Article

Nursery Propagation Systems for High-Quality Strawberry (*Fragaria × ananassa* Duch.) Plug Plant Production from Micropropagated, Soilless-Grown Mother Plants

Valentina Morresi [1,2], Franco Capocasa [1], Francesca Balducci [2], Jacopo Diamanti [2] and Bruno Mezzetti [1,*]

[1] Department of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche (UNIVPM), Via Brecce Bianche 10, 60131 Ancona, Italy; valentina.morresi@salvi.it (V.M.); f.capocasa@staff.univpm.it (F.C.)

[2] PROLAB, Società Agricola SALVI VIVAI s.s., Via Bologna 714, 44124 Ferrara, Italy; francesca.balducci@salvi.it (F.B.); jacopo.diamanti@salvi.it (J.D.)

* Correspondence: b.mezzetti@staff.univpm.it; Tel.: +390712204933

Abstract

The commercial propagation of strawberries is increasingly constrained by the incidence of both established and emerging soilborne pathogens, particularly under soil cultivation systems. Micropropagation represents an effective strategy to ensure the production of virus-free, true-to-type mother plants suitable for high-efficiency propagation. In this study, micropropagated mother plants of four short-day cultivars ('Francesca', 'Silvia', 'Lauretta', and 'Dina') and one ever-bearing advanced selection ('AN12,13,58') were cultivated under a controlled soilless system. Quantitative parameters including number of runners per plant, runner length, and number of tips per runner and per plant were assessed to evaluate propagation performance. Micropropagated mother plants exhibited a significantly higher stoloniferous potential compared to in vivo-derived mother plants (frigo plants type A), with the latter producing approximately 50% fewer propagules. Rooted tips of 'Dina' were further assessed under different fertigation regimes. The NPK 20–20–20 nutrient solution enhanced photosynthetic activity and shoot and root biomass (length, diameter, and volume via WinRHIZO analysis). These results confirm the suitability of micropropagated mother plants grown in soilless conditions for efficient, high-quality clonal propagation and support the integration of such systems into certified nursery production schemes.

Keywords: *Fragaria x ananassa*; micropropagation; mother plant; soilless cultivation; nursery production

check for updates

Academic Editors: Adrián Rodríguez-Burruezo and Radu E. Sestras

Received: 28 May 2025
Revised: 18 July 2025
Accepted: 24 July 2025
Published: 1 August 2025

Citation: Morresi, V.; Capocasa, F.; Balducci, F.; Diamanti, J.; Mezzetti, B. Nursery Propagation Systems for High-Quality Strawberry (*Fragaria × ananassa* Duch.) Plug Plant Production from Micropropagated, Soilless-Grown Mother Plants. *Horticulturae* **2025**, *11*, 888. https://doi.org/10.3390/horticulturae11080888

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Strawberry plant production is strongly driven by consumer demand for fresh, high-quality products sourced from organic systems or through agricultural practices with low environmental impact and short distribution channels [1]. According to FAO statistics, in 2023, global strawberry cultivation covered 434,977 hectares, yielding 10,485,454 tons of fruit. This expansion is largely driven by intensive breeding programs, using wide germplasm to develop genotypes adapted to various climates and cultivation systems [2]. Commercial strawberry varieties are vegetatively propagated. The growing fruit market has encouraged nurseries' investments in diverse types of clonally propagated plants, such as frigo-plants (type A-, A, waiting bed and tray plants) and fresh plants (vegetative, bare root, or rooted tips) [3,4]. These types, along with everbearing cultivars, have facilitated

out-of-season production, including autumn harvests and soil or soilless programmed cultivation [5,6]. The success and sustainability of strawberry orchards increasingly depend on the phytosanitary and phenological quality of these certified nursery plants of all newly released cultivars. Currently, nursery vegetative propagation is challenged by soilborne pathogens (e.g., *Anthracnose*, *Phytophthora fragariae*, *Colletotrichum* spp., *Neopestalotiopsis*, and *Plectosphaerella*) [7–9], especially following the ban on methyl bromide for soil disinfection [7,9]. Soilless cultivation of mother plants, for tray plants and rooted-tip, offers a promising solution. These are different from frigo and fresh in vivo propagated plants because they can be produced by using mother plants cultivated in soilless systems, and such controlled conditions contribute to reducing the risk of soil pathogen contamination and to increase the quality and homogeneity of the plants. To meet evolving market demands, further to this, a growing need for innovative solutions remains [10]. In fact, this demand for plant of high phytosanitary quality has even justified the launch of important projects aimed at developing seed propagation of strawberries through F1 hybrids between partially pure lines [11]. There are some expectations for these newly possible seed propagation systems; however, most of the strawberry industry is based on the use of clonally propagated plants. This method will continue to dominate the future of the market, simply because of the high stoloniferous capacity of the plants, combined with its plasticity in flower differentiation.

This shift towards high phytosanitary quality has also sparked research into strawberry micropropagation, which is widely recognized for producing virus-free plants [11,12], as required by certification protocols [13]. In Europe, the use of in vitro-propagated strawberry plants is restricted by regulations. According to Italy's Legislative Decree No. 18 (2 February 2021), aligned with EU Regulations 2016/2031 and 2017/625, farmers are not permitted to transplant in vitro-propagated strawberry plants directly into production fields. These plants must undergo limited micropropagation cycles followed by rooting and acclimatization. Afterward, they must be grown for three successive cultivation cycles in phytosanitary-protected environments to ensure genetic fidelity and high-quality standards [13]. Its use in later nursery stages, has been limited due to concerns about somaclonal variation, primarily caused by excessive cytokinin concentrations and repeated subculturing during in vitro stages [9]. Studies have shown that reducing cytokinin levels and limiting the number of subcultures significantly decreases the risk of phenotypic and genotypic variations [14]. As a result, many laboratories worldwide now utilize micropropagation to produce both mother plants and, in some cases, plants destined for direct cultivation [15,16]. Clearly, micropropagation cannot totally replace other in vivo plant propagation systems, but it must be considered as integrative to them.

Building on this foundation, the present study aims to present a more solid approach for extending the use of micropropagation for the production of large numbers of in vivo clean mother plants used in a soilless system that allow to propagate a very high number of tips in a shorter period. This possible new production scheme was already described in [17] for an open field and a soilless cultivation system. Here, we aimed to evaluate the stoloniferous efficiency of mother plants micropropagated from different strawberry genotypes ('AN12,13,58', 'Dina', 'Francesca', 'Lauretta' and 'Silvia'), as well as to assess the agronomical qualitative traits of the rooted tips derived from 'Dina'. The results demonstrate that micropropagated mother plants significantly increase the production of runners with high-quality tips, which were much higher compared to runners produced by in vivo propagated type A mother plants ('AN12,13,58'). A complementary experiment allowed us to explore how the propagation system not only increases the number of tips produced but also their subsequent development and potential field performance. To monitor commercial quality, the vegetative growth response of 'Dina' tip plants was tracked throughout the

entire rooting and plant growth phases. Varying levels of nutrition influenced the morpho-physiological traits of the rooted tips, highlighting another important factor in enhancing the quality of the newly propagated plants. This system constrains the nursery schedule time and grants the production of plants with a high roots quality that could facilitate farmers' transplant operations [17]. Together, these evaluations provide a comprehensive insight into the efficiency and quality outcomes of the strawberry propagation process.

2. Materials and Methods

2.1. Strawberry Mother Plant: Cultivars and Selection

The efficiency of micropropagated strawberry mother plants for nursery production of tips plants in soilless conditions was studied by measuring the stoloniferous capacity of the following strawberry genotypes derived from the breeding programs of Marche Polytechnic University: 'Francesca', 'Lauretta', 'Silvia' and 'Dina', short-days varieties, and 'AN12,13,58', an everbearing advanced selection [18], for which it was necessary to initiate rapid multiplication of the propagation material to support their commercial spread in the cultivation areas, mainly in Italy and Central Europe. For this last selection, the stoloniferous capacity of micropropagated mother plants was compared with the ones of in vivo propagated frigo mother plants (type A). Finally, the qualitative response to different fertilization protocols of the runner tips' rooting was studied for tips harvested from micropropagated mother plants of 'Dina', a low chilling everbearing. Its market is oriented towards the Southern Mediterranean areas, where it could be shipped as a rooted fresh plant for autumn cultivation in a protect field or soilless cultivation system.

