The potential of witloof chicory (*Cichorium intybus* L.) for stimulating the utilization of biomass resources derived from livestock wastes

畜産廃棄物系バイオマス資源活用に適したチコリの特性

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SUMMARY

Chicory (*Cichorium intybus* L.) is a common Compositae crop, originally developed in Mediterranean regions. It is well known that chicory contains relatively large amount of Potassium (K) not only in the edible part but also for the whole plant body, and there is a possibility to use chicory as the material for removing the extra K in the soil.

The witloof chicory has a unique production cycle that can be divided into two stages, namely root cultivation and forcing culture. The areas that are well suited for witloof root production are the cold or high-altitude regions, because it is important to expose them to cold temperatures at the late stage of cultivation for obtaining matured roots. In this way, transferring of photosynthesis products to the root can occur. Therefore, in Japan, the current main production areas are mainly in the north part of the mainland and in Hokkaido Island. The forcing culture of witloof chicory is usually conducted in an enclosed space, with 15-18°C air temperature, nearly 100% Relative Humidity (RH), under complete dark conditions, for the emergence of a discoloured etiolated head. In addition, the forcing culture of witloof chicory has a high affinity to the heat energy source with limited and unstable heat capacity.

To establish a firm base of a sustainable production system for horticultural crops, it is indispensable to make the best use of biomass resources. The organic fertilizers can be utilized for conventional cultivation system, however, they contain a relatively large amount of K comparing with nitrogen or phosphorus. Consequently, this may lead to the occurrence of problems like a K accumulation in the soils. The organic fertilizers would be utilized more

widely and efficiently in case of effective solutions able to remove accumulated salts, especially with K. Thus, and the cleaning crop concept would be one of the meaningful solutions, with ecological advantages to diminish the salt accumulation in the soils.

To produce indoor horticultural crops in cold seasons, we are dependent on combustion of fossil fuels for the bulk of heat energy source. However, the direct heat recovering system from biomass resources, which is derived from livestock wastes, can be utilized more extensively for horticultural production, once its efficiency, simplicity and space-saving capacity could be improved.

The present study demonstrated the 1) feasibility of forcing culture of witloof chicory by using the fermentation heat from composting cow manure and the 2) Effectiveness of witloof chicory crop as a remedy for salt accumulated in soils, especially K accumulation, which may be caused by an excessive application of organic fertilizers derived from livestock wastes.

In Chapter 2, an experiment was executed to investigate the feasibility of witloof chicory production with the fermentation heat of cow manure, in Hokkaido, during semi-cold and cold seasons. Forcing culture experiments were conducted in semi-cold seasons (once, from April to May, 2013) and in cold seasons (twice, March, 2014; March, 2015). In each experiment, cow compost, produced through a solid-liquid separator (water content: 72.6%), was used as heat sources. Temperatures of outside air, indoor air, compost container, forcing chambers (soil and air) and heat exchangers were recorded. Through all experiments, compost temperature was maintained up to 30°C, at which it showed the potential to be used as a heat source for the chicory forcing culture. In the semi-cold season, temperatures of forcing chambers ($6.3 \times 0.9 \times 0.65$ m) were maintained constant; the average air temperature of the forcing chamber reached 17.2°C in average, and the marketable etiolated heads (chicon) were obtained after 22 days of forcing. In the cold season, the air temperature of the forcing chamber ($3.0 \times 0.9 \times 0.65$ m) was maintained constant (10.6°C in 2014, 14.4°C in 2015, in average), and the marketable heads were obtained after 15 to 19 days. The results indicated that witloof chicory forcing culture in semi-cold and cold seasons by using cow manure fermentation heat as heat sources is indeed possible.

In Chapter 3, under a high-K stressful condition of plant cultivation in pots in a greenhouse, the K absorption capacity of witloof chicory was compared with guinea grass (*Panicum maximum* Jacq.). As K_2O application increased, the biomass of both canopy and root tended to decrease in witloof chicory; however, in guinea grass, the plant total biomass increased significantly. The root biomass of witloof chicory was always greater than that of guinea grass, indicating that witloof chicory has the potential to grow under a high-K stressful condition. The forcing culture was conducted and the marketable etiolated heads were obtained when the K application amount was less than 2,000 kg ha^{-1}. The K-uptake amount per plant of witloof chicory was kept at about 45.8 to 73.9% compared to that of guinea grass among K_2O treatments higher than 200 kg ha^{-1}. The calculation of estimated K-uptake amount per ha, as a remedy of removing K-accumulated in agricultural fields, based on the practical plant density revealed that the K-uptake capacity of witloof chicory is more than 3 times higher than guinea grass. It can be concluded that witloof chicory can be one of the possible solutions for removing considerable amounts of K from K-accumulated soils.

In Chapter 4, the aim was to evaluate the potential of witloof chicory as a remedy for K accumulated soils under practical situations. For doing this, the K absorption capacity of witloof chicory under nutrient-rich conditions were analysed, together with the forage type chicory and guinea grass. The methodology considered using modelled salt accumulated soils made by excessive application (733 t ha^{-1} in a total 2 years) of a methane fermentation digested slurry of cow manure for 2 years. Comparing to the first year, the top biomass of witloof chicory in the second year was maintained at the same level: the root biomass significantly increased, while the total plant biomass of guinea grass was significantly decreased: no significant differences were observed in the total plant biomass of forage type chicory. The K-uptake amount per plant of witloof chicory was 37.6% greater than that of guinea grass, and 73.8% more than that of forage chicory. Through a forcing culture experiment, it became clear that the negative influences of an excessive application of methane fermentation digested slurry for two years on production of etiolated heads was limited. In conclusion, the witloof chicory can be utilized for K removal from salt accumulated soils under the practical situation, concurrently with the potential of deriving agricultural incomes by producing etiolated heads for the market.

For the reasons mentioned above, it can be concluded that the witloof chicory can play an important role to enhance the best use of biomass resources derived from livestock wastes. In particular, the growing system should stimulate the practical application of organic fertilizers and the utilization of recovered heat energy derived from composting livestock wastes, for the production of horticultural crops.

CHAPTER 1

GENERAL INTRODUCTION

1. Botanical characteristics of witloof type chicory (*Cichorium intybus* L.)

Cichorium is a small genus within the Asteraceae family (tribe Lactuceae), and chicory (*Cichorium intybus* L.) (2n=8) is originally developed in Mediterranean regions (Ryder 1988). The genetic variation of this crop is relatively wider, presenting some unique types that are different from each other in appearance, utilization and chemical components (Lucchin et al. 2008). Examples of this diversity refer to: 1) witloof type, with two different growing stages, such as root production and forcing culture; 2) Radicchio Type, forming a cabbage-like head in the open field, some sub-types in this category also need two different cultivation stages; 3) Pain de Sucre type, forming an elongated head resembling the chinese cabbage (*Brassica rapa* var. *pekinensis*); 4) Root type, bred for inulin extraction; and 5) Forage type, bred to be used as forage in grasslands (Rumball 1986, Barry 1998; Rumball et al. 2003). In this thesis, the author focuses on witloof type, and this type is also known as Belgian endive in English, in Dutch as witloof or witlof, indivia in Italy, endivias in Spain, endive or chicon in France. Still its origin and the process of development of witloof type remain as a matter to be discussed further, however, it is inferred from recent studies that this type was obtained from 'Magdeburg' or 'foliosum' (Koch and Jung 1997; Van Stallen et al. 2003; De Profy et al. 2003; Van Stallen et al. 2004).

2. History of chicory production in Japan

The witloof type chicory has been developed mainly in France, Belgium and The Netherlands from the late 1800s, and it has been introduced as a premium European Winter vegetable since 1868 in Japan (Tamura 1965). In 1962, Japan External Trade Organization

(JETRO) imported sample seeds of witloof type chicory from Belgium and executed growing tests in Hokkaido. At that time, apparently the main aim was to clarify the potential of chicory root as a surrogate for coffee. By using roots obtained from the above mentioned growth tests in Hokkaido, some growers in Tokyo started to produce etiolated heads (chicons) through forcing cultivations. In 1965, a few growers in Karuizawa (Nagano Prefecture), Sayama (Saitama Prefecture), Takaza (Kanagawa Prefecture) and Yatsugatake (Yamanashi Prefecture) produced the roots of witloof chicory for an estimated total acreage of less than 4.0 ha. In 1975, Ohta and Miyawaki reported their experimental cultivation of witloof chicory in Kagawa prefectural agricultural experiment station, and the basic techniques of the witloof chicory production, from seeds to the final fresh products (chicons) was established. In 1990s, a number of studies on cultivation techniques for both root production and forcing culture were conducted mainly in the agricultural research stations of prefectural governments such as Hokkaido (Sasaki 1990; Sawaguchi 1998), Nara prefecture (Kiya et al. 1990), Aomori prefecture (Kamada et al. 1990; Iwase et al. 1990; Iwase et al. 1992) Iwate prefecture (Takahashi et al. 1991), Wakayama prefecture (Iwao et al. 1995), Fukui prefecture (Ohki 1997) and Chiba prefecture (Ando et al. 1997; Ando et al 1998). Over the past few decades, it has been recognized that several private companies and growers have tried to expand their production of witloof chicory, mainly in Gifu prefecture, Gunma prefecture and Hokkaido. The current domestic total acreage of root production can be estimated around 50 ha.

3. Growth pattern of witloof chicory

The witloof chicory has a unique production cycle, which can be divided into two stages, namely root cultivation and forcing culture (Fig. 1-1). The root shape of witloof type is similar to "Kuroda" type Carrot (*Daucus carota* subsp. *sativus*), circular in cross-section, and with a potential biomass production relatively larger than other types. The areas that are well suited

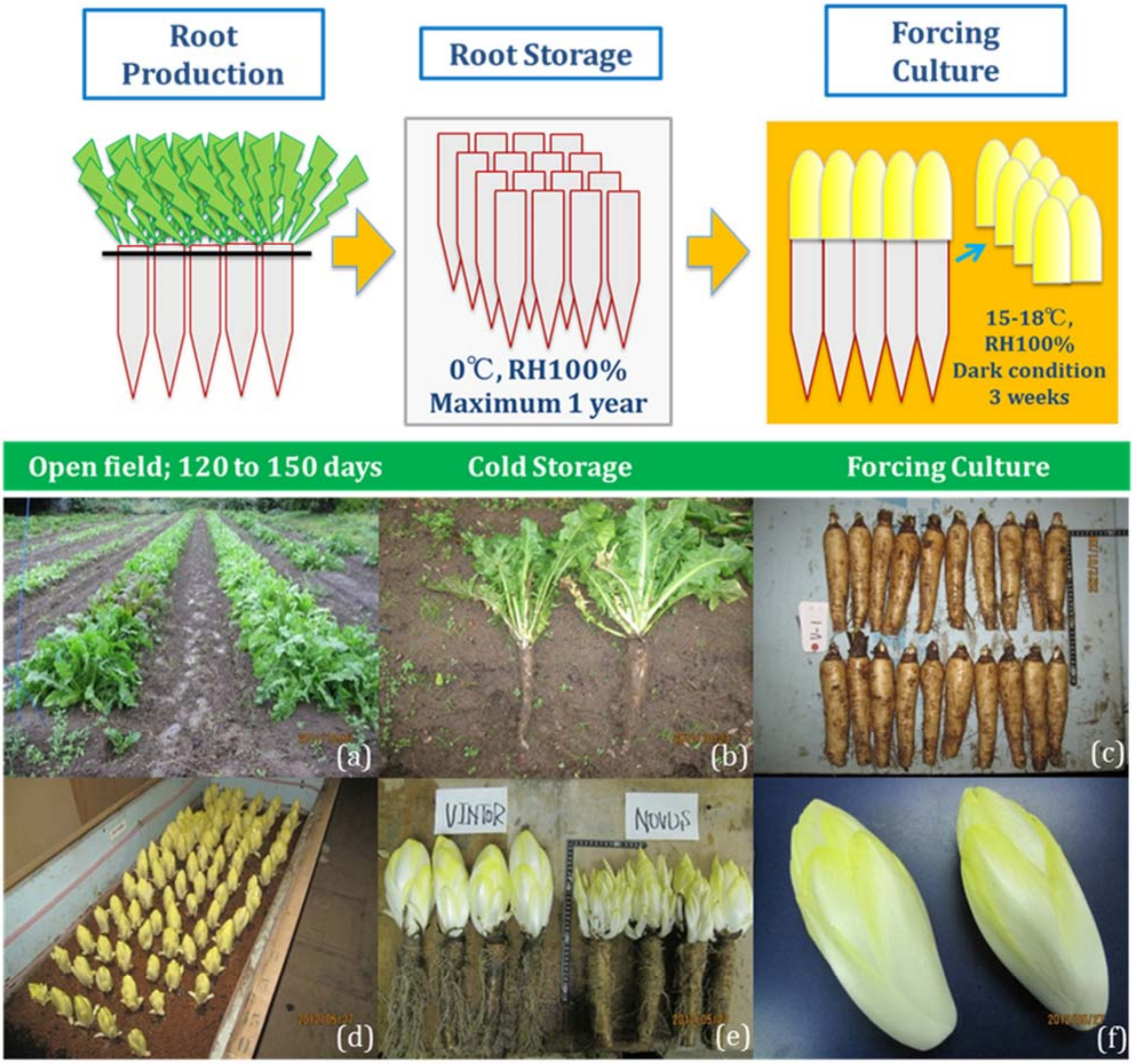

Fig. 1-1 Production cycle of witloof chicory (*Cichorium intybus* L. 'Vintor'). (a) Root production in the open field, (b) Matured roots around 125 days after sowing, (c) Roots that were cleaned and trimmed after cold storage, (d) The forcing chamber, (e) Etiolated heads (chicon) with roots at the harvesting timing (21days after starting of forcing culture) and (f) Etiolated heads (chicon).

for witloof root production are the cold and high-altitude regions when at later stages of cultivation roots are exposed to cold temperatures. Under these circumstances, plants transfer photosynthesis products to the roots, which in turn will be able to produce new leaves under forcing culture. Therefore, the current main production areas in Japan are mainly in Hokkaido, Tohoku-region, Gunma and Nagano. The chicon, an edible part of the witloof chicory, can be obtained through the forcing culture of matured roots, which were grown over 120 days in the open field, from early summer to the beginning of winter. The forcing is normally conducted with a high-density planting of roots in enclosed spaces, under dark conditions with a temperature around 15°C and with high RH (Sasaki 1990).

4. Sustainable production system for horticultural crops

In present time, the development of sustainable production techniques for horticultural crops is frequently demanded to achieve reducing environmental footprints (Prime minister of Japan and his cabinet, 2006). For contemporary horticultural production, especially for vegetables, large amount of fuels, fertilizers, production materials and agricultural chemicals are consumed. The origin of those supplies are crude petroleum, phosphate rocks and potash ores, all considered as limited resources. The argument over the risks against the future depletion of such finite resources is striking, and the development of technologies to enhance efficiency and sustainability in the utilization of these resources for horticultural production is needed.

To establish a firm base for sustainable production systems of horticultural crops, making the best use of biomass resources is indispensable. Organic fertilizers derived from food wastes, or livestock wastes as substitutes to chemical fertilizers, biomass fuels such as wood-pellet for heating production facilities, and plant plastics for packaging fresh products are *inter alia* now considered as practical options to utilize biomass resources for effective horticultural production. Some techniques have been widely developed, as the practical utilization of livestock wastes, with a broad perspectives, such as 1) utilizing livestock wastes as fertilizers or soil amendments (Katoh et al. 2012); 2) exploiting the recovered heat from combustion of methane, which derives itself from methane fermentation of livestock wastes for heating (Shimizu and Yuyama 2010; Weiland 2010); and 3) making use of heat energy that can be directly recovered from aerobic fermentation of livestock wastes during composting (Mote and Griffis 1982; Seki and Komori 1984; Klejment and Rosinski 2008; Miyatake et al. 2009; Smith and Aber 2014).

The organic fertilizers, including Methane Fermentation Digested Slurry (MFDS), deriving from livestock wastes, contain a large amount of the three main plant macronutrients (Oyanagi et al. 2004). They can be utilized practically as the main fertilizing constituents not only for organic horticultural productions but also for conventional cultivation systems. In addition to be a good source of fertilizer components, these fertilizers present the obvious advantages linked to the improvement of fertilizer-holding capacity and buffering capacity of the soils by increasing the humus (chemical merits), increasing the moisture retain-capacity and pore spaces (physical merits) and enhancing the diversity of soil microorganisms (biological merits) (National institute for rural engineering 2012).

Some problems remain in the utilization of organic materials related to environmental issues.

5. The potential of livestock wastes as fertilizers

For horticultural productions, fertilizers are quite often applied excessively. Basically, the main reasons for this fact are: 1) growers tend to prioritize the production efficiency or growth-promotion other than environment conservation; 2) less attention is given to the importance of soil analyses for setting sound fertilization strategies; and 3) it is essential for horticultural productions to keep the fertilize effect until the end of harvest because normally the edible part of horticulture crops is harvested at an immature stage (Ono and Fujii 1994; Ono 1999; Obara and Nakai 2003; Obara and Nakai 2004; Moritsuka 2009).

However, the organic fertilizers originated from livestock wastes contain relatively large amount of K compared with nitrogen or phosphorus. This unique characteristic may lead to a K accumulation in the soils when it is applied as fertilizers dosed according to nitrogen demand (Nakano et al. 2001; Katsuki and Joh 2003). The excessive accumulation of K in the soils may bring negative impacts on yield or product quality, in addition to an increased load on the environment, such as the pollution of the groundwater (Zörb et al. 2014). It should be stressed that organic fertilizers would be utilized more widely and efficiently in case of effective solutions able to remove accumulated salts, especially with K (Oizumi et al. 1979; Shimada et al. 1979; Chiba and Yokoyama 1980; Kinjyo et al. 2007).

6. Cleaning crop concept; a technique to remove accumulated nutrients from the soils by crops

"Cleaning Crop" technique is regarded as one of the efficient solutions to remove salts from the soil with lettle negative impacts to the environment (Kondo et al. 2009; Maeda et al. 2012). The concept has been widely studied globally, and the technique is called "Catch Crop" in western countries (Thorup-Kristensen et al. 2003; Constantin et al. 2010). In other words, this is the method to remove accumulated salts in the soil by growing forage or green manure grass crops, which are prone to absorb exceeded fertilizer components from the soils. Practically, the salts accumulated in the top part of the soils are going to be transferred to outside of the soil, removed from the field after cropping; conversely, the root parts are not affected. Takezawa et al. (1991) analysed the difference in the nutrient value of grass crops with the potential to be used as Cleaning Crops in the case of excessive application of K_2SO_4, $CaCO_3$

and $MgSO_4$ in greenhouse. Species under consideration were Guinea Grass (*Panicum maximum* Jacq.), sorghum (*Sorghum bicolor* L. Moench), sudangrass (*Sorghum* × *drummondi* (Nees ex. Steud.) Millsp. & Chase) and Rhodes grass (*Chloris gayana* Kunth). It is not popular to utilize those crops for feeding livestock practically, because of the risks of health problems of livestock due to the high concentration of K these crops can then contain. In particular, Guinea grass, a warm-season forage crop, has an exceptional potential to produce biomass. Some commercial varieties were bred specifically for the purpose of salt removal from the soils and are thus available nowadays. The capacity to remove accumulated nutrients from the soil by this grass has been in fact demonstrated (Kinjyo et al. 2007).

The approach related to the Cleaning Crop concept presents at the moment several problems. Firstly, by following this technique, it is difficult to utilize the potential of root parts for salt removal, given that the roots parts tend to be left in the field. Secondly, the number of available catch crops for cold regions such as Hokkaido is very limited. Thirdly, farmers do not rise their motivation toward catch crops production because no income from the catch crop is deriving. It is however appropriate to assume that the cleaning crop concept would be one of the meaningful solutions, with ecological advantages to diminish the salt accumulation in the soils.

7. Potential of witloof chicory as a remedy for salt accumulated soil, especially for K

It is well known that chicory contains relatively large amount of K not only in the edible part but also in the whole plant body (Ryder 1998; Ministry of Education, Culture, Sports, Science and Technology of Japan 2015). This fact has been exploited even by athletes, who are in European countries, commonly take chicons as a source of K; the edible part of witloof chicory is used against the suffer of muscle pains or for the delayed onset of muscle soreness. The relatively larger amount of K contained in the witloof chicory has the effect to stimulate the recovering of the damage of muscles after heavy load of training exercises, and this has been indicated as more evident from this species than from other vegetables.

Neel et al. (2002) reported that forage type chicory tends to accumulate quite a large amount of K to the plant body when grown under mineral-rich conditions. The authors emphasized growers' attention to consider the risks of the negative influence on animal health and performance if the K concentration in the plant body of forage chicory exceeds the maximum tolerable level of ruminants with considerable probability.

There is a possibility to use chicory as the material for removing the extra K in the soil, not for feed crops. However, the number of reports on the K uptake capacity of chicory is limited and detailed information is necessary for using chicory as a K-scavenger.

8. Potential of livestock wastes as heat sources for horticultural production

To produce indoor horticultural crops in cold seasons, efficient heating system is mandatory, however at present we are dependent on combustion of fossil fuels for the bulk of heat energy source. On the other hand, the development of new techniques to utilize biomass energy derived from livestock wastes as heat sources for horticultural productions is progressively widening.

A biogas plant is a system that aims to obtain heat energy and electricity by combustion of methane derived from methane fermentation of livestock wastes, food wastes and other, now commercially spread globally (Weiland 2010). However, the initial costs are excessive and the system requires a relatively large scale facility for an efficient fermentation reaction, limiting the spread of this technology.

The development of efficient techniques for horticultural production based on the utilization of heat energy recovered directly from a composting process of livestock wastes has been widely studied recently (Roland and Griffis 1982; Seki and Komori 1984; Klejment and

Rosinski 2008; Miyatake et al. 2009; Smith and Aber 2014). Such system does not require a large-scale and complex equipment; therefore, the initial costs of the plant can be reduced, giving an important benefit to small-scale horticultural productions. Nevertheless, this system has some limitations. For instance, the recoverable heat is relatively small and unstable due to its limited scale and biological (aerial micro-organism) fermentation system, which is affected by environmental conditions. Furthermore, it is difficult to reach a high efficiency in recovering the heat from the composting process of livestock wastes, due to the open-type aerobic fermentation reaction. It seems reasonable to infer that the full technique advancement of this system has not yet been well established, and therefore the keys to make this system more popular rely on overcoming the above mentioned limitations.

The direct heat recovering system from biomass resources, which is derived from livestock wastes, could be utilized more extensively for horticultural production, once its efficiency, simplicity and space-saving ability could be improved. The fact that it is difficult to miniaturize the biogas system has long been recognized and it would be sumptuous even if it succeeds.

