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Establishment of a Propagation System for Strawberry Using a Plant Factory with Artificial Lighting

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ABSTRACT

This thesis consists of four chapters of foundation research to establish an autotrophic transplant production method (ATPM), a novel propagation method for strawberry using a plant factory with artificial lighting for transplant production (T-PFAL). In ATPM, it was necessary to use smaller runner plants as propagules than mother plants in conventional propagation mehtods in order to reduce the timescale for production of new propagules and to enhance propagation rates. To maximize propagation rate in ATPM, the appropriate size of propagules was determined. As the net photosynthetic rate of runner plants after separating did not decrease beyond 15 days after fixing runner tips (DAFR), they were grown autotrophically after 15 DAFR when their crown diameter was c.a. 4.6 mm. Propagation cycles (required timescale to produce new propagules) and productivity of propagules with a 5 mm crown were shorter and greater, respectively, than those of propagules with a 4 mm crown or a 6 mm crown. These results indicate that appropriate propagules for

ATPM are runner plants with a 5 mm crown, two leaves, and a runner. In the T-PFAL, the runner plants connected with their propagules should be grown under high density and smaller container volume to increase the space efficiency as they were grown dependency on their propagules. Using nine propagules with two leaves, a runner, and a 5 mm crown by ATPM in a cultivation area of 3.6 m^2 for 365 days, we produced 3,497 strawberry transplants, which was 15 times greater than that from conventional propagation methods. Through a program for estimation of strawberry transplant production for the propagation system verified by measured results, we assured that the ATPM is an appropriate propagation method to produce transplants rapidly in a T-PFAL, which would be especially, useful when the number of propagules or propagules is limited. We established ATPM to produce strawberry transplants using smaller propagules in a T-PFAL through these researches and expected that using ATPM at upper step in the propagation and distribution system for domestic strawberry cultivars will be able to spread new cultivars quickly.

Keywords: crown diameter, *Fragaria* × *ananassa*, photosynthetic photon flux, planting distance, propagule size, transplant production, vegetative propagation

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INTRODUCTION

The most commonly cultivated strawberry, *Fragaria* × *ananassa* Duch. (2n = 8x = 56), which is classified as an herbaceous perennial, is an octoploid and a hybrid of two species, *Fragaria chiloensis* and *Fragaria virginiana* (Darrow, 1966). There are three major types of strawberry plants grown commercially: short-day, day-neutral, and everbearing plants. 'Short-day' strawberry plants initiate flower buds during short days (less than 14 $h \cdot d^{-1}$) and under low temperatures (less than 15°C), and 'Day-neutral' strawberry plants are insensitive to day length but are sensitive to temperatures above 30°C. However, 'Everbearing' strawberry plants are insensitive to both day length and high temperatures (Ruan et al., 2013). Polyploidy and allogamous behavior in *F.* × *ananassa* contribute complexity to the genome structure. Therefore, seed propagation has become unreliable in strawberry production, while limited numbers of seed-propagated cultivars are commercially available.

Strawberries are a high priority in the fruit market during the winter and spring seasons in Korea. During the early 2000s, the growing area remained rather stable at about 6,400 ha. However, the area has decreased slightly due to a reduction in the number of growers and an increase in the labor costs involved in transplanting and harvesting. Despite the decrease in the growing area, strawberry production has been maintained at approximately 20 thousand tons per year as a result of improvements in cultivation and environmental control techniques. Major strawberry cultivars popular with Korean growers and consumers were originally bred in Japan. The most popular cultivars were 'Akihime' and 'Red Pearl,' due to

their high yield and adaptability to the forcing culture cropping type, which focuses on very early or off-season production. Since Korea joined the International Union for the Protection of New Varieties of Plants (Union interationale pour la Protection des Obtentions Végétales; UPOV) in 2002, strawberry plants have become subjected to a variety of protections. For example, Korean strawberry growers have been required to pay royalties for the use of foreign cultivars since 2011. As a countermeasure against the UPOV, breeding of new domestic cultivars has been attempted and domestic cultivars such as 'Maehyang,' 'Seolhyang,' 'Jukhyang,' and 'Damhyang,' which are known to be well-adapted to forcing culture, have been registered. These cultivars have excellent fruit quality and storability; such features have resulted in a very rapid increase in the cultivation area of Korean domestic strawberry cultivars.

Since these reliable domestic cultivars were registered, a system for the production, propagation, and distribution of disease-tested stocks was implemented for distribution of virus-free or newly-cultivated plant varieties. As the system consists of five grades, (1) nuclear transplant, (2) elite transplant, (3) pre-basic transplant, (4) basic transplant, and (5) disseminative transplant, at least five years are required to distribute a new cultivar among farmers. Recently, the cultivation area of one strawberry cultivar, 'Seolhyang,' has become too large in Korea, which has led farmers and researchers to worry about the damage caused by a single cultivar. Outstanding alternative cultivars have been registered, but it takes a long time to distribute the cultivars among farmers, due to the poor efficiency of the system. The system must be made more efficient in order to distribute new cultivars more rapidly; however, this is difficult to achieve, due to the system's use of

conventional propagation methods for producing transplants. Therefore, a novel propagation method that can substantially increase the propagation rate is required in order to expedite the distribution of transplants of new cultivars.

Most commercial strawberry cultivars reproduce asexually using runners (Hancock, 1999). The runners are the clonal plants' means of vegetative propagation. They develop from the crown, and are comprised of a compressed modified stem where leaves, branch crowns, and flower clusters arise during long days with warm temperatures. Several vegetative propagation methods are used for commercial transplant production. One propagation method for bare-root plant production that has been used for a long time is to dig a field nursery-grown strawberry plant and transplant it to the production field within several days. The plants are classified as fresh-dug plants, frigo plants, and waiting-bed plants, according to their processing after digging. The fresh-dug plants are the bare-root plants just after being dug from the field, and the frigo plants are dormant plants stored for several months at -1.5^{o}C (Daugaard, 1999). The waiting-bed plants can be defined as heavy, large, multi-crowned plants, conditioned to start fruiting about five to eight weeks after planting, depending on the cultivar, planting date, and environmental conditions after planting (Kirschbaum et al., 2000). They are produced using the following steps: (1) Mother plants are planted in the nursery field. (2) Dormant single-crowned runner plants are dug and then cold-stored (-1.5 ^{o}C). (3) Cold-stored dormant plants are planted in a waiting-bed, and flowers and runners must be removed. (4) Multi-crowned waiting-bed plants are planted in cultivation areas or are cold-stored for next season. Tray plants are containerized plants produced from runner tips, which are unrooted plantlets on the tips of runners.

These are typically grown over a five-month period in 8-cell, 12-cell, or 16-cell plastic trays, with individual cell depths of 8-9 cm and an average crown diameter of 10-18 mm. Plug plants offer an alternative approach for obtaining strawberry starts for use in annual hill and high tunnel production systems (Rowley et al., 2010). While plug plants are more expensive than fresh-dug or frigo plants, they typically offer the appropriate level of initial vigor for fall planting. Plug transplants allow flexible transplantation dates, the opportunity for mechanical transplantation, and greater water utilization efficiency for transplant establishment compared to fresh bare-root plants (Bish et al., 2001).

The higher grade transplants in the distribution system are grown under very strict conditions, are propagated under careful control, and receive field inspections for major pathogenic viruses and nematodes. Micropropagation of strawberry plants is not widely used in many countries because of problems with off-types, especially complications due to a hyperflowering trait (Jemmali et al., 1995).

A plant factory with artificial lighting for transplant production (T-PFAL) is an appropriate cultivation area to propagate the transplants, because it rapidly produces disease-free (but not aseptic) transplants under appropriate environmental conditions, at low cost, and with minimal use of resources. Conventional propagation methods are not appropriate for propagation in a T-PFAL. The tray plant production method, one of the conventional propagation methods, produces transplants by separating runner plants from their mother plants when the runner plants reach a certain size, or when a specific planting time is reached (RDA, 2013). In the plug plant production method, another conventional propagation method, unrooted runner plantlets produced on the runner chains are separated from their

mother plants, and are then fixed on the cell trays and grown in a greenhouse or a plastic tunnel with saturated humidity for rooting (Durner et al., 2002). Mother plants from the first method are too big to grow in a T-PFAL using multi-layered propagation beds, due to the longer time required for growing runner plants. In the second method, two types of propagation beds or a PFAL are required to produce transplants such as cultivation systems for producing runner tips, rooting the runner tips, and growing runner plants after rooting. Therefore, a new propagation method for the T-PFAL is required.

In the vegetative propagation, it is necessary to determine the size of the propagules that can be used as starting materials for propagation to confirm production of uniform transplants and the propagation cycle which is the time required for propagules to grow and produce new propagules (Kubota and Kozai, 2001). The propagules should be runner plants that can be grown independently from their mother plants, and they should be separated from their mother plants after the development of numerous lateral roots.

We hypothesized that using smaller propagules may increase the propagation rate as a result of decreasing the propagation cycle. In order to test this hypothesis, we investigated the growth and net photosynthetic rate of the runner plants before and after separation, according to the number of days after fixing the runner tips on the growing medium, which helped to determine the appropriate separation timing for autonomous growth (Chapter I). Next, we investigated the accumulated number of transplants, the propagation cycles, and the growth of propagules and runner plants as they were affected by the crown diameter of the propagules, in order to determine the appropriate size of the propagules (Chapter II). We examined ways

in which the growth of the runner plants before separation from their propagules was affected by cell volume and photosynthetic photon flux (PPF) level, and whether the runner plants connected with their propagules could be grown under high density (Chapter III). Finally, the transplants were produced by a novel propagation method for 365 days, and the accumulated number of transplants was investigated as affected by the number of initial propagules and the propagation area, in order to verify the productivity of the strawberry propagules (Chapter IV).

LITERATURE REVIEW

Plant Factory with Artificial Lighting for Transplant Production

A plant factory with artificial lighting for transplant production (T-PFAL) is defined as a warehouse-like structure covered with opaque thermal insulators, in which ventilation is kept at a minimum and lamps are used as the sole light source for plant growth. In addition, multi-layered shelves with lamps on each shelf are used whenever possible to increase the production capacity per floor area (Kozai, 2006). Advantages of a T-PFAL over a greenhouse for producing high quality transplants include: rapid and efficient growth of transplants mainly resulting from a considerably higher light utilization efficiency of transplants produced under uniformly controlled environments in the protected area free from pest insects and pathogens and the disturbance of outside weather; higher productivity per floor area per year, mainly due to the use of multi-layered shelves with a ratio of planting area to floor area of 1.2-1.5; a high planting density per tray area; a high percentage of saleable transplants; 10-20% higher sales price due to higher quality and uniformity of transplants; 30-70% shorter production period; drastically higher utilization efficiencies of water, CO_2, fertilizers, and conditioners, resulting in little waste water to the outside; virtually no requirement of heating cost even in the winter because of its thermally insulated structure; lower comfortable working environments; and easier control of plant development such as stem elongation, flower bud initiation, bolting, and root formation (Kozai et al., 1998; Kozai et al., 1999; Kozai et al., 2000; Chun and Kozai, 2001; Kozai et al., 2004).

High electricity cost and initial investment are often mentioned as

disadvantages of the T-PFAL. However, the electricity cost for transplant production could be reduced considerably by using thermally insulated walls, multi-shelves, and advanced lighting and air conditioning systems (Kozai et al., 2004). Since only approximately 10% of the greenhouse floor area is required to produce the same number of transplants, the initial cost per annual plant production in a T-PFAL is lower than in a greenhouse.

Using the environmental control function of the T-PFAL, value-added transplants can be produced relatively easily with the following features: 1) the number of nodes below the first flower cluster of tomato plants can be set at about 8 in the summer (Ohyama et al., 2003); 2) enhanced flower bud differentiation and growth of pansy and strawberry transplants in the summer by providing relatively low temperatures (Omura et al., 2000); 3) retarded bolting of oriental spinach varieties in the summer by providing a light period shorter than the critical photoperiod (Chun et al., 2000); 4) an increased number of runner plants obtained from strawberry mother plants by CO_2 enrichment and a photoperiod of 16 $h \cdot d^{-1}$; 5) production of virus-free sweet potato transplants using single node leafy cuttings (Lok et al., 2002); 6) uniform growth of cucumber and tomato seedlings used for grafting as scions and stock plants throughout the year; 7) enhanced nursing and acclimatization of grafted cucumber, watermelon, eggplants, and tomato transplants; 8) production of vigorous Chinese cabbage, broccoli, and cabbage transplants with short but thick hypocotyls (Ohyama et al., 2001); and 9) year round production of herb, lettuce, and chicory transplants for hydroponic culture.

Size of Propagules and Environmental Conditions for Strawberry Transplant

Production

Transplant quality from mother plants used for transplant production or transplants for the production of strawberry fruits depends on their crown size, commercially. In frigo plants, transplants that have a 9-15 mm crown diameter are classified as Frigo A and transplants with a 15-18 mm crown are classified as Frigo A+ (Daugaard, 1999). In waiting bed plants, transplants with 15-18 mm crowns, 18-23 mm crowns, and more than 23 mm crowns are assessed as Cat. I, Cat. II, and Cat. III, respectively (Lopez-Galarza et al., 1997). However, they mostly grow and propagate on the nursery field and are produced by the bare-root type.

The use of plug plants grown from cuttings of runner tips has become increasingly popular with the increase in production of strawberries with substrate culture systems, due to their low cost and ease of disease control, plant management, and handling compared to conventional plants or waiting-bed plants (Yoshida and Motomura, 2011). In other words, plug transplants are small containerized plants produced from runner tips, which are an alternative to conventional field-grown strawberry transplants (Durner et al., 2002). A method for producing plug plants called tip-raised potted plants generates small containerized plants produced from runner tips, generally grown for 3-5 weeks in 50-cell plastic trays with cell depths of 5-6 cm and an average crown diameter of 8-11 mm.

When transplants were grown with four crown sizes, i.e., <0.5, 0.5-0.8, 0.8-1, and >1 cm, transplants with a crown size of > 1 cm performed better in all vegetative and reproductive parameters such as the number of crowns per plant, number of leaves per plant height, number of runners, plant spread, terminal leaf area, number of days to flower induction, and number of flowers and fruits (Diengngan et al.,

2016). Yoshida and Motomura (2011) reported that flowering was later in young and small tray plants compared to aged large plants. Takeda et al. (2004) also reported that larger transplants produced more branch crowns than smaller transplants in the fall; however, runner production in the fall was not affected by either the position on the runner or weight at the time of runner plant harvest for plug plant production.

Dijkstra et al. (1993) reported that strawberry plants grown linearly increased their yield·m^{-2} as plant density increased from 4.3 to 8.5 plants·m^{-2}; however, yield per plant decreased as the plant density increased. Perez de camacaro et al. (2004) reported that plant growth and yield per plant increased as plant spacing increased from 20 to 30 cm, but the highest harvest index and yield per square meter were obtained at the closest spacing. Paranjpe et al. (2008) reported that total and early marketable yield increased linearly as plant density increased.

Runner and floral induction occur in mutually conflicting environments (Konsin et al., 2001). Environmental factors controlling the transition from vegetative to floral growth play a key role in strawberry production. The short-day plants (June-bearing plants) initiate flower buds either under short-day conditions (less than 14 h of day length) or when temperatures are less than 15ºC. Above 15ºC, the critical photoperiod for floral induction is 8-12 h (Hancock, 1999). Floral induction was inhibited by high temperatures even in short-day conditions, with marginal short-day induction taking place at 27ºC (Sonsteby and Heide, 2006); however, this was mainly determined by photoperiod and temperature, while plant age and the duration of short-day treatment determined the number of inflorescences per plant (Verheul et al., 2006; Verheul et al., 2007).

PPF levels promote the formation of runners and runner plants from strawberries, so the rapid propagation method with high PPF levels can be feasible for the production of vigorous transplants (Kim et al., 2010). Long-term CO_2 enrichment boosted vegetative propagation of strawberry plants as well, and it is more efficient to use elevated CO_2 concentrations of up to 600-750 ppm rather than 900 ppm for greenhouse cultivation of strawberries (Chen et al., 1997; Keutgen et al., 1997).

