Studies on the autotoxicity of strawberry and beans in hydroponics with their means to overcome

(水耕イチゴおよびマメ類の自家中毒とその回避法に関する研究)

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Studies on the autotoxicity of strawberry and beans in hydroponics with their means to overcome

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Chapter 1

General Introduction

1.1 Replanting problems in succeeding cropping

Successive culture of the same crop on the same land for years cause soil sickness or replanting injuries (Hirano, 1940; Bonner and Galson, 1944; Tsuchiya, 1990) resulting reduction in both crop yield and quality. The causes of this replanting injury are difficult to diagnose and are generally a complex of interacting factors. These factors may include biotic agent such as an increase or change in the microbial complex in the soil, both beneficial and pathogenic soil microbes such as nematodes, fungi, and bacteria. Inadequate soil type, such as a heavy clay or poor soil structure, low soil fertility, or contaminated with soil pollutant may also contribute to the problem. Beside the above two factors, chemical interference in the rhizosphere soil may also attribute to replanting injuries in plants.

This phenomenon of replanting problem is evidenced in agricultural cropping system including in the production of horticultural crops (Young, 1984; Grodzinsky, 1992). It leads to resurgence of disease pest, exhaustion of soil fertility, and developing chemical interference in the rhizosphere referring to allelopathy (Takahashi, 1984; Young, 1984; Hegde and Miller, 1990). In recycled hydroponics this phenomenon is more clearly evidenced. In hydroponics the root exudates hamper the plant growth mainly by hampering water and mineral uptake. Previous studies have shown that allelochemicals released from plant roots play an important role in replant injuries of crops. Autotoxicity of root exudates is an important feature for understanding replanting problems in agroecosystem as it represents one of the largest direct inputs of allelochemicals into the rhizosphere environment with potent biological activity and great variation in chemical components (Inderjit and Weston, 2003).

1.2. Allelopathy in crop plants

Allelopathy comes from the Latin words allelon ‘of each other’ and pathos ‘to suffer’ refers to the chemical inhibition of one species by another. The ‘inhibitory’ chemical is released into the environment where it affects the development and growth of neighboring plants. Allelopathic chemicals can be present in any parts of an allelopathic plant. They can be found in leaves, flowers, roots, fruits, or stems and also in the surrounding soil. Research on the recognition and understanding of allelopathy has been well documented over the past few decades (Rice, 1984; Rizvi and Rizvi, 1992). These include the symptoms and severity of adverse effects of living plants or their residues upon growth of higher plants and crop yields, interactions among organisms, ecological significance of allelopathy in plant communities, replanting problems, problems with crop rotations, autotoxicity, and the production, isolation and identification of allelochemicals in agro ecosystem.

1.3. Autotoxicity in continuous monoculture

Autotoxicity is a phenomenon of intraspecific allelopathy that occur when a plant species releases chemical substances which inhibit or delay germination and growth of the same plant species (Putnam, 1985a; Singh et al. 1999). It been reported to occur in a number of crop plants in agro ecosystem causing serious problems such as growth reduction, yield decline and replant failures (Singh et al. 1999; Pramanik et al. 2000; Asao et al. 2003). Plants when experiences autotoxicity it releases chemicals to its rhizosphere (Singh et al. 1999) through various mechanisms such as leachation (Overland, 1966), volatilization (Petrova, 1977), root exudation (Tang and Young, 1982), and crop residue decomposition (Rice, 1984). Autotoxicity was found to be pronounced if the plants were cultivated consecutively for years on the same land or grown by hydroponic culture without renewal of nutrient solution. One of the principal causes of this autotoxic growth inhibition in the successive culture of plants has

been attributed to the effect of exuded chemicals from plant roots. Accumulations of these allelochemicals are immense in reused nutrient solution during hydroponic culture. Previously, our research group have studied the phenomenon of autotoxicity in several vegetables crops such as cucumber (Yu and Matsui, 1994; Asao et al. 1998a), taro (Asao et al. 2003), some leafy vegetables (Asao et al. 2004a), many ornamentals (Asao et al. 2007a) and including strawberry (Kitazawa et al. 2005) at the glasshouse and Plant Factory supported research facility of Experimental Research Center for Biological Resources Science, Shimane University, Matsue, Japan using hydroponic culture.

1.4. Autotoxicity in strawberry in recycled hydroponics

In strawberry this phenomenon is typically characterized by the black root rot disease (Strong and Strong, 1931), which increases gradually each year, leading to decrease in plant vigor in field condition and eventually loss of productivity (LaMondia, 2004). This complex disorder decline strawberry fruit yield greatly is not only attributed by soil born fungal pathogen (Matin and Bull, 2002; Zhao et al. 2005; Zhu et al. 1994), nematode (LaMondia, 2004), or physical factors like soil compaction, soil texture, higher rates of herbicidal application, age of seedlings etc. (Wing et al. 1995) but also exudated chemicals interference in the rhizosphere soil are associated with this disease (Kitazawa et al. 2005). The crop yield decline is therefore due to allelopathic inhibition.

Strawberry plants generally cultured through hydroponics in Japan and other developed countries and recently closed type hydroponic systems gained popularity for the production on a commercial basis. However, this managed and viable technique has the autotoxicity constraint. In strawberry autotoxicity from root exudates has been investigated in closed hydroponic culture and benzoic acid was confirmed as the most potent growth inhibitor (Kitazawa et al. 2005). Strawberry plants when experiences autotoxicity, the shoot and root

growth, number of flowers and harvested fruits per plant, and fruit enlargement reduces significantly. Elimination of growth inhibitors from recycling hydroponic cultivation system is desirable from the viewpoint of conservation-oriented agriculture. Activated charcoal (AC) supplementation was found to improve growth and yield significantly by adsorbing phytotoxic chemicals (mainly benzoic acid), from strawberry root (Kitazawa et al. 2005). Several other methods has also been found to be effective in removing/degradating the phytotoxic substances released from plant roots during autotoxicity such as degradation by microbial strain (Asao et al. 2004b) or auxin (2,4-D and NAA) supplementation (Kitazawa et al. 2007). Electrochemical methods have also been applied for degradation/oxidation of phenols and its derivatives from organic waste or pollutants by several researchers. Phenolic compounds, including phenols, catecol and hydroquinone in aqueous solution and even benzene were found to decompose when treated by electro-degradation (ED) (Fleszar and Ploszynka, 1985; Comninellis and Pulgarin, 1991; Feng and Li, 2003). Based on these results, previously ED has been tried to decompose benzoic acid (Asao et al. 2008). It was found that exogenously added benzoic acid to a nutrient solution was almost completely decomposed within 24 hours. The growth inhibitions of plantlets in the nutrient solution containing benzoic acid or in the nutrient solution used for strawberry culture were significantly ameliorated by ED application. However, complete yield recovery from non-renewed nutrient solution to renewal control was suggested through further investigations. Therefore, we studied the appropriate timing and intensity of ED treatment to maintain nutrient solution and allow vigorous plant growth in a closed hydroponic culture.

1.5. Autotoxicity in beans in recycled hydroponics

Allelopathy has been investigated in some beans such as in pea (Kato-Noguchi, 2003), velvet beans (Fujii et al. 1991), soybean (Huber and Abney, 1986; Xiao et al. 2006; Yan and Yang, 2008), and chickpea (Yasmin et al. 1999). It has been found that, in addition to common beans, several other species within the Leguminosae family contain secondary plant products that have allelopathic potential (Rice, 1984). In field experiments, it has been reported that residues and extracts of pea plants suppressed the growth and population size of several plant species (Purvis, 1990; Schenk and Werner, 1991; Tsuchiya and Ohno, 1992; Akemo et al. 2000). Phytotoxic substances in pea root exudates have been reported by several researchers (Hatsuda et al. 1963; Yu and Matsui, 1999) and, recently, pisatin has been identified as an inhibitory chemical from its shoots (Kato-Noguchi, 2003). Aqueous leachates of dry shoot of common beans that contain phenolics showed allelopathic effects on several crop species (Nava-Rodriguez et al. 2005). Autotoxicity due to root exudates found to be involved in growth reduction in soybean monocropping, which decreased plant biomass and root triphenyl tetrazolium chloride-reducing activity as well as seedlings after exposure to root exudates, exhibited higher activities of superoxide dismutase and guaiacol peroxidase (Xiao et al. 2006). The study of autotoxicity in commonly grown beans would provide useful knowledge of sustainable crop production. Thus, identification of the allelochemicals from bean root exudates, evaluation of their phytotoxicity, and their removal would facilitate the maintenance of profitable crop production. Previously, we found evidence of autotoxicity in *Lathyrus ordoratus*, a leguminous crop (Asao et al. 2007b). In this study, we investigated autotoxicity in three beans, namely, pea, common beans, and snap bean as well as their allelochemicals, using hydroponic culture.

1.6. Selection of succeeding crops for replanting field

Strategies to overcome the problems in replanting soil or reuse of hydroponic culture solution had been suggested by many researchers. For example, screening of genotypes in cucumber and many leaf vegetables has revealed that many commercial cultivars have low autotoxic potentials, while others showed strong potential (Asao et. al. 2001), grafting of resistant species as root stock in cucumber extends the harvesting period (Asao et. al. 1999a; Asao et. al. 2000), application of microbial suspension can be used to degrade phytotoxins (Asao et. al. 2004b; Caspersen et. al. 2000; Chen et. al. 2011), or removal of these residues from the soils may be an important step in overcoming soil sickness (Singh et. al. 1999). AC, with its large surface area, pore volume and polarity, has tremendous adsorption capacity for many organic compounds. In cucumber, tomato and asparagus, increased productivity has been observed after using AC in non-renewed solution culture (Asao et. al. 2003; Motoki et. al. 2006, Yu and Matsui, 1994; Yu et. al. 1993). In addition, by applying TiO_2 photocatalysis and ED in a recycling hydroponic cultivation system, autotoxicity was avoided in asparagus and strawberry (Asaduzzaman et. al. 2012; Miyama, et. al. 2009; Sunada et. al. 2008). Thus our research thrust was to find a simple, fast and easy method to overcome the replanting problems in the plants under study. Suitable succeeding crops or crop rotation has good control over problems associated with monoculture. It can limit soil sickness or autotoxicity caused by allelopathy to a greater extend (Batish et. al. 2001). Suggesting suitable crops also has a promising role in eliminating the replanting and/or soil sickness problems in aforesaid crop species.

1.7. Objectives of the present study

In our prior experiments, we have investigated the phenomenon of autotoxicity in strawberry following recycled hydroponics and identified the responsible allelochemicals that showed phytotoxicity to the donor plant species. As a means to overcome the autotoxicity in strawberry, ED method has been studied and fruit yield was recovered to about 71% compared to renewed control. However, greater yield recovery from non-renewed nutrient solution was suggested through further investigation of ED duration. As the follow up experiment, the objectives of the present study were to investigate the appropriate timing and intensity of ED treatment to maintain nutrient solution for higher yield recovery of strawberry under autotoxicity.

The production of common beans found to be declined under replanting condition. Therefore, the objectives of the present study were to investigate the autotoxicity in three beans, namely, *Pisum sativum*, *Phaseolus vulgaris*, and *Vicia faba* using hydroponic culture, identify their allelochemicals, and evaluate their phytotoxicity in seedling growth bioassay of the test plants. In addition to three beans, replanting injuries were also evidenced in asparagus and taro, whose autotoxicity has already been investigated by our research group. In asparagus, yield decline is common in older asparagus production areas due to continuous cropping leading to replanting injuries. Similarly in taro plants do not grow well if cultivated consecutively for years on the same land. In order to find out a convenient and practical tool of replanting problems in beans crop, our third study aims to select ideal succeeding crops after asparagus, taro, broad bean, garden pea and snap bean. This study also aimed to suggest bioassays methods suitable for laboratory or field conditions.

Chapter 2

Growth and yield recovery in strawberry plants under autotoxicity through electro-degradation

1. Introduction

Autotoxicity is a phenomenon of intraspecific allelopathy that occur when a plant species releases chemical substances that inhibit or delay germination and growth of the same plant species (Putnam, 1985a; Millar, 1996; Singh et al. 1999). It has been reported to occur in a number of crop plants in agroecosystem causing serious problems such as growth reduction, yield decline and replant failures (Singh et al. 1999; Pramanik et al. 2000; Asao et al. 2003). Plants when experiences autotoxicity it releases chemicals/ allelochemicals to its rhizosphere (Singh et al. 1999) through various mechanisms. These include leachation (Overland, 1966), volatilization (Petrova, 1977), root exudation (Tang and Young, 1982), crop residue decomposition (Rice, 1984; Putnam, 1985b), and pollen spreading in some plants (Curz-Ortega et al. 1988). The released chemical compounds create problems in monoculture and/or closed hydroponic culture systems as they can accumulate and inhibit the growth of the actual crop. Exposure of these allelochemicals play a multitude of ecological and physiological roles as they inhibit plant growth (Rice, 1984), alter mineral uptake (Lyu and Blum, 1990; Baziramakenga et al. 1994), disrupt membrane permeability (Baziramakenga et al. 1995), cause stomatal closure and induce water stress (Barkosky and Einhellig, 1993), influence respiration (Penuelas et al. 1996), affect photosynthesis and protein synthesis (Mersie and Singh, 1993; Rohn et al. 2002), impair hormone balance (Hollapa and Blum, 1991) and alter enzyme activities (Rohn et al. 2002; Doblinski et al. 2003). Among them, ion uptake and hydraulic conductivity (i.e., water uptake) are worse affected processes since root is the first organ to come into contact with autotoxins in the rhizosphere (Blum et al. 1999).

Closed hydroponic system has recently gained popularity for commercial production of strawberry (Takeuchi, 2000; Oka, 2002; Koshikawa and Yasuda, 2003) but this managed culture technique has the problem of autotoxicity due to accumulation of allelochemicals in the culture solution. In strawberry autotoxicity from root exudates has been investigated in closed hydroponic culture and benzoic acid was confirmed as the most potent growth inhibitor (Kitazawa et al. 2005). Strawberry plants when experiences autotoxicity, the shoot and root growth, number of flowers and harvested fruits per plant, and fruit enlargement reduces significantly. Elimination of growth inhibitors from recycling hydroponic cultivation system is desirable from the viewpoint of conservation-oriented agriculture. AC supplementation was found to improve growth and yield significantly by adsorbing phytotoxic chemicals (mainly phenolics), for example in strawberry (Kitazawa et al. 2005), taro (Asao et al. 2003), cucumber (Yu and Matsui, 1994; Asao et al. 1998a; Asao et al. 1999a; Asao et al. 2000), several leafy vegetables (Asao et al. 2004a), and some ornamentals (Asao et al. 2007). Several other methods has also been found to be effective in removing/degradating the phytotoxic substances released from plant roots during autotoxicity such as degradation by microbial strain (Asao et al. 2004b) or auxin (2,4-D and NAA) supplementation (Kitazawa et al. 2007). Electrochemical methods have also been applied for degradation/oxidation of phenols and its derivatives from organic waste or pollutants by several researchers. Phenolic compounds, including phenols, catecol and hydroquinone in aqueous solution and even benzene were found to decompose when treated by electro-degradation (Fleszar and Ploszynka, 1985; Comninellis and Pulgarin, 1991; Feng and Li, 2003). These compounds are oxidized rapidly at the anode and decompose to CO_2. Thus ED may cause the decomposition of allelochemicals; including benzoic acid exuded in the nutrient solution from plants, and could be useful tool to mitigate autotoxicity in strawberry.

Based on these results, previously ED has been tried to decompose benzoic acid (Asao et al. 2008). It was found that exogenously added benzoic acid to a nutrient solution was almost completely decomposed within 24 hours. In the ED process a titanium plate was used as cathode and a ferrite rod as anode. The growth inhibitions of plantlets in the nutrient solution containing benzoic acid or in the nutrient solution used for strawberry culture were significantly ameliorated by ED application. However, complete yield recovery from non-renewed nutrient solution to renewal control was suggested through further investigations. Therefore, the present study aimed to determine the appropriate timing and intensity of ED treatment to maintain nutrient solution and allow vigorous plant growth in a closed hydroponic culture. A modified ED machine was used to degrade allelochemicals from the culture solution of strawberry in Wagner's pot hydroponic system.

2. Materials and Methods

2.1. Verification of electrolysis condition

Electrolysis conditions were verified in a series of concentrations (0, 6.25, 12.5, 25, 50, 75, and 100%) of 'Enshi' nutrient solution (Table 1). Initially the electric current was slightly increased with the increase of nutrient solution concentration but voltages were decreased gradually. The electric current ranged from 1.75 to 1.88 amps and electric voltage form 35.7 to 8.1 volts. However, the electric current was below two amps in all the concentrations of nutrient solution. When the electric current was adjusted at two amps, then the voltage was not changed greatly from the initial. Therefore, two amps were selected as the test current for the investigations.

Table 1. Electric current (amps) and voltage (volt) at different concentrations of nutrient solution during electro-degradation (ED).

Nutrient solution concentration (%)	Initial		Adjusted	
	Amp	Volt	Amp	Volt
0.0	1.75*	35.7	2.0	40.2
6.25	1.82	21.0	2.0	22.5
12.5	1.84	16.2	2.0	17.2
25.0	1.86	12.0	2.0	12.6
50.0	1.87	9.8	2.0	10.2
75.0	1.88	8.8	2.0	9.0
100.0	1.88	8.1	2.0	8.4

*Values are the average of three readings.

2.2. Growth chamber bioassay of strawberry plantlets

Strawberry (*Fragaria* × *ananassa* Duch. var. 'Toyonoka') plantlets obtained through plant tissue culture were used for this bioassay. The virus free and healthy plantlets at 3rd - 4th leaf-stage were planted to plastic containers (17 cm × 29 cm × 9.5 cm) in the growth chamber at 20/15 ºC (day/night) with a light intensity of 74-81 $\mu molm^{-2}s^{-1}$ and a 14-hours photoperiod. The containers were filled with 3 liters of 25% standard 'Enshi' solutions (EC 0.8 dSm^{-1}). Six plantlets were planted in each container using urethane foam blocks as support. There were four types of nutrient solution viz. renewed, non-renewed, non-renewed with ED at weekly and non-renewed with ED at biweekly intervals (Fig. 1). The nutrient solutions were renewed at every week intervals (control). Nutrient solutions of ED treatments were combined in a plastic bucket and then ED was applied. During ED application the nutrient solutions were aerated continuously by air pump (NISSO, Chikara α 4000 SW, Osaka, Japan). The ED was applied for 24 hours with electric current adjusted at two amps. After completion of ED, full amount of Fe^{3+} was added in the treated nutrient solution and the amount of nutrient solution was adjusted to three liter by adding standard nutrient solution. During the growth periods the major nutrients (NO_3^-, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+}, and Fe^{3+}) content in the culture solution were adjusted as close as possible to initial concentrations on the basis of chemical analysis with ion meter (C-141, Horiba, Ltd. Kyoto, Japan) for NO_3^-, spectrophotometer (UV mini 1240, Shimadzu Corporation, Kyoto, Japan) for PO_4^{3-} and atomic absorption spectrophotometer (Z-5010, Hitachi, Tokyo, Japan) for K^+, Ca^{2+}, Mg^{2+}, and Fe^{3+}. After seven weeks, the number of leaves, crown diameter, root length, shoot fresh weight, shoot dry weight and root dry weight were measured.

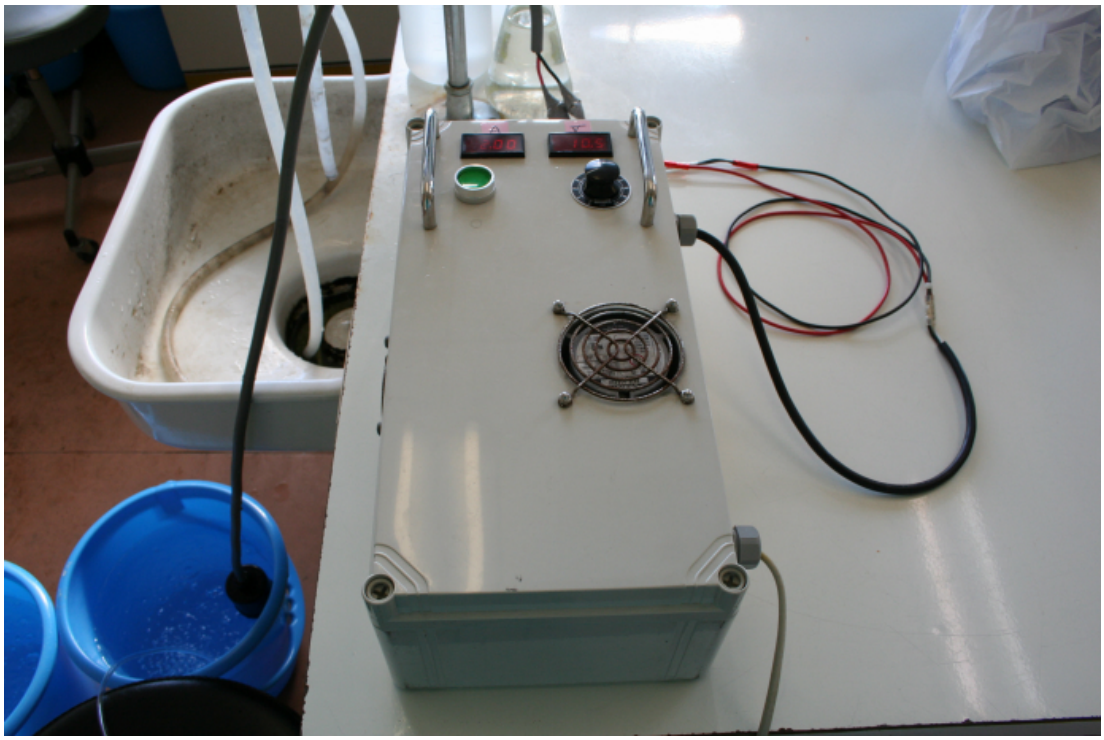

Fig. 1. Electro-degradation (ED) of non-renewed culture solution of strawberry plants grown in growth chamber. The electric current was set 2.0 amps and the electrolysis duration was 2 hours.

2.3. Electro-degradation of nutrient solution in no plant experiment

ED treatment was applied in nutrient solution following without plant experiment. In these experiments 10 liters of 25% Enshi nutrient solution were electro-degraded with ED machine used previously for 24 hours (Fig. 2). The electric current was adjusted at two amps. During the ED process nutrient solutions were aerated constantly by an air pump. Treated nutrient solutions were sampled at 0, 1, 3, 6, 9, and 24 hours. In plastic bottles 25 ml samples were collected for major nutrient analysis following the procedures described earlier. The electric voltage and temperature was recorded from ED machine, electrical conductivity was measured by EC meter (ES-51, Horiba, Ltd., Kyoto, Japan), and pH was also recorded from a pH meter (D-12, Horiba, Ltd., Kyoto, Japan) during each sampling.

Fig. 2. Electro-degradation (ED) of nutrient solution with benzoic acid with constant aeration in a without plant experiment.

2.4. Wagner's pot hydroponic system of strawberry plants

Strawberry plantlets were planted into Wagner's pot (1/5000a, NF-5, AsOne, Osaka, Japan) connected with a reservoir containing 60 liters of 25% nutrient solution in closed hydroponic system (ten plants in one line) on 16 November 2009 (Fig. 3-4). At transplanting the plantlets were at 5th - 6th leaf stage, healthy and similar in size. The Wagner's pot system includes main inlet pipes (15 mm diameter) for supply and drainage of nutrient solution between reservoir and pots, Wagner's pot with three liter capacity for planting, inlet tubes (4 mm diameter) to supply solution to the pots, and 60 liters capacity nutrient solution container with a pump (KP-101, Koshin, Kyoto, Japan). The culture solutions were prepared as renewed at every week, non-renewed entirely, non-renewed with ED at every two weeks and non-renewed with ED at every four weeks intervals. Strawberry plants after reaching active vegetative phase, ED treatments were applied on 11 January 2010. The electric current was adjusted at two amps and ED was applied for two hours. The ED machine was modified to investigate its efficacy for degradating autotoxic chemical following a Wagner's pot hydroponic system in the greenhouse. The modified machine has an electrode having a central core made of ferrite with a surface area of 65.9 cm^2 (anode) which enclosed with cylindrical tube made of titanium with a surface area of 103.7 cm^2 (cathode). The nutrient solution can pass through the electrode where ED takes place (Fig. 5-6). The machine was coupled with a digital DC power supplier (AD-8735D, AND, Japan). In the previous study, ED of autotoxic chemicals from strawberry root exudates was done by an electrode (cathode) consisting of a cylindrical titanium plate with a 180 cm^2 surface area and ferrite stick of 42 cm^2 surface area (anode) (Asao et al. 2008). The previous ED machine requires a separate pump system to flow the nutrient solution during treatment but the modified machine has the facility of attaching pump for flowing treated nutrient solution homogenously through the electrode.