In conclusion, developing an efficient heat recovering techniques from the composting of livestock wastes is required and it is one of the keys to expand the utilization of biomass origin heat sources for horticultural production.

9. Compatibility of witloof type chicory forcing culture to the heat source which has limited heat capacity with instability

The forcing culture of witloof chicory is usually conducted in an enclosed space with 15-18°C air temperature, nearly 100% RH, under complete dark conditions, for the emergence of a discoloured etiolated head. The facility for the forcing culture has to be covered by heat insulators and light blocking materials in order to maintain the inside air temperature with considerable precision. The demand of heating energy in the forcing culture is definitely smaller than other horticultural crops such as Solanaceae fruits vegetables bearing fruit vegetables grown in greenhouse, because the chicory roots can be grown at high density (more than 400 plants per m^2) during the forcing stage. The forcing culture of witloof chicory has a high affinity to the heat energy source with limited and unstable heat capacity, and there is a possibility to use composting heat in chicory forcing culture.

10. The scope of this study

Following scientific aspects to clarify the feasibility of above mentioned possible functions of witloof chicory are confronted.

1) Feasibility of forcing culture of witloof chicory by using fermentation heat of cow manure composting

The uniqueness of the required cultivation condition of forcing culture in witloof chicory stimulated the rationale of using a heat source which has limitation in heat capacity and stability, such as a heat recovering from the composting process of cow manure, a kind of biomass resources. The target of the first research in this study was to evaluate the feasibility of forcing culture of witloof chicory by using cow manure as a heat source, focusing on the direct heat recovery method by circulating antifreeze solution between composting chambers and forcing beds.

2) Effectiveness of witloof chicory as a remedy for salt accumulated soils, focusing on K accumulation that may be caused by excessive application of organic fertilizers, derives from livestock wastes

The rationale of the study was based on the following hypothesis: if the utilization of witloof chicory as a remedy for salt-accumulated soils is possible, it may bring integral benefits to growers by the removal of K from such soils. Concurrently, growers can obtain an agricultural income from the forcing culture by using roots which absorbed K from soils. Thus, the goal of the second and third experiment in the present thesis was twofold: first to clarify the potential of witloof chicory as a remediation of K accumulated in soils (K scavenger); and second to estimate yield and quality of the etiolated heads produced from chicory roots that grew under excess K conditions. The research describes the efforts to examine the canopy growth and K absorption capacity and the change of soil chemical profiles during growing periods. Two types of chicories were considered, both witloof and forage, comparing to the existing cleaning crop; Guinea grass (*Panicum maximum* Jacq.), under nutrient rich conditions, such as modelled salt accumulated field which were made by excessive application of MFDS or chemical fertilizers. Experimental forcing cultures were also conducted in order to verify the influence of stress caused by nutrient rich conditions on the yield and quality of the edible pat of witloof chicory.

CHAPTER 2

Forcing culture of witloof chicory (*Cichorium intybus* L.) using fermentation heat of cow manure

INTRODUCTION

Many investigations have focused on the use of heat and electricity generation through biogas system with regard to the usage of livestock wastes as a source of energy for agricultural production (Weiland 2010; Monteiro 2011). However, studies focusing on the direct use of recovered heat from manure fermentation are limited (Mote and Griffis 1982; Klejment and Rosinski 2008). For indoor horticultural production, especially in cold regions such as Hokkaido (Japan), vast fossil fuels are still being consumed in the cold season. Over the past decades, increased attention has been given to the search for alternative energy with a potential as practical heat source for indoor horticultural production (Miyake 1980; Kawamura et al. 2006; Fukui et al. 2009).

Through the composting process of livestock manures, it is well known that extracting heat from decomposition of organic matters is possible, and there are several studies that aimed to use such techniques as heating systems for indoor agricultural production. To use fermentation heat directly as a heat source for horticultural production, two methods have been mainly discussed: 1) transferring the heat from composting chambers to the cultivation place by heat exchanger to connect heat condensers buried in manure and heat radiators in the cultivation place (Seki and Komori 1984); and 2) having the heat recovery by a vacuum-induced aeration system which forces air throughout the manure bed. The former method does not require a complicated mechanism and less initial investments than the latter, and also presents an advantage in the efficiency of the heat recovery with less thermal loss. There are some disadvantages, however, in the workability when loading or turning the manure. The present study investigated the first method. An advantage in the second method is twofold, not only obtaining liquid fertilizer by acid-wash of vacuumed vapour from manure composting through ammonia scrubbers, but also the potential to realize both facility heating and CO_2 fertilization by using ammonia-free vacuumed vapour. The disadvantage of the second method is, however, the high costs of the initial investment (Miyatake et al. 2009; Smith and Aber 2014).

Thus far, the practical use of manure fermentation heat as a heat source for horticultural production has been limited, and it is reasonable to suppose that the main reason is the difficulty to realize an efficient heat recovery from unstable heat sources. However, it can be assumed that maybe several crops that do not require high temperature for their growth can be grown by a heat source of limited capacity.

The cultivation of witloof chicory (*Cichorium intybus* L.) can be divided into two stages, namely root production in open fields and forcing culture in dark containers. The forcing culture is usually conducted by a high-density planting of roots within heat-insulated containers, thus with a limited requirement of heat capacity. The optimum temperature range for the forcing culture is around 15°C (Sasaki 1990), relatively lower than that of typical indoor horticultural crops. Tight heads become loose and the marketable ratio of etiolated heads is normally decreased when the temperature during a forcing culture is out of the optimum range (12 to 18°C) or unstable (Huysles 1961; Ryder 1998).

Those unique characteristics stimulated the hypothesis that the forcing culture of witloof chicory can be done by a limited heat source, such as the manure fermentation as described above. The objective of the present study was to evaluate the feasibility of a forcing culture of witloof chicory by using cow manure as a heat source, focusing on the direct heat recovery method by circulating antifreeze solution between composting chambers and forcing beds.

MATERIALS AND METHODS

Forcing culture experiments were conducted in Yubari City, Hokkaido, using indoor experimental facilities (E141°58′N43°3′). Three experiments were conducted, one in semi-cold season (April to May, 2013, 22 days), and 2 in a cold season (March, 2014, 19 days, and March, 2015, 15 days).

Dairy cow manure compost and heat exchange

For each experiment, dairy cow compost (water content: 72.6%, CN ratio: 25) was produced through a solid-liquid separator (Yamashita et al. 2014). The applied amounts of compost were approximately 2 m^3 for the semi-cold season, and approximately 3 m^3 for the cold season. In the semi-cold season, the size of the compost container was 2.92 m^3 (1.8×1.8×0.9 m), and the estimated total surface area of the heat exchanger (0.7×0.45×0.03 m) buried into the compost was 0.7 m^2 (Fig. 2-1).

In the cold seasons, the size of the compost container was 5.81 m^3 (2.2×2.2×1.2 m), and total surface area of the heat exchanger buried into the compost was 2.79 m^2 (a spiral copper pipe, 2.2 cm diam., 40 m) (Fig. 2-2). The obtained heat was supplied by a circulating antifreeze solution through heat exchangers equipped in the compost chamber and polyethylene pipes (13 mm diam.), as heat radiators, which were installed in the forcing chambers (surface area of internal heat exchangers; 2.08 m^2 in 2013, 1.00 m^2 in 2014 and 2015). The antifreeze solution was circulated by water pump (UPS 25-60 180, GRUNDFOS Holding A/S, Denmark), and the water flow was regulated by a temperature indicator controller (R15, Azbil Corp., Japan). The heat exchanger and heat radiators were connected by polyethylene pipes covered by heat insulators.

In the semi-cold season in 2013, manure turnings were executed 7 days, 12 days and 18 days after starting the forcing culture. In the cold season in 2014, manure turnings were

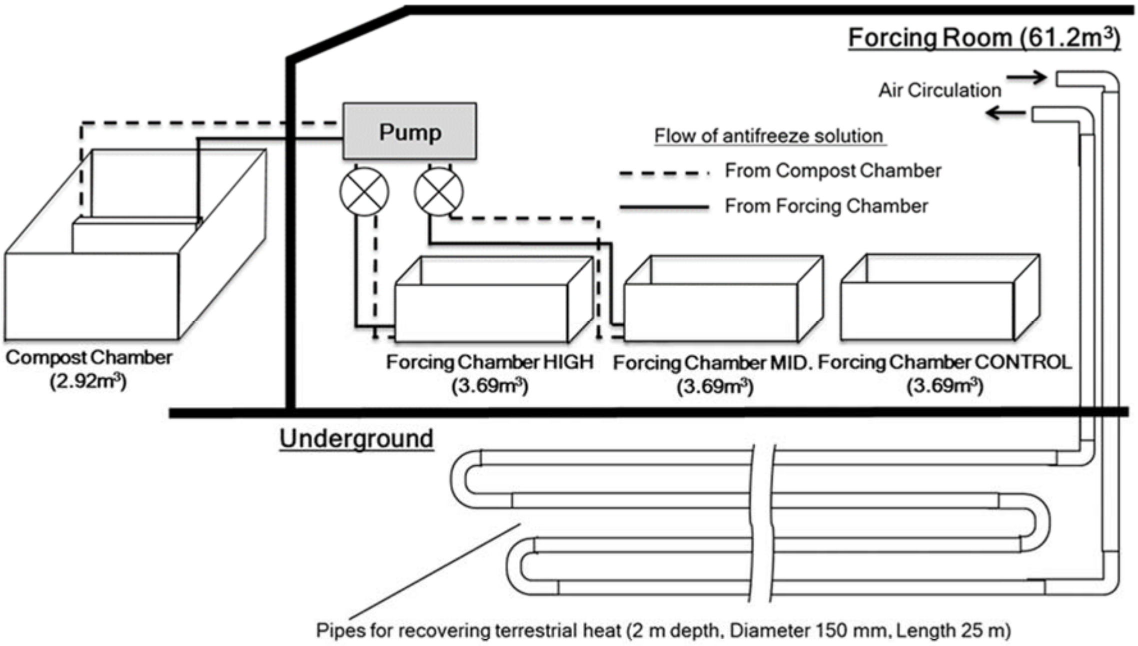

Fig. 2-1 Experimental facility used in semi-cold season, in 2013. The capacity of compost chamber was 2.92m^3 (1.8 × 1.8 × 0.9 (m)). The capacity of forcing chambers were 3.69m^3 (6.3 × 0.9 × 0.65 (m)), and those forcing chambers were installed in the forcing room (61.2m^3; 12.6 × 1.8 × 2.7 (m)). The forcing room was connected with a terrestrial heat recovery system, and the air was circulated between the forcing room and underground pipes for recovering terrestrial heat by electric fan through the system. The inside temperature of the forcing chambers was controlled manually at 3 temperatures, 19° C (HIGH), 17° C (MID.) and non-heating (CONTROL).

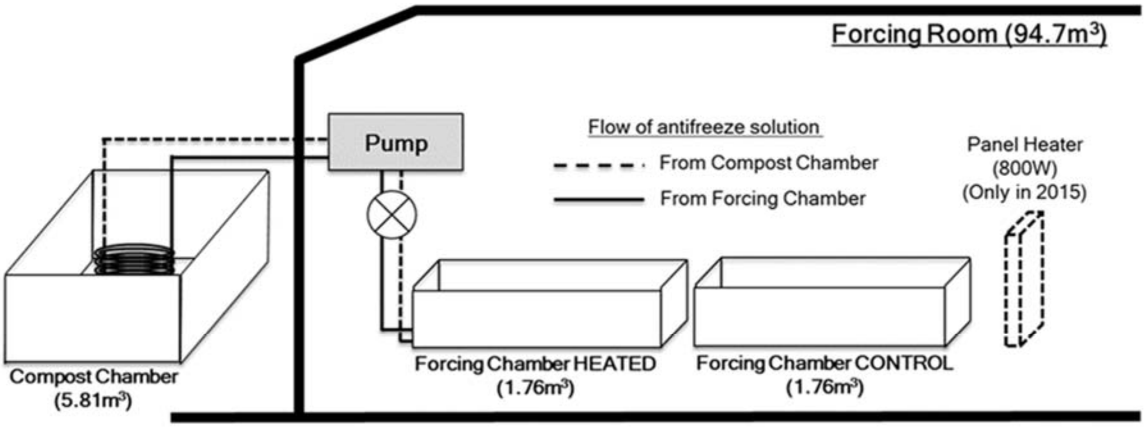

Fig. 2-2 Experimental facility in cold seasons, in 2014 and 2015. The capacity of compost chamber was 5.81m^3 (2.2 × 2.2 × 1.2 (m)). The capacity of forcing chambers was 1.76m^3 (3.0 × 0.9 × 0.65 (m)), and those forcing chambers were installed into the forcing room (94.7m^3; 12.6 × 1.8 × 2.7 (m)). A electric panel heater (800W) was used to maintain the air temperature of forcing room higher than 0°C, only in 2015. One chamber was used for the treated treatment (HEATED) and another for the non-treated control (CONTROL).

executed 6 days and 13 days after starting the forcing culture. In cold season in 2015, manure turnings were executed 9 days after starting the forcing culture.

Semi-cold season experiment, April in 2013

In the semi-cold season, three forcing chambers of same size (3.69 m^3; 6.3×0.9×0.65 m), provided of a thermo-insulator and a heat radiator, were prepared in the room (61.2 m^3; 12.6×1.8×2.7 m) of the agricultural experimental facility of the local government of Yubari City, Hokkaido. The inside temperature of the forcing chambers was controlled manually at 3 temperatures, 19°C (HIGH), 17°C (MID.) and non-heating (CONTROL), by regulating the amount of the circulating antifreeze solution with a water valve. A terrestrial heat recovery system was used (2 m depth, 25 m length) to stabilize the air temperature of the forcing room by air circulation between the room and ducts buried underground by an electric fan (Straight sirocco fan BFS-100SUC, Mitsubishi Electric Corp., Japan, air circulation speed; 1,000 m^3h^{-1}) (Yamakawa et al. 2013).

Cold season experiments, March in both 2014 and 2015

In the cold season, two forcing chambers of the same size (1.76m^3; 3×0.9×0.65 m, with a thermo-insulator and a heat radiator) were prepared in a room (94.7 m^3 11.6×3.4×2.4 m) of the agricultural experimental facility of the local government of Yubari City, Hokkaido. One chamber was used for the treated treatment (HEATED) and another for the non-treated control (CONTROL). All forcing chambers were made with 12 mm thickness wooden boards (commercially available as concrete formwork); the insides of the chambers were covered by a heat insulator, 2.5 cm thick of an extruded polystyrene foam sheet (Fig. 2-3). In 2015, an electric panel heater (Panel Heater Yumedanbo 880H (880 W), RCS Corp., Japan) was used to maintain the room air temperature above 0°C.

Fig. 2-3 Forcing chamber (Upper), heat condenser (copper pipe) in composting chamber (Middle) and heat radiator (polyethylene pine) in forcing chamber (Bottom).

Wiitloof chicory root production and planting in forcing culture

Witloof type cultivar 'Vintor' (VI) (Nunhems B.V., Nunhem, The Netherlands) was used for the forcing culture. VI roots were produced at the Experimental Farm, Field Science Center for Northern Biosphere, Hokkaido University, Sapporo (E141°20′N43°4′). In each year from 2013 to 2015, the seeds were sown directly (222,222 plants ha^{-1}) into open fields in mid-July. Roots were harvested around 145 days after sowing, in the middle of November. After washing with tap water, the harvested roots were sorted and cut into 20 cm lengths pieces (from the root shoulder to the bottom end), and stored in a refrigerator, with air temperature between 0.2 to 0.8°C, and 100% of RH, until the start of the forcing culture.

In each experiment, roots were transplanted into plastic containers (57.5×41.5×19.0 cm) which were filled with a medium used for nursery production (Takii Tanemaki Baido, Takii Co., Ltd, Japan), with a plant density of 120.0 plants m^{-2}. Roots were grown under dark conditions, with periodic irrigation. A total 15 roots were planted for each treatment in the semi-cold season, in 2013. A total 60 roots were planted for the treated plot, while 10 roots were planted as CONTROL in the cold season, in 2014. A total 60 roots were planted for the treated plot, while 20 roots were planted as CONTROL in the cold season, in 2015.

Measurement of temperature and evaluation of calorific value supplied from compost

The temperatures of the outdoor, forcing room, hot water inlet and outlet, inside forcing chambers (air and soil) and manure were recorded every hour during forcing cultivation with a data logger (midi logger GL820, Graphtec Corp., Japan), using thermocouple wires. As to the estimation of the calorific value supplied from the manure composting heat to the forcing chambers, the temperature difference of circulated antifreeze solution between the inlet and the outlet of the forcing chamber was measured, and the amount of fluid flow per minute was recorded by a liquid flow meter (Liquid flowmeter LD20-PATAAA-RC, HORIBA, Japan).

Growth characteristics of etiolated heads emerging in forcing culture

The fresh weight (FW) (before and after trimming), height, diameter and flower stalk length of etiolated heads were measured when heads were harvested in each experiment, 22 days, 19 days and 15 days after starting the forcing culture, in the semi-cold season in 2013, in the cold-season in 2014, and in the cold-season in 2015, respectively. The relative core ratio was calculated as per the core length/etiolated head length × 100. The growth of the emerged etiolated heads was measured and statistically analysed; the significant differences in mean values were calculated using Tukey-HSD test and Welch's *t* test.

RESULTS

Semi-cold season (April to May, 2013)

The air temperature of the forcing room was kept over 0°C during the forcing cultivation even though the minimum outside air temperature was -3.3°C because of the terrestrial heat exchange system (Fig. 2-4). Nevertheless, the outside air temperature averaged 4.3°C, and the manure temperature was kept above 40°C from 2 days after starting the forcing culture. The manure temperature tended to increase after 2 to 3 days of each turning (27 April, 2 May and 8 May, 2013). During the forcing period, from 20 April to 12 May, 2013, the air temperature of the inside forcing chamber was kept stable (15.2°C at the MID, 17.2°C at the HIGH, on average), especially from 7 days (27 April) to 18 days (7 May) after the start of the forcing culture. The average FW of the trimmed etiolated heads was above 210 g at the HIGH, and 170 g at the MID; these values were significantly higher than the CONTROL at the 5% probability level (Table 2-1). In the HIGH and MID treatments, the FW ratio (after/ before trimming) was at the same level of the CONTROL, however, the core ratios in them were significantly higher than in the CONTROL at the 5% probability level.

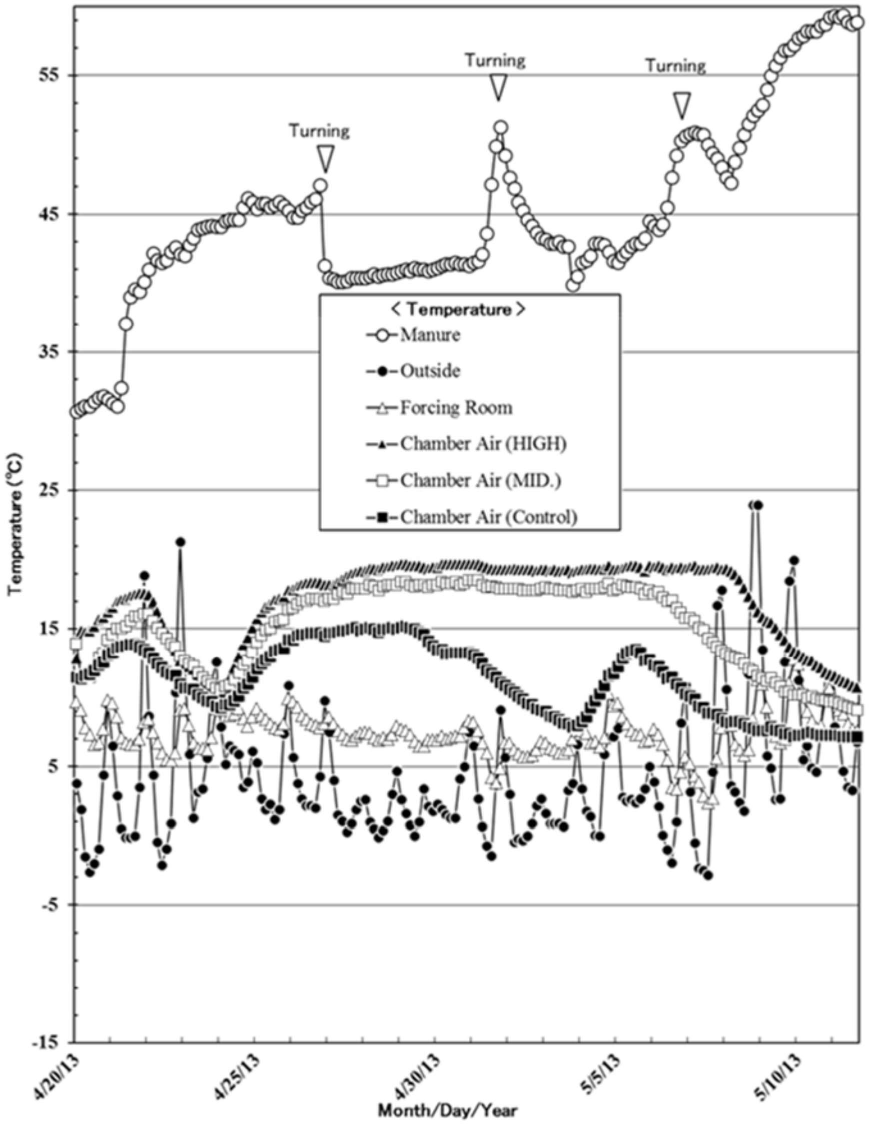

Fig. 2-4 Transition of air temperature during forcing culture (20 April to 12 May, 2013). HIGH; temperature in the "HIGH" forcing chanber, MID; the temperature in the "MID" forcing chamber and Control; the temperature in the "CONTROL" forcing chamber.

Table 2-1 Effect of forcing temperature on etiolated head yield and quality in semi-cold season, from 20 April to 12 May, 2013.