2.2. Production of Micropropagated and In Vivo Mother Plants

In June 2021, apical and lateral buds of the cultivars 'Francesca', 'Lauretta', 'Silvia', 'Dina', and the advanced selections 'AN12,13,58' were collected from the experimental field "Pasquale-Rosati" of Marche Polytechnic University for in vitro culture establishment. Explants were surface-sterilized via immersion in a 10% (v/v) sodium hypochlorite (NaOCl) solution under constant agitation (100 rpm) for 15 min [19]. Subsequently, the explants were transferred under a sterile hood and washed three times (1, 5, and 10 min) with distilled water, then placed in tubes containing in vitro medium composed of Murashige and Skoog (MS) basal salts (Duchefa Biochemie®, Haarlem, The Netherlands), which included macronutrients (NH_4NO_3 20.61 mM, KNO_3 18.79 mM, $CaCl_2{\cdot}2H_2O$ 2.9 mM, $MgSO_4{\cdot}7H_2O$ 1.5 mM, KH_2PO_4 1.25 mM), micronutrients (H_3BO_3 100.27 µM, $MnSO_4{\cdot}H_2O$ 100.00 µM, $ZnSO_4{\cdot}7H_2O$ 29.91 µM, KI 5.00 µM, $Na_2MoO_4{\cdot}2H_2O$ 1.03 µM, $CuSO_4{\cdot}5H_2O$ 0.10 µM, $CoCl_2{\cdot}6H_2O$ 0.11 µM), iron (FeNaEDTA 100.00 µM), and vitamins (thiamine-HCl 0.30 µM, pyridoxine-HCl 2.43 µM, nicotinic acid 4.06 µM, myo-inositol 554.94 µM, glycine 26.64 µM). The medium was supplemented with 30 g/L of sucrose and solidified with 7 g/L of commercial agar. The pH was adjusted to 5.7 before autoclaving. After two weeks, shoots that developed without contamination were transplanted on the same medium supplemented with 0.25 mg/L of the cytokinin 6-benzylaminopurine (BAP) to introduce lateral shoot proliferation. New, sterile explants were proliferated in tubes for 2 subcultures to better control the full sterile conditions, then they were transferred in larger glass jars to increase space for better and larger fruit proliferation. Tube and glass jar containing the explants were maintained in a growth chamber with constant temperature (±24 °C) and a 16 h photoperiod. Every three weeks, proliferating shoots were sub-cultured in MS media with the same amount of cytokinin added (0.25 mg/L) for a maximum of eight subcultures. This decision was driven by the aim to establish a stable and efficient multiplication rate, promoting shoot proliferation and reliable morphogenetic responses. According to standard micropropagation protocols, repeated subculturing over

several cycles allows for the stabilization of growth patterns and minimizes physiological or developmental anomalies that may occur in earlier stages. Moreover, maintaining the number of subcultures below ten is widely regarded as a precautionary measure to preserve genetic fidelity during clonal propagation, especially when callus formation is avoided in favor of organized shoot development. Strawberry plantlets were introduced in MS media without hormones to induce plant rooting and elongation. In February, March, and April 2022, the in vitro rooted strawberry plants were planted in alveolar containers with a substrate composed of 23.0% v/v of acid blond peat (Kekkilä®, Kekkilä Oy, Vantaa, Finland) 23.0% v/v of perlite (Gyproc®, Saint-Gobain, Courbevoie, France), 23.0% v/v of medium vermiculite (Agrical®, Yara International ASA, Oslo, Norway), and 31.0 v/v of water. The acclimatization phase was conducted in a tunnel with a temperature of 24–26 °C and a high humidity level of 80–85%. For 3–4 days after, the plantation tunnels remained closed with continuous nebulization. From the seventh to tenth days, the tunnels were gradually opened during the central hours of the day. After this stage, the strawberry plants were considered acclimatized because they had new, in vivo-formed leaves with waxes and a reactivated closure mechanism of the stomata, as well as new, hairy roots. Data on the response of the different genotypes to the proliferation, rooting, and acclimatization phases of the micropropagation cycle were not collected because the primary focus was to evaluate the propagation performance of the mother plants under in vivo conditions, allowing us to concentrate here on nursery performance for practical applications.

The in vivo propagated mother plants (category A) of the selection 'AN12,13,58' were obtained from the mother plants cultivated in the same experimental field and planted in July 2021. The stolons were harvested in January 2022 and stored as frigo-plants (at −1 °C) until planting time in the nursery together with the micropropagated plants.

2.3. Soilless Cultivation of the Mother Plants

Micropropagated and in vivo propagated mother plants were transferred in a soilless cultivation system, prepared at the Innesti Leopardi medium–tech indoor nursery (Osimo, Ancona, Italy; 43.47289°, 13.46198°) from April to September 2022. The soilless system allows for the cultivation of strawberry plants in Geotec1® (distributed by Agrochimica, Bolzano, Italy) growing bags (1.0 × 0.22 × 0.10 m) filled with coconut fiber medium extra coarse 5–29 and coconut crush fraction 10–30 mm, with average structure and pH of 5.5–6.5 fixed on a metal holder placed on the nursery bench. The holders of the bags were raised more than 2 m from the ground to promote the development in length of the stoloniferous chains, without touching the ground, to avoid risk of microbial contamination and guarantee the highest phytosanitary quality of the tips. It was used at a plantation density of 8 plants per bag, with bags 1 m long. This operation was conducted on the 13th of April for the cultivars 'Francesca', 'Lauretta', 'Silvia', and 'AN12,13,58'. The cultivar 'Dina' was transplanted on two different dates (13th of April and 5th of May); as this cultivar is more adapted to southern conditions, we considered it important to test the mother plants' stoloniferous activity in a later transplanting period. The fertilization of the soilless growing mother plant was accomplished using a micro-irrigation system (4 drippers per bag). After the transplanting, for 60 days, 5 cycles of fertigation lasting 10 min were planned. When the temperature had risen in June, the frequency of irrigation increased from 5 to 6, and it proceeded for 15 min. According to [20], for better runner growing efficiency, the nutritive solution of the mother plants was composed of an NPK ratio of 20–8–20. In the first vegetative phase, as for the nursery's farmers, 0.5 g/L of fertilizer was used (Orvital® Plant-Prod (Master Plant-Prod Inc., Brampton, ON, Canada)), containing the microelements needed to fulfill the nutritional requirements of the plants (magnesium (Mg) 0.15%, boron (B) 0.02%, chelated copper (Cu) 0.05%, chelated iron (Fe) 0.10%, chelated manganese (Mn)

0.05%, molybdenum (Mo) 0.015%, chelated zinc (Zn) 0.05%, EDTA 1.24%). After the fourth runners' emission, they were moved to a double concentration of fertilizer (1 g/L). The ECs were measured (1300 mS dm^{-1}), and the pH was adjusted (6.5). The greenhouse was equipped with an air conditioning system for controlling temperature, sunshine, and ventilation. From April to September, only the ventilation and the shading system with dark nets were activated. The length of day hours for the requested photoperiod (16 h) was naturally granted without the need for artificial illumination. The automatic system of ventilation and shading was set with sensors; opening and closing of lateral greenhouse barriers was ensured with temperatures higher than 20–25 °C. This system contributed to maintaining ambient humidity within the 60–70% range, reducing the risk of reaching humidity levels that can promote the development of fungal diseases. The greenhouse management was set to ensure the best climatic conditions to promote faster morpho-physiological development to enhance stolon emission and runner tip production in the mother plants.

2.4. Nursery Tips Production of cv 'Dina'

As 'Dina' was intended for shipment to Southern regions for soilless transplanting by September, we evaluated different nursery fertigation protocols to accelerate runner tip rooting and production.

For ensuring the highest transplanting performance of the fresh plant, we observed the qualitative response of the runner tips of 'Dina'.

Similar to refs. [20,21], we harvested the runners when their tips had initial roots (at least 12.7 mm) and almost two leaves with a surface of 2 × 4 mm^2. We selected medium, homogeneous tips with no necrotic leaves with the objective of obtaining major uniformity in the container in the following cultivation steps. The runner tips were rooted in 60-hole plastic pots filled with acid watered substrate with blond peat (Kekkilä®, Kekkilä Oy, Vantaa, Finland) and medium vermiculite (Agrical®, Yara International ASA, Oslo, Norway). The cellular turgor of the epigeal apparatus and the faster rooting process of the runner tips were provided by a sprinkler micro-irrigation. This irrigation system had been adapting for 10 days after transplanting. Thereafter, irrigation with the bar was sufficient until the development of plants ready for commercialization.

To observe the vegetative response of the 'Dina' tips, during the post-transplant acclimatization phase on the substrate, the rooted plug plants were subjected to various fertilization tests. Agroleaf power® (Everris, The Netherlands) fertilizers were used. We distinguished them based on their three different NPK macro-element ratios (NPK 12–52–5, NPK 15–31–10, and NPK 20–20–20) (Table 1). We prepared three different nutrient solutions by applying 1 g/L of water. The control solution referred to the irrigation water of the nursery. Immersion fertilization was used. The 60-hole plastic pots were submerged for 5–6 min until reaching the presumed field water capacity. This operation was repeated three times a week for a month (from 5th September to 3rd October).

