Implementation and Monitoring of Vegetative Propagation in the Context of Vertical Farms

Rong (Janet) Chao

Department of Biology, Utrecht University

General Research Profile

Dr. Vincent Truffault (Supervisor Host Institute) and Dr. Jaco Appelman (Examiner)

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Abstract

Strawberry is known as a valuable crop due to its tasty and healthy fruit. It has been shown that strawberry fruit production is susceptible to pests and diseases, and thus most of the current nurseries use multiple pesticides in strawberry plants. However, the plants stressed by chemicals do not meet the requirements at Futura Gaïa, an indoor vertical farming company producing high-quality crops in a pesticide-free and resource-efficient way. Therefore, the company has a need for strawberry plants that fit the indoor farming system. In this study, we attempt to validate agronomical recipes for strawberry asexual reproduction and develop an indoor vegetative propagation system that can effectively produce strawberry daughter plants. We followed the biomimicry design spiral to define the challenge, learn lessons from nature, and deliver innovative solutions to the problem. The vegetative propagation was monitored and compared under different light sources and nitrogen levels in fertigation. The sun light LED and the white light LED were shown to be appropriate for vegetative multiplication. Also, a fertigation solution with 8 mM nitrogen is suitable for the propagation. Furthermore, based on our observation, the problems of the propagation process were clearly defined and addressed. We created a U-shaped structure inspired by the geometries of peristomes in tropical pitcher plants that could greatly reduce damage to the daughter plants. A prototype of the vegetative propagation system integrating the bio-inspired structure was built for further examination. Our design should enable a sustainable source of healthy and pesticide-free strawberry plants for fruit production at Futura Gaïa.

Keywords: vegetative propagation, bio inspired design, indoor farming

Layman Summary

Strawberry is a popular fruit crop with high economic value around the world. In agriculture, strawberry plants are mostly multiplied through vegetative propagation. The young daughter plants generated by the mother plants can then be utilized for fruit production in large quantities. Futura Gaïa, an indoor vertical farming company providing healthy and pesticide-free crops, has recently performed extensive research on growing strawberry plants in an indoor farming system. However, the strawberry plants provided by external nurseries are normally treated with various chemicals, which fails to agree with the company's commitment. As a result, a source of healthy and pesticide-free strawberry plants is needed. In this study, we investigated the effects of light sources and nitrogen supply on vegetative propagation in strawberries. Meanwhile, we monitored the multiplication process and developed an indoor vegetative propagation system for plant production. The biomimicry design spiral was applied as a guide to define the challenge we faced, discover nature's genius, and finally come up with a bio-inspired solution to the problem. Strawberry growth is highly dependent on environmental conditions such as light, nutrients, and temperature. The effects of temperature and photoperiod have been well documented in the literature. Here, we focused on the influences of light sources and nitrogen fertigation levels. Our results showed that the sun light LED and the white light LED are proper light sources for strawberry asexual reproduction. In addition, 8 mM of nitrogen supply in fertigation is applicable to vegetative multiplication in strawberries. To make vegetative propagation more efficient, we define the goals and the problems lying in the vegetative propagation process, including growing strawberry plants from in vitro plantlets and harvesting the daughter plants. Runners and daughter plants usually become entangled when the plants are arranged in a compact manner. Thus, during the harvesting process, separating the runners from each other can damage the daughter plants, which has been stated as the main problem. After discovering models and

strategies in nature, we found a potential solution in tropical pitcher plants. The peristome of the pitfall traps is shown to possess a specialized curved surface that can raise the possibility for insects to fall into the trap. Inspired by the pitcher plants, we designed a U-shaped structure that has great potential to capture strawberry runners and separate the runners from each other naturally. Thus, the damage to the daughter plants during the process could be reduced. The bio-inspired structure was integrated into a vegetative propagation system, and a prototype of the system was constructed for future examination and validation. In conclusion, our research offers insights into the environmental conditions required for strawberry vegetative propagation, and the prototype provides a great starting point for validating a bio-inspired indoor vegetative propagation system. Our work should promote strawberry fruit production by providing a stable, low-cost, and year-round available source of healthy and pesticide-free strawberry plants.

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Contents

1. Introduction

1.1. Futura Gaïa

This internship was done at Futura Gaïa Technologies, which is an agricultural company located in Rodilhan, France. The company was founded in April 2019 with the aim of meeting the agricultural challenges of the 21st century through a unique automated soil-based vertical farming system. Based on the solution, high-quality and pesticide-free plant production is grown locally with the optimized use of water. Thus, it is ensured that healthy produce with affordable and stable prices is available all year round. To diversify the crop catalog, Futura Gaïa intends to produce tasty strawberries in a pesticide-free and resource-efficient way as other plants in the indoor farm. In the field production system, strawberry plants are usually transplanted in the spring and grow in the summer (Hernández-Martínez et al., 2023). The chilling temperature in the winter allows the plants to be ready to produce fruits in the following spring (Hernández-Martínez et al., 2023). Based on the open-field environmental conditions, Futura Gaïa has developed agronomical recipes that prepare the plants for fruit production in the indoor vertical farming system. To move into large-scale production, a source of young strawberry plants suitable to the production system is needed. The plants are expected to be healthy, pesticide-free, productive, and available year-round. However, the prepared strawberry plants provided by the current nurseries are normally treated with multiple pesticides and are only available in December and January. Also, some diseases and fungal contaminations have been found on the plants from the external supplier while the primary research was performed in the indoor vertical farming system. Therefore, Futura Gaïa wishes to produce young strawberry plants through vegetative propagation internally. An efficient way of propagating strawberry plants is required.

1.2. Plant Science Background

The strawberry we are familiar with is the garden strawberry (*Fragaria x ananassa*), a hybrid of two wild octoploid strawberries, *Fragaria virginiana* and *Fragaria chiloensis* (Hernández-Martínez et al., 2023). Instead of reproducing by seeds, they mainly propagate through stolons, which are also referred to as runners (Gupta & Gupta, 2022). The stolons grow horizontally above the ground surface and develop daughter plants on the tips in nature (Guo et al., 2021). From an agricultural production point of view, propagation through runners is a very useful method as the hybrid genotype can be maintained (Guo et al., 2021). In the field production systems, runners are generated in the summer to produce daughter plants afterward, whereas flowering and fruiting induced by sufficient chilling throughout the winter occur in the following spring (Hernández-Martínez et al., 2023). As strawberry is a popular and economically important fruit crop, control of its flower initiation and vegetative propagation has gained enormous interest over the past decades, which has been extensively reviewed by Hytönen & Kurokura (2020). In general, a strong trade-off between flowering and runnering has been found in strawberry plants due to differentiation in the axillary meristem (Hytönen & Kurokura, 2020). Inflorescences are always formed terminally from the apical meristem of the leaf rosette, and then vegetative extension of the crown is continued from the youngest axillary meristem located below the apical meristem (Andrés & Koskela, 2022). The axillary meristem can specialize into either branch crowns or stolons, which allows additional inflorescence formation or vegetative propagation, respectively (Hytönen & Kurokura, 2020). The transition from the vegetative stage to the generative stage and vice versa is highly dependent on photoperiod and temperature (Heide et al., 2013). Responses to environmental conditions vary between strawberry cultivars (Heide et al., 2013).

According to their flowering and fruiting habits, strawberries can be roughly categorized into two groups: (1) seasonal flowering (SF) genotypes, which produce flowers

and fruits only in the spring and are referred to as short-day plants in general, and (2) everbearing (EB) genotypes, which continue flowering and fruiting throughout the growing season besides the spring and are basically long-day plants (Heide et al., 2013). The critical photoperiod of flower induction is remarkably adapted to temperature (Heide et al., 2013). For example, in most SF strawberries, flowering is induced under day lengths shorter than 12-14 h while temperatures are around 18ºC (Heide et al., 2013; Hytönen & Kurokura, 2020). In addition, floral initiation is prevented at temperatures higher than 22ºC, while flowers develop independently of photoperiods when temperatures are lower than 10ºC (Heide et al., 2013; Hytönen & Kurokura, 2020). On the other hand, in EB strawberries, flowering is typically induced under long-day conditions, especially at intermediate and high temperatures (Hytönen & Kurokura, 2020). At low temperatures, flowering responses in EB strawberries are found to be day-neutral (Heide et al., 2013). In terms of vegetative development, runnering is principally inhibited under environmental conditions favored by floral induction (Hytönen & Kurokura, 2020; Koskela et al., 2016). In SF strawberries, stolon formation is enhanced by long days and high temperatures in general (Koskela et al., 2016). Also, in EB strawberries, runnering is promoted by high temperatures and various day lengths based on cultivars (Koskela et al., 2016). Overall, strawberry plants are highly responsive to the surrounding environment, especially light and temperature, which gives controlled-environment agriculture a big advantage in managing flower initiation and propagation of cloned plants.

1.3. Aims of the Internship

In this project, we aim to monitor strawberry vegetative propagation and implement a system in an indoor production context. The objectives can be divided into three parts: (1) learn about strawberry asexual reproduction and define suitable indoor agronomic recipes through both qualitative and quantitative research, (2) design a bio-inspired system for plant

production, and (3) build a prototype of the vegetative propagation system in an indoor context.

For the research part of this study, the effects of light sources and nitrogen fertigation on strawberry vegetative propagation were focused on due to their important roles in plant growth and development. We grew strawberry plants from in vitro plantlets (pathogen-free) under three different light sources and two distinct fertigations to define suitable indoor growing recipes in terms of vegetative propagation. Three cultivars known for excellent fruit yield were employed, including 'Charlotte' (EB), 'Ciflorette' (SF), and 'Gariguette' (SF). LED light sources of (i) sun light, which is the same spectra as outdoor environment, (ii) white light, which is an indoor farming light source with less intensity in wavelength 500 nm – 600 nm (green), and (iii) blue light, which is known to induce generative plant growth (Schroeter-Zakrzewska & Pradita, 2021), were applied. It has been shown that blue light can promote flowering in strawberry plants (Ye et al., 2021). Based on the trade-off between flowering and runnering in strawberry plants, we hypothesized that runnering process can be reduced under the blue light condition. In terms of fertigation, nitrogen is viewed as the most important macronutrient for runner production in strawberries (Trejo-Téllez & Gómez-Merino, 2014). Farjana et al. (2023) demonstrated that the optimal fertigation concentration of nitrogen varies among strawberry cultivars. Here, fertigation solutions with 8 mM nitrogen (Control), which is the same as the strawberry growing recipe established at Futura Gaïa, and 20 mM nitrogen (N+) were tested in our experiment. We speculated that in certain cultivars, higher fertigation nitrogen concentration would have a similar effect on strawberry plants under sun light and white light conditions, i.e. either promote vegetative propagation or reduce it. The effect of fertigation nitrogen concentration in the three cultivars could be different. The results reveal that sun light LED and white light LED are suitable light sources

for vegetative propagation. Also, the fertigation solution with 8 mM of nitrogen is appropriate for the plants under the sun light LED.

For the design part, the Biomimicry Design Spiral, which guides us to learn from nature and gain bio-inspired innovation, is used as a problem-solving tool throughout the process. The steps include defining the challenge, biologizing function and context, discovering biological strategies, abstracting design strategies, emulating nature's lessons, and evaluating fit and function (*Biomimicry Design Toolbox*, n.d.). While we faced the challenges of vegetative propagation, nature-inspired strategies were integrated into the design, along with plant science research, to produce a solution. Excitingly, we went through the process in detail and managed to come up with a three-dimensional (3D) U-shaped structure inspired by tropical pitcher plants. Moreover, a prototype integrated with the bio-inspired structure was sketched and simulated in a 3D platform. A wooden prototype was successfully built. With the prototype, we are now able to test the efficacy of the 3D-printing bio-inspired structure, monitor the vegetative propagation process of strawberries in the designed system, and further improve the system to fulfill our needs.

2. Materials and Methods

2.1. Biomimicry Design Spiral

Biomimicry is learning from nature and creating bio-inspired design to solve problems in a sustainable way. The Biomimicry Design Spiral, provided by the Biomimicry Institute (*Biomimicry Design Toolbox*, n.d.), is a tool for creating biomimetic solutions to challenges. The design spiral includes six essential elements for generating a bio-inspired design (*Biomimicry Design Toolbox*, n.d.). First, 'define the challenge' is a step that we clearly state the challenge of our focus (*Biomimicry Design Toolbox*, n.d.). A well-defined problem is critical to success. The context of the challenge and the impact we wish to make through the design also need to be considered. Second, 'biologize function and context' is to reframe the challenge in biological terms (*Biomimicry Design Toolbox*, n.d.). Translating the challenge from human perspectives to nature world enables us to find corresponding solutions more easily. Third, 'discover biological strategies' means searching for natural models that possess the functions and contexts relevant to our challenge (*Biomimicry Design Toolbox*, n.d.). The organisms or ecosystems may have adapted to the contexts and developed the strategies that help them survive. Fourth, 'abstract design strategies' is to translate the biological strategies we found into design strategies (*Biomimicry Design Toolbox*, n.d.). The translation is important to learn the features or mechanisms and integrate them into our design. Fifth, 'emulate nature's lessons' describes the process of applying insights gained from nature to our challenge (*Biomimicry Design Toolbox*, n.d.). Emulation is the heart of biomimicry, and it is also the creative part of the process. Last, 'evaluate fit and function' is to assess the design concepts and consider the feasibility of the solution (*Biomimicry Design Toolbox*, n.d.). Importantly, design is an iterative process. These key elements of biomimicry design are performed many times, and it is not necessary to follow the order in the design spiral. In this study, the steps in the biomimicry design spiral were taken to solve the design challenge of

vegetative propagation. Instead of being conducted sequentially, the steps were revisited many times during the process in order to revise the ideas and design concepts we had.

2.2. Plant Material Preparation

The garden strawberry (*Fragaria x ananassa*) in vitro plantlets were prepared in external in vitro labs. The cultivar 'Gariguette (GAR)' was provided by the Interprofessional Technical Centre for Fruits and Vegetables (CTIFL), and the cultivars 'Charlotte (CHA)' and 'Ciflorette (CIF)' were given by INVENIO. The plantlets of the three strawberry cultivars were transplanted to germination trays filled with potting soil (TBSP, 'Florentaise', France), and grown in the germination room for 12 days to enable the plantlets to root. The average temperatures were 25°C during the day and 23.5°C during the night. The average humidity was 67.5%. The plantlets were grown under the sun light LED (details see Table 1) with a photosynthetic photon flux density (PPFD) of 206.4 μmol m^{-2} s^{-1}. The photoperiod was given as 12 hours of light and 12 hours of darkness. The plantlets were sub irrigated with the germination solution (details see Table 2).

2.3. Experimental Design and Data Collection

After 12 days of rooting, the plantlets of each cultivar were divided into six groups. Every two groups of plantlets were grown under one of the three light sources, i.e. the sun light LED (Sun), the white light LED (White), and the blue light LED (Blue). Among the two groups under the same light treatment, the control fertigation solution (Control) and the solution with higher nitrogen concentration (N+) were applied respectively. The details of the light sources and the fertigation solutions can be found in Table 1 and Table 2, respectively.

The vegetative propagation parameters such as length of the first runner, number of runners per plant, and number of daughter plants per plant were recorded over time in order to understand the asexual reproduction in strawberry grown from in vitro plantlets. To compare vegetative multiplication and plant growth in strawberry plants under different environmental

conditions, the three vegetative propagation parameters and the plant growth parameters (e.g. crown diameter, number of leaves, and height) in the 5th week of the experiment were measured.

Table 1

Details of the Three Light Sources

Light Source	Sun Light LED	White Light LED	Blue Light LED
Product	(T8, Valoya, Finland)	(Backlight, VGD-LED, France)	(SpectraLine BloomLED, FloralLED, France)
PPFD	206.4 µmol m^{-2} s^{-1}	209.6 µmol m^{-2} s^{-1}	144.8 µmol m^{-2} s^{-1}
R:G:B	40:40:21	61:23:17	-
Spectrum			

Note. The product information, photosynthetic photon flux density (PPFD), Ratio between Red, Green, and Blue (R:G:B), and the spectrum of each light source are given.

Table 2

Composition of the Fertigation Solutions

Fertigation	Germination Solution	Control Solution	N+ Solution
Electroconductivity (EC)	0.46 mS cm^{-1}	0.7 mS cm^{-1}	1.3 mS cm^{-1}
pH	6.5	6.5	6.5
Fertilizer			
N	3,20	8,00	20,00
P	0,60	0,60	0,60
K	0,90	0,90	0,90
Ca	1,30	1,30	1,30

Mg	0,80	0,80	0,80
S	0,10	0,10	0,10
B	0,0000	0,0200	0,0200
Cu	0,0000	0,0007	0,0007
Fe	0,0000	0,0070	0,0070
Mn	0,0000	0,0350	0,0350
Mo	0,0000	0,0010	0,0010
Zn	0,0000	0,0005	0,0005

Note. The electroconductivity, pH, and fertilizers of the three fertigation solutions are shown.

