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Development of sweet potato culture system using water and inorganic nutrient salts effectively

Siqinbatu

2014

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Abstract of the Thesis

Introduction

In recent years, there has been a global increase in desertification and decline of water resources, including in Inner Mongolia. These phenomena are attributed to the development and need for meeting increasing food and feed needs accompanying population increase. Deforestation and removal of natural plant cover for food production by improper management, such as overgrazing, cause outflow and scattering of topsoil and accelerate water evaporation from soil. Desertification, therefore, is progressing in many regions around the world. The culture of sweet potato has advantages since this crop has greater resistance to dry weather, higher yield, and higher profit than other vegetable crops. In addition, the roots, stems, and leaves are useful as livestock feed, and as a cover plant, can help to conserve soil. Sweet potato is, therefore, useful for food production in regions facing
desertification. With the aim of developing efficient methods for sweet potato production in arid or semi-arid regions, I investigated the effects of soil water content in the root zone on the growth and yield of sweet potato grown in sandy soil. In addition, one important consideration was to construct a sustainable agricultural production system that could circulate and reuse waste resources involving drainage to reduce the load on the local environment. Recently, methane fermentation has been promoted for effective use as a resource in waste biomass. Many digestates, as byproducts from methane fermentation, are disposed following treatment based on environmental assessment. There have been very few successful attempts to use digestates containing many nutrients in the form of inorganic nutrient salts as a liquid nutrient in agriculture. In this thesis, water and nutrients derived from a methane fermentation digestate were used in a sweet potato production system because bottom irrigation is a useful method to supply liquid nutrients to the root zone. The four key findings obtained are as follows:

1) Evaluation of drought tolerance of sweet potato
In an experiment using potted plants, the effects of soil water content on leaf stomatal conductance of sweet potato were compared to four other agricultural plant species (maize, okra, cucumber and tomato) in order to examine the level of drought stress tolerance. Soil volumetric water content was highest immediately after irrigation and decreased with time. Leaf conductance decreased after irrigation in the morning of Day 1 and continued to decrease throughout Day 2 if no irrigation was applied. All plants wilted in the afternoon of Day 1 and this condition became more severe on Day 2. Sweet potato, after a period of drought stress, had the ability to quickly increase leaf conductance after re-watering. These results indicate that sweet potato is a drought-tolerant crop that is able to recover from a certain level of drought stress and is able to maintain its growth rate by using water more efficiently under semi-dry conditions.

2) Effects of soil water content on growth of sweet potato

In regions where water is insufficient for agriculture, crop culture systems with efficient water use are imperative. With the aim of developing efficient methods for sweet potato production in arid or semi-arid regions, I investigated the effect of soil water content in the root zone on the growth
and yield of sweet potato cultivated in sandy soil. Sweet potato was cultured in containers filled with sand with four different water table levels (15 cm (treatment code: D15), 20 cm (D20), 25 cm (D25), and 30 cm (D30) from the soil surface), with highest volumetric water content (45%) and CO$_2$ concentration (3.5%) at a depth of 15 cm in D15, and becoming reduced in treatments with deeper water table levels (5% and 0.8%, respectively in D30). The dry mass of tuberous roots and the whole plant was greatest in D25 and smallest in D30, and D25 > D20 > D15 > D30 for the ratio of root dry mass to whole plant dry mass. In conclusion, tuberous root production of sweet potato can be promoted by maintaining a sufficient distance of the water table from the soil surface to sustain the volumetric water content at 10 to 15% (-0.42 to -0.35 MPa water potential) in sandy soil when a bottom irrigation system is used.

3) Effects of soil CO$_2$ concentration on growth of sweet potato

I investigated the effects of increasing CO$_2$ concentration on the growth of sweet potato during its period of establishment in order to test the hypothesis that elevated CO$_2$ concentrations in the root zone associated with elevated soil water content lead to growth stagnation. Sweet potato
was cultured in containers in which CO$_2$ concentration was adjusted to represent low (1.8%), average (2.5%) or high (4.9%) concentrations using chemical agents that absorbed or released CO$_2$ to imitate the gaseous environment possibly experienced in field soil. No tuberous roots developed in the high CO$_2$ treatment. Furthermore, the dry mass of the whole plant and fibrous roots was 1.6 and 3 times greater, respectively, in the low CO$_2$ treatment than in the high CO$_2$ treatment. It is hypothesized that high CO$_2$ concentrations in soil inhibited the growth of sweet potato tuberous roots in the treatment area with high soil water content. Thus, the inhibition of sweet potato growth due to a high CO$_2$ concentration in soil is based on the inhibition of water absorption through roots.

4) Application of digestate from a methane fermentation process for supplying water and nutrients in sweet potato culture

Digestate from a methane fermentation process, which contains many nutrients and water, is a potentially useful resource for agriculture. In order to decide the appropriate strength of digestate from methane fermentation for sweet potato production, different strengths of digestate diluted with
water were applied to sweet potato plants as the water and nutrient supply in sandy soil. The impact of the diluted digestate on the growth of sweet potato was compared with that of commercial nutrient solution (Otsuka formula A). The growth rate of tuberous roots with 1/20 strength of digestate was greatest among treatments with different strengths of digestate (1/80-1/2) and commercial nutrient solution (1/4-1). Consequently, this study confirms that digestate as a byproduct from methane fermentation based on biomass waste treatment is a suitable nutrient solution for sweet potato production. Maximum yield of tuberous roots was achieved when a 20-fold dilution of digestate was used.

In conclusion, 10 to 15% volumetric water content (-0.42 to -0.35 MPa water potential) and a low CO₂ concentration (1-2%) in soil are necessary to maintain tuberous root growth of sweet potato cultured in sandy soil using a bottom irrigation system. Theoretically, this would allow of the effective use of water in regions where there is insufficient water for agriculture. Water absorption through roots was inhibited by a decline in the soil water potential and low soil water content while the growth of tuberous roots was inhibited by a decline in water absorption through roots caused by an increase in soil CO₂ concentration when soil water content
was high.

This study also confirmed that digestate from methane fermentation can supply water and nutrients for sweet potato production. I demonstrated that digestate at appropriate dilutions is suitable as nutrients for sweet potato culture resulting in similar yield as obtained from conventional culture. I suggested a sweet potato production system with a bottom irrigation method with digestate from methane fermentation, which can be applied to semiarid regions. Results of this study will contribute towards the establishment of a water and resource recycle system, including regional sustainable agricultural systems.

**Key words:** Digestate from methane fermentation, Leaf conductance, Soil CO$_2$, Soil water content, Sweet potato, Tuberous root, Yield
Chapter 1 General Introduction

In recent years, desertification has advanced in many regions as a result of increased water evaporation from soil, spillage of topsoil and overgrazing. Poor water management for food production where there is an increasing population and a decrease in the availability of water for agriculture is especially serious in semi-arid regions. In these regions, there is a dire need to effectively use water in crop production. The world’s population is experiencing massive growth, particularly in developing countries of Asia and Africa, and is expected to increase to 8.5 billion by 2025 (The United Nations, 2011). Deforestation and removal of natural plant cover for food production by improper management practices such as overgrazing cause outflow and scattering of topsoil and accelerate water evaporation from soil (Sheng et al., 2000). Desertification, therefore, is expanding in many regions.

Sweet potato can fix more energy than other crops and can grow under poor soil conditions, which has contributed to its role as a food security crop in many regions (Mok et al., 1998). The leaves contain relatively high levels of nutritional compounds, including polyphenolic compounds.
(Ishiguro and Yoshimoto, 2005; Yoshimoto et al. 2005), which have potential antioxidant properties. Sweet potato is considered to be an environmentally friendly crop, as it requires low quantities of fertilizers and other agricultural chemicals and is tolerant to short-term stressful weather conditions such as typhoons, droughts, and high rainfall (Yamakawa, 1998). Its high growth rate and yield, combined with its low requirements for fertilizer and water, particularly under poor soil conditions, give it many advantages over other crops (Kozai et al., 1996b). The tuberous roots of sweet potato contain higher levels of various vitamins, minerals, and proteins than other vegetables (Woolfe, 1992), as well as antioxidants such as β-carotene, ascorbic acid, and tocopherol, which provide protection against heart disease and cancer (Ahn et al., 1998). Sweet potato is also an important leafy vegetable (Islam et al., 1997).

With regard to sweet potato culture, Ogawa et al. (2006) reported the relationship between changes in soil moisture by different irrigation methods corresponding to growth stage and yield, and Tanaka et al. (1997) reported the relationship between water holding capacity with different soil culture methods and growth stages. These investigations observed the pF value rather than volumetric water content in soil. However, the effect of
the water table depth on the yield of sweet potato was not clear in the field (Ghuman and Lal, 1983). Moisture regimes in soil will affect the growth of sweet potato for the growth period from establishment of cuttings to harvest. Rao and Li (2003) reviewed the effects of flooding on the growth of some field crops including sweet potato and noted mainly injurious effects of waterlogging on sweet potato tuberous roots. There are not many studies on the effects of excess moisture content in soil on the growth performance of sweet potato. The yield of tuberous roots of sweet potato decrease when irrigation water is supplied in excess (Thompson et al., 1992).