Expected Temperature	Average Temperature[y]		Before Trimming	After Trimming				Etiolated Head Quality Index	
	Air (℃)	Soil (℃)	Fresh Weight (g/plant)	Fresh Weight (g/plant)	Height (cm)	Diameter (cm)	Core Length (cm)	FW Ratio (After/Before Trimming)	Core Ratio (Length/Height)
CONTROL (Non-Heating)	11.3	10.0	137.9±7.0 c[x]	88.4±5.6 c	11.5±0.34 b	5.5±0.20 b	2.7±0.18 c	0.65±0.03 a	0.24±0.01 c
MID. (17℃)	15.2	14.9	248.9±8.2 b	169.8±4.8 b	13.5±0.25 a	8.3±0.21 a	6.1±0.41 b	0.69±0.01 a	0.45±0.03 b
HIGH (19℃)	17.2	15.5	336.7±19.4 a	211.6±17.5 a	13.8±0.77 a	9.0±0.50 a	7.9±0.55 a	0.64±0.06 a	0.59±0.06 a
Tukey-HSD test[w]			**	*	*	**	**	ns	*

[z] Roots were trimmed into 20 cm lengths (shoulder to the bottom end), and sorted (452.3 g fresh weight in average).
[y] Period; 20 April, 2013- 12 May, 2013 (22 days).
[x] n=15, mean ± SE, The numbers followed by the same letter within a column are not significantly different according to the Tukey-HSD test.
[w] ns, ** and * indicate not significant, significant at p<0.01 and p<0.05, respectively.

The temperature of the forcing chambers decreased from 8 May until the end of the forcing cultivation period due to the malfunction of a pump used for the circulation of the antifreeze liquid.

Cold season (March, 2014)

The average air temperature of the forcing room was 0.9°C during the forcing cultivation while average outside air temperature was -4.2°C in March 2014 (Fig. 2-5). The manure temperature was kept above 35°C throughout the forcing period, 38.3°C on average. The same trend of the previous experiment conducted in 2013 was observed for the manure temperature, which always increased after 1 to 2 days from turning (8 March and 15 March, 2014). During the forcing period, from 2 to 21 March, 2014, the air temperature inside the forcing chamber of HEATED was kept stable at 10.6°C on average. The air temperature in the CONTROL averaged 0.5°C almost the same level as recorded in the forcing room.

The average FW of the trimmed etiolated heads was around 100 g in HEATED, with a significant difference between CONTROL and HEATED at 1% probability level (Table 2-2). The size of the etiolated heads in HEATED was relatively smaller than the commercial standard, however, the head height in HEATED was significantly higher than CONTROL at 1% probability level. The average core ratio in HEATED was below 0.2, and the FW ratio of the etiolated head (after/ before trimming) reached almost 90%, significantly higher than CONTROL at 1% probability level.

Cold season (March, 2015)

The average air temperature in the forcing room was 6.3°C during the forcing cultivation while the average outside air temperature was 0.4°C (Fig. 2-6). From 5 days after

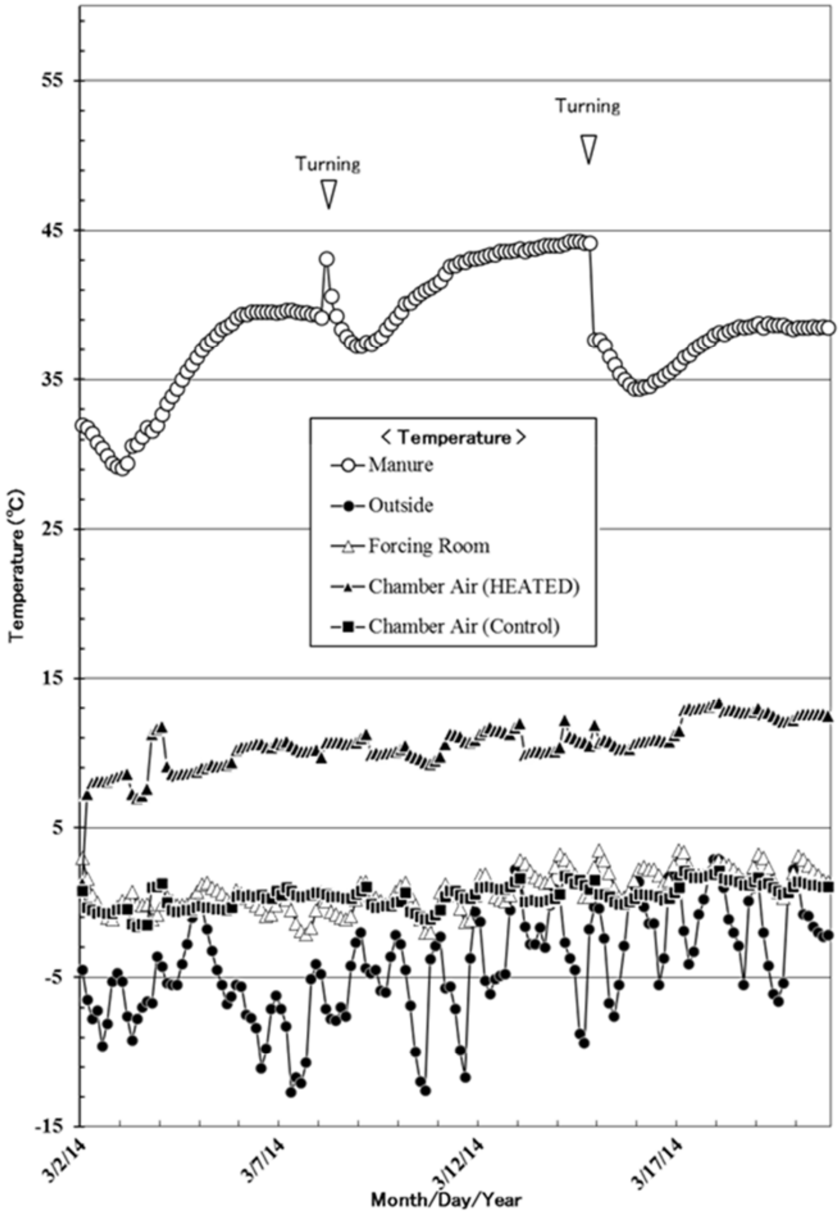

Fig. 2-5 Transition of air temperature during forcing culture (2 to 21 March, 2014).). HEATED; temperature in the "HEATED" forcing chanber and Control; the temperature in the "CONTROL" forcing chamber.

Table 2-2 Effect of forcing temperature on etiolated head yield and quality in semi-cold season, from 2 March to 21 March, 2014.

Treatment	Average Temperature[y]		Before Trimming	After Trimming				Etiolated Head Quality Index	
	Air (℃)	Soil (℃)	Fresh Weight (g/plant)	Fresh Weight (g/plant)	Height (cm)	Diameter (cm)	Core Length (cm)	FW Ratio (After/Before Trimming)	Core Ratio (Length/Height)
Control (Non-Heating)	0.5	-0.5	7.4±1.0[x]	4.0±0.4	6.3±0.3	N/A[w]	N/A[w]	0.55±0.03	N/A[w]
Heated	10.6	11.0	117.1±2.5[v]	103.9±2.2	14.0±0.1	4.9±0.1	2.6±0.1	0.89±0.00	0.19±0.00
t-test[u]			**	**	**	-	-	**	-

[z] Roots were trimmed into 20 cm lengths (shoulder to the bottom end), and sorted (208.2 ± 4.0 g fresh weight).
[y] Period; 2 Mar., 2014- 21 Mar., 2014 (19 days).
[x] n=10, mean ± SE
[w] Data were not obtained because of poor growth of chicons.
[v] *n*=60, mean ± SE
[u] ns, ** and * indicate not significant, significant at $p<0.01$ and $p<0.05$, respectively.

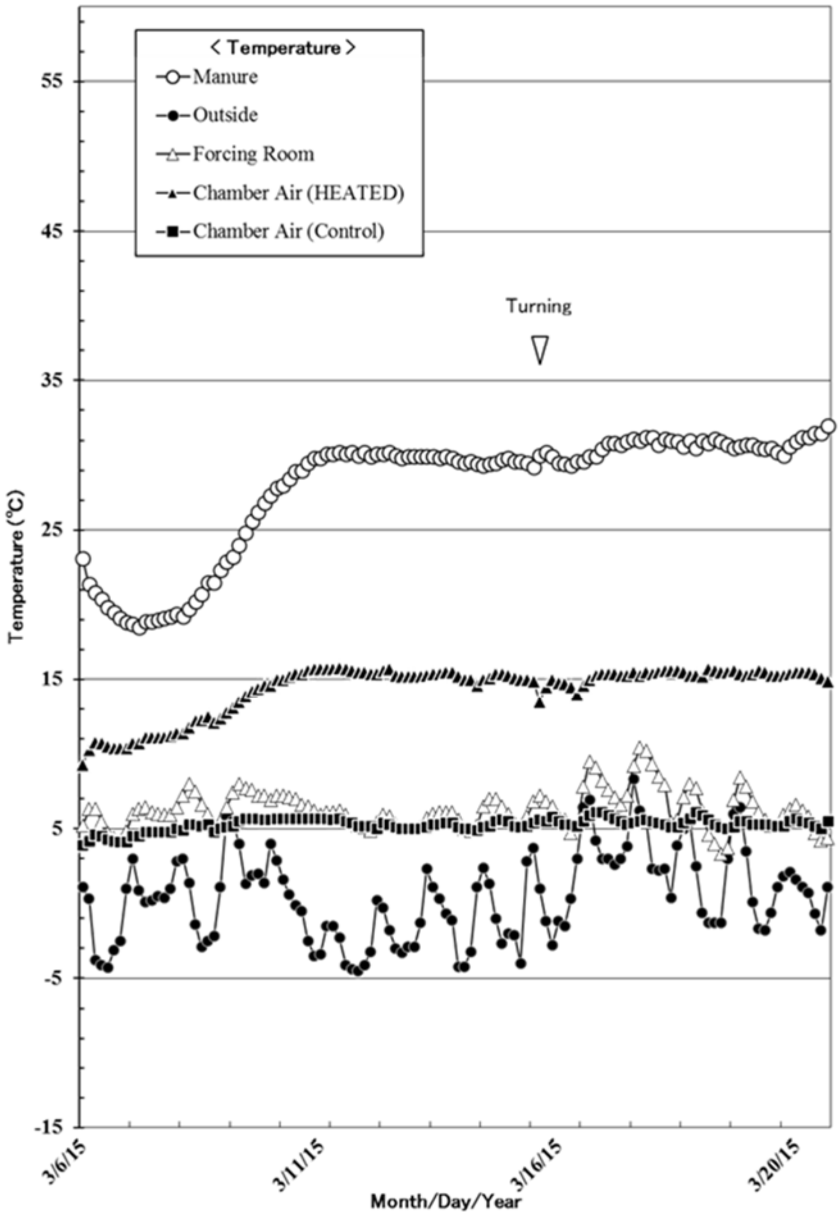

Fig. 2-6 Transition of air temperature during forcing culture (6 to 21 March, 2015). HEATED; temperature in the "HEATED" forcing chanber and Control; the temperature in the "CONTROL" forcing chamber.

starting the forcing culture, manure temperature was kept over 30°C, and averaged 27.9°C. During the forcing period, from 6 to 21 March, 2015, the air temperature inside the forcing chamber of HEATED was kept at 14.4°C on average, while that in CONTROL averaged 5.3°C.

The volume of the forcing room was 94.7 m^3 (11.6×3.4×2.4 m). The air temperature in the forcing room was kept between 3.2 to 10.7 °C by the small electric panel heater (880W), 2.1°C in average higher compared with the other room which was not provided of any heating system in the experimental facility throughout the cultivation period. The manure was turned only once during the forcing culture, 9 days after starting (15 March, 2015); however, no large increase in temperature was observed after turning. Based on the recorded data of the temperature difference between inlet and outlet of the anti-freezing liquid and flow rate, the heat energy introduced into the heat chamber per hour was evaluated as 134.7 kJ h^{-1}.

After removing the outer leaves, the average FW of the etiolated heads in HEATED was 147.3 g, significantly greater than that in CONTROL at 1% probability level (Table 2-3). Both height and diameter of the heads in HEATED were significantly higher than those in CONTROL at 1% probability level. The average core ratio in HEATED was kept lower than 0.4. The FW ratio (after/ before trimming) in HEATED was significantly higher than in CONTROL. These results showed that the temperature during forcing culture was controlled with enough stability to maintain the practical level of etiolated head quality (Fig. 2-7).

DISCUSSION

Factors obtained marketable head from the forcing culture of chicory

Marketable etiolated heads of VI were obtained in an experiment in the semi-cold season (May 2013) and in cold season (March 2015) and not in another cold season (March in 2014). In the semi-cold season experiment (2013), the outside temperature was around 0-5°C, while the temperature of the forcing room was maintained at around 5°C by a terrestrial heat

Table 2-3 Effect of forcing temperature on etiolated head yield and quality in semi-cold season, from 6 March to 21 March, 2015.

Treatment	Average Temperature[y]		Before Trimming	After Trimming				Etiolated Head Quality Index	
	Air (℃)	Soil (℃)	Fresh Weight (g/plant)	Fresh Weight (g/plant)	Height (cm)	Diameter (cm)	Core Length (cm)	FW Ratio (After/Before Trimming)	Core Ratio (Length/Height)
CONTROL (Non-Heating)	5.3	4.8	27.3±1.3 [x]	11.6±0.4	7.6±0.2	2.1±0.0	0.9±0.1	0.44±0.01	0.12±0.01
HEATED	14.4	14.7	163.0±1.9 [w]	147.3±1.7	14.3±0.1	5.9±0.0	5.3±0.1	0.90±0.00	0.37±0.00
t test [v]			**	**	**	**	**	**	**

[z] Roots were trimmed into 20 cm lengths (shoulder to the bottom end), and sorted between 4.1 to 4.4 cm diameter (195.5 ± 1.1 g fresh weight).

[y] Period; 6 Mar., 2015- 21 Mar., 2015 (15 days).

[x] n=20, mean ± SE

[w] n=60, mean ± SE

[v] ns, ** and * indicate not significant, significant at p<0.01 and p<0.05, respectively.

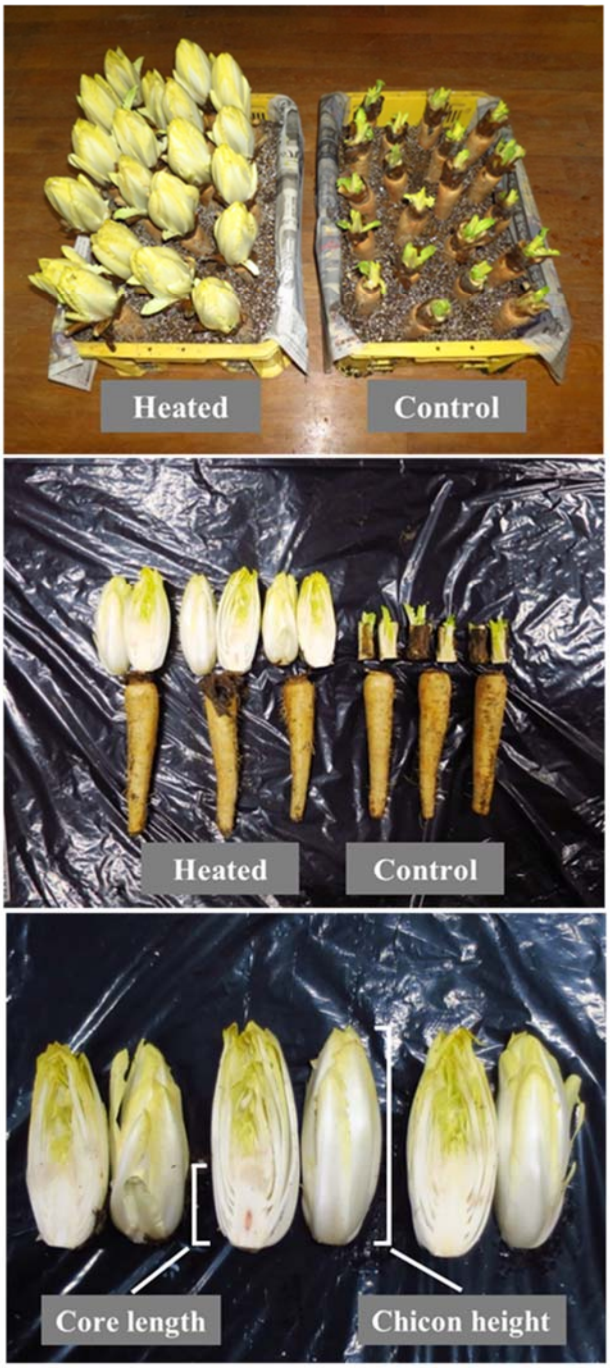

Fig. 2-7 Forcing culture within plastic container (Upper), heads and roots when harvested (15 days after starting forcing culture) (Middle) and definition of head height and core length (Bottom).

recovery system, which circulates air between the forcing room and underground pipes at 2 m depth. In addition, the air temperature in the forcing chamber was maintained at 17.2°C (HIGH) and 15.2°C (MID), in average, by supplying the exchanged heat from fermented manure compost, above 50°C. On the other hand, in the cold season experiment (2014), the temperature inside the compost was around 40°C, close to the level of the year 2013, while the air temperature inside the forcing chamber was less than 10°C unless exchanged heat from the compost was inserted into the forcing chamber. The difference in the environmental factor between the experiment in semi-cold (2013) and cold season (2014) was the temperature in the forcing room. Heat insulator boards, 20 mm in thickness, were attached at bottom, top and side of the forcing chamber; however, it was supposed that the compost heat was not enough to elevate the air temperature to 15°C inside the forcing chamber. A panel heater was set for warming the forcing room in the cold season experiment in 2015 and the temperature in forcing room increased to around 5°C. Though the temperature inside the compost was around 30°C in 2015, i.e., less than that in previous years, the air temperature inside the forcing chamber reached 15°C. Those results indicate that maintaining room air temperature above 0°C enhanced the controllability of the air temperature in the forcing chamber for the growth of etiolated heads, and for this purpose, a terrestrial heat recovery system can be considered one of the practical options.

Temperature increase in manure composting

Miyatake et al. (2009) reported that manure temperature during composting can be increased and stabilized by turning, and the obtained heat energy increased more than 1.5 times, 141 to 216 J s^{-1} m^{-3}, when the frequency of turning increased, from once a week to twice a week. With the present experiments, manure was turned every 6 to 9 days in 2013 and 2014, and the manure temperature increased after 3 to 4 days from turning. These facts indicated the importance of turning the manure to maintain its high temperature during the composting process.

Volume of compost required per heating of forcing chamber

The volume ratio of composting manure used (3.0 m^3) to the forcing chamber (1.76 m^3) was 1.70 in 2014 and 2015, and marketable etiolated heads of VI with sufficient quality were obtained by using the compost heat. From what has been discussed above, there was no report on the relationship between the compost volume and the chamber volume. It seems reasonable to conclude that the required volume of composting manure per volume of forcing chambers of witloof chicory can be estimated as at a minimum of 1.70.

Estimated energy produced by cow manure and exchanged with forcing chambers

According to the study by Seki and Komori (1984) conducted with a small heat insulated compost chamber (0.71 m^3), the recoverable heat energy from composting manure, a mixture of cow manure, poultry manure, rice bran and sawdust, was estimated to be around 836.8 kJ m^{-3} hr^{-1}. Miyatake et al. (2009) also reported that, through a vacuum-induced aeration system, the estimated recoverable heat energy from composting of manure, a mixture of milk cow manure and dried rice straw, was 216 J s^{-1} m^{-3}. Based on the result of the experiment of the present thesis in 2015, in this study, the estimated transferred heat energy into the forcing chamber per hour was calculated as 134.7 kJ h^{-1}, and the estimated produced heat from 3m^3 cow manure composting was calculated as 648.0 to 697.8 J s^{-1}. The heat exchange efficiency of this system is between 5.4 to 5.8%.

Conclusion

The witloof chicory's optimum temperature range for forcing is relatively cooler and the cultivation period is shorter, compared to other indoor horticultural crops. With the present study, it became clear that the witloof chicory forcing cultivation can be done with low heat energy, such as the fermentation heat of livestock manure, in small enclosed spaces. The results also indicate that the temperature required for witloof chicory forcing culture with small heat-insulated chambers can be managed by a low heat energy from the cow manure composting process. Besides, keeping the temperature of the forcing room at more than 5°C is preferable for warming the temperature inside the forcing chamber for the emergence of etiolated heads. Further research on commercial-scale demonstration experiments would clarify the practical feasibility of composting manure as a heat source for witloof chicory forcing production.

CHAPTER 3

Potential of witloof chicory (*Cichorium intybus* L.) as a remedy for potassium accumulated soil

INTRODUCTION

The problem with salt accumulation in horticultural crop production fields has been recently growing. In Japan, salt accumulation in agricultural fields had not become a subject of issue until the 1960s, due to it is temperate region classification, receiving sufficient precipitation to leach out salts in the soil surface and drain it into the lower layer in open fields. However, with the introduction of horticultural production both in the greenhouse and in the open field, which had progressed from the 1960s, the problem with salt accumulation in cultivation soils has been growing. The main causes of the salt accumulation problems are now known to be the excessive application of chemical fertilizers and livestock manure compost, repeated cultivation of specific crops, such as fruits and vegetables, which require heavy-manuring culture, and insulation from rainfall, especially in greenhouses.

In many cases, the salt accumulation in cropping fields is caused by an excessive application of decomposed livestock manure (Kato et al. 2012). Because decomposed livestock manure contains such large amounts of salts, mainly K salts, such as potassium chloride and potassium sulfate (Oyanagi et al. 2004), it is reasonable to suppose that the K accumulation in cropping fields is largely due to an excessive application of decomposed livestock manures.

In Japan, there have been several studies establishing effective techniques for the avoidance of salt accumulation in the cultivation soils, mainly for the indoor horticultural cultivation environment. Some studies were conducted to establish the integrated fertilization management, leaching accumulated salts by water or snow treatments, soil dressing on cultivation fields, and mixing surface and under-layered soils by deep plowing (Ono and Fujii 1994, Ono 1999). However, those techniques may lead to the pollution of groundwater and re-accumulation of salts in surface layer soils.

Several investigations have also been conducted for using forage crops and green manure crops, mainly grasses, for removing accumulated salts from cultivated field soils, both indoors and in the open field. These investigations showed the advantages of remedying salt-accumulated soils by cultivating crops (Oizumi et al. 1979; Shimada et al. 1979; Chiba and Yokoyama 1980; Yokoyama et al. 1983). Another study made clear that it is possible to utilize Guinea grass (*Panicum maximum* Jacq.) to reclaim salt accumulated soils, specifically by using this crop to remove minerals, especially nitrate and K (Kinjo et al. 2007).

At present, several commercial varieties of crops bred for removal of accumulated salts from soil are available in Japan, and it is common to describe them as 'Cleaning Crop'. However, the existing Cleaning Crop approach has several disadvantages. One is that the harvested plants cannot be used widely because of their high concentration of absorbed minerals after cultivation for the remedy of salt-accumulated soils. Another problem is that it is relatively difficult to find suitable crop species for cold regions, being most of the existing Cleaning Crops originated in tropic and sub-tropic conditions. Furthermore, it is virtually impossible for growers to obtain income from Cleaning Crop cultivation, which is aimed to remove salts from soils. It will be necessary to solve these problems to expand the use of Cleaning Crops.

