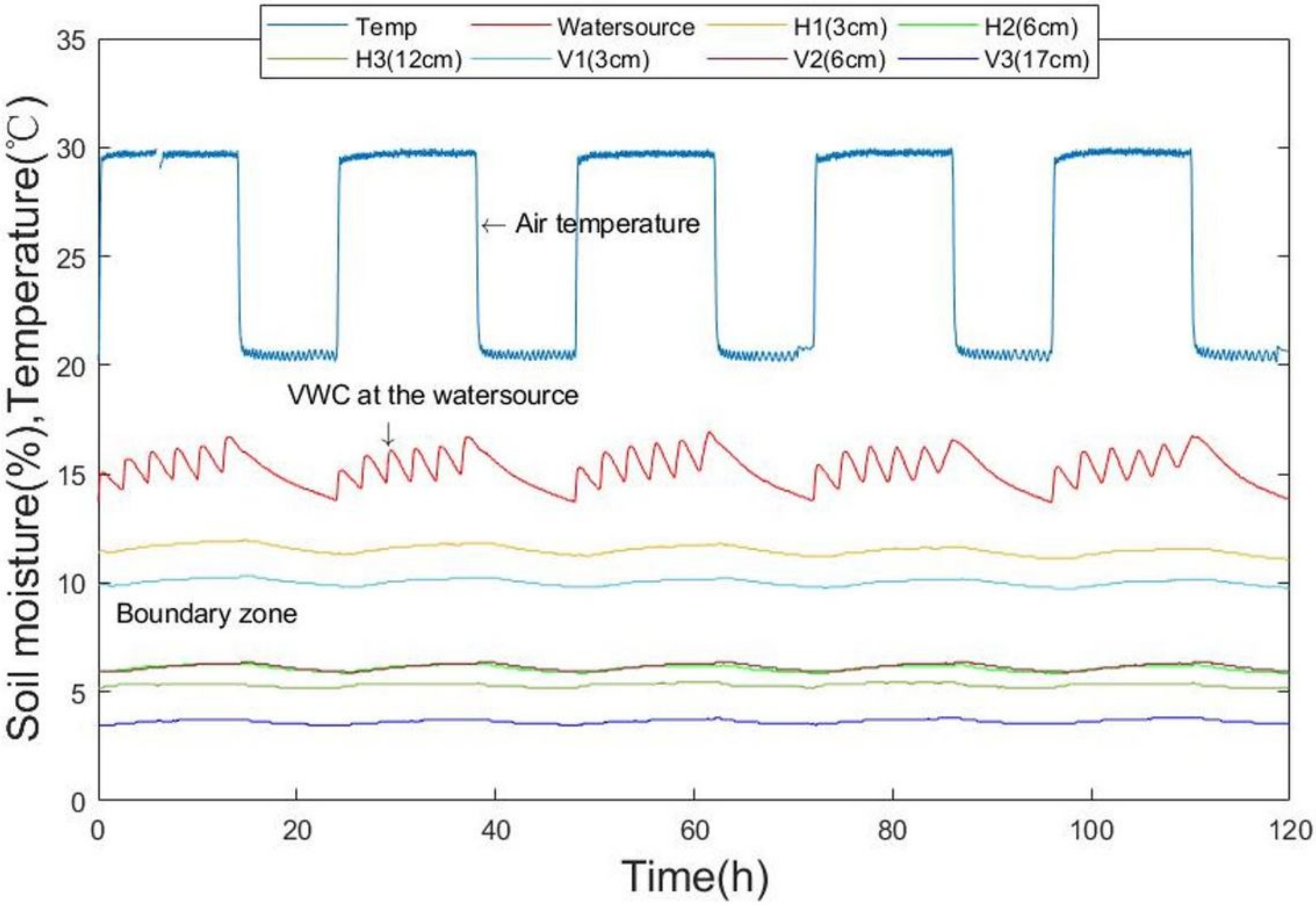


Figure 1

Soil moisture responses at each location in time series

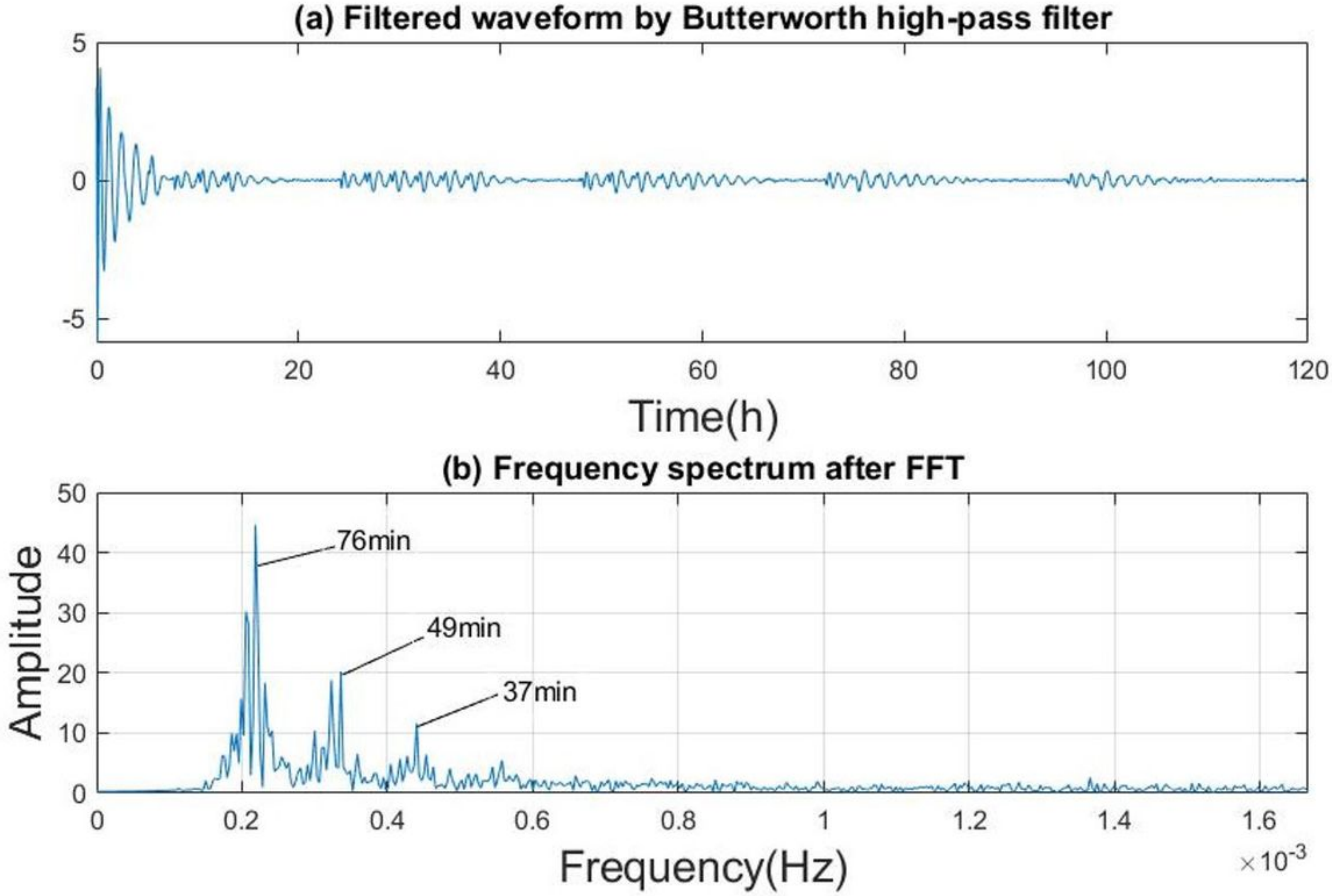


Figure 2

Frequency analysis of soil moisture dynamics at the water source. (a) Filtered waveform by the Butterworth high-pass filter. (b) Frequency spectrum after Fast Fourier transform (FFT) of the filtered moisture data