Table 1. Electrical conductivity (EC mS dm^{-1}) and pH of the fertilizer solution measured with the conductivity meter Gro Line® EC meter (Hanna Instruments Inc., Woonsocket, RI, USA). The parameters registered for the control solution (C) were the ones of the irrigation water. The 3 fertilizer solutions (Sol_1: NPK 12–52–5; Sol_2: NPK 15–31–10; Sol_3: NPK 20–20–20) were applied at a concentration of 1 g/L. Because of their distinct mineral composition, they registered different EC and pH values.

SAMPLE	Concentration (g/L)	EC (mS dm^{-1})	pH
C: CONTROL	0 g/L	0.65	7.50
Sol_1: NPK 12-52-5	1 g/L	1.26	5.72

Table 1. *Cont.*

SAMPLE	Concentration (g/L)	EC (mS dm^{-1})	pH
Sol_2: NPK 15-31-10	1 g/L	1.39	6.27
Sol_3: NPK 20-20-20	1 g/L	0.99	6.14

2.5. Data Collected to Identify the Stoloniferous Efficiency of the Different Mother Plants

With the aim to evaluate the quantitative stoloniferous efficiency of different micropropagated and in vivo strawberry genotypes, data were collected at 20, 40, and 60 days after transplanting. The parameters measured included the number of runners per plant, the length (cm) of two runners per plant, the number of tips produced by these two runners, and the total number of tips per plant. To this end, the use of these specific informative indicators permitted us to evaluate not only the plants' reproductive potential but also their meristematic activity and juvenility due to their in vitro propagation.

2.6. Qualitative Response of 'Dina' Nursery Tip Production

To evaluate the growth and development of the 'Dina' tips, several morphological and physiological parameters were recorded. These included the fresh and dry weight of leaves and petioles (g) as indicators of vegetative development and metabolic biomass. The leaf color of the oldest and youngest leaves of the tips was measured using the Konica Minolta Colorimeter (Konica Minolta, Sensing Inc., Osaka, Japan), which provides three parameters: lightness (L*), chroma (C*), and hue angle (h°). L* values range from 0 (black) to 100 (white); C* represents chroma or color intensity; h° is the hue angle, expressed in degrees, which defines the type of color (e.g., 0° = red, 90° = yellow, 180° = green, 270° = blue). The statistic data were studied considering three Minolta shutter clicks/samples.

The root characteristics of length (cm), diameter (mm), and volume (mm^3) were analyzed using WinRHIZO software, Version 2020b (Regent Instruments Inc., Quebec City, QC, Canada) after scanning. We detached these parameters to determine the plant's stability after transplanting. The fresh weights of the leaves and roots were measured with a precision analytical balance, while the dry weights were recorded after drying the samples in a forced-air oven at 70 ± 2 °C for 72 h. Each treatment was evaluated based on a total of six samples.

2.7. Statistical Analysis

The different responses of micropropagated and in vivo mother plants were analyzed by setting up a completely randomized experimental trial, with four replications per genotype, each consisting of eight plants per bag but sampling only the four central mother plants to avoid border effects (16 samples). The treatment positions in the greenhouse environment were randomized to minimize spatial bias. We counted the number of runners and tips per plant. Runner length was assessed by measuring two runners per mother plant, and for both, we counted the number of tips.

The quantitative response of the 'Dina' plug plant was evaluated across six samples for each of the four fertilization solutions during the five surveys. Rooted tips with active shoots and one new leaf were selected and analyzed.

STATISTICA software (Version 7.0, Statsoft Inc., Tulsa, OK, USA) was used for one-way ANOVA and PCA. Comparison of inter-genotypes and plant structural differences were considered statistically significant at $p < 0.05$ according to an LSD test. Given the limited number of treatments and our focus on subtle genotypic and micropropagation-related differences, the LSD test was chosen over more conservative post hoc methods such as Tukey's HSD to enhance sensitivity in detecting treatment differences, reinforcing the reliability of our comparisons. Moreover, a Principal Component Analysis (PCA)

was performed using MATLAB (ver. R2022a, MathWorks, Natick, MA, USA) and in-house functions based on existing algorithms to investigate the overall variability among genotypes. STATISTICA software (Version 7.0, Statsoft Inc., Tulsa, OK, USA) was also used to generate 3D scatter plots as an exploratory tool to support the interpretation of the principal component analysis. The trends observed in the graph were consistent with the PCA results.

3. Results and Discussion

3.1. Comparing Stoloniferous Capacity of In Vivo and Micropropagated Mother Plants of the Everbearing 'AN12,13,58' Breeding Selection

The micropropagated mother plants of the selection 'AN12,13,58' showed a 70% increase in stoloniferous capacity (Table 2). In fact, before harvesting, the in vitro mother plants had 13.42 ± 6.19 runners per plant compared to 4.06 ± 1.24 for the in vivo ones. This corresponded to a large difference in the number of tips per runner (micropropagated 4.26 ± 0.51 was twice the cold-stored 2.56 ± 1.15) and in the total tips harvested from the micropropagated mother plants (34.75 ± 12.38 tips per plant) in comparison with the cold-stored mother plants (10.24 ± 1.68 tips per plant) (Table 2–Figure 1). However, the high standard deviation observed in the number of runner tips produced by the micropropagated mother plants can be attributed to the intrinsic variability in the stoloniferous behavior of this genotype. This heterogeneity is likely influenced by both physiological factors linked to the stage of acclimatization and environmental microvariations within the cultivation system. Additionally, the asynchronous development of tips and the competition for assimilates between vegetative growth and tip formation in everbearing types may have further contributed to the observed dispersion of the data.

Table 2. Number of runners per plant, number of tips per plant, number of tips per two runners per plant, and length of two runners per plant of selection 'AN12,13,58' on three different dates from transplanting 20, 40, and 60 days (T20, T40, and T60) of in vivo and in vitro mother plants. Mean ± standard error, LSD test $p < 0.05$.

Parameters	**In Vivo Mother Plant**			**In Vitro Mother Plant**		
Days from Transplanting	**T20**	**T40**	**T60**	**T20**	**T40**	**T60**
No. of runners p^{-1}	0.75 ± 0.77 b	2.75 ± 1.06 b	4.06 ± 1.24 b	5.00 ± 1.13 a	10.42 ± 3.49 a	13.42 ± 6.19 a
No. of tips per r^{-1}	0.69 ± 0.68 b	1.67 ± 0.84 b	2.56 ± 1.15 b	1.35 ± 0.22 a	3.21 ± 0.41 a	4.26 ± 0.51 a
No. of tips p^{-1}	1.00 ± 1.26 b	4.75 ± 2.24 b	10.24 ± 1.68 b	6.75 ± 1.84 a	30.58 ± 11.01 a	34.75 ± 12.38 a
Length of runner	6.19 ± 6.90 b	48.00 ± 18.36 b	98.88 ± 20.56 b	17.08 ± 4.40 a	64.5 ± 11.88 a	107.5 ± 13.60 a

The 'AN12,13,58' breeding selection is identified as an everbearing genotype, prompting immediate flower differentiation independent of the light hours. This trait can affect the stoloniferous capacity depending on the type of plant.

In vivo type A frigo plants developed both flower buds and runners, and although emerging inflorescences were removed, these plants produced a reduced number of runners. On the contrary, the micropropagated plants showed only a vegetative habit, corresponding to the development of a much larger number of runners. For this type of plant, the condition created by the micropropagation protocols contributed to generating higher vegetative development, of high benefit for the plant nursery production. In fact, with the proposed method, a very high number of commercial tips can be produced using significantly fewer micropropagated mother plants. As a result, less linear space is required for growing bags (Table 3). This contributes to a reduction in structural costs while still achieving a great

yield of high-quantity of plants. Moreover, the greenhouse environmental conditions and the growing daylight of the summer period have highlighted the vegetative response [22].

Figure 1. Representation of the significant difference in runner and tip production between micropropagated mother plants (+50%) compared to the control 'frigo' ones of the selection 'AN12,13,58' in a soilless cultivation system 60 days from transplanting. The image serves as an example of the productive response of 3 bags containing 8 mother plants per bag.

Table 3. Number of tips per plant grown in greenhouse soilless conditions per meter (at T60), mean ± standard error, LSD test $p < 0.05$, was used for the estimation of number of runner tips produced by 8 mother plants in a one-meter bag (RT m^{-1}). In addition, using this value, we calculated the number of mother plants needed and the linear meter required (1 bag 1 m long) for the production of 100,000 strawberry plug plants of the selection 'AN12,13,58'.

Prop. System	No. Tips at T_60	Estimated RT m^{-1}	Estimated No. of Mother Plants	Estimated No. Bags and Linear Meter (8 Plants b^{-1} of 1 m)
In vivo	10.24 ± 1.68 b	82	9766	1220
In vitro	34.75 ± 12.38 a	278	2878	359

What we observed remains in line with prior studies [23,24]. The selected cultivation system promoted stolon formation and vegetative growth and inhibited the development of flower buds, which is essential for elite propagation. These factors include long photoperiods, temperature, and the use of the BAP as plant growth regulators (PGRs) during the in vitro propagation of the mother plants.