'Witloof chicory' is one of the major cultivar groups of Chicory (*Cichorium intybus* L.), and also one of the traditional vegetable in Europe. The growth cycle of witloof chicory can be divided into two phases, i.e., root production and forcing culture for the edible part (chicon) production.

We established a hypothesis that using the witloof chicory as a 'Cleaning Crop' would be effective as a remedy for salt-accumulated soils, and may bring integral benefits to growers by the removal of K from such soils. At the same time, growers may obtain an agricultural income from the forcing culture by using roots that which absorbed K from soils. It is quite likely that this cultivation technique will also be a new source of agricultural income in winter, especially in a snowy-cold region like Hokkaido, Japan. If witloof chicory absorbs much amount of K and stores considerable amount of K in the root, this crop can realize an evolutional progress as a cleaning crop in cold region.

To evaluate the possibility of having the witloof chicory, as a remedy for K-accumulated soils, we examined the influences of excessive K_2O application during root cultivation on plant growth, mineral concentration of the plant dry matter, the estimated amount of K that can be absorbed, and the transition of chemical profiles of soils, comparing all this with the representative existing 'Cleaning Crop', Guinea grass 'Soil Clean'. Additionally, we investigated the influence of an excessive K_2O application during root cultivation on the yield and the quality of etiolated heads that were obtained after a forcing culture.

MATERIALS AND METHODS

Soil material

Chemical properties of the soil used for pot culture in this study, collected in Kurisawa, Hokkaido, are shown in Table 3-1.

Plant materials and seedling establishment

Witloof chicory 'Vintor' (Nunhems B.V., Nunhem, The Netherlands) (VI) and Guinea grass 'Soil Clean' (Snow Brand Seed Co., Ltd., Sapporo, Japan) (GG) were used. The seeds

Table 3-1 Chemical profiles of the soil used for this study

Soil Type	pH (H_2O)	EC ($mS\ m^{-1}$)	Cation Exchange Capacity (me/100g)	Humus (%)	Phosphate Absorption Coefficient	Inorganic salts (mg/100g)					
						NH_4-N	NO_3-N	P_2O5	K_2O	MgO	CaO
Andisols	6.60	9.00	12.05	3.60	880.0	0.60	0.63	7.64	1.50	5.46	81.03

of these two cultivars were sown in paper pots (FS515; 5 cm diameter, 15 cm height, Nippon Beet Sugar Manufacturing Co., Ltd., Tokyo, Japan), and filled with a potting soil mix (Pot Ace; Katakura Chikkarin Co., Ltd., Tokyo, Japan) on July 1, 2012.

Pot culture with extra K_2O concentration

Root production by pot culture was conducted in a plastic greenhouse at the Experimental Farm, Field Science Center for Northern Biosphere, Hokkaido University, Sapporo, Japan, in 2012. The seedlings were transplanted into plastic pots having 4 small holes in the bottom (30.5 cm height; 30.5 cm diameter, Chubu Nozai Co., Ltd., Aichi, Japan), containing approximately 20 L Andisol soil (chemical analysis results are shown on Table 3-1), on July 24, 2012. One seedling was transplanted into each pot. Five rates of K_2O chemical fertilizer, 0 kg ha^{-1}(K0), 200 kg ha^{-1}(K200), 1,000 kg ha^{-1}(K1,000), 2,000 kg ha^{-1} (K2,000) and 5,000 kg ha^{-1}(K5,000) were applied by potassium sulphate (Hokuren Nogyo Kyodo Kumiai, Hokkaido, Japan), respectively. Hayashi et al. (2009) reported that the range of the K_2O concentration in intense cultivated greenhouses in Hokkaido was from 46.1 to 81.7 mg per 100 g soil. In fact, the range of soil K_2O concentrations at the start of cultivation was from 93.1 mg to 210.9 mg per 100 g soil, among K1,000 to K5,000. It is clear that the range of the initial level of K_2O concentration of the soil in this study is covering the range of K_2O concentration level in a practical situation. In every pot, 200 kg N, given as ammonium sulfate (JFE Chemical Corporation, Tokyo, Japan), and 100 kg P_2O_5 given as fused magnesium phosphate (Hinode Chemical Industry Co., Ltd., Kyoto, Japan), were added per ha. A total of seven plants were prepared as replications for each treatment. The irrigation was conducted to avoid that drying of the soil surface in the pots, keeping pF between 1.6 to 2.3, measured by pF meter (DIK-8333, Daiki Rika Kogyo Co., Ltd., Saitama, Japan). A manual weed removal was performed to avoid the plant competition for nutritional absorption from the soil. The layout of the pot arrangement in a greenhouse was fully randomized. A temperature and RH data logger (LR5001; Hioki E. E. Corporation, Nagano, Japan) was set in the center of the greenhouse, 1.5 m above the ground, to record the hourly air temperature and RH.

Throughout the root cultivation period, plant samples of VI were collected twice, 9 weeks and 19 weeks after transplanting (WAT). GG plants were collected only once, 9 WAT, because they grew nearly to the roof of the greenhouse 9 weeks after the transplanting and the cultivation had to be stopped.

Collected plant samples were separated into two parts, top and root shares. Plant samples were washed thoroughly with tap water to remove adhering particles from the potting soil mix. The soil in the pot was well-mixed and was sampled at each sampling and at the end of the cultivation, then sieved through a 2.0 mm mesh, and dried 2 weeks at room temperature without exposure to direct sunshine. All samples, both plants and soils, were kept in sealed plastic bags in a refrigerator at 4°C, until analysis. At each sampling timing during pot culture, plant samples were obtained from seven pots which were randomly selected. After measuring fresh weight of the sample plants, top and root separately, plants were dried in an air-circulating oven at 60°C for 2 weeks, and then the dry weight of plants was measured.

Forcing culture in witloof chicory

The roots of VI were harvested around 19 WAT after the pot culture. Collected plant samples were separated into two parts, i.e., top and root, and washed thoroughly with tap water to remove adhering particles from the potting soil mix. After measuring fresh weight (FW), Brix of roots and other growth parameters, roots were sorted and cut into 20 cm lengths (from root shoulder to the bottom end), and stored in a refrigerator, with air temperature from 0.2 to 0.8°C, and a RH of 100%, until the start of the forcing culture. In each experiment, roots were transplanted into plastic containers (57.5×41.5×19.0 cm), which were filled with a medium normally used for nursery production (Takii Tanemaki Baido, Takii Co., Ltd, Japan). A total seven roots were planted as replications for each treatment and the plant density was 120.0 plants m^{-2}. The layout of the roots in each cultivation container was fully randomized. To obtain etiolated heads, roots were grown under dark conditions, with a temperature of 16°C in average, and a RH of 100%, and the irrigation was periodically supplied. The fresh weight (before and after trimming), and the height, diameter and flower stalk length of etiolated heads were measured when those heads were harvested, 21 days after the starting of the forcing culture.

Mineral concentration analysis and K uptake amount per plant

At each sampling, from both pot culture and forcing culture five dried plants were selected randomly from each treatment, and top and root parts were crashed and ground separately. For analysing the concentration of K, Ca, Mg and P, approximately 100 mg of samples were put into individual metal-free polypropylene tubes. In each tube with tissues, 1.3M of HNO_3 (20-50 mL) was added and tissues were digested at 60°C for 1 to 2 h. the obtained solutions were filtered and diluted with 0.1M HNO_3, and the mineral element concentrations were analysed by using ICP-AES (ICPE-9000, Shimadzu Corporation, Kyoto, Japan). By using the results of the mineral concentration analysis of plant dry matter, K-uptake amount per plant was calculated separately for both top and root parts. For analysing the concentration of C and N, approximately 10 mg of samples were put into the tin capsules, and the element concentration of plant dry matter was analysed by using CN analyzer (Vario EL Ⅲ, Elementar Analysensysteme GmbH, Hanau, Germany).

Soil chemical profile analysis

Dried soils and distilled water were mixed in a ratio of 1:2.5, and then pH was determined using a pH meter (Portable pH meter D-74, Horiba Co., Ltd., Kyoto, Japan). Dried soils and distilled water were mixed in a ratio of 1:5, and then electrical conductivity (EC) was determined using EC meter (DM-37, Takemura Denki Seisakusho Co., Ltd., Tokyo, Japan). Chemical profiles of soils, collected at each sampling, were analysed by the conventional method, using commercially available integrated colorimeter system for soil analysis (ZA-Ⅱ, Fujihira Industry Co., Ltd., Tokyo, Japan). The concentration of K_2O in the soil was analysed by the Kalibor (Sodium tetraphenylborate) turbidimetric method.

Statistical analysis

Data obtained from each sampling time were statistically analysed, and significant differences of the mean values were calculated using the Tukey-HSD test. For the plant growth comparison, under both pot culture and forcing culture, and soil analysis, each value represented the mean of seven replications. For the plant dry matter mineral concentration analysis, each value represented the mean of five replications.

RESULTS

Temperature and RH during pot culture

Recorded temperature and RH are shown in Table 3-2. The results revealed that plants were exposed to a sub-zero temperature that promotes the translocation of carbohydrate substances from the top to the root part of the plant after mid-November, representing a sufficiently low temperature treatment for VI root development even within the greenhouse.

Table 3-2 Profiles of temperature and humidity in greenhouse during pot cultivation.

Growing period		Temperature (℃)	Relative Humidity (%)
24 July, 2012 - 29 November, 2012	Average	18.2	80.0
	Maximum	41.5	100.0
	Minimum	-2.5	25.1

Biomass production during pot culture

GG grew faster than VI, reaching the roof of the greenhouse 9 WAT, therefore, investigations of GG plant growth was terminated at 9 WAT. The maximum leaf lengths of VI were 31.2 cm at 9 WAT, and 35.5 cm at 19 WAT, respectively. The dry weights tended to decrease corresponding to an increase of K_2O application in VI (Fig. 3-1). Root dry weights of VI (19 WAT) were heavier than those of GG (9 WAT) at each K_2O treatment. At 19 WAT, the root dry weight of VI in K0 was the highest (99.3 g), followed by K200 (84.0 g), K1,000 (83.6 g), K2,000 (72.4 g) and K5,000 (48.2 g), and no significant difference was observed among treatments except K 5,000. However, a significant difference was not observed in the top dry weight of VI 19 WAT (K0; 33.9 g, K200; 28.5 g, K1,000; 31.6 g, K2,000; 25,2 g, and K5,000; 19.6 g). In contrast, the top dry weight of GG increased significantly when the K_2O concentration increased; the top dry weight in K5,000 was the highest (109.7 g), followed by K2,000 (106.7 g), K1,000 (97.5 g), K200 (72.6 g) and K0 (66.3 g), respectively. There was no significant difference in the root dry weight of GG among treatments at 9 WAT.

Mineral concentration on plant dry matter during pot culture

In VI, the K concentration, both of the top and root parts, increased when the K_2O application increased, and the difference in K concentration between the tops of VI was greater than that of the root part in each of the K_2O treatment at both 9 WAT and 19 WAT (Table 3-3). In VI, top K concentrations in K5,000 were always the highest at two samplings: 11.69% at 9 WAT and 8.87% at 19 WAT. In GG, the highest K concentrations were observed in K1,000 and K2,000 (5.03%, 5.03%, respectively) in the top part, and in K5,000 (2.15%) in the root part. In GG, the difference of the K concentration in the top part among treatments was smaller than that of VI.

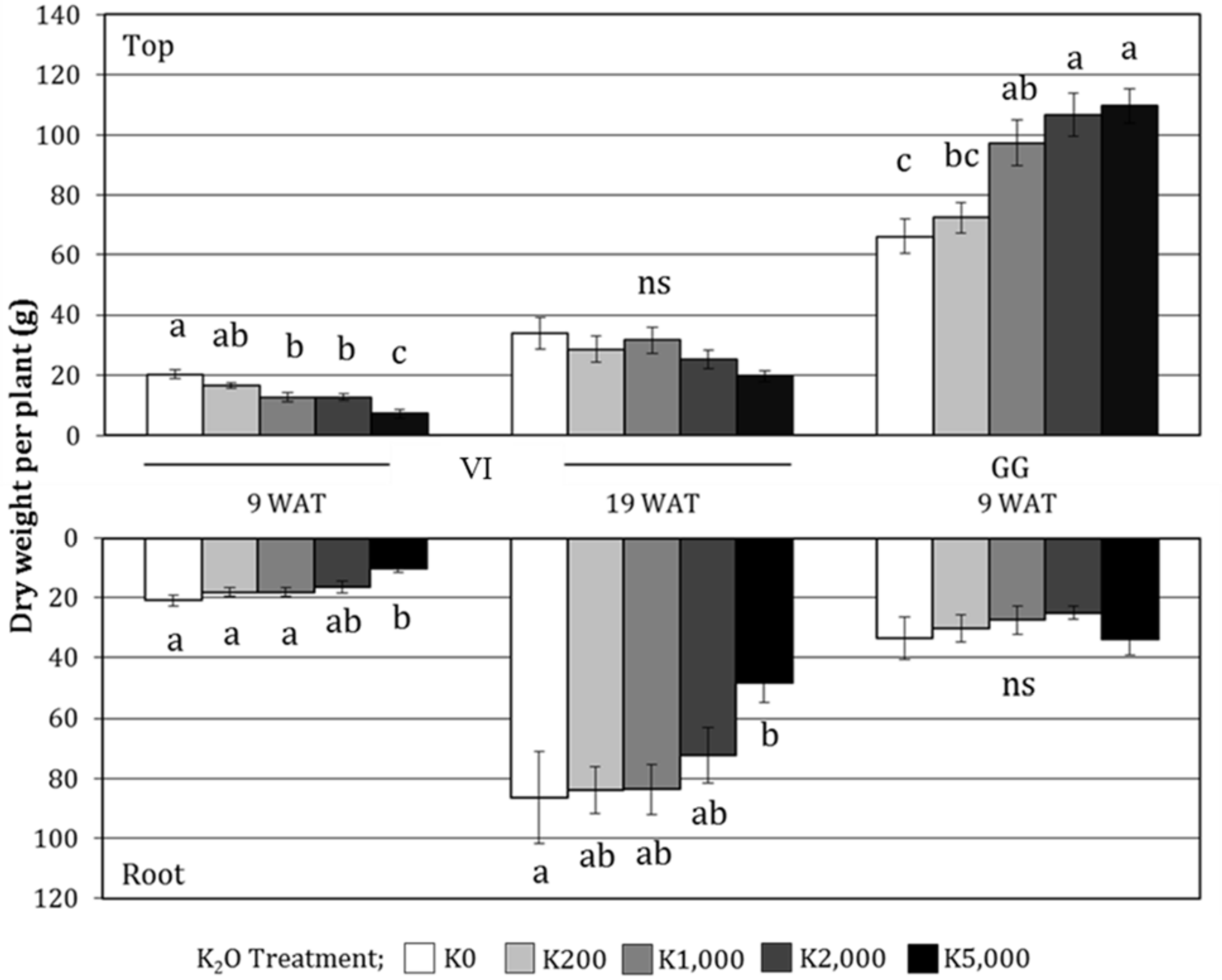

Fig. 3-1 Effect of K_2O treatment on plant dry matter at 9 weeks and 19 weeks after transplant. Values represent a mean of 7 replications ± SE. Bars with different letters within the treatments are not same by turky's HSD test at p< 0.05., ns; not significant, WAT; Weeks after transplanting.

Table 3-3 Effect of K_2O treatment on chemical profiles of plant dry matter at 9 weeks and 19 weeks after transplanting.

Plant Part	Crop	Sampling Timing	Treatment K_2O	Water Content (%)	K (%)	C (%)	N (%)	P (%)	Ca (%)	Mg (%)
Top	VI	9 Weeks	K 0	90.2±0.7 a[z]	6.50±0.43 c	37.1±0.21 a	2.79±0.08 a	0.286±0.034 a	2.24±0.17 a	0.519±0.038 a
			K 200	90.5±0.8 a	7.78±0.31 c	36.5±0.63 a	2.68±0.12 a	0.285±0.012 a	1.98±0.08 a	0.427±0.025 ab
			K 1,000	93.4±0.9 a	9.26±0.22 b	35.0±0.67 ab	2.75±0.15 a	0.293±0.022 a	1.55±0.05 b	0.386±0.009 bc
			K 2,000	91.6±1.2 a	9.94±0.40 b	35.1±0.25 ab	2.61±0.11 a	0.250±0.028 a	1.40±0.03 b	0.336±0.013 bc
			K 5,000	92.1±1.2 a	11.69±0.24 a	33.5±0.64 b	2.67±0.11 a	0.213±0.021 a	1.21±0.11 b	0.329±0.013 c
		19 Weeks	K 0	82.4±1.2 a	4.39±0.63 c	41.2±0.08 a	0.74±0.06 a	0.178±0.009 a	2.23±0.29 a	0.440±0.069 a
			K 200	80.6±0.4 a	4.63±0.25 c	41.2±0.29 a	0.78±0.07 a	0.082±0.003 b	1.95±0.12 ab	0.376±0.023 ab
			K 1,000	83.3±0.4 a	6.84±0.32 b	40.1±0.30 b	1.00±0.05 a	0.144±0.022 a	1.40±0.06 bc	0.225±0.018 c
			K 2,000	81.2±0.7 a	7.50±0.14 ab	40.8±0.15 ab	0.81±0.03 a	0.094±0.004 b	1.45±0.05 bc	0.255±0.014 bc
			K 5,000	82.2±0.8 a	8.87±0.54 a	40.4±0.15 ab	0.88±0.10 a	0.088±0.005 b	1.11±0.06 c	0.199±0.023 c
	GG	9 Weeks	K 0	81.1±1.0 a	3.31±0.50 b	42.0±0.48 a	1.43±0.34 a	0.209±0.068 a	0.409±0.036 a	0.226±0.021 a
			K 200	81.5±0.8 a	3.91±0.25 ab	41.6±0.42 a	1.48±0.24 a	0.247±0.022 a	0.360±0.017 ab	0.226±0.025 a
			K 1,000	83.7±0.5 a	5.03±0.22 a	40.4±0.29 a	1.86±0.20 a	0.328±0.040 a	0.383±0.016 ab	0.289±0.036 a
			K 2,000	83.2±0.6 a	5.03±0.48 a	40.8±0.51 a	1.78±0.26 a	0.210±0.027 a	0.331±0.026 ab	0.287±0.042 a
			K 5,000	82.8±0.5 a	4.68±0.20 ab	41.2±0.33 a	1.79±0.15 a	0.267±0.036 a	0.304±0.024 b	0.295±0.034 a
Root	VI	9 Weeks	K 0	78.2±0.8 a	1.90±0.15 b	37.0±0.33 a	3.48±0.21 a	0.175±0.021 a	0.236±0.012 a	0.112±0.004 c
			K 200	75.6±1.7 a	2.05±0.12 b	36.9±0.29 a	3.13±0.12 ab	0.163±0.008 a	0.238±0.009 a	0.118±0.004 bc
			K 1,000	67.8±3.4 ab	2.24±0.10 ab	35.9±0.25 ab	3.20±0.09 ab	0.158±0.020 a	0.211±0.006 ab	0.129±0.002 bc
			K 2,000	70.3±1.4 ab	2.57±0.12 a	36.2±0.36 ab	2.89±0.24 ab	0.160±0.021 a	0.202±0.017 ab	0.135±0.003 ab
			K 5,000	56.6±6.2 b	2.60±0.05 a	35.2±0.16 b	2.56±0.12 b	0.116±0.012 a	0.172±0.009 b	0.153±0.009 a
		19 Weeks	K 0	73.4±0.6 a	1.77±0.13 a	40.6±0.11 a	0.78±0.06 a	0.189±0.019 a	0.185±0.008 a	0.116±0.002 a
			K 200	71.0±0.4 b	1.29±0.03 b	40.7±0.09 a	0.71±0.04 a	0.080±0.005 b	0.170±0.006 ab	0.117±0.003 a
			K 1,000	73.2±0.4 a	1.70±0.08 ab	40.5±0.05 a	0.78±0.06 a	0.133±0.028 ab	0.157±0.007 b	0.125±0.007 a
			K 2,000	72.1±0.3 ab	1.86±0.08 a	40.6±0.10 a	0.73±0.09 a	0.090±0.005 b	0.148±0.006 bc	0.135±0.005 a
			K 5,000	71.8±0.5 ab	1.97±0.13 a	40.7±0.07 a	0.73±0.04 a	0.085±0.011 b	0.125±0.004 c	0.126±0.006 a
	GG	9 Weeks	K 0	34.1±6.2 a	1.13±0.16 b	39.3±0.99 a	1.61±0.78 a	0.076±0.016 a	0.422±0.048 a	0.266±0.016 a
			K 200	38.8±4.4 a	1.41±0.12 ab	39.6±0.97 a	0.75±0.08 a	0.076±0.010 a	0.368±0.041 a	0.220±0.018 ab
			K 1,000	42.4±4.0 a	1.59±0.23 ab	40.5±0.74 a	0.90±0.05 a	0.098±0.020 a	0.329±0.036 a	0.188±0.024 ab
			K 2,000	46.4±6.5 a	1.75±0.21 ab	40.7±1.02 a	0.76±0.08 a	0.071±0.013 a	0.281±0.023 a	0.144±0.009 b
			K 5,000	54.4±7.2 a	2.15±0.30 a	37.3±1.96 a	0.83±0.11 a	0.093±0.016 a	0.380±0.043 a	0.212±0.034 ab

[z] WAT; Weeks After Transplanting
[y] Values represent a mean of 5 replications ± SE. Values within a column with different letters are not same by turky's HSD test at $p <$ 0.05.

There were no significant differences among treatments with respect to the P concentration in top part in VI at 9 WAT; however, significant differences were observed at 19 WAT among treatments, and the same trends were observed in the root part. For both top and root parts of VI, the P concentration tended to slightly decrease when K_2O application increased. For GG, there were no significant differences in the P concentration at top and root among treatments at 9 WAT. Concentrations of Ca and Mg in the top part of VI decreased significantly as K_2O application increased. For GG, the disparities of top concentrations of Ca among treatments were smaller than those of VI, and no significant differences among treatment were observed in the root Ca concentration and in the top Mg concentration.

K-uptake amount per plant

In all treatments, the K-uptake amounts of GG (9 WAT) in the top were significantly higher than VI (19 WAT), and the highest amount (5.37 g/plant) was observed in K2,000, followed by K5,000, K1,000, K200 and K0 (5.14 g/plant, 4.90 g/plant, 2.84 g/plant, 2.20 g/plant, respectively) (Table. 3-4). The highest K-uptake amount (2.16 g/plant) in VI was observed at K1,000, however, that was approximately 40% of the peak K-uptake amount of GG (5.37g/plant). In contrast, in the root part, K-uptake amounts in VI (19 WAT) were always higher than those of GG among all treatments, and significant differences were observed, in comparison with GG (9 WAT), except for K5,000.

