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Enhancing yield and fruit quality of strawberry cultivars through *in vitro* propagation

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ABSTRACT

Introduction: Strawberries (*Fragaria × ananassa*) are valued for their nutritional and functional properties, including vitamins, antioxidants, and bioactive compounds. *In vitro* propagation provides a means to produce uniform, virus-free planting material, potentially enhancing plant health and fruit quality.

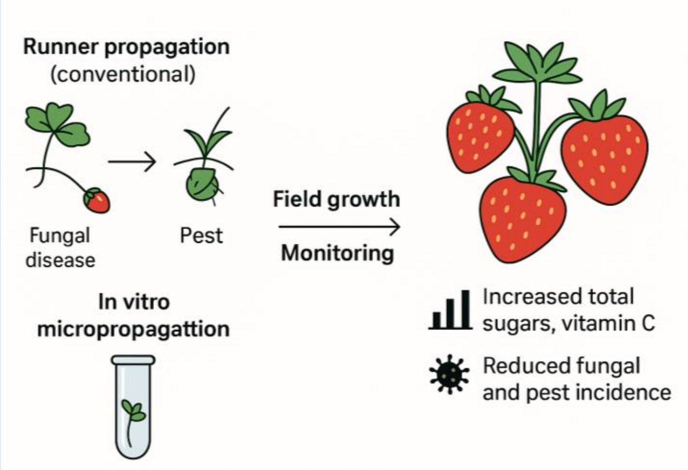
Objective: This study evaluated the effects of propagation method—conventional runner propagation versus *in vitro* micropropagation—on fungal disease incidence, pest control efficacy, morphological development, yield, and fruit biochemical composition across six commercial strawberry cultivars.

Materials and Methods: Six strawberry cultivars ('Lambada', 'Cabrillo', 'Alba', 'Murano', 'Jenny', and 'Aurora') were propagated using virus-tested *in vitro* culture and conventional methods. Field trials employed a randomized block design with three replicates of 25 plants per treatment. Five fungal pathogens and three key pests (melon aphid, onion thrips, and two-spotted spider mite) were monitored after a single pesticide application. Morphological traits, yield, and fruit quality parameters (total sugars, vitamin C, and titratable acidity) were measured. Data were analyzed statistically using Student's *t*-test ($p < 0.05$).

Results: Micropropagated plants showed markedly reduced fungal disease incidence, especially *Botrytis cinerea* (gray mold) and *Podosphaera aphanis* (powdery mildew). For the 'Lambada' cultivar, *B. cinerea* incidence decreased from 40.8 ± 8.9% to 7.4 ± 3.1%, and *P. aphanis* from 28.3 ± 6.4% to 10.1 ± 2.1%. Pest control efficacy remained higher in *in vitro* plants, sustaining >90% suppression of aphids and thrips by day 10, compared with 68–96% in conventional plants. Although control of *Tetranychus urticae* declined over time, *in vitro* plants maintained 68–88% control, compared with 48% in traditional plants. Morphological assessments showed enhanced flowering, vegetative growth, yield, and fruit size in micropropagated plants; for example, 'Cabrillo' yield rose from 2100 ± 204 g to 2800 ± 217 g per plant. Fruits from *in vitro* plants contained significantly more total sugars (+0.5–1.3 g/100 g) and vitamin C (+3.0–5.1 mg/100 g). At the same time, titratable acidity was slightly lower (except in 'Cabrillo'), resulting in a more favorable sugar-to-acid ratio.

Conclusion: *In vitro* propagation significantly improves strawberry production by reducing pathogen and pest incidence, enhancing vegetative growth and yield, and increasing key fruit quality traits. These findings support micropropagation as a sustainable strategy for producing high-quality, virus-free strawberry plants. Future work should integrate micropropagation with optimized pest management programs to address challenges, such as the persistence of two-spotted spider mites.

Keywords: strawberry (*Fragaria × ananassa*); *in vitro* propagation; micropropagation; functional food; fruit quality; yield improvement; sustainable agriculture



Graphical abstract: Enhancing yield and fruit quality of strawberry cultivars through in vitro propagation

INTRODUCTION

Strawberries (*Fragaria × ananassa* Duch.) are among the most economically critical small fruits worldwide and are valued for their high nutritional quality, flavor, and versatility [1–2]. They are rich in vitamin C, folate (vitamin B9), manganese, potassium, and dietary fiber [3–4]. They are notable for containing abundant bioactive compounds such as flavonoids, anthocyanins, and phenolic acids, which exhibit potent antioxidant and anti-inflammatory properties [5–7].