Fig. 3. Wagner's pot with capacity of 3 liters nutrient solution used for strawberry hydroponics in t he greenhouse.

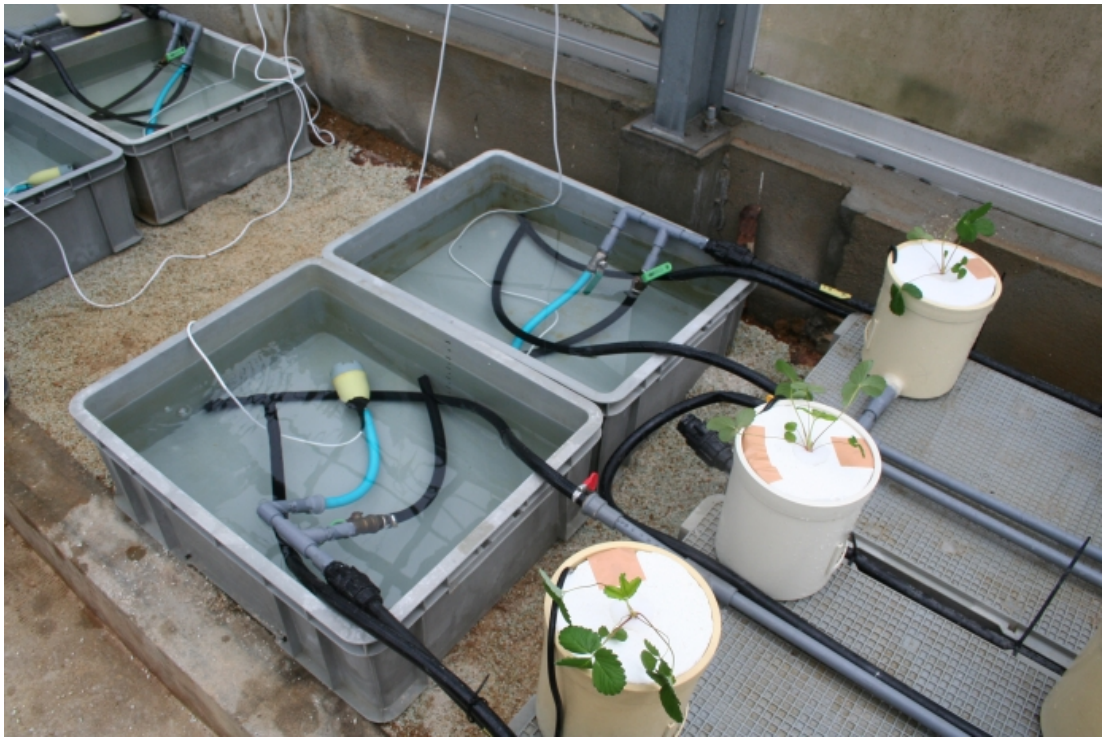

Fig. 4. Wagner's pot hydroponic system used for strawberry culture in the greenhouse.

Therefore, the modified ED machine was used for degradation of allelochemicals in Wagner's pot hydroponics of strawberry in greenhouse. The nutrient solution was circulated through the pipes for five minutes at 10 minutes intervals using an automatic pump timer (KS-1500, Iuchi, Osaka, Japan). The nutrient solutions were renewed at every four weeks interval. The concentration of major nutrients (NO_3^-, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+}, and Fe^{3+}) were analyzed following the methods and instruments used for growth chamber bioassay and adjusted at four weeks interval. The EC, pH and temperature of nutrient solution were measured at each time of treatment application. The date of anthesis were recorded for each plant to check whether there were any influences of ED on flowering of strawberry among the treatments. Pollination was aided by a soft brush at two days intervals. As the plants attained its full vegetative growth phase, leaf length and width were measured on 10 April 2010 to compare growth among the treatments. The fruits were harvested at their maturity. At each harvest fresh weight of fruits were recorded and gathered for final yield comparison among the treatments. Leaf number, leaf length and width, root length, dry weight of leaves, dry weight of crown, and dry weight of root were also recorded at final harvest.

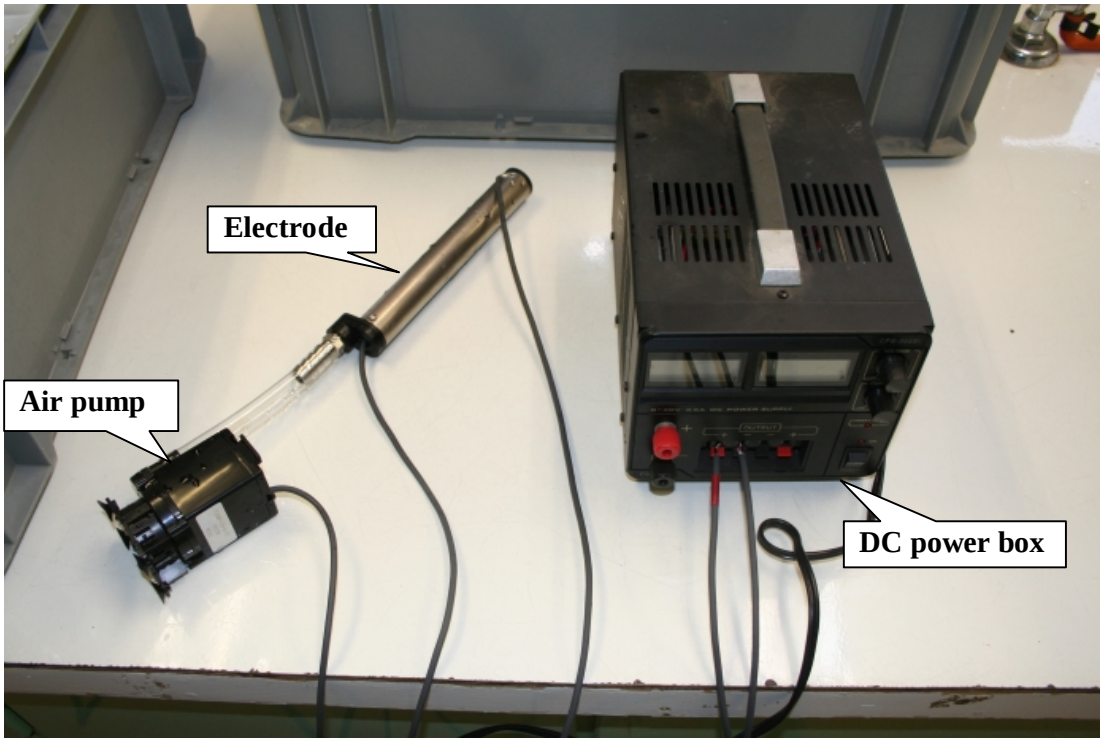

Fig. 5. Electro-degradation (ED) machine used for degradating non-renewed nutrient solution of strawberry grown in the greenhouse. The machine consists of electrode, DC power box and air pump.

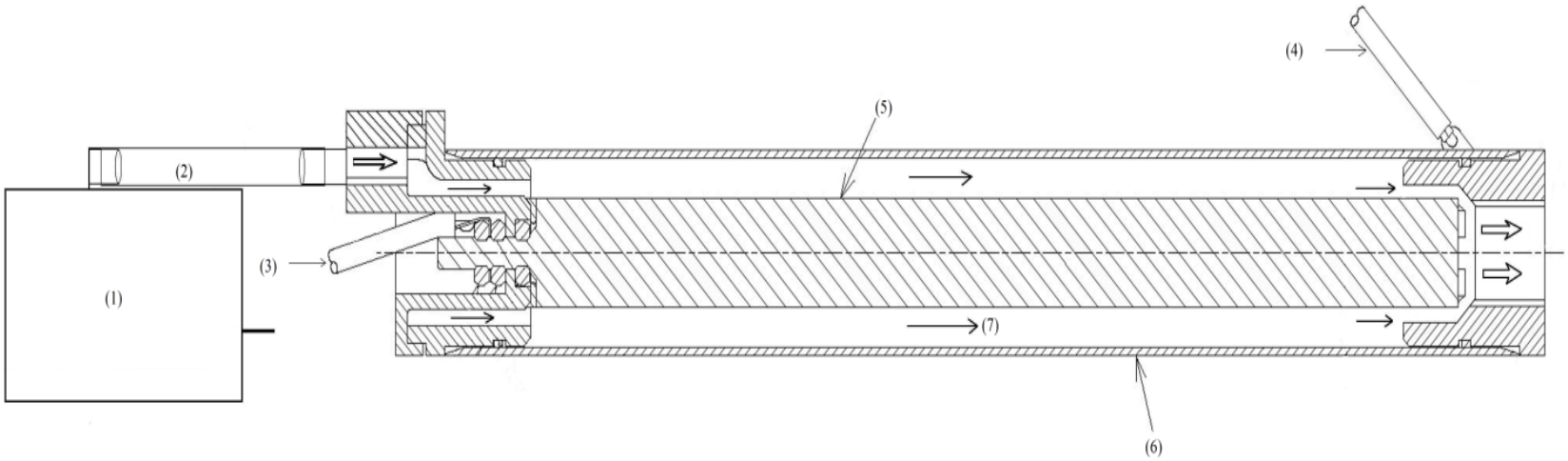

Fig. 6. Schematic diagram of the electrode used for ED treatment. Different components are as (1) pump, (2) plastic tube connecting pump with electrode, (3) anode, (4) cathode, (5) central ferrite core, (6) cylindrical titanium pipe and (7) nutrient solution flow.

2.5. Fruit quality of strawberry grown in nutrient solution with electro-degradation

Strawberry fruits were composited after each harvest and were frozen at –30ºC for subsequent analysis of soluble solids, titratable acids and ascorbic acid content. Fruit samples were kept out of freezer before analysis to obtain juice for determining the above qualities of strawberry fruits. The soluble solid content of the fruit was determined using a digital refractometer (PR-1, Atago Ltd., Japan). Titratable acid contents were determined by diluting each 2 ml aliquot of strawberry juice to 10 ml with 8 ml distilled water and added 2-3 drops of phenolphthalein then adjusted the pH to 8.2 using 0.1 N (w/v) NaOH. The quantity of NaOH (ml), and the amount for appropriate acidity was converted into citric acidity (%). Ascorbic acid content was measured with 2,4-dinitrophenylhydrazine (DNP) colorimetry. Strawberry fruit juice (0.5 ml) were taken in 50 ml test tube then 0.5 ml of 10% meta-phosphoric acid solution, 1 ml of distilled water, 1 ml of 0.03% 2,6-dichlorophenol-indophenol (DCP), 2 ml of thiourea, and 1 ml of DNP was added to the samples following three hours incubation at 37 ºC in water bath. After incubation samples 5 ml of 85% H_2SO_4 were added keeping in water cooled with iced water. After 30 minutes cooling ascorbic acid content was measured at 540 nm by spectrophotometer (U-2900, Hitachi High Technologies Corporation, Tokyo, Japan).

2.6. Mineral nutrient content in strawberry plants grown in nutrient solution with electrodegradation

Strawberry plant parts were separated into leaves, crown and roots and kept in a constant temperature oven (DKN 812, Yamato Scientific Co., Ltd. Japan) for 72 hours at 80 ºC. When the dry matter reaches constant weight, it was ground into powder with a mixer machine (National MX-X53, Japan). Samples weighing 0.5 g were mixed with 8 ml of HNO_3 and digested by microwave sample preparation system (ETHOS1, Milestone S.r.l,

Bergamo, Italy). After digestion samples were measured up to 50 ml of volumetric flask and then filtered with qualitative filter paper (Advantec Grade no. 131, 185 mm thickness). The filtered sample solutions were analyzed for mineral nutrients by atomic absorption spectrophotometer (Z-2310, Hitachi High Technologies Corporation, Tokyo, Japan).

2.7. Experimental design and statistical analysis

A randomized complete block design with three replicates was used for both growth chamber bioassay and Wagner's pot hydroponics in the greenhouse as described above. These experiments were not repeated over time due to the consistent results. The growth and yield data obtained from bioassay and Wagner's pot hydroponics of strawberry plants were subjected to analysis of variance using Tukey-Kramer test (Statcel 2 software, OMS publication, Tokorozawa, Saitama, Japan) and the treatment means were separated at 5% level of probability. The instrumental data obtained from fruit and plant matters were also analyzed for their difference among the treatments.

3. Results and Discussion

3.1. Growth chamber bioassay of strawberry plantlets

The ED of the culture solution of strawberry plantlets grown in growth chamber showed a significant influence on growth parameters (Table 2). The weekly renewed nutrient solution leads a vigorous plant growth while inhibition of vegetative growth of strawberry plantlets resulted due to non-renewed nutrient solution during the entire growth period. This growth inhibition is basically attributed by the accumulation of autotoxic root exudates in the non- renewed culture solution. In previous study, Kitazawa et al. (2005) identified benzoic acid as the most potent growth inhibitor that accumulated in strawberry culture solution. In biweekly ED application, growth was not inhibited rather recovered significantly to renewed nutrient solution except crown diameter. However, ED when applied weekly, the growths were inhibited remarkably as in non-renewed nutrient solution (Fig. 7). Shoot weight was found to be the worst affected in weekly ED of non-renewed nutrient solution and it was about 69% and 62% reduction in shoot fresh and dry weight against renewed nutrient solution. Growth retardation in weekly ED culture solution also produced fewer new leaves and shorter root length (about 54% and 45%, respectively) compared to renewed control. The major reason of this drastic growth inhibition in weekly ED culture is low concentration of Fe^{3+} in the degraded solution. In previous study it was found that 24 hours ED decreases the Fe-EDTA concentration about 10% of the initial (Asao et al. 2008). Therefore in this bioassay the effects of ED treatment were immense in weekly than biweekly intervals with the ED machine used previously. The other possible reasons of this growth retardation and timing of ED were investigated following without plant experiments.

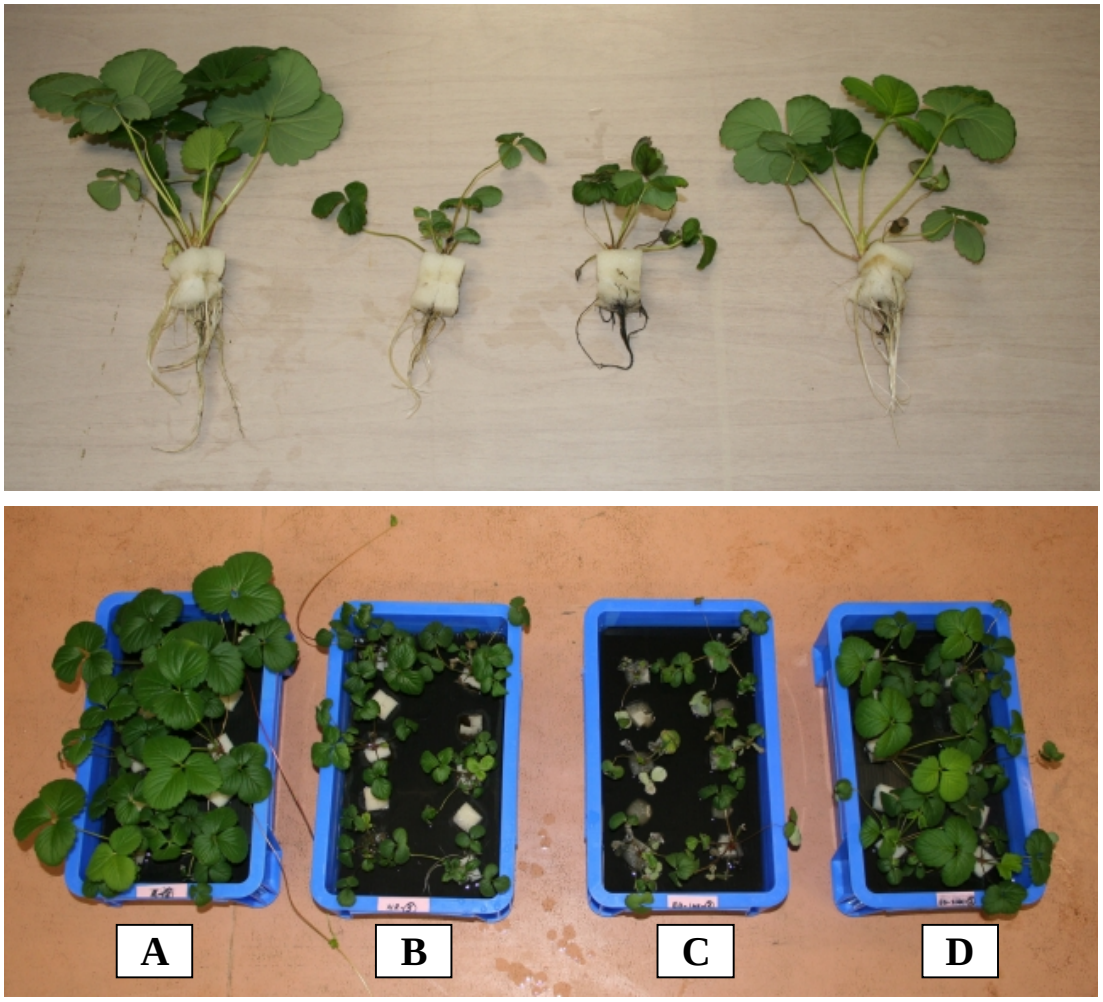

Fig. 7. Effects of electro-degradation (ED) on the growth of strawberry plantlets in growth chamber. Strawberry plants were grown in renewed (A), non-renewed (B), non-renewed with weekly ED and non-renewed with biweekly ED (D).

Table 2. Effects of ED on the growth of strawberry plantlets in growth chamber bioassay. Culture solutions were treated as renewed, non-renewed, non-renewed with weekly ED and non-renewed with biweekly ED. ED of culture solution were done by electrode used in previous experiment in 2008 (Asao et al. 2008) .

Culture solution	Number of leaves*	Crown dia. (mm)	Root length (cm)	Shoot fresh wt. (g)	Shoot dry wt. (mg)	Root dry wt. (mg)
Renewed	8.9 a†	7.2 a	14.1 ab	2.9 a	444.5 a	86.0 ab
Non-renewed	8.0 a	4.8 bc	10.6 b	2.1 a	307.8 ab	66.1 b
ED weekly	3.7 b	4.3 c	7.7 b	0.9 b	171.1 b	45.9 b
ED biweekly	8.2 a	5.9 b	15.8 a	2.4 a	390.6 a	93.9 a

*Parameters measured per plant basis.

†Values in a column followed by different letter differ significantly by Tukey's test ($P<0.01$; $n = 6$).

3.2. Electro-degradation of nutrient solution in without plant experiment

The electrolysis timing and intensity were investigated in without plant experiment (Table 3). ED of nutrient solution was conducted at two amps for 24 hours by the ED machine used previously. After 24 hours ED electrical voltage were decreased by 2.2 volts whereas the electrical conductivity was not varied with the electrolysis durations. However, the pH of solution was decreased as low as 3.1 after 24 hours of ED but it was almost not changed till 9 hours ED. This exceedingly low pH after 24 hours of ED was another important cause that leads to strong inhibitory effects on strawberry plantlets growth at weekly ED in the growth chamber bioassay (Table 2). The solution temperature also increase considerably (over 9.1 ºC form starting) after 24 hours ED compared to non ED solution. Changes in concentration of nutrients were determined and it was found that Ca^{2+} decreased (about 66%) considerably after 24 hours ED (Fig. 8). In addition to decrease in Fe^{3+} concentrations in the ED treated nutrient solution, Ca^{2+} also found to be decreased

after three hours ED. Therefore, ED of nutrient solution for 24 hours was associated with some problems, it was not only degradated the allelochemicals but also breakdown the essential nutrients like Ca^{2+} and Fe^{3+}. Considering the very low pH and decrease in Ca^{2+} and Fe^{3+} concentration in the treated nutrient solution a shorter ED duration (2 hours) was selected for Wagner's pot hydroponic system for strawberry. Moreover, short electrolysis duration can save electricity and can be feasible in the greenhouse strawberry cultures.

Table 3. Electric conditions of 24 hours ED of nutrient solution by the machine used in previous experiment in 2008 (Asao et al. 2008).

Electrolysis	Volt	EC ($mScm^{-1}$)		pH		Temperature (°C)	
duration (hours)	+*	+	–	+	–	+	–
0	14.9	0.84	0.83	6.95	6.98	14.4	14.3
1	14.3	0.84	0.83	7.20	7.30	15.8	14.5
3	13.9	0.79	0.79	7.36	7.35	18.0	14.7
6	13.6	0.72	0.82	7.26	7.28	20.4	14.5
9	13.3	0.75	0.82	6.86	7.40	22.1	14.5
24	12.7	0.79	0.82	3.13	7.26	22.6	14.2

*(+, –) indicate with or without ED.

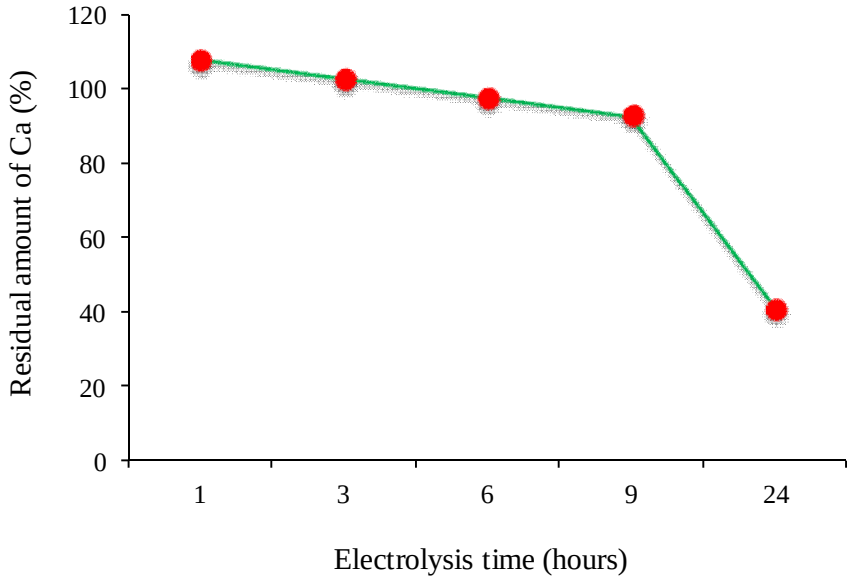

Fig. 8. Decrease in Ca^{2+} concentration in the electro-degraded nutrient solution after 24 hours by the machine used in previous experiment in 2008 (Asao et al. 2008).

3.3. Wagner's pot hydroponic system of strawberry plants

ED method was applied for recovering the autotoxicity in strawberry, which usually occurs in closed hydroponics (Kitazawa et al. 2005). Therefore, a closed hydroponic system was developed in Wagner's pot for treating the strawberry culture solution in the greenhouse. After active vegetative growth strawberry plants were started flowering. There were no significant influences of ED on the anthesis of strawberry plants but comparatively earlier flowering were observed in plants grown in non-renewed nutrient solution (Table 4). Generally plant switches its vegetative growth to reproductive when it experiences some stresses. The synthesis and exudation of allelochemicals enhanced by stress conditions that the plant encounters such as extreme temperature, drought and UV exposure (Pramanik et al. 2000; Inderjit and Weston, 2003). Thus earlier anthesis in

strawberry plants grown in non-renewed nutrient solution might be the results of allelochemical stresses which were released from its root. Delayed anthesis was occurred in plants with ED at four weeks intervals. This phenomenon might be due to degradation of allelochemicals (mainly benzoic acid) form culture solution of strawberry. Vegetative growth measurements showed differences among the culture solutions of strawberry (Table 4). Vigorous growth was observed in plants grown in renewed nutrient solution and in non-renewed nutrient solution with ED at four weeks intervals (Fig. 9-10). Although leaf lengths were not differed significantly, the leaf widths were varied among the treatments. Renewed and non-renewed nutrient solution with ED four weekly intervals produces strawberry plants with wider leaves. Larger leaves provide higher photosynthetic area that produces higher strawberry yield. Whereas non-renewed and non-renewed with biweekly ED nutrient solution produces less width leaves. The results revealed that the leaf size were decreased about 21% in non-renewed nutrient solutions than renewed but non-renewed nutrient solutions with ED produced similar sized leaves as in renewed nutrient solution.