Plant total amount of K-uptakes in GG (9 WAT) were always higher than those of VI (19 WAT), except in K0, and the highest was in K5,000 (5.87 g/plant), followed by K2,000, K1,000, K200 and K0 (5.80 g/plant, 5.34 g/plant, 3.26 g/plant and 2.57 g/plant, respectively). In K200, the plant total K-uptake of VI at 19 WAT (2.41 g/plant) was about 74% of GG at 9 WAT (3.26 g/plant), the highest ratio of VI (19 WAT) to GG (9 WAT) in the plant total K-uptake, and the lowest ratio (45.8%) was observed in K5,000 (VI; 2.69 g/plant, GG; 5.87

Table 3-4 Effect of K_2O treatment on K absorption in chicory and guinea grass.

Treatment	Plant	K absorption amount[y] (g /plant)		
		Top	Root	Plant Total
K0	VI	1.49	1.76	3.24
	GG	2.20	0.38	2.57
	t test	**	**	ns
K200	VI	1.32	1.08	2.41
	GG	2.84	0.43	3.26
	t test	**	**	*
K1,000	VI	2.16	1.42	3.58
	GG	4.90	0.44	5.34
	t test	**	**	*
K2,000	VI	1.89	1.35	3.24
	GG	5.37	0.44	5.80
	t test	**	**	**
K5,000	VI	1.74	0.95	2.69
	GG	5.14	0.73	5.87
	t test	**	ns	**

[Z] Pots of 0, 200, 1,000, 2,000 and 5,000 were fertilized with 0, 200, 1,000, 2,000 and 5,000 kg K_2O ha^{-1}, respectively.
[y] K Absorption amount = (K concentration on dry weight) × Dry weight.
[x] **, * and ns; significance at p=0.01, 0.05 and non-significance by t test (n=7).
WAT; Weeks after transplanting

g/plant). In comparison with GG (9 WAT), plant total K-uptakes of VI (19 WAT) were always higher than 55% from K0 to K2,000.

Changes of soil chemical profile during pot cultivation

The EC decreased sharply in VI after cultivation, mainly for the soils exposed to high K_2O treatments; the decreased ratio of VI in 9 weeks was larger than that of GG (Fig. 3-2-a). The highest decreases in pH were observed in every treatment of GG, even though the pH increased after the pot cultivation of VI, except in K1,000 (Fig. 3-2-b).

K_2O concentrations in soils decreased after cultivation, especially in the high K_2O application, both in VI and GG (Fig. 3-2 -c). At 9 WAT, in K5,000 (210.9 mg/100 g at transplanting), the soil K_2O concentrations decreased to 109.6 mg/100 g in VI, lower than in GG (170.9 mg/100 g). In K2,000 (131.4 mg/100 g at transplanting), VI was lower (37.4 mg/100 g) than GG (65.4 mg/ 100 g), and in K1,000 (93.9 mg/100 g at transplanting), VI (25.4 mg/100 g) was lower than GG (35.4 mg/100 g). At 19 WAT, the soil K_2O concentration of VI decreased further. After cultivation, in K200 and K0, the soil K_2O concentration of all plants decreased to the level of the original soil, between 0.6 mg/100 g to 1.5 mg/100 g. Even in K2,000 and K5,000, the K_2O concentration in the soil decreased at 30mg/100g and 60 mg/100g, respectively, in VI plant.

Forcing culture of witloof Chicory

The root FW of plants grown from K0 to K2,000 were 253- 295 g per plants; no significant difference was observed among treatments, and it was larger than that in K5,000. The average root FW, after trimmed into 20 cm length, from K0 to K2,000 were significantly larger than K5,000 at the 5% probability level (Table 3-5). The average FW of trimmed

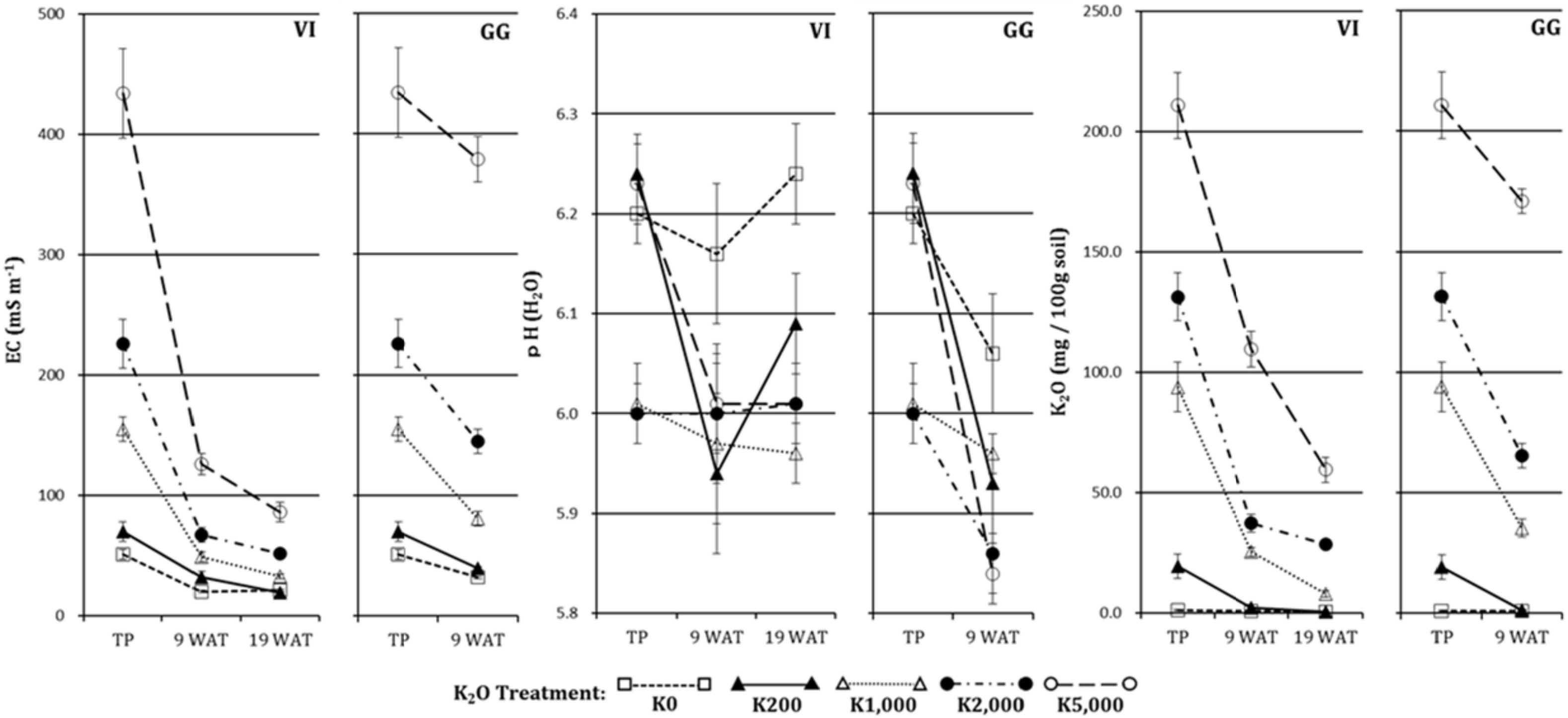

Fig. 3-2 Changes in chemical profiles of soils contained with 5 levels of potassium treatments. Values represent a mean of 7 replications ± SE. TP; Transplant. WAT; Weeks after transplanting.

Table 3-5 Effect of K_2O treatment during root cultivation on growth parameters of roots and etiolated heads after forcing culture, in witloof chicory 'Vintor'. Roots were trimmed by cutting into 20cm length.

Treatments	K_2O ($kg\ ha^{-1}$)	Roots (19 Weeks After Transplant) Fresh Weight (g/plant)	Roots Diameter (cm)	Roots Brix	Etiolated heads: Before Trimming Fresh Weight (g/plant)	After Trimming[z] Fresh Weight (g/plant)	After Trimming Height (cm) (B)	After Trimming Diameter (cm)	After Trimming Flower Stalk Length (cm) (A)	After Trimming Ratio; (A)/(B)
K 0	**0**	290.7±31.6 a[y]	5.9±0.3 a	17.9±0.2 a	231.0±25.2 a	205.6±20.1 a	14.4±1.2 ab	6.9±0.3 a	4.7±0.2 a	0.35±0.06 a
K 200	**200**	255.9±39.7 a	5.4±0.3 a	19.6± 0.3 b	190.9±33.2 ab	165.9±25.1 a	14.7±0.7 a	6.3±0.4 a	4.1±0.1 a	0.28±0.01 a
K 1,000	**1,000**	253.4±12.9 a	5.5±0.1 a	19.5± 0.4 b	170.0±7.6 ab	154.9±6.3 a	14.8±0.4 a	6.4±0.2 a	4.0±0.3 a	0.27±0.02 a
K 2,000	**2,000**	295.4±39.7 a	5.8±0.2 a	19.7± 0.4 b	198.3±42.0 ab	163.9±36.0 a	13.4±1.3 ab	6.6±1.1 a	3.9±0.3 a	0.30±0.02 a
K 5,000	**5,000**	120.3±7.7 b	4.2±0.2 b	19.8± 0.5 b	96.0±8.6 b	86.9±8.1 b	11.0±0.4 b	4.8±0.2 a	2.7±0.2 b	0.25±0.01 a

[z] Trimming; Removing outer leaves for marketing of etiolated heads.
[y] Values represent a mean of 7 replications ± SE. Values within a column with different letters are not same by turky's HSD test at $p < 0.05$.

etiolated heads, which were obtained after the forcing culture, always exceeded 150 g, except at K5,000; in addition, those were significantly higher than K5,000 at the 5% probability level. The size of the etiolated heads of all the treatments except K5,000 was at the same level of the commercial standard. No significant differences were observed among treatments in the average ratio of flower stalk (flower stalk length / height of etiolated head) at the 5% probability level.

Mineral concentration on etiolated heads

The K concentration in the dry matter of etiolated heads was observed in the range between 4.4 - 5.1% in this study. There were no significant differences among treatments with the K concentration of etiolated heads based on dry matter at the 5% probability level (Table 3-6). The dry matter concentration of N, P, Ca and Mg in etiolated heads tended to decrease when the K_2O application amount increased, and K5,000 was always the lowest among the treatments. There were no significant differences among treatments with the C concentration of the dry matter of etiolated heads at the 5% probability level.

DISCUSSION

Effect of K_2O application on plant growth during pot cultivation

At 9 WAT, the top and root dry weights of VI decreased with the increase of K_2O application, but at 19 WAT, no significant differences were observed in top growth even with a greater K_2O application (Fig. 3-1). This result indicates that VI has the potential for root cultivation under high-K_2O stressful conditions.

In the present study, it was observed that the dry weight of the top part of GG increased with the rise of the K_2O application, and no significant differences in root dry weights were

Table 3-6 Effect of K_2O treatment on chemical profiles of etiolated head dry matter after forcing culture in witloof chicory 'Vintor'.

Sampling Timing	Treatments K_2O (kg ha^{-1})	Water Content (%)	% / Dry matter					
			K	C	N	P	Ca	Mg
	0	94.5±0.2 a[y]	4.6±0.4 a	40.1±0.2 a	4.13±0.13 a	0.55±0.04 a	0.60±0.02 a	0.44±0.01 a
	200	93.9±0.4 ab	4.6±0.3 a	40.5±0.2 a	4.09±0.12 a	0.50±0.05 a	0.57±0.04 ab	0.45±0.03 a
9 WAT[z]	1,000	94.1±0.3 a	4.5±0.2 a	40.6±0.1 a	3.95±0.08 a	0.44±0.02 a	0.45±0.04 bc	0.38±0.03 a
	2,000	94.2±0.5 a	5.2±0.3 a	40.5±0.2 a	3.94±0.13 a	0.49±0.04 ab	0.40±0.05 c	0.36±0.02 ab
	5,000	92.5±0.5 b	4.4±0.2 a	40.4±0.2 a	3.19±0.15 b	0.34±0.01 b	0.21±0.02 d	0.27±0.01 b

[z] WAT; Weeks after transplanting.
[y] Values represent a mean of 5 replications ± SE. Values within a column with different letters are not same by turky's HSD test at $p< 0.05$.

found among treatments. Kinjo et al. (2007) reported that plant dry weight of GG, both top and root, increased as the application of combined chemical fertilizer was increased (N; 200 kg ha^{-1}, P_2O_5; 175 kg ha^{-1}, K_2O; 166 kg ha^{-1}, CaO; 130 kg ha^{-1}; applying 2 and 4 times each, with a total 3 treatments) after growth of 9 weeks.

In contrast, for VI, plant dry weight of both in top and root tended to be suppressed as K_2O application was increased. It is clear that there is a difference in the growth response to K_2O stressful condition between VI and GG.

Effect of K_2O application on mineral concentration on plant dry matter

The K concentration in the dry matter was enormously higher than that of Ca and Mg in VI (Table 3-3). Inthichack et al. (2012) reported that Ca and Mg concentrations in plant dry matter of lettuce, celery and cabbage decreased as K concentration increased in plant body, by the application of several types of K sources. In the present study, it was observed that concentrations of Ca and Mg in VI top dry matter decreased significantly, both in 9 WAT and 19 WAT, when the K concentration in plant dry matter increased with the increase of K_2O application (Table 3-3). In contrast, the Ca concentration in the top of GG decreased slightly as the K_2O application increased; however, no significant differences were observed in Mg concentration among treatments. In roots of all plants used, Ca concentrations in plant dry matter decreased in almost all of the cases, as with the increase of K_2O application; however, the differences in Ca concentrations among treatments were smaller than those occurring in the top parts. From these facts, there is considerable support for the existence of validity a trade-off relationship among the accumulation of K, Ca and Mg in the top part of VI under high K stressful ~~conditions~~ as reported by Inthichack et al. (2012).

Effect of K_2O application on K-uptake of witloof Chicory and Guinea grass

The K concentration in the top dry matter of VI increased as K_2O application increased, and a wide variation was recognized in Top-K concentration among the treatments, ranging from 4.4% in K0 to 8.9% in K5000. However, such concentrations in VI was higher than those occurred in GG, 4.7% in K5000 at 9 WAT. The capacity of the K accumulation in the root was limited, almost 2 mg/kg, and it was smaller than those in the top part.

When Guinea grass is used as a cleaning crop, usually its roots are not removed from the field, thus its salt absorption capacity is defined only by the amount of absorption in the top part. In the present study, the K concentration in the top dry matter of GG at the end of the pot cultivation ranged from 3.3% (K0) to 5.0% (K1,000 and K2,000), values relatively smaller than those obtained for VI, that is, 52.8% to 75.0%. However, the top K-uptake amount of GG (2.20 g per plant (K0) to 5.37 g per plant (K2,000)) was 1.48(K0) to 2.84 (K2,000) times greater than that of VI (1.49 g per plant (K0) and 1.89g per plant (K2,000)) in the same treatment, due to the large biomass present in the top part of GG. For the reasons mentioned above, VI can perform at certain level in the K absorption even if the top biomass is relatively smaller, because the K concentration in the plant dry matter of the top part of VI (19WAT) was larger than that of GG (9WAT) in all treatments.

It is well known that various kinds of plants consume large amounts of K under high K available environments, and K easily increases its concentration in the plant body to a luxurious level (Zörb et al. 2014). In this regard, witloof chicory is superior to GG, especially for its top. The total amount of K-uptake per VI plant was the highest at K1,000, 3.58 g/plant, approx. 67.0% of K uptake in that of GG in the same treatment. In other treatments, such ratios of total amount of K uptake in VI to GG were 73.9% in K200, 55.9% in K2000 (Table 3-4).

Effect of K_2O application on quality and mineral concentration of etiolated head of witloof Chicory

The negative influence of an excessive application of K during chicory root cultivation on the FW of trimmed etiolated heads after forcing culture was not observed until the K application amount did not exceed 2,000 kg ha^{-1} (131.4 mg/100 g soil at transplanting). Also, no significant difference in flower stalk ratio (flower stalk length/ height of etiolated head) was observed among the treatments at the 5% probability level, even in the excessive K application, such as with 2,000 kg ha^{-1} application. From those facts, it was recognized that the marketable etiolated heads could be obtained from the roots cultivated in the field with a high K concentration, such as 131.4 mg/100 g soil, as the level of 2,000 kg-K ha^{-1} application in this study.

No significant difference in the K concentration in the etiolated head dry matter was observed among the treatments at 5% probability level. Also, no significant differences in etiolated head dry matter concentration of N, C, P and Mg were observed among the treatments under K2,000. Although a significant difference in Ca concentration in etiolated head dry matter was observed among the treatments, the differences were not large, ranging, from 0.60% at K0 to 0.40% at K2000. It is reasonable to suppose that a high K condition during root production does not have a strong negative influence on the mineral concentration in the etiolated heads obtained after the forcing culture of roots.

Estimated K-uptake amount per ha in both witloof Chicory and Gunia Grass

Usually, the root biomass of witloof chicory increases especially in the end of growing period. In this study, root dry weights per plant of VI in all treatments increased largely at 19 WAT comparing to 9 WAT, and the root dry weight of VI (19WAT) was always larger than that of GG (9WAT) at all treatments. Also K-uptake amounts in roots of VI were always larger than that of GG at all treatments, and the ratio of K-uptake amount by root to that in the total plant ranged between 35.3% (K5,000) to 54.3% (K0).

For these reasons, it became clear that, in VI, the root contributes to a large amount of the K-uptake in the plant total. It is presumed that root of witloof chicory will be harvested from the field even when it is used as a cleaning crop because of its use in the forcing culture of the roots for obtaining etiolated heads. It is clear that both top and root parts of the witloof chicory play a part for removing extra K in the soil. Roots will be harvested for the forcing culture to obtain the etiolated heads. Therefore, salt absorption amounts in both top and root parts have to be considered when we estimate the potential of witloof chicory as a cleaning crop.

The plant total (both top and root parts) K-uptake amounts of VI ranged from 2.69 g at K5,000 to 3.58g at K1,000, per plant, and those accounted for 52.3% and 84.9% of K-uptake amount per plant of the top part of GG, at same treatment.

The plant densities in both VI and GG in the present experiment were set at the same level and only one plant was transplanted per pot, as a matter of practical convenience. However, the actual dry matter yield per ha of Guinea grass at the mowing can be estimated approximately 4.16 t under practical conditions, when sowing density was 15.0 kg per ha (Fujii et al 2005). On the other hand, the usual plant density of witloof chicory for root production is approximately 200,000 plants per ha. Based on those data, the estimated K-uptake amount per ha as a cleaning crop in VI (plant total) and GG (top part only) was calculated using the data of the K concentration on the plant dry matter (Table 3-3) and the K-uptake amount per plant (Table 3-4) which were obtained from this study, at K1,000 and K2,000. It was found from the result that the estimated K-uptake amount per ha of VI is 648.0 kg (K2,000) to 716.0 kg (K1,000) and that of GG is 208.0 kg (K1,000 and K2000) per ha, when used as cleaning crops. It should also be added that there is a certain difference between the pot experiment in this study and the practical open field cultivation in growing conditions. However, the K-uptake amount per ha of witloof chicory is 3.12 to 3.44 times larger than that of Guinea grass under the cultivation in the K accumulated soil at the practical level.

Conclusion

The VI, a witloof chicory variety 'Vintor', has the potential to be used as a remedy for K accumulated in soils, and its K-uptake capacity can be estimated 3.12 to 3.44 times larger than that of Guinea grass. The results from the present study proves clearly that marketable etiolated heads can be obtained even when the witloof chicory is used as a remedy of K accumulated in soils. It can be concluded that 'Vintor' has the potential to remove K from salt-accumulated soils. More detailed research focusing on the performance of witloof chicory as a 'Cleaning Crop', in natural conditions such as a modelled salt-accumulated field in outdoor, is needed to develop an effective remedy for salt-accumulated agriculture fields.

CHAPTER 4

The potassium absorption capacity of witloof chicory (*Cichorium intybus* L.) in modelled salt accumulated field made by excessive application of methane fermentation digested slurry

INTRODUCTION

The Methane Fermentation Digested Slurry (DS) contains sufficient nitrogen and other fertilizer components, thus several studies have been conducted on the development of practical techniques to use DS for horticultural productions (Möller and Müller 2012; Endo 2014). In practice, there are several studies for a more efficient use of DS as a fertilizer for realizing a sustainable production of various horticultural crops, such as tomato (*Solanum lycopersicum* L.), cabbage (*Brassica oleracea* L.), Komatsuna (*Brassica rapa*) and cucumber (*Cucumis sativus* L.) (Endo et al. 2002; Tokuda et al. 2010; Fujikawa and Nakamura 2010; Yoshino et al. 2012).

On the other hand, it has been recognized that, in Japan, the problem of remanence and accumulation of fertilizer components in the soil is getting conspicuous, not only for indoor fields but also for open field horticultural production, being the cause of this problem an excessive use of fertilizers, both chemical and organic (Tanimoto 1991). In particular, the organic fertilizers, such as compost and DS derived from livestock wastes, contain a high concentration of K among the three major plant macronutrients. This specific chemical constitution leads consequently to the K accumulation in the soils when we use it based on the required amount of nitrogen (Goto and Eguchi 1997; Oyanagi et al. 2002). In order to make the best use of organic fertilizers for an efficient production of horticultural crops, it is necessary to develop practical solutions which can avoid K accumulation in the soils.

The accumulation of salts, including K in the soils of agricultural fields, tends to break the balance of mineral absorption by crops. This may lead to a yield decreasing, a deterioration in quality and negative impacts to livestock animals such as grass tetany when used as a forage crop; consequently, the importance of effective solutions to evade salt accumulation in the soils has been recognized (Ito et al. 1981; Eguchi 1993). The major techniques recently used for salt removal from salt accumulated soils are: 1) excessive irrigation or flooding, including dumping the snow into the field (Aragaki et al. 1986); 2) dilution of salts by removing surface soils, soil dressing and plowing to replace surface soil with subsoil; 3) organic matter application which aims to increase chemical, physical and biological soil buffering capacity (Ikeda et al. 1994); and 4) growing a "Cleaning Crop", which has an excessive salt absorption capacity from the soils, e.g. grass for forage or green manure. The most common method with comparative ease is probably the flooding (excessive irrigation): However, it has been reported that this technique has several problems, such negative impacts on the ground water quality due to the leaching of nitrate nitrogen or sulphate ion from the surface to the deeper layers (Yanagase et al. 2005). Furthermore, researches have clarified until now that this technique can lead to the emission of a large amount of nitrous oxide, well known as a greenhouse effect gas, as well as leaching nitrate nitrogen. Therefore, to develop effective and environmental-friendly solutions which can be substituted to the flooding is requested (Sadamatsu et al. 2008).