Regular strawberry consumption has been associated with a reduced risk of cardiovascular disease, certain cancers, and metabolic disorders [8–10], thereby underscoring their classification as a functional food crop within established regulatory frameworks [11–12].

Viral, bacterial, and fungal diseases reduce plant vigor and yield, while insect pests increase production costs and complicate integrated pest management (IPM) programs [13–14]. Arthropod pests, in particular, pose significant challenges to strawberry cultivation and require targeted management strategies [15].

These issues underscore the need for alternative propagation strategies that provide high-quality, disease-free planting material. *In vitro* micropropagation offers a reliable method for producing large quantities of genetically uniform, pathogen-free plants more rapidly than traditional methods [16–18]. By eliminating systemic pathogens during culture initiation, micropropagated plants often exhibit improved vigor, greater resistance to pests and diseases, and enhanced yield and fruit quality [19–20].

Given the rising demand for strawberries in Armenia, effective propagation systems that ensure high productivity and sustainability are vital. This study compared micropropagated and traditionally propagated strawberry plants of six cultivars grown under field conditions, assessing disease incidence, pest response, morphological characteristics, yield metrics, and fruit biochemical composition to identify the benefits of micropropagation for commercial production.

Materials and Methods

Study Location: Research was conducted from 2018 to 2021 at the Scientific Center of Agrobiotechnology, ANAU. *In vitro* propagation and biochemical analyses were performed under controlled laboratory conditions, while field trials (2019–2021) were conducted in open-field plots in the Geghakert community, Armavir region, Armenia.

Plant Material: Six strawberry (Fragaria × ananassa) cultivars were selected for this study based on their origin and phenotypic traits, as summarized in Table 1. These cultivars differed in fruiting habits, yield potential, and suitability for fresh consumption or processing.

Table 1. Six *Fragaria × ananassa* cultivars were selected based on origin and phenotypic traits

Cultivar	Origin	Key Characteristics
Lambada	Netherlands	Early-ripening, short-day; high sugar content; soft texture; ideal for fresh consumption.
Cabrillo	USA	Day-neutral; vigorous growth; high yield; firm fruits suitable for storage and transport.
Alba	Italy	Short-day; large fruits; preferred for fresh markets due to flavor and appearance.
Murano	Italy	Day-neutral; morphologically similar to 'Alba'; extended fruiting period.
Aurora	Italy	High productivity; superior quality; suitable for both fresh use and processing.
Jenny	Europe	Adaptable and resilient; performs well in temperate climates.

Propagation Methods:

We used the following propagation approaches to evaluate the performance of each cultivar:

- **Micropropagated plants:** produced under sterile, virus-free conditions using tissue culture techniques.
- **Conventionally propagated plants:** obtained from runners without prior virus elimination.

Experimental Design and Field Conditions: A randomized complete block design (RCBD) with three replicates per treatment was employed. Each plot contained 25 plants per cultivar and propagation method. Standard agronomic practices were applied uniformly across all treatments.

Disease and Pest Assessment: Fungal disease incidence was determined as the percentage of infected leaves and fruits at peak infection, based on descriptions of Botrytis incidence by Elad [19]**.** The assessed pathogens included *Botrytis cinerea* (B. cinerea), *Podosphaera aphanis* (P. aphanis), *Phytophthora fragariae* (P. fragariae), *Septoria fragariae* (S. fragariae), and *Diplocarpon earlianum* (D. earlianum). Pest populations, including *Aphis gossypii*, *Thrips tabaci*, and *Tetranychus urticae*,-were recorded at 0, 3, 7, and 10 days after pesticide application according to integrated pest management principles [20] and recent monitoring protocols for spider mites [21].

Morphological and Yield Assessments*:* The number of vegetative shoots, flowers, and fruits per plant was recorded. Total fruit yield per plant was calculated by weighing all mature fruits [22].

Biochemical Analyses:

1. **Soluble sugars:** measured with a digital refractometer (°Brix) [23].
2. **Vitamin C:** measured by titration with 2,6-dichlorophenolindophenol (mg/100 g FW) [24].
3. **Titratable acidity***:* measured by titration with 0.1 N NaOH to pH 8.1 (g citric acid/100 g FW) [25].

Statistical Analysis: Data are presented as mean ± SE. Pairwise comparisons between propagation methods were performed for each cultivar using *Student's t-test* ($P < 0.05$).