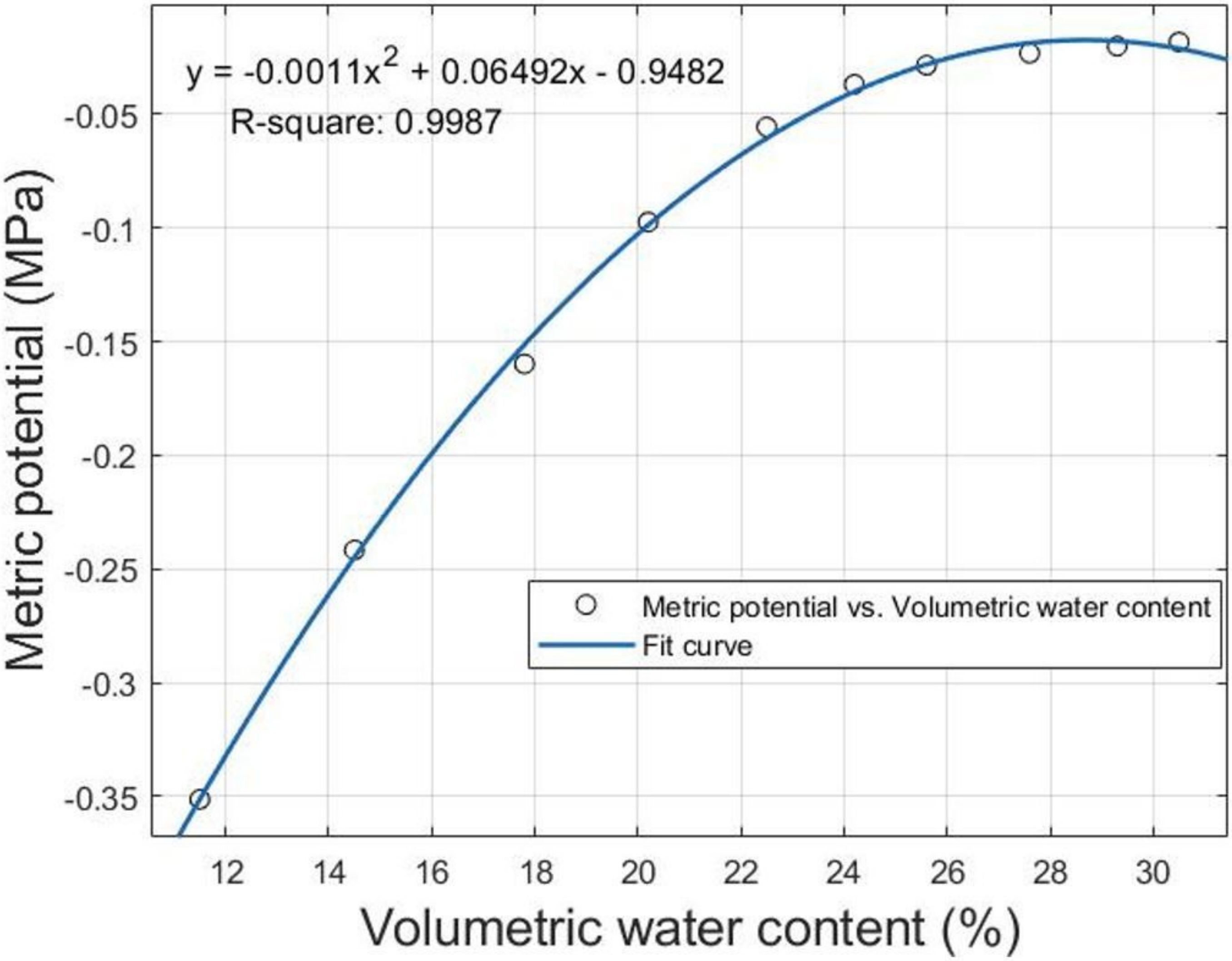


Figure 3

Relationship between VWC and water potential gradient of the experimental soil