"Cleaning Crop" technique is recognized as one of the efficient solutions to remove salts from the soil without heavy negative environmental impacts to the crops. In other words, this is the method to remove accumulated salts in the soil by the strong absorption capacity of fertilizer components in the forage or green manure grass crops (Kinjyo et al.

2007; Kondo et al. 2009; Maeda et al. 2012). Takezawa et al. (1991) reported the difference of nutrient value of grass crops which has a potential to be used as the cleaning crop for the greenhouses with an excessive application of K_2SO_4, $CaCO_3$ and $MgSO_4$, such as Guinea grass (*Panicum maximum* Jacq.), sorghum (*Sorghum bicolor* L. Moench), sudangrass (*Sorghum* × *drummondi* (Nees ex. Steud.) Millsp. & Chase) and Rhodes grass (*Chloris gayana* Kunth). However, it is clear that there is little income from the production of cleaning crops because of no cropping for cash crops, especially in greenhouse.

Chicory (*Cichorium intybus* L.) is a Compositae crop, common in wider areas in western, central and southern Europe, north Africa and some parts of Asian countries, originally developed in Mediterranean regions (Ryder 1988). The genetic variation of this crop is relatively wider, with some unique types that varies in appearance, utilization and inherent chemical components (Lucchin et al. 2008). The witloof type chicory has been developed mainly in France, Belgium and The Netherlands from the 1980's, and it also started be to introduced as premium European winter vegetable in Japan from the 1990's (Sasaki 1990). The forage type has been developped energetically from the 1980's in New Zealand, and it will expand successfully as a new forage crop that can grow well under warm dry conditions (Rumball 1986; Barry 1998; Rumball et al. 2003).

Neel et al. (2002) reported that the forage type chicory clearly has the capacity to accumulate extraordinary high K concentrations under mineral-rich conditions, not just K. The authors presumed that probably chicory can be used as a remedy for salt accumulated soils, especially for K. If chicory could be used as a remedy for the K accumulated in soils, it may bring integral benefits to growers by the removal of K from such soils. Concurrently growers may obtain an agricultural income from the forcing culture by using roots that absorbed K from soils in the case the type used is witloof. Furthermore, possibly this technique can be one of the effective solutions for the K accumulation problem caused by consecutive application of organic fertilizers such as DS and livestock manure composts.

The purpose of the present chapter was to clarify the potential of witloof chicory to be used as a cleaning crop, which can remediate the K accumulated in soils and concurrently to obtain agricultural incomes. The research described here was an effort to examine the growth, K absorption capacity, and the change of soil chemical profiles during growing periods of two types of chicories, witloof and forage, comparing the system to the typical cleaning crop, i.e., Guinea grass, in the modelled salt accumulated field that were made by an excessive application of DS. In addition, the production of etiolated heads from the chicory roots obtained from the field after receiving an excess DS was conducted. Lastly, an experimental forcing culture of witloof chicory using roots that were obtained from the modelled salt accumulated field was conducted to examine the effect of excessive DS application during the root cultivation on yield and quality of etiolated heads.

MATERIALS AND METHODS

Experimental fields

Field experiments were conducted in the Experimental Farm, Field Science Center for Northern Biosphere, Hokkaido University, Sapporo, Japan (E141°20' N43°04').

Methane Fermentation Digestion Slurry (DS) application

The DS produced from the biogas plant for the treatment of dairy cow waste in Hokkaido University was used, and its chemical profiles are shown in the Table 4-1. The DS was applied into the experiment field mentioned below once in the first year (the second week of June, 2013) and twice in the second year (the second week of June and the first week of July, 2014). The amount of application in the first year was 200 t ha^{-1} and that in the second year

Table 4-1 Fertilizer components of digested slurry from the biogas plant in Hokkaido University (3 years average from 2006 until 2008).

Fertilizer components	TN	NH_4-N	P_2O_5	K_2O	Water Content	pH
Amount (mg/kg)	3,023	1,700	896	4,615		7.5
Concentration (%)	0.30%	0.17%	0.09%	0.46%	94.8	

was 533t ha^{-1}, for a total applied amount in the two years of 733 t ha^{-1} (Table 4-2). An injector of liquid fertilizer was used to prevent the runoff of the DS from the experimental plot (Fig. 4-1) (Iida et al. 2009). The total applied amount for two years of N, P_2O_5 and K_2O were 2,216 kg ha^{-1}, 657 kg ha^{-1} and 3,383 kg ha^{-1}, respectively. The average applied amount pf K_2O per year was approx. 1,100 kg ha^{-1}, and this was around 10 times higher than the conventional level for root production of witloof type chicory.

Plant materials

Witloof chicory 'Vintor' (Nunhems B.V., Nunhem, The Netherlands) (VI), forage chicory 'Puna Ⅱ' (PGG Wrightson Seeds Ltd., Christchurch, New Zealand) (P2) and Guinea grass 'Soil Clean' (Snow Brand Seed Co., Ltd., Sapporo, Japan) (GG) were used. The seeds of these three species were sown directly by hand in the modelled K-accumulated soil by excessive application of DS, two weeks after the DS application. Manual thinning of extra seedlings was conducted three weeks after sowing. The plant density of these three crops was set at the same level (0.1 m × 0.45 m, 222,222 plants ha^{-1}) after the thinning. The field experiment was conducted for two cropping seasons, i) June 2013 to November 2013 and ii) June 2014 to November 2014. Four plots per each plant material, VI, P2 and GG, with a Control (without growing any plant) were prepared, with a plot size of 5.0 m^2 (2.0 m × 2.5 m). The plots were arranged with four replications.

Sample collection during the open field experiment

Plant samples of each crop were collected three times, at 6, 12 and 18 weeks after sowing (WAS). Collected plant samples were separated into two parts, top and root shares, and each samples were washed thoroughly with tap water to remove adhering particles from the

Table 4-2 Actual application amount by fertilizer component.

Application Period	Total Amount (t/ha)	Application Amount (kg/ha)			
		TN	NH_4-N	P_2O_5	K_2O
1^{st} Year	200	604.6	340.0	179.2	923.0
2^{nd} Year	533	1,611.3	906.1	477.6	2,459.8
Total	**733**	**2,215.9**	**1,246.1**	**656.8**	**3,382.8**

Fig. 4-1 An injector which was used for methane fermentation digested slurry application (Left), the surface of the experimental field just after the DS application (Center) and the field shot on 23rd August, 2014 (42 days after sowing) (Right).

field. At each sampling, fresh weights of the top and root parts were measured separately by using 10 plant samples randomly selected from each plot. Samples were dried in an air-circulating oven at 60°C for 2 days, and then the dry weights were measured. The soil samples were collected from three different depths, 0.15 m, 0.3 m and 0.8 m from the surface, at the same time of plant sampling. An iron pipe, 50 mm in diameter, was inserted into soil for collecting soil samples from 0.3 m and 0.8 m depth, and all holes where the samples were collected were plugged carefully. Soil samples were collected from 5 different places in each plot and those were mixed to make one representative sample of each plot. The collected sample soils were sieved through a 2.0 mm mesh, and then dried 2 weeks at room temperature without exposure to direct sunshine. All samples, both plants and soils, were kept in sealed plastic bags within in refrigerator at 4°C, until analysis.

Forcing culture in witloof chicory

A forcing experiment was conducted to clarify the effect of excessive DS application during root production on the yield and quality of etiolated heads by comparing the roots that were grown under conventional condition. VI roots for Control were grown with conventional applications of chemical fertilizers (N - P_2O5 - K_2O = 100 kg ha^{-1} – 100 kg ha^{-1} – 100 kg ha^{-1}) at the above-mentioned experiment. The cultivation period was the same as the plants produced in the field with an excess application of DS (Treatment (DS)). The plot size was 1.35 m^2, and the matured roots of both Control and Treatment (DS) were collected 140 days after sowing in the second season. Collected plant samples were separated into two parts, top and root shares, and washed thoroughly with tap water to remove adhering particles from the field. After the roots were sorted and cut into 0.2 m lengths (from root shoulder to the bottom end), the fresh weight (FW) and other growth parameters of both top and root parts were measured and stored in refrigerator, with air temperature of 0.2 to 0.8°C and a RH of 100%, for 83 days until the start of the forcing culture (Fig. 4-2). The experiment was conducted with four replications.

Roots were transplanted into plastic containers (575×415×190 mm) which were filled with a medium used for the nursery production (Takii Tanemaki Baido, Takii Co., Ltd, Japan). All collected roots from all replications were planted into the plastic containers with plant density of 84.0 plants per m^2. To obtain etiolated heads, roots were grown under dark conditions (15.9°C in average, 100% of RH), with period irrigations. After the forcing culture, the etiolated heads were cut from the roots, and the outer leaves removed to obtain trimmed heads. The fresh weight (before and after trimming), height, diameter and flower stalk length of etiolated heads were measured at 22 days after starting the forcing culture.

Mineral concentration analysis and K uptake amount per plant

At each sampling in both field experiment and forcing culture, the obtained dried plant samples, top and root parts, were crashed and ground separately. For each experiment, samples of 10 plants that were collected from the same plot were mixed into one sample for mineral analysis. For analysing the concentration of K, Ca, Mg and P, approximately 100 mg of samples were inserted into individual metal-free polypropylene tubes. 1.3M HNO_3 (20-50 mL) was added to each tube,and tissues were digested at 60°C for 1 to 2 h. Obtained solutions were filtered and diluted with 0.1M HNO_3 and the mineral element concentrations were analysed by using ICP-AES (ICPE-9000, Shimadzu Corporation, Kyoto, Japan). Using the results of mineral concentration analysis of the plant dry matter, the K absorption amount per plant was calculated separately for both top and root parts.

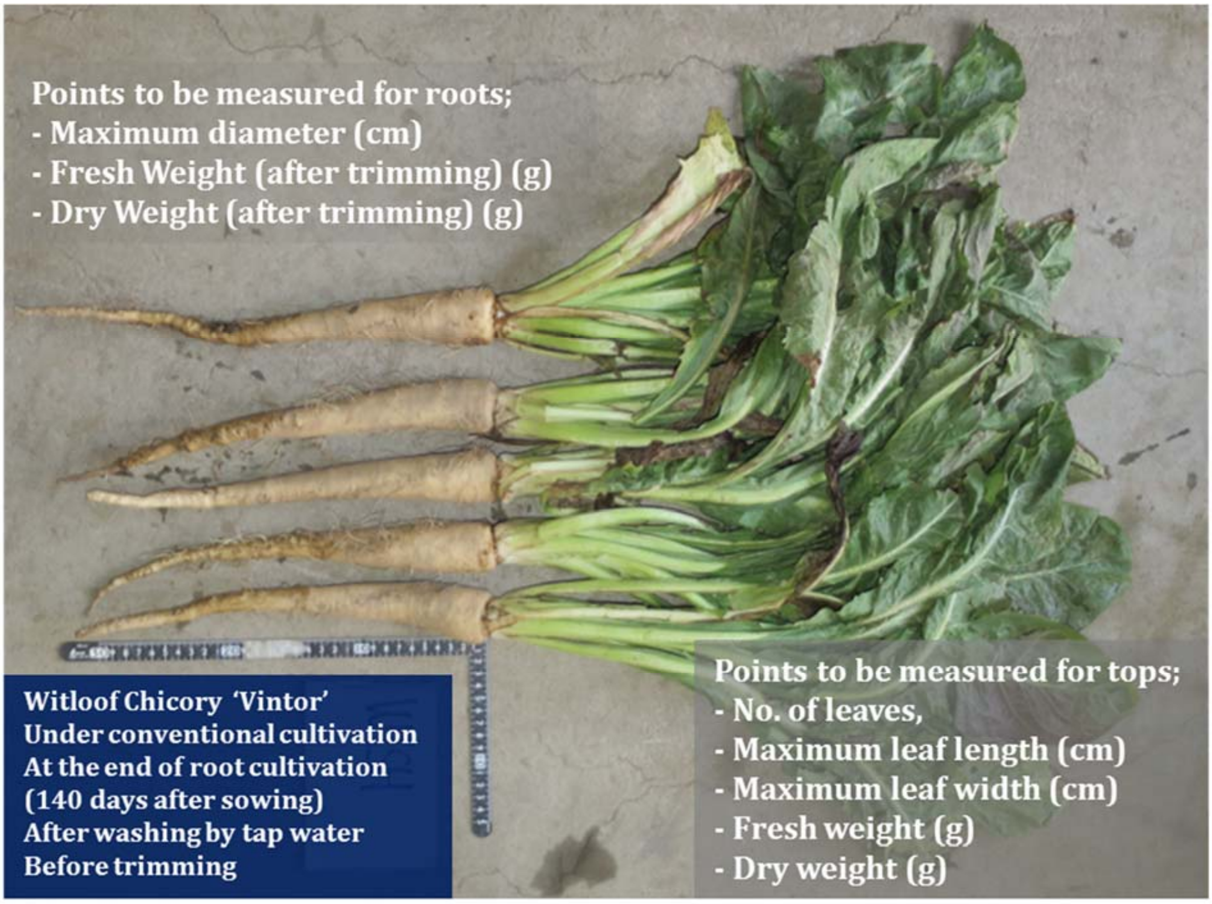

Fig. 4-2 Roots at the end of open field cultivation and the measurement items in both top and root.

Soil chemical profile analysis

Dried soils and distilled water were mixed in a ratio of 1:5, and then electrical conductivity (EC) was determined using EC meter (DM-37, Takemura Denki Seisakusho Co., Ltd., Tokyo, Japan). Chemical profiles of major fertilizer components such as K_2O of soils that were collected at each sampling were analysed by the conventional method, using commercially available integrated colorimeter system for soil analysis (ZA-Ⅱ, Fujihira Industry Co., Ltd., Tokyo, Japan).

Statistical analysis

Data obtained from each sampling were statistically analysed, and significant differences of the mean values were calculated using Tukey-HSD test and Welch's *t* test. For the plant growth comparison, under both pot culture and forcing culture, and the soil analysis, the values represented the mean of seven replications. For the plant dry matter mineral concentration analysis, the values represented the mean of five replications.

RESULTS

Climate condition during field experiment

The summary of climate conditions (10 days average air temperature and 10 days total precipitations) during each experiment period are shown in Fig. 4-3: 3rd July, 2013 to 9th November, 2013, 13th June, 2014 to 8th November, 2014, including Long Term Average (LTA) (30 years; from 1984 to 2014) was shown in Fig. 4-2. The precipitation in the experiment periods, especially in August and September was considerably larger than the LTA.

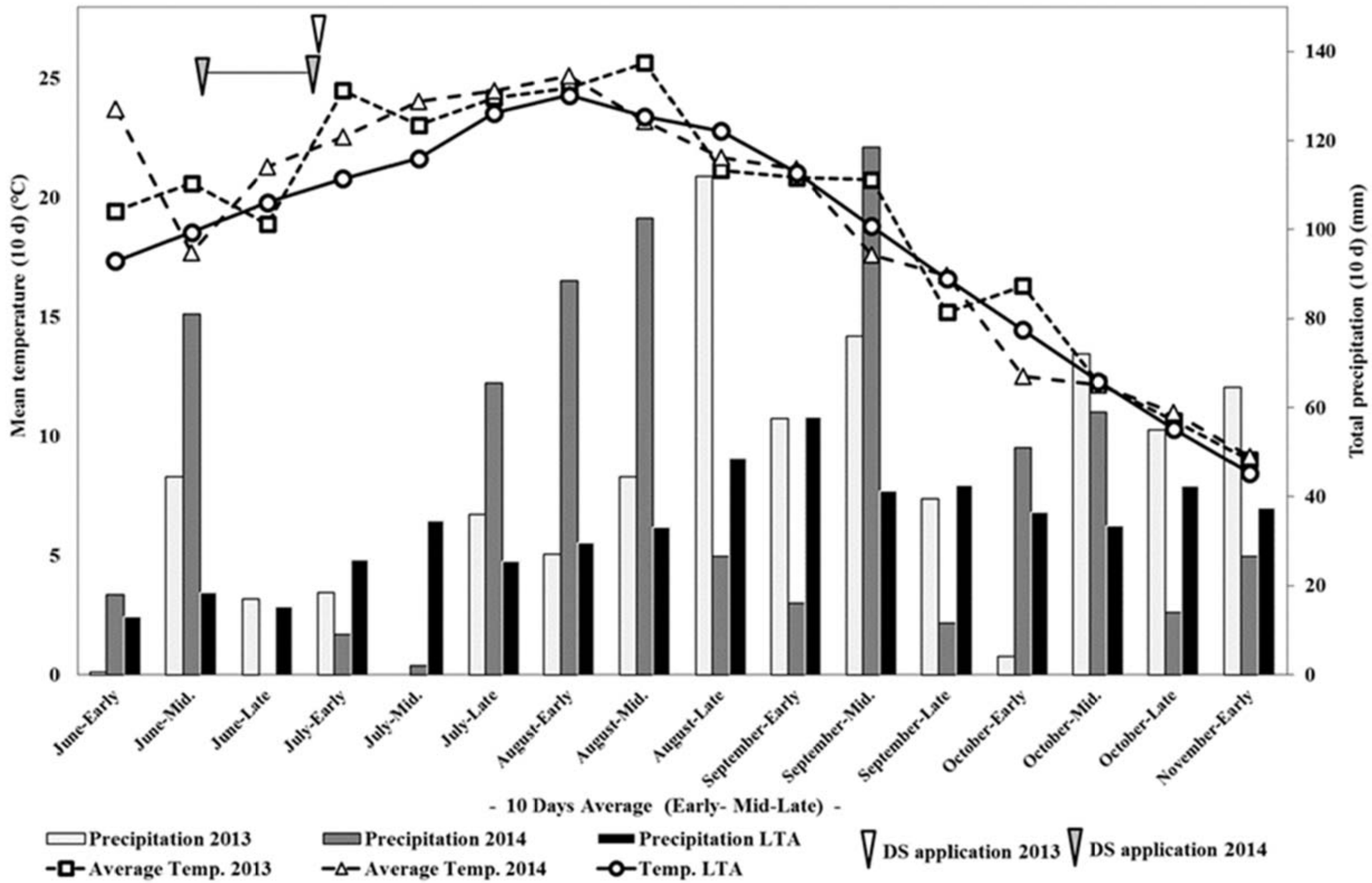

Fig. 4-3 Outline of average 10 days air temperature and 10 days total precipitation during the field experimental period (from 3rd July to 9th November in 2013 and from 13th June to 8th November in 2014) including Long Term Average (LTA) (from 1984 to 2014) (source: Japan meteorological Agency), DS; Methane Fermentation Digested Slurry.

Biomass production during field experiments

The top dry matter of GG at the end of the field experiments (18 WAS), in both years was always greater than in the two types of chicories (Fig. 4-4). The top dry matter of GG at 18 WAS in the second year decreased approx. 50% comparing to that of 18 WAS in the first year. However, a significant difference between the first year and the second year in root dry matter of GG at 18 WAS was not found. In contrast, the root dry matter of VI at 18 WAS in the second year (28.3 g //plant) was significantly larger than that in the first year (20.0g) (p<0.01). There were no significant differences in top dry matters at 12 WAS and 18 WAS in VI between the years of experiments. In each sampling and among the years of experiments, there were no significant differences in P2 plant dry matter, both in top and root parts.

Mineral concentration on plant dry matter during field experiment

The results clearly showed that the K concentration of the two types of chicories in both top and root parts is significantly greater than that of GG at all samplings time throughout the experimental periods (p<0.05) in the second year (Fig. 4-5 (a)). At 18 WAS in the second year, the top dry matter K concentration in VI was the greatest (8.92%), 2.4 times higher than that of GG's (3.74%) and 1.2 times higher than that of P2's (7.27%). The root dry matter of GG was significantly smaller than that of the two types of chicories (p<0.05). The same trends in the results of the first year were observed in the second year.

The top Ca concentrations of P2 tended to be the greatest at every sampling, except 12 WAS of the second year, and that of GG were always significantly smaller than that of two types of chicories (p<0.05) (Fig. 4-5 (b)). The root dry matter Ca concentration of VI was significantly smaller than GG at 18 WAS in the second year (p<0.05). The dry matter Mg concentration in both top and root parts of GG was significantly larger than that of VI and P2 throughout the growing period in the second year (p<0.05) (Fig.4-5(c)). No significant

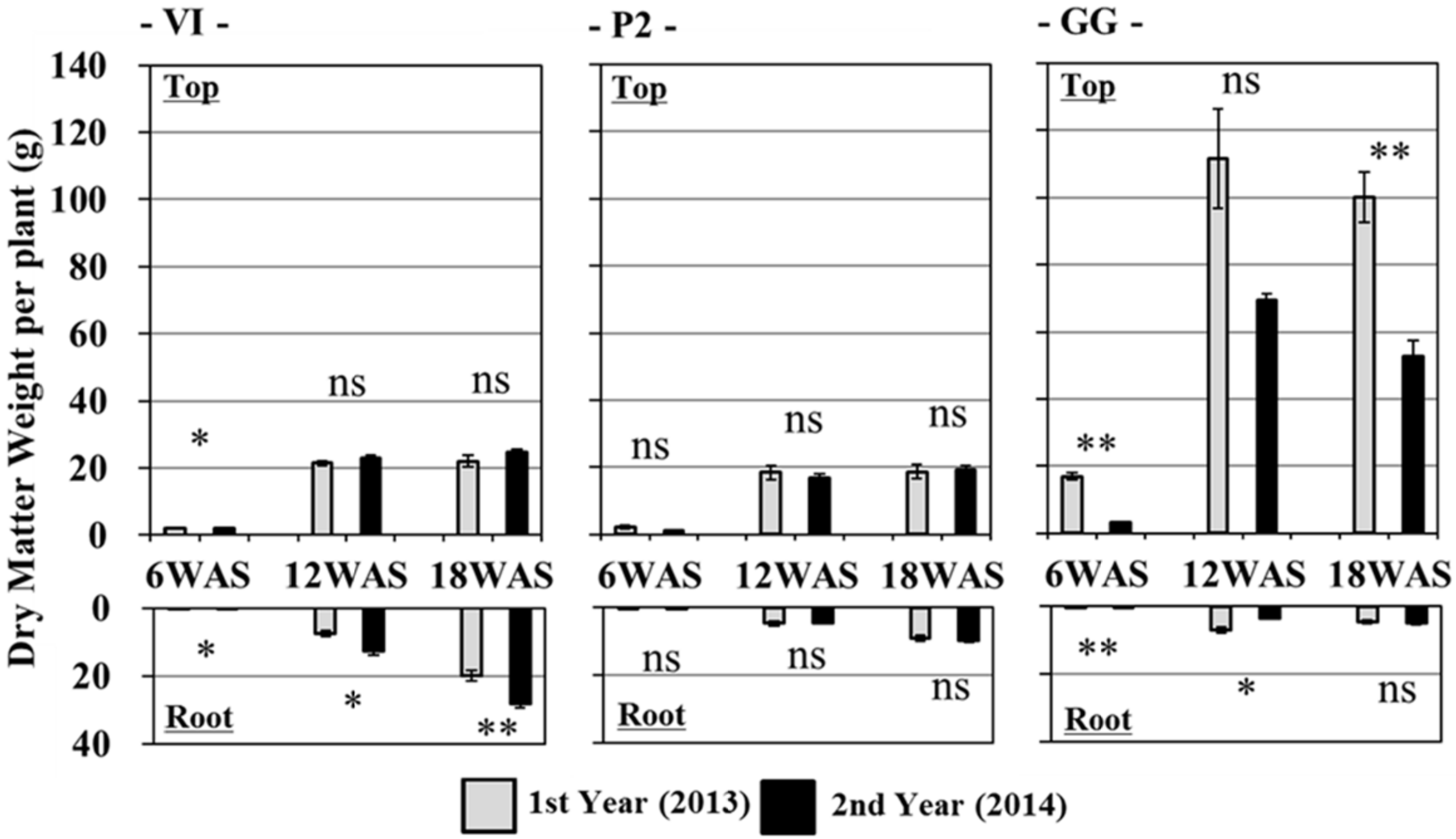

Fig. 4-4 Comparison of plant dry matter weights between the first year and the second year, by plant type. The bars represent a mean of 4 replications ± SE. The bars with different letters are not same by Welch's *t* test (**; $p<0.01$, *; $p<0.05$, ns; not significant). WAS; Weeks after sowing.