2.4. Statistical Analysis

Regarding changes of parameters over time, the means, the standard error of the mean (SEM), and sample size (N) of the three vegetative propagation parameters at different time points were calculated and presented in line charts using GraphPad Prism 9 software. In terms of comparison of the plants under different environmental conditions, the data of the vegetative propagation parameters and the plant growth parameters from the week 5 were processed in GraphPad Prism 9. The means, the standard deviation (SD), and sample size (N) were calculated and shown in bar charts. Two normality tests (e.g. Shapiro-Wilk test and Kolmogorov-Smirnov test) were conducted in GraphPad Prism 9 to test the normality of the data. The Krudkal-Wallis tests of the data of each parameter in each cultivar were performed using R Studio to compare the difference among the six groups. The p-value and the letters indicating the significant differences were generated in R studio. The letters were then noted in the bar charts generated in GraphPad Prism 9 manually. The recovery percentage was calculated by dividing the number of recovered daughter plants into the number of total daughter plants. The recover percentage over time was presented in line chart through GraphPad Prism 9. The principal component analysis (PCA) was carried out based on vegetative propagation parameters and growth parameters in GraphPad Prism 9.

2.5. Prototype Construction

The 3D sketch of the bio-inspired U-shaped structure was illustrated in Autodesk and adjusted in Tinkercad. The bio-inspired structures and the control structure were generated by 3D printers in FabLab and a private 3D printer. The 3D model of the prototype was created in Tinkercad. The prototype was constructed with wood and rebar and covered with waterproof paint.

3. Results

To develop an efficient way of vegetatively reproducing strawberry plants, the Biomimicry Design Spiral was applied in this study. The results are shown sequentially based on the steps of the design spiral while the process was performed in a non-linear way as described in Section 2.1. There are three main outcomes throughout the process: (1) plant growth and asexual reproduction under different treatments were observed and analyzed scientifically, (2) a 3D shape inspired by pitcher plants was generated, and (3) a prototype of the vegetative propagation system was built for further examination.

3.1. Define the Challenge: I. Scientific Research

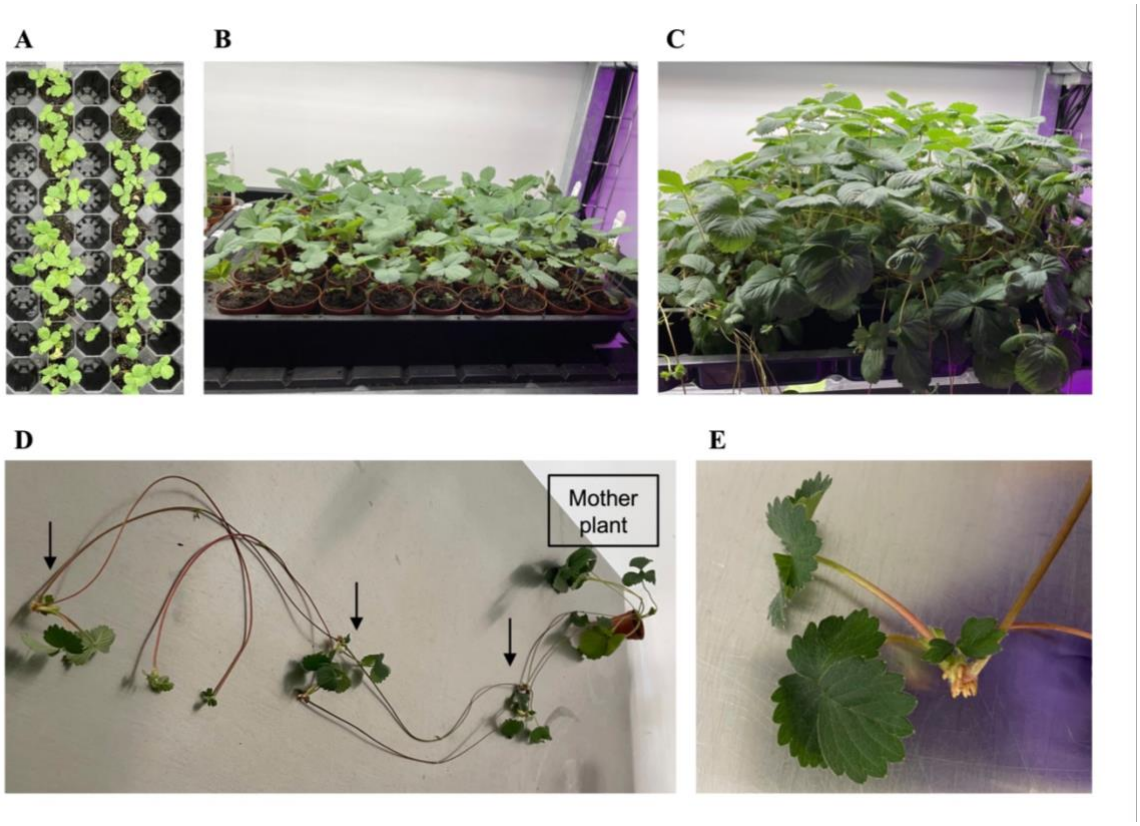
The task of asexual propagation in an indoor context was defined from the perspectives of both plant science and industrialization. On the one hand, taking an agronomy point of view, a series of experiments, measurements, and analyses were conducted in order to learn how to efficiently multiplicate and optimize the recipe for strawberry vegetative growth. On the other hand, in order to find the root causes of the problem, the propagating process was dissected concerning an industrialization aspect, especially the human resources. In terms of plant science, to understand the vegetative growth of strawberry plants, we set up six environmental conditions which are the combination of three different light sources (i.e. sun light (Sun), white light (White), and blue light (Blue)) and two types of fertigation solutions (i.e. control solution (Control) and solution with higher nitrogen concentration (N+)). Plantlets of three strawberry cultivars, i.e. Gariguette (GAR), Charlotte (CHA), and Ciflorette (CIF), obtained by in vitro propagation were divided into six groups and grown under designated conditions. Many parameters of plant growth and vegetative propagation were noted during the experiments.

3.1.1. How Do Strawberry Plants Grow and Form Daughters from in Vitro Plantlets?

Plant growth over time was observed. Figure 1A to 1C display the development of the strawberry plants under sun light and N+ fertigation conditions, from prepared in vitro plantlets (week 0) to robust and luxuriant strawberry plants (week 8). As can be seen in Figure 1C, runner sprouting had started after eight weeks of the treatments. Each mother plant can produce multiple runners, and several daughter plants can be formed on the same runner, which is visible in Figure 1D. New leaves and little roots are generated in daughter plants while they are connected to the mother plant, which is given in Figure 1E.

Figure 1

Growth and Vegetative Propagation of Strawberry Plants



Note. (A) Prepared in vitro plantlets of CHA (n = 18) with small leaves in week 0 of the treatments are shown. (B) Strawberry plants of GAR (n=18), CHA (n=18), and CIF (n=18) grown under sun light and N+ conditions in week 3 are demonstrated. New leaves have been formed. (C) The same tray of strawberry plants as (B) but in week 8. The plants are luxuriant,

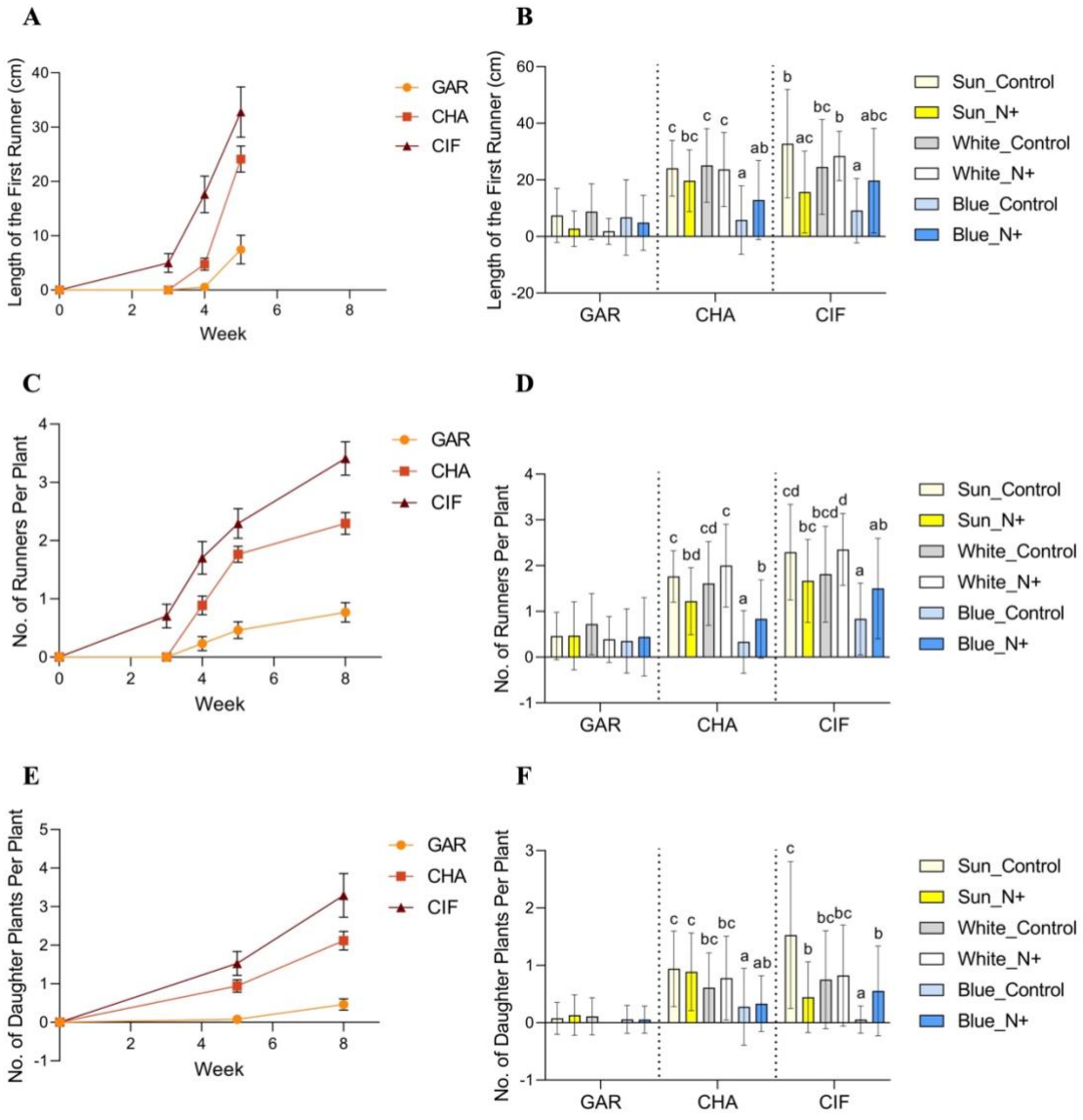
and the runners have been sprouting and extending. (D) One strawberry plant of CHA from the tray shown in (C) in week 11. Two runners with a total of five daughter plants (as indicated by arrows) can be observed. (E) A daughter plant with two expanded leaves and some small roots is especially shown.

3.1.2. How Many Daughter Plants Can a Mother Plant Produce Over Time?

To be aware of the efficiency of daughter plant production concerning time, (i) the length of the first runner (ii) the number of runners per plant, and (iii) the number of daughter plants per plant were monitored in the course of time. Under sun light and control fertigation conditions, runner sprouting starts in the 3rd week of the experiment in CIF, which was one week earlier than the other two cultivars (Figure 2A and 2C). Runners in CIF extended dramatically since the 3rd week of the treatments while the lengths of the runners in CHA had a huge increase from the 4th week to the 5th week, as can be seen in Figure 2A. Over time, more runners and daughter plants were formed (see Figure 2C and 2E). After two months of growth, the average number of runners per mother plant was 0.8 in the GAR, 2.3 in the CHA, and 3.4 in the CIF. Meanwhile, on average, two daughter plants per mother plant in the CHA and more than three daughters per plant in the CIF were found after 8 weeks of growth (see Figure 2E). The length of runners and the number of runners both affect the number of daughter plants that can be produced. The more runners and the longer length of runners a mother plant generates, the more daughter plants are produced. Thus, similar ranks of the three cultivars can be found in Figure 2A, 2C, and 2E. Among the three cultivars, CIF has the highest average first runner lengths, average number of runners, and average number of daughter plants, while GAR has the lowest values of the three vegetative propagation parameters (see Figure 2A, 2C, and 2E).

Figure 2

Strawberry Vegetative Propagation in GAR, CHA, and CIF Cultivars



Note. Vegetative propagation parameters in GAR, CHA, and CIF under the sun light and the control fertigation were observed and noted over time, including (A) the length of the first runner, (C) the number of runners per plant, and (E) the number of daughter plants per plant. Data from the 5th week in (A), (C), and (E) were analyzed and compared among the six environmental conditions in (B), (D), and (F), respectively. Data in (A), (C), and (E) represent means with SEM in both directions among 18 mother plants (biological replicates). Data in

(B), (D), and (F) represent means with SD on both sides among 18 mother plants (biological replicates). The letters indicating the significant differences can only be applied within each cultivar. A summary of statistical analyses can be found in Appendix A.

3.1.3. What Are the Suitable Environmental Conditions for Vegetative Propagation?

To understand the impacts of light sources and nitrogen fertigation on strawberry's vegetative propagation, data of the three parameters collected in the 5th week were analyzed and compared among six groups under different environmental conditions (e.g. Sun_Control, Sun_N+, White_Control, White_N+, Blue_Control, and Blue_N+) in each cultivar. In terms of light sources, comparisons of vegetative propagation parameters among the sun light LED, white light LED, and blue light LED, were conducted. No significant difference among the six groups in GAR was shown in the vegetative propagation parameters (Figure 2B, 2D, and 2F). In CHA and CIF, among the groups irrigated with the control solution, the first runners of the mother plants grown under sun light and white light are significantly longer than the ones under blue light treatment, which is given in Figure 2B. Notably, similar patterns can be found in the number of runners per plant and the number of daughter plants per plant. As can be seen in Figure 2D and 2F, in both CHA and CIF, significantly more runners and daughters were generated in the groups of Sun_Control and White_Control, compared to the ones in Blue_Control.

Besides the effects of different light sources, the influence of nitrogen concentration in fertigation was analyzed. In our experiment, fertigation solutions with 8 mM nitrogen (Control) and 20 mM nitrogen (N+) were tested. A visible difference was found between the mother plants with control fertigation and the ones with N+ fertigation. Mother plants with the higher fertigation nitrogen concentration were generally more compact and in darker green, compared to the ones with the control fertigation (as can be observed from Appendix B). According to the data in Figure 2B, 2D, and 2F, higher nitrogen fertigation had the

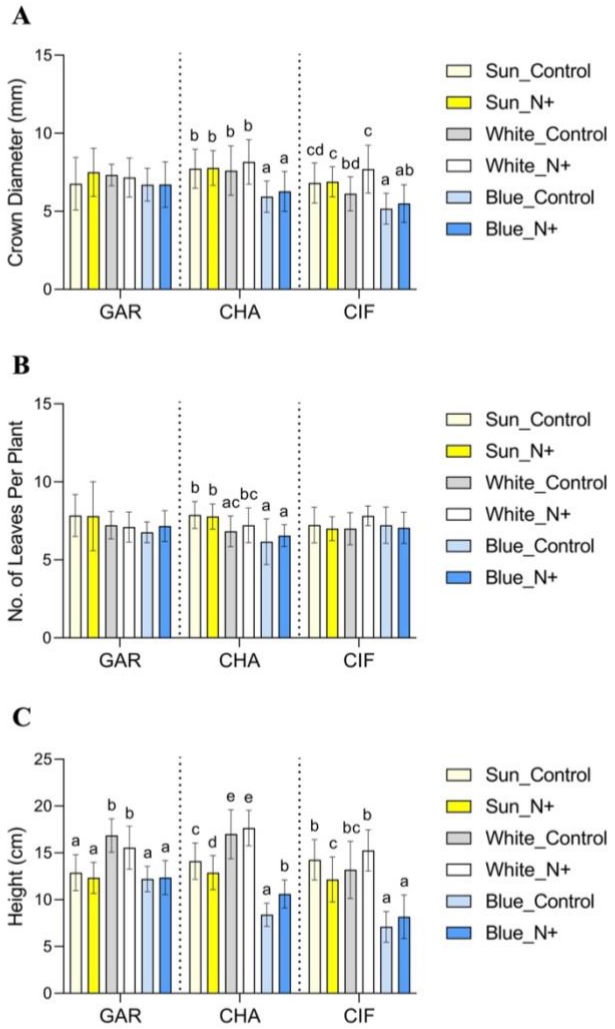
opposite effect on the vegetative reproduction of the strawberry plants grown under the sun light, compared to the ones grown under the blue light. For instance, in the CHA under the sun light treatment, the N+ fertigation group produced significantly fewer runners in comparison with the control fertigation group (see Figure 2D). Conversely, in the CHA under the blue light treatment, significantly more runners were identified in the N+ fertigation group, compared to the control fertigation group (as demonstrated in Figure 2D). Likewise, significantly fewer daughter plants were produced by the CIF mother plants under sun light and N+ fertigation, compared with the ones under sun light and control fertigation (Figure 2F). On the contrary, N+ fertigation led to a significantly higher number of daughter plants in the CIF mother plants grown under blue light, compared with the ones with control fertigation (Figure 2F). Interestingly, there is no significant difference in the number of daughter plants among the three light sources when the CIF mother plants are treated with the higher nitrogen fertigation solution (as can be observed in Figure 2F). Notably, under the white light condition, no significant difference between the control group and the N+ group can be found in the three cultivars, as given in Figure 2B, 2D, and 2F.