Fig. 9. Effects of electro-degradation (ED) on the growth of strawberry at pre-flowering stage.

Table 4. Effects of ED on the anthesis and vegetative growth of strawberry plants in Wagner's pot hydroponic system. Culture solutions were treated as renewed, non-renewed, non-renewed with ED at two weeks and non-renewed with ED at four weeks intervals.

Culture solution	Date of anthesis (month/day)*	Vegetative growth	
		Leaf length (mm)	Leaf width (mm)
Renewed	2/22	222.2	194.4 a†
Non-renewed	2/20	215.4	162.2 b
ED biweekly	2/23	225.7	178.6 ab
ED four weekly	2/26	228.2	198.3 a
	NS‡	NS	

*Strawberry plantlets were transplanted on 16 November 2009 and anthesis was observed on February 2010.

†Values in a column followed by different letter differ significantly by Tukey's test ($P<0.05$; $n = 10$).

‡Non significant.

Fig. 10. Growth of strawberry plants in Wagner's pot hydroponics in the greenhouse. One line consists of ten pots of renewed, ED biweekly and ED four weekly (above) and non-renewed, ED four weekly and renewed nutrient solutions (bellow).

The growth and yield of strawberry plants were significantly influenced by the culture solutions used (Table 5). Renewed and four weekly ED of non-renewed nutrient solution produced higher dry weights of leaf and root than non-renewed and non-renewed with biweekly ED. Leaf number per plants, maximum leaf length and width, root length, crown dry weights were higher in renewed nutrient solution and non-renewed with four weekly ED followed by non-renewed and its biweekly ED. This indicates during autotoxicity, growth in strawberry plants were recovered by ED treatment. In cucumber, it was found that plant growth was inhibited by its own root extract and exudates while improved by removal of these substances from the rhizosphere (Yu and Matsui, 1994; Asao et al. 1998a; Yu et al. 2000). The number of fruits in non renewed nutrient solution and biweekly electro-degraded solution decreased to 13% and 27%, respectively compared with renewed control. Whereas, there were no significant difference in fruit number between renewed and non-renewed nutrient solution having four weekly ED. The fruit yield of strawberry in non-renewed nutrient solution and its biweekly ED treatment were decreased about 25% compared with control (renewed four weekly). The complete recovery (about 99%) of fruit yield was resulted in plants grown in non-renewed nutrient solution with four weekly ED. In biweekly ED of non-renewed nutrient, Ca^{+2} and Fe^{3+} were broken in addition to degradation of allelochemicals from the strawberry plant roots. Biweekly ED is more intense compared to four weekly and therefore, contain lower concentration of major nutrients like calcium and iron in the culture solution. As a result strawberry plants were found to be retarded in these culture solutions. In previous study, ED of non renewed nutrient solution, strawberry fruit yield recovered about 71% to renewed control (Asao et al. 2008) and complete yield recovery was not achieved due to decrease in Fe^{3+} in the nutrient solution. In the present Wagner's pot hydroponics, appropriate timing and intensity of ED were applied and therefore complete recovery of fruit yield was obtained.

Table 5. Effects of ED on the growth and yield of strawberry plants in Wagner's pot hydroponic system. Culture solutions were treated as renewed, non-renewed, non-renewed with ED at two weeks and non-renewed with ED at four weeks intervals.

Culture solution	Number of leaves*	Leaf length (cm)	Leaf width (cm)	Root length (cm)	Dry weight of leaves (g)	Dry weight of crown (g)	Dry weight of root (g)	Number of harvested fruits	Fruit yield (g)
Renewed	60.4	32.7	20.0 a†	37.4	47.3 a	2.4	7.5 a	30.8 a	295.0 a
Non-renewed	58.8	28.3	15.9 b	35.2	32.2 b	1.6	5.5 b	26.7 ab	223.5 bc
ED biweekly	58.8	30.5	18.4 a	37.5	35.7 b	2.1	5.7 b	22.5 b	220.6 c
ED four weekly	55.9	30.3	19.7 a	34.4	40.0 ab	2.2	6.0 ab	30.3 a	290.7 ab
	NS‡	NS		NS		NS			

*Parameters measured on per plant basis.

†Values in a column followed by different letter differ significantly by Tukey's test ($P<0.05$; $n = 10$).

‡Non significant.

3.4. Fruit quality of strawberry grown in nutrient solution with electrodegradation

The quality of strawberry fruits were not differed significantly in the culture solutions either renewed and/or non-renewed (Table 6). Soluble solid content, citric acid acidity, and ascorbic acid content in the strawberry fruits were not varied among the culture solutions used. Therefore, ED of nutrient solution did not left any detrimental effects on the fruit quality in strawberry.

Table 6. Soluble solid content, citric acid and ascorbic acid content in fruits of strawberry grown in nutrient solution with ED; fruits harvested at stage I (2010/3/23~2010/4/30), stage II (2010/5/1~2010/5/15), and stage III (2010/5/16~2010/6/8). Culture solutions were treated as renewed, non-renewed, non-renewed with ED at two weeks and non-renewed with ED at four weeks intervals.

Culture solution	Soluble solid content (%)			Citric acidity (%)			Ascorbic acid (ppm)		
	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III
Renewed	9.1	9.0	9.8	0.53	0.54	0.61	630.6	583.2	587.2
Non-renewed	9.1	9.4	9.6	0.41	0.46	0.43	632.1	635.0	780.2
ED biweekly	8.6	8.9	9.1	0.35	0.39	0.46	816.9	611.9	602.5
ED four weekly	8.6	8.2	9.1	0.58	0.51	0.62	671.6	662.2	701.4
	NS*	NS	NS	NS	NS	NS	NS	NS	NS

*Non significant.

3.5. Mineral nutrient content in strawberry plants grown in nutrient solution with electro-degradation

Calcium and iron contents were determined in the plant parts strawberry as these two minerals were found to be decreased from the nutrient solution due to ED (Table 7). Calcium contents in non-renewed nutrient solution and its biweekly ED were decreased significantly in all plant parts except in crown compared to renewed and non-renewed nutrient solution with four weekly ED. Calcium uptake in plants grown in non-renewed nutrient solution was inhibited by the accumulated autotoxic chemicals whereas in case of biweekly ED of non-renewed solution, it was due to the breakdown of Ca^{2+} in the nutrient solution by the ED. Iron content in strawberry plants were differed significantly among the culture solution used (Table 7). In leaves and crown Fe^{3+} content were decreased in non-renewed and/or ED nutrient solution while in root it was decreased only in non-renewed nutrient solution. In previous study it was found that 10% Fe-EDTA degradated from nutrient solution due to ED (Asao et al. 2008).

Table 7. Mineral nutrient content in strawberry plants grown in nutrient solution with ED. Culture solutions were treated as renewed, non-renewed, non-renewed with ED at two weeks and non-renewed with ED at four weeks intervals.

Culture solution	Ca (mg plant^{-1})			Fe (mg plant^{-1})		
	Leaves	Crown	Root	Leaves	Crown	Root
Renewed	0.42 a*	0.029 a	0.063 a	0.062 a	0.0092 a	0.062 b
Non-renewed	0.26 c	0.020 b	0.043 b	0.039 c	0.0052 b	0.042 c
ED biweekly	0.23 c	0.027 a	0.045 b	0.038 c	0.0052 b	0.064 ab
ED four weekly	0.31 b	0.028 a	0.062 a	0.054 b	0.0065 b	0.080 a

*Values in a column followed by different letter differ significantly by Tukey's test ($P<0.01$; $n = 10$).

The previous study concluded that ED treatment of nutrient solution could mitigate autotoxicity plants in closed hydroponic culture and recovered fruit yield upto 71% of control. However, further studies were suggested to determine the appropriate timing and intensity of ED treatment to maintain the nutrient solution and allow vigorous plant growth. In this present follow up study, we found complete recovery in fruit yield of strawberry plants grown in closed hydroponic system where culture solutions were allowed to ED treatment before reuse. In the growth chamber bioassay strawberry plantlets showed retarded growth in non-renewed and/or its weekly ED whereas it was recovered in biweekly applications. Therefore, electro-degraded nutrient solutions were investigated following without plant experiments. It was found that ED of nutrient solution was associated with degradation of Fe-EDTA (about 10%) and low concentration of Ca^{2+} (about 66% of the initial) in the treated culture solution. The disadvantages of longer duration ED were decrease in solution pH as low as 3.1 and increase in temperature. Therefore, shorter duration (two hours) ED treatments were applied in the greenhouse experiments following Wagner's pot hydroponics. ED machine used for previous experiments was modified for allowing the treated solution passed continuously through the electrode. Results obtained from Wagner's pot experiment showed that growth and yield (about 99% to renewed control) of strawberry plants were improved in non-renewed nutrient solution having ED at four weeks interval. Therefore, in practice, application of ED to non-renewed nutrient solution in closed hydroponic system for two hours at every four weeks intervals would recover autotoxicity in strawberry.

4. Summary

The appropriate timing and intensity of ED of nutrient solution to recover strawberry autotoxicity in closed hydroponic system was investigated during spring 2010 in glasshouse of Experimental Research Center for Biological Resources Science, Shimane University. In growth chamber bioassay, growth of strawberry plantlets were inhibited greatly in non-renewed nutrient solution due to the accumulation of autotoxic root exudates compared to nutrient solution renewed weekly (control). Plants grown in non-renewed nutrient solution electro-degraded weekly intervals also showed growth inhibition whereas biweekly ED improved its growth. This growth inhibition in weekly ED nutrient solution was found to be attributed by the degradation Fe-EDTA (about 10%) and low concentration of Ca^{2+} in culture solution. Electrolysis durations were evaluated in without plant experiments. It was found that low pH (3.13) and increased temperature were two major constraints for longer ED duration (24 hours). Therefore, considering electric voltage, EC and pH, shorter ED duration (two hours) was selected for further experiments. Two hours ED treatment of nutrient solution certainly can save energy and be feasible for greenhouse applications. In greenhouse, strawberry plants were grown in Wagner's pot hydroponic system where nutrient solutions were either renewed four weekly or not-renewed entirely. The non-renewed nutrient solutions were electro-degraded either biweekly or four weekly. Growth and yield of strawberry plants was decreased in non-renewed nutrient solution and its biweekly ED treatment but it was improved in non-renewed nutrient solution when ED applied at four weeks intervals. It was found that fruit yield of strawberry plant was completely recovered (≈ 99%) in non-renewed nutrient solution with ED at every four weeks, whereas, in our previous study the recovery was 71% compared to renewed nutrient solution. Therefore, we recommend application of ED to non-renewed nutrient solution for two hours at every four weeks intervals to avoid autotoxicity in strawberry in a closed hydroponic system.

Chapter 3

Autotoxicity in beans and their allelochemicals

1. Introduction

Beans are grain legumes that belong to the family Leguminosae, which includes food and forage legumes. Bean plants are cultivated primarily for their seeds, which are harvested at maturity and are rich in protein and energy. They are used either for animal feed or for human consumption. The major grain legumes are *Pisum sativum, Vicia faba, Lens culinaris, Glycine max, Phaseolus vulgaris*, *Lupinus* spp., and *Cicer arietinum*. These grain legumes are generally intercropped with cereals to enhance crop yield, increase nitrogen use efficiency, and reduce weed infestation and the occurrence of plant disease (Willey, 1979; Jensen, 1996; Hauggaard-Nielsen et al. 2001, 2008). Among the grain legumes, some edible beans are used as vegetables and intensively cultivated in the same farmland year after year. The production of these common bean plants and other perennial legumes declines in replanting conditions owing to autotoxicity, a form of intraspecific allelopathy that occurs when a plant species releases chemical substances that inhibit or delay germination and growth of the same plant species (Putnam, 1985b; Miller, 1996; Singh et al. 1999). Allelopathy has been investigated in some beans such as in *Pisum sativum* (Kato-Noguchi, 2003), *Mucun pruriens* (Fujii et al. 1991), *Glycine max* (Huber and Abney, 1986; Xiao et al. 2006; Yan and Yang, 2008), and *Cicer arietinum* (Yasmin et al. 1999). L-DOPA and cynamidine has been found to be potential allelochemicals identified in *Mucuna pruriens* and *Vicia villosa*, respectively (Fujii, 2003). It has been found that, in addition to common beans, several other species within the Leguminosae family contain secondary plant products that have allelopathic potential (Rice, 1984). In field experiments, it has been reported that residues and extracts of pea plants suppressed the growth and population size of several plant species (Purvis, 1990; Schenk and Werner, 1991; Tsuchiya and Ohno, 1992; Akemo et al. 2000). Phytotoxic substances in *Pisum sativum* root exudates have been reported by several researchers (Hatsuda et al. 1963;

Yu and Matsui, 1999) and, recently, pisatin has been identified as an inhibitory chemical from its shoots (Kato-Noguchi, 2003). Aqueous leachates of dry shoot of *Phaseolus vulgaris* that contain phenolics showed allelopathic effects on several crop species (Nava-Rodriguez et al. 2005). Autotoxicity due to root exudates found to be involved in growth reduction in *Glycine max* monocropping, which decreased plant biomass and root triphenyl tetrazolium chloride-reducing activity as well as seedlings after exposure to root exudates, exhibited higher activities of superoxide dismutase and guaiacol peroxidase (Xiao et al. 2006).

Successive culture of the same crop on the same land for years cause soil sickness or replanting injuries (Hirano, 1940; Bonner and Galson, 1944; Tsuchiya, 1990) resulting reduction in both crop yield and quality. This phenomenon is evidenced in agricultural cropping system especially in the production of horticultural crops (Young, 1984; Grodzinsky, 1992). It leads to resurgence of disease pest, exhaustion of soil fertility, and developing chemical interference in the rhizosphere referring to allelopathy (Takahashi, 1984; Young, 1984; Hegde and Miller, 1990). Similar to successive culture, in closed hydroponics phytotoxic chemicals accumulated in the culture solution leading to the occurrence of autotoxicity and it was investigated in *Cucumis sativus* (Yu and Matsui, 1994, 1997), *Citrullus lanatus* (Kushima et al. 1998; Hao et al. 2007), *Colocasia esculenta* (Asao et al. 2003), *Fragaria ananassa* (Kitazawa et al. 2005), *Solanum lycopersicum* (Yu and Matsui, 1993), and *Lactuca sativa* (Lee et al. 2006). In this phenomenon, root exudates hamper the plant growth mainly by hampering water and mineral uptake. Previous studies have shown that allelochemicals released from plant roots play an important role in replant injuries of crops. Autotoxicity of root exudates is an important feature for understanding replanting problems in agroecosystem as it represents one of the largest direct inputs of allelochemicals into the rhizosphere environment with potent biological activity and great variation in chemical components (Inderjit and Weston, 2003). The synthesis and exudation of allelochemicals, along with increased overall production of root exudates, is typically

enhanced by stress conditions that the plant encounters such as extreme temperature, drought and UV exposure (Pramanik et al. 2000; Inderjit and Weston, 2003). The removal of the inhibitory chemicals from soils or culture solution can permit continued crop cultivation in the same land for years. Hydroponic culture technique has the facility of trapping and isolating the chemicals released through plant roots. Elimination of these growth inhibitors from recycling culture solution is desirable from the viewpoint of conservation-oriented agriculture. Therefore, many researchers suggested addition of AC to the culture solution to improve growth and yield significantly by adsorbing organic compounds (mainly phenolics), for example in *Fragaria ananassa* (Kitazawa et al. 2005), *Colocasia esculenta* (Asao et al. 2003), *Cucumis sativus* (Yu and Matsui, 1994; Asao et al. 1998a; Asao et al. 1999a; Asao et al. 2000), several leafy vegetables (Asao et al. 2004a), and some ornamentals (Asao et al. 2007a).

The study of autotoxicity in commonly grown beans would provide useful knowledge of sustainable crop production. Thus, identification of the allelochemicals from bean root exudates, evaluation of their phytotoxicity, and their removal would facilitate the maintenance of profitable crop production. Previously, we found evidence of autotoxicity in *Lathyrus ordoratus*, a leguminous crop (Asao et al. 2007b); in this study, we investigated autotoxicity in three beans, namely, *Pisum sativum*, *Phaseolus vulgaris*, and *Vicia faba* as well as their allelochemicals, using hydroponic culture. The phytotoxicity of the identified allelochemicals was evaluated using seedling growth bioassay of the test plants.

2. Materials and methods

2.1. Plant materials

Bean plants viz. *Pisum sativum* cv. Kurume-yutaka, *Phaseolus vulgaris* cv. Taibyou-morokko, and *Vicia faba* cv. Nintoku-1-sun were used in this experiment.

2.2. Plant cultivation either with or without AC

Seeds of the beans under study were germinated on vermiculite on a plastic tray with tap water. The seedlings were transplanted to plastic containers (50 cm × 60 cm × 21 cm) in the greenhouse of Shimane University (Fig. 1). Twelve plants were planted in each container and three containers were used for each treatment (plants either with or without AC) following a randomized block design. The container was filled with 50 liters of 75% Enshi nutrient solution with electrical conductivity (EC) of 2.0 dS m^{-1} (Hori, 1966). Full-strength nutrient solution contains the following amounts of salts 1000 $liters^{-1}$ of tap water: 950 g of $Ca(NO_3)_2.4H_2O$; 810 g of KNO_3; 500 g of $MgSO_4.7H_2O$; 155 g of $NH_4H_2PO_4$; 3 g of H_3BO_3; 2 g of $ZnSO_4.7H_2O$; 0.05 g of $CuSO_4.5H_2O$; 0.02 g of $NaMoO_4$; and 25 g of NaFeEDTA. The nutrient solution in the containers were continuously aerated (3.8 liter min^{-1}) using air pumps with two small air filters each packed with 100 g of AC (Type Y-4P, 4-8 mesh, Ajinomoto Fine Techno Co., Kawasaki, Japan). The same aeration system was maintained for the nutrient solution without AC. The AC was used to trap the chemicals exuded from the plants and was replaced by fresh AC at 2-week intervals until the end of the experiment for efficient adsorption of the chemicals. The used AC was either immediately extracted with alkaline methanol or stored at 4 ºC for later extraction. $FeSO_4 \cdot 7H_2O$ (0.75 g) was added to each solution container at 2-day intervals since the AC that absorbed Fe-EDTA and Fe^{2+} was rapidly oxidized to Fe^{3+} and less available for the plants. During cultivation, the water level of the solution containers was kept constant by regularly adding tap water. Nutrient concentrations (NO_3^-, PO_4^{2-}, K^+, Ca^{2+}, Mg^{2+}, and Fe^{3+}) in the solution were adjusted as close

as possible to the initial concentration at 2-week intervals on the basis of chemical analyses with an atomic absorption spectrometer (AA-630, Shimadzu Co., Kyoto, Japan), a spectrophotometer (UVmini-1240, Shimadzu Co., Kyoto, Japan), and an ion meter (D-23, Horiba, Kyoto, Japan). The pH of the nutrient solutions ranged from 5.7 to 7.1 irrespective of either with or without AC addition. At the end of the experiment, plant length, fresh and dry mass of shoots, dry mass of roots, root length, numbers of pods and seeds, and fresh mass of pods and seeds were recorded.

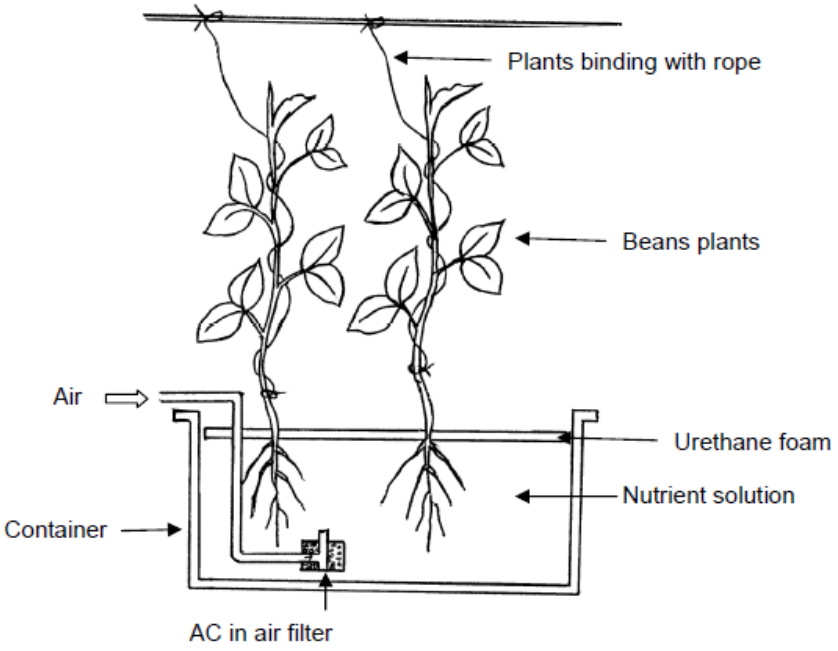

Fig. 1. Hydroponic system used for bean plants cultivation

2.3. GC-MS analysis of root exudates adsorbed in AC

The AC used to trap the exudates (organics) were desorbed three-times using 200 ml 1:1 (v/v) methanol (100 ml):0.4 M aqueous NaOH (100 ml) (Pramanik et al., 2001). Each batch of AC (200 g) was gently shaken with the mixture for 12 h at room temperature (25 ºC) with an electric shaker (20 rpm). The three extracts (600 ml) were combined and filtered through Whatman (No. 6) filter paper. The filtrates were neutralized with 6 M HCl and concentrated to 25 ml in a rotary vacuum evaporator at 40 ºC. Organic compounds in the concentrate were then extracted according to Yu and Matsui (1993). The concentrated AC-extract was adjusted to pH 2.0 with 4 M HCl, extracted three times with 35 ml of refined diethyl ether (DE), and a further three times with 35 ml of ethyl acetate (EA). DE2 and EA2 were the pooled DE and EA extract fractions (105 ml), respectively at pH 2.0. DE2 and EA2 fractions were dried over anhydrous $CaSO_4$ and concentrated to 5 ml each in a rotary evaporator at 40 ºC. Both concentrated fractions (DE2 and EA2) extracted from the AC were analyzed using a gas chromatograph coupled to a mass spectrometer (GC-MS, Hitachi M-80B, Hitachi, Tokyo, Japan) before or after methylation with diazomethane from *N*-methyl-*N*-nitoso-*p*-toluene sulfonamide. An aliquot of each concentrated fraction (1 or 2 ml) was diluted in 50 ml ether, treated with diazomethane and concentrated to 5 µl in a rotary evaporator then in a N_2 stream in a water bath at 35 ºC. One microliter of the concentrated sample was injected into a GC-MS with a capillary column (0.25 mm × 60 m) of TC-5 (GL Science, Tokyo, Japan). Helium was used as the carrier gas at a pressure of 78.4 kN m^{-2}. The column was held initially at 100 ºC for 2 minutes and then raised at 5 ºC $min.^{-1}$ to a final temperature of 260 ºC for 10 minutes. The injector temperature was held at 270 ºC. The ionization voltage and temperature in the electron impact (EI) mode were 70 eV and 250 ºC, respectively.