Roles of a Runner for Runner Plant Production

Clonal plants are capable of producing asexual offspring, such as strawberry runner plants, that remain attached to their mother plants at least until they root (Pitelka and Ashmum, 1985; Klimes et al., 1997). Stolons, or runners in the strawberry, function not only to emerge new runner plants but also to transfer the mother plant's resources to the runner plants until they are completely independent with their own roots (Savini et al, 2008). Clonal growth in strawberries permits a mother plant to transport water, nutrients, and photosynthates to its runner plant, which can increase the probability that the runner plants will survive (Schmid, 1990; Oborny and Bartha, 1995). Runner plants often remain attached to the mother plants after they root, resulting in a connection between the mother plants and runner plants to share resources, and resource sharing can increase their combined survival and growth (Price and Marshall, 1999). Resource sharing between connected plants within clones is especially likely to increase performance when the plants are grow in contrasting environmental conditions such as physical heterogeneity or competition (Oborny et al., 2000; Pennings and Callaway, 2000; Saitoh et al., 2002;

Roiloa and Retuerto, 2005). The connected plants can increase their overall performance by translocating one resource from a plant with a high supply to the other with a low supply. When the availabilities of two resources are negatively correlated, plants may reciprocally exchange one resource for the other (Roiloa et al., 2007). Reciprocal resource sharing may allow connected plants to survive in an environment where all of the microsites are lethally deficient in different resources (Alpert and Mooney, 1986).

When a plant given low light availability and high N availability is connected to a plant given high light and low N, the first one will accelerate growth of the root and the second one will accelerate growth of the shoot. This specialization to acquire resources is called 'division of labor' (Alpert and Stuefer, 1997; Hutchings and Wijesinghe, 1997; Stuefer, 1998). Division of labor within connected plants depends upon internal transport of some signal between the plants (Stuefer et al., 2004). Given that acquisition of an abundant resource is likely to be more economical than acquisition of a scarce one, the division of labor is likely to increase the overall efficiency and hence the performance of the connected plants in habitats where connected plants experience contrasting availabilities of different resources.

Advantages of resource sharing in heterogeneous environments could be increased by the properties of clonal plants; however, Alpert (1999) reported that resource sharing might actually be disadvantageous due to the cost of maintaining connections and exporting resources from rich to poor in homogeneous environments. A simple conceptual model of plant performance as a function of internal resource supply and environmental resource availability suggests that

resource sharing between plants within clones is likely to be disadvantageous in homogeneous habitats and advantageous in heterogeneous ones. Increased growth of the runner plants must exceed their mother plants' lost growth (Caraco and Kelly, 1991). In other words, translocation of a limiting resource can reduce the growth of mother plants while increasing the growth of runner plants. Younger ramets, runner plants in the strawberry, appeared to attract slightly greater amounts of resources from connected older ramets, mother plants in the strawberry (Friedman and Alpert, 1991; Savini et al., 2008; Alpert, 1996). Flow proceeds from an established plant to a younger and usually smaller plant because physiological integration implies more than passive diffusion of energy and nutrients from areas with a higher to lower concentration until the system equilibrates. The greater size of the mother plants allows them to have larger internal stores and greater rates of resource acquisition (Caraco and Kelly, 1991). Nitrogen may move mainly in the xylem, where it must follow the bulk flow of water. As older plants generally have more extensive roots, potential water gradients might exist from mother plants to runner plants (Alpert and Mooney, 1986). Therefore, energy and nutrients tend to move from an older to a younger plant in the case of clonal plants because they diffuse from higher to lower concentration until the system equilibrates, and energy and nutrient pools are typically larger in older plants compared to younger plants.

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CHAPTER I

Growth of Runner Plants as Affected by Separation Time from Propagules

ABSTRACT

The suitable size of runner plants based on separation time from their propagules was determined to maximize the propagation rate of strawberry transplants in a plant factory with artificial lighting (PFAL). When runner tips with unfolded bracts were fixed on growing medium, the first true leaf, the first runner and the roots of runner plants appeared at 6-10 days after fixing (DAF), and their shoot and root dry weights significantly increased at 6 and 10 DAF, respectively. The T/R ratio of the runner plants was the greatest at 10 DAF among those until 30 DAF. The net photosynthetic rate of runner plants at 9, 11, and 13 DAF decreased after separation, while that at 15, 17, and 19 DAF did not. Runner plants could grow independently and autotrophically at 15 DAF under a controlled environment in a PFAL. The runner plants separated from their propagules at 15, 20, and 25 DAF were successfully grown until 30 DAF to be used as propagules for the next propagation generation. At 30 DAF, the root dry weight of the runner plants separated from the propagules at 15 DAF was smaller than that of the runner plants separated at 20, 25, and 30 DAF, whereas the dry weights of leaves and runners were not significantly different. The use of small propagules could reduce the propagation cycles, but there was no significant difference in the propagation cycle

when the runner plants were separated at 15 or 20 DAF due to the relatively insufficient growth of runner plants separated at 15 DAF. These results indicate that the runner plants separated at 20 DAF that had two true leaves and a c.a. 5.3-mm crown diameter would be suitable for use as propagules for the next propagation cycle in ATPM in a PFAL for strawberry propagation.

INTRODUCTION

Vegetative propagation using runner plants is a typical method used for production of strawberry transplants (Hartmann et al., 2011). Runner plants should be separated from their stock plants or propagules to be used as transplants when they can grow independently. Unrooted runner tips should be fixed on a growing medium that has suitable moisture and temperature to develop their root systems (Saito et al., 2008). Chun (2016) reported that young runner tips with only one unfolded bract could develop root systems in growing medium if the environment was properly controlled.

In conventional propagation methods, a runner plant with a well-developed root system is separated after its crown diameter reaches a certain size. RDA (2013) recommended that they should be separated when their crown diameter reaches 9-13 mm 40-60 days after the runner tips are fixed on growing medium. Durner et al. (2002) reported that runner plants could be separated as early as 20-35 days after fixation, since they might have well developed root systems.

While runner plants are attached to propagules before developing their own root systems, they have to grow by receiving assimilates, water, and mineral nutrients,

etc., from their propagules (Roiloa et al., 2007; Savini et al., 2008; Stuefer et al., 2004), and the growth of the propagules is reduced (Salzman and Parker. 1985). Even after establishing their own root systems, runner plants still receive these resources if they are attached to their propagules (Alpert, 1999), resulting in photomixotrophic growth.

A novel propagation method was conceived with a hypothesis that separating runner plants from their propagules early could promote autotrophic growth of the runner plants and minimize growth decline of propagules (Chun et al., 2012). In this method, runner plants are separated to be used as propagules in the next propagation generation when their sizes reach the initial size of their propagules. Using smaller propagules might shorten the timescale for each runner plant to reach the initial size of their propagules, which would result in a greater propagation rate. Therefore, the optimal size of propagules for use in successive propagation generation, which is mainly dependent on when the runner plants are separated from their propagules, should be determined.

To determine the appropriate separation timing that can grow autotrophically, we investigated growth and the net photosynthetic rate of the runner plants before and after separation according to days after fixing the runner tips on the growing medium. We also investigated propagation cycles of the runner plants used as subsequent stock plants in order to confirm the effect of the separation timing of the runner plants from their stock plants on the propagation rates.

MATERIALS AND METHODS

Plant materials and environmental conditions

Strawberry plants (*Fragaria×ananassa* Duch. cv. Maehyang) that had a crown diameter of ca. 9 mm and 3-4 leaves and planted into plastic pots (ø 70 mm) filled with commercial growing medium (Plant World; NongwooBio Co. Ltd., Suwon, Korea) were selected and placed in a T-PFAL. Runner tips generated from those plants were fixed on 32-cell cutting plug trays (150 mL/cell) filled with the commercial growing medium when their bracts were still unfolded (8-12 days after runner emergence) for development of adventitious roots and formation of runner plants. They were grown in a T-PFAL with nine 32-W cool white fluorescent lamps (TLD32W830RS, Philips Electronics, The Netherlands) for each bed and the PPF level above the empty bed was 230 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The photoperiod was set at 16 h/day and the air temperature was maintained at 27°C/23°C during the photo-/dark periods. The CO_2 concentration was set at 800 $\mu mol \cdot mol^{-1}$. The stock plants and runner plants were sub-irrigated with Yamazaki nutrient solution for strawberries (Yamazaki, 1978; pH 6.0 and EC 0.7 $dS \cdot m^{-1}$) for 10 minutes, once a day.

Growth and net photosynthetic rates of runner plants before and after separation

The eight runner plants generated from the plants were cultivated until 30 DAF in the T-PFAL. We measured the numbers of leaves and runners, crown diameter, leaf area, dry weights, and top/root ratios (T/R ratio) of the runner plants at 3, 6, 10, 15, 20, 25, and 30 DAF. Leaf areas were measured by leaf area meters (Li-3100; LI-COR, Lincoln, NE, USA) and dry weights were measured on oven-dried (72 h at 80^{o}C) materials. We also calculated the relative growth rate (RGR) of the shoots and roots of the runner plants between 3 and 6, 6 and 10, 10 and 15, 15 and 20, 20

and 25, and 25 and 30 DAF using averages of dry weights according to the following an equations reported by Kozai (2016).

$$\mathrm{RGR} = \frac{1}{W1} \times \frac{W2 - W1}{T2 - T1}$$

where W_1 and W_2 are dry weights on T_1 and T_2, respectively.

At 9, 11, 13, 15, 17, and 19 DAF, the photosynthetic rates of the four runner plants were measured before and after they were separated from the strawberry plants. The photosynthetic rate on the primary fully-expanded leaf was measured using a clear chamber bottom (6400-08; LI-COR) mounted on an infrared CO_2/H_2O analyzer (LI-6400 Portable Photosynthesis System; LI-COR) under a PPF of 230 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The conditions in the measurement chamber were maintained as follows: a flow rate of 500 $\mu mol \cdot s^{-1}$, CO_2 concentration of 800 $\mu mol \cdot mol^{-1}$, RH of 60%, and air temperature of 24ºC.

The eight runner plants were separated from the strawberry plants at 10, 15, 20, and 25 DAF, and they were subsequently cultivated in the T-PFAL. At 30 DAF, we measured the numbers of leaves and runners, crown diameters, leaf areas, and shoot and root dry weights of the runner plants separated at 15, 20, 25, and 30 DAF (or 15, 10, 5, and 0 days after separation, respectively). Data for the runner plants separated at 10 DAF were not acquired, because all of them were withered.

Propagation efficiency of runner plants as propagules

The runner plants were classified into four groups: 4.5-4.7, 5.2-5.4, 6.0-6.2, and

7.0-7.2 mm crown diameters based on the separation timing (at 15, 20, 25, and 30 DAF; Fig. 1) and used as propagules grown in the T-PFAL in order to investigate the propagation efficiency as affected by the initial size of propagules. Before the experiment, all of the runners generated from the propagules were removed. Five propagules were placed in a low and four replications of four treatments were arranged with a completely randomized design. When the crown diameter of subsequent runner plants reached the initial size of their propagules, they were separated and timescales from placing propagules to separation of the first, second, and third runner plants were measured.

Data analysis

The experimental data were statistically analyzed by the statistical analysis system (SAS 9.2, SAS Institute Inc., Cary, NC, USA) using Duncan's multiple range tests. The treatment differences were considered to be significant at a 95% confidence level.

RESULTS AND DISCUSSION

Figure I-1 shows the growth of runner plants at 3, 6, 10, 15, 20, 25, and 30 DAF. The first unfolded leaf, a runner, and roots appeared on the runner plants from 6 to 10 DAF. The shoot and root dry weights significantly increased between 6 and 10

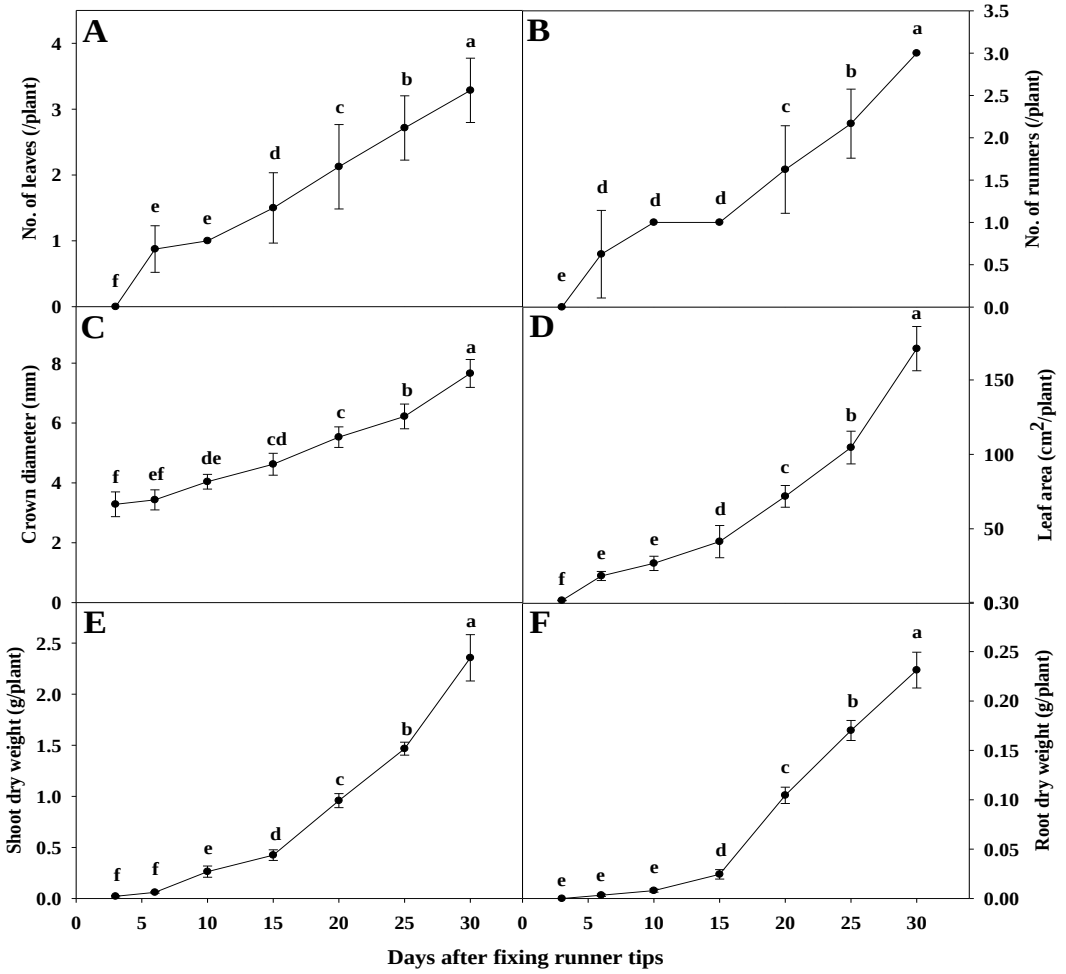

Fig. I-1. Time course of numbers of leaves (A) and runners (B), crown diameter (C), leaf area (D), and dry weights of shoot (E) and root (F) of runner plants as affected by days after fixing runner tips. Means above each point followed by the same letters are not significantly different according to Duncan's multiple range test at $p < 0.05$. Vertical bars show standard deviation of the means (n = 8).

DAF, while the shoot RGR of the runner plants 3-6 and 6-10 DAF (0.60 and 0.83 d^{-1}, respectively) was greater than those after 10 DAF (0.10-0.20 d^{-1}), and the root RGR before 20 DAF (0.34-0.65 d^{-1}) was greater than those 21-30 DAF (0.06-0.15 d^{-1}). The T/R ratios were the greatest at 10 DAF and gradually decreased after then (Fig. I-2). Nishizawa and Hori (1986) reported that strawberry leaves rarely export assimilates to other parts before they are fully unfolded. In this study, the first true leaf was fully unfolded after 10 DAF. Until 10 DAF, the runner plants needed assimilates transferred from their propagules to develop the first leaf and roots because they could not autotrophically produce assimilates yet. Moreover, they were not able to uptake sufficient water and minerals due to poor development of the root system. We concluded that the runner plants should not be separated from their propagules before 10 DAF in a T-PFAL.