RESULTS AND DISCUSSION

Effect of the Propagation Method on Fungal Disease Incidence in Strawberry: The propagation method significantly influenced strawberry susceptibility to fungal pathogens. Micropropagated plants consistently showed a lower incidence of five major pathogens under field conditions (*S. fragariae, P. fragariae, D. earlianum, P. aphanis,* and *B. cinerea*) (Table 2). These results agree with previous studies demonstrating that micropropagation produces pathogen-free planting material, which reduces vulnerability to infections transmitted by vectors, airborne spores, and soil-borne inoculum [26]. Techniques such as meristem culture and cryotherapy effectively eliminate latent viruses, improve plant health, and enhance overall field performance [27]. Furthermore, the use of clean, pathogen-free plants has been shown to significantly reduce *P. fragariae* incidence in field-grown strawberries [28–29].

Table 2. Effect of propagation method on disease incidence (%) in different strawberry cultivars (mean ± SE, n = 3).

Cultivar (Origin)	Propagation Method	S. fragariae (%) (mean ± SE)	P. fragariae (%) (mean ± SE)	D. earlianum (%) (mean ± SE)	P. aphanis (%) (mean ± SE)	B. cinerea (%) (mean ± SE)
Lambada (Netherlands)	Conventional	25.3 ± 2.0^{a}	7.8 ± 0.6^{a}	4.7 ± 0.6^{a}	28.3 ± 6.4^{a}	40.8 ± 8.9^{a}
	In vitro	12.4 ± 1.1^{b}	2.4 ± 0.3^{b}	3.1 ± 0.5^{b}	10.1 ± 2.1^{b}	7.4 ± 3.1^{b}
Cabrillo (USA)	Conventional	28.6 ± 2.5^{a}	6.3 ± 0.5^{a}	8.2 ± 0.8^{a}	24.4 ± 7.1^{a}	37.3 ± 6.7^{a}
	In vitro	10.8 ± 0.8^{b}	5.1 ± 0.6^{b}	3.2 ± 0.4^{b}	11.3 ± 3.0^{b}	6.8 ± 2.4^{b}
Alba (Italy)	Conventional	18.1 ± 1.6^{a}	5.4 ± 0.5^{a}	6.4 ± 0.5^{a}	18.6 ± 4.1^{a}	29.4 ± 4.8^{a}
	In vitro	9.5 ± 0.8^{b}	3.1 ± 0.3^{b}	3.1 ± 0.4^{b}	10.5 ± 2.8^{b}	10.3 ± 2.4^{b}
Murano (Italy)	Conventional	11.8 ± 1.5^{a}	6.8 ± 0.7^{a}	5.4 ± 0.6^{a}	30.1 ± 8.2^{a}	35.8 ± 6.9^{a}
	In vitro	7.5 ± 0.6^{b}	4.4 ± 0.4^{b}	2.4 ± 0.4^{b}	10.8 ± 3.2^{b}	7.5 ± 2.6^{b}
Jenny (Europe)	Conventional	18.8 ± 1.6^{a}	5.1 ± 0.6^{a}	7.1 ± 0.8^{a}	26.1 ± 6.1^{a}	29.6 ± 5.7^{a}
	In vitro	10.1 ± 0.6^{b}	3.8 ± 0.4^{b}	4.8 ± 0.4^{b}	10.8 ± 3.1^{b}	10.0 ± 2.6^{b}
Aurora (Italy)	Conventional	18.6 ± 1.7^{a}	5.6 ± 0.7^{a}	8.6 ± 0.9^{a}	31.4 ± 7.8^{a}	35.4 ± 7.1^{a}
	In vitro	13.5 ± 1.1^{b}	2.8 ± 0.3^{b}	5.2 ± 0.7^{b}	10.7 ± 2.9^{b}	11.5 ± 2.9^{b}

Different superscript letters within each cultivar indicate significant differences between propagation methods according to the *Student's t*-test ($p < 0.05$).

Among the six cultivars tested, the greatest reductions were observed for *B. cinerea* and *P. aphanis*. For example, in 'Lambada,' *B. cinerea* incidence decreased from 40.8 ± 8.9% in conventionally propagated plants to 7.4 ± 3.1% in micropropagated plants, while *P. aphanis* declined from 28.3 ± 6.4% to 10.1 ± 2.1%. Similar trends were observed across the other cultivars, highlighting the effectiveness of micropropagation in reducing fungal disease incidence. In contrast, the reduction in *S. fragariae* incidence for the cultivar 'Aurora' was modest compared with other cultivars, suggesting that genotype-specific factors may influence disease tolerance. These findings underscore the phytosanitary benefits of micropropagation, including reduced pathogen transmission, minimized microbial contamination, and faster field establishment. Wider adoption of this approach could lower fungicide requirements and contribute to sustainable strawberry production.