Besides the three most relevant vegetative propagation parameters, strawberry plant growth parameters such as crown diameter, number of leaves per plant, and plant height were analyzed and compared among the six groups of environmental conditions. Based on Figure 3A, bigger crowns were formed in strawberry plants grown under sun light and white light except GAR. In terms of leaf number, more leaves were observed in the CHA strawberry plants under the sun light treatment, compared with the ones under the blue light treatment (Figure 3B). Notably, a pattern similar to the results of vegetative propagation parameters can be found in the data on plant height. As shown in Figure 3C, under sun light and white light, strawberry plants grew higher than the ones under blue light, except GAR under sun light. Based on Figure 3C, plant height in CHA under sun light was reduced when higher nitrogen

fertigation was applied. Meanwhile, an opposite effect can be found in CHA under blue light, i.e. higher nitrogen fertigation causes a rise in plant height (Figure 3C).

Figure 3

Strawberry Growth Parameters in GAR, CHA, and CIF Cultivars



Note. Three growth parameters in the 5th week were analyzed and compared among the six environmental conditions, including (A) crown diameter, (B) number of leaves per plant, and (C) plant height. Data represent means with SD on both sides among 18 mother plants (biological replicates). The letters indicating the significant differences can only be applied within each cultivar. A summary of statistical analyses can be found in Appendix C.

Moreover, a principal component analysis (PCA) was performed based on vegetative propagation parameters and growth parameters listed in Table D1 in Appendix D. Based on the loadings plot, two main clusters of parameters can be observed (Figure D1 in Appendix D). The three vegetative propagation parameters mentioned are in one cluster while the three growth parameters belong to another cluster (Figure D1 in Appendix D). The PCA results reveal that blue light has a great impact on strawberry plants among the other light sources (Figure D2 in Appendix D). In addition, strawberry plants treated with the two fertigation solutions have similar distributions in the PC score plots (Figure D2 in Appendix D).

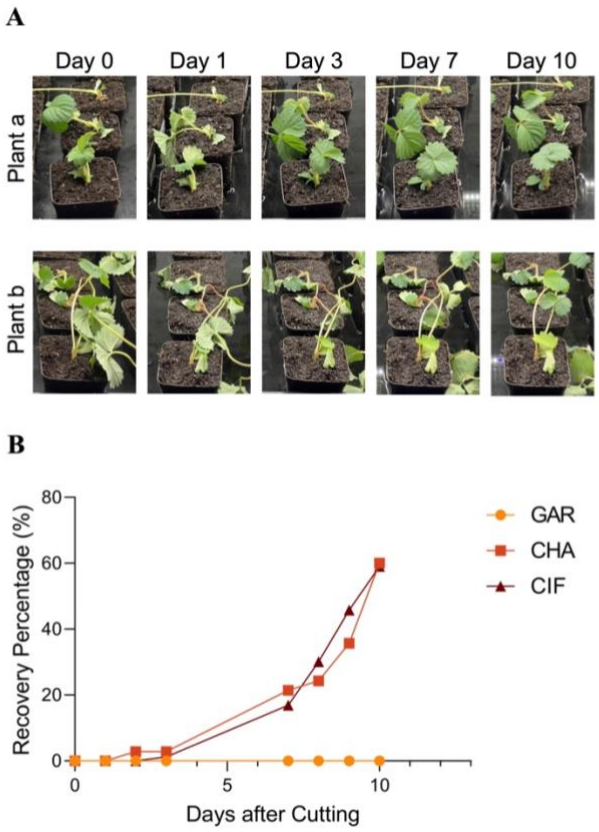
In brief, our results show that the sun light LED and the white light LED are both appropriate light sources for vegetative propagation. The nitrogen fertigation concentration of 8 mM is suitable for asexual reproduction in strawberry plants grown under the sun light.

3.1.4. Do the Daughter Plants Recover Well After Being Harvested?

To determine whether the vegetative propagation was successful or not, we separated the daughter plants from the runners and analyzed if they could survive transplantation. The daughter plants of two environmental groups (i.e. the Sun_Control group and the White_Control group) were harvested on the 8th week and left recovering for 10 days. The daughter plants looked green and healthy on the day of the harvesting as they were disconnected from the mother plants for merely a few hours (see Figure 4A). After one day, the daughter plants were fallen and dehydrated (Figure 4A). Notably, we found that the number of days needed for daughter plants to get back varied according to plant size. As can be seen in Figure 4A, the shorter daughter plant (i.e. Plant a) recovered within 3 days while the one with elongated leaves (i.e. Plant b) revived after 10 days. However, not all the daughter plants survived the transplantation. We found that only around 60% of daughter plants survived 10 days after cutting in both CHA and CIF as can be seen in Figure 4B.

Figure 4

Daughter Plants Recovery After Harvesting



Note. The recovery of daughter plants after being harvested is demonstrated. (A) In both the small daughter plant (Plant a) and the big daughter plant (Plant b), the recovery progress from harvesting (Day 0) to the 10th day after cutting (Day 10) is shown. (B) The recovery percentage among all daughter plants harvested along with time is displayed. In total, 15 GAR, 70 CHA, and 83 CIF daughter plants were harvested and analyzed. The exact recovery percentages are listed in Appendix E.

3.2. Define the Challenge: II. Industrialization

3.2.1. What Are the Final Goals of an Indoor Vegetative Propagation System?

To further investigate the challenge of vegetative reproduction concerning industrialization, we went through many steps such as determining the final goals, summarizing the existing barriers, and brainstorming the functions that would allow us to

achieve the goals. First, in regard to building a strawberry asexual propagation system that is in line with the commitment of Futura Gaia (as mentioned in the Introduction section), three main goals and a description of each goal were defined. The following are the ambitions we wish to accomplish through the vegetative propagation system at the end of the innovation and validation process, which will not be completely implemented in this project but serve as the guidelines for the design process instead.

The Final Goals

1. To allow sustainable production of daughter plants.

   That is to say, the system needs to (1) be available for long-term production, and (2) be adaptive to different plant sizes, different lengths of runners, and varied daughter plant sizes during the growing and harvesting process.
2. To provide sufficient daughter plants that are well-developed and homogeneous.

   This means that the environmental conditions such as light, humidity, and temperature in the system have to be spatially uniform for all mother plants and potential daughter plants.
3. To be resource-efficient during the production process.

   More exactly, (1) damage to the plants must be kept to a minimum, and (2) the use of time, space, and human resources should be optimized during the multiplication procedure.

3.2.2. What Are the Problems of Current Vegetative Propagation Systems?

Next, we looked back on the observations that have been made along with the plant science experiments and summarized the current difficulties that keep us away from the goals. At the growing stage, two types of plant arrangements were tested. In the beginning, strawberry plantlets were transplanted to pots with a diameter of 5.5 centimeters, and every 54 pots were placed in one tray densely. The compact trays were space-efficient, and the plants

were in good condition as the plants grew bigger (see Figure 1B and 5A). However, many problems emerged when the runner sprouting started. Runners extended between the lush leaves in all directions, which made it hard to distinguish one plant from another (as illustrated in Figure 5A and Figure 5B). Moreover, separating the plants became even more complicated while daughter plants attached to the runners were produced. The daughter plants were mostly stuck in the leaf tangles as their newly formed leaves and roots with relatively fixed shapes intertwined with the leaves of the mother plants easily. To harvest the daughter plants, we need to first isolate each mother plant and untangle the runners. The plants concentrated in each tray (as shown in Figure 5B) have to be rearranged and divided into multiple trays (as demonstrated in Figure 5C). We found that the daughter plants and the runners can be easily damaged during the manual separating process, which might be one of the reasons for the low recovery percentage of daughter plants after harvesting (as shown in Figure 4B). Also, it took approximately half an hour for one person to separate 10 plants from a full tray without destroying them, which is very time-consuming. From the environmental conditions aspect, we assumed that the humidity in the center of the jungle was much higher than normal as some contamination of fungi was found. Besides the messy jungle of the plants, we found that some daughter plants started to develop their root system and even fixed themselves to the soil as they touched the soil in the pots of the mother plants or the fertigation at the bottom of the tray.

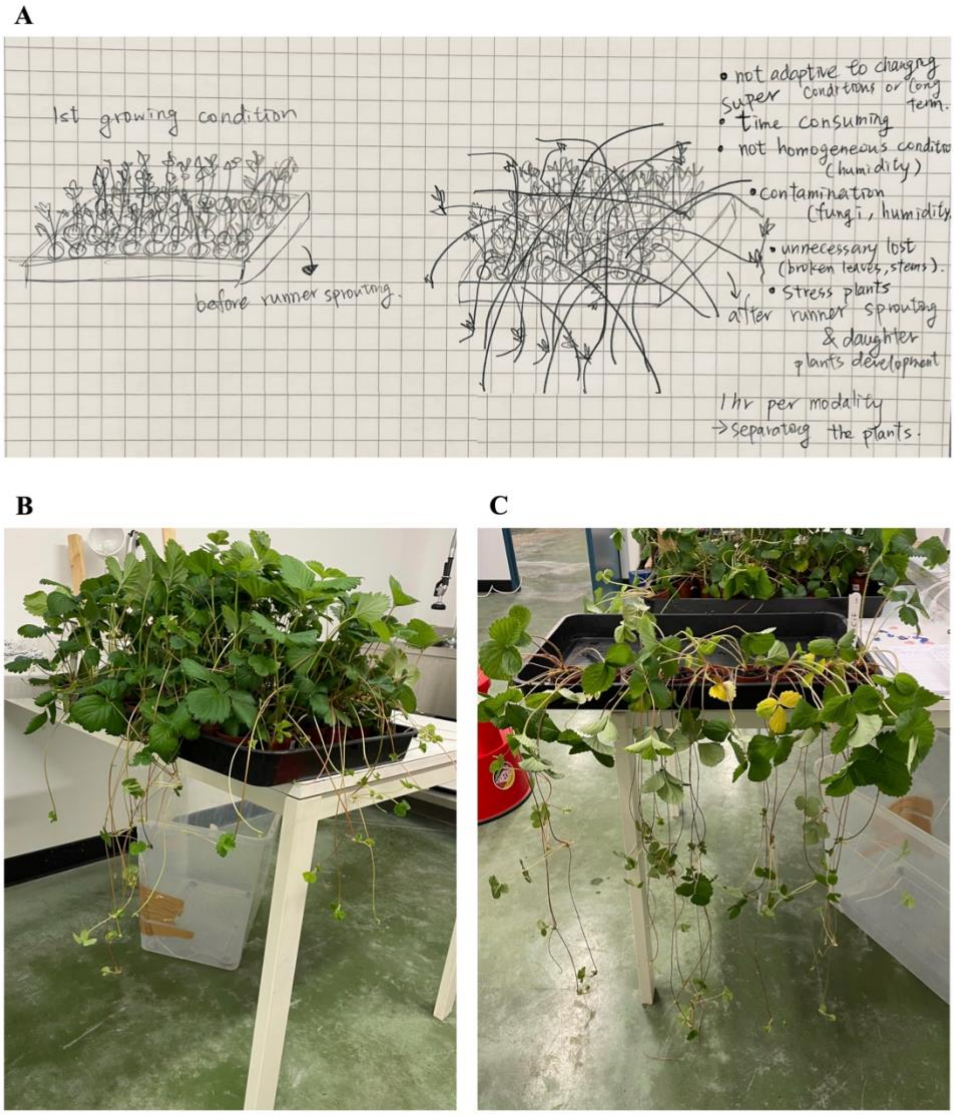
In general, none of the three final goals were fulfilled while the strawberry plants were growing under the compact arrangement (current vegetative propagation system 1; Figure 5). First, the growing system did not realize Goal 1 as it was not adaptive to different stages during the dynamic process. The complications of organizing the plants raise the difficulties of organizing the plants in a long-term period. Second, Goal 2 failed as not all of the daughter plants were homogeneous. The daughter plants that were in contact with the soil or the

fertigation had advanced their root system development, which might lead to unequal developmental stages among the others. Also, the humidity in the center of the tray might differ from the periphery. Last, even though the compact arrangement is space-efficient, Goal 3 was not completely achieved. The process of organizing the plants is too complicated and time-consuming, which makes the system inefficient from a time perspective. Also, the unnecessary loss of the daughter plants due to the damage during the manipulation decreases the resource efficiency in the plant material aspect.

To reduce the amount of time spent on separating the plants, we tried to place the plants in two or three rows and manually organize the runners in the same direction, which is the current vegetative propagation system 2, as demonstrated in Figure 6. In this way, we saved time in organizing the plants and reduced the damage to the daughter plants and the runners. Also, daughter plants have less chance to touch the soil or fertigation. However, we found that the leaves of the mother plants were not in good condition due to the manipulation. Some of the runners that hung down from the mother plant lay on the leaf petioles, which is displayed in Figure 6A and 6B. The weight of the runners forced the leaves to bend to the side instead of growing towards the light (as can be seen in Figure 6C). Furthermore, the daughter plants and the runners can still be damaged when they are pulled away from others. Therefore, another system for vegetative propagation that fits the final goals is needed.

Figure 5

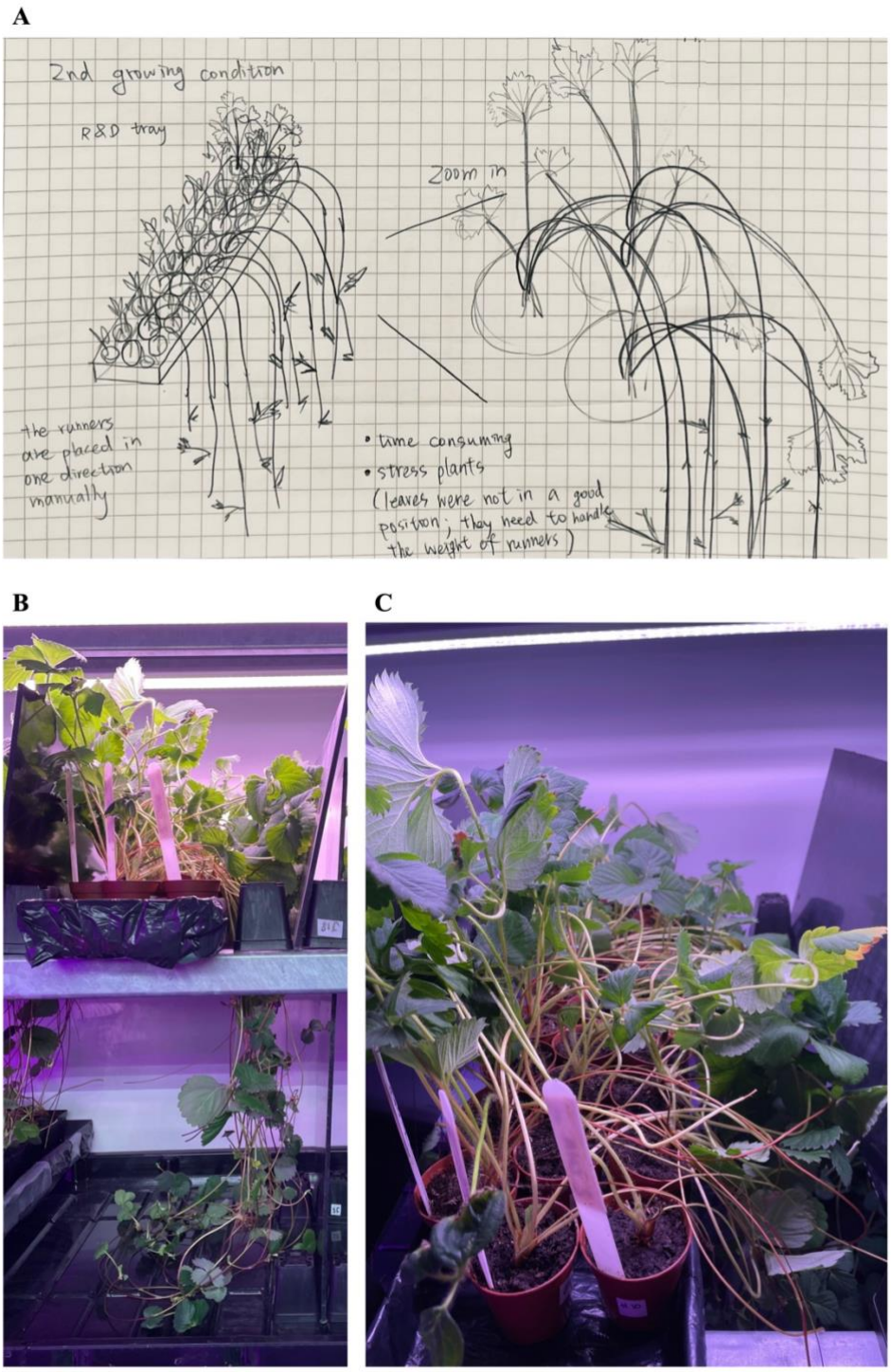
Current Vegetative Propagation System 1



Note. (A) Drawings of current vegetative propagation system 1. Strawberry plants without runners are illustrated on the left-hand side. The dense cluster of strawberry plants after the runner sprouting started is displayed on the right-hand side. (B) A tray with compact strawberry plants (n = 54) endowed with numerous runners. (C) A row of strawberry plants (n = 9) separated from the tray in (B). Runners were placed on one side manually.