3.2. *Stolonifer Capacity of Micropropagated Mother Plants of Different Genotypes*

Acclimatized micropropagated strawberry mother plants of the short-days cultivars, when transplanted in Geotec 1® (distributed by Agrochimica, Bolzano, Italy) bag substrate, immediately started to differentiate new stoloniferous chains, without any flowers. The first runner emission was observed two weeks later, and the emission of the highest number was detected after 12 weeks (corresponding to early June). This response was associated with increased temperatures and solar radiation, two important factors to promote plant vigor [20]. After 40 days, some genotypes already showed a different stoloniferous capacity (Table 4). 'AN12,13,58' and 'Dina' recorded 10.42 ± 0.41 and 6.72 ± 0.19 runners per plant, significantly higher than the number counted from the mother plants of the other examined cultivars. At this time, in particular, the everbearing selection differed for the highest production of runners and tips per runner. This difference was associated with the early emission of new short runners from the secondary buds of the main stoloniferous chains.

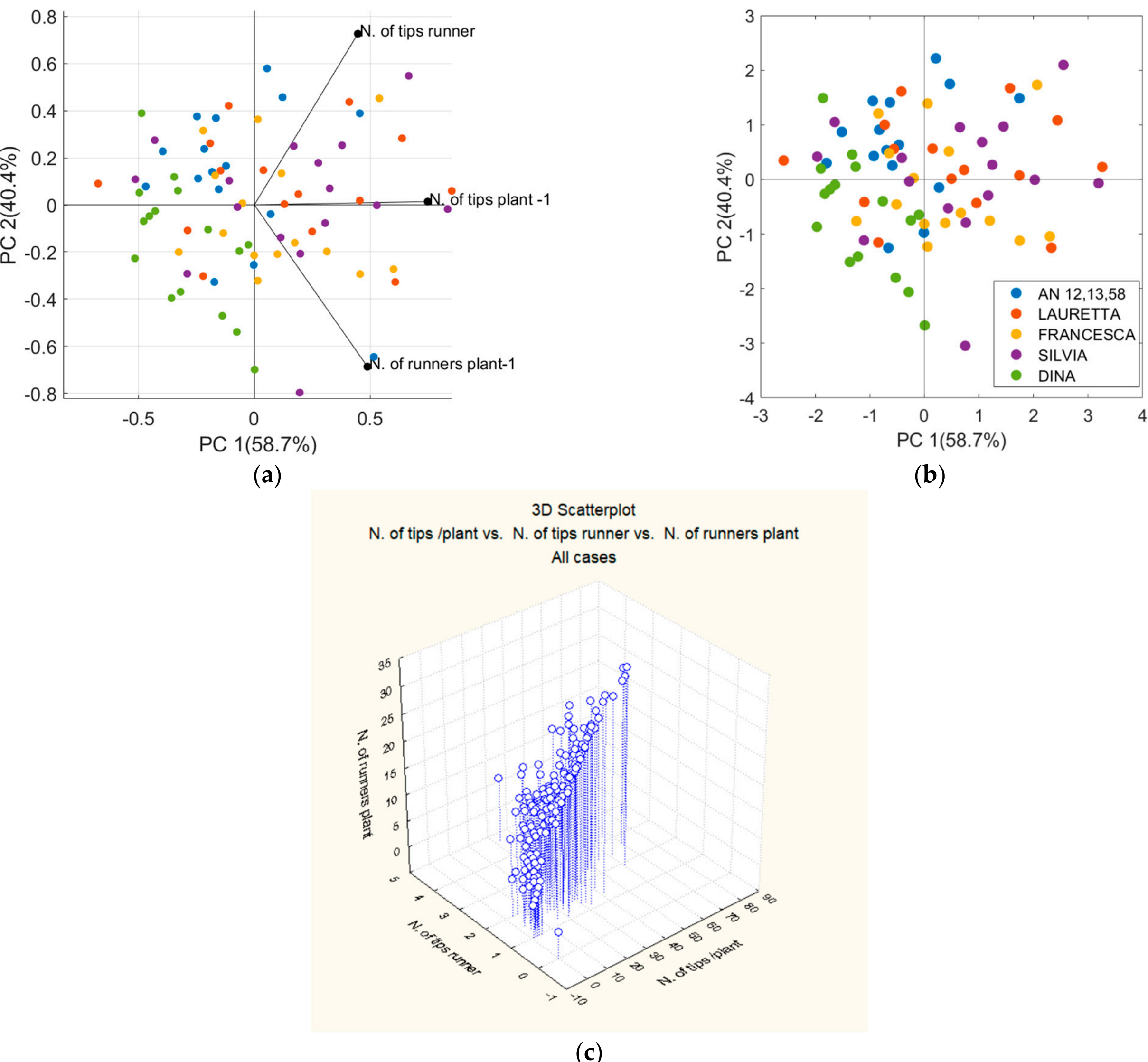
Meanwhile, after 60 days from transplanting, micropropagated mother plants of the short-day cultivars 'Francesca' and 'Silvia' produced a significantly higher number of runners per plant ('Francesca' 17.56 ± 1.33, 'Silvia' 17.25 ± 1.94) and tips per runner ('Francesca' 3.81 ± 1.33, 'Silvia' 4.06 ± 2.35), then followed by 'Lauretta', 'Dina' and 'AN12,13,58' (Table 4). In fact, at harvest, the highest number of runners, tips per runner, and per plant were counted for micropropagated mother plants of 'Silvia' and 'Francesca', followed by 'Lauretta' (Table 4). The different number of tips per runner was also related to the different length of the runners measured at the harvesting ('AN12,13,58' 107.5 ± 13.62 cm; 'Dina' 94.59 ± 26.16 cm; 'Francesca' 95.56 ± 19.02 cm; 'Lauretta' 88.08 ± 17.29 cm; 'Silvia' 138.28 ± 38.65 cm). This result confirms that the production of stoloniferous chains, as well as their length, is determined by the characteristics of the genotype, which, depending on the type of vegetative vigor, can influence the production of runners to a greater or lesser extent [4].

Table 4. Number of runners per plant, number of tips per plant, and number of tips per two runners per plant for five different strawberries genotypes on three different dates from transplanting, 20, 40, and 60 days (T20, T40, and T60), in a soilless cultivation system. Mean ± standard error, LSD test $p < 0.05$. ns = not significant.

Genotypes	No. of Runners Plant^{-1}			No. of Tips Per Runner^{-1}			No. of Tips Plant^{-1}		
	T20	T40	T60	T20	T40	T60	T20	T40	T60
'AN12,13,58'	5.00 ± 0.22 a	10.42 ± 0.41 a	13.4 ± 1.69 ns	1.35 ± 0.22 a	3.21 ± 0.41 a	4.03 ± 1.69 a	6.75 ± 1.84 a	30.58 ± 11.01 a	34.75 ± 12.38 ab
'Dina'	1.34 ± 0.21 c	6.72 ± 0.19 bc	15.16 ± 2.35 ns	0.91 ± 0.39 c	1.70 ± 0.70 b	3.50 ± 1.55 b	1.41 ± 0.91 d	12.97 ± 10.28 b	26.84 ± 6.98 b
'Francesca'	0.75 ± 0.58 c	2.63 ± 0.30 c	17.56 ± 1.33 ns	0.69 ± 0.48 d	1.29 ± 0.30 c	3.81 ± 1.33 a	0.75 ± 0.58 d	3.50 ± 1.79 b	43.81 ± 13.40°
'Lauretta'	2.44 ± 0.39 b	3.88 ± 0.70 bc	15.56 ± 1.55 ns	1.02 ± 0.08 bc	1.43 ± 0.25 bc	3.81 ± 1.94 a	2.50 ± 0.97 c	5.50 ± 1.75 b	45.06 ± 20.46°
'Silvia'	3.19 ± 0.08 b	4.94 ± 0.25 b	17.25 ± 1.94 ns	1.19 ± 0.21 ab	1.60 ± 0.19 bc	4.06 ± 2.35 a	3.88 ± 1.59 b	8.00 ± 2.22 b	47.19 ± 18.74°

To further investigate the relationships among genotypes based on stoloniferous traits—namely the number of runners per plant, number of tips per plant, and number of tips per runner—a Principal Component Analysis (PCA) was conducted (Figure 2). The biplot projection onto the first two principal components (explaining 58.7% and 240.4% of the total variance, respectively) showed a clear separation between the high-performing cultivars ('Francesca', 'Silvia', and 'Lauretta') and the less vigorous ones (e.g., 'Dina' and 'AN12,13,58') (Figure 2a). The spatial distribution of the genotypes confirmed that vegetative growth capacity was strongly influenced by genetic background. The short-day cultivars 'Silvia' and 'Francesca' clearly grouped apart from the others, supporting their potential suitability for high-efficiency runner production systems (Figure 2b). In addition, the 3D plot provided a complementary view of the data structure, confirming that a greater number of runners per plant was generally associated with a higher total number of tips. The clustering pattern observed in the 3D plot suggests the presence of morpho-agronomic profiles among genotypes, which are further explored through principal component analysis (Figure 2c). The total number of runner tips produced by the mother plants is important information for better planning the nursery operative work. The highest average number of tips produced per plant was achieved with eight plants per meter of growing bag, optimizing the space for qualified strawberry vegetative propagation. As an example, using data from the experimental trials, the number of tips produced per meter and number of mother plants and linear meters needed to produce 100,000 commercial tips was estimated depending on genotype (Table 5). With the 60-day cultivation of 8 mother plants per bag, 1 m in length, 'Silvia', 'Lauretta', and 'Francesca' went beyond the production of 300 runner tips per linear meter, followed by 'AN12,13,58' and 'Dina'. The highest number of tips

produced per plant has a beneficial effect for the nursery, reducing the number of mother plants, so even the linear meters of growing bags produced the same number of total runner tips (Table 5). This difference in reduction of plants, materials, and space can bring clear economic benefit to the nursery company.