2.4. Bioassay

The bioassay was carried out according to the method of Asao et al. (1998b). In nature, plant roots exudates allelochemical at a low concentration in the rhizosphere and it depends greatly on the environmental factors. In case of *Cucumis sativus*, the exudation rates varied markedly with the kind of acids, temperatures, and photoperiods, ranging from 0.2 to 4.17 $mg \cdot day^{-1} \cdot plant^{-1}$ (Pramanik et al. 2000). We prepared a series of aqueous solutions of the identified allelochemicals at concentration of 0 (control), 50, 100, 200 and 400 µM with a 75% Enshi nutrient solution (EC 2.0 dS m^{-1}). To determine the most inhibitory concentration, high concentrations (400 µM) of the allelochemicals were bioassayed for their phytotoxicity. The inhibitions of the test solution were assayed by their effect on bean seedlings. Each treatment was replicated 10 times. Test solutions were added to 420 ml flasks wrapped with black polyethylene to avoid direct light on the roots of test plants. The selected plants were transplanted to each flask with urethane foam as support. We planted the bean plants in such a way that roots were inserted into the nutrient solution inside the flask keeping the shoot outside. Urethane foam blocks were used for holding the plants tight and upright at the neck of the flask. The planted flask was placed in a growth chamber at 25 ºC with a light intensity of 74-81 µmol s^{-1} m^{-2} and 16 h photoperiod. To minimize the effect of aeration and the microbial degradation of organic compounds (Sundin and Waechter-Kristensen, 1994) on the bioassay, we renewed the test solutions in the planted flask at every 3 or 4 days. The plants were grown for two weeks and then the number of leaves, maximum leaf length and width, plant length, root length, the fresh and dry mass of shoots, and dry mass of roots were measured.

2.5. Statistical analysis

The growth and yield data obtained from bioassay and hydroponics of bean plants were compiled and analyzed for statistical differences among the treatments and means were separated by analysis of variation with Tukey-Kramer test and t-test (Statcel 2 software, OMS publication, Tokorozawa, Saitama, Japan) at $P < 0.05$.

3. Results and Discussion

3.1. Growth and yield of Pisum sativum, Phaseolus vulgaris, and Vicia faba in hydroponics

The growth and yield of the above three beans were significantly affected by the AC addition in the culture solution (Table 1). In *Pisum sativum*, the plant length, shoot fresh mass, shoot dry mass, and root dry mass declined significantly in the plants grown without AC compared with those grown with AC. The reductions were about 79%, 61%, 67%, and 59% of the values without AC addition, respectively. Similarly, the addition of AC to the nutrient solution greatly improved growth in *Pisum sativum* (Yu and Matsui, 1999). In *Phaseolus vulgaris*, growth was severely retarded in terms of the plant length, shoot fresh mass, shoot dry mass, and root length and dry mass (reductions of about 82, 72, 69, 89, and 66%, respectively), when cultivated without AC. A similar pattern of reduction in growth parameters of *Vicia faba* also appeared. However, there was no significant difference in root length and root dry mass between plants cultivated with and without AC addition. Growth inhibition of the beans without AC addition might be due to the endogenous chemicals from root exudates in the culture solution. In a previous study, the aqueous extracts from leguminous crop residues found to be phytotoxic to crop plants, the aqueous extracts of alfalfa shoots, reduced the germination of alfalfa to 35% and radish to 80% at higher concentrations (Nakahisha et al. 1993). In addition, alfalfa plant extracts significantly affected root growth and morphological differentiation of alfalfa and barnyard grass with increasing concentration, resulting in reduction of their biomass in the presence of either autotoxic or allelopathic compounds (Chon et al. 2002). In other studies, growth inhibition was recovered by the supplementation of AC to nutrient solution or soil, which removed the phytotoxic chemicals from root exudates of *Cucumis sativus* and *Lomandra longifolia* (Asao et al. 1999b; Asao et al. 2007b).

The yields of the bean plants under investigation were significantly affected by the non-renewed nutrient solution either with or without AC addition (Table 1). In control (without AC) plants, the number of pods, their fresh mass, the number of seeds, and fresh mass of seeds in *Pisum sativum* were reduced by half (to about 52%, 52%, 53%, and 54% of the values with AC, respectively). However, the number of pods and pod fresh mass in *Phaseolus vulgaris*, and the number of pods in *Vicia faba* were decreased significantly to about 67%, 64%, and 49%, respectively in without AC supplementation compared to with AC. The results showed that the addition of AC in nutrient solution improved the yield of the beans by adsorbing organic compounds or allelochemicals released by roots. Activated carbon was found to adsorb allelopathic plant exudates in the soil with only small effects on soil nutrients (Callaway and Aschehoug, 2000).

Table 1. Growth and yield of three beans either with or without AC addition in hydroponics.

Beans	AC[z]	Plant length (cm)	FM[y] of shoot (g)	DM of shoot (g)	Root length (cm)	DM of root (g)	No. of pod plant^{-1}	FM of pods plant^{-1} (g)	No. of seeds plant^{-1}	FM of seeds plant^{-1} (g)
Pisum sativum	–	176.2	512.1	141.0	–[w]	41.2	36.2	288.9	199.5	118.5
	+	223.0	838.8	211.2	–	70.3	69.9	557.0	374.5	220.8
		*[x]	*	*		*	**	**	**	**
Phaseolus	–	39.4	56.1	9.7	72.4	3.89	9.8	74.3	–	–
vulgaris	+	48.0	78.4	14.0	81.7	5.85	14.3	116.3	–	–
		**	**	**	**	**	**	**		
Vicia faba	–	74.1	73.4	13.4	57.3	6.0	3.9	–	–	–
	+	88.1	96.2	17.5	60.6	6.2	7.9	–	–	–
		**	*	*	NS	NS	*			

[z] AC added (+), non-AC (–).

[y] Fresh mass *(*FM), Dry mass (DM).

[x] Significant at the 1% (**) and 5% levels (*), and not significant (NS) by t-test.

[w] No data.

Decrease of yield in the non-renewed nutrient solution and its reversal by supplementation of AC were found in *Cucumis sativa* (Asao et al. 1998a), *Colocasia esculenta* (Asao et al. 2003), and *Fragaria ananassa* (Kitazawa et al. 2005) hydroponics. By its very large surface-to-volume ratio, AC has long been known as an adsorbent of organic compounds in soils (Zackrisson et al. 1996), and has been used in greenhouse and field experiments (Prati and Bossdorf, 2004; Callaway et al. 2005; Kulmatiski and Beard, 2006). In laboratory conditions, addition of AC to the medium for plant tissue cultures was found to improve growth by adsorbing toxic metabolites (Wang and Huang, 1976). In field experiments, activated carbon has been shown to adsorb phenols released by *Empetrum hermaphroditum* vegetation and to eliminate the inhibitory effects of *E. hermaphroditum* on tree seedling establishment and growth (Zackrisson and Nilsson, 1992; DeLuca et al. 2002; Thoss et al. 2004).

3.2. Allelochemicals in the root exudates of Phaseolus vulgaris and Vicia faba

Root exudates from *Phaseolus vulgaris and Vicia faba* were analyzed and a number of compounds were detected (Table 2). Benzoic, salicylic, and malonic acids were identified in the root exudates of *Phaseolus vulgaris* whereas lactic, benzoic, *p*-hydroxybenzoic, vanillic, adipic, succinic, malic, glycolic, and *p*-hydroxyphenylacetic acids were detected in the root exudates of *Vicia faba*. In *Vicia faba*, it has been found that phenolic compounds were rapidly released from the emerging root and the amount of phenolics in exudates peaked the first day after seed germination (Bekkara et al. 1998); in another study, salicylic acid was identified as an allelochemical from its root exudates (Schulz and Friebe, 1999). Benzoic, cinnamic, *p*-hydroxybenzoic, 3,4-dihydrobenzoic, vanillic, *p*-coumaric, and sinapic acids were identified from the phytotoxic acidic fraction of the root exudates of *Pisum sativum* (Yu and Matsui, 1999). Several investigations have been

conducted to identify the allelochemicals responsible for autotoxicity in *Glycine max*. Exogenously supplied p-coumaric acid (≥0.25 μM) induced premature cessation of root growth, and increased peroxidase activity and lignin content in it (Zanardo et al. 2009). Allelochemicals like benzoic, vanillic, cinnamic, and ferulic acids showed inhibition in P uptake of *Glycine max* (Baziramakenga et al. 1997); likewise, benzoic acid and *trans*-cinnamic acid reduced root and shoot dry biomass, lowered the amounts of P, K, Mg, Mn, Cl^-, and SO_4^{2-} and reduced leaf chlorophyll content (Baziramakenga et al. 2005). The germinating seeds were responsible for a large portion of the total aliphatic and aromatic acid exudation of seedling plant grown aseptically for 14 days and lactic acid was the predominant aliphatic acid detected in *Pisum sativum* and *Hordeum vulgare* root exudates, whereas malic acid was the predominant acid found in *Pisum sativum, Gossypium* spp. and *Hordeum vulgare* seed exudates (Kovacs, 1971).

Table 2. The allelochemicals identified in the exudates of *Phaseolus vulgaris and Vicia faba* adsorbed on AC added in the nutrient solution.

Allelochemicals	*Phaseolus vulgaris*	*Vicia faba*
Lactic acid	–[z]	+
Benzoic acid	+	+
p-Hydroxybenzoic acid	–	+
Vanillic acid	–	+
Adipic acid	–	+
Succinic acid	–	+
Malic acid	–	+
Glycolic acid	–	+
Salicylic acid	+	+
Malonic acid	+	–
p-Hydroxyphenylacetic acid	–	+

[z] Detected (+) and not detected (–).

3.3. Bioassay with the identified allelochemicals

Seedling growth bioassays were carried out to evaluate the allelopathic potential of the identified chemicals at several concentrations. The phytotoxic effects of the allelochemicals were assayed for growth parameters of *Phaseolus vulgaris and Vicia faba* at several concentrations (Tables 3 and 4). In *Phaseolus vulgaris* grown in benzoic acid solution, the number of leaves, maximum leaf width, shoot fresh mass, and shoot dry mass were significantly reduced to 67, 83, 78, and 84% compared with those of the control, respectively, even at a low concentration (50 µM). Salicylic and malonic acids decreased the number of leaves, shoot fresh mass, and shoot dry mass in snap bean compared with those of the control. *Vicia faba* shoot length, fresh mass, and transpiration rates were affected by salicylic acid at concentrations higher than 3.5 µM after long-term treatments and it was found that guard cells in epidermal peels exhibited a high sensitivity at concentrations as low as 0.001 µM, resulting in stomatal closing. HPLC analysis of methanolic extracts from roots and leaves revealed the presence of free salicylic acid and a metabolite, the amount of which increased with time in plants previously incubated with a medium containing salicylic acid (Barbara et al. 1992).

In *Vicia faba*, benzoic acid at 50 µM significantly reduced root length, and shoots fresh and dry mass to 89, 83, and 81% those of the control, respectively. Adipic and *p*-hydroxyphenylacetic acids decreased root length to 87 and 88% of that of the control, respectively. When three-day-old soybean seedlings were cultivated in nutrient solution containing ferulic or vanillic acid (0.1 to 1 µM) for 48 hours, both compounds (at 0.5 and 1 µM) decreased root length, fresh mass, and dry mass and increased phenylalanine ammonia-lyase contents (Herrig et al. 2002).

Table 3. Effects of the identified phenolic acids at different concentrations on the growth of *Phaseolus vulgaris.*

Allelochemicals	Conc. (μM)	No. of leaves plant^{-1}	Max. leaf length (mm)	Max. leaf width (mm)	Plant length (mm)	Root length (mm)	FM of shoot (g)	DM of shoot (g)	DM of root (g)
None (control)	0	4.2a[z]	110.3a	133.2a	180.8a	141.8a	5.19a	0.55a	0.11a
Benzoic acid	50	2.8b	107.2a	111.0b	173.7a	147.0a	4.05b	0.46b	0.09a
	100	2.8b	98.3b	107.8b	148.0b	146.6a	4.07b	0.43b	0.09a
	200	2.7b	90.2b	90.8b	145.5b	149.2a	3.71b	0.41b	0.08a
	400	2.2c	82.3b	82.5b	132.0c	141.8a	3.44c	0.35c	0.09a
Salicylic acid	50	3.2b	112.5a	126.7a	182.7a	144.2a	4.23b	0.49b	0.11a
	100	2.5c	107.8a	107.8b	148.3b	120.2b	3.34c	0.44b	0.10a
	200	2.3c	89.0b	97.7b	152.8b	125.2b	3.37c	0.31c	0.09a
	400	2.5c	60.5c	65.5c	122.2c	114.2c	3.13c	0.26c	0.06c
Malonic acid	50	3.0b	123.5a	130.2a	178.8a	127.3b	4.45b	0.50b	0.11a
	100	3.0b	122.5a	128.5a	152.2b	125.3b	4.35b	0.46b	0.11a
	200	3.0b	125.2a	125.7a	157.5b	127.2b	4.21b	0.47b	0.11a
	400	3.0b	93.2b	90.3b	145.0b	121.0b	3.87b	0.42b	0.09a

[z] Values in a column followed by a different letter differ significantly by Tukey's test (p=0.05; n=10).

Table 4. Effects of the identified phenolic acids at different concentrations on the growth of *Vicia faba*.

Allelochemicals	Conc. (μM)	No. of leaves per plant	Max. leaf length (mm)	Max. leaf width (mm)	Plant length (mm)	Root length (mm)	FM of shoot (g)	DM of shoot (g)	DM of root (g)
None (control)	0	5.9a[z]	53.0a	102.8a	256.4a	175.8a	6.36a	0.63a	0.32a
Lactic acid	50	6.0a	55.4a	110.7a	249.3a	176.9a	6.18a	0.66a	0.38a
	100	5.9a	56.9a	113.0a	256.0a	176.6a	6.49a	0.64a	0.34a
	200	5.7a	54.6a	105.9a	257.4a	186.6a	6.52a	0.65a	0.34a
	400	5.5a	54.8a	103.7a	267.0a	182.4a	6.95a	0.65a	0.32a
Benzoic acid	50	5.9a	52.6a	105.4a	225.6a	156.1b	5.29b	0.51b	0.34a
	100	6.4a	57.5a	102.1a	252.1a	154.4b	5.41b	0.48b	0.24b
	200	5.9a	56.6a	105.0a	260.6a	151.8b	5.48b	0.45b	0.23b
	400	5.1a	37.9b	86.0b	209.9b	152.3b	4.69b	0.44b	0.18b
p-Hydroxybenzoic acid	50	5.6a	53.8a	103.6a	255.9a	177.5a	6.15a	0.69a	0.35a
	100	5.9a	56.4a	99.5a	262.4a	181.8a	6.22a	0.62a	0.32a
	200	5.8a	50.4a	101.0a	245.8a	168.5a	5.86a	0.69a	0.40a
	400	5.8a	51.3a	98.8a	248.1a	169.4a	5.71a	0.68a	0.37a
Vanillic acid	50	5.9a	49.6a	99.6a	258.8a	184.3a	6.28a	0.68a	0.37a
	100	5.9a	54.3a	108.8a	251.6a	176.9a	6.14a	0.66a	0.37a
	200	6.3a	51.6a	95.3a	256.3a	139.4b	5.95a	0.68a	0.32a
	400	6.3a	50.3a	106.1a	245.4a	145.1b	6.04a	0.70a	0.40a
Adipic acid	50	6.1a	54.3a	99.8a	274.8a	152.6b	6.95a	0.70a	0.36a
	100	6.1a	52.3a	93.5a	261.5a	152.1b	5.89a	0.69a	0.35a
	200	6.1a	51.9a	93.3a	244.4a	145.6b	5.75a	0.63a	0.28a
	400	5.8a	41.9b	100.1a	240.5a	143.3b	5.42b	0.49b	0.24b
Succinic acid	50	5.8a	55.9a	99.4a	276.8a	175.6a	6.45a	0.59a	0.30a
	100	5.9a	56.1a	100.4a	261.0a	176.8a	5.99a	0.59a	0.37a
	200	6.0a	56.9a	98.5a	277.4a	174.9a	6.01a	0.58a	0.30a
	400	6.1a	56.9a	103.6a	269.8a	170.1a	6.07a	0.59a	0.29a
Malic acid	50	6.0a	57.0a	109.1a	273.0a	185.5a	6.77a	0.73a	0.42a
	100	6.1a	54.5a	102.6a	259.8a	176.9a	6.31a	0.65a	0.37a
	200	5.6a	52.1a	105.5a	243.5a	175.1a	5.89a	0.64a	0.39a
	400	5.8a	49.4a	103.9a	253.3a	141.5b	6.02a	0.72a	0.33a
Glycolic acid	50	6.0a	51.4a	119.6a	266.1a	191.4a	6.06a	0.63a	0.23b
	100	6.0a	56.6a	106.8a	273.5a	184.3a	6.45a	0.60a	0.24b
	200	6.1a	55.5a	102.8a	272.0a	182.4a	6.58a	0.62a	0.23b
	400	5.8a	53.8a	100.1a	271.9a	191.3a	6.08a	0.59a	0.21b
p-Hydroxyphenylacetic acid	50	5.9a	50.6a	105.0a	276.9a	154.1b	6.11a	0.66a	0.31a
	100	6.0a	55.3a	100.9a	264.0a	155.3b	6.12a	0.58a	0.28a
	200	6.1a	56.1a	105.6a	254.8a	141.8b	6.20a	0.56a	0.28a
	400	6.0a	55.6a	99.0a	247.9a	146.0b	6.25a	0.63a	0.28a

[z] Values in a column followed by a different letter differ significantly by Tukey's test (p=0.05; n=10).

The above results clearly indicate that the root exudates from bean plants create autotoxicity in non-renewed culture solution (without AC), which leads to retardation in growth and the poor yield. This growth and yield retardation was significantly improved by the addition of AC in the non-renewed culture solution. The potent allelochemicals were detected as benzoic acid, salicylic acid, and malonic acid in *Phaseolus vulgaris*; in *Vicia faba*, they were benzoic acid, adipic acid, glycolic acid, and *p*-hydroxyphenylacetic acid.

4. Summary

The autotoxicity of *Pisum sativum*, *Phaseolus vulgaris*, and *Vicia faba* were investigated in hydroponics either with or without activated charcoal (AC) addition. Growth and yield of the three beans were significantly reduced when grown in the culture solution without AC addition. In *Pisum sativum* plants grown in non-renewed culture solution without AC, the number of pods, pod fresh mass, number of seeds, and seed fresh mass were reduced by about half compared with those with AC. The number of pods plant^{-1} and fresh mass of pods^{-1} plant in *Phaseolus vulgaris*, as well as pod number in *Vicia faba*, were decreased significantly to 49~67% without AC addition. The identified allelochemicals were benzoic, salicylic, and malonic acids in the root exudates of *Phaseolus vulgaris* and lactic, benzoic, *p*-hydroxybenzoic, vanillic, adipic, succinic, malic, glycolic, and *p*-hydroxyphenylacetic acids in *Vicia faba*. Bioassay of the identified allelochemicals revealed that benzoic, salicylic, and malonic acids significantly reduced the growth of *Phaseolus vulgaris* even at low concentrations. In *Vicia faba*, benzoic acid at 50 μM significantly reduced root length, and shoots fresh and dry mass by over 81% of those of the control, whereas adipic and *p*-hydroxyphenylacetic acids decreased root length to 87 and 88% of that of the control, respectively.

Chapter 4

Selection of ideal succeeding crops after asparagus, taro and beans replanting field in seedling growth bioassay

1. Introduction

Successive culture of same crop on the same land for years cause soil sickness or replanting injuries (Rice, 1984; Tsuchiya, 1990) resulting in reduction in both crop yield and quality. This phenomenon is evident in agricultural cropping systems including the production of horticultural crops (Grodzinsky, 1992; Tsuchiya, 1990). It leads to resurgence of disease pest, exhaustion of soil fertility and developing chemical interference in the rhizosphere referring allelopathy (Hedge and Miller, 1990; Komada, 1988; Takahashi, 1984). Replanting problems in continuous cropping usually suggested a chemical interference from previous crops or their residues in the soil. Among the possible reasons of this natural complex phenomenon, self-allelopathy or autotoxicity has often suggested (Asao and Asaduzzaman, 2012; Asao et. al. 2003). Allelopathic effects from crop residues and root exudates have been extensively studied in vegetable crops [alfalfa (Chung et. al. 2011; Miller, 1983; Nakahisa et. al. 1993, 1994), asparagus (Hartlung et. al. 1983; Young, 1984; Young and Chou, 1985), cucumber (Yu and Matsui, 1994; Yu and Matsui, 1997), watermelon (Hao et. al. 2007; Kushima et. al. 1998), taro (Asao et. al. 2003), strawberry (Kitazawa et. al. 2005), tomato (Yu and Matsui, 1993), lettuce (Lee et. al. 2006), and beans (Asaduzzaman and Asao, 2012)]. Among the above horticultural crops, replanting problem due to continuous cropping of asparagus, taro and some beans has been investigated extensively. In asparagus, production in the former asparagus fields (replanting) is less profitable than that in fresh fields without a history of asparagus crops (new planting). This phenomenon is common in older asparagus production areas and is known as the replanting problem (Hartung and Stephen, 1983; Lake et. al. 1993;

Young and Chou, 1985). Therefore, growth inhibition due to continuous cropping of asparagus has become a major problem in the older asparagus production areas. Similarly, taro plants do not grow well if cultivated consecutively for years on the same land (Takahashi, 1984). Rotation with other crops for at least 3-years, in combination with organic matter and soil disinfectants has been suggested to improve the yield of taro. However, even in fixed crop rotation system, there was a great difference in the growth and yield of taro plants. This depends upon the kinds of crops in rotation and their order of rotation. Common beans such as broad bean, garden pea and snap bean are also intensively cultivated in the same farmland year after year and therefore, production of these beans declines in replanting conditions owing to autotoxicity (Miller, 1996; Putnam, 1985a; Singh et. al. 1999). In field experiments, it has been reported that residues and extracts of pea plants suppressed the growth and population size of several plant species (Akemo et. al. 2000).

Strategies to overcome the problems in replanting soil or reuse of hydroponic culture solution had been suggested by many researchers. For example, screening of genotypes in cucumber and many leaf vegetables has revealed that many commercial cultivars have low autotoxic potentials, while others showed strong potential (Asao et. al. 2001), grafting of resistant species as root stock in cucumber extends the harvesting period (Asao et. al. 1999d; Asao et. al. 2000), application of microbial suspension can be used to degrade phytotoxins (Asao et. al. 2004b; Caspersen et. al. 2000; Chen et. al. 2011), or removal of these residues from the soils may be an important step in overcoming soil sickness (Singh et. al. 1999). Activated Charcoal, with its large surface area, pore volume and polarity, has tremendous adsorption capacity for many organic compounds. In cucumber, tomato and asparagus, increased productivity has been observed after using Activated Charcoal in non-renewed solution culture (Asao et. al. 2003; Motoki et. al. 2006, Yu and Matsui, 1994; Yu et. al. 1993). In

addition, by applying TiO_2 photocatalysis and electro-degradation in a recycling hydroponic cultivation system, autotoxicity was avoided in asparagus and strawberry (Asaduzzaman et. al. 2012; Miyama, et. al. 2009; Sunada et. al. 2008). Thus our research thrust was to find a simple, fast and easy method to overcome the replanting problems in the plants under study. Suitable succeeding crops or crop rotation has good control over problems associated with monoculture. It can limit soil sickness or autotoxicity caused by allelopathy to a greater extend (Batish et. al. 2001). Suggesting suitable crops has promising role in eliminating the replanting and/or soil sickness problems in aforesaid crop species.

In this study, we evaluated the growth performances of 67 vegetable crop cultivars through seedling growth bioassay using used nutrient solution of asparagus and also replanting soil of asparagus, taro and three beans. This study also aimed to suggest (i) the possible succeeding crops after asparagus, taro, broad bean, garden pea and snap bean and (ii) bioassays methods suitable for laboratory or field conditions.

2. Materials and Methods

2.1. Plant materials

Seeds of 67 cultivars of 42 vegetable crop species from 14 families were used in the bioassay of once used culture solution of asparagus (*Asparagus officinalis* cv. Welcome), and replanting soil of asparagus, taro (*Colocasia esculenta* cv. Ishikawa-wase), broad bean (*Vicia faba* cv. Nintoku-1-sun), garden pea (*Pisum sativum* cv. Kurumey-utaka) and snap bean (*Phaseolus vulgaris* cv. Taibyou-morokko) (Table 1). Seeds of different vegetable cultivars were collected from the leading seed companies in Japan such as Sakata Seed Co. Yokohama, Takii & Co. Ltd. Kyoto, Kaneko Seeds Co. Ltd. Gunma, Tohoku Seed Co. Ltd. Utsunomiya, and Nakahara Seed. Co. Ltd. Fukuoka.

2.2. Autotoxic compounds of asparagus, taro, and beans

Many potential autotoxic compounds have been identified from the aqueous extracts of dried asparagus roots or root exudates of taro, broad bean, garden pea, and snap bean (Table 2). Growing of these crop species continuously on the same land create replanting injuries leading to yield decline. Therefore, bioassays were designed using used nutrient solution or replanting soil of these crops.