O₂ deficiency in the root environment can cause injuries to roots and inhibit plant growth. Root extension for many plant species in solution culture experiments is, however, generally kept at a normal rate when the O₂ partial pressure is as low as 100 hPa, which is almost half of the atmospheric O₂ concentration (Drew, 1983). Negative effects of high soil CO₂ concentration on the growth of sweet potato have been reported qualitatively (Kitaya et al., 1992; Islam et al., 1997, 1998a). Information about the impact of high soil CO₂ observed in cultivated fields on plant growth is, however, very limited, especially for tuberous root growth of
sweet potato. In addition, there is no information clearly accounting for the effect of soil CO$_2$ on plant growth, distinguishing it from the effect of soil water content.

Siqinbatu et al. (2013) examined the growth of sweet potato in sandy soil with different water contents and gas components. They reported that elevated soil CO$_2$ concentrations in the root zone associated with elevated soil volumetric water content suppressed growth and tuberous root development. Managing water supply to ensure sufficient water content and low CO$_2$ concentrations in sandy soil are important in sweet potato production. Compared to potato, sweet potato has greater resistance to dry weather, higher yield, and higher profitability. In addition, the roots and leaves are useful as livestock feed, and the roots help in soil conservation. Sweet potato is useful for food production in regions facing desertification. The soil and weather conditions in Inner Mongolia region are conducive for sweet potato culture, and this crop is useful for the agricultural ecological system there.

In this study, for a system that could produce sweet potato effectively in dry conditions, such as in semiarid regions where available water is
insufficient for agriculture, was considered since sweet potato is produced globally. Soil water content and the gas component in sandy culture were considered when designing the bottom irrigation system.

The construction of a local sustainable agriculture production that uses circulated waste resources through a drainage system to reduce the environment load was another objective of this study. Recently, methane fermentation by using industrial waste as biomass resources is being promoted.

Most digestates as by-products from methane fermentation are discharged through treatment based on environmental assessment (Iwashita et al., 2010). Characteristic The digestate from methane fermentation is characterized by many nitrogen- and potassium-containing components but few phosphorus-containing components. Most of these nitrogen-containing components are in the form of ammonia (Nakamura et al., 2011). Digestates that include many inorganic nutrient salts could serve as liquid nutrient constituents in agriculture but such use is still limited.
The supply of water and nutrients derived from methane fermentation digestates for sweet potato production was studied because a bottom irrigation system is available to supply nutrient liquids to the root zone.
Chapter 2 Evaluation of drought tolerance of sweet potato

2.1 Abstract

In an experiment using potted plants, the effects of soil water content on leaf stomatal conductance of sweet potato were compared to four other agricultural plant species (maize, okra, cucumber and tomato) in order to examine the level of drought stress tolerance. Soil volumetric water content was highest immediately after irrigation and decreased with time. Leaf conductance decreased after irrigation in the morning of Day 1 and continued to decrease throughout Day 2 if no irrigation was applied. All plants wilted in the afternoon of Day 1 and this condition became more severe on Day 2. Sweet potato, after a period of drought stress, had the ability to quickly increase leaf conductance after re-watering. These results indicate that sweet potato is a drought-tolerant crop that is able to recover from a certain level of drought stress and is able to maintain its growth rate by using water more efficiently under semi-dry conditions.
2.2 Introduction

Various perspectives of drought tolerance in sweet potato have been studied (Prabawardani et al., 2004; van Heerden et al., 2008). These studies were conducted on long-term drought tolerance that corresponded to water supply levels that involved rainfall in practical field culture.

To date, physiological properties or parameters have not been reported for drought tolerance of sweet potato under changing volumetric water content in soil. Therefore, I planned a watering schedule that involved no watering and re-watering for 3 days continuously after ordinary culture for 50 days. Of the objective was to confirm physiological parameters related to drought tolerance in sweet potato, including PPFD, temperature, humidity deficit, volumetric water content and leaf conductance were measured over time. In order to evaluate the drought stress tolerance of sweet potato through leaf conductance caused by variations in soil water content, data was compared with that of four other agricultural plants (maize, okra, cucumber and tomato).
2.3 Materials and Methods

2.3.1 Measurements of leaf gas exchange rates and leaf conductance in sweet potato (preliminary experiment)

Sweet potato (*Ipomoea batatas* (L.) Lam, cv. ‘Kokei No. 14’) were grown for 148 days, from June 16 to November 10, 2013, in a plastic house with fully opened side windows under a simulated dry condition at Osaka Prefecture University. Leaf conductance as well as net photosynthetic and transpiration rates were measured using a portable photosynthesis and transpiration measurement system (LI6400, LI-COR Inc., city, USA).

2.3.2 Evaluation of drought tolerance of sweet potato

Five agricultural crops, sweet potato (cv. ‘Kokei No. 14’), maize (*Zea mays* L., ‘Canberra 86’), okra (*Abelmoschus esculentus* Moench, ‘Green Star’), cucumber (*Cucumis sativus* L. ‘Hokushin’) and tomato (*Solanum lycopersicum* L., ‘Momotaro’), were grown for 53 days, from May 8 to June 29, 2010, in the same plastic house as in the preliminary experiment. Each plant were planted in a plastic pot (18 cm diameter, 20 cm in height)
filled with sandy soil. A half-strength standard nutrient solution (A-type Otsuka formula, Otsuka Chemical Co., city, Japan) and water were applied to the soil surface every morning. The air temperature and vapor pressure deficit were measured using a temperature and humidity data logger (TR-72; T&D Co., city, Japan) at 1 m above the floor surface. PPFD was measured using a photometer (ISA-3151, T&D Co., city, Japan).

Ten plants for each species were grown and three of these were treated to drought and re-watering treatments. Soil volumetric water content and leaf conductance were monitored periodically. Environmental factors such as air temperature, humidity deficit, and PPFD were monitored continuously. The soil volumetric water content of each pot was measured using a soil moisture meter (TDR100; Campbell Scientific Ltd., city, USA) at a depth of 0-15 cm from the soil surface. The measured value was then compared against the value calculated using a gravimetric method.

Leaf conductance was used as an index of the photosynthetic and transpiration rates. In a preliminary experiment, we found that the leaf conductance measured on the abaxial or adaxial leaf surface was significantly related to that of the whole leaf, accounting for 62% and 38%
of the whole leaf, respectively (Fig. 2.1). The ratio of leaf conductance on the abaxial leaf surface to that on the adaxial leaf surface was nearly 2:1. There was also a strong correlation between the conductance of the whole leaf surface and the conductance of the abaxial and adaxial leaf surfaces. Thus, it was confirmed that the conductance of the whole leaf surface could be accurately estimated using measurements of conductance of the abaxial leaf surface. Leaf conductance was, therefore, measured on the abaxial leaf surfaces of leaves using a steady state porometer (LI-1600; LI-COR Inc., city, USA), and these measurements were then used as an index for the gas exchange ability of leaves. PPFD was also monitored at the same time, as it is an important environmental factor positively affecting leaf conductance in general (Siqinbatu et al., 2013).

At the end of the experiment, the number of leaves (mean ± SD) was 12 ± 1, 52 ± 8, 11 ± 2, 36 ± 5, and 190 ± 20, for sweet potato, maize, okra, cucumber, and tomato, respectively, and the corresponding lengths of the stem were 1.22 ± 0.21 m, 1.08 ± 0.07 m, 1.24 ± 0.09 m, 0.95 ± 0.12 m, and 1.27 ± 0.25 m.
Fig. 2.1. Relationships between leaf conductance on the whole leaf surface and leaf conductance on the abaxial (○) and adaxial (●) leaf surface of sweet potato under variable photosynthetic photon flux density (PPFD) conditions.
2.4 Results and Discussion

In the preliminary experiment, the relationship between net photosynthetic rate and leaf conductance as well as the relationship between transpiration rate and leaf conductance in sweet potato was linear (Fig. 2.2). Such a relationship indicates that leaf conductance can be used as an indicator of the net photosynthetic and transpiration rates in sweet potato leaves. Figure 2.3 indicates the relationship between volumetric water contents and the water potential in sandy soil used in this research. The water potential declined according to decrease in the volumetric water contents.