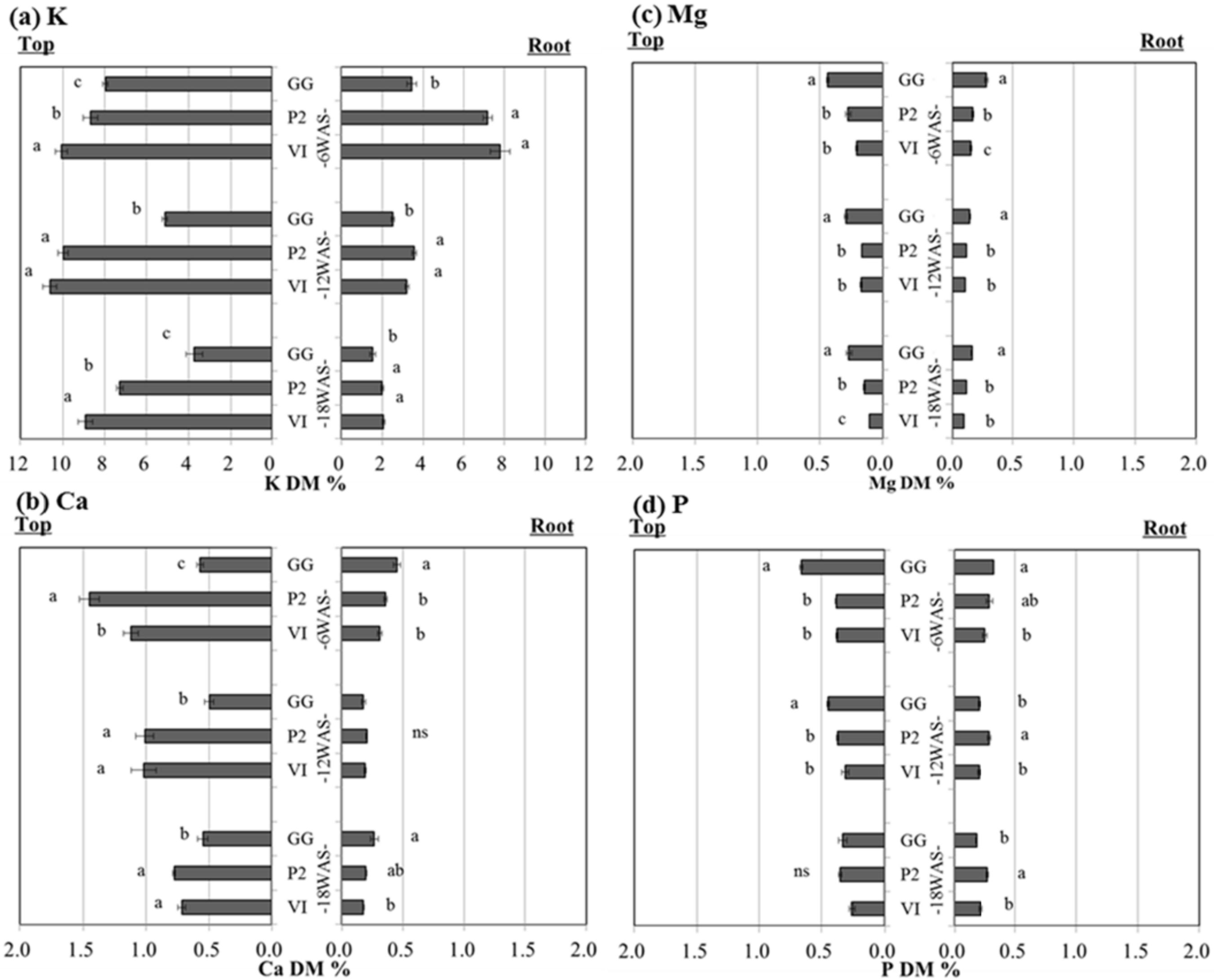

Fig. 4-5 Effect of excessive application of methane fermentation digested slurry on chemical profiles of plant dry matter at 6 WAS, 12 WAS and 18 WAS in the second experiment year. The bars represent a mean of 4 replications ± SE. The bars with different letters are not same by Tukey-HSD test at 5% level (p<0.05). ns; not significant. WAS; Weeks after sowing.

differences in the top dry matter P concentrations were observed among plants at 18 WAS in the second year; in addition, there was no significant difference between VI and GG in the root dry matter P concentration at 18WAS in the second year (Fig. 4-5 (d)).

K absorption amount per plant

The estimation of the plant total K absorption amount of GG (3.96 g) in the first year was significantly greater than that of the two types of chicories (VI; 2.22 g, P2; 1.63 g) ($p<0.05$). However, that of VI in the second year (2.87g) was significantly greater than that of P2 (1.60g) and GG (2.02g) (Table 4-3). In the second year, the top K absorption amount of P2 (1.41 g) was significantly smaller than of VI (2.20 g) and of GG (1.95 g) ($p<0.05$). However, VI (0.58 g) had the greatest in root K absorption amount, and significant differences were observed comparing P2 (0.19g) and GG (0.07g) ($p<0.05$).

Soil chemical profile

The chemical profile analysis results at the starting of the first year of experiment is shown in Table 4-4. With regard to the EC level during the field experiment, at the end of the first year, Control (26.4 mS m^{-1}) was significantly greater than VI (16.0 mS m^{-1}) and GG (16.4 mS m^{-1}) at 0.8m depth. At the end of the second year, at 0.3m depth and 0.8m depth, the EC in Control were greater than the other treatments, although no significant differences were observed among them at 0.15m depth (Fig. 4-6(a)).

The K_2O concentrations of VI at the end of the first year of the experiment were significantly smaller than of the Control in all layers (0.15 m, 0.3 m and 0.8 m) ($p<0.05$), and only in VI (107.0 mg per 100g soil) they were significantly smaller than in the Control at 0.15m depth (83.7 mg per 100g soil) ($p<0.05$). However, at the end of the second year, no significant differences in all layers and, among treatments were found (Fig. 4-6(b)).

Table 4-3 K accumulation per plant in Chicory and Guinea Grass.

Year	Crops	K accumulation amount^{z} (g /plant) Top	Root	Plant Total
First Year	VI	1.80 b^{y}	0.42 a	2.22 b
	P2	1.46 b	0.18 b	1.63 b
	GG	3.85 a	0.11 b	3.96 a
Second Year	VI	2.20 a	0.58 a	2.78 a
	P2	1.41 b	0.19 b	1.60 b
	GG	1.95 a	0.07 c	2.02 b

z K Accumulation amount = K concentration (%) × Dry matter weight.
y Different letters indicate significant differences among treatments by Tukey's test (n=4) at 5% level.
x Sampling date; 9 Nov., 2013, 8 Nov., 2014

Table 4-4 The soil chemical profiles before the starting of field experiments (25th May, 2013).

Soil Type	Depth (m)	pH (H_2O)	EC ($mS\ m^{-1}$)	Cation Exchange Capacity (me/100g)	Humus (%)	Phosphate Absorption Coefficient	T-N (%)	T-C (%)	NH_4-N (mg/100g)	NO_3-N (mg/100g)	P_2O_5 (mg/100g)	K_2O (mg/100g)	MgO (mg/100g)	CaO (mg/100g)
	0.15	5.72	10.6	22.7	7.19	1094.8	0.208	3.28	0.43	1.26	130.5	76.6	38.2	302
Andisols	**0.3**	5.76	8.6	24.8	6.74	1087.0	0.197	3.02	0.91	0.83	123.0	63.9	38.8	303
	0.8	6.10	5.8	16.4	1.67	877.2	0.073	0.44	0.25	0.38	13.7	58.3	46.6	269

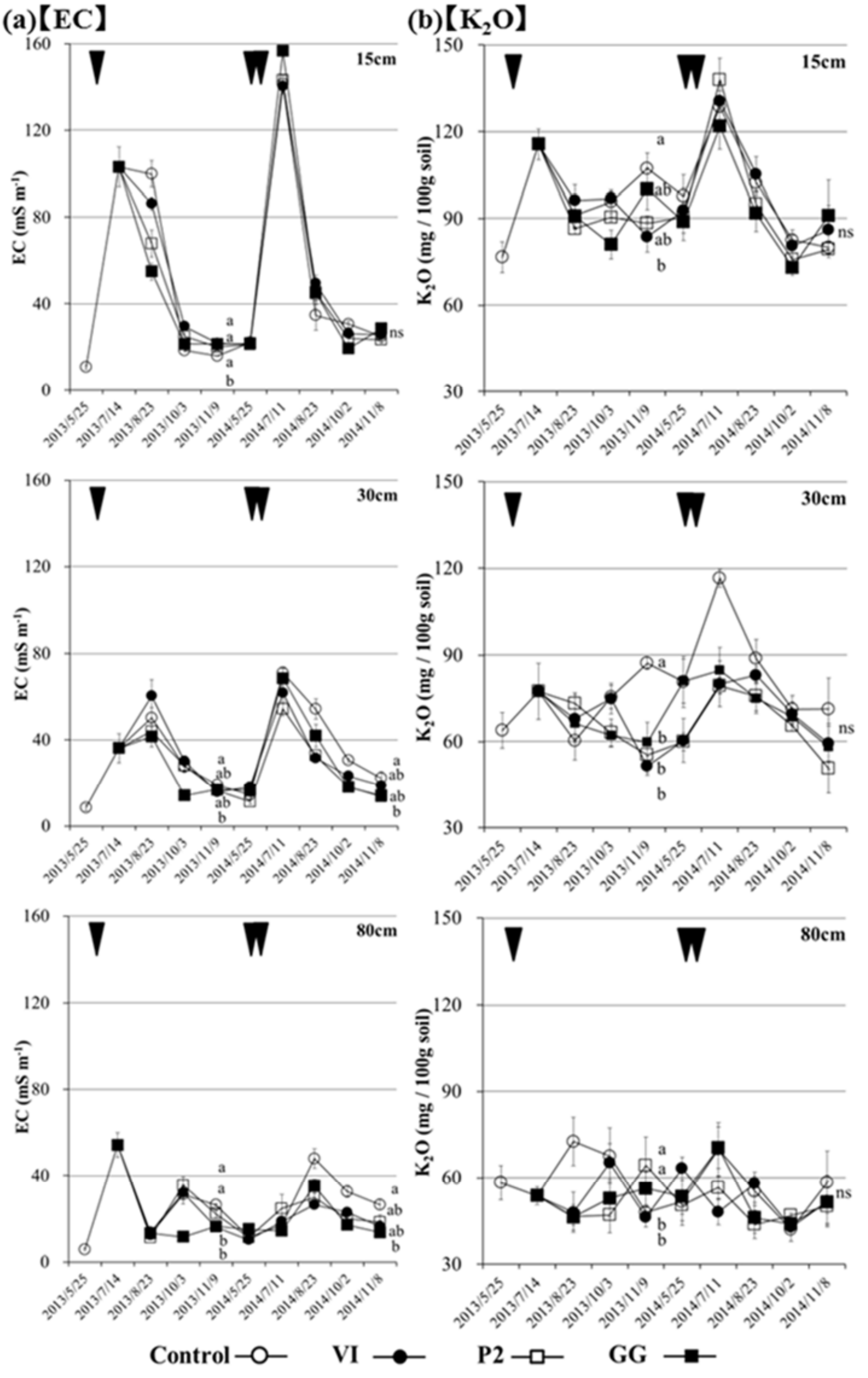

Fig. 4-6 The transition of soil chemical profiles. The marks represent a mean of 4 replications ±SE. The symbol marks with different letters are not same by Tukey-HSD test at 5% level (ns; not significant). VI; Witloof chicory 'Vintor', P2; Forage chicory 'PunaII', GG; Guinea grass 'Soil clean'.

Forcing culture of witloof chicory

The number of leaves in Treatment (DS) was significantly larger than Control ($p<0.05$) (Table 4-5). However, there was no significant difference in the top FW and root FW before starting the forcing culture between Treatment (DS) and Control. There was no significant difference in etiolated head FW before trimming between control and Treatment (DS) (Table 4-6). However, the FW of trimmed etiolated heads in Control was significantly greater than that of Treatment (DS) ($p<0.05$) and consequently the ratio of etiolated head FW after/before trimming in Control was significantly larger than that of Treatment (DS) ($p<0.01$). Table 4-6 also reveals that there were no significant differences in size of etiolated heads, height and diameter, and core length among treatments.

DISCUSSIONS

Effect of excessive application of DS on plant growth of witloof chicory

No significant decreases in the plant total biomass comparing the end of the first year and the end of the second year was found between two types chicories. In addition, the root biomass of witloof type increased approx. 41.5% from the first year to the second year (1^{st} year (18WAS): 20.0 g per plant; 2nd year (18 WAS): 28.3g per plant) (Fig. 4-4). Such results are highly suggestive of hitherto the tolerance of two types of chicory, both witloof and forage types, against nutrient-rich conditions made by the excessive application of DS to the soils, more than 10 times higher than the normal application. Those facts can be interpreted as there is a fair chance for witloof chicory to obtain matured roots that can be utilized for forcing culture in nutrient-rich conditions which are made by an excessive continuous application of organic fertilizers.

Table 4-5 Effect of excessive application of methane fermentation digested slurry on growth parameters of chicory roots.

Treatment	Top			Root[z]	
	Number of Leaves	Leaf length (cm)	Top FW (g)	Diameter (cm)	FW (g)
Control (Conventional)	9.4±0.4 [y]	55.3±3.7	110.4±21.1	3.5±0.1	128.1±6.4
Treatment (DS)	10.9±0.3	60.4±1.5	144.9±8.7	3.5±0.1	111.2±11.8
t test [w]	*	ns	ns	ns	ns

Roots were trimmed into 0.2m length (shoulder to the bottom end)

[y] n=4, mean ± SE

[x] ns, **, * indicate not significant, significant at p<0.01 and p<0.05, respectively

Table 4-6 Effect of excessive application of methane fermentation digested slurry on yield and quality of etiolated heads.

Treatment	Before Trimming	After Trimming				Etiolated Head Quality Index	
	Fresh Weight (g/plant)	Fresh Weight (g/plant)	Height (cm)	Diameter (cm)	Core Length (cm)	FW Ratio (After/Before Trimming)	Core Ratio (Length/Height) (%)
Control (Conventional)	211.5±8.9	183.6±4.6	18.6±0.5	6.5±0.3	1.1±0.2	0.88±0.03	6.0±1.0
Treatment (DS)	176.8±13.5	129.8±21.2	18.7±0.4	6.2±0.3	1.1±0.0	0.70±0.07	5.7±0.1
t test [w]	ns	*	ns	ns	ns	**	ns

[z] Period; 21 Feb., 2015- 15 Mar., 2015 (22 days)
[y] After storage in refrigerator
[x] n=4, mean ± SE
[w] ns, **, * indicate not significant, significant at p<0.01 and p<0.05, respectively

Sergio et al. (2012) investigated the salinity tolerance of chicory. They found that chicory is able to germinate and grow further in saline conditions, and they suggested that this unique characteristic maybe highly relate to its antioxidative responses. Arshi et al. (2006) reported that a $CaCl_2$ application is probably able to reduce the negative effects of a NaCl application on plant growth of chicory. In the future, the mechanism of chicory's tolerance against salinity or nutrient-rich conditions also needs to be discussed in more details.

K Absorption capacity of witloof chicory

Throughout the present study, the total K absorption amount by VI plant under nutrient-rich condition was larger than that by GG, which is widely known as a practical solution for removing salts from salt accumulated soils (Table 4-3). At the end of the second season, the plant total K absorption amount of VI was approx. 37.6% larger than that of GG, and 73.8% larger than that of forage type chicory (P2). Such results reveal that witloof chicory is a good candidate to absorb excessive K from K accumulated soils.

Neel et al. (2002) investigated the salinity stress tolerance of chicory, and reported that this crop can be used as a nutrient mop in fields where excess soil nutrients are a problem. Throughout their experiments, the K concentration in the dry matter of chicory plant was ranging from 14.0% to 15.9% when the nutrient solution containing $Ca(NO_3)_2$, NH_4NO_3, KH_2PO_4, KNO_3 and $MgSO_4$ was applied excessively (the range of ionic strength was 90 mS m^{-1} to 1,200 mS m^{-1}). The plant dry matter decreased linearly as ionic strength of soils increased, however, Neel's experiment focused only on forage type chicory. The study presented in this thesis investigated the capacity of the K absorption for two types of chicories, both witloof type and forage type, and the obtained results suggested that nutrient-rich conditions made by a continuous excessive DS application may cause an increase of plant dry matter K concentration of chicory. However, a negative influence was also observed: a different reaction occurred according to the type of crop. In fact, in VI, the plant total K absorption amount increased from the first year to the second year, but that of P2 decreased. Rengel and Damon (2008) assessed the relationship between genotypes and efficiency of K uptake and use in various crops, and they suggested that the relationship might be influenced by the morphological differences in the root system. Applying their approach in future studies would give better insights into the difference in the K absorption capacity among the two types of chicories.

The change of soil chemical profiles during the field experiment

As it can be seen from Fig. 4-6(b), the K_2O concentration at the end of the first year in VI was the lowest among the treatment, and VI was also the only treatment that had significant differences from the Control both at 0.15m and 0.3m depths. Based on the fact that the K absorption amount per plant in VI was significantly the greatest (p<0.05), it may be presumed that the soil K_2O concentration of VI at the end of the second year was also the lowest, even though significant differences in the soil K_2O concentration among treatments at the end of the second year could not be observed. The seasonal total precipitation (from the first week of July to the first week of November) in the first year was 594.0 mm and that in the second year was 581.5 mm. These values are greater (28.4% and 25.6%, respectively) than those of the long term average (same period, 30 years; from 1984 to 2014) (Fig. 4-3). It is probably reasonable to suppose that the soil K_2O in the experiment field has been reduced by leaching caused by a relatively large precipitation during the experimental period.

The Effect of Excessive DS Application during Root Production on the Yield and Quality of Etiolated Head

There were no significant differences in the top FW and root FW between Control and Treatment (DS) before the forcing culture (Table 4-5). Furthermore, there were no significant differences in the FW of etiolated heads, with the outer leaves, which were obtained after the forcing culture as shown in Table 4-6. However, the FW of trimmed etiolated heads, without outer leaves, differed significantly among treatments ($p<0.05$); FW in Control was approx. 41% greater than that in Treatment (DS) (Control; 183.6 g per plant, Treatment (DS); 129.8 g per plant).

The fresh weight of marketable etiolated heads is usually more than 120g; the FW obtained in etiolated heads from the roots grown in the field with excessive DS application was recognized as marketable. However, an excessive application of DS in the root production stage (366.5 t ha^{-1} in yearly average) has a significant effect on the quality of the etiolated head. After the forcing culture, the outer leaves of the etiolated head in Treatment (DS) were relatively opened and twisted, and sometimes they developed an internal tip burn (Fig. 4-7). Zamaniyan et al. (2012) reported that an increase of K:Ca ratio in the nutrient supply during root production may cause physiological disorders of the etiolated heads such as brown pith, hole pith and tip burn. In present study, in the VI plot, the K_2O concentrations in the shallow layers of soils increased before the second season, however, that of CaO decreased 9.5 - 13.0 % in 0.15 and 0.3m depth soil (Data not shown). Zamabiyam et al. (2012) also reported that there is a possibility to change the K:Ca ratio into unsuitable conditions. It is likely to say that the main reasons for the low marketable ratio and the low quality of etiolated head in Treatment (DS) are probably the increase of K:Ca ratio in the soils during the root production by an excessive DS application.

CONCLUSIONS

A major goal of this research has been to investigate the potential of witloof chicory to be used as a cleaning crop which can remediate the K accumulated in soils and concurrently to obtain agricultural incomes. The results from the present study demonstrated that; 1) the

Fig. 4-7 Forcing chamber with heat insulators and heat exchangers (Upper), the Roots with etiolated heads at the end of forcing experiment (22days after starting) (Middle) and the etiolated heads after trimming, C; Control, T; Treatment (DS) (Bottom).

witloof chicory has enough tolerance against nutrient-rich conditions, especially those of high K_2O accumulations, caused by an excessive application of DS (733t ha^{-1} in 2 years total); 2) the potential of witloof chicory in the K absorption from the K accumulated in soils is relatively high, and its K absorption capacity is approx. 37.6% larger than existing cleaning crops such as Guinea grass; and 3) the negative impacts of nutrient-rich conditions during root production on the yield and quality of edible part (etiolated heads) cannot be ignored completely but it is still commercially valuable.

CHAPTER 5

GENERAL DISCUSSION

1. The potential of witloof chicory for stimulating utilization of recovered heat from composting livestock wastes as a heat source

1-1. Pilot experiment to clarify the potential of fermentation heat of cow manure as a heat source for witloof chicory forcing culture

Composting heat is generated in the process of decomposition of cow manure. However, such heat has not been used in the greenhouse production because of the small amount of the heat energy and the difficulty in recovering and transferring of such heat to the necessary places.