Figure 6

Current Vegetative Propagation System 2



Note. (A) Drawings of current vegetative propagation system 2. Strawberry plants (n = 26) arranged in rows with runners in one direction are illustrated on the left-hand side. A zoom-in view of the crossed runners and leaves is shown on the right-hand side. (B) Current vegetative

propagation system 2 with runners placed in one direction. (C) A zoom-in view of (B). The runners (red and thin) and leaf petioles (green and thick) are overlapping, and the leaves hang down due to the weight of the runners and/or leaves on them.

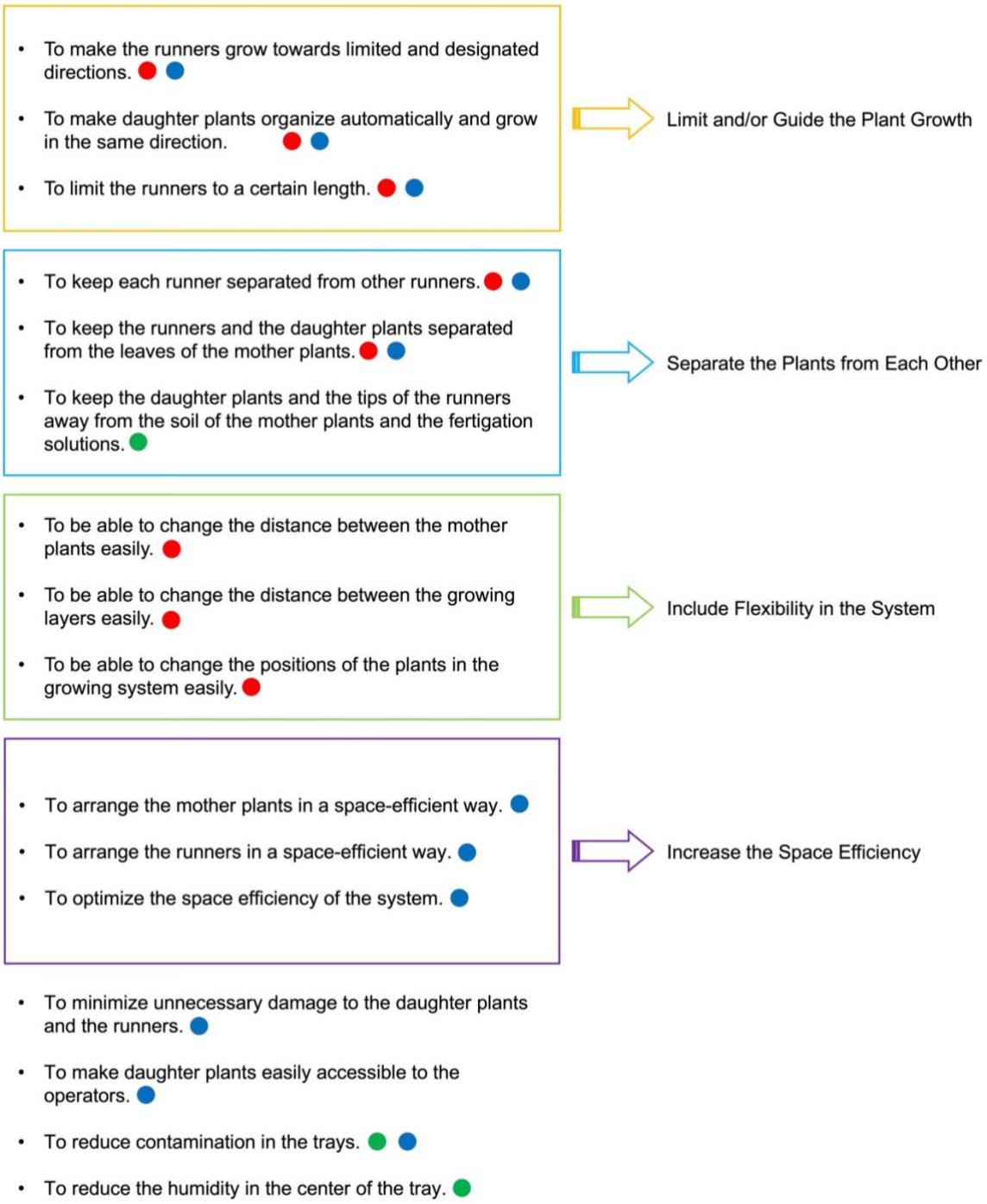
3.2.3. What Are the Functions that Can Help Us Achieve the Goals?

After understanding the trouble with asexual reproduction, we brainstormed the potential functions we may need in order to solve the problems and/or get closer to the final goals. The brainstorm results are categorized by the similarity and the related final goals as demonstrated in Figure 7. In general, there are four main functions that might be able to bring us towards the final goals: (1) limit and/or guide the plant growth, (2) separate the plants from each other, (3) include flexibility in the system, and (4) increase the space-efficiency. The first and the second functions aim to modify the plants directly, which makes them more relevant to the vegetative propagation process. On the other hand, the third and the fourth functions hope to increase the lifespan of the system and raise the value of the system from an economic aspect. To improve the vegetative propagation system and test its viability, we decided to begin with the first and the second functions in this project. The following steps of the design process center around the two functions with a mid-term goal of protecting the daughter plants and runners from damage. Here the outcome of the "Define the Challenge" step can be summarized below to continue the next step.

- *Design Question: How might we protect strawberry daughter plants and runners from damage during an indoor vegetative propagation process?*
- *Functions: limit and/or guide the plant growth; separate the plants from each other*
- *Context: indoor; control or N+ fertigation; sun light or white light LED*

Figure 7

The Outcome of Brainstorming Potential Functions



Note. The results of the brainstorming session are described. The circle symbols indicate the final goals that the function might be able to fulfill. The red circle '●' represent the Goal 1, the green circle '●' stands for the Goal 2, and the blue circle '●'means the Goal 3. Most potential functions were categorized into four main functions: (1) limit and/or guide the plant

growth, (2) separate the plants from each other, (3) include flexibility in the system, and (4) increase the space-efficiency.

3.3. Design the System

3.3.1. Biologize Function and Context

Before looking for solutions in nature, we first translated the functions and context defined in the previous step into biological terms. We moved back and forth between the "Biologize" step and the "Discover" step. Different questions were formed and applied to discover a wide range of biological strategies. Several biologized questions that intend to solve the same problem are formed and listed below.

Biologized Questions:

- *How does nature move on solids?*
- *How does nature move in the air?*
- *How does nature orientate in the terrestrial environment?*
- *How does nature modify size in the terrestrial environment?*
- *How does nature modify its position in the terrestrial environment?*
- *How does nature avoid overlapping in the terrestrial environment?*
- *How does nature capture solids in the terrestrial environment?*

3.3.2. Discover Biological Strategies

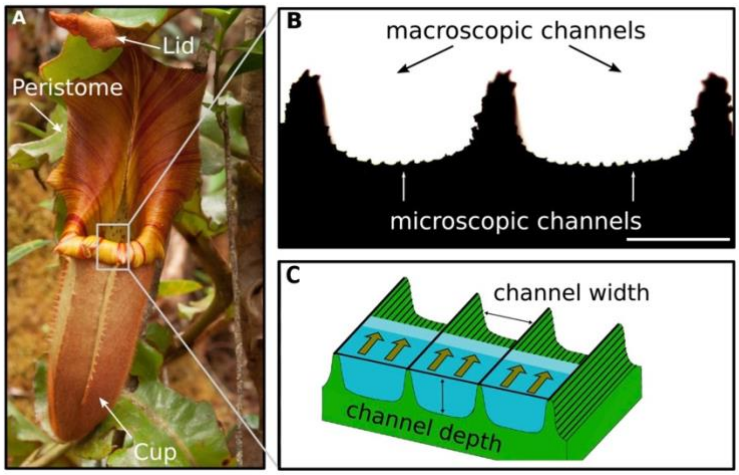
Based on the biologized questions, we looked for suitable biological strategies through the Ask Nature website and scientific literature. Many natural models regarding the functions stated in the questions were found. For example, ant lions make holes with a steep slope in the sand to capture their prey (Devetak et al., 2020); carnivorous pitcher plants have specialized leaves that can trap insects (Mithöfer, 2022); sensitive plants fold their leaves when they are attacked by insects (Hagihara et al., 2022). Interestingly, we found that the biological strategy

in tropical pitcher plants (*Nepenthes*) properly fits the functions and context we need for our design.

The tropical pitcher plant (*Nepenthes*) is a genus of carnivorous plants that capture animal prey to obtain additional nutrients (Mithöfer, 2022). A *Nepenthes* pitfall trap consists of the lid, the pitcher rim (also called peristome), and the cup as can be seen in Figure 8A (Labonte et al., 2021). The specialized pitcher rim has been found to play a key role in passive prey capture in *Nepenthes* (Bohn & Federle, 2004; Labonte et al., 2021). The pitcher rim with specialized ridges is a slippery surface, which can cause insects to aquaplane when they arrive on the rim (Moulton et al., 2023). The prey that aquaplanes on the rim will then fall into the cup and be digested (Moulton et al., 2023). It has been shown that the sliding is a result of both surface properties and geometry of the peristome (Labonte et al., 2021; Moulton et al., 2023). The macroscopic and microscopic ridges on the peristome surface have been noted to enhance the efficacy of the traps as they make the surface super hydrophilic and able to stabilize thin water films where aquaplaning happens (see Figure 8B) (Labonte et al., 2021). The macroscopic ridges restrict lateral water spreading and guide water along the inward and outward directions (see Figure 8C) (Labonte et al., 2021). Meanwhile, the microscopic ridges ensure the stability of water films between the insect pad and the rim (Labonte et al., 2021).

Figure 8

Tropical Pitcher Plants and Peristome Surface Properties



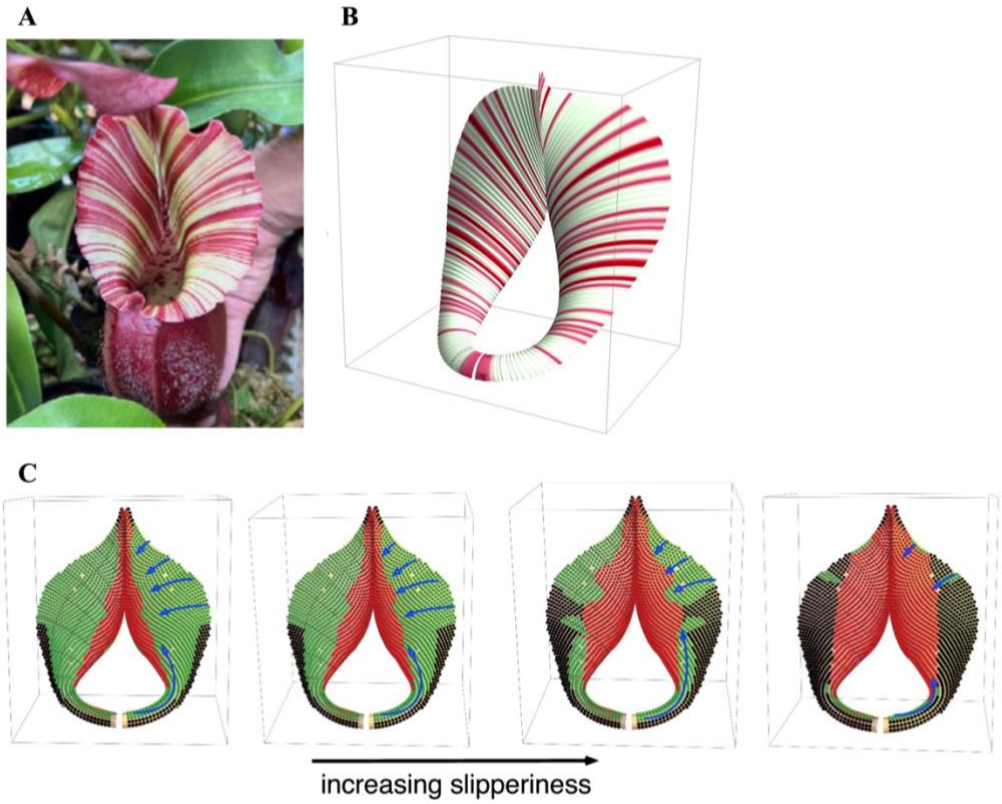
Note. (A) A passive pitfall trap of tropical pitcher plants, including the lid, the peristome, and the cup. (B) A light microscopy image of a cross-section of the peristome surface with a scale bar of 100 μm. The slippery surface property of the peristome is due to the specialized macroscopic channels and microscopic channels. (C) The specialized ridges guide water flow along with the direction of the channels. The figures are adapted from Labonte et al. (2021).

On the other hand, a novel mathematical model linking the peristomes' geometries to prey capture was provided by Moulton et al. (2023) (see Figure 9A and 9B). Under the force of gravity, the surface stability varies in different positions on the peristome (Moulton et al., 2023). Unstable positions can be further divided into two groups: (1) positions that cause the insect to slide into the pitcher and (2) positions that make the insect slide out (see Figure 9C) (Moulton et al., 2023). The total unstable area becomes larger in response to the higher slipperiness of the surface (see Figure 9C) (Moulton et al., 2023). Interestingly, the fall-out area is always smaller than the fall-in area under different surface slipperiness (see Figure 9C) (Moulton et al., 2023). It has been suggested that there is a trade-off between prey capture and peristome production (Moulton et al., 2023). For example, flaring along the bottom rim is not

found in nature as the increased area does not contribute to prey capture but instead allows the prey to slide off (Moulton et al., 2023).

Figure 9

Peristome Geometries of Tropical Pitcher Plants



Note. (A) A flared peristome of *Nepenthes veitchii*. (B) A mathematical reconstruction of the flared peristome in (A). (C) Stability and capture properties of the flared peristome as slipperiness is increased. Stable positions are shown as green points while unstable positions are illustrated with red points and black points. Specifically, the red points are positions that lead the prey to fall in, while the black points are positions that cause the prey to fall out. The figures are adapted from Moulton et al. (2023).

In general, tropical pitcher plants have developed biological strategies in order to capture their prey and survive in nutrient-poor environments: (1) the specialized ridges on the peristome enhance the surface slipperiness and increase the prey capture of the pitch, and (2)

the optimized geometric shape of the peristome improves the efficacy of the prey capture. Their strategies match the biologized questions about moving on solids and capturing solids in the context of the terrestrial environment. Therefore, we chose the tropical pitcher plant as our biological model and continued to design process.

3.3.3. Abstract Design Strategies

Before emulating the biological model, we considered the strategies in a general way. Based on the biological strategies found in tropical pitcher plants, the design strategies were described as (1) macroscopic and microscopic structures on the surface keeping the surface slippery, and (2) specialized geometries ensuring unstable surface areas.

3.3.4. Emulate Nature's Lessons

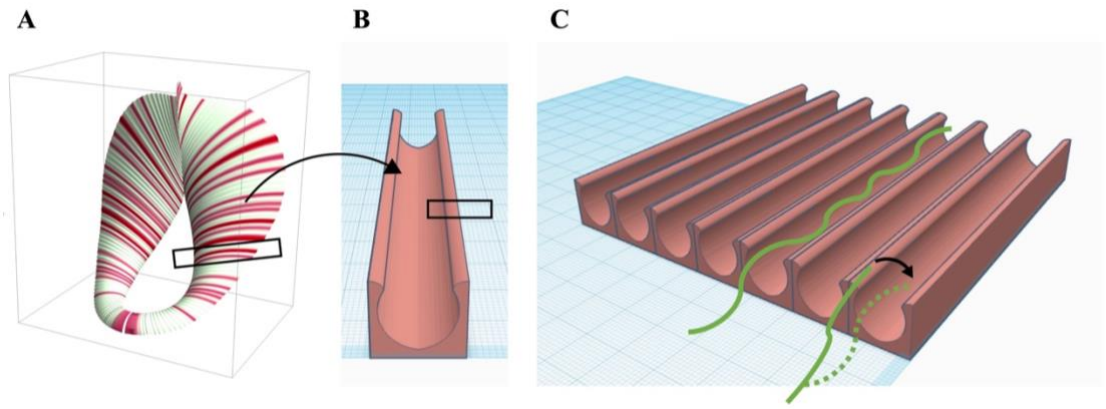
Here we looked back to the design question and the functions we intended to accomplish. In order to protect strawberry daughter plants and runners from damage, functions such as (1) limit and/or guide the plant growth and (2) separate the plants from each other were listed in previous steps. Our biological model, tropical pitcher plants, can direct the insects located at the surface of the peristome into the trap. More precisely, insects on the outer rim and the ones on the inner rim are guided inward and outward, respectively. Therefore, we tried to apply the design strategies in the pitcher plants to the strawberry propagation system. As the specialized ridges are micrometer-sized, it might be complicated to implement the surface structures at the beginning of the design process. Considering the feasibility and complexity, we decided to emulate the pitcher plants' geometries to develop a structure that can separate the runners from each other and guide the runners to grow in a certain direction.