In the drought tolerance experiment, there was a transition of leaf conductance of sweet potato, maize, okra, cucumber and tomato and volumetric water content in the soil (Fig. 2.4a-e). Fig. 2.4f shows the transition in air temperature, humidity deficit and PPFD. The mean air temperature was 38.2°C ± 2.1°C (mean ± SD) and the absolute humidity deficit was 30.1 ± 6.2 g m⁻³ from 08:00 to 16:00 during the experimental period (Fig. 2.4f). Soil volumetric water content was highest immediately after irrigation and decreased with time. Volumetric water content peaked
at around 25% at 08:00AM after 30 min. of watering (Day 1) and declined almost linearly to around 10% at 13:00 PM and it was around 8% at 16:00 PM (Fig. 2.5a). Volumetric water content in soil on the no-watering Day 2 declined to around 8~1% in all crops, which almost withered (Fig. 2.5b). Volumetric water content in soil on re-watering Day 3 had nearly the same tendency as Day 1. Leaf conductance decreased from 0.7 cm s$^{-1}$ at the maximum to 0.1 cm s$^{-1}$ at the minimum in all crops (Fig. 2.4a-e). Leaf conductance decreased after irrigation in the morning of Day 1 and continued to decrease throughout Day 2 if plants were not irrigated. Wilting was observed in all plants by the afternoon of Day 1 and became more severe on Day 2. Leaf conductance and withering after re-watering in sweet potato and maize (Fig. 2.4a,b) recovered rapidly (Fig. 2.5c) compared to gumbo, cucumber and tomato (Fig. 2.4c,d,e). Leaf conductance after re-watering in sweet potato and maize were highest compared to the other crops and recovered rapidly to reach the level of Day 1.

Consequently, leaf conductance in sweet potato and maize following re-watering after a day of water stress showed a speedy recovery compared to okra, tomato and cucumber. These results indicate that sweet potato is a
drought-tolerant crop that is able to recover from a certain level of drought stress and is able to maintain its growth rate by using water more efficiently under semi-dry conditions.
Fig. 2.2. Relationships between leaf conductance and the net photosynthetic or transpiration rates in sweet potato leaves measured at different PPFDs.

Net photosynthetic rate (µmol CO₂ m⁻² s⁻¹)

Transpiration rate (mmol H₂O m⁻² s⁻¹)

Leaf conductance (mm s⁻¹)

PPFD (µmol m⁻² s⁻¹)

- 600
- 900
- 1200

y = 0.9097x + 5.4911
R² = 0.8125

y = 0.3452x + 1.7896
R² = 0.849
Fig. 2.3. Relationship between volumetric water content and the water potential.

The volumetric water content were measured using a soil moisture meter (TDR100; Campbell Scientific Ltd., city, USA) and the water potential were measured using a tension meter (DP2-80; TAKEMURA Co., Japan).
Fig. 2.4. Change in leaf conductance and soil volumetric water content for each crop (a-e) and fluctuations in PPFD, air temperature and humidity deficit over 3 days. Arrows indicate irrigation time at 07:30. Error-bars for leaf conductance ($n = 5$) and soil volumetric water content ($n = 3$) represent standard deviation.
Fig. 2.5. Photograph under effect of presence of water supply in culture of sweet potato and several crops (experiment 1). No. under each photograph shows ① Okra, ② Tomato, ③ Cucumber, ④ Maize and ⑤ Sweet potato, respectively.
2.5 Conclusions

Leaf conductance in re-watering after sweet potato and maize were received water stress during a day showed speedy recovery compared to okra, tomato and cucumber. These results indicate that sweet potato is a drought-tolerant crop that is able to recover from a certain level of drought stress and is able to maintain its growth rate by using water more efficiently under semi-dry conditions. This evidence confirmed that sweet potato is a drought stress-tolerance crop which could efficiently use available water in semi-arid regions.
Chapter 3 Effects of soil water content on growth of sweet potato

3.1 Abstract

In regions where water is insufficient for agriculture, crop culture systems with efficient water use are imperative. With the aim of developing efficient methods for sweet potato production in arid or semi-arid regions, I investigated the effect of soil water content in the root zone on the growth and yield of sweet potato cultivated in sandy soil. Sweet potato was cultured in containers filled with sand with four different water table levels (15 cm (treatment code: D15), 20 cm (D20), 25 cm (D25), and 30 cm (D30) from the soil surface), with highest volumetric water content (45%) and CO₂ concentration (3.5%) at a depth of 15 cm in D15, and becoming reduced in treatments with deeper water table levels (5% and 0.8%, respectively in D30). The dry mass of tuberous roots and the whole plant was greatest in D25 and smallest in D30, and \( D_{25} > D_{20} > D_{15} > D_{30} \) for the ratio of root dry mass to whole plant dry mass. In conclusion, tuberous root production of sweet potato can be promoted by maintaining a sufficient distance of the water table from the soil surface to sustain the
volumetric water content at 10 to 15% (-0.42 to -0.35 MPa water potential) in sandy soil when a bottom irrigation system is used.

3.2 Introduction

In recent years, desertification is advance in many regions by increased water evaporation from soil, spillage of topsoil and overgrazing in some region according to destructive development and disordered water use for the food production due to population increase and decrease of water for agriculture especially has been serious in semi-arid region. In these regions, effectively use of water in the crop production has demanded. The world population is being experienced massive growth, particularly in the developing countries of Asia and Africa, and it is expected to increase to 8.5 billion by 2025 (The United Nations, 2011). Deforestation and removal of the natural plant cover for food production by improper managements such as overgrazing cause outflow and scattering of topsoil and accelerate water evaporation from soil (Sheng, et al., 2000). Desertification, therefore, is progressing in many regions.
Sweet potato culture has advantages of more resistance to dry weather, higher yield, and higher profit compared to other vegetable crops. In addition, the roots, stems, and leaves are useful as livestock feed, and a property as a cover plant helps soil conservation. Sweet potato is, therefore, useful for food production in regions facing desertification. With regard to sweet potato culture, Ogawa et al. (2006) reported the relationship between the change in soil moisture by different irrigation methods corresponding to growth stage and the yield, and Tanaka et al. (1997) reported the relationship between water holding capacity with different soil culture methods and the growth of sweet potato. However, the effect of the water table depth on the yield of sweet potato was not clear in the field (Ghuman and Lal, 1983).

In this study, I investigated the effect of water content in the root zone on the growth and yield of sweet potato culture in sandy soil in order to develop efficient methods for sweet potato production in arid or semi-arid regions.
3.3 Materials and Methods

Sweet potato cuttings were planted in containers filled with sand in 2009 and 2010. The culture experiment was conducted in a plastic house with fully opened side windows at Osaka Prefecture University, under conditions that imitated those experienced in dry conditions.

The experiment was conducted for 155 days from June to November and was replicated twice (in 2009 and 2010). Four plastic bottomless culture containers (30 × 75 × 30 cm deep each) were filled with sandy soil to a depth of 30 cm and set in a water tank (130 × 80 × 20 cm deep) (Fig. 3.1). Ten sweet potato (Ipomoea batatas (L.) Lam, cv. ‘Beniazuma’, ‘Kokei No. 14’) cuttings were planted in two lines at 15 cm intervals in each container. A nutrient solution was then supplied to the soil surface every day for 2 weeks. After 2 weeks, when the roots had developed, one side of the water tank was lifted to a height of 15 cm, so that the distance between the water table and the soil surface was 15 cm, 20 cm, 25 cm, and 30 cm for each of the four containers. The nutrient solution was half-strength of a standard nutrient solution (Otsuka formula A, Otsuka Chemical Co., Japan). The
nutrient solution was supplied continuously from the bottom of the container as shown in Fig. 3.1.

The volumetric water content was also measured at depths of 15 cm, 20 cm, 25 cm, and 30 cm from the soil surface for every water table treatment using a soil moisture meter (TDR100; Campbell Scientific Ltd., city, USA). The O₂ concentration in the soil was measured at depths of 10 cm and 20 cm from the soil surface for every water table treatment using an O₂ meter (OXY-4; Pre Sens Co., city, Germany). The CO₂ concentration in the soil was measured by inserting tubes to depths of 10 cm and 20 cm from the soil surface and using a syringe to sample soil gas for every water table treatment. These samples were then analyzed using gas chromatography. Leaf conductance in the abaxial leaf was measured for 30 leaves in each treatment using a steady state porometer (LI-1600; LI-COR Inc., USA), as outlined by Siqinbatu et al. (2013), and the measurement was used as an index for net leaf photosynthesis and transpiration. Measurements were made at 9:00–18:00 h on August 6, 24 and 25 in 2010.