Evaluation of Pest Control Efficacy: The susceptibility of strawberry plants to three major pests—melon aphid (*Aphis gossypii*), onion thrips (*Thrips tabaci*), and two-spotted spider mite (*Tetranychus urticae*)—was evaluated following a single pesticide application. *A. gossypii* was treated with Confidor® 200 SL (Bayer CropScience, Germany), containing 200 g/L imidacloprid, applied at 0.5 mL/L water. *T. urticae* was treated with Vertimec® 1.8 EC (Syngenta, Switzerland), containing 18 g/L abamectin, at a rate of 1 L/ha. Pest populations were monitored on both conventionally and *in vitro* propagated strawberry plants at 0, 3, 7, and 10 days post-treatment. Infestations developed under natural field conditions without artificial inoculation.

Initial observations showed higher *A. gossypii* densities on conventionally propagated plants, whereas *in vitro*-derived plants maintained consistently lower infestations (Figure 1). For example, in the ‘Jenny’ cultivar, conventional plants averaged 32 ± 4.2 aphids per leaf, compared with 6 ± 1.0 *in vitro*; similar results were seen in ‘Aurora’ (30 ± 5.4 vs. 7 ± 1.0). Comparable trends were noted for *T. tabaci* and *T. urticae*, although *T. urticae* was generally more difficult to control, consistent with its rapid reproductive cycle and known resistance mechanisms. By day 3, pesticide efficacy against *A. gossypii* ranged from 82% to 100% across cultivars. By day 10, *in vitro*-derived plants exhibited significantly higher suppression rates (93–100%) than conventionally propagated plants (68–96%). For example, in the ‘Murano’ cultivar, control efficacy reached 96% *in vitro* compared with 76% in conventional plants. These results suggest that *in vitro* propagation not only reduces initial pest pressure but also enhances treatment outcomes. This may be attributed to improved plant vigor, genetic and physiological uniformity, and the absence of latent infections in *in vitro* plants, which collectively promote more effective pesticide uptake and limit pest establishment.

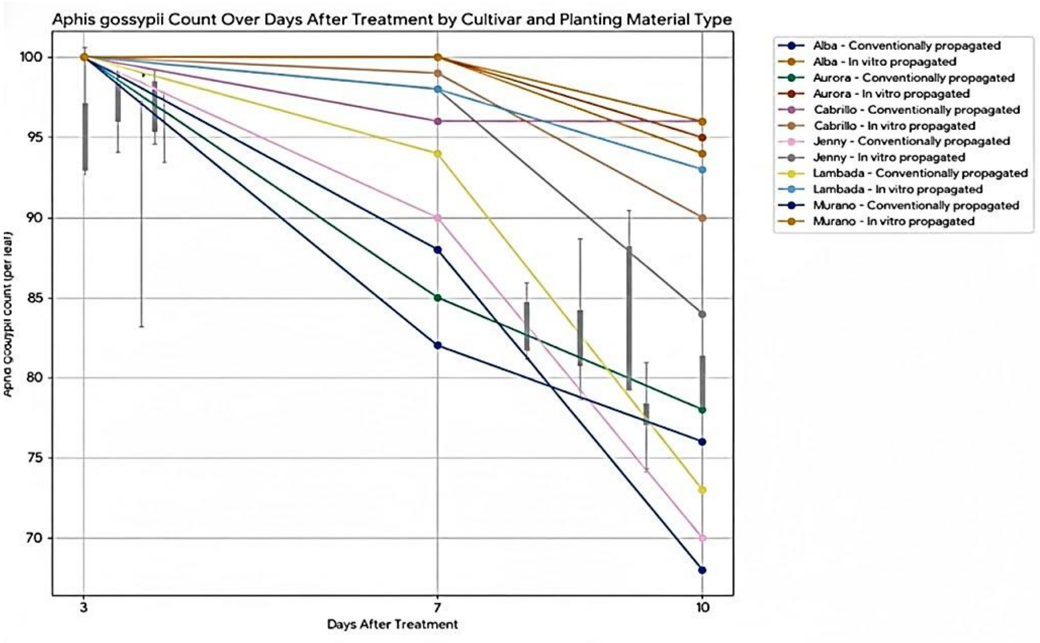


Figure 1. Aphid count per leaf over 10 days following Imidacloprid (Confidor® 200 SL) treatment on melon aphid (Aphis gossypii) in conventionally and in vitro propagated strawberry cultivars

Figure 2 illustrates treatment efficacy against onion thrips (*Thrips tabaci*) infestation across strawberry cultivars, comparing conventionally propagated and micropropagated plants over a 10-day observation period. The results consistently show that micropropagated plants maintained higher and more sustained control throughout the study. While both propagation types showed strong initial suppression at 3 days post-treatment (96–100%), efficacy in conventionally propagated plants declined more rapidly by days 7 and 10. For example, in the ‘Murano’ cultivar, control efficacy in conventionally propagated plants dropped to 64% by day 10, whereas micropropagated plants-maintained control levels above 84%. These findings highlight the influence of the propagation method on treatment outcomes.