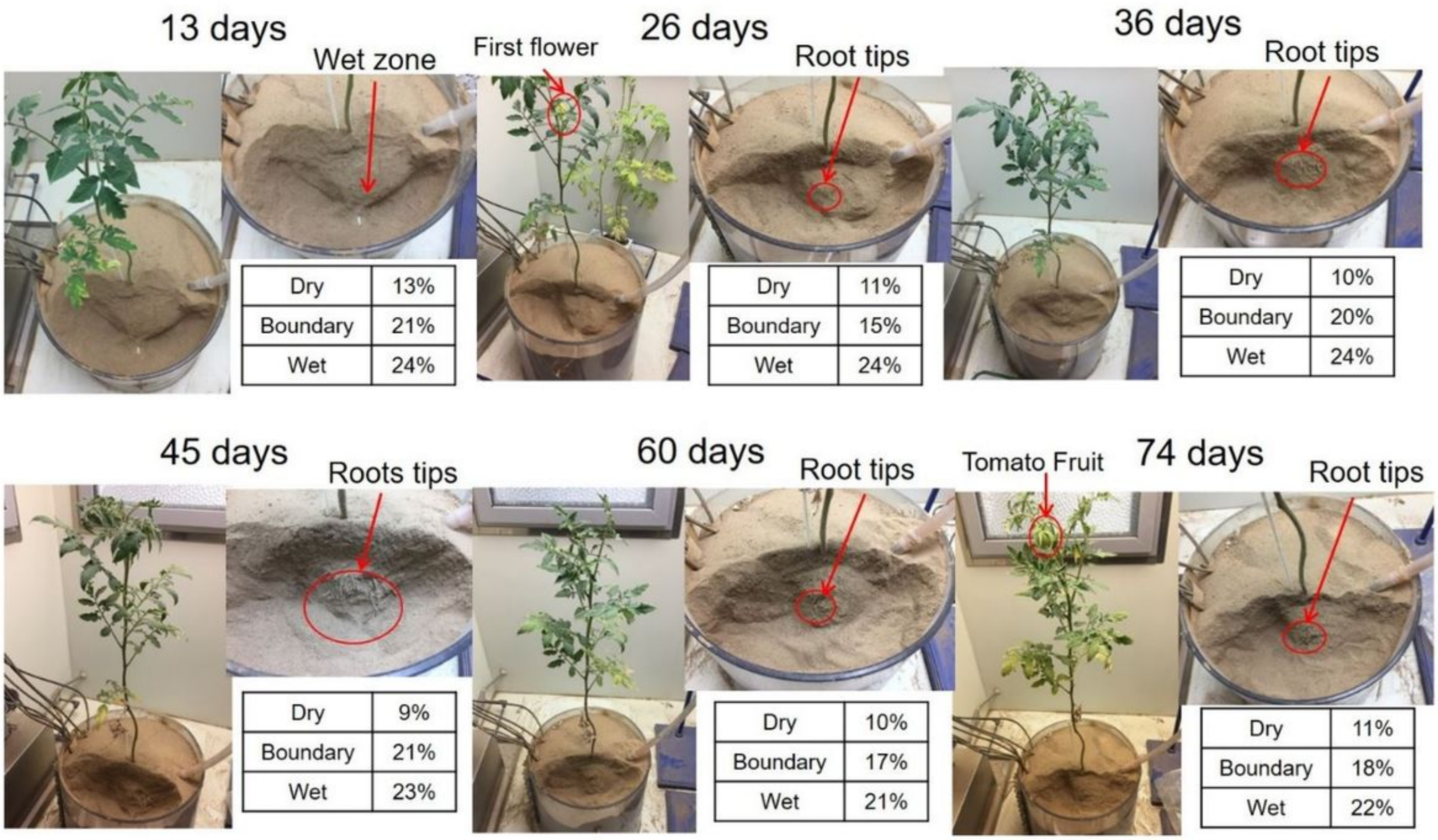


Figure 4

Root behavior at the wet/dry soil boundary for Pot 1 on various days after transplanting.