Table 1. Crops and varieties used in bioassays

Family	Vegetable crops	Scientific names	Cultivars
Alliaceae	Onion	*Allium cepa* L.	'Aton'
	Welsh onion	*Allium fistulosum* L.	'Hakata-kuro-negi'
	Welsh onion	*Allium fistulosum* L.	'White Star'
Apiaceae	Carrot	*Daucus carota* L.	'Koigokoro'
	Carrot	*Daucus carota* L.	'Dr. Carotene 5'
	Celery	*Apium graveolens* L.	'Top Seller'
	Mitsuba	*Cryptotaenia japonica*	'Siroguki-mitsuba'
	Parsley	*Petroselinum crispum* L.	'Curly Paramount'
Araceae	Taro	*Colocasia esculenta* Schott.	'Ishikawa-wase'
Asteraceae	Aster	*Kalimeris pinnatifida*	'Stera-rose'
	Burdock	*Aretium lappa*	'Hagobou'
	Chrysanthemum	*Chrysanthemum indicum* L.	'Kikujirou'
	Lettuce	*Lactuca sativa* L.	'Bancyuu-red-fire'
	Lettuce	*Lactuca sativa* L.	'Shato'
	Lettuce	*Lactuca sativa* L.	'Great Lakes 366'
Brassicaceae	Broccoli	*Brassica oleracea* var. *botrytis* L.	'Castle'
	Broccoli	*Brassica oleracea* var. *botrytis* L.	'Syasuta'
	Cabbage	*Brassica oleracea* var. *capitata* L.	'Wakamine'
	Cabbage	*Brassica oleracea* var. *capitata* L.	'Green-ball-sougetsu'
	Cabbage	*Brassica oleracea* var. *capitata* L.	'Early Ball'
	Cabbage	*Brassica oleracea* var. *capitata* L.	'Kiyoshi'
	Cabbage	*Brassica oleracea* var. *capitata* L.	'Terukichi'
	Cauliflower	*Brassica oleracea* var. *botrytis* L.	'Snow Crown'
	Chinese cabbage	*Brassica pekinensis* L.	'Yuuki'
	Chinese cabbage	*Brassica pekinensis* L.	'Kigokoro 365'
	Chinese cabbage	*Brassica pekinensis* L.	'Daifuku'
	Kale	*Brassica oleracea* L.	'Aojiruyoukeru'
	Kale	*Brassica oleracea* L.	'Aojirukeru'
	Komatsuna	*Brassica rapa* var. *komatsuna* L.	'Rakuten'
	Mizuna	*Brassica rapa var. japonica*	'Kyoumizore'
	Pak-choi	*Brassica rapa* var. *chinensis* L.	'Cyouyou'
	Pak-choi	*Brassica rapa* var. *chinensis* L.	'Tyoukou'
	Takana	*Brassica juncea* var. *integrifolia*	'Yanagawa-oosyukuyou-takana'
	Takana	*Brassica juncea* var. *integrifolia*	'Miikeo-obachirimen-takana'
	Takana	*Brassica juncea* var. *integrifolia*	'Yanagawa-suikou'
	Turnip	*Brassica rapa var. rapa* L.	'Swan'
	Turnip	*Brassica rapa var. rapa* L.	'Tsuda-kabu'
Chenopodiaceae	Spinach	*Spinach oleracea* L.	'Puraton'
	Spinach	*Spinach oleracea* L.	'Okame'
Cruciferae	Radish	*Raphanus sativus* L.	'Tibyou-soubutori'
Cucurbitaceae	Cucumber	*Cucumis sativus* L.	'Nankyoku-1gou'
	Cucumber	*Cucumis sativus* L.	'Natu-suzumi'
	Kanpyou	*Lagenaria siceraria* (Molina) Standl.	'Kachidoki-2gou'
	Melon	*Cucumis melo* L.	'Arse-night-natukei-2gou'
	Pumpkin	*Cucubita moschata* L.	'Tetukabuto'
	Tougan	*Benincasa hispida* (Thunb).	'Lion-tougan'
	Tougan	*Benincasa hispida* (Thunb).	'Daimaru-tougan'
	Water melon	*Citrullus lanatus* (Thunb).	'Ibuki'
	Water melon	*Citrullus lanatus* (Thunb).	'Shimaou-max-k'
	Yuugao	*Lagenaria siceraria* (Molina) Standl.	'Don K'
Fabaceae	Broad bean	*Vicia faba* L.	'Nintoku'
	Garden pea	*Pisum sativum* L.	'Taibyou-morokko'
	Garden pea	*Pisum sativum* L.	'Kurumey-utaka'
	Soybean	*Glycine max* L.	'Natunokoe'
	Soybean	*Glycine max* L.	'Tankurou'
	Soybean	*Glycine max* L.	'Nou-hime'
	Soybean	*Glycine max* L.	'Sayamusume'
	Soybean	*Glycine max* L.	'Kiyomidori'
Graminae	Sweet corn	*Zea mays* L.	'Launcher 82'
Lamiaceae	Shiso	*Perilla frutescens* var. *japonica* L.	'Aosiso'
	Shiso	*Perilla frutescens* var. *japonica* L.	'Houkou-aosiso'
Lilliaceae	Asparagus	*Asparagus officinalis* L.	'Welcome'
Malvaceae	Morohair	*Corchorus olitorius* L.	'Moroheiya'
	Okra	*Abelmoschus esculentus* L.	'Green Star'
Solanaceae	Eggplant	*Solanum melongena* L.	'Senryou-2gou'
	Sweet pepper	*Capsicum annuum* L.	'Wonder Bell'
	Tomato	*Solanum lycopersicum* L.	'Momotarou'

Table 2. Autotoxic compounds found in test plants

Plant species	Autotoxic compounds	References
Asparagus (*Asparagus officinalis* L.)	Ferulic, iso-ferulic, malic, citric, fumaric, and caffeic acids	Hartung et. al., 1990
Taro (*Colocasia esculenta* Schott.)	Lactic, benzoic, m-hydroxybenzoic, p-hydroxybenzoic, vanillic, succinic, and adipic acids	Asao et. al., 2003
Broad bean (*Vicia faba* L.)	Lactic, benzoic, p-hydroxybenzoic, vanillic, adipic, succinic, malic, glycolic, and p-hydroxyphenylacetic acids	Asaduzzaman and Asao, 2012
Garden pea (*Pisum sativum* L.)	Benzoic, cinnamic, vanillic, p-hydroxybenzoic, 3,4-dihydroxybenzoic, p-coumaric, and sinapic acids	Yu and Matsui, 1999
Snap bean (*Phaseolus vulgaris* L.)	Benzoic, salicylic, and malonic acids	Asaduzzaman and Asao, 2012

2.3. Preparation of used nutrient solution of asparagus

The experiments were conducted in the greenhouses at Experimental Research Center for Biological Resources Science of the Shimane University. Asparagus cv. 'Welcome' seedlings (14 days old and 30 cm height) were planted in plastic containers (54 cm × 34 cm × 20 cm) filled with 50 liters of 75% standard 'Enshi' nutrient solution with electrical conductivity (EC) of 2.0 dS/m (Fig. 1). The full strength 'Enshi' nutrient solution contains the following amount of salts per 1000 L of tap water: 950 g $Ca(NO_3)_2 \cdot 4H_2O$; 810 g of KNO_3; 500 g of $MgSO_4 \cdot 7H_2O$; 155 g of $NH_4H_2PO_4$; 3 g of H_3BO_3; 2 g of $ZnSO_4 \cdot 7H_2O$; 2 g of $MnSO_4 \cdot 4H_2O$; 0.05 g of $CuSO_4 \cdot 5H_2O$; 0.02 g of Na_2MoO_4; 25 g of NaFe-EDTA (Hori, 1966). Eighteen seedlings were planted in each container supported with four urethane foam (23 mm × 23 mm × 27 mm) blocks and nutrient solution was circulated 24 h by pumps (KP-101, Koshin, Kyoto, Japan) with automatic timer (KS-1500, Iuchi, Osaka, Japan). Asparagus culture was

continued for 2-months, then culture solutions were collected, which was referred to as 'used nutrient solution' of asparagus. This once used nutrient solution of asparagus was passed through Activated Charcoal (Type Y-4P 4-8 mesh, Ajinomoto Fine Techno Co. Kawasaki, Japan) at 20 g per 10 L solution, which is referred to as 'used solution with AC addition'. Concentration of major nutrients were adjusted to 75% nutrient solution based on the chemical analyses with Compact NO_3^- meter (B-343, Horiba, Ltd. Kyoto, Japan) for NO_3^-, Spectrophotometer (U-2900, Hitachi, Tokyo, Japan) for PO_4^{3-} and Polarized Zeeman Atomic Absorption Spectrophotometer (Z-2310, Hitachi, Tokyo, Japan) for K^+, Ca^{2+}, Mg^{2+} and Fe^{3+}.

2.4. Bioassay with used nutrient solution of asparagus

Ten asparagus seedlings of 18-days old were transplanted into a plastic container (29 cm × 17 cm × 9 cm) with urethane foam as support. There were four types of nutrient solutions: New solution either with or without AC and Used solution either with or without AC. Three liters of each nutrient solution was added to each container (4.0 L capacity). The containers were placed in growth chamber at 25/20 ºC (day/night) under fluorescent light with intensity of 74-81 μmol/m^2/s and 12 h photoperiod. The seedlings were grown for 3-weeks and then fresh weight of shoot and root lengths per plant were measured. Seedlings of 30 different vegetable cultivars other than asparagus were also transplanted as per the methods described above. In this case, there were 3-types of nutrient solutions viz., (i) new nutrient solution without AC (Activated Charcoal), (ii) used nutrient solution with AC or (iii) used nutrient solution without AC.

Fig. 1. Preparation and bioassay of used nutrient solution of asparagus. The above figure shows the state of asparagus culture before collection of used nutrient solution and the bellow shows the performance of asparagus seedling in the growth chamber bioassay.

2.5. Preparation of asparagus, taro, broad bean, garden pea and snap bean replanting soil

Field soils from cultivable land and previously not cropped with either asparagus, taro, broad bean, garden pea or snap bean were collected in plastic containers with 60 or 30 kg capacity for creating replant soil. Two years old asparagus seedlings were collected from Takii and Co. Ltd., Kyoto, Japan for use in this study. The seedlings were planted in plastic containers with 30 kg soil and further cultured for 2-years. The soils were incorporated with asparagus root zone and were ground into fine and friable for using as medium of growth for bioassay. The soil thus created was referred to as 'asparagus replanting soil'. Ten taro plantlets were planted in plastic containers with 60 kg of field soil. The culture was continued for 2-years for creating replanting soil of taro. Shoot and corms of taro were harvested and soils were collected and pulverized to make friable for creating 'taro replanting soil'. The seeds of broad bean, garden pea and snap bean were sown in plastic containers with 30 and 60 kg field soil. At maturity beans were harvested and shoots were removed leaving roots in the soil. The cultured soils of each bean were collected, ground into friable for using as growth medium in the respective bioassay. Therefore, soils thus created were referred to as 'replanting soil of broad bean, garden pea or snap bean'.

During the culture period, all plants were supplied with water and nutrient solution for their normal growth and development. Weeding was done as and when necessary. The replant soils of each five plant species were amended with AC at 20 g per 10 kg soils. Thus, we prepared the replanting soil with AC for each plant. The field soil collected from the same land was used as a reference soil to compare the growth performance of the test plants in replanting soil with or without AC (control).

2.6. Bioassay with asparagus and taro replanting soil following direct seed sowing method

Seeds of test vegetables crops were sown in plastic cell trays (285 mm × 285 mm × 57 mm, 16 or 20 cells) in the greenhouse of Shimane University (Fig. 2). The cells were filled with asparagus or taro replanting soil amended with or without AC. Seeds of each test crop species were sown in each cell with either replanting soil with or without AC and there were five cells for each specie. The treatments were replicated thrice using three different cell trays. Watering was done regularly to retain moisture in the soil substrate. The cultivation was continued for 3-4 weeks depending on the germination and seedling growth speed of test crop species. At the end of bioassay, number of leaves, maximum leaf length and width, plant height, and fresh weight of shoot per plant were measured.

2.7. Bioassay with broad bean, garden pea and snap bean replanting soil following seedling transplanting method

Seeds of test vegetable crops were sown in cell trays (54 cm × 28 cm × 4.3 cm, 128 cells) with vermiculite (Fig. 3). Comparatively larger seeds were sown in cell trays with bigger cells (57 mm × 48 mm × 45 mm, 32 cells). At transplanting stage, the seedlings were washed in tap water to remove vermiculite and then transplanted in cell trays (285 mm × 285 mm × 57 mm, 16 or 20 cells). Only radish seeds were sown directly as it is root vegetable. Seedlings were transplanted in broad bean, garden pea or snap bean replanting soil amended with or without AC. One seedling of each crop species was sown per cell with replanting soil either with or without AC and there were five cells for each species and they were replicated thrice using three different cell trays. Watering was done regularly to retain moisture in the soil substrate. The culture was continued for 3-4 weeks depending on the growth speed of different test crop species. At the end of bioassay number of leaves, maximum leaf length and width, plant height, and fresh weight of shoot per plant were measured.

2.8. Statistical analysis

The growth data of each cultivar obtained from each bioassay in nutrient solution, direct seed sowing or seedling transplanting method were compiled and analyzed for statistical differences among the treatments and means were separated by analysis of variation with Tukey-Kramer test and *t*-test (Statcel 2 Software, OMS publication, Tokorozawa, Saitama, Japan) at $P < 0.05$.

Fig. 2. Overview of direct seed sowing bioassay using replanting soil of asparagus, taro and new soil (control as not cultured with either asparagus or taro). Seed of different vegetables were sown directly into the soil to evaluate their growth performance.

Fig. 3. Over view of the seedling growth bioassay using replanting soil of asparagus, taro and three beans in the greenhouse. The above figure shows the seeds in germinating cell trays and the bellow shows the seedling transplanting in the replanting soil.

3. Results and Discussion

Bioassays are integral part of allelopathic studies. The most widely used bioassay to test allelopathic activity is seed germination (Putnam and Tang, 1986; Rice, 1984) however; sensitivity is normally higher in growth bioassays than in germination bioassays (Leather and Enhellig, 1985; Wardle et. al. 1991). Therefore, we have conducted six bioassays using once used nutrient solution of asparagus and replanting the soil of asparagus, taro and three beans to understand their comparative suitability.

3.1. Solution culture bioassay in used nutrient solution of asparagus

Nutrient solution bioassay showed that growth of test species was mostly inhibited, while, few were unaffected due to asparagus used nutrient solution without AC. In case of asparagus, fresh weights of shoot and root length per plant were decreased significantly in replanting soil without addition of AC compared to AC addition (Table 3 and Fig. 4). There were no significant growth differences between plants grown in used nutrient solution with addition of AC and plants grown in new nutrient solution with or without AC. There were no significant differences in growth of asparagus seedlings when grown in new nutrient solution with or without addition of AC.

Nutrient solutions once used for asparagus in hydroponics either with or without AC addition showed significant effects on the shoot fresh weight of thirty vegetable crop species under this study (Fig. 5). Shoot fresh weight of cucumber, garden pea, komatsuna, melon, pak-choi cv. ‘Tyoukou’, parsley and soybean (except cv. ‘Tankurou’) did not differ significantly, hence, these species can be planted after asparagus culture without having allelopathic inhibition by allelochemicals.

Table 3. Effects of nutrient solutions used for asparagus in hydroponics on the growth of asparagus seedlings

Asparagus culture solution	Activated Charcoal addition	Shoot fresh weight/plant (g)	Root length/plant (mm)
New	−	0.63 a	203.9 a
New	+	0.73 a	209.2 a
Used	−	0.50 b	176.4 b
Used	+	0.56 a	182.8 a

'−' and '+' indicates with or without addition of AC in the asparagus culture solution.

Values in a column followed by different letters are significant according to Tukey's test ($P < 0.05$; $n = 10$).

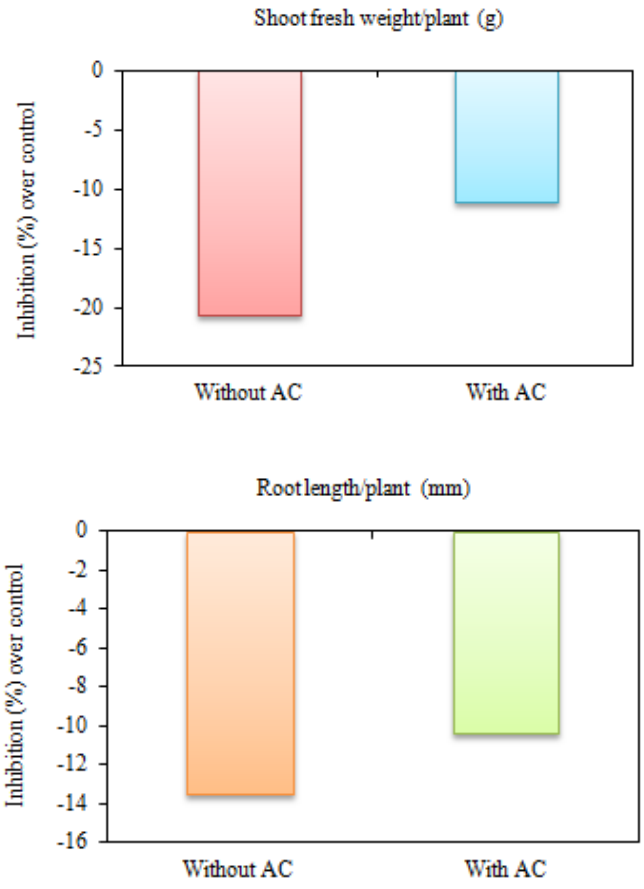

Fig. 4. Inhibitory effects of used nutrient solution of asparagus on shoot fresh weight and root elongation.

Growth of asparagus, aster, cabbage (cv. ‘Wakamine’, ‘Green-ball-sougetsu’ and ‘Kiyoshi’), Chinese cabbage, eggplant, lettuce cv. ‘Banchu-red-fire’, mizuna, okra, soybean cv. ‘Tankurou’, spinach cv. ‘Platon’, sweet pepper, takana cv. ‘Yanagawa-suikou’, tomato, water melon cv. ‘Shimaou-max-k’ and welsh onion cv. ‘Hakata-kuro-negi’ cultivars declined significantly in used nutrient solution than new nutrient solution. On the other hand, this growth inhibition was recovered when plants were grown in used nutrient solution with AC addition except in cabbage (‘Wakamine’ and ‘Kiyoshi’), eggplant, mizuna, okra, soybean (‘Tankurou’), spinach (‘Platon’), and welsh onion (‘Hakata-kuro-negi’). The shoot fresh weight of Chinese cabbage cv. ‘Yuuki’ was significantly decreased in used asparagus nutrient solution even after AC addition, its exact reason was not understood. These results suggested that crops species and also their cultivars should be considered during the selection of succeeding crops of asparagus replanting field to avoid allelopathic injuries.

Growth stimulation in cabbage cv. ‘Early Ball’ and lettuce cv. ‘Shato’ was observed, when grown in used asparagus nutrient solution without AC addition. Perhaps it was due to the stimulatory effects of allelochemicals at lower concentrations (Rice, 1986; Rizvi and Rizvi, 1992). The functional activity of an allelochemical depends on its concentration and time exposure to the test plants. So, it is possible that the quality and quantity of root exudates in nutrient solution in absence of AC might not be sufficient to inhibit growth in these vegetable species, but rather their growth was stimulated. In lettuce, stimulated growth was observed for 2,4-dichlorobenzoic acid even at a concentration of 100 µmol/liter (Pramanik et. al. 2000), although the acid strongly inhibited the cucumber growth at 2 µmol/liter concentration (Asao et. al. 1999c). Bioassay using used nutrient solution of asparagus was studied for whole-plant allelochemical(s) interactions (Enhellig et. al. 1982; Liu and Lovett, 1993). Use of hydroponic nutrient solution enables simulation of naturally release of allelochemicals for chemical interference from living donor plants on living receiver plants (Olofsdotter et. al. 1995;

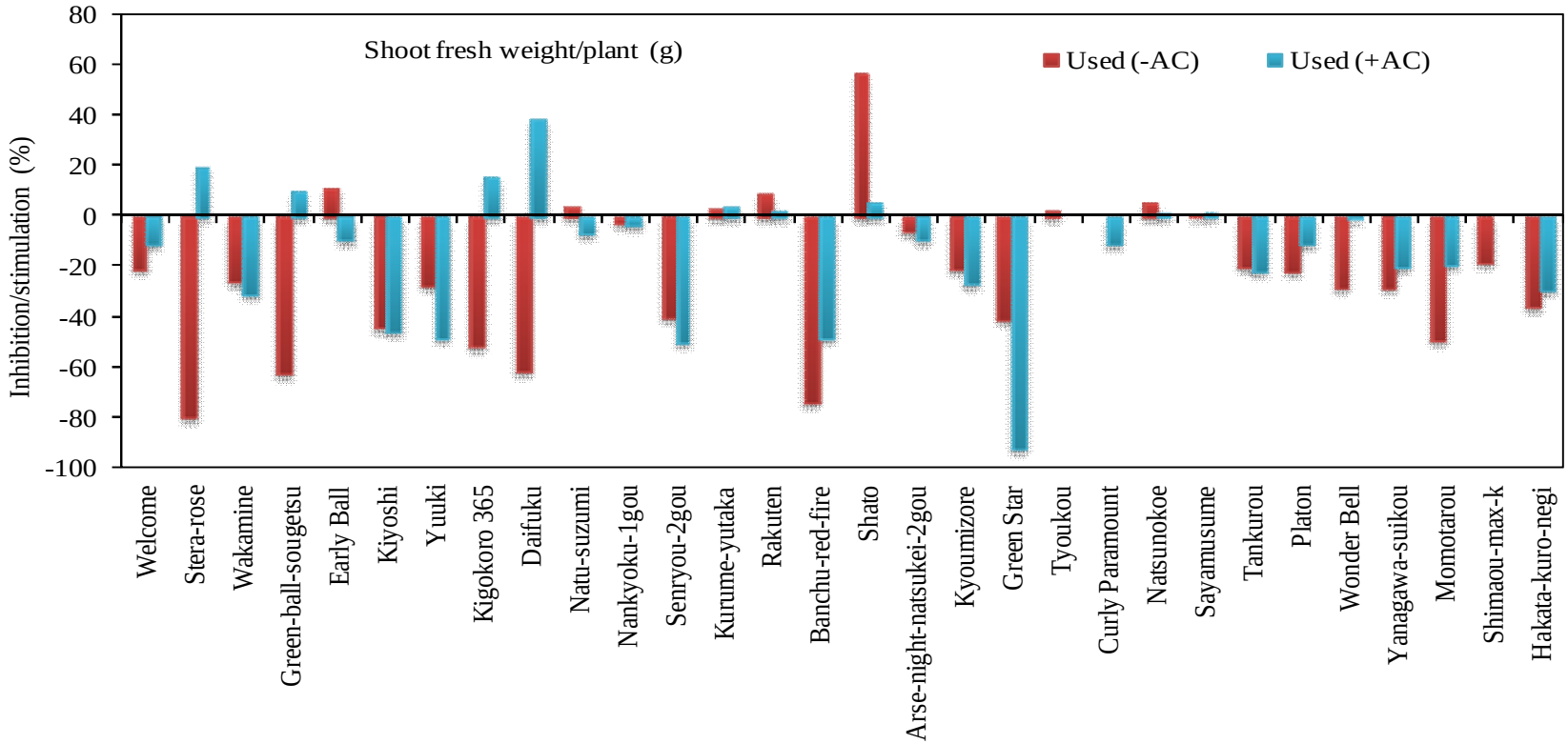

Fig. 5. Effects of nutrient solutions used for asparagus in hydroponics and the addition of AC on the fresh weight of shoot in different vegetable crop cultivars.

Wu et. al. 2000, 2001) and therefore, growth responses of test species were clear. However, results obtained in nutrient solution bioassay may not represent the field condition. Therefore, we have conducted bioassay using replanting soil of plants under study to minimize the gap between laboratory and field conditions.