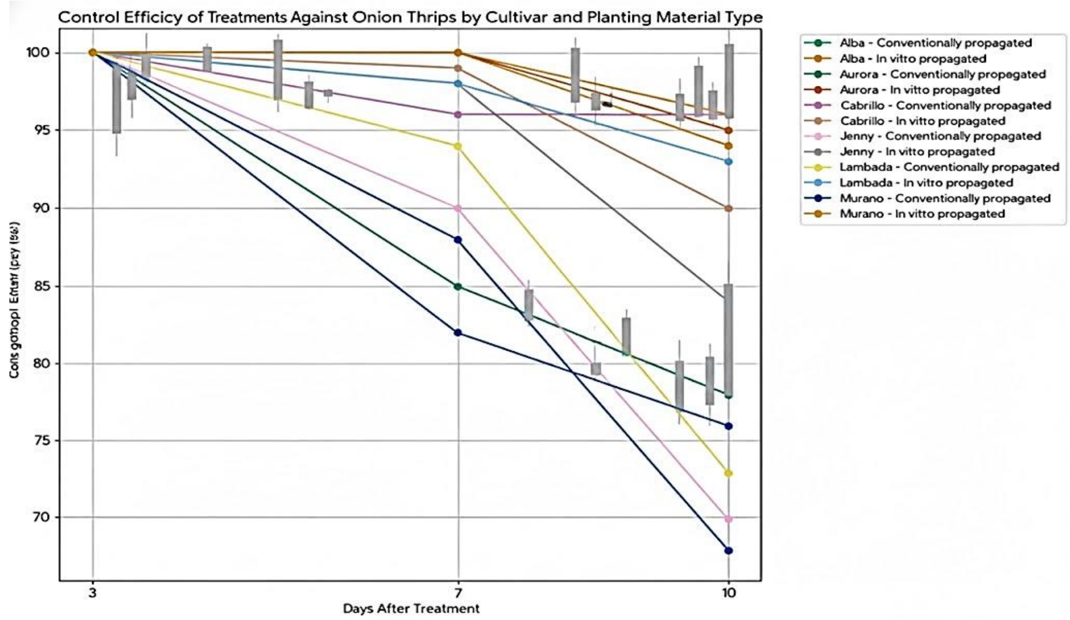


Figure 2. Comparative control efficacy (%) of Imidacloprid (Confidor® 200 SL) against onion thrips (Thrips tabaci) in conventionally and in vitro propagated strawberry cultivars over a 10-day period.

The sustained suppression observed in *in vitro*-derived plants may be attributed to their uniform morphology, higher vigor, and absence of latent biotic stresses, which can enhance pesticide uptake and reduce pest establishment. These traits are crucial for maintaining control against *T. tabaci*, a persistent pest in strawberry cultivation.

Figure 3 shows treatment efficacy against two-spotted spider mite (*Tetranychus urticae*) infestation. Spider mite counts per leaf were recorded over 10 days following treatment, comparing conventionally propagated and *in vitro* propagated strawberry cultivars.