The net photosynthetic rate of the runner plants separated at 9 and 11 DAF (Pn_9 and Pn_11, respectively) decreased gradually after separation, and they almost wilted in one day (Fig. I-3). The Pn_13 decreased 60 minutes after separation, but did not wilt. The Pn_15, Pn_17, and Pn_19 were maintained even before and after separation. Wilting of the runner plants separated at 9 and 11 DAF might have been caused by their dependence on stock plants as mentioned earlier. The greater T/R ratio and poor root development might have been the reasons for the decrease in Pn_13 after separation. Savini et al. (2008) reported that unrooted runner plants under sufficient light conditions used water and minerals from their propagules to grow, even though they could photosynthesize. The runner plants separated at 13 DAF could photosynthesize (Fig. I-3), but might not have been able to uptake sufficient water and minerals via their roots, because root initiation was

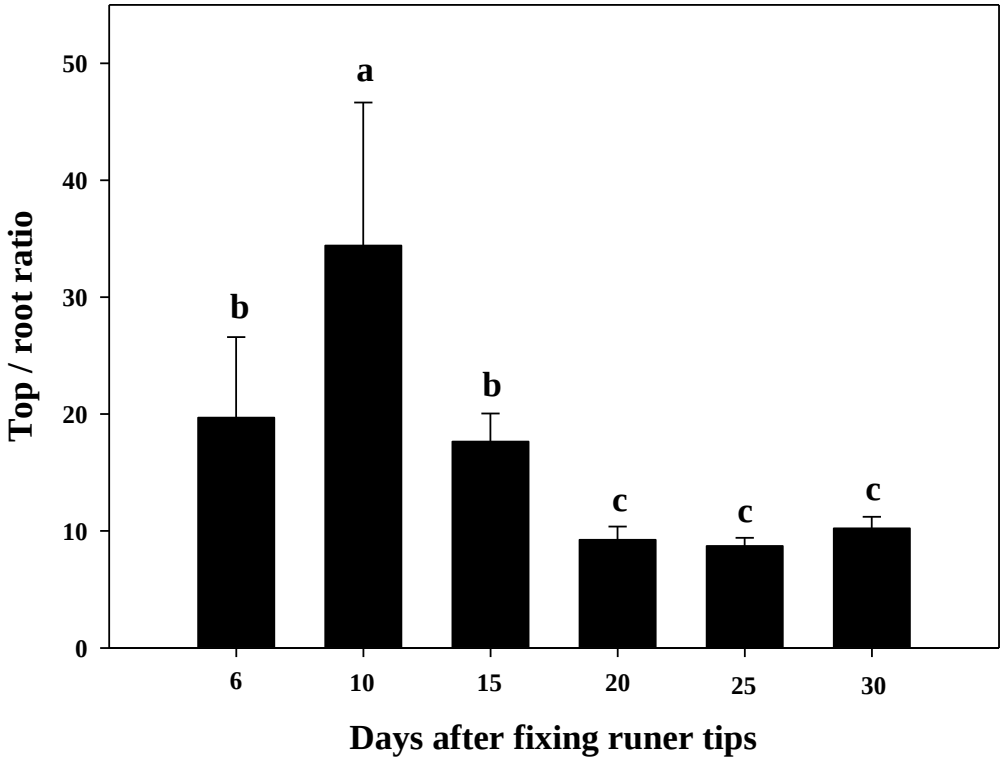

Fig. I-2. Top/root ratio of runner plants as affected by days after fixing runner tips. Means above each bar followed by the same letters are not significantly different according to Duncan's multiple range test at $p < 0.05$. Vertical bars show standard deviation of the means (n = 8).

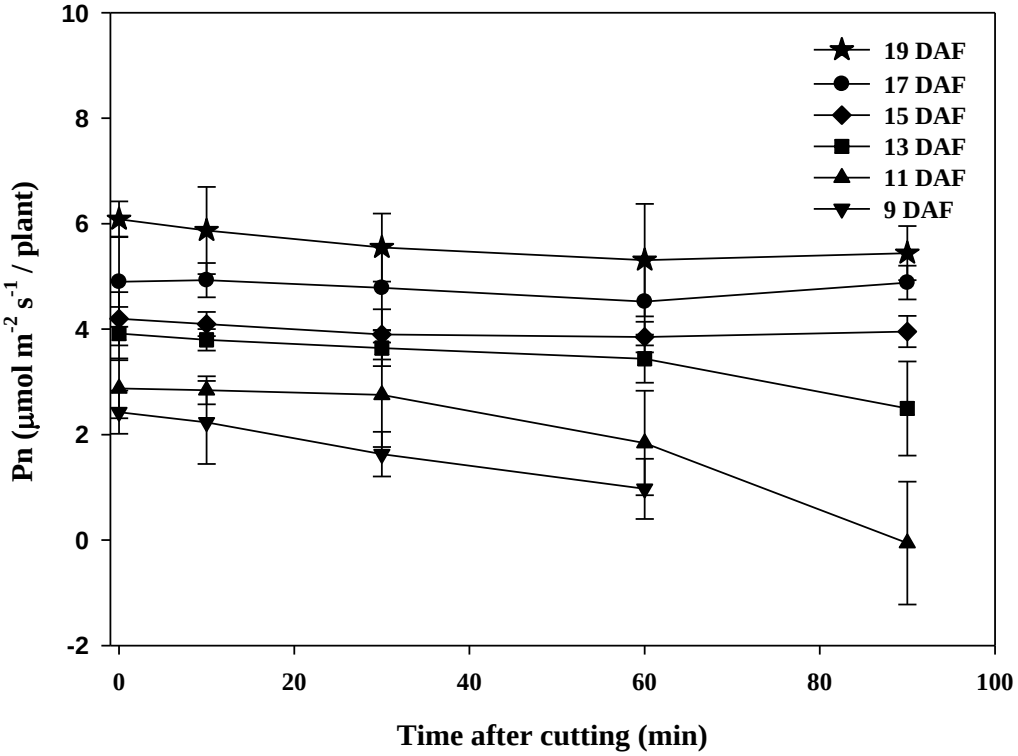

Fig. I-3. Time course of net photosynthetic rate of runner plants as affected by days after fixing runner tips before and after separating from the stock plants. Net photosynthetic rate at 90 minutes of runner plants 9 days after fixing could not measure because they were wilted. Vertical bars show standard deviation of the means (n = 4).

later than shoot initiation in runner plants (Fig. I-1). The T/R ratio was the greatest at 10 DAF and then gradually decreased until 20 DAF (Fig. I-2). Beyond 15 DAF, the runner plants might have developed a large enough root system to uptake sufficient water and minerals. Therefore, the runner plants could separate from their stock plants as early as 15 DAF in the ATPM in a T-PFAL.

The runner plants separated at 10 DAF were wilted after separation (Table I-1) as previously shown in Figure 3. The number of leaves and runners, the leaf area, and the dry weights of the leaves and runners of the runner plants were not significantly different when affected by separation time. The crown diameter, dry weights of stems (a crown and petioles) and roots, and T/R ratio of runner plants separated at 15 DAF were significantly smaller than those separated at 20, 25, and 30 DAF. Runner plants between 16 and 20 DAF showed a greatest root RGR (0.65 d^{-1}) until 30 DAF in previous results calculated from Figure 1F. Reduction of root growth of the runner plants separated at 15 DAF might have been caused by exclusion of resources from their stock plants when their root growth rate was the highest.

The propagation cycles to produce first, second, and third runner plants were shortened as runner plants were separated earlier for use as propagules; however, those of runner plants separated at 15 and 20 DAF were not significantly different (Fig. I-4). We assumed that small size propagules may shorten their propagation cycles if they can grow after separating from their elder propagule. The runner plants separated at 15 DAF could be too small to be used as propagules due to the relatively smaller root system and greater T/R ratio resulting in slightly insufficient growth compared to those separated at 20 DAF. Therefore, we concluded that the

Table I-1. Growth of runner plants 30 days after fixing runner tips as affected by separating time.

	Number of leaves	Number of runners	Crown diameter (mm)	Leaf area (cm^2 / plant)	Dry weight (g/plant)				T/R ratio
					Leaf	Runner	Stem[z]	Root	
S10[y]	-[x]	-	-	-	-		-	-	-
S15	3.8a[w]	2.9a	6.8b	152.5a	0.93a	0.83a	0.42b	0.17b	12.5a
S20	3.5a	2.6a	7.3a	135.1a	0.90a	0.80a	0.43b	0.21a	10.2b
S25	3.3a	2.8a	7.8a	168.0a	1.01a	0.98a	0.49a	0.22a	11.7ab
S30	3.2a	3.0a	7.6a	149.1a	0.85a	0.93a	0.54a	0.23a	9.9b

[z]Petioles + crown.

[y]Separating runner plants from the strawberry plants 10, 15, 20, 25 and 30 days after fixing runner tips.

[x]All the plants withered.

[w]Means within each column followed by the same letters are not significantly different according to Duncan's multiple range test at $p < 0.05$.

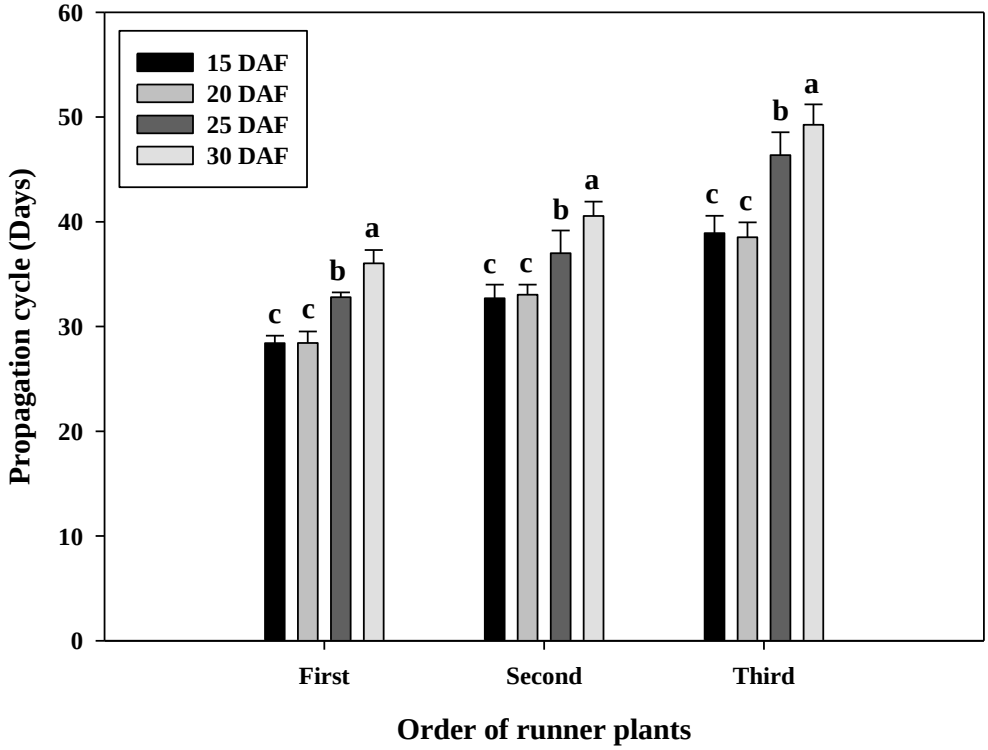

Fig. I-4. Timescales from placing propagules to separating the first, second and third runner plants as affected by runner plants used as propagules separated 15, 20, 25, and 30 days after fixing runner tips. The runner plants of next generation were separated from their propagules when their crown diameters were reached to initial one of propagules. Vertical bars show standard errors of the means from four replications. Means above each bar followed by the same letters are not significantly different according to Duncan's multiple range test at $p < 0.05$. Vertical bars show standard errors of the means from four replications (n = 20).

runner plants separated at 20 DAF with c.a. 5.3-mm crown diameter were suitable as propagules for the autotrophic transplant production method in a T-PFAL.

The timescales to produce three runner plants were used as propagation generation in this study. While the propagules produced three runner plants, they kept growing and resulted in a c.a. 10-mm crown diameter similar to that of transplants for fruit production or mother plants for conventional propagation methods (Durner et al., 2002; RDA, 2013). Producing three runner plants per propagules in each propagation generation could improve production efficiency, because the propagules that are used can be reused as the transplants or mother plants and not discarded like they are after propagation in most conventional methods.

The results indicated that early fixation of runner tips results in shorter separation time of runner plants, while their sizes are much smaller than in conventional propagation methods. Using the runner plants separated at 20 DAF with a c.a. 5-mm crown diameter as propagules may improve the propagation rate by reducing the propagation cycles in the autotrophic transplant production method using a T-PFAL. We confirmed that strawberry transplants could be produced more rapidly and efficiently using small size propagules under controlled environmental conditions.

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CHAPTER II

Propagation Rate of Propagules as Affected by Crown Diameter

ABSTRACT

The appropriate propagule size was determined in order to maximize the propagation rate of strawberry transplants in an autotrophic transplant production method using a plant factory with artificial lighting for transplant production. During 90 days after planting initial propagules, propagation cycles (T_{PC}; required timescale to produce new propagules; sum of the time from planting propagules to emergence of runners and the time from emergence of runners to separation of runner plants) of first, second, and third runner plants produced from propagules with a 5 mm crown (CD5) were shorter than those produced from propagules with a 4 mm crown (CD4) or a 6 mm crown (CD6). In second and third runner plants, the time from planting propagules to emergence of runners increased as propagule crown diameter decreased; however, the time from emergence of runners to separation of a runner plant decreased as crown diameter decreased. Fluctuation of T_{PC} with propagation generation in CD5 was smaller than those in CD4 and CD6. After separating runner plants from their propagules, number of leaves in CD5 fluctuated less than those in CD4 and CD6. The accumulated number of transplants in CD5 was greater than those in CD4 and in CD6. These results indicate that propagules having a ca. 5 mm crown and two leaves are appropriate for the novel production method in a plant factory.

INTRODUCTION

Production of high quality transplants has been emphasized to achieve high yield and quality of the final products in vegetable production. For this, many techniques have been proposed, one of which is transplant production using a plant factory with artificial lighting (T-PFAL; Kozai, 2006). The T-PFAL is a system to produce disease-free transplants using minimum resources in an airtight facility with thermally well-insulated roof and wall. Moreover, it is possible to rapidly produce uniform transplants by manipulating environmental conditions in this system. In order to apply these advantages to vegetative propagation, Chun et al. (2012) developed a new vegetative propagation method.

The autotrophic transplant production method (ATPM) was developed based on strawberry transplant production with the T-PFAL using vegetative propagation (Chun, 2016). Conventional propagation methods are inappropriate for propagation in the T-PFAL. In the tray plant production method, one of the conventional propagation methods, transplants are produced by separating runner plants from their propagules when the runner plants reach a certain size or planting time is reached (RDA, 2013). In a plug plant production method, another of the conventional propagation method, unrooted runner plantlets produced on the runner chains are separated from their propagules, fixed on cell trays, and grown in a greenhouse or a plastic tunnel with saturated humidity for rooting (Durner et al., 2002). Propagules or stock plants of the first method are too big to grow in T-PFAL using multi-layered propagation beds. The second method needs more than two

types of propagation beds or PFALs to produce transplants, such as cultivation systems for producing runner tips (wider space between layers), rooting the runner tips (narrower space between layers and high relative humidity), and growing runner plants after rooting (narrower space between layers). In contrast, the propagules in the ATPM are smaller in size and are produced using only one type of PFAL. Moreover, less waste is produced from ATPM than from convention methods because the propagules terminated propagation in the ATPM can be used as transplants for the conventional methods, while those in the conventional methods are thrown out.