3.2. Direct seed sowing bioassay of asparagus and taro replanted soil

The conventional bioassay methods used in studying plant-plant interactions does not involve soil and involve an artificial soil substrate. Hence numerous phytotoxins secreted by autotoxic plants were isolated. However, the full complexity of interactions that occur in the natural rhizosphere is eliminated in such a system, and so the obtained results should be analyzed with caution. Indeed, studies conducted without using soil might not reproduce the conditions that are needed for the expression of such allelochemicals in nature (Inderjit and Callaway, 2003). It is also desired that the bioassay should have a relatively rapid response time and the requirements for set-up, including the handling of the plant material, should be simple. This will reduce the cost and labor requirements and increase the ease of treatment application and reproducibility. Overall, an effective bioassay should require minimal equipment and space although it depends on the types of assay and the objectives of study. Therefore, we considered seedling growth bioassay using cell trays which has been widely used to measure the effects of allelopathic compounds retained in the rhizosphere soil of the test species. The bioassays include direct seed sowing in replanting soil of asparagus and taro. Results of these studies showed both inhibitory and stimulatory effects of replanting soil of asparagus, taro and beans on the growth and development of different vegetable crop cultivars.

3.2.1. Asparagus replanted soil

Forty-four vegetable crops species were directly sown in replanted soil of asparagus amended with or without AC. Plant growth was significantly affected by the replanted soil, while it improved with the added AC (Fig. 6). Performances of test plants were evaluated as percent comparing the growth of plants grown without AC (control) with those grown in with AC (Fig. 7). Different plants responded differently to the addition of AC in replanted soil of asparagus in direct seed sowing method. The results indicated that fresh weight in cabbage cv. 'Terukichi', carrot cv. 'Koigokoro', Chinese cabbage cv. 'Yuuki' and 'Kigokoro 365', chrysanthemum, kanpayu, komatsuna, taro and tomato was significantly decreased. Contrarily, it was stimulated in asparagus, broccoli cv. 'Castle', kele cv. 'Aojiruyoukeru', and water melon cv. 'Ibuki' when grown in replanted soil without AC. Chinese cabbage cv. 'Yuuki' and 'Kigokoro 365' (~ 54 and 57%, respectively), taro (~ 48%) and tomato (~ 59%) showed most sensitivity to autotoxicity from asparagus replanting culture in terms of decrease in shoot weight. Number of leaves, maximum leaf length and width, and plant height also declined significantly in some plants grown without AC than with AC (Table 4).

3.2.2. Taro replanted soil

Growth performance of forty vegetable crops species were also evaluated through direct sowing method in replanted soil of taro with or without addition of AC (Fig. 8 and Table 5). The growth of only five plants species was inhibited when grown in replanted soil of taro without AC addition. Shoot fresh weight was decreased about 60, 65, 47, 73, 60, and 64% in burdock, cabbage cv. 'Early Ball', carrot cv. 'Koigokoro', celery, chrysanthemum, and turnip, respectively. Most of the plants under this study were not affected by the allelopathic injuries due to replanting soil of taro.

Fig. 6. Direct seed sowing bioassay using replanting soil of asparagus, taro, and three beans. The above figure shows seedlings growth performance of soybean, pumpkin and soybean, and bellow shows the seedling growth performance of taro.

Moreover, plant growth in terms of fresh weight of shoot was increased significantly in Chinese cabbage cv. ‘Daifuku’, and in soybean cv. ‘Sayamusume’ when grown in replanted taro soil. In above two bioassays, growth performances of test species in replanting soil were mostly unaffected, while few were inhibited significantly (Fig. 7 and 8). That is most of the vegetable crop species under this study can be planted in the field that has been cultured repeatedly with taro. It is also clear that soil bioassay is less sensitive than bioassay using nutrient solution. Moreover in seed germination bioassay delay in emergence under field conditions could additionally be a response of changed environmental factors and response of allelopathic compounds. Therefore, obviously there is need for developing field techniques for separating the effects of allelopathy from other factors.

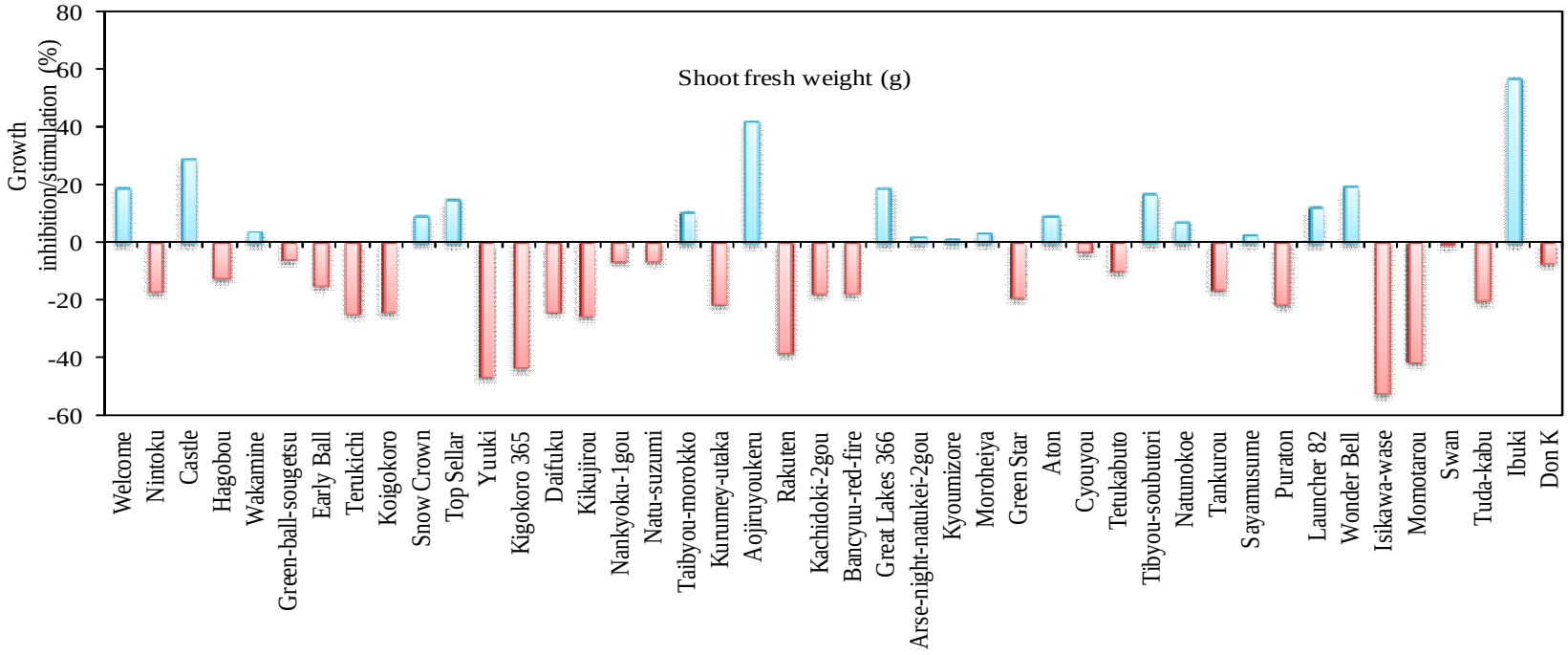

Fig. 7. Shoot fresh weight inhibition/stimulation (%) of different vegetable cultivars over with AC and without AC addition in asparagus replanting soil through direct seed sowing method.

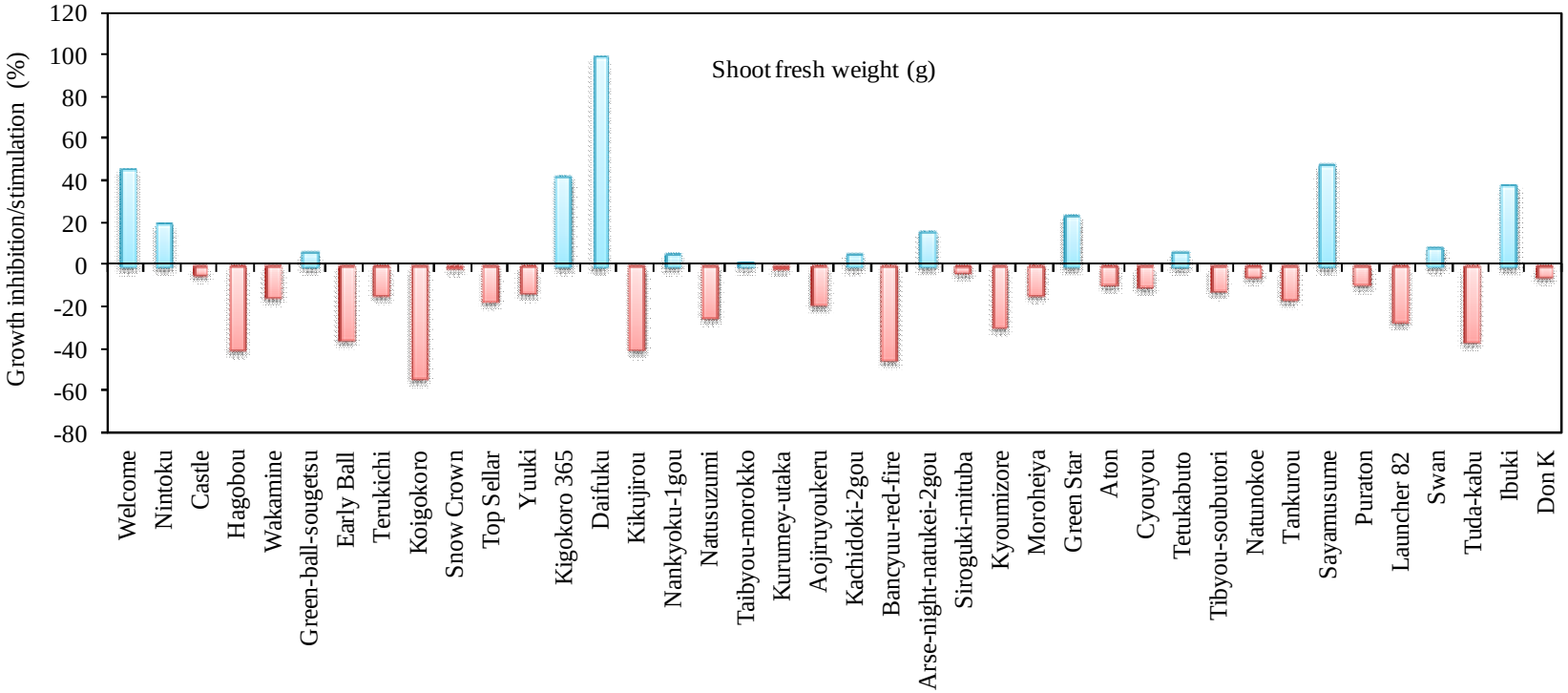

Fig. 8. Shoot fresh weight inhibition/stimulation (%) of different vegetable cultivars over with AC and without AC addition in taro replanting soil through direct seed sowing method.

Table 4. Effects of 'asparagus replanted soil' treated with or without Activated Charcoal on growth performance of directly sown vegetable crops. Growth performance (%) = Growth in absence of AC/Growth in presence of AC × 100.

Vegetables	Cultivars	No. of leaves	Size of leaves		Plant height
			Length	Width	
Asparagus	Welcome	–	–	–	107.1^{ns}
Broad bean	Nintoku	81.5^{*}	88.8^{ns}	81.0^{ns}	84.0^{*}
Broccoli	Castle	94.6^{ns}	121.2^{**}	109.3^{ns}	124.7^{**}
Burdock	Hagobou	114.8^{*}	105.8^{ns}	106.6^{ns}	107.6^{ns}
Cabbage	Wakamine	94.9^{ns}	105.5^{ns}	104.9^{ns}	100.8^{ns}
Cabbage	Green-ball-sougetsu	96.2^{ns}	99.8^{ns}	97.0^{ns}	96.2^{ns}
Cabbage	Early Ball	98.2^{ns}	95.6^{ns}	94.3^{ns}	93.3^{ns}
Cabbage	Terukichi	102.4^{ns}	91.2^{ns}	92.2^{ns}	94.5^{ns}
Carrot	Koigokoro	100.0^{ns}	89.0^{ns}	78.3^{*}	86.8^{*}
Cauliflower	Snow Crown	101.3^{ns}	113.1^{**}	118.2^{*}	103.7^{ns}
Celery	Top Sellar	98.9^{ns}	–	–	–
Chinese cabbage	Yuuki	98.4^{ns}	78.9^{**}	79.0^{**}	81.4^{**}
Chinese cabbage	Kigokoro 365	91.1^{ns}	83.5^{*}	86.3^{ns}	85.2^{*}
Chinese cabbage	Daifuku	99.8^{ns}	97.1^{ns}	103.4^{ns}	98.1^{ns}
Chrysanthemum	Kikujirou	84.1^{*}	84.6^{**}	70.7^{**}	86.1^{*}
Cucumber	Nankyoku-1gou	100.0^{ns}	105.1^{ns}	98.8^{ns}	101.8^{ns}
Cucumber	Natu-suzumi	112.0^{ns}	98.0^{ns}	106.3^{ns}	100.0^{ns}
Garden pea	Taibyou-morokko	105.0^{ns}	99.7^{ns}	107.6^{ns}	105.1^{ns}
Garden pea	Kurumey-utaka	115.9^{ns}	129.0^{ns}	114.3^{ns}	125.3^{ns}
Kale	Aojiruyoukeru	104.6^{ns}	112.2^{ns}	117.5^{ns}	108.3^{ns}
Kamotsuna	Rakuten	80.8^{**}	77.1^{*}	82.4^{ns}	80.7^{*}
Kanpyou	Kachidoki-2gou	107.7^{ns}	94.6^{ns}	104.3^{ns}	93.3^{ns}
Lettuce	Bancyuu-red-fire	100.3^{ns}	106.3^{ns}	96.5^{ns}	168.8^{*}
Lettuce	Great Lakes 366	80.0^{ns}	104.7^{ns}	96.5^{ns}	105.7^{ns}
Melon	Arse-night-natukei-2gou	98.0^{ns}	100.5^{ns}	116.2^{ns}	100.1^{ns}
Mizuna	Kyoumizore	103.2^{ns}	97.8^{ns}	104.8^{ns}	96.9^{ns}
Morohair	Moroheiya	88.0^{ns}	95.8^{ns}	100.8^{ns}	106.9^{ns}
Okra	Green Star	99.8^{ns}	95.8^{ns}	89.4^{*}	102.6^{ns}
Onion	Aton	100.0^{ns}	–	–	105.1^{ns}
Pakchoi	Cyouyou	98.4^{ns}	98.1^{ns}	100.0^{ns}	97.0^{ns}
Pumpkin	Tetukabuto	104.2^{ns}	99.8^{ns}	104.3^{ns}	96.3^{ns}
Radish	Tibyou-soubutori	101.2^{ns}	111.7^{ns}	121.2^{*}	109.5^{ns}
Soybean	Natunokoe	106.3^{ns}	114.9^{ns}	118.4^{ns}	98.0^{ns}
Soybean	Tankurou	91.8^{ns}	94.1^{ns}	88.8^{ns}	91.9^{ns}
Soybean	Sayamusume	100.0^{ns}	110.4^{ns}	111.0^{ns}	90.0^{ns}
Spinach	Puraton	95.2^{ns}	101.8^{ns}	101.8^{ns}	98.5^{ns}
Sweet corn	Launcher 82	100.0^{ns}	107.0^{ns}	110.0^{ns}	100.9^{ns}
Sweet pepper	Wonder Bell	105.7^{ns}	106.0^{ns}	–	–
Taro	Isikawa-wase	86.4^{ns}	66.2^{**}	63.2^{**}	65.6^{**}
Tomato	Momotarou	95.2^{ns}	72.9^{ns}	89.9^{ns}	79.3^{**}
Turnip	Swan	94.9^{ns}	106.2^{ns}	101.1^{ns}	104.8^{ns}
Turnip	Tuda-kabu	93.8^{ns}	89.3^{ns}	92.0^{ns}	90.0^{ns}
Water melon	Ibuki	155.6^{**}	139.9^{*}	122.7^{ns}	133.6^{*}
Yuugao	Don K	84.8^{ns}	96.1^{ns}	101.0^{ns}	96.1^{ns}

'–' indicates no data. $^{ns, *, **}$ Non-significant or significant at $P < 0.05$, 0.01%, respectively according to *t*-test (n = 15).

Table 5. Growth performances of vegetable crops in direct seed sowing bioassay in Taro replanted soil with or without Activated Charcoal. Growth performance (%) = growth in absence of AC/growth in presence of AC × 100.

Vegetables	Cultivars	No. of leaves	Size of leaves		Plant height
			Length	Width	
Asparagus	Welcome	–	–	–	108.8^{ns}
Broad bean	Nintoku	113.7^{ns}	107.2^{ns}	98.0^{ns}	104.9^{ns}
Broccoli	Castle	103.6^{ns}	102.2^{ns}	94.8^{ns}	106.4^{ns}
Burdock	Hagobou	101.8^{ns}	84.8^{ns}	67.8^{**}	78.6^{*}
Cabbage	Wakamine	103.0^{ns}	87.9^{ns}	89.1^{ns}	88.1^{ns}
Cabbage	Green-ball-sougetsu	108.3^{ns}	93.8^{ns}	102.6^{ns}	91.7^{ns}
Cabbage	Early Ball	90.3^{ns}	80.3^{*}	75.1^{**}	84.0^{*}
Cabbage	Terukichi	94.9^{ns}	94.3^{ns}	91.5^{ns}	101.8^{ns}
Carrot	Koigokoro	69.4^{ns}	70.2^{*}	62.5^{**}	75.1^{**}
Cauliflower	Snow Crown	100.6^{ns}	95.3^{ns}	85.4^{ns}	99.9^{ns}
Celery	Top Sellar	96.9^{ns}	–	–	–
Chinese cabbage	Yuuki	110.5^{ns}	89.5^{*}	90.3^{ns}	91.2^{ns}
Chinese cabbage	Kigokoro 365	120.1^{**}	116.7^{ns}	117.0^{ns}	112.6^{ns}
Chinese cabbage	Daifuku	127.4^{**}	137.5^{*}	134.0^{ns}	132.4^{*}
Chrysanthemum	Kikujirou	88.7^{*}	76.9^{*}	70.3^{**}	79.5^{**}
Cucumber	Nankyoku-1gou	105.4^{ns}	106.9^{ns}	101.4^{ns}	102.2^{ns}
Cucumber	Natusuzumi	96.9^{ns}	87.1^{ns}	87.7^{ns}	88.1^{ns}
Garden pea	Taibyou-morokko	99.7^{ns}	99.4^{ns}	104.7^{ns}	102.3^{ns}
Garden pea	Kurumey-utaka	94.2^{ns}	119.8^{ns}	101.8^{ns}	94.9^{ns}
Kale	Aojiruyoukeru	101.3^{ns}	88.2^{ns}	94.3^{ns}	94.4^{ns}
Kanpyou	Kachidoki-2gou	97.4^{ns}	101.0^{ns}	106.7^{ns}	109.1^{ns}
Lettuce	Bancyuu-red-fire	95.7^{ns}	78.4^{ns}	76.4^{ns}	75.7^{ns}
Melon	Arse-night-natukei-2gou	106.7^{ns}	99.8^{ns}	101.7^{ns}	119.8^{ns}
Mitsuba	Siroguki-mituba	100.0^{ns}	102.9^{ns}	99.1^{ns}	113.3^{ns}
Mizuna	Kyoumizore	88.5^{ns}	84.9^{ns}	94.0^{ns}	84.3^{ns}
Morohair	Moroheiya	90.1^{ns}	92.9^{ns}	91.7^{ns}	84.3^{ns}
Okra	Green Star	104.3^{ns}	98.2^{ns}	97.6^{ns}	104.3^{ns}
Onion	Aton	96.4^{ns}	–	–	101.0^{ns}
Pakchoi	Cyouyou	100.8^{ns}	89.0^{ns}	93.4^{ns}	90.8^{ns}
Pumpkin	Tetukabuto	102.7^{ns}	103.7^{ns}	109.0^{ns}	101.4^{ns}
Radish	Tibyou-soubutori	91.2^{ns}	95.7^{ns}	96.8^{ns}	96.5^{ns}
Soybean	Natunokoe	117.2^{*}	100.6^{ns}	101.8^{ns}	110.0^{ns}
Soybean	Tankurou	102.3^{ns}	97.8^{ns}	84.4^{ns}	91.8^{ns}
Soybean	Sayamusume	100.0^{ns}	147.5^{ns}	150.2^{*}	118.7^{**}
Spinach	Puraton	93.3^{ns}	96.7^{ns}	84.0^{ns}	99.3^{ns}
Sweet corn	Launcher 82	91.0^{ns}	90.0^{ns}	93.8^{ns}	103.0^{ns}
Turnip	Swan	115.8^{ns}	96.6^{ns}	102.8^{ns}	99.6^{ns}
Turnip	Tuda-kabu	91.2^{ns}	80.6^{ns}	76.2^{*}	84.5^{ns}
Water melon	Ibuki	125.0^{ns}	101.8^{ns}	109.8^{ns}	126.0^{ns}
Yuugao	Don K	93.8^{ns}	91.7^{ns}	95.9^{ns}	89.1^{ns}

'–' indicates no data. $^{ns, *, **}$ Non-significant or significant at $P < 0.05$, 0.01%, respectively according to *t*-test (n = 15).

3.3. Seedling transplanting bioassay of broad bean, garden pea and snap bean replanted soil

In direct seed sowing bioassay of asparagus and taro replanting soil, the cause of lower seedling emergence is difficult to understand, it might be either due to non-viable seed or allelochemicals inhibition. Thus, we have compared direct seed sowing bioassay with seedling transplanting method using replanting soil of three beans. In practice, most of the vegetable crop seedlings are usually transplanted rather than directly sown in the field. Therefore, seedling transplanting bioassay would be a more practical tool for selecting crop species in successive culture (Fig. 9 and 10).

3.3.1. Broad bean Replanted soil

Seedling growth performance of 55- vegetable crop species were evaluated by seedling transplanting in replanted soil of broad bean, garden pea and snap bean with or without addition of AC (Fig. 11-13). The test vegetable crop species responded differently to the replanted soil of these three beans. In broad bean replanting soil bioassay (Fig. 11 and Table 6), fresh weights of carrot cv. 'Dr. Carotene 5', komatsuna, pumpkin, soybean cv. 'Natunokoe', turnip, and welsh onion cv, 'White Star', were decreased in without AC addition replanted soil that is their growth performance were better in replanted soil with AC. Most of the plants fresh weights were unaffected in replanted soil of broad bean although other growth variables increased or decreased significantly. Some crop species showed stimulated growth in terms of shoot fresh weight when grown in replanted soil without AC, such as broccoli cv. 'Castle', Chinese cabbage cv. 'Yuuki', lettuce cv. 'Shato', melon, morohair, okra, and tomato. Thus the crop species whose growth was unaffected or stimulated can be cultured as succeeding crop.

Fig. 9. Seedling growth bioassay using replanting soil of asparagus, taro, and three beans. The above figure shows corn seedlings growth and bellow shows the seedling growth of Chinese cabbage.

3.3.2. Garden pea Replanted soil

Growth performances were either inhibited or stimulated significantly when transplanted in replanted soil of garden pea (Fig. 12). Decreased growth performance in terms of shoot fresh weights were recorded from cabbage cv. 'Wakamine' and 'Terukichi', carrot cv. 'Dr. Carotene 5', cauliflower, Chinese cabbage cv. 'Yuuki', kale cv. 'Aojirukeru', lettuce cv. 'Shato', mitsuba, soybean cv. 'Tankurou', and tomato. Other growth variables such as number of leaves, maximum leaf length and width, and plant height of these plants were also decreased when grown in replanted soil without AC addition (Table 7). On the other hand, the shoot fresh weight of some vegetable crop species such as celery, Chinese cabbage cv. 'Daifuku', onion, soybean cv. 'Nou-hime', welsh onion cv. 'White Star' were stimulated when grown in replanted soil, which was attributed to improve growth variables.