The temperature and relative humidity at the experimental site were measured every 10 min during the culture periods at 1 m above the ground
using a thermo recorder (TR-72U; T&D Co., Japan). The temperature of the soil in the culture containers was determined at depths of 5 cm and 15 cm from the soil surface for the 15 cm and 30 cm water table treatments. The air temperature and relative humidity in the plastic house were 28.9 ± 4.1°C (mean ± SD) and 54 ± 13%, respectively, in 2009, and 31.5 ± 4.5°C and 76 ± 11%, respectively, in 2010. The soil temperature at depths of 5 cm and 15 cm from the soil surface was 28.1 ± 3.3°C and 26.9 ± 1.4°C, respectively, in 2009 and 2010. Thermal images of plants were captured using an infrared thermography system (TH9100; NEC-San-ei Co., Japan) to compare the leaf temperatures and thus the transpiration ability of plants. The emissivity value was set at 0.93 following the findings of Jones (1999). The images were taken at 10:00-14:00 h on August 11, 12 and 13 in 2010.

Fresh and dry weights of each part of the sweet potato plant were measured after harvesting. To measure the dry weight (dry mass), plants were dried at 80°C in an oven for more than 4 days after fresh weight measurements.


**Statistical analysis**

A multiple comparison test (Tukey-Kramer) was performed to compare the means of growth parameters among treatments. Least significant differences (LSD) were used to determine significant differences at P<0.05.
Fig. 3.1. Experimental system for culturing sweet potato with different water table levels in sandy soil in 2009 and 2010.
3.4 Results

The air temperature and relative humidity in the plastic house were 28.9 ± 4.1°C (mean ± SD) and 54 ± 13%, respectively, in 2009, and 31.5 ± 4.5°C and 76 ± 11%, respectively, in 2010. The soil temperature at depths of 5 cm and 15 cm from the soil surface was 28.1 ± 3.3°C and 26.9 ± 1.4°C, respectively, in both 2009 and 2010. The soil volumetric water content in D15, D20, D25 and D30 showed the following ranges in the 10-20 cm soil layer below the soil surface: 34-46%, 21-36%, 10-15%, and 5-9%, respectively. The volumetric water content of the soil was greatest when the water table was 15 cm below the soil surface, and decreased as the water table lowered (Fig. 3.2).

CO₂ concentration was highest in deeper soil (Fig. 3.3) and, for any given depth, was greatest when the water table was 15 cm below the soil surface and decreased as the water table lowered. The highest CO₂ concentration was 3.5% at a depth of 20 cm when the water table was 15 cm below the soil surface. In contrast, O₂ concentration was lower in deeper soil (Fig. 3.4) and, for any given depth, was lowest when the water table was 15 cm below the soil surface and increased as the water table
lowered. The lowest O₂ concentration was 17.2% at a depth of 20 cm when the water table was 15 cm below the soil surface. Mean soil CO₂ concentration was 2.8%, 2.1%, 1.4%, and 0.9%, and mean soil O₂ concentration was 18.1%, 18.7%, 19.2%, and 19.7% in D15, D20, D25, and D30, respectively. The soil CO₂ concentration increased and the soil O₂ concentration decreased as the water table lowered, showing positive/negative liner relationships between CO₂/O₂ concentrations and volumetric water content.

Fig. 3.2. Profiles of volumetric water contents in sandy soil with different water table levels in 2010. Horizontal error-bars indicate standard deviations (n = 5).
Fig. 3.3. Profiles of CO$_2$ concentrations in sandy soil with different water table levels in 2010. Horizontal error-bars indicate standard deviations ($n = 9$).

Fig. 3.4. Profiles of O$_2$ concentrations in sandy soil with different water table levels in 2010. Horizontal error-bars indicate standard deviations ($n = 9$).
Figures 3.5 and 3.6 show the dry weight of leaves, stems, fibrous roots, and tuberous roots of sweet potato plants cultured in sandy soil with different water table levels in 2010. The dry weights of the whole plant and tuberous roots were greatest when the water table was 25 cm below the soil surface and smallest when it was 30 cm deep. Similar results were obtained in 2009 and 2010, although growth was slightly lower in 2009 than in 2010 due to lower temperatures and relative humidity.

The highest ratio of tuberous root dry weight to whole plant dry weight, 0.55, was observed when the water table was 25 cm below the soil surface and smallest when the water table was 15 cm. On the other hand, the ratios of dry weight of fibrous roots and shoots (leaves + stems) to whole plant dry weight were 0.19 and 0.68, respectively. Both were highest when the water table was 15 cm below the soil surface. In addition, they were 0.05 and 0.48, respectively, and both lowest when the water table was 30 cm below the soil surface.
Fig. 3.5. Dry weights of different segments of sweet potato (cv. Beniazuma) cultured in sandy soil with different water tables levels in 2010.

Least significant difference (LSD) was determined at the 5% level by Tukey-Kramer multiple comparison test. Vertical error-bars indicate standard deviations of whole plant dry weights ($n = 10$).
Fig. 3.6. Dry weights of different segments of sweet potato (*cv. Kokei No. 14*) cultured in sandy soil with different water tables levels in 2010.

Least significant difference (LSD) was determined at the 5% level by Tukey-Kramer multiple comparison test. Vertical error-bars indicate standard deviations of whole plant dry weights (*n* = 10).
Tuberous roots tended to form on lateral roots from the first node just under the soil surface in each water table treatment as shown in Fig. 3.7 and Fig. 3.8. These photos also show that tuberous root formation was promoted in the 25 cm water table and fibrous root formation tended to be promoted at 15 cm and at 20 cm. The plants of the 30 cm water table had the longest fibrous roots.

Leaf conductance was measured as an index of the photosynthetic and transpiration ability at various photosynthetic photon flux densities (PPFDs). Leaf conductance was significantly smaller \( (P<0.05) \) when the water table was 30 cm below the soil surface than other water table levels at PPFDs ranging from 500 to 1500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (Fig. 3.9.1 and Fig. 3.9.2). There was no significant difference among other water table levels. Leaf conductance was significantly greater \( (P<0.05) \) when the water table was 25 cm below the soil surface than 15 and 20 cm at PPFDs ranging from 1000 to 2000 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). The water content of the shoots tended to be smallest when the water table was 15 cm below the soil surface (Table 1).

The thermal image of the canopy of sweet potato plants grown in sandy soil at different water table levels shows a higher canopy temperature in
D30 compared with other levels (Fig. 3.10). The mean temperatures of 60 leaves measured for three days were $31.4 \pm 1.5^\circ C$ (mean ± SD), $31.8 \pm 1.3^\circ C$, $31.5 \pm 1.1^\circ C$, and $35.1 \pm 1.2^\circ C$, respectively, in D15, D20, D25 and D30.

![Representative underground parts of sweet potato (cv. Beniazuma) cultured in sandy soil with different water table levels in 2010.](image)

Fig. 3.7. Representative underground parts of sweet potato (cv. Beniazuma) cultured in sandy soil with different water table levels in 2010.
Fig. 3.8. Representative underground parts of sweet potato (cv. *Kokei No. 14*) cultured in sandy soil with different water table levels in 2010.
Fig. 3.9.1 Leaf conductance as affected by photosynthetic photon flux density (PPFDs) for sweet potato (cv. Beniazuma) cultured in sandy soil with different water table levels in 2010.
Fig. 3.9.2 Leaf conductance as affected by photosynthetic photon flux density (PPFDs) for sweet potato (cv. **Kokei No. 14**) cultured in sandy soil with different water table levels in 2010.
Fig. 3.10. Representative thermal image of sweet potato cultivated in sandy soil with different water table levels: 15 cm (D15), 20 cm (D20), 25 cm (D25), and 30 cm (D30) below soil surface. The images were taken at 14:00 on August 11, 2010. The mean temperature of each area was determined with 30 leaves in the different water table treatments. Air temperature: 40.6°C; Humidity deficit: 36.9 g m⁻³.
Table 3.1. Water contents (expressed as % of fresh weight) of sweet potato plants cultured in sandy soil with different water table levels in 2010.

<table>
<thead>
<tr>
<th>Water table</th>
<th>Shoot</th>
<th>Tuberous roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 cm</td>
<td>80.4</td>
<td>70.7</td>
</tr>
<tr>
<td>20 cm</td>
<td>83.6</td>
<td>69.2</td>
</tr>
<tr>
<td>25 cm</td>
<td>84.6</td>
<td>69.8</td>
</tr>
<tr>
<td>30 cm</td>
<td>83.8</td>
<td>68.9</td>
</tr>
<tr>
<td>LSD.</td>
<td>3.7</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Least significant difference (LSD) was determined at the 5% level by Tukey-Kramer multiple comparison test. (n = 10).
3.5 Discussion

Sandy soil with a water table depth of 25 cm below the soil surface was suitable for producing tuberous roots (Fig. 3.4 and Fig. 3.5). Although the high water content of the root zone soil with the water table depth of 15 cm accelerated the growth of fibrous roots and the shoot, it had a negative effect on the formation of tuberous roots. This fact indicates that wet soil is inappropriate for the growth of sweet potato and confirmed the result of Thompson et al. (1992), who reported that the yield of sweet potato tuberous roots decreased as the amount of irrigation water increased. Ghuman and Lal (1983) reported that poor soil aeration due to an adverse soil moisture regime under a high water table or temporary flooding conditions lowered tuber yield of sweet potato. Pardales and Escalante (1978) reported that the formation of tuberous roots depended largely on the water table level in soil and that fewer marketable tubers were produced at shallower water tables.