Kubota and Kozai (2002) reported that the number of transplants was higher with a shorter propagation cycle in a vegetative propagation. The propagation cycle required timescale to produce new propagules (TPC) was affected by size of propagules. In a vegetative propagation method for sweet potato using a T-PFAL, there are two types of T_{PC}; timescales for cutting and for stock plant bases (Kubota and Kozai, 2002). In ATPM for strawberry production, there are three types of T_{PC} because a propagule produces three runner plants that are then used in conventional methods as transplants or stock plants due to their similar size (Chun, 2016). TPC in the ATPM is sum of a time from placing propagules to emergence of runners (TPE) and a time from emergence of runners to separation of a runner plant from the propagule (TES). Because emergence of the runners can be promoted from propagules with surplus resource pools (Alpert, 1991), We hypothesized that as the size of the propagules decreases, TES shortens; however, TPE may increase.

To determine the appropriate size of propagules for ATPM, we investigated propagation cycles, growths of propagules and runner plants, and accumulated

number of transplants produced in a T-PFAL for 90 days as affected by crown diameter of the propagules.

MATERIALS AND METHODS

Strawberry plants (*Fragaria* × *ananassa* Duch. cv. Maehyang) for propagules were selected based on crown diameter (4.0-4-2, 4.9-5.1, and 5.9-6.1 mm) and named CD4, CD5, and CD6, respectively. These respective plants had two leaves and one runner, two leaves and two runners, and three leaves and two runners. We planted them into 150 mL containers using 32-cell cutting plug tray (Bumnong Co. Ltd., Jeongeup, Korea) filled with commercial medium (Plant World; Nongwoo Bio Co. Ltd., Suwon, Korea) and placed them in a T-PFAL. They were produced runner plants for 90 days.

Runner tips generated from the propagules were fixed on the 32-cell cutting plug tray filled with the commercial medium for development of adventitious roots and formation of runner plants when their bracts were unfolded. However, in case their bracts had been already unfolded, they were fixed on medium trays regardless of their size. Crown diameter of runner plants derived from the runner tips was measured every day, and the runner plants were separated from their propagules when their crown diameter reached initial crown diameter in each group. When the runner plants were separated, we measured numbers of leaves and runners of them, placed them on the propagation bed, and cultivated them as subsequent propagules for the next generation. We also measured T_{PC} (sum of TPE and TES).

The propagules that produced three runner plants were released from the T-

PFAL, and we measured number of leaves, crown diameter, and dry weight of them. When the number of propagules in the T-PFAL exceeded the maximum number of propagules that can be cultivated in the propagation area (3.6 m^2; 160 propagules), the newly formed propagules were also released from the T-PFAL. We counted the number of runner plants produced in the T-PFAL every day until 90 DAP.

The propagules and runner plants were grown in the T-PFAL with 32W cool white fluorescent lamps (TLD32W830RS, Philips Electronics, The Netherlands) for each bed, and the PPF level above the bed was 230 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Air temperature during photo/dark-period was maintained at 27°C/23°C, and the photo-period and CO_2 concentration were 16 h and 800 $\mu mol \cdot mol^{-1}$, respectively. Closed irrigation system was applied to the plant factory used in this study and plants were sub-irrigated with Yamazaki nutrient solution for strawberries (Yamazaki, 1978; pH 6.0 and EC 0.7 $dS \cdot m^{-1}$) for 10 minutes, once a day.

We statistically analyzed the experimental data using SAS 9.2 (SAS Institute Inc., Cary, NC, USA) with Duncan's multiple range tests. Treatment differences were considered significant at a level of 5% probability

RESULTS AND DISCUSSION

The first, second and third T_{PC} (1^{st} TPC, 2^{nd} TPC, and 3^{rd} TPC) in CD5 were smaller than those in CD4 and CD6 (Fig. II-1). The first, second and third TPE (2^{nd} TPE and 3^{rd} TPE) decreased as crown diameter of initial propagules increased; however, the second and third TES (2^{nd} TES and 3^{rd} TES) increased as the crown diameter increased. The 1^{st} TES in CD5 and CD6 were smaller than their 2^{nd} TES

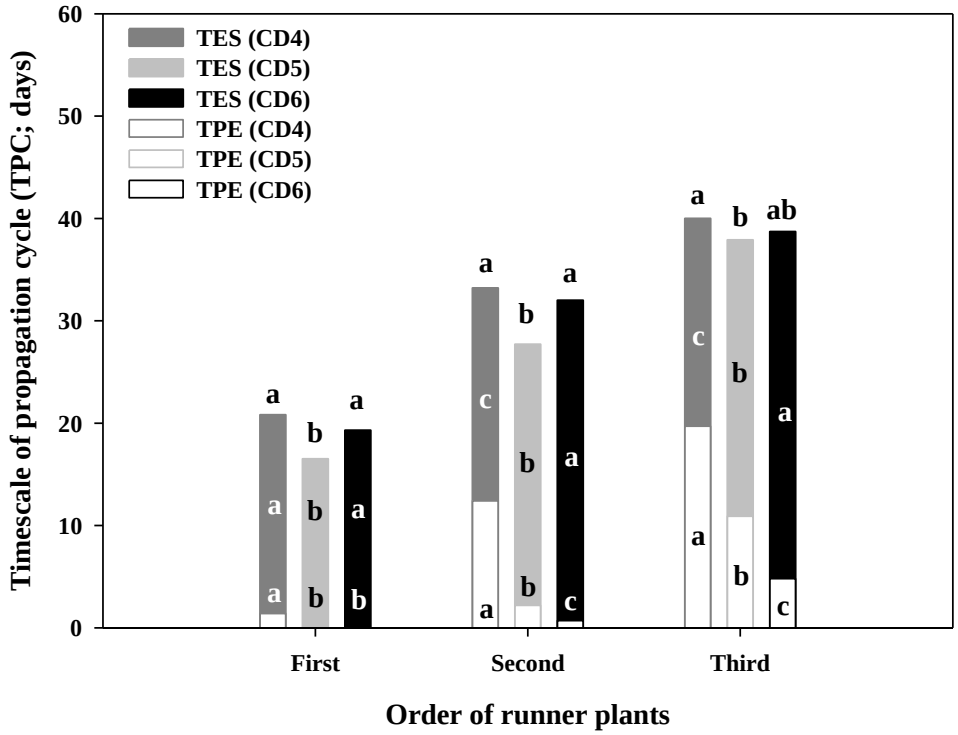

Fig. II-1. Timescales of propagation cycle (TPC), from planting propagules to emergence of runners (TPE) and from emergence of runners to separation of runner plants (TES) as affected by crown diameter in a plant factory with artificial lighting for transplant production for 90 days. Means above each bar followed by the same letters are not significantly different according to Duncan's multiple range test at $p < 0.05$.

and 3rd TES; however, that in CD4 was similar to the 2nd TES and 3rd TES. All of runner plants used as plant materials already had a runner at least. However, the bract of first runners in CD5 and CD6 had already unfolded before fixing runner tips whereas that in CD4 had not. Therefore, TES may lengthen as crown dimeter of propagules increases in case that runners of the propagules emerge at the same time.

Results in Chapter Ⅰ showed that 1st, 2nd, and 3rd TPCs of propagules with 5.3 mm or 6.1 mm crown diameters were 28, 33, and 39 days or 33, 37, and 46 days, respectively, and those were greater than the TPCs in the present study. In the previous experiment, the runner plants used as propagules after removing any attached runners. For that reason, their TPEs might be greater than those in the present study, which might be why their TPCs were greater than those in the present study.

Until 90 DAP, runner plants in the sixth propagation generation (PG6) had been produced, and propagules in PG4 were released from the T-PFAL due to producing three runner plants (Fig. II-2). TPCs in PG1 were smaller than those in other PG. Results in Chapter Ⅰ showed that crown growth rate of runner plants increased as resource support from their propagules increased. Because runner plants used as plant materials and initial propagules in PG1 were produced from stock plants with ca. 9 mm crown diameter, they might receive more resources and their crown growth might be faster than those in other PG considering their smallest number of leaves (Table II-1). Therefore, a larger resource pool of propagules in PG1 might shorten the TPCs.

The TPCs of CD6 increased as PG progressed; however, those of CD5 were

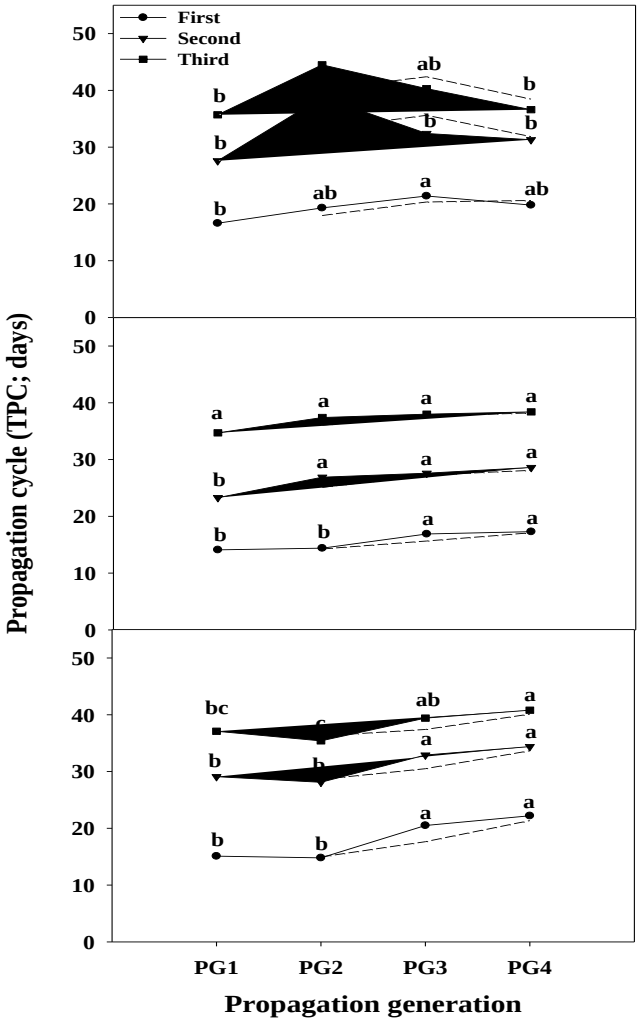

Fig. II-2. Time course of timescales of propagation cycles (TPC; solid line) and their moving averages (dash line) as affected by crown diameter in a plant factory with artificial lighting for transplant production for 90 days. Means above each point followed by the same letters are not significantly different according to Duncan's multiple range test at $p < 0.05$.

Table II-1. Numbers of leaves and runners of runner plants separated from their propagules as affected by propagation generation and their crown diameter 90 days after planting initial propagules.

			Leaves		Runners	
	TC[z]	PG[y]	No. of leaves	N-P[x]	No. of runners	N-P
Crown diameter	CD4		1.7c[w]	0.7	0.9c	-0.1
	CD5		2.2b	0.2	1.5b	-0.4
	CD6		3.2a	0.8	2.1a	0.0
Propagation generation	CD4	P[v]	1.0c	-	1.0a	-
		1G	1.5b	0.5	0.9a	-0.1
		2G	1.6ab	0.6	0.8a	-0.2
		3G	1.8a	0.8	0.9a	-0.1
		4G	1.8a	0.8	1.0a	0
	CD5	P	2.0b	-	1.9a	-
		1G	2.0b	0.0	1.4b	-0.5
		2G	2.1ab	0.1	1.5b	-0.4
		3G	2.2ab	0.2	1.5b	-0.4
		4G	2.3a	0.3	1.5b	-0.4
	CD6	P	2.4c	-	2.1ab	-
		1G	2.9b	0.5	2.1ab	0.0
		2G	3.0b	0.6	2.2ab	0.1
		3G	3.3a	0.9	2.3a	0.2
		4G	3.4a	1.0	1.8b	-0.3

[z] Treatment code.

[y] Runner plants of *i*th propagation generation.

[x] Subtract number of leaves or runners in initial propagules from that in their runner plant of *i*th PG.

[w] Means within each column followed by the same letters are not significantly different according to Duncan's multiple range test at $p < 0.05$.

[v] Numbers of leaves and runners of Initial propagules.

similar from PG2 to PG4. The 2nd TPC and 3rd T_{PC} of CD4 in PG2 were greater than those in other generations. When runner plants were separated from their propagules and used as new propagules, numbers of leaves and runners of them increased as crown diameter of propagules increased (Table II-1). As PG progressed, the number of leaves of them increased but number of runners did not. These results show that propagules in CD5 might be appropriate for ATPM due to a stable propagation cycles and number of leaves in their runner plants, unlike those in CD4 and CD6.

Growth of propagules producing three runner plants increased as initial propagule crown diameter increased; however, the growths in CD4 and CD5 were not significantly different except fresh weight of leaves (Table II-2). The 3rd T_{PC} in CD4 was ca. 2 days longer than that in CD5; however, the time from fixing runner tips with an unfolded bract to separating the runner plants from their propagules in CD4 was at least 5 days shorter than that in CD5 according to the result of Chapter Ⅰ. In other words, the propagules in CD4 reached this growth stage ca. 3 day earlier than those in CD5. In CD4, timescale that propagules were connected with three runner plants (T = 1st TPC – 3rd TPE) was shorter (1.1) than those in CD5 (5.6) and CD6 (14.5; Fig. 1). Caraco and Kelly (1991) reported that growth of propagules could be reduced by support to grow their runner plants. The earlier separation of runner plants could explain the higher growth rate of propagules in CD4 than those in CD5.

For 90 days, then number of transplants produced from nine propagules in CD5 was the greatest (445), followed by those in CD6 (323) and CD4 (308), in that order (Fig. 3). This result might be because T_{PC}s of CD5 were shorter than those

Table II-2. Growth of propagules producing three runner plants as affected by their crown diameter 90 days after planting initial propagules.

Treatment code	No. of leaves	Crown diameter (mm)	Fresh weight (g/plant)			Dry weight (g/plant)			T/R ratio
			Leaf	Crown	Root	Leaf	Crown	Roots	
CD4	6.9b[z]	10.4b	12.0c	2.0b	3.3b	2.84b	0.31b	0.32b	10.0b
CD5	6.9b	10.6b	13.5b	2.2b	3.6ab	3.12b	0.39ab	0.35b	10.4ab
CD6	7.7a	11.1a	16.2a	2.7a	3.9a	3.91a	0.43a	0.41a	10.8a

[z]Means within each column followed by the same letters are not significantly different according to Duncan's multiple range test at $p < 0.05$.

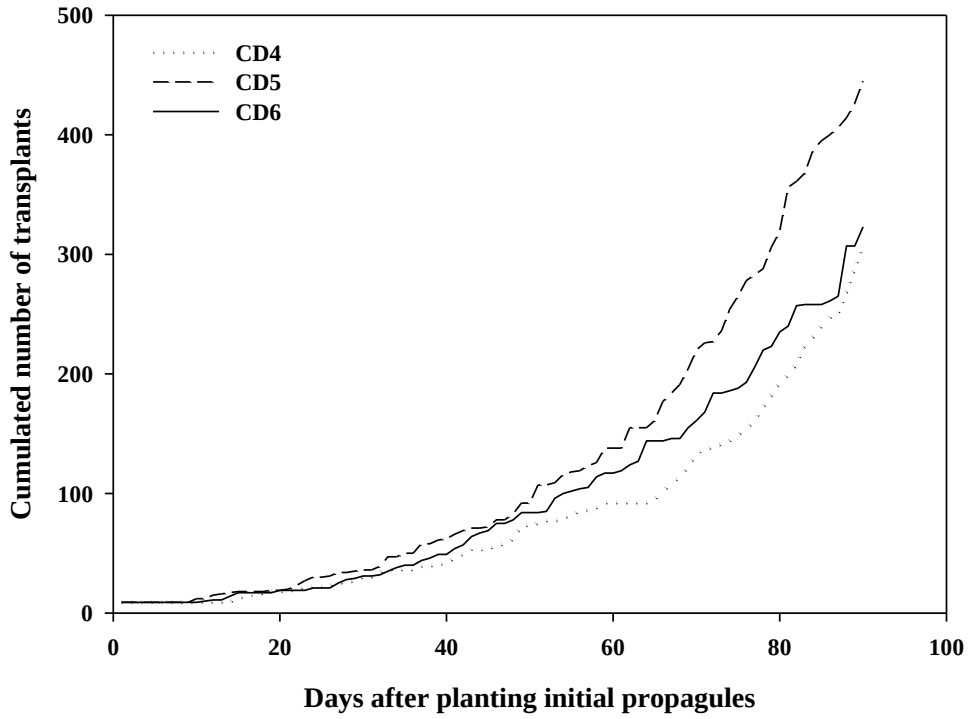

Fig. II-3. Time course of cumulated numbers of transplants as affected by crown diameter of propagules produced in a plant factory with artificial lighting by autotrophic transplant production method for 90 days.

of CD4 and CD6, as shown in Figure II-1. A new runner appeared only when propagules had sufficient or surplus resources (Alpert, 1991), and the propagules were sources for growth of runner plants, allowing them to grow under unsuitable environmental conditions (Savini et al., 2008). The propagules in CD4 might have smaller resource pools for runner emergence than those in CD5 and CD6. Those in CD6 seemed to have sufficient resource pools to produce runners; however, consumption of their resources was higher than those in CD4 and CD5 due to longer timescale that propagules were connected with three runner plants.