3.3.3. Snap bean Replanted soil

In case of seedling growth bioassay with replanting soil of snap bean, both inhibitory and stimulatory effects were observed on the growth of several vegetable crop species (Fig. 13 and Table 8). The seedling growth was significantly inhibited in cabbage cv. 'Green-ball-sougetsu', kale cv. 'Aojirukeru', komatsuna cv. 'Rakuten', lettuce cv. 'Shato', mizuna cv. 'Kyoumizore', spinach cv. 'Puraton', and sweet pepper cv. 'Wonder Bell' when grown in replanted soil of snap bean. Growth variables such as number of leaves, maximum leaf length and width, and plant height were also affected by the replanted soil with or without AC addition. It was found that shoot fresh weight in cabbage (~ 55%) and spinach (~ 45%) was severely affected due to replanted soil of snap bean. While some vegetable crop species such as Chinese cabbage cv. 'Yuuki', lettuce cv. 'Bancyuu-red-fire', morohair cv. 'Moroheiya', onion cv. 'Aton', spinach cv. 'Okame' and turnip cv. 'Swan' showed better growth performance in replanting soil of snap bean without AC compared to with AC.

Fig. 10. Seedling growth bioassay using replanting soil of asparagus, taro, and three beans. The above figure shows eggplant seedlings growth and bellow shows the seedling growth of sweet pepper.

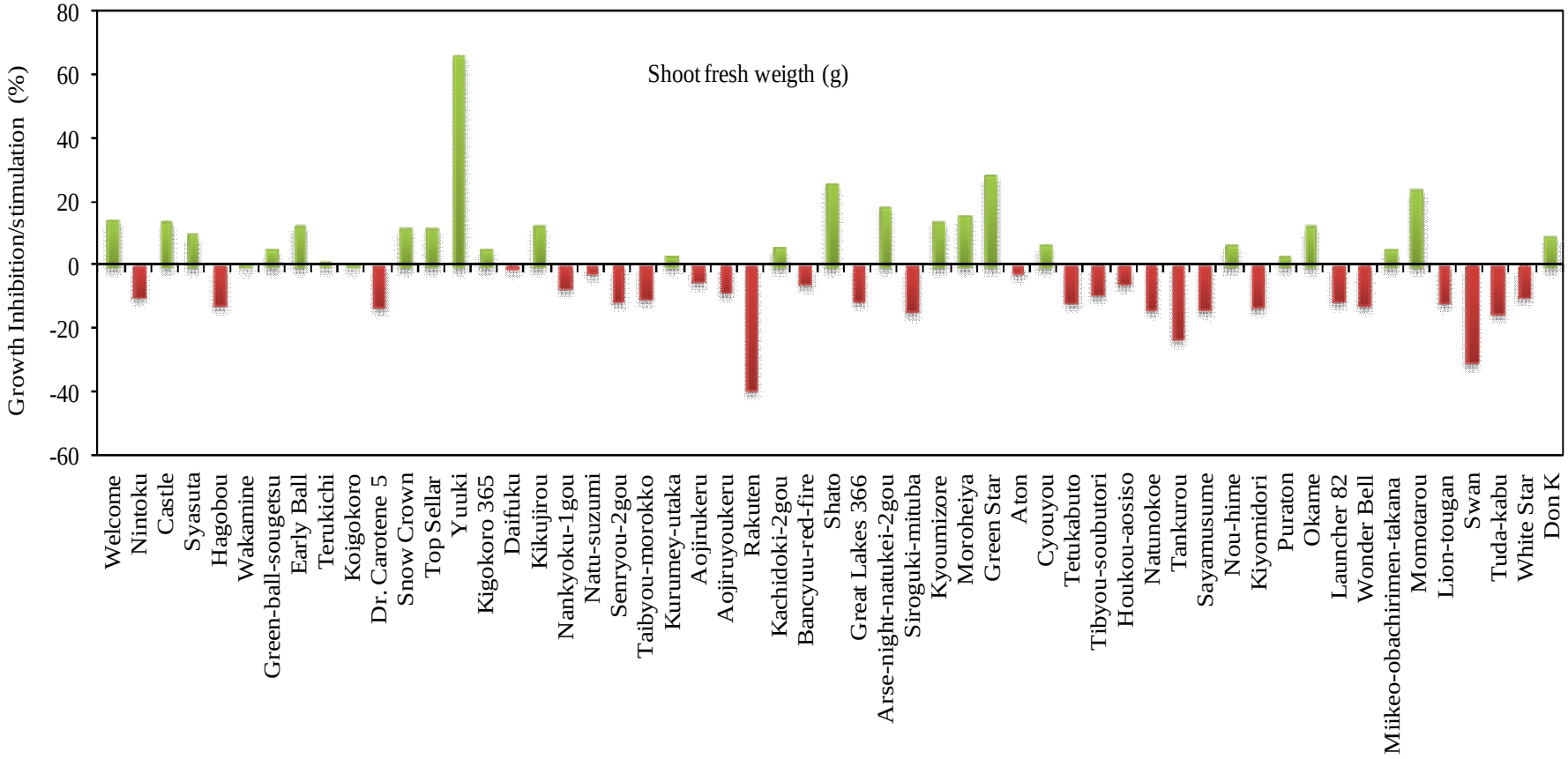

Fig. 11. Shoot fresh weight inhibition/stimulation (%) of different vegetable cultivars over with AC and without AC addition in broad bean replanting soil through seedling transplanting method.

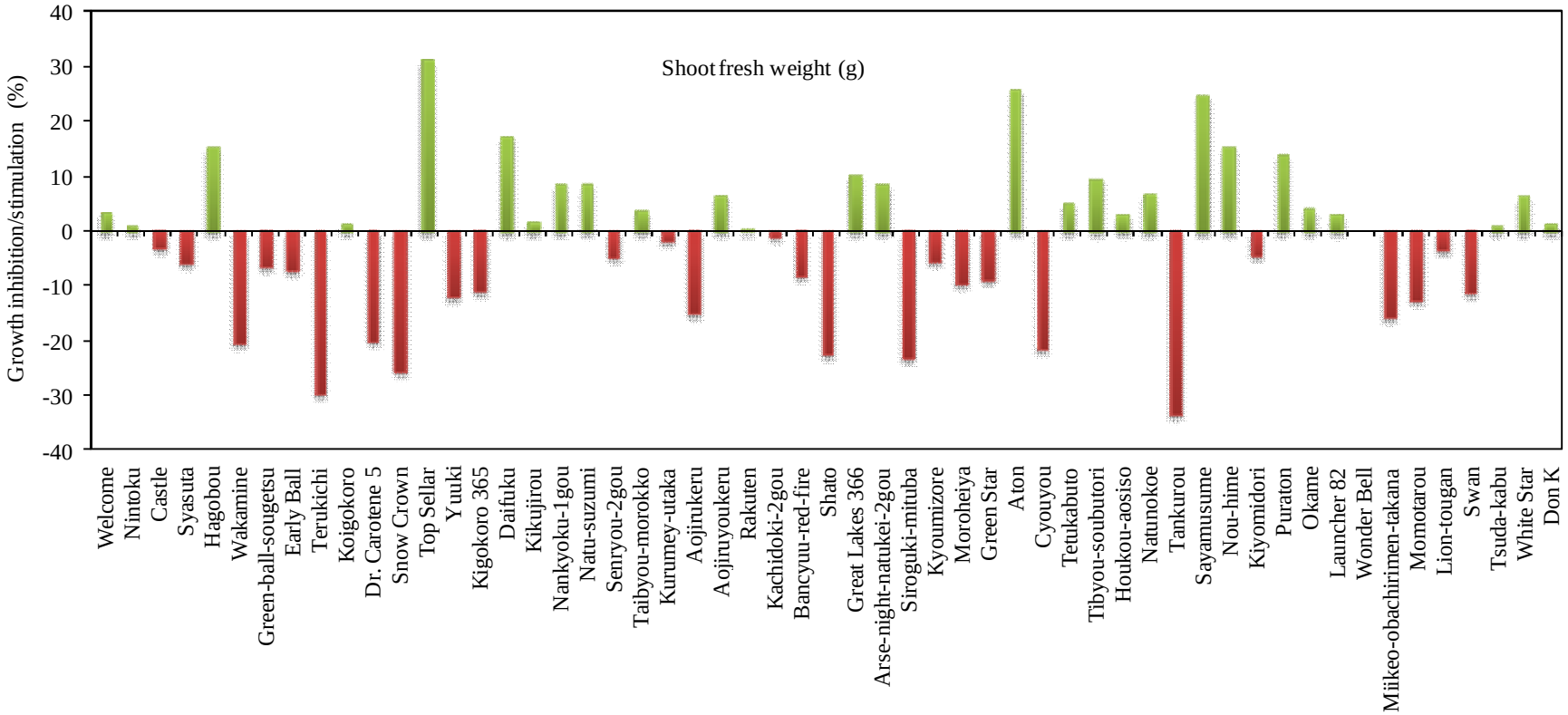

Fig. 12. Shoot fresh weight inhibition/stimulation (%) of different vegetable cultivars over with AC and without AC addition in garden pea replanting soil through seedling transplanting method.

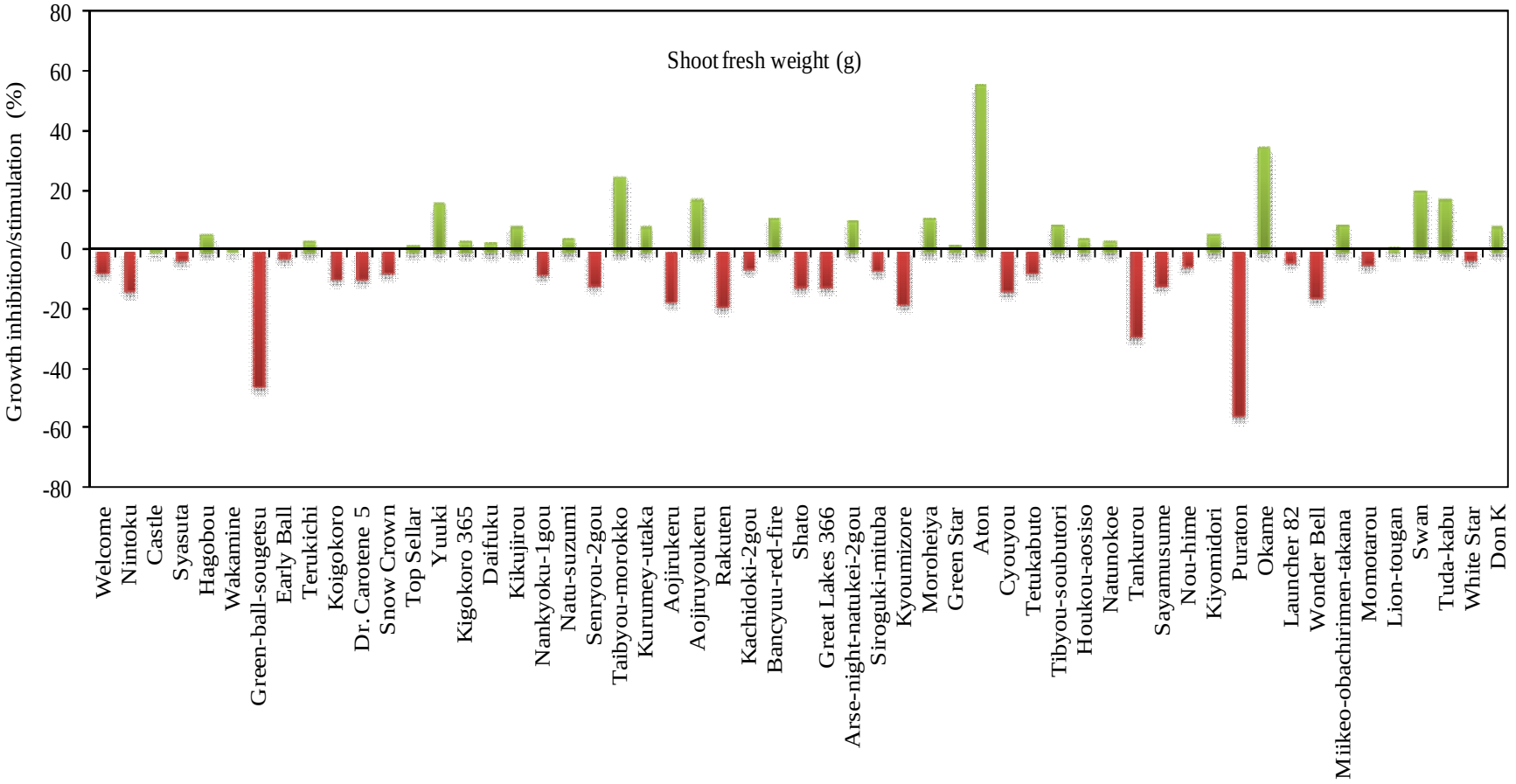

Fig. 13. Shoot fresh weight inhibition/stimulation (%) of different vegetable cultivars over with AC and without AC addition in snap bean replanting soil through seedling transplanting method.

Table 6. Growth performances of vegetable crops in seedling transplanting bioassay in 'Broad bean replanted soil' with or without AC. Growth performance (%) = growth in absence of AC/growth in presence of AC × 100.

Vegetables	Cultivars	No. of leaves	Size of leaves		Plant height
			Length	Width	
Asparagus	Welcome	–	–	–	97.9^{ns}
Broad bean	Nintoku	109.9^{ns}	101.3^{ns}	101.9^{ns}	86.9^{ns}
Broccoli	Castle	97.4^{ns}	103.3^{ns}	106.4^{ns}	106.9*
Broccoli	Syasuta	102.1^{ns}	103.1^{ns}	98.8^{ns}	105.8^{ns}
Burdock	Hagobou	100.0^{ns}	94.7^{ns}	97.2^{ns}	90.3*
Cabbage	Wakamine	102.7^{ns}	101.9^{ns}	103.2^{ns}	100.3^{ns}
Cabbage	Green-ball-sougetsu	97.0^{ns}	87.6**	100.0^{ns}	93.7^{ns}
Cabbage	Early Ball	105.0^{ns}	102.2^{ns}	104.5^{ns}	107.5^{ns}
Cabbage	Terukichi	107.7^{ns}	105.5*	99.7^{ns}	98.7^{ns}
Carrot	Koigokoro	101.7^{ns}	101.8^{ns}	93.0^{ns}	101.2^{ns}
Carrot	Dr. Carotene 5	100.0^{ns}	101.2^{ns}	96.1^{ns}	–
Cauliflower	Snow Crown	102.6^{ns}	105.0^{ns}	103.9^{ns}	105.7^{ns}
Celery	Top Sellar	104.5^{ns}	109.1*	124.7**	–
Chinese cabbage	Yuuki	106.8^{ns}	128.3**	121.8**	123.6**
Chinese cabbage	Kigokoro 365	108.3^{ns}	99.7^{ns}	103.8^{ns}	104.7^{ns}
Chinese cabbage	Daifuku	95.3^{ns}	103.1^{ns}	100.0^{ns}	102.7^{ns}
Chrysanthemum	Kikujirou	94.5^{ns}	101.8^{ns}	109.1^{ns}	98.2^{ns}
Cucumber	Nankyoku-1gou	100.0^{ns}	95.7^{ns}	97.8^{ns}	95.2^{ns}
Cucumber	Natu-suzumi	97.3^{ns}	92.7*	99.7^{ns}	90.4*
Eggplant	Senryou-2gou	101.4^{ns}	98.1^{ns}	104.7^{ns}	94.4^{ns}
Garden pea	Taibyou-morokko	100.0^{ns}	105.4^{ns}	90.0^{ns}	106.5^{ns}
Garden pea	Kurumey-utaka	102.0^{ns}	94.4^{ns}	76.2^{ns}	89.9^{ns}
Kale	Aojirukeru	104.2^{ns}	102.9^{ns}	99.6^{ns}	101.4^{ns}
Kale	Aojiruyoukeru	90.9^{ns}	103.2^{ns}	94.0^{ns}	104.9^{ns}
Kamotsuna	Rakuten	111.1**	82.4**	82.7**	84.3**
Kanpyou	Kachidoki-2gou	100.0^{ns}	94.5^{ns}	97.2^{ns}	104.2^{ns}
Lettuce	Bancyuu-red-fire	98.7^{ns}	91.6*	98.4^{ns}	93.8^{ns}
Lettuce	Shato	94.0^{ns}	110.1*	118.0^{ns}	–
Lettuce	Great Lakes 366	100.0^{ns}	95.1^{ns}	90.5**	92.7^{ns}
Melon	Arse-night-natukei-2gou	124.0**	102.8^{ns}	109.1*	116.3**
Mitsuba	Siroguki-mituba	109.8^{ns}	104.8^{ns}	92.2^{ns}	99.7^{ns}
Mizuna	Kyoumizore	104.8^{ns}	106.5^{ns}	114.6**	104.1^{ns}
Morohair	Moroheiya	114.6**	106.4*	107.4*	111.2**
Okra	Green Star	110.3^{ns}	115.3**	117.4**	118.6**
Onion	Aton	86.5*	–	–	98.7^{ns}
Pakchoi	Cyouyou	90.4^{ns}	102.8^{ns}	104.3^{ns}	99.8^{ns}
Pumpkin	Tetukabuto	100.0^{ns}	88.2**	94.6**	82.6**
Radish	Tibyou-soubutori	100.0^{ns}	104.2^{ns}	103.3^{ns}	97.0^{ns}
Sisso	Houkou-aosiso	100.0^{ns}	99.0^{ns}	100.0^{ns}	95.1^{ns}
Soybean	Natunokoe	100.0^{ns}	94.7^{ns}	101.7^{ns}	94.5^{ns}
Soybean	Tankurou	81.8^{ns}	99.1^{ns}	97.3^{ns}	99.1^{ns}
Soybean	Sayamusume	85.7^{ns}	102.1^{ns}	102.2^{ns}	105.0^{ns}
Soybean	Nou-hime	96.0^{ns}	101.8^{ns}	104.2^{ns}	104.6^{ns}
Soybean	Kiyomidori	90.9^{ns}	106.9^{ns}	105.3^{ns}	94.7^{ns}
Spinach	Puraton	94.5^{ns}	99.2^{ns}	99.7^{ns}	–
Spinach	Okame	111.2*	97.6^{ns}	89.4^{ns}	–
Sweet corn	Launcher 82	95.0^{ns}	104.6^{ns}	109.9^{ns}	96.4^{ns}
Sweet pepper	Wonder Bell	101.3^{ns}	94.8^{ns}	101.8^{ns}	95.6^{ns}
Takana	Miikeo-obachirimen-takana	96.6^{ns}	99.2^{ns}	105.3^{ns}	–
Tomato	Momotarou	100.0^{ns}	106.9^{ns}	100.3^{ns}	110.4^{ns}
Tougan	Lion-tougan	115.4^{ns}	96.8^{ns}	103.1^{ns}	95.4^{ns}
Turnip	Swan	96.8^{ns}	75.8**	81.2**	77.9**
Turnip	Tuda-kabu	107.3^{ns}	100.4^{ns}	97.8^{ns}	100.9^{ns}
Welsh onion	White Star	117.6*	–	–	105.1^{ns}
Yuugao	Don K	100.0^{ns}	103.4^{ns}	105.9^{ns}	107.9^{ns}

'–' indicates no data. $^{ns, *, **}$ Non-significant or significant at $P < 0.05$, 0.01%, respectively according to *t*-test (n = 15).

Table 7. Growth performances of vegetable crops in seedling transplanting bioassay in 'Garden pea replanted soil' with or without AC. Growth performance (%) = growth in absence of AC/growth in presence of AC × 100.

Vegetables	Cultivars	No. of leaves	Size of leaves		Plant height
			Length	Width	
Asparagus	Welcome	–[x]	–	–	112.9[ns]
Broad bean	Nintoku	88.7[ns]	100.0[ns]	102.8[ns]	103.0[ns]
Broccoli	Castle	102.7[ns]	96.4[ns]	96.3[ns]	101.3[ns]
Broccoli	Syasuta	100.0[ns]	96.5[ns]	92.7[*]	97.1[ns]
Burdock	Hagobou	96.9[ns]	105.8[ns]	106.6[ns]	102.9[ns]
Cabbage	Wakamine	94.9[ns]	98.8[ns]	91.5[*]	101.4[ns]
Cabbage	Green-ball-sougetsu	100.0[ns]	95.0[ns]	93.4[ns]	97.7[ns]
Cabbage	Early Ball	102.3[ns]	107.2[ns]	101.5[ns]	103.9[ns]
Cabbage	Terukichi	91.7[ns]	95.7[ns]	90.6[**]	95.9[ns]
Carrot	Koigokoro	96.5[ns]	99.9[ns]	94.9[ns]	98.7[ns]
Carrot	Dr. Carotene 5	91.2[ns]	96.2[ns]	83.1[**]	–
Cauliflower	Snow Crown	106.7[ns]	90.0[ns]	91.6[ns]	93.6[ns]
Celery	Top Sellar	114.3[**]	111.2[*]	119.0[**]	–
Chinese cabbage	Yuuki	98.7[ns]	90.9[**]	112.4[ns]	97.0[ns]
Chinese cabbage	Kigokoro 365	95.8[ns]	99.3[ns]	103.8[ns]	101.2[ns]
Chinese cabbage	Daifuku	100.0[ns]	107.0[ns]	115.7[**]	108.7[**]
Chrysanthemum	Kikujirou	121.9[**]	104.2[ns]	93.9[ns]	101.6[ns]
Cucumber	Nankyoku-1gou	102.8[ns]	105.0[ns]	107.4[ns]	109.5[ns]
Cucumber	Natu-suzumi	105.6[ns]	103.4[ns]	103.1[ns]	119.9[**]
Eggplant	Senryou-2gou	97.0[ns]	100.1[ns]	94.4[ns]	104.6[ns]
Garden pea	Taibyou-morokko	94.1[ns]	107.5[ns]	94.8[ns]	126.1[ns]
Garden pea	Kurumey-utaka	96.2[ns]	102.2[ns]	78.9[ns]	93.0[ns]
Kale	Aojirukeru	93.9[ns]	93.2[ns]	87.5[**]	96.1[ns]
Kale	Aojiruyoukeru	96.6[ns]	100.2[ns]	93.0[ns]	109.2[ns]
Kamotsuna	Rakuten	92.1[ns]	105.3[ns]	101.5[ns]	103.1[ns]
Kanpyou	Kachidoki-2gou	94.7[ns]	100.0[ns]	100.6[ns]	104.7[ns]
Lettuce	Bancyuu-red-fire	95.2[ns]	104.9[ns]	97.3[ns]	105.1[ns]
Lettuce	Shato	97.0[ns]	99.6[ns]	92.4[ns]	–
Lettuce	Great Lakes 366	105.5[ns]	96.2[ns]	86.6[ns]	93.2[ns]
Melon	Arse-night-natukei-2gou	100.0[ns]	110.6[ns]	102.9[ns]	107.1[ns]
Mitsuba	Siroguki-mituba	89.1[ns]	92.3[ns]	88.7[**]	92.6[ns]
Mizuna	Kyoumizore	106.3[ns]	101.8[ns]	98.0[ns]	98.8[ns]
Morohair	Moroheiya	102.1[ns]	92.0[*]	93.3[ns]	96.7[ns]
Okra	Green Star	91.4[ns]	89.9[**]	88.1[**]	93.2[ns]
Onion	Aton	115.6[*]	–	–	111.6[ns]
Pakchoi	Cyouyou	97.5[ns]	95.9[ns]	95.9[ns]	94.0[ns]
Pumpkin	Tetukabuto	97.3[ns]	101.4[ns]	105.1[ns]	112.0[*]
Radish	Tibyou-soubutori	107.8[ns]	107.0[ns]	104.7[ns]	103.8[ns]
Sisso	Houkou-aosiso	100.0[ns]	91.1[ns]	99.4[ns]	97.9[ns]
Soybean	Natunokoe	114.3[ns]	111.3[ns]	99.5[ns]	113.3[*]
Soybean	Tankurou	66.7[ns]	91.6[ns]	102.6[ns]	102.2[ns]
Soybean	Sayamusume	107.1[ns]	115.8[ns]	115.1[ns]	111.4[ns]
Soybean	Nou-hime	104.2[ns]	121.9[**]	106.6[ns]	110.3[*]
Soybean	Kiyomidori	110.0[ns]	93.5[ns]	95.5[ns]	99.0[ns]
Spinach	Puraton	102.7[ns]	100.8[ns]	86.3[*]	–
Spinach	Okame	96.8[ns]	105.6[ns]	107.9[ns]	–
Sweet corn	Launcher 82	97.5[ns]	99.5[ns]	99.4[ns]	104.8[ns]
Sweet pepper	Wonder Bell	106.0[ns]	106.0[ns]	105.3[ns]	98.6[ns]
Takana	Miikeo-obachirimen-takana	93.0[ns]	92.2[ns]	98.8[ns]	–
Tomato	Momotarou	100.2[ns]	96.4[ns]	97.7[ns]	94.8[ns]
Tougan	Lion-tougan	86.2[ns]	85.3[*]	92.9[ns]	102.9[ns]
Turnip	Swan	100.0[ns]	92.7[ns]	112.3[ns]	92.2[*]
Turnip	Tsuda-kabu	103.5[ns]	106.9[ns]	96.9[ns]	106.9[ns]
Welsh onion	White Star	97.6[ns]	–	–	107.3[ns]
Yuugao	Don K	94.7[ns]	112.0[ns]	101.3[ns]	109.9[ns]

'–' indicates no data. [ns, *, **] Non-significant or significant at $P < 0.05$, 0.01%, respectively according to *t*-test (n = 15).