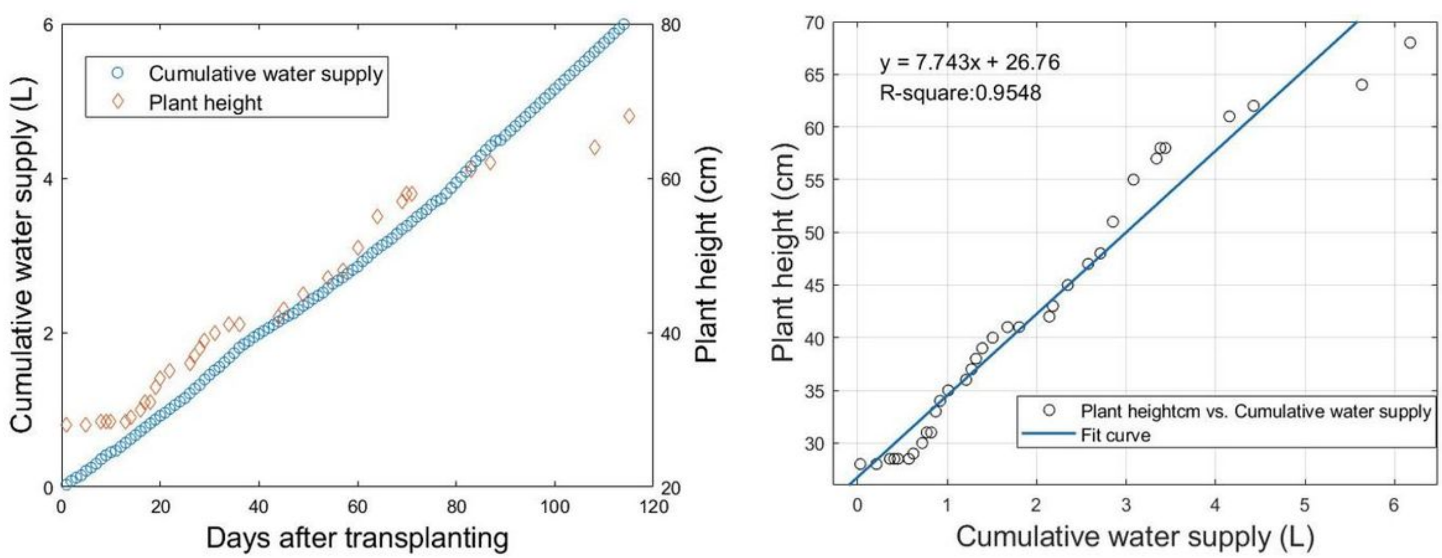


Figure 5

Relationship between crop growth and water supply

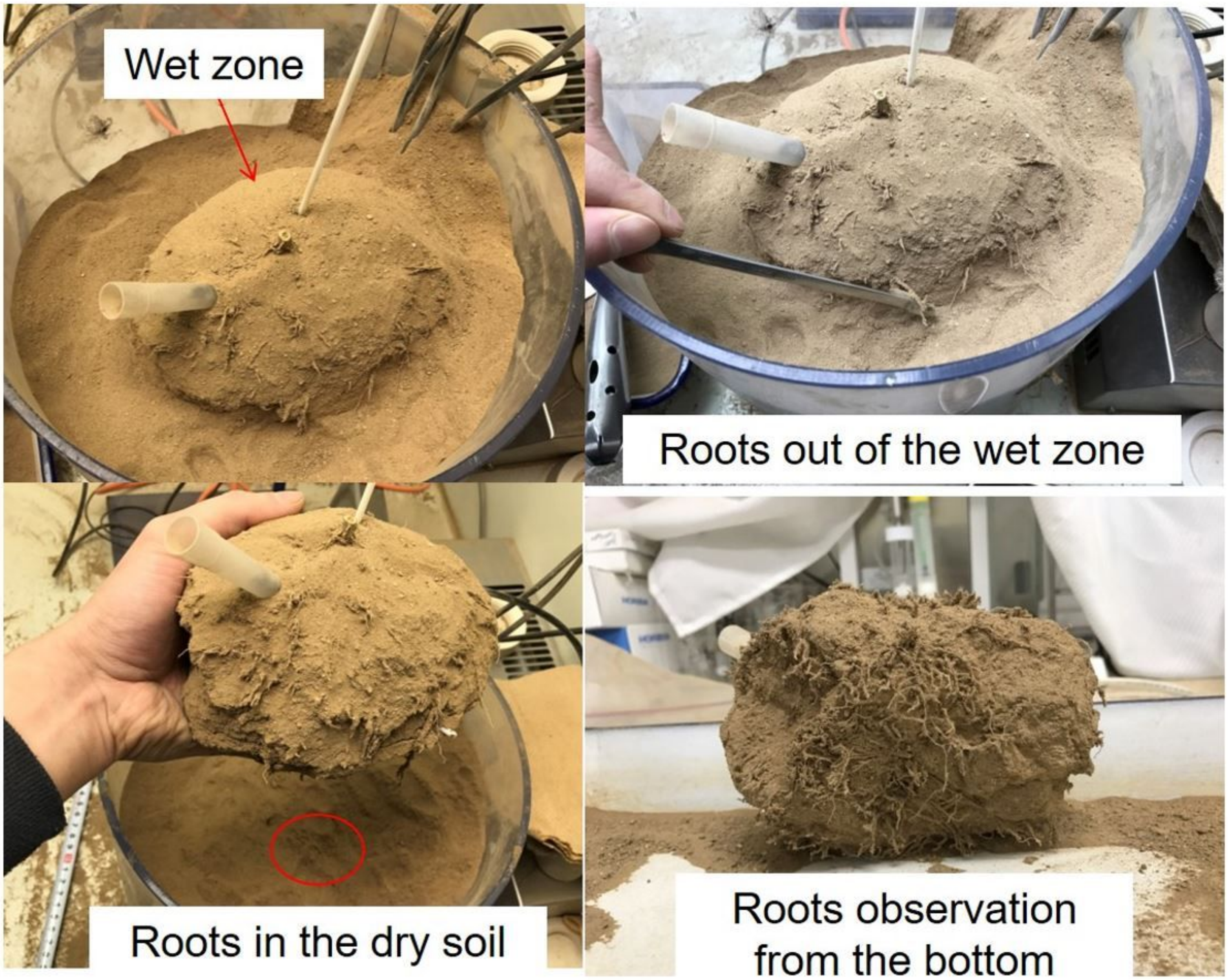


Figure 6

Observation of root distribution of Pot 2 by taking out the wet zone from the soil at the end of the experiment