The presented results indicate that propagation cycles and transplant production rate in the ATPM are affected by initial propagule size, and propagules in CD5 having a ca. 5 mm crown, two leaves, and one runner seem to be appropriate for ATPM to rapidly produce uniform transplants in a T-PFAL because propagation cycles can increase when propagules is bigger or smaller than that size.

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CHAPTER III

Growth of Runner Plants Grown in a Plant Factory as Affected by Light Intensity and Container Volume

ABSTRACT

Transplant production in a plant factory with artificial lighting provides several benefits; rapid and uniform transplant production, high production rate per unit area, and production of disease free transplants production. To improve the growth of runner plants when strawberry transplants are produced in a plant factory, we conducted two experiments to investigate the effect of different light intensity for propagules and runner plants on the growth of runner plants, and the effect of different container volume for runner plants on their growth. When the propagule and runner plants were grown under nine different light conditions composed of three different light intensities for each propagule and runner plants, increasing the light intensity for propagules promoted the growth of runner plants. However, the growth of runner plants was not enhanced by increasing the light intensity for runner plants under same light intensity condition for propagules. I also cultivated runner plants using plug trays with four different container volumes for 20 days, and found that using plug trays with lager container volume did not enhance the growth of runner plants. Forty five days after separating, the growth of runner plants used as propagules was not significantly different based on the distance between

them. These results indicate that providing optimal condition for propagules, rather than the runner plants, is more important for increasing the growth of the runner plants and that the efficiency of strawberry transplant production in a plant factory can be improved by decreasing light intensity or container volume for runner. Arranging propagules and runner plants at 60 and 30 mm intervals, respectively, improves the production rate of strawberry transplants using the autotrophic transplant production method in a T-PFAL.

INTRODUCTION

A form of asexual reproduction in strawberries is clonal growth, in which runner plants derive from runners and remain attached to their propagules until their roots are sufficient to support themselves (Alpert and Mooney, 1986). The runner plants originate at runner tips; their leaves grow first, and then their roots and runners are formed (Nishizawa, 1994; Saito et al., 2008). The runners between the propagules and the runner plants transfer resources from the propagules to the runner plants to support growth (Roiloa et al., 2007). When propagule and runner plants grow in heterogeneous environments, they share resources with each other to procure insufficient resources in their respective environments (Alpert and Stuefer, 1997).

The runners in short-day cultivars grown in Korea are generated under long day conditions and high temperatures (Verheul et al., 2006), and their production rates increase under high photosynthetic photon flux (PPF) and CO_2 concentrations

(Chen et al., 1997; Kim et al., 2010). Using a plant factory with artificial lighting that specializes in transplant production, in which environmental conditions can be easily controlled, is appropriate for rapid and year-round transplant production. However, it is difficult to apply the conventional strawberry propagation methods to a plant factory because the size of propagules used in the conventional propagation method is too large. Kozai (2016) suggested that the appropriate height of transplants produced in a plant factory should be less than 15 cm to maintain adequate air current and relative humidity, while the height of propagules used in the conventional propagation method is approximately 25 cm and the length of their leaf blade and petiole are ca. 5 and 20 cm, respectively (Kim et al., 2010).

To overcome the limitations of the conventional propagation methods for transplants production in a plant factory with multi-layered shelves, Chun et al. (2012) developed a propagation method called the autotrophic transplant production method (ATPM). This method is performed as follows: (1) propagules whose size (crown diameter: ca. 5 mm) is smaller than that for conventional methods (9-13 mm; RDA, 2013) are used, (2) runner tips that have unfolded their bract (generated from the propagules) are fixed on medium in 150 mL pots, (3) runner plants derived from the runner tips are separated from their propagules when their crown diameter reaches the initial size of the propagules (ca. 20 days after placing propagules), and (4) they are then used as new propagules for next propagation cycle.

We hypothesized that, if all of the runner plants' nutrients and water can be received from propagules and unfavorable environmental conditions for runner

plants do not affect their growth while they are still connected with their propagules, the efficiency of transplant production by ATPM in a plant factory can be improved by decreasing the light intensity or container volume for the runner plants. To confirm these hypotheses, we investigated (1) the effect of different light intensities applied to both stock and runner plants on the growth of runner plants and (2) the effect of different container volumes for runner plants on the growth of runner plants. We also investigated the growth of propagules as affected by planting distance between propagules to determine the shortest planting distances for propagules and runner plants.

MATERIALS AND METHODS

Plant materials

Strawberry plants (*Fragaria* × *ananassa* Duch. cv. Maehyang) used for propagules were selected based on crown diameter of 5.0 ± 0.5 mm. Selected propagules had two compound leaves and one runner with an unfolded bract. They were planted into 150 mL containers using a 32-cell cutting plug tray (Bumnong Co. Ltd., Jeongeup, Korea) filled with commercial medium (Plant World; Nongwoo Bio Co. Ltd., Suwon, Korea).

Growth of propagules and runner plants as affected by PPF level

Six propagules, planted in 150 mL containers, were arranged in a row on the

bed and their runners were fixed in 150 mL containers filled with commercial medium for the formation of runner plants. The distance between plants in a row was 120 mm and between rows was 240 mm. Nine different treatments composed of three different light intensities were applied to both stock and runner plants; PPF 100 (104.8 ± 6.9 μmol·m^{-2}·s^{-1}), 200 (192.5 ± 5.6 μmol·m^{-2}·s^{-1}), and 400 (391.7 ± 12.8 μmol·m^{-2}·s^{-1}). Light intensity was controlled by changing the number of 32W cool white fluorescent lamps or using tinted films. Air temperature during the light/dark-period was maintained at 27°C/23°C, and the photo-period and CO_2 concentration were 16 h and 800 μmol·mol^{-1}, respectively. A closed irrigation system was used in the plant factory and plants were sub-irrigated with Yamazaki nutrient solution for strawberries (Yamazaki, 1978; pH 6.0 and EC 0.7 dS·m^{-1}) for 10 minutes once a day. At 20 days after placing (DAP), six stock and runner plants from each treatment were selected, and the number of compound leaves and runners, crown diameter, leaf area, and dry weight were measured.

Growth of runner plants as affected by container volumes

Six propagules, planted in 150 mL containers, were arranged in a row on the bed and the distance between plants in a row was 120 mm. We set four treatments with different container volumes (21, 34, 73, and 150 mL) using 32, 50, 72, and 128-cell cutting plug trays (Bumnong Co. Ltd., Jeongeup, Korea) for runner plants. Runner tips from propagules were fixed into containers with different volumes and cultivated for 20 days in a plant factory. The light intensity and photo-period were 210 μmol·m^{-2}·s^{-1} and 16 h, respectively. Air temperature during the light/dark-

period was maintained at 27°C/23°C and CO_2 concentration was 800 μmol·mol^{-1}. A closed irrigation system was used in the plant factory and plants were sub-irrigated with Yamazaki nutrient solution for strawberries (Yamazaki, 1982; pH 6.0 and EC 0.7 dS·m^{-1}) for 10 minutes once a day. At 20 DAP, 15 runner plants from each treatment were selected, and the number of compound leaves and runners, crown diameter, leaf area, and dry weight were measured. To investigate the subsequent growth of runner plants after being separated from their propagules, ten runner plants separated at 20 DAP were transplanted into plastic pots (ø 90 mm) filled with the commercial medium and grown for 35 days in a greenhouse located in Suwon, Korea (E 127.0º, N 37.3º). Thirty five days after transplanting, the number of compound leaves and runners, crown diameter, leaf area, and dry weight were measured.

Growth of propagules as affected by planting distance

Runner plants grown in a row adjacent to another row of propagules while they were connected with their own propagules were used as new propagules in the T-PFAL. The PPF in the shade beside the propagules where the runner plants were grown was 40–150 μmol · m^{-2} · s^{-1}. Five new propagules were placed in a row and spaced 60, 120, or 180 mm apart. The distance between rows was 300 mm, and the environmental conditions for growth of the new propagules and their runner plants were the same as those for growth of the stock plants. At 45 DAP the propagules on the propagation beds in the T-PFAL, we measured the numbers of leaves and

runners, crown diameters, leaf areas, and dry weights of the propagules.

Data analysis

The experimental data was analyzed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA) with Duncan's multiple range tests. Treatment differences were considered significant at a level of 5% probability.

RESULTS AND DISCUSSION

Growth of propagules and runner plants as affected by PPF levels

Table III-1 and III-2 show the growth of propagules and runner plants, respectively, as affected by PPF level at 20 DAP. The growth of both stock and runner plants was greatest when both were grown under 400 μmol·m^{-1}·s^{-1}, and those increased as PPF elevated. The growth of the runner plants was affected by the PPF of their propagules regardless of their own PPF level. The dry weight of leaves in the propagules was affected by PPF regardless of their runner plants' PPF levels; however, their root, runner, and total dry weights were affected by the PPFs of both the stock and their runner plants. When considering the sum of the PPF applied to both stock and runner plants {600 (400 + 200 or 200 + 400; propagule + runner plant), 500 (400 + 100 or 100 + 400), 300 (200 + 100 or 100 + 200)}, the total dry weight of stock and runner plants increased as the PPF of the propagules increased. Kim et al. (2010) reported that increasing PPF levels promoted the formation of

Table III-1. Growth and development of propagules as affected by photosynthetic photon fluxes for each propagules and runner plants 20 days after placing the propagules in a plant factory.

PPF		No. of leaves	No. of runners	Crown diameter	Dry weight (g / plant)				
P[z]	R[y]	(/plant)	(/plant)	(mm)	Leaf[x]	Crown	Root	Runner	Total
P400	P400	5.0a[w]	4.0a	7.1a	1.56a	0.09a	0.26a	0.42a	2.35a
	P200	4.5abc	2.7b	6.7a	1.38b	0.09a	0.22b	0.21b	1.90b
	P100	4.2bc	2.2bc	6.7a	1.38b	0.08ab	0.21b	0.16c	1.83bc
P200	P400	4.5abc	1.8bcd	6.3a	1.26b	0.08ab	0.21b	0.13cd	1.68c
	P200	4.2bc	1.2cde	6.4a	1.09c	0.08ab	0.20b	0.10de	1.47d
	P100	4.7ab	1.8bcd	6.2ab	1.06c	0.08ab	0.17c	0.07ef	1.39de
P100	P400	4.7ab	0.8de	6.1ab	0.98cd	0.08ab	0.17c	0.05fg	1.27e
	P200	4.2bc	0.8de	6.1ab	1.01c	0.08ab	0.14d	0.02g	1.26e
	P100	4.0c	0.5e	5.5b	0.85d	0.07b	0.11d	0.02g	1.06f
Significance									
	P	ns[v]	**	**	**	ns	**	**	**
	R	*	ns	ns	ns	ns	**	**	*
	S X R	*	*	**	**	ns	**	**	**

[z]PPF levels for propagules.

[y]PPF levels for runner plants.

[x]Leaf blades + petioles.

[w]Means within each column followed by the same letters are not significantly different according to Duncan's multiple range test at $p < 0.05$.

[v]ns: non-significant; *: significant at $p < 0.05$; **: significant at $p < 0.01$.

Table III-2. Growth and development of runner plants as affected by photosynthetic photon fluxes for each propagules and runner plants 20 days after placing the propagules in a plant factory.

PPF		No. of leaves	No. of runners	Crown diameter	Dry weight (g / plant)				
P[z]	R[y]	(/plant)	(/plant)	(mm)	Leaf[x]	Crown	Root	Runner	Total
P400	P400	2.6a[w]	2.2a	6.1a	0.66a	0.06a	0.14a	0.28a	1.15a
	P200	2.3a	1.8ab	5.7ab	0.61a	0.06ab	0.13ab	0.23ab	1.04ab
	P100	2.2a	1.7abc	5.7ab	0.59a	0.05abc	0.12b	0.24ab	1.00b
P200	P400	2.3a	1.5abc	5.6ab	0.47b	0.05abc	0.09c	0.23ab	0.83c
	P200	2.3a	1.5abc	5.6ab	0.46b	0.05abc	0.08cd	0.23ab	0.81c
	P100	2.5a	1.2bc	5.3bc	0.45b	0.05bcd	0.07de	0.20b	0.78c
P100	P400	2.3a	1.3bc	5.5bc	0.39bc	0.05cd	0.06ef	0.12c	0.62d
	P200	2.2a	1.2bc	5.1bc	0.38bc	0.05cd	0.05f	0.11c	0.59d
	P100	2.1a	1.0c	4.8c	0.34c	0.04d	0.03g	0.10c	0.51d
Significance									
	P	ns[v]	**	**	**	**	**	**	**
	R	ns	ns	ns	ns	ns	ns	ns	ns
	S X R	ns	**	ns	**	**	**	**	**

[z]PPF levels for propagules.

[y]PPF levels for runner plants.

[x]Leaf blades + petioles.

[w]Means within each column followed by the same letters are not significantly different according to Duncan's multiple range test at $p < 0.05$.

[v]ns: non-significant; *: significant at $p < 0.05$; **: significant at $p < 0.01$.

runners and runner plants in a closed transplant production system until 280 μmol·m^{-2}·s^{-1}. In this study, the number and dry weight of runners derived from runner plants were not significantly affected by the runner plants' PPF level. However, the number and dry weight of runners from propagules increased as their PPF level increased. In clonal plants, such as strawberry, assimilates and nutrients tend to move from older to younger plants because they diffuse from higher to lower concentrations until the system equilibrates, and assimilate and nutrient pools of older plants are greater than those of younger plants (Caraco and Kelly, 1991).

Figure III-1 shows that proportion of each organ in total dry weight of the propagules. Proportion of leaves decreased as the PPFs applied to both stock and runner plants increased while that of crown decreased as PPF for propagules increased. Strawberry plants connected to each other may overcome light deficiency by translocating assimilates from a plant with a high supply to a plant with a low supply by enhancing the leaf growth of the plants exposed to higher light intensity (Roila et al., 2007). However, assimilates produced by the runner plants might be used for establishment of them until 20 DAP as they could not grow independently, and they might attract assimilates and nutrients from connected propagules due to younger than the propagules (Alpert, 1996; Friedman and Alpert,1991; Savini et al., 2008).

As assimilates increase under higher light intensities (Kinet et al., 1985) and runner plants under lower PPF might attract more assimilates from their propagules, the proportional weight of leaves in the propagules might be higher when the PPF applied to the runners is lower.