Table 8. Growth performances of vegetable crops in seedling transplanting bioassay in 'Snap bean replanted soil' with or without AC. Growth performance (%) = growth in absence of AC/growth in presence of AC × 100.

Vegetables	Cultivars	No. of leaves	Size of leaves		Plant height
			Length	Width	
Asparagus	Welcome	–	–	–	127.2*
Broad bean	Nintoku	83.3^{ns}	95.2^{ns}	96.0^{ns}	94.6^{ns}
Broccoli	Castle	100.0^{ns}	102.4^{ns}	97.0^{ns}	99.3^{ns}
Broccoli	Syasuta	97.9^{ns}	102.1^{ns}	98.1^{ns}	99.4^{ns}
Burdock	Hagobou	100.0^{ns}	103.3^{ns}	105.6^{ns}	98.1^{ns}
Cabbage	Wakamine	108.1^{ns}	105.5^{ns}	102.0^{ns}	104.9^{ns}
Cabbage	Green-ball-sougetsu	111.1^{ns}	79.1*	77.8**	83.6*
Cabbage	Early Ball	102.5^{ns}	100.2^{ns}	92.3^{ns}	102.6^{ns}
Cabbage	Terukichi	93.8^{ns}	109.0^{ns}	103.1^{ns}	111.7**
Carrot	Koigokoro	101.9^{ns}	99.8^{ns}	97.5^{ns}	101.5^{ns}
Carrot	Dr. Carotene 5	98.0^{ns}	94.2^{ns}	85.7**	–
Cauliflower	Snow Crown	98.0^{ns}	100.8^{ns}	103.2^{ns}	99.1^{ns}
Celery	Top Sellar	103.1^{ns}	93.3*	110.2^{ns}	–
Chinese cabbage	Yuuki	109.0*	99.1^{ns}	101.2^{ns}	99.4^{ns}
Chinese cabbage	Kigokoro 365	97.3^{ns}	95.7^{ns}	97.4^{ns}	104.3^{ns}
Chinese cabbage	Daifuku	98.7^{ns}	102.6^{ns}	104.0^{ns}	104.3^{ns}
Chrysanthemum	Kikujirou	104.6^{ns}	112.6^{ns}	112.9*	103.7^{ns}
Cucumber	Nankyoku-1gou	97.2^{ns}	100.0^{ns}	107.0^{ns}	102.5^{ns}
Cucumber	Natu-suzumi	100.0^{ns}	102.2^{ns}	110.2**	104.8^{ns}
Eggplant	Senryou-2gou	92.1^{ns}	96.5^{ns}	90.1*	90.7*
Garden pea	Taibyou-morokko	94.1^{ns}	114.6^{ns}	91.6^{ns}	103.7^{ns}
Garden pea	Kurumey-utaka	100.0^{ns}	134.2*	77.2^{ns}	73.3^{ns}
Kale	Aojirukeru	98.0^{ns}	92.1^{ns}	93.5^{ns}	92.1^{ns}
Kale	Aojiruyoukeru	91.3^{ns}	120.1*	120.4*	107.6^{ns}
Kamotsuna	Rakuten	100.0^{ns}	83.9**	88.1**	86.0**
Kanpyou	Kachidoki-2gou	100.0^{ns}	97.5^{ns}	93.4^{ns}	90.4^{ns}
Lettuce	Bancyuu-red-fire	98.9^{ns}	102.3^{ns}	111.6^{ns}	100.1^{ns}
Lettuce	Shato	97.1^{ns}	97.6^{ns}	101.1^{ns}	–
Lettuce	Great Lakes 366	105.3^{ns}	93.2^{ns}	90.3*	93.1^{ns}
Melon	Arse-night-natukei-2gou	112.0^{ns}	115.8**	109.8^{ns}	115.5**
Mitsuba	Siroguki-mituba	105.1^{ns}	106.1^{ns}	90.1*	94.3^{ns}
Mizuna	Kyoumizore	97.6^{ns}	93.8^{ns}	91.9**	95.3^{ns}
Morohair	Moroheiya	106.7*	100.9^{ns}	106.3^{ns}	100.8^{ns}
Okra	Green Star	96.3^{ns}	95.2^{ns}	94.7^{ns}	102.6^{ns}
Onion	Aton	119.4**	–	–	115.0**
Pakchoi	Cyouyou	91.8*	101.4^{ns}	100.9^{ns}	100.3^{ns}
Pumpkin	Tetukabuto	100.0^{ns}	97.0^{ns}	102.0^{ns}	94.6^{ns}
Radish	Tibyou-soubutori	102.1^{ns}	101.5^{ns}	106.1^{ns}	95.2^{ns}
Sisso	Houkou-aosiso	100.0^{ns}	90.8*	96.7^{ns}	93.6^{ns}
Soybean	Natunokoe	100.0^{ns}	98.9^{ns}	109.8^{ns}	97.3^{ns}
Soybean	Tankurou	112.5^{ns}	81.1^{ns}	86.0^{ns}	85.1^{ns}
Soybean	Sayamusume	104.1^{ns}	99.4^{ns}	97.4^{ns}	105.5^{ns}
Soybean	Nou-hime	95.8^{ns}	101.9^{ns}	98.8^{ns}	100.8^{ns}
Soybean	Kiyomidori	100.0^{ns}	117.5^{ns}	109.2^{ns}	110.2^{ns}
Spinach	Puraton	81.7**	85.9**	83.5*	–
Spinach	Okame	98.9^{ns}	114.1*	120.5*	–
Sweet corn	Launcher 82	89.5^{ns}	93.7^{ns}	90.8^{ns}	99.4^{ns}
Sweet pepper	Wonder Bell	98.0^{ns}	92.7^{ns}	89.5^{ns}	98.5^{ns}
Takana	Miikeo-obachirimen-takana	94.2^{ns}	100.3^{ns}	110.7^{ns}	–
Tomato	Momotarou	96.0^{ns}	96.2^{ns}	90.2^{ns}	101.7^{ns}
Tougan	Lion-tougan	91.7^{ns}	107.7^{ns}	102.2^{ns}	115.2^{ns}
Turnip	Swan	94.2^{ns}	114.9**	109.0^{ns}	115.5**
Turnip	Tuda-kabu	107.4^{ns}	101.5^{ns}	91.3^{ns}	112.3**
Welsh onion	White Star	97.4^{ns}	–	–	109.5*
Yuugao	Don K	93.8^{ns}	105.1^{ns}	106.9^{ns}	102.1^{ns}

'–' indicates no data. $^{ns, *, **}$ Non-significant or significant at $P < 0.05$, 0.01%, respectively according to *t*-test (n = 15).

From all bioassays it was found that cultivars of the same species (broccoli, cabbage, carrot, Chinese cabbage, kale, lettuce, soybean, spinach and turnip) responded differently in used nutrient solution or in replanting soil with or without AC addition. These results suggested that susceptibility to autotoxicity or allelopathy might occur not only due to the differences in species (Asao et. al., 2001) but also due to its cultivars. When the test species were transplanted into replanting soil, the responses were more conspicuous than previous bioassays. In some species either growth inhibition or stimulation was significant but most of the test species were unaffected in replanting soil (Fig. 11-13). Due to the difficulty of separating competitive from allelopathic interactions under field conditions (Miller, 1969), allelopathic studies have been based mainly upon biological assays conducted under laboratory or controlled conditions. In our studies we have planted each plant to each cell to minimize the resource competition. However, we could not consider the interactions such as synergism, antagonism or additive effects in soil bioassay. Recently He et al. (2012) studied the separation of allelopathy from resource competition using rice/barnyard grass mixed-cultures using hydroponics and results showed that the competitive ratio as one rice accession PI312777 plant was more competitive than 2 barnyard grass plants. The allelopathic effects of PI312777 were much more intense than the resource competition in rice/barnyard grass mixed cultures.

We compared growth performance of test cultivars in replanting soil against AC amended replanting soil as control. AC is widely used to adsorb the accumulated phytotoxic chemicals for the culture solution and improve the growth and yield in strawberry (Kitazawa et. al. 2005), taro (Asao et. al. 2003), cucumber (Asao et. al. 1999a; Asao et. al. 2000; Asao et. al. 1998b; Yu and Matsui, 1994), several leafy vegetables (Asao et. al. 2004a) and some ornamentals (Asao et. al. 2007). In a field replanted with asparagus, AC incorporation increased the fresh weight of rhizomes and storage roots (Motoki et. al. 2006). Although

recent research showed undesired side effects of activated carbon for testing allelopathy in invasive plant by changing the substrate chemistry substantially (Weißhuhna and Pratib, 2009), in a short period soil bioassay has no detrimental effects on soil properties.

In seedling growth bioassay, we evaluated the growth of 67- vegetable crops cultivars, using used nutrient solution of asparagus and also replanting in soil of asparagus, taro, broad bean, garden pea and snap bean to find suitable succeeding crops (Table 9). Three methods of bioassay viz., (i) ussed nutrient solution, (ii) direct seed sowing and (iii) seedling transplanting in the replanting soil of the test species were evaluated. Asparagus used nutrient solution bioassay has most sensitivity to seedling growth but this result may not represent the field condition. Therefore, bioassays using replanting soil of asparagus were also done. In case direct seed sowing method, no or delayed germination was main problem, while the seedling transplanting method was similar to field conditions. Thus, we suggested that nutrient solution bioassay to select test crop species in laboratory condition and seedling transplanting bioassay in greenhouse condition are ideal.

Table 9. Lists of vegetable crops which can be successfully grown as preceding crop of asparagus, taro and three beans

Preceding crops	Succeeding crops	Selection methods
Asparagus	cabbage cv. 'Early-ball', cucumber, garden pea, komatsuna, lettuce cv. 'Shato', melon, pak-choi cv. 'Tyoukou', parsley and soybean (except cv. 'Tankurou').	**Seedling growth bioassay in used nutrient solution of asparagus**
	asparagus, broccoli cv. 'Castle', cabbage cv. 'Wakamine', cauliflower, celery, garden pea cv. 'Taibyou-morokko', kale cv. 'Aojiruyoukeru', lettuce cv. 'Great Lakes 366', melon, mizuna, morohair, onion, radish, soybean cv. 'Natunokoe' and 'Sayamusume', sweet corn, sweet pepper and water melon cv. 'Ibuki'.	**Direct seeding bioassay in asparagus replanting soil**
Taro	asparagus, broad bean, cabbage cv. 'Green-ball-sougetsu', Chinese cabbage cv. 'Kigokoro 365' and 'Daifuku', cucumber cv. 'Nankyoku-1gou', garden pea cv. 'Taibyou-morokko', kanpyou, melon, okra, pumpkin, soybean cv. 'Sayamusume', turnip cv. 'Swan', and water melon cv. 'Ibuki'.	**Direct seeding bioassay in taro replanting soil**
Broad bean	asparagus, broccoli cv. 'Castle' and 'Syasuta', cabbage, carrot cv. 'Koigokoro', cauliflower, celery, Chinese cabbage cv. 'Yuuki' and 'Kigokoro 365', chrysanthemum, garden pea cv. 'Kurumey-utaka', kanpyou, lettuce cv. 'Shato', melon, mizuna, morohair, okra, pak-choi cv. 'Cyouyou', soybean cv. 'Nou-hime', spinach cv. 'Puraton' and 'Okame', takana cv. 'Miikeo-obachirimen-takana', tomato, and yuugao.	**Seedling transplanting bioassay in broad bean replanting soil**
Garden pea	asparagus, broad bean, burdock, carrot cv. 'Koigokoro', celery, Chinese cabbage cv. 'Daifuku', chrysanthemum, cucumber cv. 'Nankyoku-1gou' and 'Natu-suzumi', garden pea cv. 'Taibyou-morokko', kale cv. 'Aojiruyoukeru', komatsuna, lettuce cv. 'Great Lakes 366', melon, onion, pumpkin, radish, sisso cv. 'Houkou-aosiso', soybean (except cv. 'Tankurou'), spinach cv. 'Okame', sweet corn, sweet pepper, turnip cv. 'Tsuda-kabu', welsh onion cv. 'White Star' and yuugao.	**Seedling transplanting bioassay in garden pea replanting soil**
Snap bean	broccoli cv. 'Castle', burdock, cabbage cv. 'Wakamine' and 'Terukichi', celery, Chinese cabbage, chrysanthemum, cucumber cv. 'Natu-suzumi', garden pea, kale cv. 'Aojirukeru', lettuce cv. 'Bancyuu-red-fire', melon, morohair, okra, onion, radish, sisso cv. 'Houkou-aosio', soybean cv. 'Natunoke' and 'Kiyomidori', spinach cv. 'Okame', takana cv. 'Miikeo-obachirimen-takana', tougan cv. 'Lion-tougan', turnip, and yuugao.	**Seedling transplanting bioassay in snap bean replanting soil**

4. Summary

Sixty seven cultivars of 42 vegetable crop species from 14 families were tested in seedling growth bioassay using the used nutrient solution of *Asparagus officinalis* L. and replanting soil of *Asparagus officinalis* L., *Colocasia esculenta* Schott., *Vicia faba* L., *Pisum sativum* L. and *Phaseolus vulgaris* L. to select possible succeeding crops. Replanting problems due to continuous cropping is typically a chemical interference from previous crops or their residues in the soil and autotoxicity has been often suggested as one of the possible reasons. Growth performances of succeeding crops were assayed using once used nutrient solution and/or replanting soil of these crops. Bioassay using asparagus used nutrient solution with or without AC suggest the tested cultivar of cucumber, garden pea, komatsuna, melon, pak-choi cv. 'Tyoukou', parsley, soybean (except cv. 'Tankurou'), cabbage cv. 'Early Ball' and lettuce cv. 'Shato' as succeeding crops. Bioassay using replanting soil with or without Activated Charcoal suggested that most of the cultivars tested can be planted after asparagus, taro, and three beans (*Vicia faba* L., *Pisum sativum* L. and *Phaseolus vulgaris* L.) with little adverse effects. Among the three methods of bioassay used (i). Nutrient solution, (ii). Direct seed sowing and (iii). Seedling transplanting in replant soil; the nutrient solution bioassay proved more sensitive than replanting soil bioassay. However, results of nutrient solution bioassay may not be reproducible in the field condition. Therefore, seedling transplanting method can be used as an easy and practical bioassay method to select succeeding crops for fields with replanting problems.

Chapter 5

General summary

In growth chamber bioassay, strawberry plants grown in non-renewed nutrient solution and electro-degraded (ED) weekly intervals showed growth inhibition whereas biweekly ED improved its growth. This growth inhibition in weekly ED nutrient solution was found to be attributed by the degradation Fe-EDTA (~ 10%) and low concentration of Ca^{2+} in culture solution. In without plant experiments, low pH (3.13) and increased temperature were two major constraints for longer ED duration (24 hours). Therefore, considering electric voltage, EC and pH, shorter ED duration (two hours) was selected for further experiments. In green house study, growth and yield of strawberry plants was decreased in non-renewed nutrient solution and its biweekly ED treatment but it was improved in non-renewed nutrient solution when ED applied at four weeks intervals. It was found that fruit yield of strawberry plant was completely recovered (≈ 99%) in non-renewed nutrient solution with ED at every four weeks, whereas, in our previous study the recovery was 71% compared to renewed nutrient solution. Therefore, we recommend application of ED to non-renewed nutrient solution for two hours at every four weeks intervals to avoid autotoxicity in strawberry in a closed hydroponic system.

The autotoxicity of *Pisum sativum*, *Phaseolus vulgaris*, and *Vicia faba* were investigated in hydroponics either with or without AC addition. In *Pisum sativum* plants grown in non-renewed culture solution without AC, the number of pods, pod fresh mass, number of seeds, and seed fresh mass were reduced by about half compared with those with AC. The number of pods $plant^{-1}$ and fresh mass of $pods^{-1}$ plant in *Phaseolus vulgaris*, as well as pod number in *Vicia faba*, were decreased significantly to 49~67% without AC addition. The identified

allelochemicals were benzoic, salicylic, and malonic acids in the root exudates of *Phaseolus vulgaris* and lactic, benzoic, *p*-hydroxybenzoic, vanillic, adipic, succinic, malic, glycolic, and *p*-hydroxyphenylacetic acids in *Vicia faba*. Bioassay of the identified allelochemicals proved that benzoic, salicylic, and malonic acids significantly reduced the growth of *Phaseolus vulgaris* even at low concentrations. In *Vicia faba*, benzoic acid at 50 µM significantly reduced root length, and shoots fresh and dry mass by over 81% of those of the control, whereas adipic and *p*-hydroxyphenylacetic acids decreased root length to 87 and 88% of that of the control, respectively.

Sixty seven cultivars of 42 vegetable crop species from 14 families were tested in seedling growth bioassay using the used nutrient solution of *Asparagus officinalis* L. and replanting of *Asparagus officinalis* L., *Colocasia esculenta* Schott., *Vicia faba* L., *Pisum sativum* L. and *Phaseolus vulgaris* L. to select possible succeeding crops. Growth performances of succeeding crops were assayed using once used nutrient solution and/or replanting soil of these crops. Bioassay using asparagus used nutrient solution with or without AC suggest the tested cultivar of cucumber, garden pea, komatsuna, melon, pak-choi cv. 'Tyoukou', parsley, soybean (except cv. 'Tankurou'), cabbage cv. 'Early Ball' and lettuce cv. 'Shato' as succeeding crops. Bioassay using replanting soil with or without AC suggested that most of the cultivars tested can be planted after asparagus, taro, and three beans (*Vicia faba* L., *Pisum sativum* L. and *Phaseolus vulgaris* L.) with little adverse effects. Among the three methods of bioassay (i) used nutrient solution, (ii) direct seed sowing and (iii) seedling transplanting in replant soil; the nutrient solution bioassay proved more sensitive than replanting soil bioassay. However, results of nutrient solution bioassay may not be reproducible in the field condition. Therefore, seedling transplanting method can be used as an easy and practical bioassay method to select succeeding crops for fields with replanting problems.

Summary in Japanese

グロースチャンバーでのバイオアッセイにおいて，培養液非交換または毎週電気分解処理することによりイチゴは生育抑制を示したが，2 週毎の電気分解では生育が改善した．この毎週の電気分解処理による生育抑制は，培養液中のキレート鉄の分解と低濃度のカルシウムイオンに原因があることが明らかになった．イチゴを用いない実験で，低いｐＨ（3.13）と温度上昇が長時間の電気分解（24 時間）を制限する大きな要因であった．よって，電圧，ＥＣ，ｐＨを考慮し，短時間（2 時間）の電気分解処理が以後の実験に選ばれた．ガラス室での実験で，イチゴの生育と収量は培養液非交換や 2 週毎の電気分解処理で減少したが，培養液非交換において 4 週毎に電気分解処理を行うと改善した．イチゴの果実収量は，以前の実験ではその回復が培養液非交換と比べて７１％であったが，培養液非交換においても 4 週毎の電気分解処理で完全に（９９％近く）回復することが明らかになった．以上より，閉鎖系養液栽培システムにおけるイチゴの自家中毒回避のために 4 週間毎に 2 時間，非交換培養液に電気分解処理をすることをすすめる．

エンドウ，インゲンマメ，ソラマメの自家中毒について養液栽培における活性炭処理の有無によって検討した．培養液非交換で活性炭無添加におけるエンドウでは，莢数，莢重，種子数，そして種子重は活性炭添加区と比べて半分に減少した．インゲンマメの 1 株当たりの莢数，莢の生体重は，ソラマメと同様に活性炭添加区の４９から６７％に有意に減少した．インゲンマメの根の滲出物から同定された物質は安息香酸とマレイン酸，ソラマメからは乳酸，安息香酸，*P*-ヒドロキシ安息香酸，バニリン酸，アディピック酸，コハク酸，リンゴ酸，グリコール酸，*P*-ヒドロキシフェニール酢酸であった．同定されたアレロパシー物質のバイオアッセイにより，

安息香酸，サリチル酸，リンゴ酸が低濃度でインゲンマメの生育を有意に抑制したことを明らかにした．ソラマメでは，アディピック酸や *P*-ヒドロキシフェニール酢酸が根長を対照区と比べてそれぞれ８７，８８％まで低減させた一方，50 μMの安息香酸が根長，地上部の生体重および乾物重を対照区と比べて８１％以上抑制した．

アスパラガス，サトイモ，ソラマメ，エンドウ，インゲンマメを栽培した後の次作物を選ぶために，14 科，42 種，67 品種の野菜が，アスパラガス培養残液，アスパラガス，サトイモ，ソラマメ，エンドウ，インゲンマメの連作土を用いた苗生育バイオアッセイでテストされた．次作物の生育能力が 1 作使用された培養液や連作土を用いて検証された．アスパラガス培養残液に活性炭添加有無によって行われたバイオアッセイは，次作物として，キュウリ，エンドウ，コマツナ，メロン，チンゲンサイ‘長陽’，パセリ，‘たんくろう’以外のダイズ，キャベツ‘アーリーボール’，レタス‘シャトー’を指示した．活性炭添加有無によるアスパラガス培養残液を用いたバイオアッセイは，検定した多くの作物がほとんど影響もなく，アスパラガス，サトイモ，そして 3 種のマメ類（ソラマメ，エンドウ，インゲンマメ）の後に植えることができることを示した．3 種類のバイオアッセイの方法（ⅰ．培養残液，ⅱ．連作土に直播，ⅲ．連作土に苗を定植）で，培養残液を用いたバイオアッセイが連作土を用いるよりも，より反応性が高いということが判明した．しかし，培養残液の用いたバイオアッセイの結果は，圃場条件で行ったものを再現していないかもしれない．したがって，苗を移植する方法は，連作障害を抱えている圃場に対する次作物を選ぶために簡易で実用的な方法として用いることができる．

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Conference proceedings

1. **Asaduzzaman, M.**, Md. Fuad Mondal, Y. Kobayashi, K. Isogami, M. Tokura and T. Asao. Effects of nutrient solution lacking potassium nitrate on the growth and fruit quality of melon. *Proceedings of the Japanese Society for Horticultural Science,* 11, 2, 446, Sep., 2012.

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List of publications

1. **M. Asaduzzaman** and T. Asao. Autotoxicity in beans and their allelochemicals. *Scientia Horticulturae*, 134, 26-31. February 2012. (*The corresponding content is presented in Chapter 2*).

2. **M. Asaduzzaman**, Y. Kobayashi, K. Isogami, M. Tokura, K. Tokumasa and T. Asao. Growth and yield recovery in strawberry plants under autotoxicity through electrodegradation. *European Journal of Horticultural Science*, 77, 2, 58-67. April 2012. (*The corresponding content is presented in Chapter 3*).

3. **M. Asaduzzaman**, M. Fuad Mondal, T. Ban and T. Asao. Selection of ideal succeeding crops after asparagus, taro and beans replanting field in seedling growth bioassay. *Allelopathy Journal* 32, 1, 1-22. July 2013. (*The corresponding content is presented in Chapter 4*).

List of sub publications

1. **Md. Asaduzzaman**, Yutaro Kobayashi, Md. Fuad Mondal, Takuya Ban, Hitoshi Matsubara, Fumihiko Adachi and Toshiki Asao. Growing carrots hydroponically using perlite substrates. *Scientia Horticulturae* 159, 113-121. July 2013.

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