A pitcher-plant-inspired structure was designed after careful consideration regarding the question, strategies, and context. Based on the geometries of the pitcher plant species with flared peristome, we applied the curved surface of the peristome to the top of a U-shaped

structure on both sides to make the rim of the U-shaped structure an unstable surface (see Figure 10). Here only the inner part of the peristome, the unstable positions that cause points to fall in the trap, was mimicked. The curved surface of the inner peristome was duplicated and flipped horizontally to make a symmetrical curved surface on the top of the U-shaped structure. Many of the U-shape structures were placed together to make a piece that can take up the runners from the mother plants (see Figure 10C). Theoretically, the runner tips that touch the U-shape structure would behave similarly to the insects that arrive on the peristome of pitcher plants, that is to say, the runners would fall in the U-shape gap, as illustrated in Figure 10C. Eventually, we speculated that the runners would be guided to grow along the U-shape structure, as displayed in Figure 10C.

Figure 10

The U-shaped Structure Inspired by Tropical Pitcher Plants



Note. (A) The mathematical reconstruction of the flared peristome of *Nepenthes veitchii*, which is adapted from Moulton et al. (2023). (B) A 3D model of a U-shaped structure. The curved surface of the peristome was applied to the top of the U-shaped structure. The rectangle in (A) indicates the part of the peristome that was implemented as the rim surface of the bio-inspired design, which is presented by the rectangle in (B). (C) A combination of many U-shaped structures to perform its function. Green lines represent runners in strawberry

plants. Theoretically, the runner slides off when it touches the curved surface, and it grows along and within the U-shaped gap.

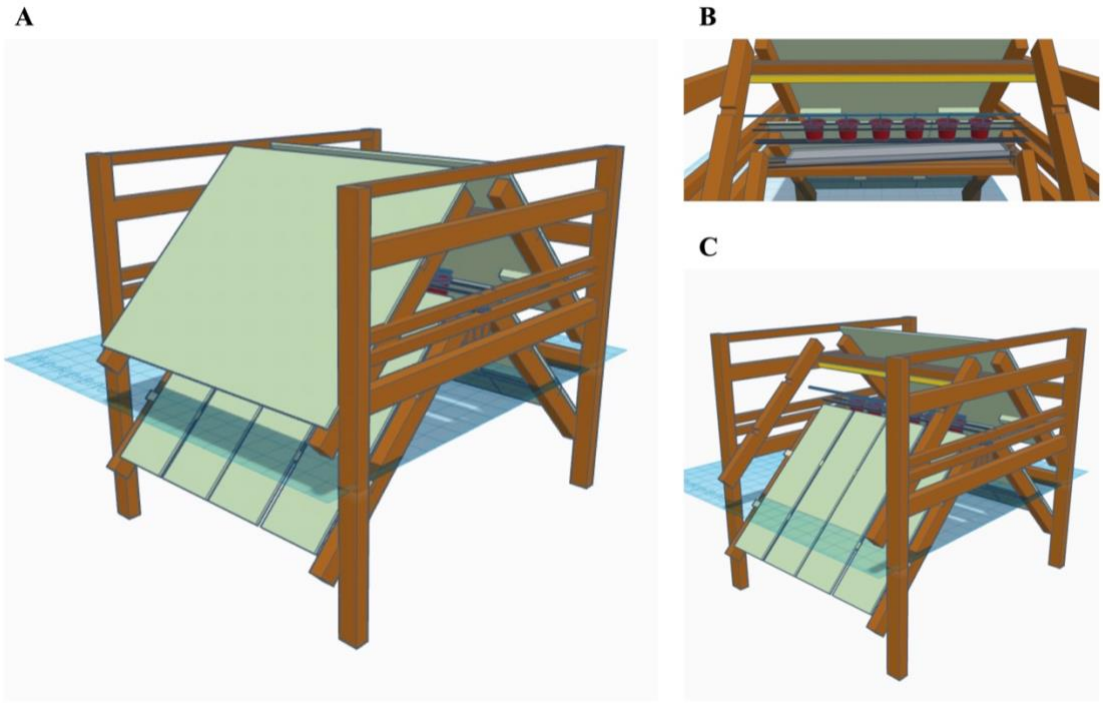
3.4. Build the Prototype (Evaluate Fit and Function)

After generating the bio-inspired design that has great potential to solve the challenge, we sketched a prototype for a vegetative propagation system that includes the bio-inspired structure. A 3D model of the prototype was established as can be seen in Figure 11A. In the middle of the prototype, light sources and one row of strawberry plants can be placed (Figure 11B). When the plants are arranged as one next to one another, the row can contain ten strawberry plants in the pots which are ten centimeters in diameter. Two one-meter-long panels are set on both sides of the strawberry plants to secure the U-shaped structure and support the runners (Figure 11A). The panels are designed to be removable to ease the process of managing the plants in the middle and harvesting the daughter plants. Also, the panels next to the strawberry plants are separated into multiple pieces to enable different experiments to be conducted at the same time and enhance the adaptability of the prototype. The length of the panels was determined based on the observation that a runner with three daughter plants is approximately one meter in length. The angle of the panels was decided according to peristomes characteristics and space efficiency. An angle of around 45 degrees to perpendicular has been found to be the most efficient peristome orientation for prey capture, even under different friction coefficients (Moulton et al., 2023). Meanwhile, when the peristome is grown horizontally, it needs to be very slippery to make the whole surface unstable area (Moulton et al., 2023). Due to the gravity, almost nothing can be captured when the peristome stands vertically (Moulton et al., 2023). As we only emulated the geometric of the peristome instead of the specialized structure that leads to the slippery property, we set the panels at an angle of 45 degrees to the vertical to ensure the “capture” of runners under less slippery conditions. Also, the 45-degree angle enables the optimal size of the prototype

regarding its height and width when the length of the panel is fixed. The prototype in Figure 11 is a unit of the vegetative propagation system. In the long term, we would like to implement multiple layers of the prototype to maximize space efficiency. Thus, in the prototype, the 45-degree panels of the upper layer are included in order to have the same airflow and humidity for strawberry plants as in the final system (multiple layers).

Figure 11

The 3D model of the Vegetative Propagation Prototype



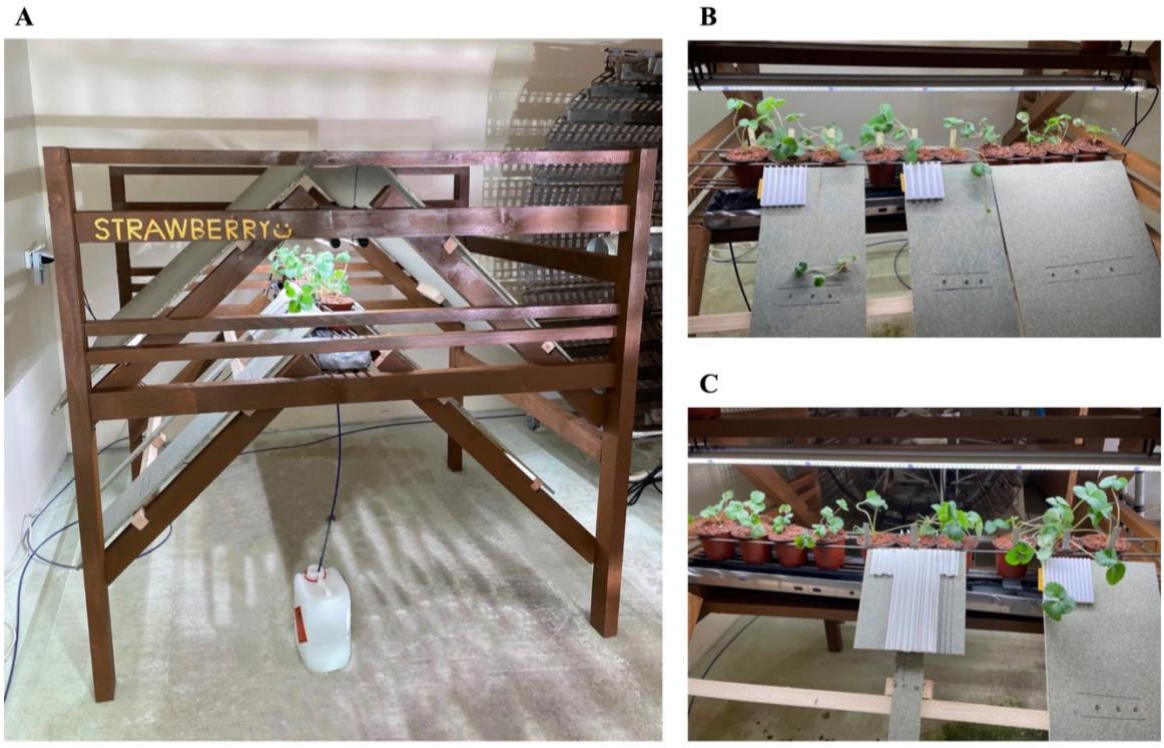
Note. (A) A prototype is sketched in a 3D model. The structure in brown is designated to be built in wood, the lines in black are for steel rods, and the green planes represent waterproof panels attaching the bio-inspired structure. (B) One row of strawberry plants can be grown in the middle of the prototype. (C) The panels are removable.

After generating the 3D model of the prototype, we built a wooden prototype with a size of 160 cm (length) x 135 (width) cm x 150 cm (height). As can be seen in Figure 12, the strawberry plants are placed on the steel rods in the middle of the prototype. On the 45-degree panels next to the strawberry plants, four types of structures were attached to test the efficacy

of the bio-inspired design for ‘capturing’ the runners, separating the runners from each other, and guiding the runners along the U-shaped structure. Before fully covering the panel with the structures, we first tested small pieces of the structure. On the one side of the prototype, we secured two 3D printing bio-inspired structures (10 cm x 10 cm) on the panel: (1) one with multiple one-centimeter-width U-shaped gaps and (2) the other one with many U-shaped gaps in 2 cm width (see Figure 12B). On the other side of the prototype, two control structures were attached to the panel: (1) a combination of L-shaped polyvinyl chloride (PVC), with a gap is 1 cm in width, and (2) a 3D printing V-shaped structure (10 cm x 10 cm) with a width of around 0.7 cm in each gap (see Figure 12C).

Figure 12

The Wooden Prototype Integrated with 3D printing Bio-inspired Structure



Note. (A) The wooden prototype was built based on the 3D model. (B) Two 3D printing bio-inspired structures are attached to the panels. One is with gaps around 1 cm in width (left), and the other is with gaps around 2 cm in width (right). (C) Two control structures are placed on the panels. One is a combination of several L-shaped PVC (left) with one-centimeter-width

gaps, and the other is a 3D printing V-shaped structure with gaps approximately 0.7 cm in width (right).

We selected some mother plants from the previous experiment and moved them to the prototype to test the vegetative propagation process. These plants are healthy and have been recorded to produce many runners during the growing process. The morphological stages of the plants when they were placed in the prototype (Day 0) are listed in Appendix F. After 12 days of growing, one runner was observed as can be seen in Figure 13. Over time, we found that the runners did not grow as expected. Instead of touching the structure through runner tips at the beginning of the runner sprouting process, the runner elongated first and then fell on the structure, which is given in Figure 13. In general, more experiments on testing the bio-inspired structure and validating the prototype are needed in the future.

Figure 13

Observations of Runner Behavior in the Strawberry Plants in the Prototype

Note. The growth of the runners and the number of days after placing the plants in the prototype were recorded. After 12 days of growth, the first runner finally formed. On the 15th day, the body of the runner touched the structure. On the 18th day, the runner grew toward the outside of the prototype. Part of the runner was fully in the gap of the bio-inspired structure after 20 days of growth.

4. Discussion

Strawberry production has been facing numerous challenges, including chilling requirements, extreme weather, pests, and diseases (Hernández-Martínez et al., 2023). To avoid damage caused by pests and diseases, numerous pesticides are commonly applied to strawberry plants in current nurseries. In addition, some fungi contaminations and diseases were found in the strawberry plants from the external nursery. To ensure consistent strawberry fruit production at Futura Gaïa, a source of young strawberry plants that are healthy, pesticide-free, productive, and available all year round is needed. In this study, we managed to produce healthy and pesticide-free strawberry plants through vegetative propagation. The mother plants were grown from in vitro plantlets to minimize the potential pathogens, and the daughter plants were produced through the runnering process successfully. The encouraging outcome allows us to continue improving the efficiency of the vegetative propagation process by altering environmental conditions and innovating a suitable bio-inspired system.

4.1. Validation of Vegetative Propagation Agronomic Recipes

In an indoor farming system, a suitable recipe regarding the environmental conditions (e.g. light, temperature, water, nutrients, and humidity) is essential for efficient production. To optimize the vegetative propagation process and produce strawberry daughter plants efficiently, we set up temperature and photoperiod based on previous literature and analyzed the effects of multiple light sources and different nitrogen fertigation concentrations.

4.1.1. Light Sources

In terms of light sources, our results indicated that sun light LED and white light LED are suitable light sources for vegetative propagation in strawberry plants. At the same time, blue light has a negative effect on vegetative propagation in strawberry plants. These results are in agreement with our hypothesis that blue light could hinder runnering in strawberry plants. In other words, blue light not only promotes floral initiation in strawberry plants (Ye et

al., 2021) but also suppresses the runnering process in the mother plants. The suggestion aligns with current understanding of the trade-off between flowering and runnering in strawberry plants caused by axillary meristem differentiation (Hytönen & Kurokura, 2020). The success in vegetative propagation in strawberry plants grown under sun light LED and white light LED enables us to apply these light sources in the prototype and future system for asexual reproduction.

Future work should further examine the link between light spectra composition and vegetative propagation. Light conditions, such as light intensity, photoperiod, and light spectra composition, have quite an impact on plant growth and development (Wei et al., 2021). The influence of photoperiod on floral induction and runnering process is well documented as summarized in Section 1.2. Also, the effect of light intensity on strawberry vegetative propagation in controlled environments has been examined by Xu & Hernández (2020) and Zheng et al. (2019). It has been demonstrated that more daughter plants can be produced when the mother plants are grown under higher light intensity (X. Xu & Hernández, 2020). The plants of a day neutral strawberry cultivar 'Albion,' that were prepared from daughter plants, grown under a PPFD of 450 μmol m^{-2} s^{-1} were shown to produce 7.3 daughter plants per week per stock plant, while the plants under a PPFD of 250 μmol m^{-2} s^{-1} were demonstrated to produce 5.1 daughter plants per week per stock plant (X. Xu & Hernández, 2020). In our results, a PPFD of 206.4 μmol m^{-2} s^{-1} was applied regarding the sun light condition, and 2.1 CHA daughter plants per mother plant and 3.3 CIF daughter plants per mother plant were produced after two months of cultivation, which includes the growing time from in vitro plantlets. The data would be more comparable if the plants were grown to a bigger size. In terms of light spectra composition, some researchers have examined how LED light spectra affect strawberry flower initiation, fruit yield, and fruit quality in controlled environments, which has been significantly reviewed by Warner et al. (2021). Besides flowering, few studies

have focused on the runnering process in strawberry plants (Naznin et al., 2016; Wu et al., 2009). For instance, Naznin et al. (2016) demonstrated that strawberry plants grown under LED light with a 10:1 red:blue light ratio can produce more runners (around 3.5 runners per plant every four weeks), compared with the plants under a 19:1 or 5:1 red:blue light ratio (about 3 runners per plant every four weeks). In our case, the plants grown under the sun light (R:G:B is 40:40:21) produced approximately 2.5 runners per plant four weeks after runner sprouting. It would be more valuable to keep the plants for a longer period and observe the average runner production in the long term. The relation between light spectra composition and vegetative multiplication should be investigated more in the future. The efficiency of vegetative propagation could be further increased if the exact light spectra composition needed for asexual reproduction is validated.

4.1.2. Nitrogen Fertigation

In terms of nitrogen fertigation, our results demonstrated that a nitrogen fertigation concentration of 8 mM is appropriate for vegetative propagation in 'Charlotte' and 'Ciflorette' strawberry plants. The results did not match our speculation that higher nitrogen concentration (20 mM) has the same effect on vegetative propagation in mother plants under sun light and white light conditions, compared with the control nitrogen concentration (8 mM). Instead, we found that higher nitrogen concentration has a negative effect on vegetative propagation in mother plants under the sun light treatment, but no effect was noticed in the plants under the white light treatment. As sun light LED and white light LED are validated as appropriate light sources for vegetative propagation, 8 mM nitrogen fertigation together with the two light sources can be implemented in future experiments for vegetative propagation.