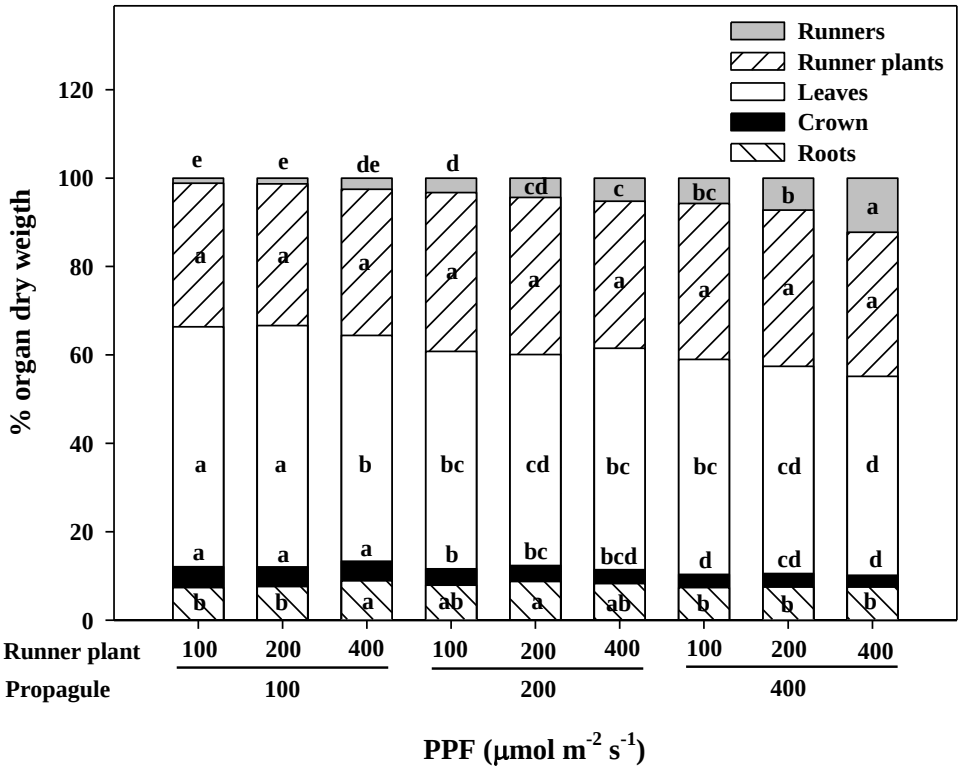

Fig. III-1. Proportion of each organ in the total dry weight of propagules as affected by the light intensity applied to propagules and runner plants 20 days after placing in a plant factory. Letters a-e indicate significant differences according to Duncan's multiple range test at $p < 0.05$.

Proportion of runner enhanced as the PPFs on both the stock and runner plants increased (Fig. III-1), and the dry weight of their runners also promoted as both PPFs increased (Table III-1). However, proportion of the runner plants was not significantly different by both PPF levels, while total dry weight of them increased when only the PPF for propagules increased (Table III-2). Runner growth depends on the amount of surplus resources for propagules because a new runner appears only when plants have sufficient resources (Alpert, 1991). Therefore, propagation rate will enhance as PPFs on both the stock and runner plants until ca. 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and runner plants under unfavorable environment conditions such as low light intensity by shading can be grow sufficiently by receiving assimilates or nutrients from propagules growing under favorable environment conditions.

Growth of runner plants as affected by container volumes

We found that the growth of runner plants was dependent on the light intensity applied to the propagules but not on the light intensity applied to the runner plants. We hypothesized that using small volume containers for runner plants, which would decrease the distance between stock and runner plants and might decrease the light intensity for runner plants due to shading, would not negatively affect their growth. To confirm this hypothesis, we investigated the effect of different container volumes on the growth of runner plants. The numbers of leaves and runners, crown diameter, leaf area, and dry weight of runner plants were not significantly affected by container volume (Table III-3). We fixed runner tips whose bract had just unfolded and where the root had not yet been established. The roots emerged after

approximately 6 DAP and grew as much as possible until being separated from the propagule 20 DAP (Chapter 1). Until this point, they might receive water and mineral nutrients from their propagules due the immaturity of their own roots (Alpert and Mooney, 1986; Friedman and Alpert, 1991). Moreover, as strawberry is clonal, runner plants connected to their propagules can overcome stress induced by environmental conditions through support from the propagules (Caraco and Kelly, 1991). In particular, a potential water gradient may exist from a propagule to a runner when the runner plant has not rooted (Alpert and Mooney, 1986). As roots of the runner plants grew for just 14 days and runner plants can receive water from their propagules, those grown in the smallest containers (21 mL/cell) did not show a reduction in growth due to the small container volume.

Runner plants separated from their propagules 20 DAP were transplanted into pots and grown 35 days after transplanting. Table III-4 shows growth and development of runner plants 35 days after transplanting. The growth of runner plants after transplanting into larger containers was not reduced by decreasing the container volume before separating. Therefore, until 20 DAP, runner plants connected with their propagules can be grown in 21 mL containers, which will both reduce the quantity of medium consumed and increase space efficiency when strawberry transplants are produced in a plant factory.

Growth of propagules as affected by planting distance

The growth of runner plants under the shade of propagules before separation was not significantly affected by the distance between propagules 45 DAP when

Table III-3. Growth of runner plants as affected by container volumes 20 days after placing the propagules in a plant factory.

Cell volume[z]	No. of leaves	No. of runners	Crown diameter (mm)	Leaf area (cm^2/plant)	Dry weight (g/plant)				
					Leaf	Runner	Stem[y]	Root	Total
21mL	2.7a[y]	1.2a	5.24a	59.9a	0.30a	0.25a	0.15a	0.05a	0.74a
34mL	2.7a	1.2a	5.26a	62.9a	0.30a	0.25a	0.15a	0.05a	0.76a
73mL	2.6a	1.3a	5.11a	63.0a	0.30a	0.24a	0.16a	0.06a	0.76a
150mL	2.8a	1.3a	5.27a	66.7a	0.31a	0.24a	0.15a	0.06a	0.76a

[z]Volume of the runner plant container while connected to their propagules.

[y]Petiols + a crown.

[x]Means within each column followed by the same letters are not significantly different according to Duncan's multiple range test at $p < 0.05$.

Table III-4. Growth of runner plants 35 days after transplanting in a green-house as affected by container volumes during connecting on their propagules.

Cell volume[z]	No. of leaves	No. of runners	Crown diameter (mm)	Leaf area (cm^2/plant)	Dry weight (g/plant)				
					Leaf	Runner	Stem[y]	Root	Total
21mL	6.9a[x]	3.0a	9.41a	385.2a	1.56a	1.81a	0.40a	0.71a	4.23a
34mL	6.8a	3.2a	9.06a	366.2a	1.42a	1.76a	0.37a	0.63a	3.81a
73mL	6.9a	3.3a	9.30a	369.0a	1.38a	1.56a	0.35a	0.68a	3.86a
150mL	6.6a	3.1a	9.13a	357.1a	1.39a	1.58a	0.40a	0.61a	4.24a

[z]Volume of the runner plant container while connected to their propagules.

[y]Petiols + a crown.

[x]Means within each column followed by the same letters are not significantly different according to Duncan's multiple range test at $p < 0.05$.

they themselves were used as propagules (Table III-5). Even at 60 mm, the space between the cells of a normal square 32-cell plug tray and the narrowest planting distance in the present study did not inhibit the growth of runner plants, which indicates that much greater planting density can be applied when using the ATPM in a T-PFAL than when using conventional propagation methods (200–350 mm; Crisp et al., 1988; Duner et al., 2002; Hancock, 1999). Therefore, the minimum planting distances for propagules and runner plants on a propagation bed in a T-PFAL were 60 mm and 30 mm, respectively (Fig. III-2).

In this study, we confirmed that the growth of runner plants is primarily affected by the environmental conditions of the propagules, and not the conditions (light intensity, container volume) of the runner plants prior to separation. Based on these results, the efficiency of strawberry transplant production by ATPM in a plant factory can be dramatically improved by reducing the resources provided to runner plants (i.e. light energy, medium quantity, space utilization etc.) prior to separation.

Table III-5. Growth of propagules as affected by distance between propagules on the propagation beds in a plant factory with artificial lighting 45 days after separating from their propagules.

Distance between propagules (mm)	No. of leaves	No. of runners	Crown diameter (mm)	Leaf area (cm^2/plant)	Dry weight (g/plant)			
					Leaf	Stem[z]	Root	Runner
60	6.5a[y]	3.2a	10.73a	422.4a	3.50a	0.45a	0.41a	0.66a
120	7.0a	3.6a	11.23a	446.7a	3.88a	0.47a	0.41a	0.58a
180	6.7a	4.0a	11.21a	432.3a	3.89a	0.51a	0.50a	0.54a

[z]Petiols + a crown.

[y]Means within each column followed by the same letters are not significantly different according to Duncan's multiple range test at $p < 0.05$.

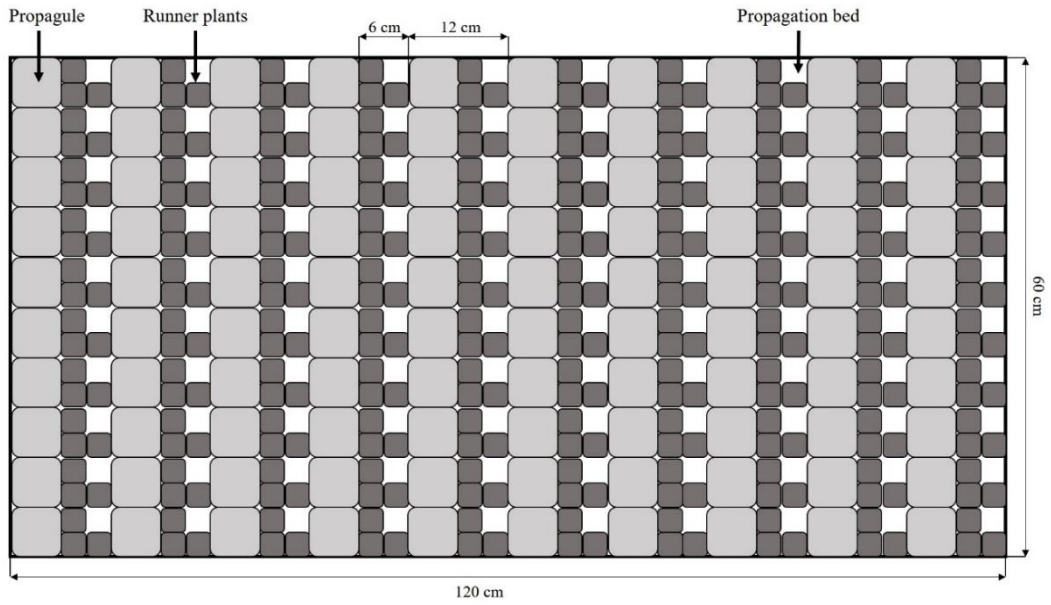

Fig. III-2. Arrangement plan of propagules and runner plants on a propagation bed in a plant factory with artificial lighting for transplant production based on planting distances for propagules (60 mm) and runner plants (30 mm).

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CHAPTER IV

Productivity of Autotrophic Transplant Production Method for Strawberry Transplants production in a Plant Factory with Artificial Lighting

ABSTRACT

To verify the productivity of the autotrophic transplant production method (ATPM), a novel propagation method in a plant factory with artificial lighting for transplant production (T-PFAL), strawberry transplants were produced by the ATPM for 365 days and the number of transplants was simulated using a program for the ATPM as affected by number of initial propagules and propagation area. 3,497 transplants were produced by the ATPM over 365 days with nine propagules in a cultivation area of 3.6 m^2 in the T-PFAL. The cumulated number of transplants (CNT) increased exponentially before reaching the maximum number of propagules in the propagation area; however, it continued to increase linearly after reaching the maximum number of propagules. When the simulated results were fit with the measured results, the propagation cycle timescales from planting propagules to producing the first, second, and third runner plants were 15, 27, and 43. The CNT produced from 5, 10, and 20 initial propagules in a cultivation area of 36 m^2 over 365 days were simulated by the verified program along with the propagation cycles, and these values were 27,970, 30,010, and 31,900, respectively.

The slopes of the linearly increasing section were equal regardless of the number of propagules, although the exponentially increasing section of the 20 initial propagules was the shortest. The simulated CNT from nine initial propagules in 18 and 72 m^2 over 365 days were 15,950 and 55,940. The exponentially increasing section in 72 m^2 was the longest and the slope of the linearly increasing section was the highest among the cultivation areas. These results indicate that the ATPM is an appropriate propagation method to produce transplants rapidly in a T-PFAL, especially, in case that the number of propagules or propagules is limited.

INTRODUCTION

Programs for the production of certified disease-free plants have been developed in major strawberry production countries for preventing spread of diseases during propagation and supplementation of high quality propagules (Broome and Goff, 1986). In terms of certified strawberry plants, there are several different grading systems which include nuclear transplants, elite transplants, pre-basic transplants, basic transplants, and disseminative transplants under the Korean system (Chun, 2016). In 2016, required numbers of transplants for each step were 100 nuclear transplants, 4,000 elite transplants, 100,000 pre-basic transplants, 2,000,000 basic transplants, and 34,000,000 disseminative transplants, respectively.

Higher grade transplants are grown under very strict conditions and they are propagated under careful control. However, the low propagation rate of transplants by use of conventional propagation methods is a major problem for rapid

distribution of certified plants as 40 transplants are produced from a propagule per year in the conventional methods (Savini et al., 2008).

A plant factory with artificial lighting for transplant production (T-PFAL) is a thermally insulated warehouse-like structure in which disease-free transplants can be produced rapidly under a controlled environment in a protected area free from pest insects/pathogens and the disturbance of outside weather (Kozai, 2006). Although conventional methods can be used for upper-level transplant production in the program, e.g., the elite transplants and pre-basic transplants; these methods are not appropriate for use in a T-PFAL. Therefore, Chun et al. (2012) developed a novel propagation method, named autotrophic transplant production method (ATPM), for strawberry propagation in a T-PFAL.

The ATPM differs from conventional methods in terms of the size of propagules. The propagules for the ATPM are smaller than those for conventional methods for propagation in a T-PFAL with multi-layer propagation beds. Results in Chapter I and II showed that runner plants with a crown diameter of approximately 5 mm and two leaves were appropriate for use as propagules in the ATPM due to shorter propagation cycles (timescales from planting propagules to producing the first, second, and third runner plants) and greater production amount of transplants. Moreover, propagation cycles in the ATPM were shorter and more replicative than those in conventional methods. Runner plants produced from propagules in the ATPM were used as propagules for the subsequent propagation generation (timescale from planting a propagule to producing three runner plants from the propagule), while runner plants in the conventional methods were only used as

transplants due to just one propagation generation.

Kubota and Kozai (2001) reported that the propagation rate of transplants can be precisely controlled and predicted using modeling and simulation; however, the accuracy of predicting the number of transplants produced may be decreased after many repetitions of propagation generations.

When producing transplants using a T-PFAL, the number of transplants produced should be calculated because propagation space is more limited than in a greenhouse or field. As the required numbers of elite transplants and pre-basic transplants to be produced in provincial agriculture research centers during one season are usually decided, a T-PFAL for the ATPM could be designed based on these numbers to reduce the initial investment costs for a facility. Moreover, the elite transplants and pre-basic transplants should be produced from nuclear transplants that are generated by tissue culture to eliminate viruses and other pathogens (Chun, 2016). In tissue culture, one meristem induces one plant because of problems with variant types such as the hyperflowering trait as reported by Jemmali et al. (1995), and thus the number of initial propagules is also limited. Moerover, efficiency of the propagation and distribution system can be improved through reducing time for dissemination of disease-free or new cultivar transplants if pre-basic or basic transplants can be produced from 100 nuclear transplants for a year.

To verify the productivity of strawberry propagules in the ATPM, we produced transplants by the ATPM for 365 days and compared the accumulated number of transplants to the number simulated by the program. We also evaluated strawberry

transplant production by ATPM as affected by the number of initial propagules and propagation area by the program to predict production of strawberry transplants, and determined required cultivation areas to produce pre-basic and basic transplants from 100 nuclear transplants for a year.

MATERIALS AND METHODS

Transplants production during one year

Strawberry plants (*Fragaria* × *ananassa* Duch. cv. Maehyang) for propagules were selected based on crown diameter of ca. 5 mm in this study. Selected propagules had two compound leaves and one runner with an unfolded bract. We planted them into 150 mL containers using 32-cell cutting plug tray (Bumnong Co. Ltd., Jeongeup, Korea) filled with commercial medium (Plant World; Nongwoo Bio Co. Ltd., Suwon, Korea).