(**c**)

Figure 2. Principal Component Analysis (PCA) of micropropagated mother plants. (**a**) Correlation circle showing the contribution of number of runners and tips per plant and number of tips per runner to PC1 and PC2. (**b**) PCA score plot of genotypes projected on the factor plane defined by PC1 and PC2. The 3D scatter plot (**c**) shows the distribution of all experimental cases based on three morphological parameters: number of runner tips per plant (*x*-axis), number of tips per runner (*y*-axis), and number of runners per plant (*z*-axis). This graphical representation highlights the overall variation among the observations and supports the PCA results by illustrating the correlation structure among the variables considered.

Table 5. Number of tips per plant grown in greenhouse soilless conditions per meter (at T60), mean ± standard error, LSD test $p < 0.05$, was used for the estimation of number of runner tips produced by eight mother plants in a one-meter bag (RT m^{-1}). In addition, using this value, the number of mother plants needed and the linear meter required (1 bag 1 m long) was calculated for the production of 100,000 strawberry plug plants of the selections 'AN12,13,58' and 'Dina', 'Francesca', 'Lauretta', and 'Silvia'.

Genotypes	No. of Tips at T_60	Estimated RT m^{-1}	Estimated No. of Mother Plant	Estimated No. Bags and Linear Meter (8 Plants b^{-1} of 1 m)
'AN12,13,58'	34.75 ± 12.38 ab	278	2878	359
'Dina'	26.84 ± 6.98 b	215	3726	465
'Francesca'	43.81 ± 13.40 a	350	2286	285
'Lauretta'	45.06 ± 20.46 a	360	2219	277
'Silvia'	47.19 ± 18.74 a	378	2119	264

3.3. Stolonifer Capacity of Micropropagated Mother Plant of cv. 'Dina' Transplanted on Two Dates

Short-day cultivars adapted to low-chilling climates are generally planted in the field in late summer–early autumn. Also, this type of cultivation is now increasing interest in plug-fresh plants. The success of the new propagation system depends on the precise timing of each cycle step, tailored to the specific cultivar and desired planting time. For this reason, using the cultivar 'Dina', another experiment was set up, differing the transplanting of the micropropagated plants on 5th May, about one month after the first plantation (13th April). The late transplanting did not reduce the high vegetative plant vigor of the micropropagated mother plants, confirming the high efficiency in the production of runners and tips per runners per plant (Table 6). In fact, both cases, after 60 days, granted the average production of 15 runners per plant with an average of 3 tips per runner and a total number of at least 30 stolons per plant (Table 6).

Table 6. Number of runners per plant, number of tips per plant, number of tips per two runners per plant, and length of two runners per micropropagated mother plant of cultivar 'Dina' transplanted on two different dates (April 13th and May 5th) three different periods from transplanting: 20, 40, and 60 days (T20, T40, and T60). Mean ± standard error, LSD test $p < 0.05$. ns = not significant.

Parameters	April 13th			May 5th		
Days from Transplanting	T20	T40	T60	T20	T40	T60
No. of runners p^{-1}	1.13 ± 0.81 ns	3.19 ± 0.91 b	15.13 ± 6.13 ns	1.56 ± 0.73 ns	10.25 ± 3.59 a	15.19 ± 4.49 ns
No. of tips per r^{-1}	0.75 ± 0.45 b	1.13 ± 0.14 b	3.38 ± 1.89 ns	1.06 ± 0.25 a	1.99 ± 0.43 a	4.25 ± 1.89 ns
No. of tips p^{-1}	1.13 ± 0.81 ns	3.69 ± 1.35 b	33.25 ± 7.54 ns	1.69 ± 0.95 ns	22.25 ± 5.72 a	29.06 ± 5.79 ns
Length of two runner	5.88 ± 4.64 b	29.91 ± 11.31 b	75.03 ± 10.76 b	9.75 ± 5.59 a	58.38 ± 18.81 a	101.13 ± 11.78 a

An experimental trial carried out with 'Chandler' and 'Sweet Charlie' strawberry cultivars [24] demonstrates that temperatures between 35 and 40 °C inhibit the vegetative growth of the runners, whereas temperatures ranging from 15 to 20 °C and 25 to 30 °C are more effective in promoting strawberry plant vegetative development [25]. The temperature measurements during the greenhouse cultivation period showed the following pattern: in April and the beginning of May, the minimum temperature was 6 ± 2 °C, while the maximum reached 15 ± 2 °C. The mother plants on the first transplanting date (13/04/2022) started the runner production only after the 20th of May, when the thermometer measured a minimum of 13 ± 2 °C and a maximum of 25 ± 2 °C. In fact, with these climate conditions, the mother plants of the second planting (05/05/2022) showed the

development of a greater number of runners and a longer length. 'Dina' is a short-day variety studied for the southern cultivation systems. The rising temperature and longer daylight hours, combined with 1 g/L of 20–8–20 fertilization, increased the stoloniferous efficiency. The data were collected pre-runner harvesting, ensuring the same greenhouse linear meter efficiency (Table 7) (Figure 3).

Table 7. Number of tips per plant grown in greenhouse soilless conditions: meter (at T60), mean ± standard error, LSD test $p < 0.05$, was used for the estimation of number of runner tips produced by eight mother plants in a one-meter bag (RT m^{-1}). In addition, using this value, the number of mother plants needed and the linear meter required (1 bag 1 m long) to produce 100,000 strawberry plug plants of the 'Dina', considering its efficiency on two different transplanting dates, was calculated. ns = not significant.

Transplanting Dates	No. of Tips at T_60	Estimated RT m^{-1}	Estimated No. of Mother Plants	Estimated No. of Bags and Linear Meter (8 Plants b^{-1} of 1 m)
13/04/2022	33.25 ± 7.54 ns	266	3007	376
05/05/2022	29.06 ± 5.79 ns	232	3441	430

Figure 3. Micropropagated mother plants of 'Dina' transplanted on two dates: 13/04/2022 and 05/05/2022. This figure demonstrates that there were no significant differences between the two treatments after 60 days from transplanting.

3.4. Four Fertilization Strategy: Plant Architecture of 'Dina' Plug Plants' Epigeal Apparatus

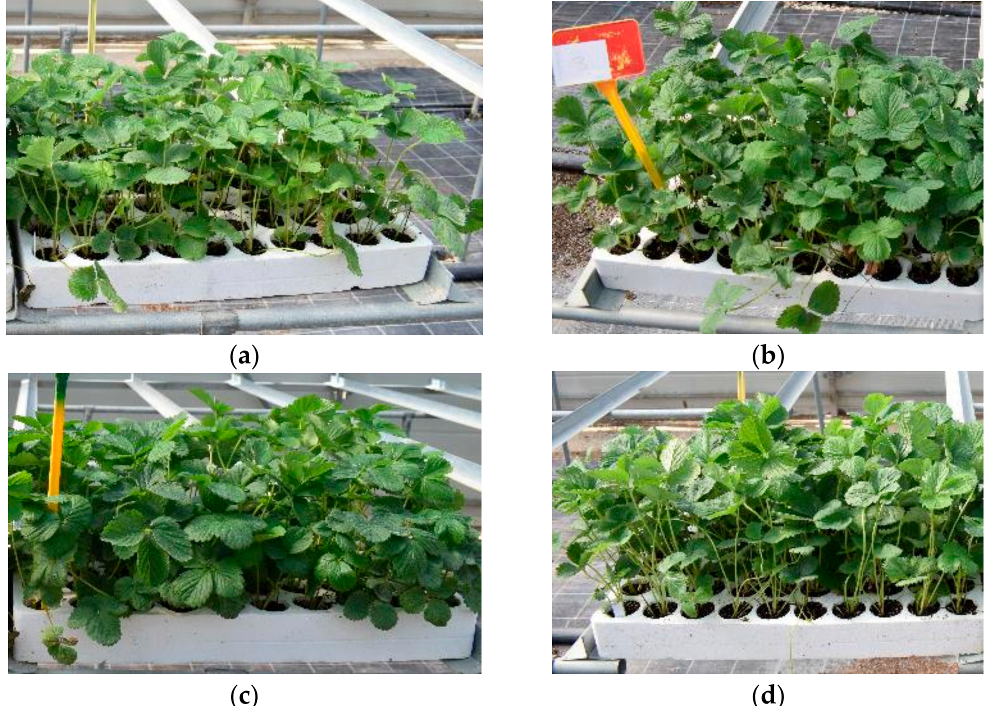
From these analyses, we collected data on the photosynthetic activity of petiole and leaves of the 'Dina' plug plants. For this, we analyzed three parameters of the fresh and dry weight of the green vegetative part and L*C*h° (lightness, chroma value, and hue angle) of the three leaf blades from three different leaves.