In the future, it would be of interest to examine an optimal nitrogen amount for vegetative propagation among a wider range of nitrogen fertigation concentrations (e.g. from 0 mM to 30 mM). Strawberries have been shown to be very responsible to nitrogen fertilizer

in terms of plant growth (Farjana et al., 2023b). Studies have indicated that appropriate amounts of nitrogen are crucial to proper vegetative growth and asexual reproduction in strawberry plants (Farjana et al., 2023b; Trejo-Téllez & Gómez-Merino, 2014). Additional nitrogen does not lead to more efficient plant growth and vegetative propagation (Farjana et al., 2023b). For instance, 10 mM nitrogen fertigation concentration has been shown to be optimal for daughter plant production in cultivar 'Altaking,' compared with 0 mM, 5 mM, 15 mM, and 20 mM (Farjana et al., 2023b). The optimal concentration of nitrogen fertigation is dependent on cultivars (Farjana et al., 2023b). Further work is needed to explore the consequences of different nitrogen concentrations, besides the two conditions examined in this study (i.e. 8 mM and 20 mM), for asexual reproduction in the cultivars we chose. An optimal nitrogen concentration for vegetative propagation can then be further validated.

4.1.3. Interaction Between Light Quality and Nitrogen Supply

Moreover, there is a need to understand the impacts of the interaction between light spectra composition and nitrogen amount on asexual reproduction in strawberry. Much attention has been paid to the individual effects of nitrogen availability and light on plant growth and resource allocation in control environment agriculture (Liang et al., 2022). Besides their individual impacts, an interaction between light spectra composition and fertigation solution has been reported (J. Xu et al., 2021). Several studies have focused on the effects of light spectra composition and nitrogen fertigation on plant growth, flowering, runnering, and fruit production in strawberries (Farjana et al., 2023b; Trejo-Téllez & Gómez-Merino, 2014; Warner et al., 2021). Excessive nitrogen fertilizer has been known to delay flowering time in agriculture (Yuan et al., 2016). Blue light has been reported to overcome the delayed flowering caused by the overapplication of nitrogen fertilizer in Arabidopsis (Yuan et al., 2016). Also, light signals have been shown to regulate nitrogen uptake and assimilation by enhancing the expression of related genes in the Arabidopsis (Sakuraba & Yanagisawa, 2018).

Our results reveal that the influence of nitrogen supply on vegetative propagation is not consistent among light sources of sun light LED, white light LED, and blue light LED. The results also demonstrated that vegetative propagation in mother plants under the blue light treatment was not hampered when the plants were provided with higher nitrogen fertigation concentration. It would be beneficial to investigate the interaction between light quality and nitrogen availability in the future. Hence, the limit on asexual reproduction in strawberries under various combinations of light spectra compositions and nitrogen fertigation concentrations could be further understood. The environmental conditions optimized for vegetative propagation in strawberries could then be defined.

4.2. Creation of the First Prototype for Bio-inspired Vegetative Propagation System

4.2.1. Efficacy of the U-shaped Structure Inspired by Tropical Pitcher Plants

Through the biomimicry design spiral, we successfully created the U-shaped structure inspired by peristome geometries in tropical pitcher plants to deal with the tangle of runners and daughter plants. The bio-inspired structure was then integrated into the wooden prototype for vegetative propagation. Currently, the bio-inspired U-shaped structure and the control V-shaped structure were placed in the prototype for further examination. We aimed to see the efficacy of different structures for separating the runners and guiding their growth. In the prototype, mother plants were the ones that produced many runners during the experiment in the first part of the study. We assumed that these mother plants are productive in terms of runners and daughter plants. However, there were only two new runners during the 20-day observation, and some of the mother plants had started flowering. Only one of the runners grew toward the structure which allowed us to monitor the effectiveness of the structure. More runners are needed to evaluate the bio-inspired structure.

To further examine the bio-inspired structure, we suggest two possible directions. First, future work should focus on how to extend the runner sprouting period in mother plants.

The agronomic recipe applied to mother plants was for vegetative propagation instead of floral initiation. However, many of the mother plants for the previous experiment, which had been grown for four months under a vegetative propagation recipe, had started to flower. We assumed that the flowering mother plants were no longer suitable for vegetative propagation in the prototype. Thus, further work is needed to extend the vegetative propagation cycle. Environmental conditions that can hold mother plants at the vegetative phase should be explored. Once the runnering period is continued, more runners will be produced. The interactions between the runners and the U-shaped bio-inspired structure could be monitored and examined widely. Second, besides testing the bio-inspired structure with strawberry plants naturally along with plant growth in the prototype, it would be beneficial to set up a runner sliding test apart from the prototype to understand the possible behavior of runners that touch the bio-inspired structure. For example, runners could be lifted in the air and then dropped on the bio-inspired structure, which could be done manually or through a simple device. The interaction between the runners and the structure could be recorded by camera. Quantitative and qualitative research could be conducted to examine the potential efficacy of the bio-inspired structure in terms of separating the runners. In this way, the structure could be examined and improved separately before the final test through the real plant growth in the prototype. Also, the validation time could be shortened.

4.2.2. Economic Cost

From the economic aspect, theoretically, the cost of daughter plants produced in our vegetative propagation system is lower than the price of in vitro plants from an external laboratory. Currently, there are multiple types of plant materials we can choose from, including runner, unprepared plant, prepared plant, and in vitro plant. The prepared plant can be used for fruit production immediately, while the rest will need extra time and effort before entering the fruiting phase. Current prices of ‘Gariguette’ strawberry for each runner,

unprepared plant, prepared plant, and in vitro plant are 0.17 euros, 0.29 euros, 0.55 euros, and 1.165 euros, respectively. We assume that the mother plants in our prototype can be as productive as the ones grown in the current system. Also, each batch of mother plants is assumed to produce runners and daughter plants continuously for one year. In our vegetative propagation system, the cost of each daughter plant is 0.85 euros. The calculation details can be found in Appendix G. The prices of runners, unprepared plants, and prepared plants are lower than the cost of our daughter plants. However, these plant materials are not ideal as they are normally treated with multiple pesticides and may bring pathogens to our indoor farming system. Regarding in vitro plants, the only type of plant materials that meet our requirements (i.e. pathogen-free and pesticide-free) are more expensive than other plant materials. Notably, the cost of our daughter plants is lower than the in vitro plants from an external lab. In other words, our vegetative propagation system has great potential to raise the economic benefits of strawberry fruit production by reducing its plant material cost.

4.2.3. Potential of the Vegetative Propagation System

For the next stage, it would be of interest to upgrade the vegetative propagation system based on the possibilities that have emerged in this project. Here we summarize some proposals that have been made but have not been applied in the prototype during the design process. Firstly, the specialized ridges on the peristome surface in tropical pitcher plants could be further investigated and implemented to improve the efficacy of runner capture in the U-shaped structure. For the first step, only the peristome geometric surface in the pitcher plants was integrated into the current bio-inspired structure considering feasibility and complexity. Nonetheless, the strategy of enhancing surface slipperiness by macroscopic and microscopic ridges has not been ruled out. It would be useful to explore the effects of the specialized ridges on the runner capture and involve the strategy to improve the efficacy of the bio-inspired structure.

Secondly, we recommend revisiting the main functions, especially the ones that were put on hold at the initial stage. While defining the challenge, we listed four main functions (see Section 3.2.3.) that could help us solve the problem based on the brainstorming result. In the current design, we focused on the two main functions, which are (1) limiting and/or guiding the plant growth, and (2) separating the plants from each other, and tried to protect strawberry daughter plants and runners from damage, which was stated as design question. For the next stage, besides minimizing unnecessary plant damage, performing the other two functions of (1) including flexibility in the system and (2) increasing the space efficiency in the vegetative propagation system is an exciting challenge for future studies. With these two functions, the lifespan of the vegetative propagation system could be extended, and the economic value of the system could be raised.

Lastly, future work should evaluate the vegetative propagation system regarding the final goals defined at the beginning of the study. Here are the three final goals: (1) to allow sustainable production of daughter plants, (2) to provide sufficient daughter plants that are well-developed and homogeneous, and (3) to be resource-efficient during the production process. A vegetative propagation system fulfilling the final goals would enable Futura Gaïa to have its own strawberry plant source that is stable, adequate, and efficient even in the long term.

4.3. The Prospects

In this project, we conducted plant science research on strawberries and established an early-stage vegetative propagation system from scratch. We have successfully grown strawberry plants from in vitro plantlets and produced young strawberry plants through vegetative propagation. Also, an agronomic recipe for asexual reproduction has been validated. Furthermore, we designed a bio-inspired structure based on the existing problems and potential functions required in the multiplication process. Moreover, a prototype for a

vegetative propagation system has been built eventually. Importantly, our system can lower the cost of plant materials for strawberry fruit production. Nevertheless, further work is needed to examine the efficacy of the bio-inspired structure and improve the prototype. Our study provides a great starting point for the validation of the vegetative propagation system. Besides the research and design work, we applied for the innovation lump-sum provided by Suave Urban Agriculture. Excitingly, our application based on the bio-inspired vegetative propagation system has been approved, and we will have 20,000 euros in funding for improving and industrializing the prototype.

In the future, the vegetative propagation system could also be applied to other stolon-forming or rhizome-forming species. For example, *Gynostemma pentaphyllum*, another species that has been investigated at the company, can propagate vegetatively through rhizomes or cuttings. The harvesting problem in *Gynostemma pentaphyllum* is similar to that in strawberries. Their stems can grow quickly but twist together easily. Thus, the bio-inspired structure may also help to separate the stems in *Gynostemma pentaphyllum* and improve the propagation efficiency.

4.4. Learning Outcomes of the Internship

During this internship, I gained valuable working experience and learned many hard skills and soft skills, which prepared me to enter the job market. The knowledge of current agricultural challenges and indoor farming systems has been acquired. Research skills such as data analysis, writing, and time management were practiced. As the atmosphere in the agronomy team was very positive, it was a perfect place for me to learn soft skills such as teamwork and communication. The potential economic value is usually considered while the research is performed. Thus, I also got a chance to learn about budgeting.

Also, during the project, I found some clues to the questions that I had in mind, which can help me develop my future career in bio-inspired design. Learning from nature has been

described in many different terms such as biomimicry, bio-inspiration, biomimetics, nature-inspired innovation, etc. The value behind has been spread for decades. Theoretically, bio-inspired innovation can lead to sustainable and beneficial solutions to human challenges. However, from my point of view, the current impact of biomimicry on human society is less than expected. Thus, I have been trying to figure out the gap between the ideal biomimicry concept and the needs of individuals, groups of people, companies and organizations, and the whole human society. This project was the first time I had to deal with a real task in the industry and try to create value through bio-inspired design. Viewing biomimicry design spiral as a problem-solving tool provides a good link between the biomimicry world and industry. Based on the problem-solving point of view, we would focus on tackling the problem instead of creating an ideal biomimicry design. The combination of scientific research and problem analysis was a success in defining the challenge. However, the examination and validation of the design might be one of the main obstacles to solving the problem in a short time. Thus, the evaluation step should be further adapted to industry requirements. For example, some management frameworks such as Scrum and Toyota Kata could be integrated into the design process. In general, I have gained incredible working experience and new insights into the biomimicry future during this internship.

5. Conclusion

In this study, we have achieved many objectives such as (1) producing healthy and pesticide-free strawberry plants, (2) validating a suitable agronomic recipe for vegetative propagation, (3) creating a bio-inspired structure that could ease the vegetative propagation process, and (4) establishing a vegetative propagation prototype integrating the bio-inspired structure. Our work should provide a great starting point for further examination and validation of an indoor vegetative propagation system. A sustainable and stable source of healthy and pesticide-free strawberry plants can be established at Futura Gaïa.

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Appendix A. Statistical Analyses of Vegetative Propagation Results

In terms of vegetative propagation, we monitored three parameters including the length of the first runner, the number of runners per plant, and the number of daughter plants per (mother) plant. In Figure 2A, 2C, and 2E, the three parameters were recorded over time. The means, the standard error of the mean (SEM), and sample size (N) of the three parameters over time are shown in Table A1, A2, and A3.

Table A1

Statistics of Length of the First Runner Over Time

Cultivar	GAR			CHA			CIF		
Week	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
0	0	0	18	0	0	18	0	0	18
1									
2									
3	0	0	18	0	0	18	4,971	1,754	17
4	0,538	0,327	13	4,750	1,095	18	17,618	3,352	17
5	7,462	2,652	13	24,118	2,388	17	32,794	4,650	17

Note. A summary of the means, the standard error of the mean (SEM), and sample size (N) are given. The unit for the length of the first runner is centimeter.

Table A2

Statistics of Number of Runners Per Plant Over Time

Cultivar	GAR			CHA			CIF		
Week	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
0	0	0	18	0	0	18	0	0	18
1									
2									
3	0	0	13	0	0	18	0,706	0,206	17
4	0,231	0,122	13	0,889	0,159	18	1,706	0,281	17
5	0,462	0,144	13	1,765	0,136	17	2,294	0,254	17
6									
7									
8	0,769	0,166	13	2,294	0,187	17	3,412	0,285	17

Note. A summary of the means, the standard error of the mean (SEM), and sample size (N) are demonstrated.

Table A3

Statistics of Number of Daughter Plants Per Plant Over Time

Cultivar	GAR			CHA			CIF		
Week	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
0	0	0	18	0	0	18	0	0	18
1									
2									
3									
4									
5	0,077	0,077	13	0,941	0,160	17	1,529	0,311	17
6									
7									
8	0,462	0,144	13	2,118	0,241	17	3,294	0,567	17

Note. A summary of the means, the standard error of the mean (SEM), and sample size (N) are shown.

In addition, the data of the three parameters from the 5th week of the experiment were analyzed, which are given in Figure 2B, 2D, 2F. The normality tests and the Kruskal-Wallis test were conducted to compare the vegetative propagation in strawberry plants under six different environmental conditions. The means, the standard deviation (SD), and sample size (N) of the data of length of the first runner among the six groups are given in Table A4. The results of the normality tests of length of the first runner in the three cultivars (e,g. GAR, CHA, and CIF) are displayed in Table A5, A6, and A7, respectively. Regarding the number of runners per plant, the means, the SD, and the N are shown in Table A8. The results of the normality tests in the three cultivars are given in Table A9, A10, and A11, respectively. In terms of the number of daughter plants per plant, the means, the SD, and the N are shown in Table A12. The results of the normality tests in the three cultivars are demonstrated in Table

A13, A14, and A15, respectively. The p-values of the Krudkal-Wallis tests for the three parameters are shown in Table A16.

Table A 4

Statistics of Length of the First Runner in Week 5

Modality	Sun_Control			Sun_N+			White_Control		
Cultivar	Mean	SD	N	Mean	SD	N	Mean	SD	N
GAR	7,462	9,562	13	2,767	6,253	15	8,778	9,883	18
CHA	24,118	9,845	17	19,694	10,932	18	25,111	13,001	18
CIF	32,794	19,171	17	15,694	14,469	18	24,594	16,762	16

Modality	White_N+			Blue_Control			Blue_N+		
Cultivar	Mean	SD	N	Mean	SD	N	Mean	SD	N
GAR	1,806	4,541	18	6,765	13,301	17	4,889	9,690	18
CHA	23,667	13,087	18	5,833	12,073	18	12,889	14,012	18
CIF	28,441	8,705	17	9,139	11,390	18	19,750	18,507	18

Note. A summary of the means, the standard deviation (SD), and sample size (N) are displayed. The unit for the length of the first runner is centimeter.

Table A 5

Normality Tests Results of Length of the First Runner in GAR in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,7325	0,5137	0,8269	0,4578	0,5752	0,5871
P value	0,0012	<0,0001	0,0037	<0,0001	<0,0001	<0,0001
Passed normality test (alpha=0,05)?	No	No	No	No	No	No
P value summary	**	****	**	****	****	****
Kolmogorov-Smirnov test						
KS distance	0,3209	0,4112	0,2137	0,3602	0,4592	0,4153

P value	0,0007	<0,0001	0,0292	<0,0001	<0,0001	<0,0001
Passed normality test (alpha=0,05)?	No	No	No	No	No	No
P value summary	***	****	*	****	****	****
Number of values	13	15	18	18	17	18

Note. A summary of the two normality tests results is given.

Table A 6

Normality Tests Results of Length of the First Runner in CHA in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,8988	0,8972	0,8341	0,9488	0,5249	0,8571
P value	0,0649	0,0514	0,0048	0,4059	<0,0001	0,011
Passed normality test (alpha=0,05)?	Yes	Yes	No	Yes	No	No
P value summary	ns	ns	**	ns	****	*
Kolmogorov-Smirnov test						
KS distance	0,1838	0,2151	0,2285	0,1142	0,4633	0,2101
P value	>0,1000	0,0272	0,0138	>0,1000	<0,0001	0,0347
Passed normality test (alpha=0,05)?	Yes	No	No	Yes	No	No
P value summary	ns	*	*	ns	****	*
Number of values	17	18	18	18	18	18

Note. A summary of the two normality tests results is shown.

Table A 7

Normality Tests Results of Length of the First Runner in CIF in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,9476	0,8709	0,946	0,9497	0,807	0,8846
P value	0,4199	0,0184	0,4292	0,4526	0,0019	0,0312
Passed normality test (alpha=0,05)?	Yes	No	Yes	Yes	No	No
P value summary	ns	*	ns	ns	**	*
Kolmogorov-Smirnov test						
KS distance	0,1409	0,2144	0,1776	0,1233	0,2112	0,1713
P value	>0,1000	0,0281	>0,1000	>0,1000	0,0329	>0,1000
Passed normality test (alpha=0,05)?	Yes	No	Yes	Yes	No	Yes
P value summary	ns	*	ns	ns	*	ns
Number of values	17	18	16	17	18	18

Note. A summary of the two normality tests results is demonstrated.