When the runner plants were placed as propagules on the propagation bed in the T-PFAL, their runner tips were fixed on the medium trays regardless of the size of the runner tips once the tips' bracts had been unfolded. The initial propagules in every group had more than one runner tip fixed on the medium. The crown diameter of the runner plants derived from the runner tips was measured every two or three days and the runner plants were separated from their propagules when their crown diameter reached the initial crown diameter. The separated runner plants were placed in an area for propagules on the bed and cultivated as propagules in the next propagation generation. The number of separated runner plants was measured when

their crown diameter was reached.

The propagules that produced three runner plants were released from the T-PFAL. When the number of propagules in the T-PFAL exceeded the maximum number of propagules that could be cultivated in the propagation area (3.6 m^2; 160 propagules), the new propagules that was runner plants just separated from their propagules were also released from the T-PFAL. The number of propagules and runner plants released were each measured.

The propagules, propagules, and runner plants were grown in a T-PFAL with a 32W cool white fluorescent lamp (TLD32W830RS, Philips Electronics, The Netherlands) for each bed and the PPF level above the bed was 230 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Air temperature during photo/dark-period was maintained at 27°C/23°C, and the photo-period and CO_2 concentration were 16 h and 800 $\mu mol \cdot mol^{-1}$, respectively. Closed irrigation system was applied to the plant factory used in this study and plants were sub-irrigated with Yamazaki nutrient solution for strawberries (Yamazaki, 1978; pH 6.0 and EC 0.7 $dS \cdot m^{-1}$) for 10 minutes once a day.

Description of the program to predict production of strawberry transplants for the ATPM

Figure IV-1 shows a flow chart of the program to predict production of strawberry transplants for the ATPM. The variables in this program were propagation period (T_P), propagation cycle of the first, second, and third runner plans {$T_{PC\ (i\text{-}1)}$, $T_{PC\ (i\text{-}2)}$, and $T_{PC\ (i\text{-}3)}$}, cultivation area (maximum number of propagules placed in the cultivation area; N_{MP}), and the initial number of propagules

(N_{IP}). These variables should be set first in order to run the program. First, the number of days after placing the initial propagules is compared to T_P to determine when propagation should end. The age of propagule (A) with the date of separating them is compared with $T_{PC\ (i\text{-}1)}$, $T_{PC\ (i\text{-}2)}$, and $T_{PC\ (i\text{-}3)}$ when T is smaller than T_P, and a runner plant is counted in the production amount when A is equal to $T_{PC\ (i\text{-}j)}$. Then, the runner plant and its propagule are evaluated separately. The propagule is appraised as either a non-activated propagule and released from the propagation area for shipping out or cold storage when A is equal to $T_{PC\ (i\text{-}3)}$, or it is appraised as an activated propagule and maintained in the propagation area for propagation. Runner plants are appraised as an activated runner plant and planted into the propagation area as new propagules when the number of propagules in the propagation area is smaller than N_{MP}. Otherwise, they are appraised as a non-activated runner plant and released from the propagation area for shipping out.

We ran the program to determine propagation cycles through fitting with measured results for 365 days and the results were compared to the measured results by regression analysis. The accumulated number of transplants over 365 days as affected by N_{IP} and N_{MP} was simulated by the program. The required propagation areas to produce pre-basic and basic transplants from 100 nuclear transplants for a year were also determined.

Data analysis

The experimental data was statistically analyzed by statistical analysis software (SAS 9.2, SAS Institute Inc., Cary, NC, USA) using Duncan's multiple range tests.

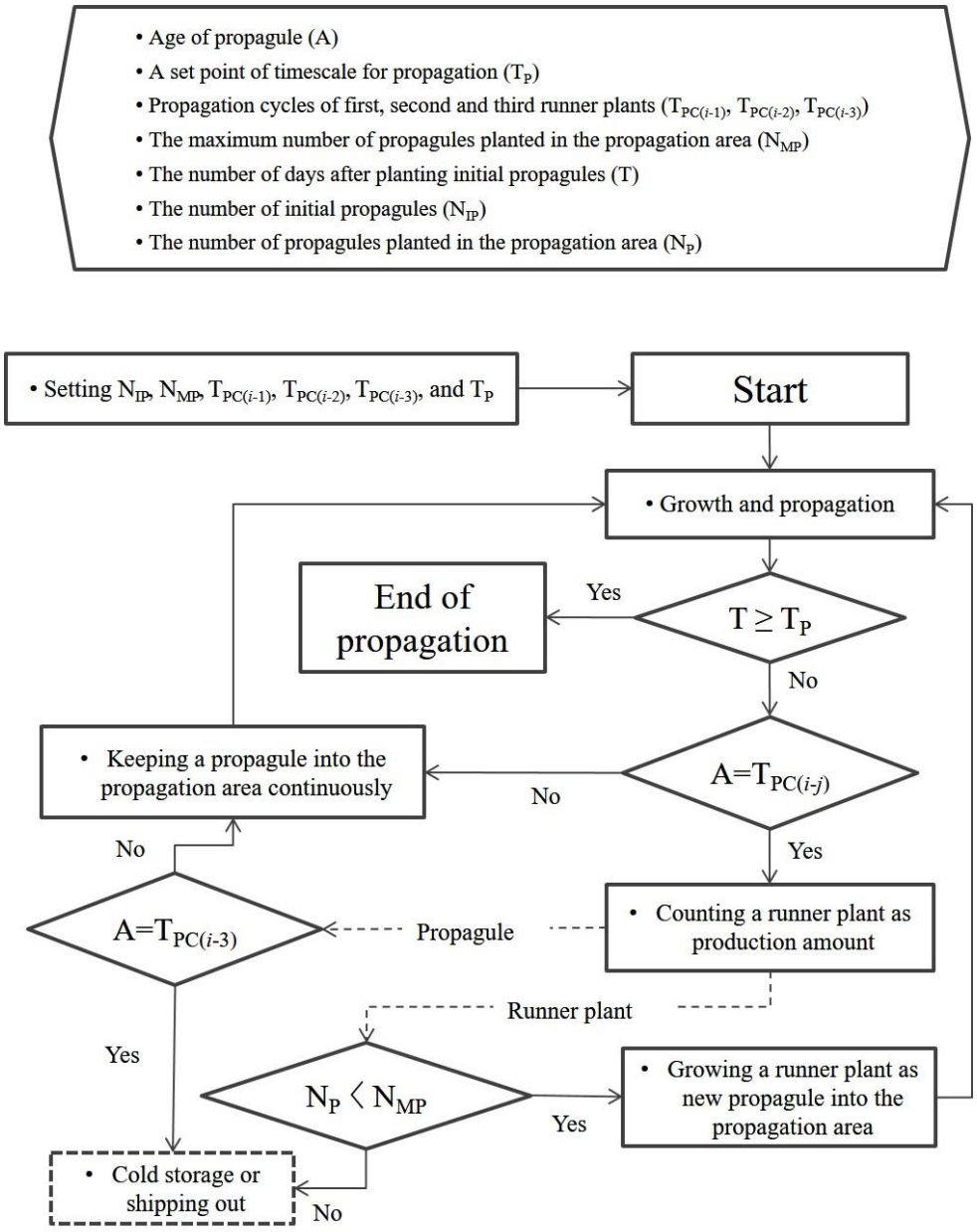

Fig. IV-1. A flow chart of a program to predict production of strawberry transplans for autotrophic transplant production method.

The treatment differences were considered to be significant at a level of 5% probability. Regression analysis was conducted using SigmaPlot^{TM} software (Systat Software, San Jose, CA, USA) to estimate the coefficient of determination.

RESULTS AND DISCUSSION

The cumulated number of transplants (CNT) produced in a T-PFAL by the ATPM over 365 days was 3,497 plants (Fig. IV-2). The number of transplants increased exponentially before N_P reached N_{MP}; however, the number increased linearly after N_P reached N_{MP}. At 35 DAP, propagules producing three runner plants were released from the T-PFAL. Runner plants were also released at 71 DAP due to limitations in the propagation area in the T-PFAL. The number of propagules and runner plants released were 1,193 and 2,144, respectively. Before N_P reached N_{MP}, the runner plants just separated from their propagules were used as new propagules in the next PG; however, the third runner plants were used as propagules after N_P reached N_{MP} because the propagules were released after producing their third runner plant. Therefore, the number of runner plants released was approximately twice as high as the number of propagules released.

We fit the simulated results so they were close to the measured results and determined propagation cycles of 15, 27, and 43 (Fig. IV-3). The CNT simulated by the program was 3,452, which was smaller than the measured CNT. The linearly and exponentially increasing sectors of the simulated result were similar to the

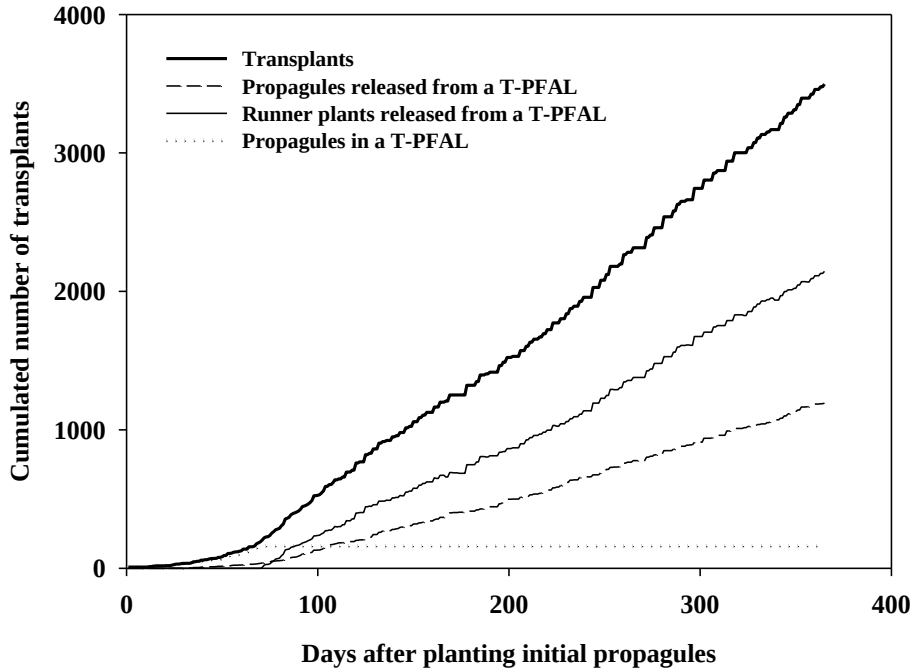

Fig. IV−2. Time course of cumulated numbers of transplants, propagules in a plant factory using artificial lighting (PFAL) and transplants released from a PFAL produced by autotrophic transplant production method. The transplants were annually produced from nine initial propagules in 3.6 m^2 propagation area (maximum capacity number of propagules: 160).

measured result.

Figure IV-4 shows a scatter plot of the transplants produced over 365 days measured and simulated by the prediction program and simple linear regression. The linear regression equation was y = 1.007x – 3.547 which indicates that the measured and simulated results are very similar. Moreover, the coefficient of determination value for the model was 0.99. Therefore, we concluded that the program is appropriate to predict the number of transplants produced by the ATPM in a T-PFAL.

Using the program, we investigated the productivity of ATPM as affected by the number of initial propagules (N_{IP}) and cultivation area which was N_{MP} (Fig. IV-5). The CNT produced from 5, 10, and 20 initial propagules (IP) in 36 m^2 over 365 days was 27,970, 30,010, and 31,900, respectively. The CNT produced from 20 IP over 365 days was 1.14 times higher than the number produced from 5 IP even though the N_{IP} was four times bigger. The N_{MP} produced from 5, 10, and 200 IP was 1,600 plants; however, the timescale needed to reach the N_{MP} produced from 20 IP was 114 DAS which was shorter than the timescale needed from 5 and 10 IP (148 and 131 DAS, respectively). The slopes of the linearly increasing section were the same regardless of the N_{IP}. The CNT produced from 10 IP in a cultivation area of 18 and 72 m^2 were 15,950 and 55,940, respectively. The transplants produced in 18 and 72 m^2 were about 53% smaller and 186% higher than those produced in 36 m^2 whereas the cultivation areas were 50% smaller and 200% higher. The N_{MP} produced in 18 and 72 m^2 were 800 and 3,200 plants. The timescale required to reach these N_{MP} values were 114 and 148 DAS and the timescale increased as the

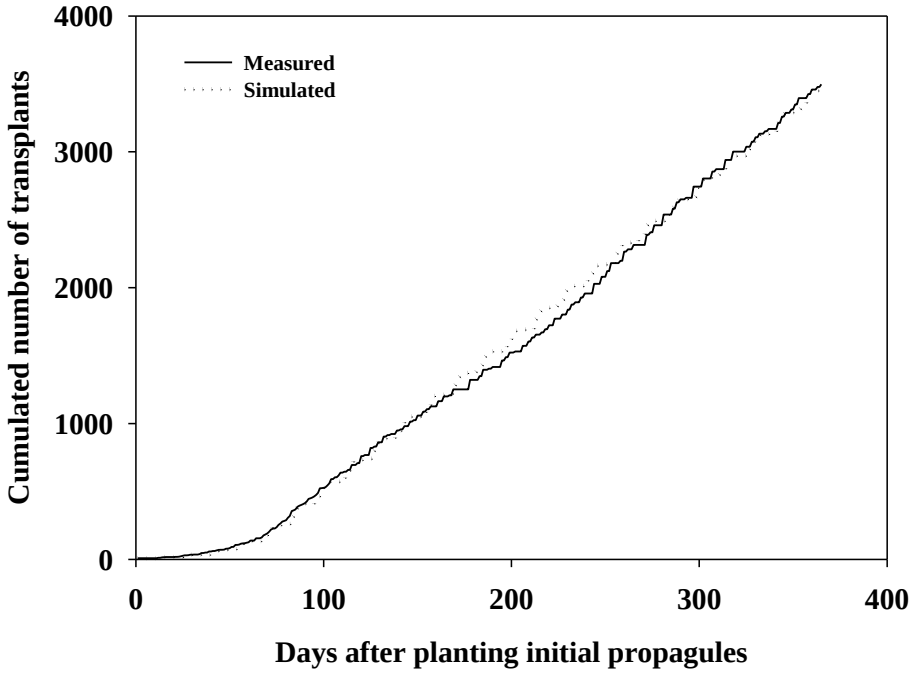

Fig. IV−3. Time course of cumulated numbers of transplants measured for 365 days and simulated by a program to predict number of transplants for autotrophic transplant production method. The transplants were annually produced from nine initial propagules in 3.6 m^2 propagation area (maximum capacity number of propagules: 160) and the simulated result was also simulated by the same conditions as measured one.

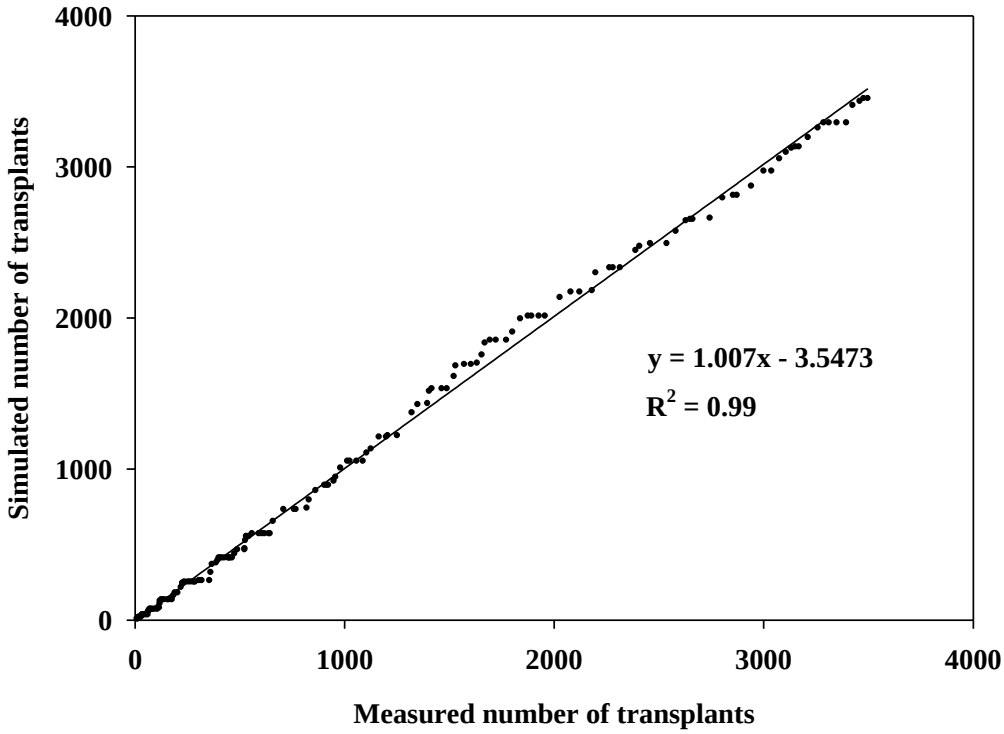

Fig. IV−4. Measured and simulated numbers of transplants for 365 days at same time. Simulated results were drived from a program to predict number of transplants for autotrophic transplant production method. The transplants were annually produced from nine initial propagules in 3.6 m^2 propagation area (maximum capacity number of propagules: 160) and the simulated result was also simulated by the same conditions as measured one. Propagation cycles used for the program were 15, 27 and 43. The coefficients of determination were estimated by regression analysis.