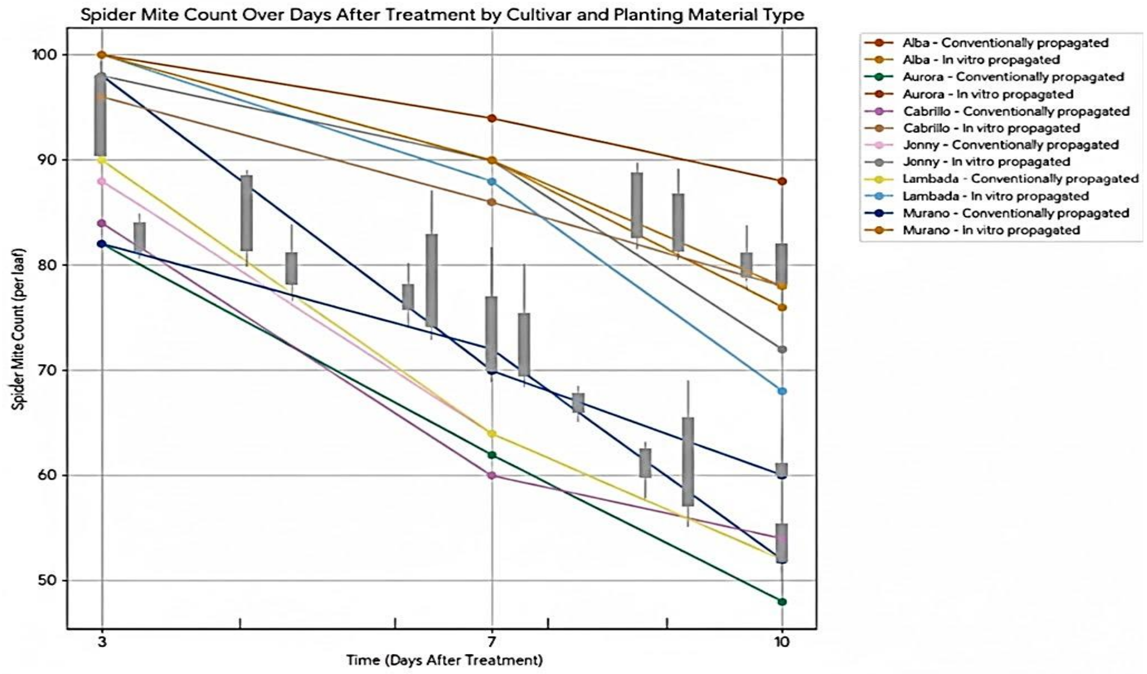


Figure 3. Spider mite count per leaf over 10 days following treatment in conventionally and in vitro propagated strawberry cultivars.

Pest Evaluation: Among the three pests evaluated, the two-spotted spider mite (*Tetranychus urticae*) was the most challenging to manage. Treatment efficacy on day 3 was high, ranging from 82% to 100% across all cultivars and propagation methods; however, control declined over time, particularly in conventionally propagated plants. This pattern reflects the biology of *T. urticae*, characterized by rapid reproduction and a well-documented capacity to develop resistance to pesticides [30-31]. Micropropagated plants consistently exhibited higher and more sustained levels of control across all cultivars. While both propagation types achieved similar efficacy by day 3, conventional plants showed a markedly steeper decline by days 7 and 10. For instance, in the 'Aurora' cultivar, control efficacy fell to 48% in conventional plants by day 10, whereas *in vitro*-derived plants maintained 68–88% suppression.

Pest control outcomes also varied among species: *Aphis gossypii* was most effectively controlled, followed by *Thrips tabaci*, while *T. urticae* remained the most persistent. Previous studies report that micropropagation improves plant vigor and uniformity [32], potentially enhancing pest resistance and increasing pesticide uptake. Additionally, anatomical and physiological differences between plants can influence pest susceptibility [33], suggesting that micropropagation may indirectly contribute to more effective pest management.

Biomorphological Assessment and Yield Performance: *In vitro* propagation significantly enhanced morphological traits and yield performance across all strawberry cultivars (Table 3). Compared with conventional controls, *micropropagated* plants produced more flowers and vegetative organs, yielded more per plant, and had higher average fruit weight ($p < 0.05$, *t-test*).

These improvements align with previous research indicating that micropropagation enhances vegetative vigor, reproductive development, and physiological uniformity [34-35]. Increased floral and vegetative growth likely contributed to better fruit set and nutrient absorption, resulting in higher yields. For instance, Murano and Alba observed significant increases in flower production and vegetative organs, which were linked to higher yields and larger fruit sizes. The average fruit weight increased by 20–83% in *micropropagated* plants, with Cabrillo reaching 78 g per fruit.

Table 3. Biomorphological characteristics and yield performance of strawberry cultivars.