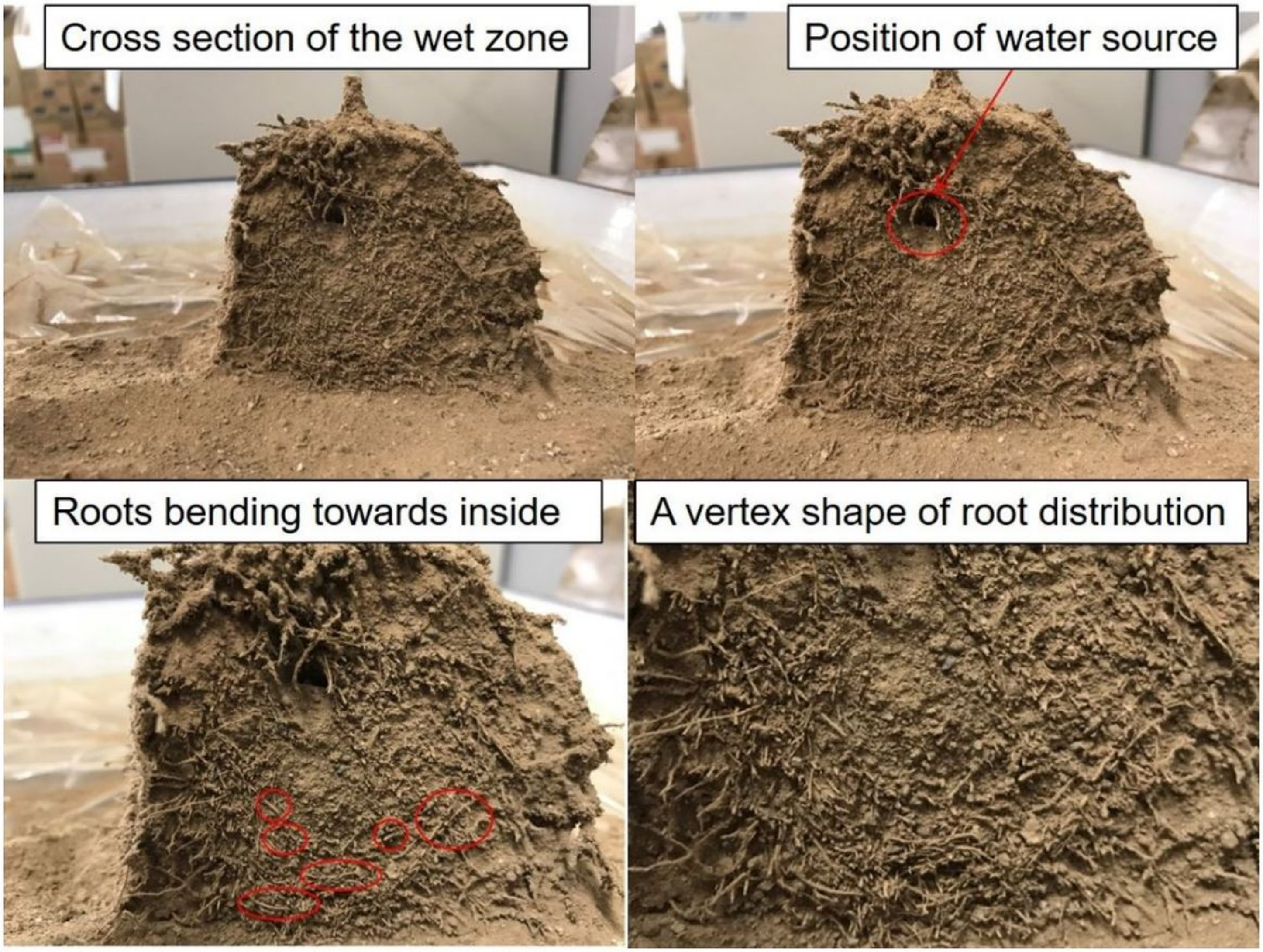
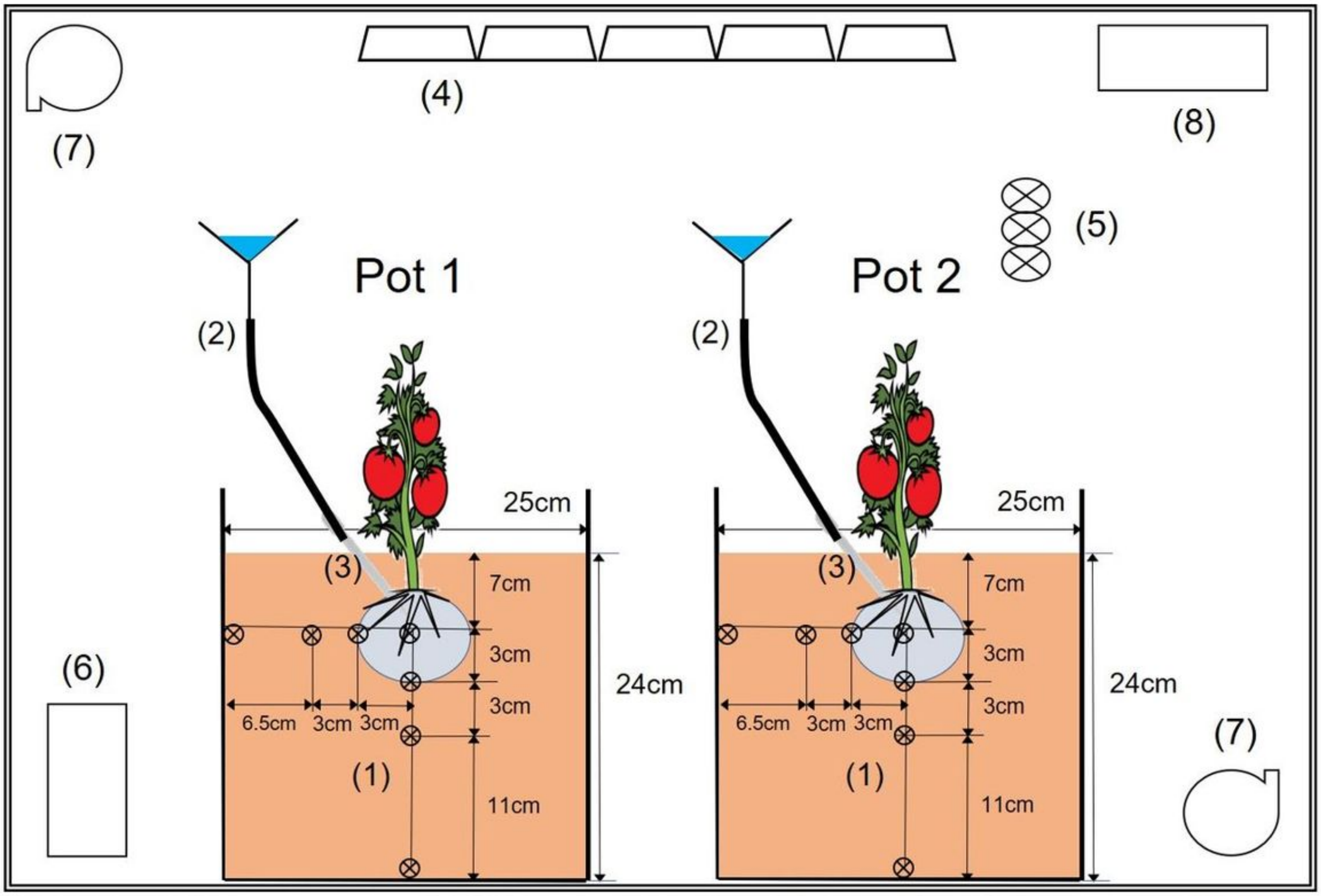


Figure 7

Root skeleton at the cross-section of the wet zone.