Table A 8

Statistics of Number of Runners Per Plant in Week 5

Modality	Sun_Control			Sun_N+			White_Control		
Cultivar	Mean	SD	N	Mean	SD	N	Mean	SD	N
GAR	0,462	0,519	13	0,467	0,743	15	0,722	0,669	18
CHA	1,765	0,562	17	1,222	0,732	18	1,611	0,916	18
CIF	2,294	1,047	17	1,667	0,907	18	1,813	1,047	16

Modality	White_N+			Blue_Control			Blue_N+		
Cultivar	Mean	SD	N	Mean	SD	N	Mean	SD	N

GAR	0,389	0,502	18	0,353	0,702	17	0,444	0,856	18
CHA	2,000	0,907	18	0,333	0,686	18	0,833	0,857	18
CIF	2,353	0,786	17	0,833	0,786	18	1,500	1,098	18

Note. A summary of the means, the standard deviation (SD), and sample size (N) are shown.

Table A 9

Normality Tests Results of Number of Runners Per Plant in GAR in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,6457	0,6629	0,7876	0,6242	0,56	0,6003
P value	0,0002	0,0001	0,001	<0,0001	<0,0001	<0,0001
Passed normality test (alpha=0,05)?	No	No	No	No	No	No
P value summary	***	***	**	****	****	****
Kolmogorov-Smirnov test						
KS distance	0,3516	0,4016	0,2721	0,392	0,4572	0,4205
P value	<0,0001	<0,0001	0,001	<0,0001	<0,0001	<0,0001
Passed normality test (alpha=0,05)?	No	No	No	No	No	No
P value summary	****	****	**	****	****	****
Number of values	13	15	18	18	17	18

Note. A summary of the two normality tests results is displayed.

Table A 10

Normality Tests Results of Number of Runners Per Plant in CHA in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+

Shapiro-Wilk test						
W	0,7331	0,8018	0,8256	0,885	0,5438	0,8148
P value	0,0003	0,0016	0,0036	0,0317	<0,0001	0,0025
Passed normality test (alpha=0,05)?	No	No	No	No	No	No
P value summary	***	**	**	*	****	**
Kolmogorov-Smirnov test						
KS distance	0,3681	0,2449	0,331	0,2778	0,4643	0,2563
P value	<0,0001	0,0056	<0,0001	0,0007	<0,0001	0,0028
Passed normality test (alpha=0,05)?	No	No	No	No	No	No
P value summary	****	**	****	***	****	**
Number of values	17	18	18	18	18	18

Note. A summary of the two normality tests results is given.

Table A 11

Normality Tests Results of Number of Runners Per Plant in CIF in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,876	0,8836	0,8093	0,8711	0,804	0,8333
P value	0,0275	0,0301	0,0036	0,0229	0,0017	0,0047
Passed normality test (alpha=0,05)?	No	No	No	No	No	No
P value summary	*	*	**	*	**	**
Kolmogorov-Smirnov test						

KS distance	0,2794	0,2544	0,3211	0,2616	0,2444	0,2867
P value	0,001	0,0032	0,0001	0,0031	0,0057	0,0004
Passed normality test (alpha=0,05)?	No	No	No	No	No	No
P value summary	**	**	***	**	**	***
Number of values	17	18	16	17	18	18

Note. A summary of the two normality tests results is shown.

Table A 12

Statistics of Number of Daughter Plants Per Plant in Week 5

Modality	Sun_Control			Sun_N+			White_Control		
Cultivar	Mean	SD	N	Mean	SD	N	Mean	SD	N
GAR	0,077	0,277	13	0,133	0,352	15	0,111	0,323	18
CHA	0,941	0,659	17	0,889	0,676	18	0,611	0,608	18
CIF	1,529	1,281	17	0,444	0,616	18	0,750	0,856	16

Modality	White_N+			Blue_Control			Blue_N+		
Cultivar	Mean	SD	N	Mean	SD	N	Mean	SD	N
GAR	0,000	0,000	18	0,059	0,243	17	0,056	0,236	18
CHA	0,778	0,732	18	0,278	0,669	18	0,333	0,485	18
CIF	0,824	0,883	17	0,056	0,236	18	0,556	0,784	18

Note. A summary of the means, the standard deviation (SD), and sample size (N) are demonstrated.

Table A 13

Normality Tests Results of Number of Daughter Plants Per Plant in GAR in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,311	0,4128	0,3731	Invalid input data	0,2622	0,2527

P value	<0,0001	<0,0001	<0,0001		<0,0001	<0,0001
Passed normality test (alpha=0,05)?	No	No	No		No	No
P value summary	****	****	****		****	****
Kolmogorov-Smirnov test						
KS distance	0,5323	0,5143	0,5233	1	0,537	0,5376
P value	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
Passed normality test (alpha=0,05)?	No	No	No	No	No	No
P value summary	****	****	****	****	****	****
Number of values	13	15	18	18	17	18

Note. A summary of the two normality tests results is given.

Table A 14

Normality Tests Results of Number of Daughter Plants Per Plant in CHA in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,7985	0,803	0,7516	0,8018	0,466	0,6007
P value	0,0019	0,0017	0,0003	0,0016	<0,0001	<0,0001
Passed normality test (alpha=0,05)?	No	No	No	No	No	No
P value summary	**	**	***	**	****	****
Kolmogorov-Smirnov test						
KS distance	0,3003	0,2875	0,2945	0,2449	0,4943	0,4207
P value	0,0003	0,0004	0,0002	0,0056	<0,0001	<0,0001

Passed normality test (alpha=0,05)?	No	No	No	No	No	No
P value summary	***	***	***	**	****	****
Number of values	17	18	18	18	18	18

Note. A summary of the two normality tests results is shown.

Table A 15

Normality Tests Results of Number of Daughter Plants Per Plant in CIF in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,8883	0,6991	0,7783	0,8146	0,2527	0,6995
P value	0,0434	<0,0001	0,0014	0,0032	<0,0001	<0,0001
Passed normality test (alpha=0,05)?	No	No	No	No	No	No
P value summary	*	****	**	**	****	****
Kolmogorov-Smirnov test						
KS distance	0,1898	0,3759	0,2602	0,2443	0,5376	0,3719
P value	>0,1000	<0,0001	0,0049	0,0081	<0,0001	<0,0001
Passed normality test (alpha=0,05)?	Yes	No	No	No	No	No
P value summary	ns	****	**	**	****	****
Number of values	17	18	16	17	18	18

Note. A summary of the two normality tests results is given.

Table A 16

The Krudkal-Wallis Test Results of Vegetative Propagation Data from Week 5

	Cultivars		
Vegetative Propagation Parameters	GAR	CHA	CIF
Length of the first runner	0,1889	9,4x10^-5	0,0005
Number of runners per plant	0,3911	3,3x10^-7	0,0001728
Number of daughter plants per plant	0,7322	0,004146	0,0002719

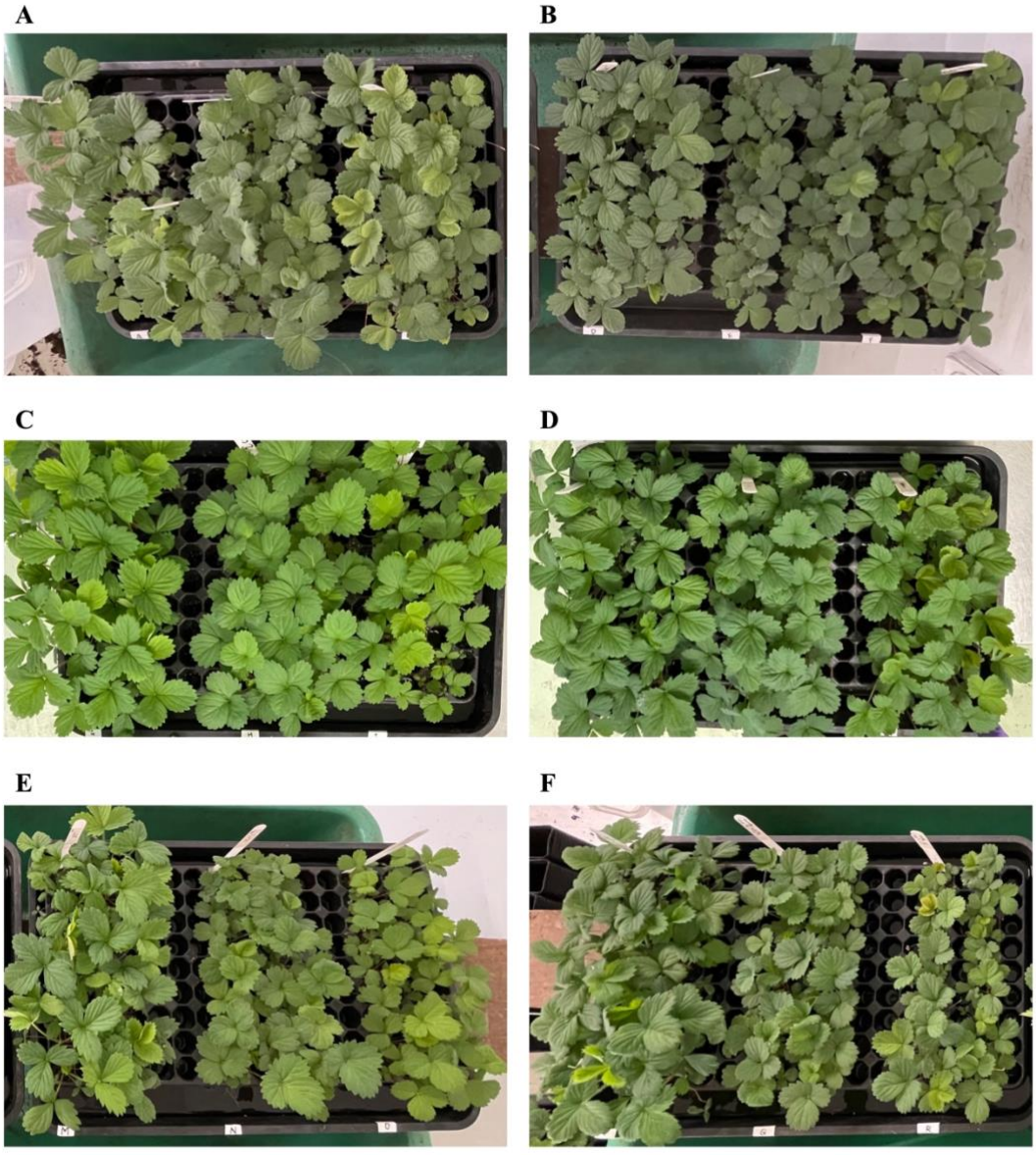
Note. The p-value of the Krudkal-Wallis test of each vegetative propagation parameter in each cultivar is shown.

Appendix B. Morphology of Each Modality in Week 3

Plant morphology of each modality was recorded in Week 3, as given in Figure B1. The 18 modalities include three cultivars under six groups of environmental conditions.

Figure B 1

Morphology of GAR, CHA, and CIF Under Six Environmental Conditions in Week 3



Note. An overview of plant morphology in the 3rd week of the experiment. In each picture, the plants are GAR, CHA, and CIF, from the left to the right. Each modality was planted in two rows of 9, which is 18 plants (biological replicates) in total. Strawberry plants grown under the sun light condition are shown in (A) and (B). The plants in (A) were treated with control

nitrogen level of fertigation, and ones in (B) was irrigated with the N+ fertigation solution. Strawberry plants raised under the white light LED are shown in (C) and (D). Likewise, the former displays the strawberries under the control nitrogen fertigation, and the latter shows the plants under the N+ fertigation condition. In (E) and (F), the plants grown under the blue light are demonstrated. The ones irrigated with the control nitrogen fertigation are shown in (E), while the ones treated with the N+ fertigation solution are given in (F).

Appendix C. Statistical Analyses of Strawberry Plant Growth Results

Besides the three parameters of vegetative propagation, three plant growth parameters (e.g. crown diameter, number of leaves per plant, and height) in the 5th week of the experiment were measured and analyzed as can be seen in Figure 3. The normality tests and Kruskal-Wallis test were performed to learn the plant growth difference caused by the six environmental groups. In terms of the crown diameter, the means, the standard deviation (SD), and sample size (N) are shown in Table C1. The normality tests results are demonstrated in three tables, which are table C2, C3, and C4, based on the cultivars. Regarding the number of leaves per plant, general statistics are given in Table C5, while the results of the normality tests are illustrated in Table C6, C7, and C8. In Table C9, the means, SD, and N of the plant height data are displayed. According to the cultivars, the normality tests results of the plant height are shown in Table C10, C11, and C12. A summary of the Kruskal-Wallis tests results is given in Table C13.

Table C 1

Statistics of Crown Diameter in Week 5

Modality	Sun_Control			Sun_N+			White_Control		
Cultivar	Mean	SD	N	Mean	SD	N	Mean	SD	N
GAR	6,769	1,691	13	7,500	1,549	16	7,333	0,686	18
CHA	7,735	1,251	17	7,778	1,114	18	7,611	1,577	18
CIF	6,824	1,286	17	6,889	0,963	18	6,125	1,088	16

Modality	White_N+			Blue_Control			Blue_N+		
Cultivar	Mean	SD	N	Mean	SD	N	Mean	SD	N
GAR	7,167	1,249	18	6,706	1,047	17	6,722	1,447	18
CHA	8,167	1,425	18	5,944	0,998	18	6,278	1,274	18
CIF	7,706	1,532	17	5,167	0,985	18	5,500	1,200	18

Note. A summary of the means, the standard deviation (SD), and sample size (N) are demonstrated.

Table C 2

Normality Tests Results of Crown Diameter in GAR in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,9229	0,7491	0,78	0,9342	0,86	0,8199
P value	0,2741	0,0006	0,0008	0,2301	0,0153	0,0029
Passed normality test (alpha=0,05)?	Yes	No	No	Yes	No	No
P value summary	ns	***	***	ns	*	**
Kolmogorov-Smirnov test						
KS distance	0,1697	0,3141	0,2789	0,1642	0,1989	0,2558
P value	>0,1000	0,0002	0,0007	>0,1000	0,0726	0,0029
Passed normality test (alpha=0,05)?	Yes	No	No	Yes	Yes	No
P value summary	ns	***	***	ns	ns	**
Number of values	13	16	18	18	17	18

Note. A summary of the two normality tests results is given.

Table C 3

Normality Tests Results of Crown Diameter in CHA in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,9036	0,905	0,9231	0,9006	0,8728	0,8647
P value	0,0781	0,0703	0,1465	0,0588	0,0198	0,0146

Passed normality test (alpha=0,05)?	Yes	Yes	Yes	Yes	No	No
P value summary	ns	ns	ns	ns	*	*
Kolmogorov-Smirnov test						
KS distance	0,1968	0,1987	0,2085	0,1652	0,3	0,2471
P value	0,079	0,0584	0,0374	>0,1000	0,0001	0,0049
Passed normality test (alpha=0,05)?	Yes	Yes	No	Yes	No	No
P value summary	ns	ns	*	ns	***	**
Number of values	17	18	18	18	18	18

Note. A summary of the two normality tests results is given.

Table C 4

Normality Tests Results of Crown Diameter in CIF in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,9179	0,856	0,9223	0,9325	0,9136	0,9462
P value	0,1361	0,0105	0,1839	0,2392	0,0994	0,369
Passed normality test (alpha=0,05)?	Yes	No	Yes	Yes	Yes	Yes
P value summary	ns	*	ns	ns	ns	ns
Kolmogorov-Smirnov test						
KS distance	0,2604	0,2681	0,2332	0,1539	0,2106	0,1719
P value	0,0033	0,0013	0,02	>0,1000	0,0338	>0,1000
Passed normality test (alpha=0,05)?	No	No	No	Yes	No	Yes

P value summary	**	**	*	ns	*	ns
Number of values	17	18	16	17	18	18

Note. A summary of the two normality tests results is given.

Table C 5

Statistics of Number of Leaves in Week 5

Modality	Sun_Control			Sun_N+			White_Control		
Cultivar	Mean	SD	N	Mean	SD	N	Mean	SD	N
GAR	7,846	1,345	13	7,800	2,210	15	7,222	0,878	18
CHA	7,882	0,857	17	7,778	0,808	18	6,833	0,985	18
CIF	7,235	1,147	17	7,000	0,767	18	7,000	1,033	16

Modality	White_N+			Blue_Control			Blue_N+		
Cultivar	Mean	SD	N	Mean	SD	N	Mean	SD	N
GAR	7,111	0,963	18	6,765	0,664	17	7,167	0,985	18
CHA	7,222	1,114	18	6,167	1,465	18	6,556	0,705	18
CIF	7,824	0,636	17	7,222	1,166	18	7,056	0,998	18

Note. A summary of the means, the standard deviation (SD), and sample size (N) are demonstrated.