Cultivar	Propagation Method	Flowers per Plant	Vegetative Organs per Plant	Yield per Plant (g)	Average Fruit Weight (g)
Lambada	Conventional	30.3 ± 2.6^{b}	11.6 ± 2.1^{b}	760 ± 76^{b}	18 ± 1.2^{b}
	In vitro	36.1 ± 2.8^{a}	17.4 ± 3.4^{a}	920 ± 81^{a}	25 ± 2.2^{a}
Cabrillo	Conventional	32.2 ± 3.8^{b}	12.8 ± 1.7^{b}	2100 ± 204^{b}	65 ± 5.1^{b}
	In vitro	41.8 ± 4.8^{a}	16.5 ± 1.9^{a}	2800 ± 217^{a}	78 ± 6.3^{a}
Alba	Conventional	25.4 ± 3.7^{b}	12.6 ± 1.8^{b}	750 ± 91^{b}	30 ± 3.3^{b}
	In vitro	39.3 ± 3.9^{a}	20.3 ± 2.1^{a}	1120 ± 101^{a}	55 ± 4.1^{a}
Murano	Conventional	34.4 ± 2.9^{b}	11.4 ± 1.4^{b}	860 ± 65^{b}	25 ± 2.7^{b}
	In vitro	48.1 ± 4.7^{a}	15.0 ± 2.0^{a}	1010 ± 79^{a}	38 ± 3.7^{a}
Jenny	Conventional	32.8 ± 1.9^{b}	14.0 ± 1.6^{b}	780 ± 79^{b}	25 ± 2.9^{b}
	In vitro	44.5 ± 4.7^{a}	18.0 ± 1.9^{a}	1100 ± 101^{a}	45 ± 4.1^{a}
Aurora	Conventional	30.1 ± 2.8^{b}	17.3 ± 2.0^{b}	900 ± 87^{b}	40 ± 3.7^{b}
	In vitro	39.3 ± 3.7^{a}	25.2 ± 2.8^{a}	1200 ± 91^{a}	60 ± 6.2^{a}

Values represent mean ± SE of three replicates ($n = 3$). Different superscript letters within each cultivar and trait indicate significant differences between propagation methods according to the *Student's t*-test ($p < 0.05$).

Such enhancements also promote synchronized crop cycles and facilitate efficient field management, as the uniform plant architecture resulting from *in vitro* propagation supports mechanized operations and consistent harvest timing [34]. Overall, the data confirm that *in vitro* propagation not only ensures clonal fidelity but also confers agronomic advantages, reinforcing its value for high-yield strawberry production.

Fruit Quality: Table 4 presents the fruit quality parameters of strawberry cultivars propagated via conventional and *in vitro* methods. Across all cultivars*, in vitro* propagation consistently resulted in higher total sugar and vitamin C content, while titratable acidity was generally lower. These differences were statistically significant ($p < 0.05$, *t-test*), as indicated by distinct letter groupings

Table 4. Fruit quality parameters of strawberry cultivars propagated via conventional and *in vitro* methods.

Cultivar	Propagation Method	Total Sugars (g / 100 g FW)	Vitamin C (mg / 100 g FW)	Titratable Acidity (g / 100 g FW)
Lambada	Conventional	7.1 ± 0.3^b	58.1 ± 1.7^b	0.85 ± 0.05^a
	In vitro	8.2 ± 0.4^a	62.2 ± 2.0^a	0.72 ± 0.04^b
Cabrillo	Conventional	6.3 ± 0.1^b	65.3 ± 2.1^b	1.10 ± 0.02^a
	In vitro	6.8 ± 0.2^a	70.4 ± 2.3^a	1.01 ± 0.03^a
Alba	Conventional	6.9 ± 0.2^b	64.2 ± 1.6^b	0.68 ± 0.02^a
	In vitro	8.2 ± 0.3^a	68.5 ± 2.0^a	0.61 ± 0.01^b
Murano	Conventional	7.3 ± 0.3^b	72.0 ± 1.5^b	0.64 ± 0.03^a
	In vitro	8.3 ± 0.2^a	76.3 ± 2.1^a	0.56 ± 0.02^b
Jenny	Conventional	6.4 ± 0.2^b	66.2 ± 1.0^b	0.73 ± 0.03^a
	In vitro	6.9 ± 0.1^a	69.1 ± 1.6^a	0.64 ± 0.02^b
Aurora	Conventional	7.2 ± 0.2^b	62.0 ± 1.1^b	0.70 ± 0.03^a
	In vitro	8.3 ± 0.2^a	65.4 ± 1.2^a	0.61 ± 0.04^b

Values represent mean ± SE of three replicates ($n = 3$). Different superscript letters within each cultivar indicate significant differences between propagation methods according to the *Student's t*-test ($p < 0.05$).