3.4.1. Leaf Fresh and Dry Weight of 'Dina' Plug Plants

The fresh weight of leaves and petioles highlighted significant differences between the three fertilization treatments only after 14 and 28 days (Table 8) (Figure 4). All of the fertilized plug plants reacted with high vegetative vigor with the emission of new shoots. This phenomenon occurred more evidently for the samples with the nutrient solution NPK 12–52–5 (8.90 ± 0.49 g) and NPK 20–20–20 (8.27 ± 1.53 g). As shown in Figure 4, the plants treated with the nutrient solution exhibited greater shoot development compared to those fertilized with NPK 15–31–10 and the control. The plants used it to stretch for space and light competition. The dry weight provided the same proportional data to the fresh one (nutrient solution 1, NPK 15–52–5 1.59 ± 0.04 g; nutrient solution 3, NPK 20–20–20 1.54 ± 0.37 g) (Table 8). These findings confirm that the adopted nursery system successfully produced plants with a high nutritional reserve, suitable for field transplanting.

Table 8. Data of L*C*h° (L*: lightness; C*: Chroma; h° hue angle) values calculated using Konica Minolta colorimeters and leaf and petiole fresh and dry weight (g). These samples were analyzed every seven days after three cycles of fertilization using four different nutritive solutions (C: irrigation water; Sol_1: NPK 12–52–5; Sol_2: NPK 15–31–10; Sol_3: NPK 20–20–20). Mean ± standard error, LSD test $p < 0.05$. ns = not significant.

Parameters	Fertilization	T0	T7	T14	T21	T28
Lightness (L*)	C	38.06 ± 2.40 ns	36.34 ± 1.44 ns	36.36 ± 2.82 a	36.73 ± 2.20°	36.54 ± 2.24 a
	Sol_1	38.61 ± 2.45 ns	33.65 ± 1.82 ns	35.63 ± 2.02 b	32.30 ± 2.73 bc	34.38 ± 2.02 b
	Sol_2	39.40 ± 3.01 ns	34.97 ± 1.84 ns	36.16 ± 2.80 b	34.23 ± 3.09 b	34.55 ± 2.12 b
	Sol_3	39.70 ± 3.46 ns	34.47 ± 2.01 ns	36.09 ± 2.45 b	31.88 ± 2.13 c	33.40 ± 2.07 b
Chroma (C*)	C	25.38 ± 4.43 ns	22.20 ± 1.83 ns	24.52 ± 4.67 ns	25.67 ± 2.85°	25.59 ± 3.41 a
	Sol_1	24.45 ± 4.4 ns	22.05 ± 3.20 ns	24.47 ± 3.45 ns	19.44 ± 3.20 c	19.41 ± 2.82 bc
	Sol_2	27.65 ± 4.75 ns	22.52 ± 1.92 ns	23.48 ± 2.47 ns	19.75 ± 2.44 bc	20.87 ± 3.54 b
	Sol_3	26.69 ± 5.0 ns	20.31 ± 2.72 ns	23.83 ± 3.15 ns	21.71 ± 2.35 b	18.21 ± 3.09 c
Hue angle (h°)	C	126.38 ± 1.61 ns	127.83 ± 1.11 ns	127.01 ± 1.82 ns	126.18 ± 1.81 b	126.86 ± 1.52 b
	Sol_1	126.97 ± 1.53 ns	128.03 ± 1.32 ns	126.31 ± 1.47 ns	128.94 ± 1.17°	130.15 ± 1.74 a
	Sol_2	125.70 ± 1.89 ns	127.71 ± 1.30 ns	127.46 ± 0.81 ns	128.65 ± 1.59°	128.71 ± 1.52 c
	Sol_3	126.06 ± 1.92 ns	128.94 ± 1.38 ns	127.31 ± 1.17 ns	127.99 ± 0.95°	129.97 ± 0.91 a
leaf petiole fresh weight (g)	C	1.95 ± 0.42 ns	3.38 ± 0.37 ns	4.45 ± 1.11 ab	5.59 ± 1.22 ns	5.71 ± 0.98 b
	Sol_1	2.00 ± 0.37 ns	2.94 ± 0.39 ns	4.05 ± 0.51 b	7.58 ± 1.01 ns	8.90 ± 0.49 a
	Sol_2	2.43 ± 0.27 ns	2.86 ± 0.37 ns	3.13 ± 0.43 b	6.49 ± 1.28 ns	6.05 ± 1.40 b
	Sol_3	1.90 ± 0.18 ns	4.02 ± 0.73 ns	3.92 ± 0.81 a	7.67 ± 0.73 ns	8.27 ± 1.53 a
leaf petiole dry weight (g)	C	0.36 ± 0.10 b	0.65 ± 0.09 ns	0.87 ± 0.24 ab	1.14 ± 0.30 ns	1.30 ± 0.26 ab
	Sol_1	0.41 ± 0.11 ab	0.50 ± 0.07 ns	0.68 ± 0.13 b	1.41 ± 0.16 ns	1.59 ± 0.04 a
	Sol_2	0.50 ± 0.06 a	0.52 ± 0.06 ns	0.58 ± 0.09 b	1.23 ± 0.25 ns	1.05 ± 0.31 b
	Sol_3	0.38 ± 0.04 ab	0.69 ± 0.15 ns	0.67 ± 0.15 a	1.38 ± 0.21 ns	1.54 ± 0.37 a



(**a**) (**b**) (**c**) (**d**)

Figure 4. Rooted tips of 'Dina' subjected to the four experimental treatments: control (**a**), nutrient solution 1 with NPK ratio 15–52–5, (**b**) nutrient solution 2 with NPK ratio 15–31–10, (**c**) and nutrient solution 3 with NPK ratio 20–20–20 (**d**) after 6 fertigations (T14).

3.4.2. L*C*h° Values of Three Leaves of 'Dina' Rooted Tips

Leaf color was quantitatively evaluated using a Konica Minolta colorimeter, enabling non-destructive, in vivo measurement of the green pigmentation in the leaves of rooted 'Dina' strawberry plug plants. Across all treatments, the leaves exhibited a uniform dark green color with average values of L* = 32.30 ± 2.73, C* = 20.31 ± 2.72, and h° = 126.27 ± 1.73 (Table 8). These values are consistent with those reported by Keskin et al. [26] for the 'Camarosa' strawberry cultivar, where similar L*C*h° metrics were associated with high foliar nitrogen levels.

Other studies conducted on horticultural varieties demonstrate a direct relation between the chlorophyll content and the L*C*h° values; for example, on lettuce [27] and pepper leaves [28]. Other scientific studies have highlighted the correlation between the colorimeter data and the concentration of primary pigments such as carotenoid for apricot [29] and orange juice [30], and lycopene for tomato [31,32].

The uniformity of the recorded L*C*h° values, 'Dina' rooted tips, including those from non-fertilized samples, suggests an absence of nutritional deficiencies across treatments and a generally high phytosanitary quality. Moreover, the elevated color indices in unfertilized plantlets pointed to a well-developed morpho-physiological status and full photosynthetic competence, comparable to that of the fertilized counterparts. These findings underscore the efficacy of the micropropagation protocol in producing vigorous, healthy plantlets suitable for successful acclimatization and transplantation.