In the present study, with a simple recovering system of composting heat from cow manure, the composting heat was introduced into forcing culture box (bed) for chicory and marketable etiolated heads (chicon) were obtained in cold season, winter and early spring, without oil heating.

The most important reason of the success for the etiolated head production with composting heat was the environmental conditions for the leaf emergence in chicory. Usually, chicory root emerges the leaves at around 15°C under dark and with high humidity, almost 100% RH. Such environment was established in an enclosed space that was easy to set up. In addition, only 2 or 3 weeks to emerge etiolated head after planting were necessary. Thus, a large amount of heat energy was not required to reach the etiolated heads. Requiring a small amount of necessary heat energy is supposed to be a good advantage for the production system of horticultural crops with low fossil carbon. The present technique will be applied into the crops that grow under not-high temperature and no sunshine, such as for instance the Japanese

Udo (*Aralia cordata*), yellow chinese chive (*Allium tuberosum*), white asparagus (*Asparagus officinalis*) and so on.

Furthermore, some improvement was necessary to conduct the composting system in the material and the system.

First, the rapid composting. Compost was produced with cow manure through strong solid-liquid separating system and the water content was around 75% in the compost. The temperature inside the compost increased rapidly in such water content (Yamashita et al, 2014) and the inside temperature of the compost reached more than 40°C about 5 days after setting the experiment place.

Second, the restriction of heat loss from the heating chamber and heat exchanger. Zhao (2015) gave details on the application of various heat recovery methods from a compost process of solid wastes, including livestock wastes. There are also studies focusing on the development of an efficient heat recovery system, which are adoptable to live stock wastes (Seki and Komori 1983, 1984, 1985, 1986, 1995; Bari and Koenig 2001; Irvine et al. 2010). Seki and Komori (1992) studied the advantages of the enclosed vertical type composting facility. Such facility can recover the heat from the humid hot air that can be collected in its top part. The authors compared this method to the conventional methods, such as the exchanging the heat by heat condensers that are embedded into the squared flat compost chamber, and they emphasized the importance of the heat loss prevention from the facility for an efficient heat recovery.

Third, the room temperature for setting the forcing chamber. Because of the simple equipment used in the present study, the temperature inside the forcing chamber was affected by the room temperature even if heat insulator boards were attached at top, bottom and side of the chamber. In the case of chicory, more than 5°C are needed for warming at around 15°C the chamber in winter. There are some methods for warming 5°C inside the forcing room, and terrestrial heat is one of the renewable ways. Yamakawa et al (2013) reported the terrestrial heat recovery system is of great utility for improving the stability of the air temperature in the forcing chamber in warm seasons.

In the thesis, air circulating system was performed between the forcing room and underground (2m depth) in the semi-cold experiment in a research facility for development of melon production in Yubari city, Hokkaido. However, such a system was not tested in the winter experiment in the school facility that was used for the all experiments reported in the present thesis. The minimum temperature is about 5°C at 3 m depth during winter in the experimental location, Yubari city (not shown). The room temperature would be maintained at around 5°C if the circulating system were established between the forcing room and the underground, at 3 m of depth.

1-2. Economic value of heat energy used for forcing culture

Based on the results from the second experiment reported in Chapter 2 (year 2014), the economic value of the heat energy used for heating the forcing chambers was estimated based on the hypothesis that the temperature in the forcing chamber could be managed by an electric hot wire (No Den cable, Nihon Noden Inc., Tokyo, Japan), exactly the same as if it were realized by using the fermentation heat of a cow manure compost. The required electric energy can be estimated based on the formula (Nippon Noden Inc. 2005) below mentioned with the temperature difference between Heated and Control in the second experiment in Chapter 2 (Fig. 2-5).

$$\text{Required Electric Energy (kW)} = \{\text{Space } (m^2) \div 3.30578 \times 40 \times \text{Temperature Difference } (^\circ C)\} \div 860$$

The space of the forcing chamber was approximately 2.7 m^2, and the average temperature difference during the experiment between Heated and Control was 10.2°C. Based on the above mentioned formula, the calculated required electric energy per hour in average was 0.386kW; the total required electric energy throughout the experiment period can be estimated as 174.8kW. Based on the electricity rate structure of the Hokkaido Electric Power Co. Inc. (December, 2016), the estimated total costs that can be required to obtain the same temperature trend as in the second experiment in Chapter 2 was 5,457.9 Japanese Yen (JPY), including basic charge (1,000.4 JPY, Charge for the first stage; 2,824.8 JPY, Charge for the second stage; 1,628.7 JPY, excluding surcharges for fuel and renewable energy promotion). It seems reasonable to conclude that 5,457.9 JPY is the estimated economic value of the heat energy that could make use of the heating for the forcing chamber in the second experiment in Chapter 2.

2. The potential of witloof chicory for enhancing the utilization of biomass resources derived from livestock wastes as fertilizers

2-1. Pilot experiments to clarify the potential of witloof chicory as a remedy for salt accumulated soils, especially for K accumulation

Chapter 3 described the result of an experiment designed to collect the basic information on the growth potential of witloof chicory and its K absorption capacity under a high-K stressful condition.

The pot experiment was conducted in a greenhouse, and K_2O was applied at an excessive level (0 to 5,000 kg ha^{-1}) by using chemical fertilizer. As the K_2O application increased, the plant total biomass increased significantly in Guinea grass species, a typical plant used for salt removing from the soil. The biomass of both top and root parts of the plant tended to decrease in witloof chicory (Fig. 3-1). However, the root biomass of witloof chicory was always greater than that of Guinea grass, indicating that witloof chicory has the potential to grow under a high K stressful condition. It became clear that the K-uptake amount per plant of witloof chicory was kept at about 45.8 to 73.9% compared to that of Guinea grass in K_2O treatments higher than 200 kg ha^{-1}. The calculation of the estimated K-uptake amount per ha in witloof chicory based on the practical plant density was more than 3 times higher than Guinea grass. The marketable etiolated heads that were obtained from chicory roots grown at K_2O application were less than 2,000 kg ha^{-1}.

Chapter 4 showed the results of an outdoor experiment designed to investigate the growth potential of witloof chicory under a nutrient-rich stressful condition, made by an excessive application of a methane fermentation digested slurry. To evaluate the potential for remedy of K accumulated soils among witloof chicory, forage chicory and Guinea grass were used. The K-uptake amount per plant of witloof chicory was the highest, 37.6% greater than that of Guinea grass, and 73.8% greater than that of forage chicory. In addition, the negative influences of an excessive methane fermentation digested slurry application on the etiolated heads was small.

One of the main problems to expand the utilization of organic fertilizers, such as methane fermentation digested slurry, is the accumulation of K in the soils when it is applied based on the amount of nitrogen supplied. From the experiments in Chapter 3 and Chapter 4, several points were clarified namely; i) the witloof chicory can be utilized for the K removal from salt accumulated soils; ii) witloof chicory has a practical tolerance against stressful conditions with nutrition-rich soils especially for K; and iii) the negative impact by nutrition-rich soil conditions on the yield and quality of etiolated heads obtained after forcing culture of roots is limited. These results are highly suggestive and stimulate the utilization of organic fertilizers for growing the witloof chicory by assisting the maintenance of the balance in soil fertilizer components, concurrently with deriving an income for the growers through the etiolated head production.

2-2. The K absorption capacity of witloof chicory

From the results of the experiment described in Chapter 4, it became clear that the K absorption amount of witloof chicory is larger than that of the typical cleaning crop, the Guinea grass. Furthermore, the results from the experiment in Chapter 3 validated the practicability of witloof chicory as a K scavenger for the K accumulated in soils. From what it has been discussed above, it can be concluded that this unique function of witloof chicory can be counted on as a practical solution to overcome one of the impediments to make the best use of organic fertilizers, that is, the accumulation of K in the soils.

At the same time, it must be recognized that there were large differences in the biomass production, especially in the root part, and in the K absorption capacity under high-nutrient stressful conditions among two different types of the same crop, namely in the witloof type and the forage type (Fig. 3-1, Fig. 4-3, Table, 3-4, Table 4-3). The results mentioned above are of great interest, because they indicate that the morphological differences, especially in the root part, is one of the main reasons for the differences. In fact, as shown in the Fig. 5-1, witloof chicory has a vertically long, straight shape of taproot, while the forage chicory has a strongly branched, horizontally wider root. Theoretically, the surface area of the branched root system can be wider than that of the root system with a smooth straight shape.

Cassan et al. (2008) suggested that the nitrogen use efficiency of witloof chicory during the root production could be genotype dependent. The authors also pointed out that further breeding effort may enlarge the genetic variability of the species for the nitrogen metabolism and it can expand the geographical adoptability of this crop. Chicory is present with many types in appearance and growth characteristic; each type has different morphological characteristics, production cycles and destinations.

Root chicory is one of them. Root chicory has been used for extracting fructans such as inulin and oligofructose or producing coffee substitute, and current commercially available

Fig. 5-1 Plant samples at the end of field experiment in November, 2014. Gunia Grass (Left), Witloof Chicory (Center) and Forage Chicory (Right).

fructans are mostly supplied by the root chicory, which are mainly grown and processed in north part of Europe and also in France and North Italy (Frese et al. 1991; Baert and Van Bockstaele 1993; Bais and Ravishanker 2001). According to a report from Lucchin et al. (2008), some recent studies have investigated to clarify whether crossing the root chicory with leaf chicory can enhance the genetic basis of root chicory breeding or not. The authors concluded that leaf chicory might not contribute to a speedy progress in root chicory; however, there has been no specific study aimed to improve the biomass production performance or the K absorption capacity of witloof chicory obtained by crossing other segments of this crop.

Barcaccia et al. (2016) reported that the thickness and the length of the main root are one of the main traits for Radicchio breeding. This is because relatively long periods of root storage is required, 2 or 3 months, for the Radicchio production, or even for crossing or being used for seed production. Wang and Wu (2015) reported that i) the K Utilization Efficiency (KUE) of plants is highly dependent on the K uptake capacity of the roots, ii) the KUE of the plants is dependent on the K transport and translocation capacity in plant tissues and organs. The authors emphasized the importance of optimizing the plant root architecture for improving KUE. They also stressed that the larger root volume increases the root surface area, which may significantly enhance the nutrient absorption, and the keys for improvements are increasing the length and the density of lateral roots and root hairs. Rengel and Damon (2008) suggested that increasing the surface area of contact between roots and soil is the key to improve K absorption capacity. K-efficient genotypes could have a relatively larger portion of thin roots in the whole root system compared with K-inefficient genotypes. Lastly, the authors also suggested the importance of the quantity and of the structure of root hairs in contributing to the K uptake capacity, by the enlargement of root surface area. Theoretically, the surface area of the branched root system can be wider than that of the root system with smooth straight shape; however, the result obtained from the experiment in Chapter 4 differs from this hypothesis.

Further research on broadening the morphological variations of witloof chicory would be helpful to understand the reason for the differences in the K absorption capacity among two types, witloof and forage, and this will also be supportive to enhance the potential of witloof chicory to stimulate the general application of organic fertilizer.

2-3. The potential of witloof chicory as a cleaning crop or a catch crop

Thorup-Kristensen et al. (2003) mentioned that the effect of a catch crop can depend strongly on the species chosen. They also suggested some points to be considered when a species should be chosen as a catch crop, namely; i) the speed of the establishment and growth rate; ii) the rooting depth; iii) the kill date (incorporation date); iv) the fixing capacity of nutritional components; and v) the chemical component of the plant material (e.g. C/N ratio, lignin contents and contents of water-soluble compounds).

Through the experiment described in Chapter 4, the difference in biomass productivity among witloof chicory, forage chicory and Guinea grass was investigated (Fig. 4-3). It became clear that witloof chicory can grow without any problems with sowing in spring, growing under a subarctic wet climate condition (Sapporo city, Hokkaido, Japan). The K fixing capacity (= absorption capacity) in witloof chicory per plant was also conducted at the practical level in the experiments in Chapter 3 and Chapter 4 (Table 3-4, Table 4-3). However, the results of the detailed analysis related to the plant dry matter concentrations of major elements at the end of the pot experiments with an excessive K application clearly indicated that it is not preferable to use the plant dry matter, which can be obtained after cropping for K-removal from soils, as green manure (Table 3-3). Despite this, still witloof chicory has numerous advantages as a winter cash crop in cold regions; it can also be used as a remedy for K accumulated soils.

Pirhofer-Walzl et al. (2013) investigated the N accessibility in different soil layers; the forage type chicory ‘Puna II ’ was confirmed as a crop with deep-rooting characteristics in terms of soil ^{15}N-access, and it seemed that it could reach to 1.2 m depth. In the experiment described in Chapter 4, the soil K_2O concentration at the end of the 1st year of cultivation was lower in witloof chicory than in forage chicory and Guinea grass at the all layers, from 0.15 m to 0.8 m (Fig. 4-5). On the basis of the above facts, there is a considerable validity in the prediction that witloof chicory has a relatively deep root system which is required as a cleaning crop.

2-4. The effects of high-nutrition condition, especially for the stressful condition with excessive K, during root production on root yield and the quality of etiolated head

In the present study, the roots of witloof chicory were grown with different level of K application amounts by using only chemical fertilizers (in the pot experiment in Chapter 3) or together with other fertilizer components, by an excessive application of methane fermentation digested slurry derived from cow manure (in the open field experiment in Chapter 4).

In the pot experiment described in Chapter 3, the biomass of both top and root parts was decreased when the application amount of K_2O increased and the application amounts of N and P_2O_5 were kept at same level for all treatments (Fig. 3-1). In contrast, in the open field experiment in Chapter 4, the root biomass in witloof chicory increased when the accumulation of fertilizer components (not only for K_2O but also for other main components such as N or P_2O_5) progressed by an excessive application of a methane fermentation digested slurry for 2 years (Fig. 4-3).

However, the trends of effects of the above mentioned treatments during the root production on the yield and quality of etiolated heads were similar in two experiments, even through they had different factors under investigation: K_2O application only in pot experiment, and excessive application of methane fermentation digested slurry in open field experiment. The similarities were the negative impacts on yield and quality of etiolated heads that increased when the application of K_2O or methane fermentation digested slurry was increased (Table 3-

5, Table 4-6). It may be helpful to consider some important factors of the difference of witloof chicory's responses to the above mentioned treatments both in 2 stages, such as root production and forcing. The methane fermentation digested slurry contains relatively larger amounts of NH_4-N and P_2O_5, and this fact may deserve careful attention (Table 4-1).

Although studies have been made on the effects of nutrient and environmental conditions during storage and forcing culture on the yield or quality of etiolated heads, the number of studies focusing on the effects of nutrient differences on edible parts is limited (de Wilde 1971; Tan and Corey 1990; van den Ende et al. 1998; de Rihck and Schrevens 1998; Krebsky and de Proft 2000; Hoang et al. 2004; Babik et al. 2009).

Ameziane et al. (1997) investigated the effect of the nitrate and phosphate nutrition on chicory tap root development and quality of etiolated heads. Based on the results of their experiments, they suggested the following points: i) root production stage: the regulation of nitrogen during root production may have a large negative impacts on root growth, and the impacts of the phosphate regulation on root growth is very limited; ii) forcing stage: the negative effect of larger amount of both nitrogen and phosphate applications during root production on etiolated head yield and quality is large; however, the lack of phosphate during the root growth has strong positive effects on yield and quality of etiolated heads. The authors also emphasized that the outer leaves of etiolated heads tended to be open and twisted when the nutrition balance during the root production was broken, and this was exactly a similar trend observed in the present thesis, in the experiment described in Chapter 4 (Fig. 4-6).

In the present study, the author investigated the effects of excessive application of K on both root growth and etiolated head quality and yield, however, the effects of combined excessive application of major fertilizer components on vegetative growth and edible parts' quality is still open discussion, and this remains as a matter to be discussed further for improvement of income level of witloof chicory cultivation which is aiming to reclaim the balance of mineral concentration in the soils simultaneously.

2-5. Estimation of agricultural income from the cultivation of witloof chicory introduced for removing accumulated salts, especially for K

Green manure plants with a large biomass, such as the Guinea grass, have been used to remove accumulated fertilizer components or salts from the soils. However, it is difficult to obtain an agricultural income from selling the fresh products of the above mentioned crops after being used as a phytoremediation of soils, because of several risks, such as; i) nitric acid toxicosis, because these crops absorb a large amount of nitric acid when they are grown to remove accumulated nitrogen from the soils; and ii) physiological disorders, such as grass tetany, because these plants accumulate a large amount of K in their plant body. The ratio of K/(Ca+Mg) increases significantly when they are used as a cleaning crop (Ito et al. 1981; Takezawa 1991); iii) no income is usually obtained from the production of green manure plants.

From the results of the experiments presented in Chapter 3 and Chapter 4, it was found that it is possible to produce witloof chicory roots even when they are grown with an excessive amount of K (by chemical fertilizers) or a methane fermentation digested slurry. There was not a significant negative impact on the yield or quality of the obtained roots. In addition, the quality of the etiolated heads, obtained after the forcing culture, could be kept at the same level of the commercially available fresh products even if the size of the etiolated heads decreased by approximately 20%. The agricultural income can be obtained when it is possible to utilize witloof chicory as a cleaning crop or catch crop and this is impossible with existing cleaning crops.

Additionally, it is also important to mention the estimation of the agricultural income from the cultivation of witloof as a cleaning crop or a catch crop. Therefore a brief calculation of the estimated economic value of fresh products (chicon: the etiolated head) per acreage (ha) was performed. The estimated ecomonic value was obtained under the growing condition as a cleaning crop based on the results in Chapter 4. For a precise estimation, the plant density was calculated based on the common practice in Japan currently (222,222 plants ha^{-1}). The results of the root production efficiency ratio (86.7%) and of the etiolated head production ratio (70.0%) in the experiment in Chapter 4 were used for the calculation (Table 4-5, Table 4-6). The estimated ex-grower's price (estimated as 50% of retail price) was calculated as 116.7 JPY per product, based on the retail price of Saladcosmo Inc. (Nakatsugawa, Japan).

The estimated total yield of etiolated heads per was 20,030 kg, while the estimated agricultural gross income per ha was 18.01 million JPY. It became clear that the income was apparently exceeding that of other major open field horticultural crops in Hokkaido such as Carrot, Radish, Cabbage and Onion (2.03, 2.32, 2.19 and 2.54 million JPY, respectively) (Ministry of Agriculture, Fishery and Forestry, 2007). It was found from the results that the agricultural income can be obtained with the witloof chicory production for the purpose of removing the accumulated fertilizer components or salts, especially for K from the soils.

3. The future prospect of witloof chicory for Hokkaido

3-1. Hokkaido is the suitable place for chicory production

With regard to the production of witloof type chicory in Hokkaido region, it has been started from the early 1990's in Otoineppu town, Hokuto city (a surrounding area of Hakodate), Nakafurano town and Tanno town (Sato 2000), and still grown actively in those areas even today. In fact, the growers in Hokkaido region can obtain well matured roots because of its unique climate condition, especially for the low air temperature in October and November when the roots mature and they require cool treatments for efficient translocation of carbon hydrate substances from the top to the root, and it stands to sense that Hokkaido is the suitable place for witloof production

On the other hand, we can see the larger potential of this crop for industrial purposes. Inulin has been utilized as a water-soluble dietary fiber and a substitute for fat, and chicory roots contain a large amount of inulin, a kind of polymer of the fructose (Kikuchi et al. 2004). The history of chicory production in Hokkaido is relatively longer than other European vegetables, because of its potential as a source of inulin or fructose. In fact, Itsumi and Tomita (1943) tried to grow chicory roots in the experimental field of Hokkaido University in 1942, and they investigated the efficient extract method of inulin from chicory roots by using inulase derived from *Penicillium* fungi. Recently, commercial production of chicory as a source of functional sugars, especially for inulin and fructose, is gradually expanding in Hokkaido, and sugar-manufacturing companies have investigated to make its cultivation system efficient. Nippon Beet Sugar Mfg. Co.,Ltd. (Tokyo, Japan) have investigated the cultivation system of chicory, especially for the root type, for inulin extraction, from early 1990's, and they have also demonstrated that chicory can be grown by using existing cultivation system for sugar beet (*Beta vulgaris* ssp. *vulgaris*), especially for transplanting growing system (Kikuchi et al. 2000). Also researchers in Hokkaido or the north part of the main land of Japan have recently reported about the nature and control methods of specific fungal diseases of chicory, and it is likely to say that the basics of chicory cultivation in Hokkaido is getting established recently (Yamauchi et al. 2005; Takahashi et al. 2009; Lan et al. 2013).

3-2. The potential of chicory as a crop that can be substitute to sugar beet in Hokkaido

Kajiyama (2011) reported that the yield of sugar beet root in Hokkaido in 2030 could be estimated 10.6 to 11.7% greater and the sugar yield in 2030 could be estimated around 2.9 to 6.8% greater than those in the midterm average (from 1986 to 2006), even the average air temperature will increase 1.3 to 2.9°C for coming 15 years. However, the yield of sugar beet per ha in Hokkaido in 2016 (53,400 t) was around 20% smaller than that in 2015 (66,800 t), and the acreage of sugar beet in Hokkaido decreased steadily for recent 15 years (69,200 ha in 2000, 62,600 ha in 2010 and 58,800 ha in 2015) (Ministry of Agriculture, Fishery and Forestry 2017). It has been recognized that the main factors of the decrease of sugar beet acreage in Hokkaido are i) the aging problem of growers, ii) the labor shortage, iii) the decrease of income level in beet production (Hokkaido local government, Agriculture Department, Food Safety Promotion Bureau, Agriculture Production Promotion Division, 2011). It seems that there is much profound truth in the prediction that the sugar beet acreage in Hokkaido might be decreased for future. It is no exaggeration to say that the motivation of growers for beet cultivation will be decreased further if the domestic sugar market will be opened internationally, sane as what was happened in European countries for the last several decades.

From this viewpoint, it is quite likely that chicory can be used as a substitution crop to sugar beet when its acreage will be decreased in future in Hokkaido, and this is also what was happened in European countries in the past. This change of cropping items may improve the productivity of agricultural industries in Hokkaido because high added-value extracts can be obtained from chicory, namely inulin and fructan that are more commercially valuable than conventional sugars.

Through this study, it became clear that witloof chicory has a practical capacity to remove K from the K accumulated soils efficiently. It is quite likely that this unique function of chicory can be utilized when it would be introduced as a substitute crop to the sugar beet. A continuous examination of the root chicory's capacity of K absorption from the K accumulated soils would strengthen the importance of both types of chicories, witloof type and root type, in Hokkaido.

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