The O₂ concentration in the soil was 18.1-19.6% throughout. A decrease in O₂ concentration in the soil has been shown to inhibit the growth of many plant species. For example, soil O₂ concentration at 1.5, 2.5 and 5%
resulted in 40, 25 and 17\% retardation of tuberous root dry mass growth of sweet potato (Watanabe et al., 1968), which can partly account for the poor performance of sweet potato in waterlogged soil. The soil $O_2$ concentration in this study was not very low to cause any negative effects on plant growth. According to Chua and Kays (1981), low oxygen in the root zone promoted the formation of fibrous roots of sweet potato. Promotion of the development of fibrous roots in Fig. 3.5 and Fig. 3.6 might be due to a low $O_2$ concentration (Fig. 3.4), when the water table was 15 cm below the soil surface. On the other hand, negative effects of high soil $CO_2$ on plant growth have been demonstrated in previous studies. $CO_2$ concentrations in soil increase with increasing soil water content due to suppression of soil aeration, which may explain the poorer growth and development of tuberous roots when the water content of the rooting substrate was higher in this study. Thus, the suppression of tuberous root growth is likely to be caused by an increase in $CO_2$ concentration as a result of poorer aeration of sandy soil at higher water contents.

Pardales and Yamauchi (2003) reported that the growth (elongation) of the fibrous roots of sweet potato was greatly suppressed by soil water deficiency. Our results in Fig. 3.5, Fig. 3.6, Fig. 3.7 and Fig. 3.8 partly
confirmed the result of Pardales and Yamauchi (2003), but the low soil water content associated with the low water table level caused some fibrous roots to elongate. In addition, our results proved that a very high CO$_2$ concentration in the root zone also suppressed fibrous root growth as well as tuberous root growth, even when the soil water content was sufficient for plant growth.

Leaf conductance was lowest in D30 but was mostly similar in D15, D20 and D25 at PPFDs ranging from 200 to 1500 µmol m$^{-2}$ s$^{-1}$ (Fig. 3.9). Thermal image of the canopy of sweet potato plants grown in sandy soil at different water table levels shows a higher canopy temperature in D30 compared with other levels (Fig. 3.10). The mean temperature of 60 leaves measured for three days was 31.4 ± 1.5°C (mean ± SD), 31.8 ± 1.3°C, 31.5 ± 1.1°C, and 35.1 ± 1.2°C, respectively, in D15, D20, D25 and D30. The thermal images and mean leaf temperatures clearly indicated higher leaf temperatures in D30 than in D15, D20 and D25. This evidence indicates that a decline in soil volumetric water content in the root zone, corresponding to a low water table, caused the depression of transpiration in D30. This phenomenon was also confirmed by the suppression of leaf conductance in D30 (Fig. 3.9).
The shoot water content was lowest (Table 1) even under the highest volumetric water content (Fig. 3.2) a water table level of 15 cm. This fact could be evidence for the suppression of water absorption by a high CO$_2$ concentration in the root zone, because the CO$_2$ concentration in soil was highest in the water table level of 15 cm (Fig. 3.3). Leaf conductance was lower when the water table was 15 and 20 cm bellow the soil surface than at 25 cm at high PPFDs, presumably due to higher CO$_2$ concentrations in soil. Siqinbatu et al. (2013) outlined that there is an appropriate volumetric water content for sweet potato tuberous root development, which would be the best combination of sufficient water content for promoting water absorption of roots and a moderately low CO$_2$ concentration for avoiding the suppression of water absorption by roots. Consequently, manipulation of the water content in soil is important to control not only water supply but also the soil gas composition for promoting tuberous root production in sweet potato.
3.6 Conclusion

In this study, sandy soil with a water table depth of 25 cm below the soil surface was found to be suitable for producing tuberous roots with a bottom irrigation system. Tuberous root production of sweet potato can be promoted by maintaining a sufficient distance of the water table from the soil surface to sustain the soil volumetric water content at 10 to 15% (-0.42 to -0.35 MPa water potential) and soil CO$_2$ concentration low in the root zone. It is thought that growth inhibition in the case of low soil water content resulted from inhibition of water absorption through a root based on the decline of the water potential at the periphery of the root. Tuberous roots did not form in the D30 treatment and it was suggested that the growth of sweet potato tuberous roots was inhibited caused by a rise in CO$_2$ concentration in soil as the soil water content increased.
Chapter 4 Effects of soil CO$_2$ concentration on growth of sweet potato

4.1 Abstract

I investigated the effects of increasing CO$_2$ concentration on the growth of sweet potato during its period of establishment in order to test the hypothesis that elevated CO$_2$ concentrations in the root zone associated with elevated soil water content lead to growth stagnation. Sweet potato was cultured in containers in which CO$_2$ concentration was adjusted to represent low (1.8%), average (2.5%) or high (4.9%) concentrations using chemical agents that absorbed or released CO$_2$ to imitate the gaseous environment possibly experienced in field soil. No tuberous roots developed in the high CO$_2$ treatment. Furthermore, the dry mass of the whole plant and fibrous roots was 1.6 and 3 times greater, respectively, in the low CO$_2$ treatment than in the high CO$_2$ treatment. It is hypothesized that high CO$_2$ concentrations in soil inhibited the growth of sweet potato tuberous roots in the treatment area with high soil water content. Thus, the inhibition of sweet potato growth due to a high CO$_2$ concentration in soil is based on the inhibition of water absorption through roots.
4.2 Introduction

Root injuries and plant growth inhibition are induced by O\textsubscript{2} deficiency in the root environment. Root extension for many plant species in solution culture experiments is, however, generally kept at a normal rate at an O\textsubscript{2} partial pressure as low as 100 hPa, which is almost half of the atmospheric O\textsubscript{2} concentration (Drew, 1983). Negative qualitative effects of high soil CO\textsubscript{2} concentration on the growth of sweet potato have been reported (Kitaya et al., 1992; Islam et al., 1997, 1998a). Islam et al. (1997, 1998a and 2000) demonstrated that tuberous root growth of sweet potato was promoted when plastic pipes with holes, rice or wheat straws, rice husks, etc. were placed into the root zone of soil ridges, as these promoted soil gas ventilation and thus decreased CO\textsubscript{2} concentration to less than 0.5% in soil. Results of these previous studies indicate that soil aeration can considerably affect tuberous root formation of sweet potato, as was confirmed by this study. Information about the impact of high soil CO\textsubscript{2} observed in cultivated fields on plant growth is, however, very limited, especially for sweet potato tuberous root growth. In addition, there is no information clearly accounting for the effect of soil CO\textsubscript{2} on plant growth
distinct from the effect of soil water content. I studied the effect of rising soil CO$_2$ concentration on the growth of sweet potato.

### 4.3 Materials and Methods

Sweet potato (cv. ‘Beniazuma’) plants were cultured for 123 days from June to October in 2010 in a plastic house at Osaka Prefecture University. Sweet potato cuttings, which planted one in each of 15 cylindrical vinyl chloride pots (8.3 cm in diameter and 60 cm deep) filled with sand (Fig. 4.1), were grown for 2 weeks. After 2 weeks, vessels containing either sodium hydrogen carbonate (to absorb CO$_2$) or calcium hydroxide (to produce CO$_2$) were placed 7 cm from the bottom of each pot to regulate the CO$_2$ concentration to 1.8%, 2.5% and 4.9% in the root zone. The bottom of each pot was sealed with a water layer. Five pots were used in each CO$_2$ concentration treatment. Nutrient solution was applied periodically to the soil surface, as outlined for Experiment 1 above. We supplied CO$_2$ to the sand soil from the bottom of the cylindrical container in order to increase the CO$_2$ concentration in the root zone. There were no significant increase in atmospheric CO$_2$ and no effects on photosynthesis and transpiration, because the CO$_2$ release rate from the soil surface was negligibly small and
the air ventilation rate was sufficiently large for rapid dispersion inside the plastic house with fully opened side windows.