3.4.3. Root System of 'Dina' Plug Plants

WinRHIZO software (Version 2020b, Regent Instruments Inc., Quebec City, QC, Canada) analysis revealed a steady increase in root length, ranging from 100 to 600 cm, and volume, from 200 to 1000 cm^3, during the vegetative growth phase of the tips. The root diameter was constantly above 0.4 and 0.5 mm during all the fertilization tests (Table 9). NPK 20–20–20 fertilizer and irrigation only with water seemed to be the more effective for root length and volume. The NPK 12–52–10 and NPK 15–31–10 did not contribute to a well-rooted 'Dina' plug plant compared to the first two. Despite this, the statistical data does not demonstrate significant differences among the three parameters for each treatment. The limitations of WinRHIZO in scanning fine roots are acknowledged by other researchers [4,33]. To address this, we used a coloring solvent to improve fine root visualization. Furthermore, to reduce the loss of fine roots, we recommend avoiding washing the samples in running water. No significant differences were observed in the fresh and dry weight of the roots on days 21 and 28 of the treatment (Table 9). Despite this, A previous study [34] demonstrated that 0.40 ± 0.20 g of fresh root weight is sufficient for deficit irrigation of plug plants after transplanting to the field. Root fresh weight, for each fertilization protocol, is sufficient to assess the quality of the strawberry plants produced under the different treatments. Consequently, the dry weight measurements for each treatment indicate a root system with adequate volume and sufficient nutrient content, thereby ensuring greater plant autonomy and improved agronomic quality in the propagated 'Dina' plants.

Table 9. Measured root fresh and dry weight, root length (cm), diameter (mm), and volume (mm^3) collected using WinRHIZO software. These samples were analyzed every seven days after three cycles of fertilization using four different nutritive solutions (C: irrigation water; Sol_1: NPK 12-52-5; Sol_2: NPK 15-31-10; Sol_3: NPK 20-20-20). Mean ± standard error, LSD test $p < 0.05$. ns = not significant.

Parameters	Fertilization	T0	T7	T14	T21	T28
Root length (cm)	C	97.61 ± 16.25 ns	208.53 ± 59.63 ns	285.10 ± 71.94 ns	626.89 ± 149.15 ns	649.19 ± 198.47 ns
	Sol_1	120.64 ± 38.29 ns	162.07 ± 57.27 ns	208.12 ± 44.15 ns	548.40 ± 39.49 ns	499.36 ± 150.95 ns
	Sol_2	105.32 ± 10.13 ns	162.57 ± 47.18 ns	236.58 ± 37.30 ns	498.08 ± 34.08 ns	462.30 ± 131.85 ns
	Sol_3	110.29 ± 14.97 ns	258.89 ± 29.39 ns	281.59 ± 31.07 ns	728.65 ± 46.88 ns	651.80 ± 130.86 ns
root diameter (mm)	C	0.42 ± 0.06 ns	0.44 ± 0.01 ns	0.44 ± 0.03 ns	0.37 ± 0.02 ns	0.43 ± 0.04 ns
	Sol_1	0.43 ± 0.03 ns	0.42 ± 0.01 ns	0.47 ± 0.05 ns	0.40 ± 0.02 ns	0.50 ± 0.04 ns
	Sol_2	0.47 ± 0.06 ns	0.42 ± 0.02 ns	0.42 ± 0.02 ns	0.43 ± 0.04 ns	0.46 ± 0.05 ns
	Sol_3	0.42 ± 0.04 ns	0.45 ± 0.05 ns	0.41 ± 0.03	0.43 ± 0.08 ns	0.47 ± 0.03 ns
root volume (mm^3)	C	137.25 ± 33.95 ns	313.00 ± 89.91 ns	432.75 ± 76.53 ab	686.75 ± 269.70 ns	986.75 ± 453.22 ns
	Sol_1	175.50 ± 63.38 ns	216.75 ± 65.95 ns	359.25 ± 122.45 b	685.75 ± 69.48 ns	993.00 ± 442.57 ns
	Sol_2	187.00 ± 36.23 ns	222.75 ± 50.78 ns	325.25 ± 81.72 b	718.00 ± 162.08 ns	759.50 ± 255.58 ns
	Sol_3	156.50 ± 48.35 ns	409.75 ± 85.09 ns	376.00 ± 87.05 a	937.25 ± 180.65 ns	1132.25 ± 354.25 ns
root fresh weight (g)	C	0.08 ± 0.05 b	0.30 ± 0.16 ns	0.68 ± 0.39 ns	0.78 ± 0.30 ns	1.21 ± 0.54 ns
	Sol_1	0.24 ± 0.08 a	0.27 ± 0.10 ns	0.36 ± 0.13 ns	0.91 ± 0.22 ns	1.30 ± 0.42 ns
	Sol_2	0.24 ± 0.09 a	0.23 ± 0.07 ns	0.34 ± 0.17 ns	1.01 ± 0.36 ns	1.08 ± 0.57 ns
	Sol_3	0.16 ± 0.04 ab	0.46 ± 0.17 ns	0.49 ± 0.12 ns	1.19 ± 0.26 ns	1.37 ± 0.50 ns
root dry weight (g)	C	0.03 ± 0.02 ns	0.05 ± 0.02 ns	0.09 ± 0.06 ns	0.14 ± 0.05 ns	0.22 ± 0.10 ns
	Sol_1	0.05 ± 0.02 ns	0.03 ± 0.01 ns	0.04 ± 0.02 ns	0.11 ± 0.02 ns	0.66 ± 1.02 ns
	Sol_2	0.04 ± 0.01 ns	0.04 ± 0.01 ns	0.04 ± 0.01 ns	0.13 ± 0.02 ns	0.12 ± 0.06 ns
	Sol_3	0.04 ± 0.01 ns	0.05 ± 0.01 ns	0.05 ± 0.02 ns	0.14 ± 0.03 ns	0.20 ± 0.07 ns

4. Conclusions

This study demonstrates that the use of micropropagated mother plants significantly improves the yield and quality of runners and tips, leading to the production of vigorous, virus-free plug plants. The proposed soilless cultivation system, using elevated bags containing sterile substrates, confirms its suitability for high-density nursery production with no disease impact and high propagation efficiency. This approach guarantees a high quantitative and qualitative response. Different transplanting dates, under optimal conditions, do not significantly impact the stoloniferous efficiency of cultivars either with low or high chilling requirements. These results confirm the potential future impact of micropropagated mother plants on enhancing strawberry nursery production. From a producer's perspective, the runner emission and the higher rate of runner tips observed in micropropagated mother plants are key parameters for evaluating the technical and economic competitiveness of a micro-plant system. While this innovative strategy offers several agronomic advantages, practical considerations must also be taken into account. The initial investment in micropropagation facilities and soilless infrastructure may be relatively high compared to traditional field systems. However, these costs may be offset by the increased production efficiency, the reduced need for the linear meters of greenhouse, and the higher sanitary status of the plants. For commercial nurseries, the use of clean, genetically uniform starting material can lead to more predictable production cycles and may improve compliance with certification schemes, especially in export-oriented markets. Nonetheless, several challenges persist, including legislative barriers within the EU and the necessity for farmers to gain confidence in technology, particularly concerning the proper planning and management of the in vitro and in vivo generation.

The different vegetative quality responses, corresponding to diverse fertigation solutions of plug plants, offer solutions to improve the quality of plants in the rooting and growth phase and therefore a better service to the farmer. Fresh and dry biomass are relevant indicators of 'Dina' plug plants quality. They are secondary in terms of direct commercial impact because they reflect the final performance of individual plants rather than the propagation system's overall capacity to generate marketable units.

This technique supports innovation in propagation scheduling and certification systems by offering a reliable source of genetically uniform, virus-free material. However, structural investments and legislative updates are required to facilitate broader adoption. Future studies should evaluate the agronomic performance of resulting fruiting plants to validate commercial acceptability in terms of yield, morphology, and organoleptic traits. Overall, the proposed protocol represents a scalable and sustainable strategy for modernizing strawberry nursery production.