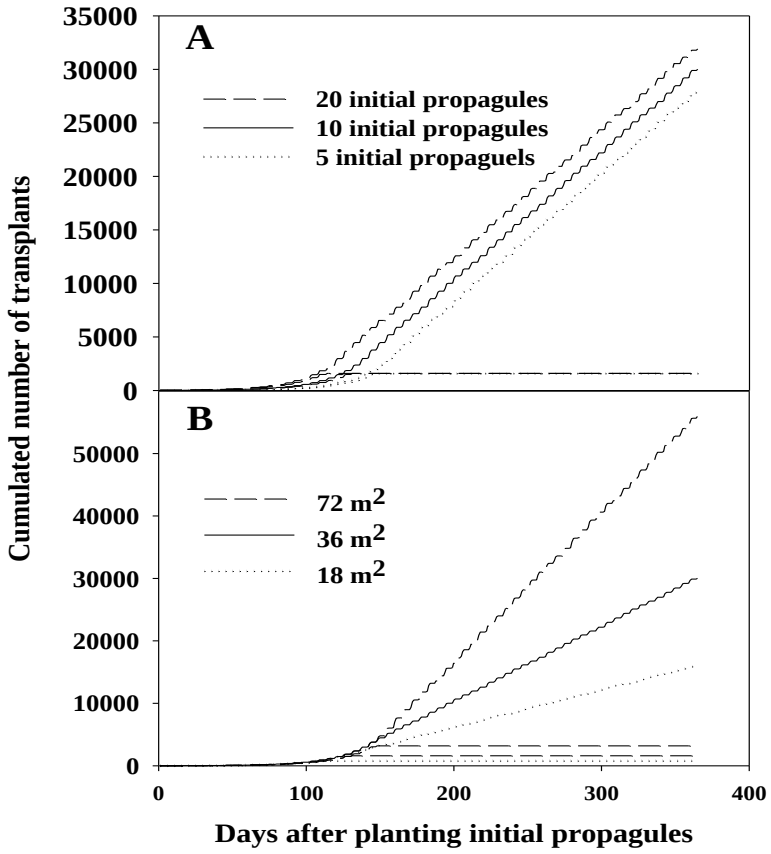

Fig. IV−5. Time courses of cumulated numbers of transplants simulated by a program to predict number of transplants for autotrophic transplant production method as affected by number of initial propagules (A) and propagation area (B). The solid lines are simulated results by propagation cycles (15, 27 and 43) and ten propagules in 36 m^2 propagation area (maximum capacity number of propagules: 1,600) and dash and dotted lines are results simulated by increasing and reducing propagation area or number of initial propagules by two times the result of solid line.

propagation area was expended. The propagation rates of the linearly increasing section of 18, 36, and 72 m^2 were approximately 59, 120, and 240 plants·d^{-1} and the difference between them was similar to differences in the propagation area. In other words, the wider the propagation area, the longer the timescale needed to reach the start of the linearly increasing section and the higher the slope of the linearly increasing section. Therefore, the increment of the CNT as an increment of cultivation area was higher than that as an increment of the N_{IP}.

Required propagation areas to produce 100,000 runner plants (pre-basic transplants) and 2,000,000 runner plants (basic transplants) from 100 propagules (100 nuclear transplants) were 115 m^2 and 3,130 m^2, respectively (Fig IV-6). The propagation area difference between producing 100,000 and 2,000,000 runner plants was ca. 27 times, but those accumulated numbers of transplants was 20 times. In case of using three modules {a module: eight beds; 8.0 m x 0.6 m x 3.0 m (L x W x H); width for every aisles: 1.0 m} for production of 100,000 runner plants, there needed 58 m^2 to build a T-PFAL. Propagation area for 2,000,000 runner plants was 1,144 m^2, ca. 20 times higher than that for 100,000 runner plants, in case of using 27 modules {a module: eight beds; 24.0 m x 0.6 m x 3.0 m (L x W x H); width for every aisles: 1.0 m}. Therefore, timescale for propagation and distribution system for domestic cultivars in Korea can be shortened not only two years but also three years if the T-PFAL can be built large.

Our results indicate that the ATPM is an appropriate propagation method to produce strawberry transplants rapidly in a T-PFAL, and it could be a suitable method to enhance the propagation and distribution system for domestic strawberry

cultivars in Korea in case that the number of propagules or propagules is limited, especially.

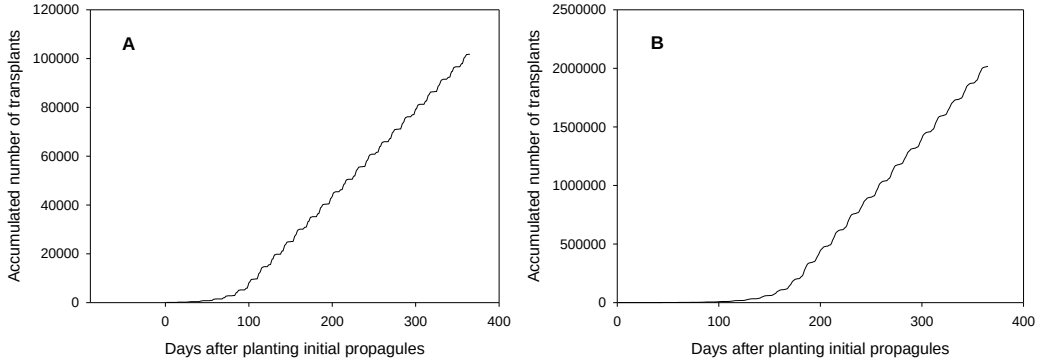

Fig. IV−6. Time courses of accumulated numbers of transplants simulated by a program to predict number of transplants for autotrophic transplant production method as affected by propagation area 115 (A) and 3,130 m^2 (B) to produce 100,000 and 2,000,000 transplants, respectively, from 100 initial propagules for a year.

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CONCLUSION

Chapter I demonstrated that early fixation of runner tips induced rapid root emergence in runner plants and led to much smaller propagules than conventional propagation methods. Runner plants should not be separated from their propagules until 15 DAF in a T-PFAL, as they cannot yet photosynthesize and cannot survive on their own roots. However, separating runner plants and propagules at 20 DAF based on a 5.3-mm crown diameter may improve propagation rates by shortening propagation cycles. Root growth of runner plants separated from propagules at 15 DAF was reduced due to cessation of resources from their propagules during higher root growth rate even though the propagation cycles of runner plants separated 15 and 20 DAF were not significantly different.

Chapter II indicated that propagules in CD5 having a ca. 5-mm crown and two leaves may be appropriate for ATPM, which will produce uniform transplants rapidly in a T-PFAL. At 90 DAF, the greater number of accumulated transplants was produced from nine propagules in CD5 than CD4 and CD6. Average propagation cycle length and change in propagation cycles as progress of propagation generation of the propagules were less in CD5 than in CD4 and CD6.

In Chapter I, runners from runner plants were removed when the runner plants were used as propagules, whereas those in Chapter II were left attached to their runner plants. Propagation cycles of propagules with a 6.0-mm crown diameter in Chapter I were greater than those in Chapter II. Runners that occurred before

planting propagules are shortened propagation cycles even though they are burden when their runner plants are just separated from the propagules.

Chapter III demonstrated that it is more important to control the environmental conditions of propagules than of runner plants in order to increase the growth of runner plants and the propagation rate. Arranging propagules and runner plants at 60 and 30 mm intervals, respectively, improves the production rate of strawberry transplants using the autotrophic transplant production method in a T-PFAL. The runner plants can grow on the 128-cell cutting plug tray when attached to their propagules; however, to use runner plants as a propagule, they need to be transplanted to a 32-cell cutting plug tray because a 128-cell tray has insufficient volume to grow as propagules. The 32-cell cutting plug tray was an appropriate container volume for runner plants, which could reduce labor cost and simplify the work process.

Strawberry transplants were produced via ATPM in a T-PFAL based on the results of Chapters II and III for 365 days and the number of transplants produced is presented in Chapter IV. The productivity of ATPM was much greater than that of the conventional methods and it can produce transplants rapidly with a small number of initial propagules.

This thesis was the first attempt to produce strawberry transplants in a PFAL; ATPM using a T-PFAL is a novel propagation method for rapid production of disease-free transplants. Using this technique for upper step in the propagation and distribution system for domestic strawberry cultivars will be able to spread new cultivars quickly.

In order to apply ATPM to the strawberry transplant production, two studies must be carried out. (1) Cold storage techniques for runner plants that are released immediately from the T-PFAL should be developed in order to producing transplants by ATPM all year round because strawberry transplants are used as mother plants to produce transplants or transplants to produce fruits for ca. 5 months (mother plants: February to April; transplants: September to October in early and standard forcing cultures). (2) Appropriate environmental conditions in the T-PFAL to enhance productibity of ATPM and (3) appropriate light sources, such as LED (fluorescent lamps in this study), in the T-PFAL to enhance energy efficiency should be also determined in the future. The results of this and subsequent studies will contribute more to sustainable development of the strawberry industry.

ABSTRACT IN KOREAN

본 논문은 4장의 딸기 연구로 구성되어 있다. 제1장에서는 육묘 전용 인공광 이용형 식물공장을 이용한 새로운 증식법인 독립영양채묘법을 이용한 딸기 묘의 증식률을 극대화 시킬 수 있는, 증식체로부터 자묘의 분리 시기를 기반으로 한 증식체의 적정 크기를 구명했다. 포엽이 전개된 런너팁을 배지에 고정했을 때, 완전 전개된 첫 본엽, 첫 런너 및 뿌리가 런너팁 고정 후 6-10일 (DAF)에 나타났고, 지상부와 지하부 건물증은 각각 6 및 10 DAF에 증가했다. 30 DAF까지 자묘의 T/R률은 10 DAF일 때 가장 높았다. 9, 11 및 13 DAF 에 채묘한 자묘의 광합성률은 채묘 이후에 감소하는 반면, 15, 17 및 19 DAF 에 채묘된 자묘는 그렇지 않았다. 자묘는 인공광 이용형 식물공장 내 환경제어를 통해 15 DAF 이후로는 독립적이고 자주적인 생장이 가능했다. 15, 20 및 25 DAF 에 채묘된 자묘는 10 DAF 에 채묘된 것과 달리 30 DAF까지 다음 증식 세대의 증식체로서 성공적으로 생장했다. 30 DAF에서, 15 DAF에 채묘된 자묘의 뿌리 건물중이 20, 25 및 30 DAF에 채묘된 자묘의 것 보다 작은 반면 엽 및 런너의 건물중은 유의한 차이가 없었다. 작은 크기의 증식체의 사용은 증식사이클을 줄일 수 있었지만 15 DAF에 채묘한 자묘의 생장이 상대적으로 충분하지 못했기 때문에 15 및 20 DAF에 채묘한 증식체의 증식사이클은 유의한 차이가 없었다. 제2장에서는 육묘 전용 인공광 이용형 식물공장에서 독립영양채묘법을 이용한 딸기묘의 증식 효율 극대화를 위한 적정 증식체를 구명하였다. 관부직경이 5mm인 증식체에서 채묘된 첫 번째, 두 번째 및 세 번째 자묘의

증식사이클 {증식체 배치부터 런너 발생까지 기간 (TPE)과 러너 발생부터 자묘 채묘까지 기간의 합 (TES)}은 관부직경이 4 및 6 mm인 증식체의 것보다 짧았다. 두 번째 및 세 번째 자묘에서, TPE는 증식체의 관부직경이 감소할수록 증가했으나 TES는 감소했다. 관부직경이 5 mm인 증식체의 증식세대별 증식 사이클의 변동은 관부직경이 4 및 6 mm인 증식체의 것보다 적었고, 채묘 후 자묘의 엽수의 변화도 관부직경이 5 mm인 증식체가 관부직경이 4 및 6 mm인 증식체보다 적었다. 증식체 배치 후 90일까지 누적 딸기묘 생산량은 관부직경이 5 mm인 증식체가 관부직경이 4 및 6 mm인 증식체보다 많았다. 제3장에서는 식물공장에서 딸기 묘 증식 시 자묘의 생육을 증진시키기 위해 증식체와 자묘의 다른 광환경에 대한 효과와 자묘의 다른 용기에 따른 효과를 조사하는 연구를 수행했다. 증식체와 자묘 각각 3수준의 광량을 조합한 9수준의 다른 광조건에서 증식체와 자묘를 재배했을 때, 증식체의 광량이 증가함에 따르 자묘의 생육은 증가했으나 증식체의 광량이 동일한 상황에서는 자묘의 광량이 증가해도 자묘의 생육은 차이가 없었다. 또한 4수준의 재배용기에 자묘를 20일간 재배했는데 용기 크기에 상관없이 자묘의 생육이 유사한 것을 확인하였다. 제4장에서는 육묘 전용 인공광 이용형 식물공장에서의 독립영양채묘법의 생산성을 확인하기 위해 365일간 딸기묘를 독립영양채묘법으로 증식시켰고, 독립영양채묘법 전용 생산량 예측 프로그램을 이용하여 최초 증식체 개수 및 면적에 따른 생산량을 예측하였다. 9개의 증식체를 3.6 m^2에서 365일간 독립영양채묘법으로 증식해서 3,497개의 정식묘를 생산했다. 증식 중 증식체의 실 개수가 재배면적의 최대 수용 증식체 개수에 도달하기 전까지는 누적 정

식묘 생산량이 기하급수적으로 증가했으나 최대 수용 개수에 도달한 이후에는 선형으로 증가했다. 실측값과 예측값을 맞췄을 때 증식사이클은 15, 27 및 43일이었다. 재배 면적 36 m^2에서 최초 증식체 개수가 5, 10 및 20개 일 때 누적 정식묘 생산량은 각각 27,970, 30,010 및 31,900개로 예측되었다. 선형 증가 구간의 기울기는 최초 증식체 개수에 상관없이 동일했으나 기하급수적인 증가 구간은 최초 증식체 개수가 많을수록 짧았다. 10개의 증식체로 재배 면적 18 및 72 m^2 에서 365일간 생산된 누적 정식묘 생산량은 각각 15,950 및 55,940개로 예측되었다. 재배 면적이 증가할수록 기하급수적인 증가 구간이 길었고, 선형 구간의 기울기가 높았다. 이 결과들을 통해서 육묘 전용 인공광 이용형 식물공장에서 독립영양채묘법으로 증식 시 관부 직경이 약 5 mm, 엽수 2매인 증식체가 정식묘를 안정적으로 빨리 생산하는데 적합한 것으로 판단되며, 적정환경 환경 조성 시 자묘보다 증식체를 중식으로 제어를 하는 것이 자묘의 생육 증진에 중요한 것으로 보여진다. 독립영양채묘법은 육묘용 인공광 이용형 식물공장에서 딸기 묘를 급속 증가시키며 증식체가 제한된 상황에서 더욱 알맞기 때문에 신품종 육성 또는 조직배양을 통한 묘 갱신 시 초기 단계의 증식률 증가에 적합한 것으로 사료된다.