(1) Soil moisture sensors, (2) Water supply tube, (3) Pipette, (4) Fluorescent lamp, (5) Environmental sensors, (6) (7) (8) Temperature/humidity conditioning equipment

Figure 8

Experimental system

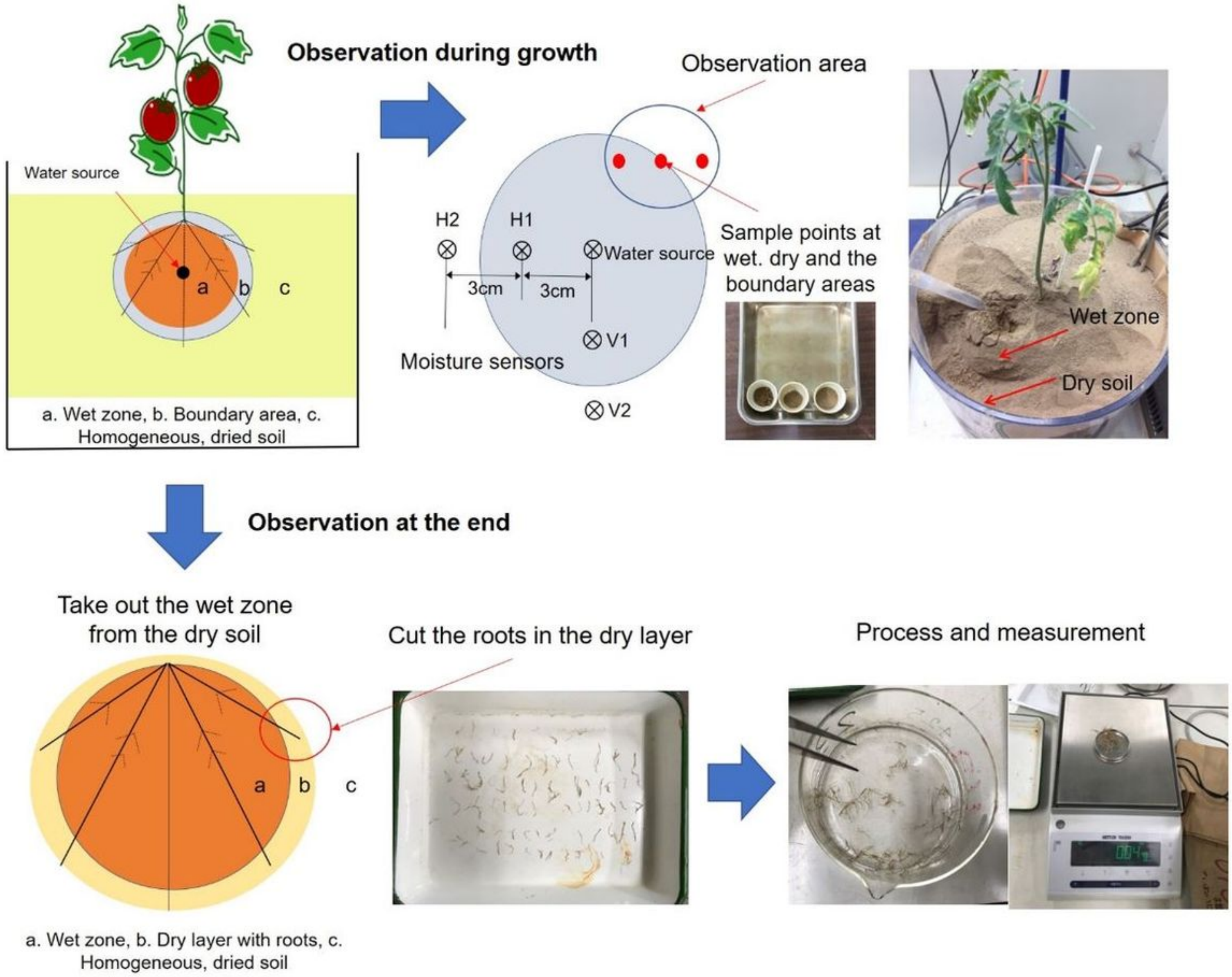


Figure 9

Observation of root distribution during and after plant growth period

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FFTresultsforeachsensor.xlsx
- soilwaterdynamicsduringtheexperimentalperiod.xlsx