Author Contributions: Conceptualization, V.M., F.C., and B.M.; methodology, V.M. and F.C.; software, V.M.; validation, B.M. and F.C.; formal analysis, V.M. and F.B.; investigation, V.M. and F.B.; resources, J.D. and F.C.; data curation, V.M. and F.C.; writing—original draft preparation, V.M.; writing—review and editing, B.M., F.C., and J.D.; visualization, B.M. and J.D.; supervision, F.C., F.B., and B.M.; project administration, B.M.; funding acquisition, B.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Union's Horizon 2020 research and innovation program, BreedingValue project, Grant Agreement No. 101000747.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: This work was carried out in order to achieve the master's thesis of the first author. We would like to express our gratitude to the agricultural company Innesti Leopardi for providing their facilities and their structures.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Sparacino, A.; Ollani, S.; Baima, L.; Oliviero, M.; Borra, D.; Rui, M.; Mastromonaco, G. Analyzing strawberry preferences: Best–worst scaling methodology and purchase styles. *Foods* **2024**, *13*, 1474. [CrossRef] [PubMed]
2. Prohaska, A.; Petit, A.; Lesemann, S.; Rey-Serra, P.; Mazzoni, L.; Masny, A.; Sánchez-Sevilla, J.F.; Potier, A.; Gaston, A.; Klamkowski, K.; et al. Strawberry phenotypic plasticity in flowering time is driven by the interaction between genetic loci and temperature. *J. Exp. Bot.* **2024**, *75*, 5923–5939. [CrossRef]
3. Hytönen, T.; Kurokura, T. Control of flowering and runnering in strawberry. *Hortic. J.* **2020**, *89*, 96–107. [CrossRef]
4. Judd, L.A.; Jackson, B.E.; Fonteno, W.C. Advancements in root growth measurement technologies and observation capabilities for container-grown plants. *Plants* **2015**, *4*, 369–392. [CrossRef]
5. Senger, E.; Osorio, S.; Olbricht, K.; Shaw, P.; Denoyes, B.; Davik, J.; Predieri, S.; Karhu, S.; Raubach, S.; Lippi, N.; et al. Towards smart and sustainable development of modern berry cultivars in Europe. *Plant J.* **2022**, *111*, 1238–1251. [CrossRef]
6. Capocasa, F.; Balducci, F.; Mazzoni, L.; Marcellini, M.; Pergolotti, V.; Mezzetti, B. Preliminary results of different strawberry cultivars in multi-cropping soilless cultivation. *Acta Hortic.* **2021**, *1309*, 579–584. [CrossRef]
7. Rahman, M.; Samtani, J.; Baker, A. New Insights in the Detection and Management of Major Foliar and Root Diseases of Strawberries. *Preprints* **2023**. [CrossRef]
8. Dale, A.; Hughes, B.R.; Donnelly, D. The role of micropropagation in producing specific pathogen-tested plants. *HortScience* **2008**, *43*, 74–77. [CrossRef]
9. Freeman, S. Detection and characterization of soilborne diseases of strawberry and their management. In *ISHS—X International Strawberry Symposium (ISS2025) Book of Abstracts*; Yancheng, China, 2025; (Book of Abstracts, publisher not formally listed.).
10. Milinkovic, M.; Merriman, P.R.; Greenhalgh, F.C.; Mattner, S.W.; Horstra, C.B. Evaluation of soil-less systems for strawberry transplant production in Australia. *Acta Hortic.* **2017**, *1176*, 53–64. [CrossRef]

11. Yamamoto, E.; Kataoka, S.; Shirasawa, K.; Noguchi, Y.; Isobe, S. Genomic selection for F1 hybrid breeding in strawberry (*Fragaria × ananassa*). *Front. Plant Sci.* **2021**, *12*, 645111. [CrossRef]
12. Neri, J.C.; Meléndez-Mori, J.B.; Tejada-Alvarado, J.J.; Vilca-Valqui, N.C.; Huaman-Huaman, E.; Oliva, M.; Goñas, M. An optimized protocol for micropropagation and acclimatization of strawberry (*Fragaria × ananassa* Duch.) variety 'Aroma'. *Agronomy* **2022**, *12*, 968. [CrossRef]
13. Quiroz, K.A.; Berríos, M.; Carrasco, B.; Retamales, J.B.; Caligari, P.D.; García-Gonzáles, R. Meristem culture and subsequent micropropagation of Chilean strawberry (*Fragaria chiloensis* (L.) Duch.). *Biol. Res.* **2017**, *50*, 20. [CrossRef]
14. Boxus, P.; Damiano, C.; Brasseur, E.; Ammirato, D.A.; Evans, P.V.; Sharp, W.R.; Yamada, Y. *Handbook of Plant Cell Culture*; Macmillan Publishing Company: New York, NY, USA, 1984.
15. Boxus, P. Review on strawberry mass propagation. *Acta Hortic.* **1988**, *265*, 309–320. [CrossRef]
16. Mukherjee, E.; Gantait, S. Strawberry biotechnology: A review on progress over past 10 years. *Scien-626 Tia Hortic.* **2024**, *338*, 113618. [CrossRef]
17. Capocasa, F.; Balducci, F.; Marcellini, M.; Bernardini, D.; Navacchi, O.; Mezzetti, B. Comparing nursery behavior, field plant yield and fruit quality of in vitro and in vivo propagated strawberry mother plants. *Plant Cell Tissue Organ Cult.* **2019**, *136*, 65–74. [CrossRef]
18. Balducci, F.; Marcellini, M.; Schrey, S.; Linnemannstöns, L.; Capocasa, F.; Mezzetti, B.; Mazzoni, L. "Francesca", "Lauretta", "Silvia" and "Dina": Four new strawberry cultivars for northern and southern European cultivation conditions from the Marche Polytechnic University breeding programme. *Acta Hortic.* **2021**, *1309*, 205–208. [CrossRef]
19. Sabbadini, S.; Marcellini, M.; Mezzetti, B.; Capocasa, F. Establishing micropropagation protocols for new strawberry (*Fragaria × ananassa*) breeding lines. *Acta Hortic.* **2021**, *1309*, 573–578. [CrossRef]
20. Rowley, D.; Black, B.; Drost, D. Strawberry Plug Plant Production. USU Extension Publication Horticulture/High Tunnels/2010-02pr. 2010. Available online: https://digitalcommons.usu.edu/psc_facpub/511/ (accessed on 23 July 2025).
21. Durner, E.F.; Poling, E.B.; Maas, J.L. Recent advances in strawberry plug transplant technology. *HortTechnology* **2002**, *12*, 545–550. [CrossRef]
22. Xu, X. Optimizing Environmental Parameters for Precision Indoor Propagation of Day-Neutral Strawberry. Master's Thesis, North Carolina State University, Raleigh, NC, USA, 2019. [CrossRef]
23. Al-Madhagi, I. The habit of strawberry flowering is the key for runner propagation, where the photoperiod is the main environmental factor-A review. *Adv. Hortic. Sci.* **2023**, *37*, 433–449. [CrossRef]
24. Li, Y.; Hu, J.; Wei, H.; Jeong, B.R. A long-day photoperiod and 6-benzyladenine promote runner formation through upregulation of soluble sugar content in strawberry. *Int. J. Mol. Sci.* **2020**, *21*, 4917. [CrossRef]
25. Kadir, S.; Sidhu, G.; Al-Khatib, K. Strawberry (*Fragaria × ananassa* Duch.) growth and productivity as affected by temperature. *HortScience* **2006**, *41*, 1423–1430. [CrossRef]
26. Keskin, M.; Sekerli, Y.E.; Gunduz, K. Influence of leaf water content on the prediction of nutrient stress in strawberry leaves using chromameter. *Int. J. Agric. Biol.* **2018**, *20*, 2103–2109. [CrossRef]
27. León, A.P.; Viña, S.Z.; Frezza, D.; Chaves, A.; Chiesa, A. Estimation of Chlorophyll Contents by Correlations between SPAD-502 Meter and Chroma Mete. *Commun. Soil Sci. Plant Anal.* **2007**, *38*, 2877–2885. [CrossRef]
28. Madeira, A.C.; Ferreira, A.; de Varennes, A.; Vieira, M.I. SPAD meter versus tristimulus colorimeter to estimate chlorophyll content and leaf color in sweet pepper. *Commun. Soil Sci. Plant Anal.* **2003**, *34*, 2461–2470. [CrossRef]
29. Ruiz, D.; Egea, J.; Tomás-Barberán, F.A.; Gil, M.I. Carotenoids from new apricot (*Prunus armeniaca* L.) varieties and their relationship with flesh and skin color. *J. Agric. Food Chem.* **2005**, *53*, 6368–6374. [CrossRef]
30. Meléndez-Martínez, A.J.; Vicario, I.M.; Heredia, F.J. Application of tristimulus colorimetry to estimate the carotenoids content in ultrafrozen orange juices. *J. Agric. Food Chem.* **2003**, *51*, 7266–7270. [CrossRef]
31. Arias, R.; Lee, T.C.; Logendra, L.; Janes, H. Correlation of lycopene measured by HPLC with the L*, a*, b* color readings of a hydroponic tomato and the relationship of maturity with color and lycopene content. *J. Agric. Food Chem.* **2000**, *48*, 1697–1702. [CrossRef]
32. Hyman, J.R.; Gaus, J.; Foolad, M.R. A rapid and accurate method for estimating tomato lycopene content by measuring chromaticity values of fruit puree. *J. Am. Soc. Hortic. Sci.* **2004**, *129*, 717–723. [CrossRef]
33. Jeffery, R.P.; Simpson, R.J.; Lambers, H.; Orchard, S.; Kidd, D.R.; Haling, R.E.; Ryan, M.H. Contrasting communities of arbuscule-forming root symbionts change external critical phosphorus requirements of some annual pasture legumes. *Appl. Soil Ecol.* **2018**, *126*, 88–97. [CrossRef]
34. Hochmuth, G.; Cantliffe, D.; Chandler, C.; Stanley, C.; Bish, E.; Waldo, E.; Legard, D.; Duval, J. Containerized strawberry transplants reduce establishment-period water use and enhance early growth and flowering compared with bare-root plants. *HortTechnology* **2006**, *16*, 46–54. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.