Leaf conductance in the abaxial leaf measured using a steady state porometer (LI-1600; LI-COR Inc., USA) was measured for 30 leaves in each treatment as outlined by Siqinbatu et al. (2013), and the measurement was used as an index for net leaf photosynthesis and transpiration. Measurements were taken between 09:00 and 18:00 h on August 24 and 25 in 2010. Thermal images of plants were captured using an infrared thermography system (TH9100; NEC-San-ei Co., Japan) to compare the leaf temperatures and thus the transpiration ability of plants. The emissivity value was set at 0.93 following the findings of Jones (1999). The images were taken at 10:00-14:00 h on August 11, 12 and 13 in 2010.
Chemical agents for regulating CO$_2$ concentrations
Absorbent agent: Sodium hydrogen carbonate
Producing agent: Calcium hydroxide

Water supply from surface of sandy soil
Sampling points for soil gases
Cylindrical container (8.3 cm in diameter and 60 cm in depth)

Sweet potato
Sandy soil (48 cm in depth)
Water (5 cm in depth)

Fig. 4.1. Experimental system for culturing sweet potato with different CO$_2$ concentrations in sandy soil.
4.4 Results

The air temperature and relative humidity in the plastic house were 29.2 ± 6.6°C (mean ± SD) and 64 ± 15%, respectively, during the experimental period. The mean CO₂ concentrations in the soil were 1.8%, 4.9%, and 2.5% for the low and high CO₂ treatments, and the control, respectively, during the experimental period. The corresponding O₂ concentrations in the soil were 18.8%, 15.5%, and 17.1%, respectively. O₂ concentration and volumetric water content in the soil was 16-19% and 15% at all times, and temperatures were similar throughout the experimental period.

Figure 4.2 shows the dry weight of leaves, stems, fibrous roots, and tuberous roots of sweet potato plants cultured in sandy soil with different CO₂ concentrations. No tuberous roots formed in plants in the high CO₂ treatment. The dry weights of the whole plant and the fibrous roots in the low CO₂ treatment were 1.6 and 3 times greater, respectively, than those in the high CO₂ treatment (Fig. 4.2). There was no significant difference in the dry weights of organs between the low CO₂ treatment and the control (Fig. 4.3). The ratio of shoot (leaves + stems) dry weight to whole plant dry weight was 0.86 and highest in the high CO₂ treatment. The ratio of fibrous
root dry weight to whole plant dry weight was 0.14 and lowest in the high CO$_2$ treatment. Growth of the entire root system, including tuberous roots, was suppressed when exposed to a high concentration of CO$_2$ in the root zone.
Fig. 4.2. Dry weights of different segments of sweet potato cultured in sandy soil with different CO$_2$ concentrations.

Least significant difference (LSD) was determined at the 5% level by Tukey-Kramer multiple comparison test. Vertical error-bars indicate standard deviations ($n = 5$).
High CO$_2$ (4.9%)  Control (2.5%)  Low CO$_2$ (1.8%)

Fig. 4.3. Representative underground parts of sweet potato cultured in sandy soil with different CO$_2$ concentrations.
Leaf conductance was significantly lower ($P<0.05$) in plants exposed to the high CO$_2$ treatment than the low CO$_2$ treatment and the control at PPFDs ranging from 500 to 1500 $\mu$mol m$^{-2}$ s$^{-1}$ (Fig. 4.4). There was no significant difference in conductance between the low CO$_2$ treatment and the control (Fig. 4.4).

Figure 4.5 shows thermal images of sweet potato plants cultivated in high or low CO$_2$ treatments. The images clearly showed higher leaf temperatures of plants in the high CO$_2$ treatment than in the low CO$_2$ treatment. The mean temperature of 10 representative leaves was 34.8°C in the high CO$_2$ treatment and 31.9°C in the low CO$_2$ treatment. The transpiration rate was estimated to be smaller in the high CO$_2$ treatment than in the low CO$_2$ treatment.
Fig. 4.4. Leaf conductance as affected by photosynthetic photon flux density (PPFD) for sweet potato cultured in sandy soil with different CO$_2$ concentrations. Temperature: 38.3 ± 2.7°C; Relative humidity: 38 ± 7%.
Fig. 4.5. Thermal images of sweet potato plants cultivated in sandy soil with different CO₂ concentrations. The images were taken at 14:00 h on August 11, 2010. Air temperature: 40.6°C; Relative humidity: 30%.
4.5 Discussion

A high level of CO$_2$ in soil was likely to have caused the observed decrease in growth (Fig. 4.2). Such depression of biomass production may be caused by inhibiting water absorption in the roots due to elevated CO$_2$ in soil. The water content of the shoot was lowest in the water table level of 15 cm with the highest volumetric water content in third chapter from the above. It has also been previously found that the absorption of water and nutrients by roots of wheat, maize, and rice was suppressed by a high CO$_2$ concentration in the root zone (Chang and Loomis, 1945).

The O$_2$ concentration in the soil was 16-19% throughout. The soil O$_2$ concentration in this study was not very low to cause any negative effects on plant growth. Soil CO$_2$ more than 2.5% reduced the root biomass of creeping bentgrass (Bunnell et. al., 2002). It has been shown that growth of bamboo (Wei et al., 2005) and carrot (Islam et al., 1998b) were suppressed when the CO$_2$ concentration in soil increased to 0.5–2%. Growth of sweet potato was also suppressed when the soil CO$_2$ increased to 0.5–2% (Kitaya et al., 1992; Islam et al., 1997, 1998a). Bouma et al. (1997), however,
reported that growth of bean (*Phaseolus vulgaris*) was not affected by soil CO$_2$ concentration in a range of 0.06-2%.

Leaf conductance decreased with increasing CO$_2$ concentration to 4.9% in soil (Fig. 4.4). Leaf conductance was lowest when the water table was at the lowest level (30 cm below the soil surface) in third chapter from the above, presumably due to insufficient soil water content. Gas exchange in leaves would also decrease as the CO$_2$ concentration in soil increased. Since there is generally a positive relationship between net photosynthetic rate and leaf conductance, this means that increased CO$_2$ in the root zone would also suppress photosynthesis in leaves, and thus plant biomass production.

Gas exchange in leaves also decreases as the CO$_2$ concentration in the soil increases. It was estimated from thermal images that the transpiration rate decreased as the root zone CO$_2$ concentration increased (Fig. 4.5). Since there is generally a positive relationship between net photosynthetic rate and leaf conductance, this means that increased CO$_2$ in the root zone would also suppress photosynthesis in leaves, and thus plant biomass production. Consequently, to promote tuberous root production in sweet potato, the water content in the soil can be manipulated to control the gas
composition in the soil. The tuberous root biomass decreased with increasing CO$_2$ concentrations from 0.5\% to 2.5\% in a study that assessed the effect of soil CO$_2$ on the development of sweet potato tuberous roots (Siqinbatu et al., 2013). The tuberous root biomass in this experiment would be plotted on this curve and the extension.

### 4.6 Conclusion

I studied, in a series of experiments, the effect of rising soil CO$_2$ concentration on the growth of sweet potato during its establishment period in order to confirm the hypothesis that elevated CO$_2$ concentrations in the root zone associated with elevated soil water contents could lead to growth stagnation in sweet potato. I substantiated that high CO$_2$ concentration in soil inhibits the growth of sweet potato tuberous roots in the treatment area with high soil water content which was indicated in third chapter from the above. It was demonstrated that the growth inhibition of sweet potato due to high CO$_2$ concentration in soil is based on inhibition of water absorption through roots.
Chapter 5 Application of digestate from a methane fermentation process for supplying water and nutrients in sweet potato culture in sandy soil

5.1 Abstract

Digestate from a methane fermentation process, which contains many nutrients and water, is a potentially useful resource for agriculture. In order to decide the appropriate strength of digestate from methane fermentation for sweet potato production, different strengths of digestate diluted with water were applied to sweet potato plants as the water and nutrient supply in sandy soil. The impact of the diluted digestate on the growth of sweet potato was compared with that of commercial nutrient solution (Otsuka formula A). The growth rate of tuberous roots with 1/20 strength of digestate was greatest among treatments with different strengths of digestate (1/80-1/2) and commercial nutrient solution (1/4-1). Consequently, this study confirms that digestate as a byproduct from methane fermentation based on biomass waste treatment is a suitable nutrient solution for sweet potato production. Maximum yield of tuberous roots was achieved when a 20-fold dilution of digestate was used.
5.2 Introduction

In agriculture, there is a desire to reform traditional agricultural systems to allow for sustained agricultural production based on resource circulation of exhaustible resources by substituting for the application of fertilizer and agrochemicals and thus decreasing the environmental load. The disposal of industrial waste from the livestock industry into agricultural and food production areas has increased, and this is problematic. The circulation of industrial waste derived from methane fermentation by could serve as a valuable agricultural resource.