Table C 6

Normality Tests Results of Number of Leaves in GAR in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,9176	0,6732	0,8786	0,9145	0,7921	0,9136
P value	0,2327	0,0001	0,0247	0,1035	0,0016	0,0994
Passed normality test (alpha=0,05)?	Yes	No	No	Yes	No	Yes
P value summary	ns	***	*	ns	**	ns

Kolmogorov-Smirnov test						
KS distance	0,197	0,264	0,211	0,2319	0,2855	0,2106
P value	>0,1000	0,006	0,0332	0,0115	0,0007	0,0338
Passed normality test (alpha=0,05)?	Yes	No	No	No	No	No
P value summary	ns	**	*	*	***	*
Number of values	13	15	18	18	17	18

Note. A summary of the two normality tests results is given.

Table C 7

Normality Tests Results of Number of Leaves in CHA in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,8716	0,8691	0,8728	0,9241	0,9185	0,8384
P value	0,0233	0,0172	0,0198	0,1527	0,1214	0,0056
Passed normality test (alpha=0,05)?	No	No	No	Yes	Yes	No
P value summary	*	*	*	ns	ns	**
Kolmogorov-Smirnov test						
KS distance	0,2604	0,275	0,2338	0,2018	0,1737	0,2914
P value	0,0033	0,0009	0,0103	0,0508	>0,1000	0,0003
Passed normality test (alpha=0,05)?	No	No	No	Yes	Yes	No
P value summary	**	***	*	ns	ns	***
Number of values	17	18	18	18	18	18

Note. A summary of the two normality tests results is given.

Table C 8

Normality Tests Results of Number of Leaves in CIF in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,9156	0,8183	0,932	0,7848	0,8441	0,8499
P value	0,1245	0,0028	0,2619	0,0013	0,0069	0,0084
Passed normality test (alpha=0,05)?	Yes	No	Yes	No	No	No
P value summary	ns	**	ns	**	**	**
Kolmogorov-Smirnov test						
KS distance	0,2283	0,2222	0,1875	0,3152	0,3032	0,2444
P value	0,0188	0,0191	>0,1000	<0,0001	0,0001	0,0057
Passed normality test (alpha=0,05)?	No	No	Yes	No	No	No
P value summary	*	*	ns	****	***	**
Number of values	17	18	16	17	18	18

Note. A summary of the two normality tests results is given.

Table C 9

Statistics of Height in Week 5

Modality	Sun_Control			Sun_N+			White_Control		
Cultivar	Mean	SD	N	Mean	SD	N	Mean	SD	N
GAR	12,885	1,907	13	12,333	1,665	15	16,861	1,781	18
CHA	14,118	1,935	17	12,889	1,819	18	17,000	2,607	18
CIF	14,271	2,160	17	12,161	2,410	18	13,188	3,049	16

Modality	White_N+			Blue_Control			Blue_N+		
Cultivar	Mean	SD	N	Mean	SD	N	Mean	SD	N
GAR	15,556	2,294	18	12,218	1,354	17	12,367	1,814	18
CHA	17,656	1,892	18	8,400	1,238	18	10,617	1,503	18
CIF	15,265	2,196	17	7,111	1,632	18	8,178	2,331	18

Note. A summary of the means, the standard deviation (SD), and sample size (N) are demonstrated.

Table C 10

Normality Tests Results of Height in GAR in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,9474	0,9736	0,9271	0,9648	0,9352	0,9402
P value	0,559	0,9079	0,1728	0,6954	0,2656	0,2917
Passed normality test (alpha=0,05)?	Yes	Yes	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns	ns	ns
Kolmogorov-Smirnov test						
KS distance	0,1343	0,1126	0,1978	0,1245	0,1726	0,1712
P value	>0,1000	>0,1000	0,061	>0,1000	>0,1000	>0,1000
Passed normality test (alpha=0,05)?	Yes	Yes	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns	ns	ns
Number of values	13	15	18	18	17	18

Note. A summary of the two normality tests results is given.

Table C 11

Normality Tests Results of Height in CHA in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,9181	0,9648	0,9385	0,9445	0,9808	0,9505
P value	0,137	0,6958	0,2726	0,3456	0,9579	0,4323
Passed normality test (alpha=0,05)?	Yes	Yes	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns	ns	ns
Kolmogorov-Smirnov test						
KS distance	0,1867	0,1402	0,162	0,1723	0,1025	0,1661
P value	>0,1000	>0,1000	>0,1000	>0,1000	>0,1000	>0,1000
Passed normality test (alpha=0,05)?	Yes	Yes	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns	ns	ns
Number of values	17	18	18	18	18	18

Note. A summary of the two normality tests results is given.

Table C 12

Normality Tests Results of Height in CIF in Week 5

Test for normal distribution	Sun & Control	Sun & N+	White & Control	White & N+	Blue & Control	Blue & N+
Shapiro-Wilk test						
W	0,976	0,9463	0,9777	0,9375	0,9311	0,8879
P value	0,9118	0,3699	0,9423	0,2898	0,2029	0,0355

Passed normality test (alpha=0,05)?	Yes	Yes	Yes	Yes	Yes	No
P value summary	ns	ns	ns	ns	ns	*
Kolmogorov-Smirnov test						
KS distance	0,1336	0,1552	0,1114	0,1605	0,2015	0,1673
P value	>0,1000	>0,1000	>0,1000	>0,1000	0,0516	>0,1000
Passed normality test (alpha=0,05)?	Yes	Yes	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns	ns	ns
Number of values	17	18	16	17	18	18

Note. A summary of the two normality tests results is given.

Table C 13

The Krudkal-Wallis Test Results of Plant Growth Data from Week 5

	Cultivars		
Vegetative Propagation Parameters	GAR	CHA	CIF
Crown diameter	0,6245	1,7x10-6	5,4x10^-7
Number of leaves per plant	0,2078	5,4x10-6	0,08415
Height	2,1x10^-9	2,8x10^-16	1,4x10^-12

Note. The p-value of the Krudkal-Wallis test of each plant growth parameter in each cultivar is shown.

Appendix D. Principal Component Analysis (PCA)

Based on the vegetative propagation parameters and growth parameters (as listed in Table D1) of strawberry plants measured in the 5th week of the experiment, a principal component analysis (PCA) was conducted. The loadings plot is given in Figure D1. The PCA plots are illustrated in Figure D2.

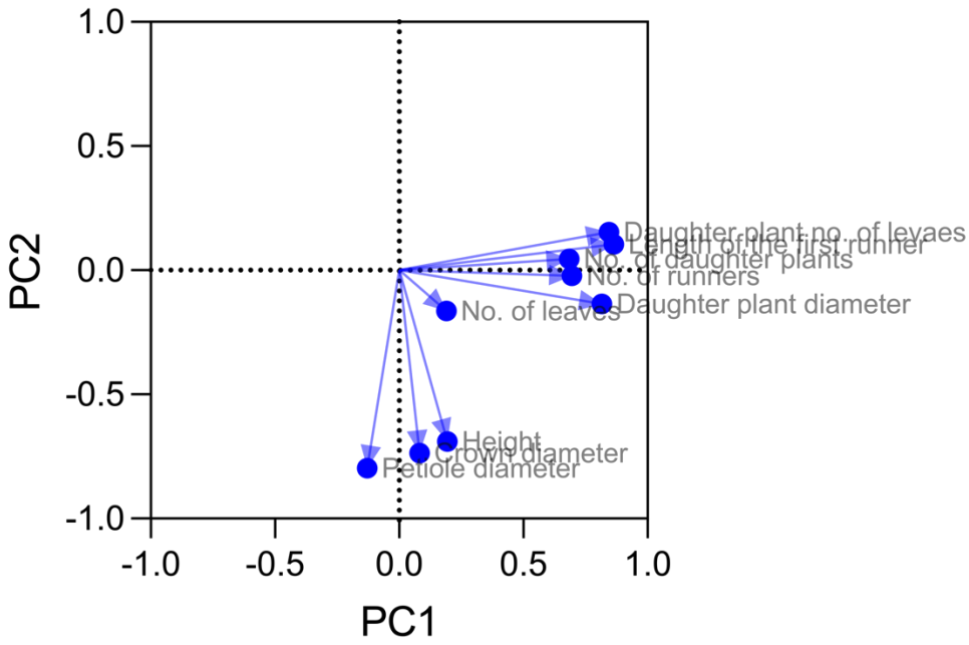
Table D 1

Vegetative Propagation Parameters	Plant Growth Parameters
Length of the first runner	Crown diameter
Number of runners per plant	Number of leaves per plant
Number of daughter plants per plant	Height
Number of leaves of the first daughter plant	Petiole diameter of the longest leaf
Crown diameter of the first daugther plant	

Note. An overview of the parameters used in the PCA.

Figure D 1

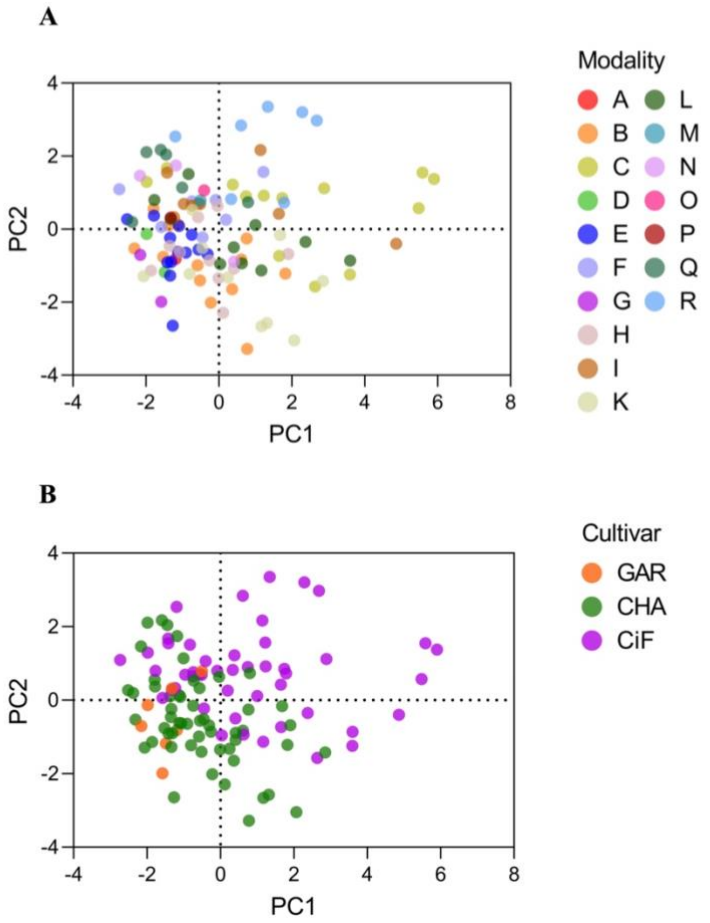
The Loading Plot of Nine Parameters Measured in Week 5

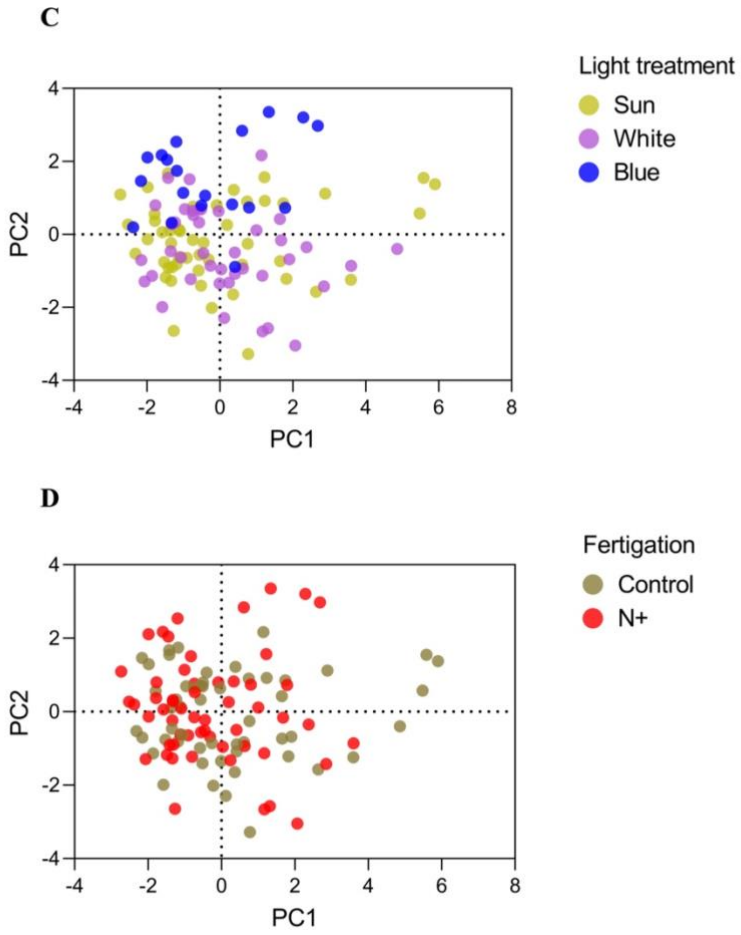


Note. The loading plot. The ‘Daughter plant diameter’ means the crown diameter of the first daughter plant on the first runner of each mother plant. Likewise, the ‘Daughter plant no. of leaves’ indicates the number of leaves of the first daughter plant in each mother plant. The number of runners per plant is represented by ‘No. of runners.’ Meanwhile, the number of leaves per plant is given as ‘No. of leaves.’ The petiole diameter of the longest leaf in each plant is represented by ‘petiole diameter.’

Figure D 2

The PCA Plots of the Strawberry Plants Under Six Environmental Conditions in Week 5





Note. The PCA plots of the 18 modalities are demonstrated. (A) The 18 modalities are illustrated in different colors. (B) The three cultivars are shown in three different colors. (C) The strawberry plants grown under different light resources (e.g. the sun light LED, the white light LED, and the blue light LED) are noted in three distinct colors. (D) The strawberry plants are demonstrated in different colors according to the nitrogen levels of the fertigations.

Appendix E. Recovery of Daughter Plants After Harvesting

The number of days required for daughter plants to recover from being separated from runners was recorded for each daughter plants. The recovery percentage was calculated and shown in Figure 4. The number of total daughter plants and recovered daughter plants on each day during the 10 days recovery period are given in Table E1.

Table E 1

Daughter Plant Recovery

	GAR			CHA			CIF		
Days	RD	TD	RP(%)	RD	TD	RP(%)	RD	TD	RP(%)
0	0	15	0	0	70	0	0	83	0
1	0	15	0	0	70	0	0	83	0
2	0	15	0	2	70	2,86	0	83	0
3	0	15	0	2	70	2,86	1	83	1,20
7	0	15	0	15	70	21,43	14	83	16,87
8	0	15	0	17	70	24,29	25	83	30,12
9	0	15	0	25	70	35,71	38	83	45,78
10	0	15	0	42	70	60,00	49	83	59,04

Note. The daughter plant recovery during the 10 days after harvesting is shown. The number of recovered daughter plants (RD), the number of total daughter plants (TD), and the recovery percentage (RP) are listed.

Appendix F. Initial Morphological Stages of the Strawberry Plants in the Prototype

After building the prototype, we chose 10 mother plants from the previous experiment and placed them in the prototype for further examination. The morphological stages of the mother plants are listed in Table F1.

Table F 1

Initial Morphological Stages of the Mother Plants in the Prototype

Position	Cultivar	History Information			Current Morphology		
		Light Source	Fertigation	No. of Daughter Plants	No. of Leaves	No. of Runners	No. of Daughter Plants
1	CHA	*	Germination	0	5	0	0
2	CHA	*	Germination	0	5	0	0
3	CHA	*	Germination	0	5	0	0
4	CHA	*	Germination	0	5	0	0
5	CHA	Sun light	Control	2	4	1	0
6	CHA	Sun light	Control	1	4	0	0
7	CHA	Sun light	Control	1	3	0	0
8	CHA	Sun light	Control	1	4	1	1
9	CHA	Sun light	Control	3	4	0	0
10	CHA	Sun light	Control	3	3	0	0

Note. The history information and current morphology of the 10 mother plants are listed. The mother plants at position 1 to 4 were irrigated with germination solution, the rest were irrigated with the control fertigation solution, which is the one with 8 mM nitrogen. The number of daughter plants, number of leaves, and the number of runners in each mother plant are given. *The light source of strawberry plants in position 1 to 4 had changed from sun light LED to no light (only the light of the surroundings).

Appendix G. The Estimated Cost of the Daughter Plant Production in the Prototype

The costs of the daughter plant production in the prototype were estimated based on the vegetative propagation results in the experiment. Here we assume that the wooden prototype can last for two years. Also, the mother plants can continue producing daughter plants for one year. In total, 10 mother plants can be placed in the prototype with three LED light bars, and approximately 124 daughter plants can be produced from the one-layer prototype. The costs of the daughter plant production are listed in Table G1.

Table G 1

Estimated Costs of the Daughter Plant Production in the Prototype

	€ Per Year	€ Per Daughter Plant
Total costs	105,77	0,85
Plant material	20	0,16
Prototype building	36,11	0,29
Environmental conditions	49,66	0,40
• light	33,02	0,27
• Air conditioning	16,51	0,13
• Fertilizer	0,06	0,00

Note. An overview of the daughter plant production in the prototype is shown. The total costs consist of three parts, including the plant material, prototype building, and environmental conditions. The costs of light, air conditioning, and fertilizer during the vegetative propagation process were calculated and added up as the costs of environmental conditions.