In vitro propagation significantly increased total sugars across all cultivars, with mean gains ranging from 0.5 to 1.3 g/100 g FW compared with conventionally propagated plants (Table 4). The highest sugar content was observed in 'Murano' and 'Aurora' (8.3 g/100 g FW each), while the lowest was recorded in 'Cabrillo' (6.8 g/100 g FW). Vitamin C content was also enhanced under *in vitro* conditions, increasing by 3.0–5.1 mg/100 g FW, with 'Murano' reaching the highest value (76.3 mg/100 g FW). Titratable acidity generally decreased in micropropagated fruits (0.07–0.72 g/100 g FW), although 'Cabrillo' maintained relatively high acidity (1.01 g/100 g FW) across propagation methods. These changes increased the sugar-to-acid ratio, producing a sweeter and a more balanced flavor profile, consistent with reported patterns of sugar and organic acid dynamics during strawberry ripening [36–37]. The observed improvements in sugar and vitamin C content may reflect enhanced carbohydrate metabolism and antioxidant biosynthesis under controlled propagation conditions, aligning with current understanding of fruit quality regulation through metabolic, transcriptional, and hormonal control of sugar, organic acid, and ascorbic acid biosynthesis [38–40].

Overall, these results indicate that *in vitro* propagation positively influenced key fruit quality traits, enhancing sweetness and nutritional value while moderating acidity. Improved sugar and vitamin C accumulation may result from better carbohydrate partitioning, stronger source–sink relationships, and increased antioxidant metabolism induced by *in vitro* conditions [37]. In addition to providing genetically uniform, disease-free planting material, *in vitro* propagation improved fruit quality, supporting commercial production and fresh-market acceptance.

Beyond fruit quality, micropropagation conferred notable benefits in plant health and disease resistance. The study demonstrated that *in vitro* propagation significantly enhanced resistance to major fungal pathogens and improved pest control efficacy, providing mechanistic evidence that pathogen-free, physiologically uniform plants supported more effective pesticide uptake and limited pest establishment. Micropropagation further modulated biochemical traits in a cultivar-specific manner, highlighting interactions between propagation method and genotype. Collectively, these findings integrate disease resistance, pest management, yield, and nutritional quality into a single propagation strategy [36–40].

Practical Implications: Certified, pathogen-free micropropagated strawberry plants offer multiple benefits, including increased pathogen resistance, improved pest management, higher yield, and enhanced fruit quality with greater sugar and vitamin C content. These advantages support faster adaptation and rooting, minimize fungicide use, and provide uniform, high-quality planting material. Adoption of *in vitro* propagation can be applied in breeding programs, large-scale nursery operations, and IPM strategies. Overall, this approach provides a scalable pathway for sustainable, high-yield, nutrient-rich strawberry production for both commercial and fresh-market purposes.

Future Directions:

- **Mechanistic studies:** Elucidate physiological and biochemical processes underlying improved pest resistance and fruit quality.
- **Protocol optimization:** Refine tissue culture techniques across cultivars and environmental conditions.
- **Commercial integration:** Incorporate *in vitro* propagation into breeding programs and production pipelines.

These approaches will contribute to the development of propagation and cultivation technologies for high-yield strawberry cultivars, supporting sustainable agriculture and innovations in the field of functional foods.

CONCLUSION

In vitro propagation of strawberry plants enhances the production of high-quality planting material by providing clean, vigorous plants with lower initial pest and disease incidence. Micropropagated plants also demonstrated rapid vegetative growth, improved yield, and superior fruit quality. Although the common spider mite remains a persistent challenge, integrated management strategies were effective in reducing its impact. Biochemical analysis of the fruits showed increased levels of soluble sugars and vitamin C, while acidity remained within a sensorially acceptable range. Overall, *in vitro* propagation represents a reliable approach for producing strong, healthy plants and nutritionally enriched strawberry fruits.

Abbreviations: B. cinerea: *Botrytis cinerea*; P. fragariae: *Phytophthora fragariae*; S. fragariae: *Septoria fragariae*; P. aphanis: *Podosphaera aphanis*; D. earlianum: *Diplocarpon earlianum*; T. urticae: *Tetranychus urticae*; A. gossypii: *Aphis gossypii*; T. tabaci: *Thrips tabaci*; FW: Fresh Weight; SE: Standard Error; t-test: Student's t-test.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Authors' Contributions: AV – conceptualization, study design, supervision, editing; AS – conceptualization, methodology, validation, writing–review and editing; MG – conceptualization, methodology, writing–review and editing; MH – experimental setup, data collection, writing–review and editing; YM – conceptualization, methodology, resources, editing; KD – experimental setup, data collection; KT – experimental setup, data collection, writing–review and editing; AB – data curation, resources, writing–review and editing; AA – data curation, resources; MM – writing–review and editing; AM – writing–review and editing; GM – data curation, biochemical analysis, writing–original draft preparation, writing–review and editing. All authors read and approved the final manuscript.

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