Methane fermentation by using biomass resources as industrial waste has recently been promoted. Most digestates as by-products from methane fermentation are discharged through treatments based on environmental assessment (Iwashita et al., 2010). The effective use of digestate from a methane fermentation, which contains many nutrients, as liquid fertilizer has only seen a small application in agriculture (Yuyama et al., 2007). These digestates consist primarily of nitrogen and potassium components with little phosphorus, the nitrogen primarily being in the form of ammonia, serving thus as a fast-acting fertilizer as NK nutrients (Nakamura et al.,
2011). Efficient use of digestate is necessary when applied as a nutrient solution based on an appropriate application design and water regimen (Somayama et al., 2008), also taking into account fertilizer costs. However, the properties of digestate differ from those of raw material biomass. The nitrification of NH$_4$-N in digestates may proceed speedily compared to chemical fertilizer. Since digestate involve intact raw materials, initial solid-liquid separation is necessary (Agriculture General Center of Chiba prefecture).

Studies on the available use as liquid nutrient of digestates in agriculture exist for *Brassica campestris* and Spinachess (Agriculture Food Industry Technology General Organization), *B. campestris*, sunflower and radish (Koyama, Kato) and Coiza and wheat (Sibata and Inoue). In all cases, digestate had an almost equal effect with the control, a chemical nutrient.

Matsuo et al. (2011) reported that digestate from methane fermentation was as effective as chemical nutrients in sweet potato culture and that application time and digestate concentration had no effect; consequently, these digestates could be applied to sweet potato culture as a single dose of digestate. Marianna. Makadi et al. (2012) reported that the kernel or bud
yield of soybean increased depending on the amount of digest applied. Moreover, the yield of feed corn increased by applying digest more than water supply. The Agriculture General Research Center of Chiba prefecture (2008) reported that tomato grown with concentrated digest or with conventional water-based chemical nutrients showed equal quality and yield. Horima et al. (2007) reported that the same amount of digestate and chemical nutrients had an equal effect on the dry mass yield of feed corn. The application of digestates to paddy rice has been studied in some methane fermentation facilities. The application of digestates in these studies was based on ridge top supply to application time of conventional nutrient.

Digestates include many inorganic nutrient salts and could be used as liquid nutrient in agriculture but there are still limited applications. In addition, the construction of a sustainable agricultural production system that is able to circulate waste resources to reduce the negative impacts on the local environmental need to be established. I aimed to develop a technology that could effectively use digestate from methane fermentation, supplied as water and nutrients, for sweet potato culture. In order to obtain fundamental information on the optimum concentration of digestate for
sweet potato culture, different strengths of digestate, diluted with water, were applied to sweet potato plants as a water and nutrient supply in sandy soil.

5.3 Materials and Methods

5.3.1 Determination of suitable digestate concentration

Sweet potato (*Ipomoea batatas* (L.) Lam, cv. ‘Beniazuma’) was grown for 144 days from June to November and was replicated twice (in 2011 and 2012), in a plastic house with fully opened side windows under a simulated dry condition at Osaka Prefecture University. Seedlings were planted in bottomless plastic pots (20 cm in diameter, 35 cm height) filled with sea sand to imitate fine sand in the semi-arid region of Inner Mongolia (Fig. 5.1). Plants were grown regularly for 2 weeks after the beginning rooting. Digestate from a methane fermentation (following MFD) was diluted to 1/2, 1/10, 1/20, 1/40 and 1/80 strength with water to provide a nutrient solution that could be supplied to plants. Also, as a comparison, a commercial nutrient solution (Otsuka nutrient solution A formula, following CNS) was diluted to 1/1, 1/2 and 1/4 strength with water. Each pot was place inside a
water tank and each nutrient solution was added into a water tank to a depth of 10 cm such that a water table of 25 cm below the soil surface was formed (Fig. 5.2 and Fig. 5.3).

**Fig. 5.1.** The culture experiment system of sweet potato (Top view).
Electrical conductivity (EC) and pH were measured using an ECTester 11+ (1772522, AZUWAN Co., city, country) and a pH meter (pH/ION METER D-53, HORIBA Co., city, country), respectively. The concentrations of ions in the nutrient solution were measured with an ion-chromatograph (LC-10Advp, SHIMAZU Co., city, country). Relative chlorophyll content in leaves was measured with a chlorophyll meter (SPAD-502 Plus, KONIKA-MINORUTA Co., city, country). The growth of sweet potato was determined 130 days after planting each year.

Fig. 5.2. The culture experiment system of sweet potato (Side view).
Fig. 5.3. Photograph of culture experiment system of sweet potato (June 14, 2012).
5.3.2 Practical examination of sweet potato cultured with digestate

I examined a practical culture system that used digestate from methane fermentation based on the experimental results above mentioned. Sweet potato (*Ipomoea batatas* (L.) Lam, cv. ‘Beniazuma’) was grown for 148 days, from June 16 to November 10, 2013, in a plastic house with fully opened side windows under a simulated dry condition at Osaka Prefecture University. Plants were cultured in two culture containers (4.5 m (L) × 0.3 m (W) × 0.3 m (H)) which were filled with sea sand to imitate fine sand in the semi-arid region of Inner Mongolia. Sweet potato was also planted around the container to prevent surrounding effects. The 20-fold diluted digestate (EC: 0.15 S m$^{-1}$) was supplied from the container bottom as a water table 25 cm below the soil surface (Fig. 5.4). Mean temperature and mean humidity deficit were 35.9°C and 28.6 g m$^{-3}$, respectively during the culture period. EC and pH were measured using an ECTester 11+ (1772522, AZUWAN Co.) and a pH meter (pH/ION METER D-53, HORIBA Co.), respectively. The growth of sweet potato plants was determined 134 days after planting each year.
Fig. 5.4. Effects of nutrients in sandy soil on growth of sweet potato in the ridge with bottom irrigation.
5.4 Results and Discussion

5.4.1 Determination of suitable digestate concentration

The air temperature and relative humidity in the plastic house were 33.9 ± 5.1°C (mean ± SD) and 50 ± 16%, respectively, in 2009, and 35.5 ± 4.3°C and 66 ± 12%, respectively, in 2010.

EC, pH and various ion concentrations of the original digestate from methane fermentation (MFD) and the commercial nutrient solution (CNS) are shown in Table 5.1. The concentration of cations and anions was higher in MFD than in CNS. Also, the concentration of potassium, ammonium and phosphorous ions in MFD was about 9, 70 and 5-fold higher, respectively, than in CNS. The EC of each nutrient solution decreased depending on the dilution ratio.

Table 5.2 shows the results for the growth of sweet potato grown in 2011 and 2012. The pH of the nutrient solution was 6.0-6.7 and 8.0-8.2, respectively, in CNS and MFD, regardless of the dilution. The pH of nutrient solutions based on MFD was higher than that of nutrient solutions
based on CNS. The dry mass of tuberous roots in 2011 was greater in the MFD×1/20 treatment. Dry mass decreased as the dilution ratio increased. On the other hand, the dry mass of tuberous roots was greater in the CNS×1/2 treatment. The dry mass of tuberous roots in the MFD×1/20 treatment was 1.3-fold higher than that in the CNS×1/2 treatment. The dry mass of tuberous roots was greater in the MFD×1/20 treatment and decreased as the dilution of MFD decreased in 2012. The dry mass of leaves, stems and lateral roots in the MFD×1/20 treatment tended to increase relative to other nutrient treatments. The relative chlorophyll content in leaves (SPAD value) was high and tuberous roots did not form in the MFD×1/20 treatment. Tuberous roots were larger in the MFD×1/20 treatment than in the CNS×1/2 treatment (Fig. 5.5).

Table 5.1 Properties of original methane fermentation digestate (MFD) and commercial nutrient solution (Otsuka formula A) (CNS).

<table>
<thead>
<tr>
<th>Solution</th>
<th>EC (mS cm⁻¹)</th>
<th>pH</th>
<th>NO₃⁻ (mg/L)</th>
<th>NH₄⁺ (mg/L)</th>
<th>K⁺ (mg/L)</th>
<th>Na⁺ (mg/L)</th>
<th>PO₄³⁻ (mg/L)</th>
<th>SO₄²⁻ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS</td>
<td>2.52</td>
<td>6.7</td>
<td>230</td>
<td>34</td>
<td>368</td>
<td>238</td>
<td>134</td>
<td>224</td>
</tr>
<tr>
<td>MFD</td>
<td>31.00</td>
<td>8.1</td>
<td>—</td>
<td>2787</td>
<td>3311</td>
<td>447</td>
<td>638</td>
<td>332</td>
</tr>
</tbody>
</table>
Table 5.2 Solution pH, relative chlorophyll contents (SPAD values) of leaves and dry weights of sweet potato plants cultured in different solution treatments in 2011 and 2012.

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatments</th>
<th>pH</th>
<th>SPAD</th>
<th>Dry weight (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td>2011</td>
<td>MFD×1/80</td>
<td>8.0</td>
<td>38</td>
<td>8.4 ± 4</td>
</tr>
<tr>
<td></td>
<td>MFD×1/40</td>
<td>8.2</td>
<td>40</td>
<td>19.2 ± 13</td>
</tr>
<tr>
<td></td>
<td>MFD×1/20</td>
<td>8.2</td>
<td>50</td>
<td>27.7 ± 4</td>
</tr>
<tr>
<td></td>
<td>CNS×1/4</td>
<td>6.5</td>
<td>43</td>
<td>23.8 ± 11</td>
</tr>
<tr>
<td></td>
<td>CNS×1/2</td>
<td>6.5</td>
<td>45</td>
<td>23.7 ± 7</td>
</tr>
<tr>
<td></td>
<td>CNS×1</td>
<td>6.7</td>
<td>41</td>
<td>11.4 ± 5</td>
</tr>
<tr>
<td></td>
<td>LSD</td>
<td>5.3</td>
<td>13.6</td>
<td>35.8</td>
</tr>
<tr>
<td>2012</td>
<td>MFD×1/20</td>
<td>8.2</td>
<td>48</td>
<td>62.6 ± 20</td>
</tr>
<tr>
<td></td>
<td>MFD×1/10</td>
<td>8.1</td>
<td>32</td>
<td>30.5 ± 8</td>
</tr>
<tr>
<td></td>
<td>MFD×1/2</td>
<td>8.2</td>
<td>23</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>LSD</td>
<td>12.8</td>
<td>25.7</td>
<td>35.9</td>
</tr>
</tbody>
</table>

LSD: Least significant difference at 5% level by Tukey-Kramer multiple comparison test. Each data indicates the mean and standard deviation (n = 6).
Fig. 5.5 Roots of sweet potato cultured in different solution treatments in 2011 and 2012.

Consequently, digestate as a byproduct from methane fermentation based on biomass waste treatment could be used as a nutrient solution for sweet potato production. Maximum yield of tuberous roots was achieved when the digestate was diluted 20 times.
5.4.2 Practical examination of sweet potato cultured with digestate

Figure 5.6 indicates relationship between volumetric water content and the water potential when a 20-fold dilution of digestate was supplied in sandy soil. The water potential declined according to decrease in the volumetric water contents in sandy soil and the water potential in the same volumetric water content was lower when diluted digestate was supplied compared with supplying only water.

In Fig. 5.7, the dry weight of each part and the whole plant of sweet potato were significantly greater when digestate solution was used than when commercial nutrient solution was used. These results indicate that digestate is effective for the growth of sweet potato on ridges with a bottom irrigation system. Therefore, it was expected that sweet potato culture supplied with water and nutrients derived from digestates is possible in fields of Inner Mongolia consisting of sandy soil with a fine particle distribution. Fig. 5.8 indicates that the formation of tuberous roots was greater when digestate solution was applied rather than commercial nutrient.
solution, supporting the results of Fig. 5.7. Thus, digestate used in this study serves as a useful nutrient solution for sweet potato culture.

In the present experiment, tuberous root yield of sweet potato was 2.48 kg m\(^{-2}\) at a planting density of 3 plants m\(^{-2}\). This tuberous root yield was the mean tuberous roots yield (2.50 kg m\(^{-2}\)), at the stranded planting density (3-4 plants m\(^{-2}\)) of same species in Japan.
Fig. 5.6 Relationship between the volumetric water content and the water potential when a 20-fold dilution of digestate was supplied in sandy soil.

The volumetric water content were measured using a soil moisture meter (TDR100; Campbell Scientific Ltd., city, USA) and the water potential were measured using a tension meter (DP2-80; TAKEMURA Co., Japan).
Fig. 5.7. Effects of nutrients in sandy soil on dry weight of sweet potato in ridge with bottom irrigation.

Vertical error-bars indicate standard deviations of the whole plant \((n = 7)\).
Fig. 5.8. Representative underground parts of sweet potato cultured in the ridge with bottom irrigation.
5.6 Conclusion

Digestate as a byproduct from methane fermentation based on biomass waste treatment and that included inorganic nutrient salts was tested as liquid nutrient in sweet potato culture. This digestate was confirmed as a suitable nutrient solution for sweet potato production. I demonstrated that digestate at appropriate dilutions is suitable as nutrients for sweet potato culture resulting in similar yield as obtained from conventional culture. Maximum yield of tuberous roots was achieved when the digestate was diluted 20 times. I suggested a sweet potato production system with a bottom irrigation method with digestate from methane fermentation, which can be applied to semiarid regions. Results of this study will contribute towards the establishment of a water and resource recycle system, including regional sustainable agricultural systems (Fig. 5.9).
Fig. 5.9. Schematic diagram of sandy soil culture of sweet potato based on bottom irrigation system in with digestate from methane fermentation.
Chapter 6 Concluding remarks

In regions where water is insufficient for agriculture, crop culture systems with efficient water use are imperative. With the aim of developing efficient methods for sweet potato production in arid or semi-arid regions, I investigated the effects of soil water content in the root zone on the growth and yield of sweet potato grown in sandy soil. In addition, a sustainable agricultural production system that could circulate and reuse waste resources. There have been very few successful attempts to use digestate containing many nutrients in the form of inorganic nutrient salts as a liquid nutrient in agriculture. In this thesis, water and nutrients derived from a methane fermentation digestate were also used in a sweet potato production system because bottom irrigation will be a useful method to supply liquid nutrients to the root zone.

The effects of soil water content on leaf stomatal conductance of sweet potato were compared to four other agricultural plant species (maize, okra, cucumber and tomato) in order to examine the level of drought stress tolerance. Sweet potato, after a period of drought stress, had the ability to quickly increase leaf conductance after re-watering. These results indicate
that sweet potato is a drought-tolerant crop that is able to recover from a certain level of drought stress and is able to maintain its growth rate by using water more efficiently under semi-dry conditions.

The effect of soil water content in the root zone on the growth and yield of sweet potato cultivated in sandy soil was investigated. Sweet potato was cultured in containers filled with sand with four different the water potential levels, -0.23, -0.29, -0.39, and -0.64 MPa, the dry mass of tuberous roots and the whole plant was greatest at -0.39 MPa water potential and smallest at -0.64 MPa, and -0.39 MPa plot > -0.29 MPa plot > -0.23 MPa plot > -0.64 MPa plot for the ratio of root dry mass to whole plant dry mass.

The effects of increasing CO₂ concentration on the growth of sweet potato during its period of establishment was investigated in order to test the hypothesis that elevated CO₂ concentrations in the root zone associated with elevated soil water content lead to growth stagnation. No tuberous roots developed in the high CO₂ treatment. Furthermore, the dry mass of the whole plant and fibrous roots was 1.6 and 3 times greater, respectively, in the low CO₂ treatment than in the high CO₂ treatment.
Digestate from a methane fermentation process, which contains many nutrients and water, is a potentially useful resource for agriculture. In order to decide the appropriate strength of digestate from methane fermentation for sweet potato production, different strengths of digestate diluted with water were applied to sweet potato plants as the water and nutrient supply in sandy soil. Consequently, this study confirms that digestate as a byproduct from methane fermentation based on biomass waste treatment is a suitable nutrient solution for sweet potato production. Maximum yield of tuberous roots was achieved when a 20-fold dilution of digestate was used.

In this study, it was found that a volumetric water content in soil of 10 to 15% (-0.42 to -0.35 MPa water potential) and a low CO\textsubscript{2} concentration (1-2%) are necessary for sustaining the development of tuberous roots of sweet potato in sandy culture that uses a bottom irrigation system. This would allow for the effective use of water in regions where water is insufficient for agriculture. Water absorption through roots was inhibited by a decline in the soil water potential and low soil water content while the growth of tuberous roots was inhibited by a decline in water absorption through roots caused by an increase in soil CO\textsubscript{2} concentration when soil
water content was high. This study also confirmed that digestate from methane fermentation can supply water and nutrients for sweet potato production. I demonstrated that digestate at appropriate dilutions is suitable as nutrients for sweet potato culture resulting in similar yield as obtained from conventional culture. I suggested a sweet potato production system with a bottom irrigation method with digestate from methane fermentation, which can be applied to semiarid regions. Results of this study will contribute towards the establishment of a water and resource recycle system, including regional sustainable agricultural systems.
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References


Ogawa, H., Kakehashi, Y., Inoue, M., Tanabe, K. and Otani, H., 2006: Influence that transition of soil moisture exert on amount and quality


Yamakawa, O., 1998: Development of new cultivation and utilization system for sweet potato toward the 21$^{st}$ century. In: “Proceedings of
International Workshop on Sweet Potato Production System Toward the 21st Century”. Kyushu National Agricultural Experiment Station, Miyazaki, Japan, pp. 273-283.
