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Research Article
Comparative Growth of Red *Chrysanthemum* Plantlets in Micropropagation and Microponic Systems

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Abstract

Background and Objective: *Chrysanthemum* is one of the most important ornamental plants worldwide, yet conventional propagation methods remain inefficient; therefore, microponics (a system integrating micropropagation with hydroponics) offers strong potential for producing high-quality planting materials at scale. This study evaluates the efficiency of microponic and micropropagation systems for *in vitro* propagation of the red cultivar of *Chrysanthemum*× *morifolium*, focusing on optimizing medium composition, plant growth regulator levels, medium volume and culture density and comparing plantlet performance during nursery acclimatization. **Materials and Methods:** *In vitro* shoots, measuring 1-1.5 cm in length and consisting of two nodes, were used as explants. Experiments were arranged in a completely randomized design with five replicates. Data were statistically analyzed using Analysis of Variance (ANOVA) with IRRISTAT 5.0, SPSS 20.0 and Microsoft Excel to identify the optimal culture conditions for each system and to compare growth parameters of plantlets during nursery conditions with treatment means compared by DMRT at the 5% significance level. **Results:** The optimal conditions for the microponic system were obtained using liquid 1/2MS medium supplemented with 0.1 ppm naphthaleneacetic acid (NAA), with a medium volume of 20 mL and a culture density of nine shoots per vessel. Under these conditions, plantlets exhibited the highest shoot height (2.03 cm), fresh mass (0.17 g), 100% root initiation, 3.89 roots per explant (0.64 cm in length) and 4.26 leaves after two weeks of culture. In contrast, the optimal micropropagation medium was solid MS supplemented with 0.3 ppm NAA, 0.5 g/L activated charcoal (AC), 30 g/L sucrose, with the same culture density that used in the microponic system. Plantlets in this system reached a shoot height of 3.14 cm, a fresh mass of 0.20 g, 100% root initiation, 4.87 roots (3.44 cm in length) and five leaves. After 90 days in the nursery, micropropagated plantlets exhibited greater height, a higher number of leaves and earlier flowering, whereas microponic-derived plantlets developed larger leaves, thicker shoots and wider canopy diameters. **Conclusion:** The micropropagation system provided optimal conditions for *in vitro* shoot growth and promoted earlier flowering, while the microponic system improved acclimatization and post-transplant performance of red cultivar of *Chrysanthemum*× *morifolium* plantlets.

Key words: Canopy diameter, microponic system, micropropagation, red *Chrysanthemum*, shoot diameter

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chrysanthemum (*Chrysanthemum*×*morifolium*), commonly known as "the Queen of the East", is one of the most important ornamental plants worldwide. It is widely cultivated for both the cut flower and potted plant markets due to its diverse forms, attractive colors and long vase life. *Chrysanthemum* can be propagated through both sexual and asexual methods[1]. However, commercial propagation is predominantly achieved through vegetative means such as root suckers or terminal cuttings. Although these conventional methods are simple and inexpensive, they are often slow and highly susceptible to viral and microbial infections, which results in reduced yield and plant quality[2].

The *in vitro* culture method has been developed to address the increasing demand for the production of disease-free, genetically uniform and rapidly propagated plantlets. These systems have proven effective in producing high-quality plant materials with stable genetic fidelity and reliable microbial control. However, these systems still face several limitations, including high production costs, contamination risks and poor acclimatization ability of plantlets after transfer to *ex vitro* conditions. These challenges mainly arise because *in vitro*-grown plantlets often exhibit low physiological quality-characterized by thin leaves, underdeveloped stomata, a weak cuticular layer, high humidity and limited CO_2 availability during culture[2,3].

To overcome these constraints, the microponic system integrated approach combining micropropagation and hydroponics-offers a promising alternative. This system reduces the reliance on gelling agents such as agar, enhances gas exchange, provides more uniform nutrient distribution and promotes natural root development, thereby minimizing physiological stress and improving survival rates during post-*in vitro* acclimatization.

Only a limited number of studies have investigated the microponic system to date. The research group led by Hahn was among the first to introduce and develop this system for *Chrysanthemum* in 1998 and 2000, as reported by Nhut *et al*.[4]. Since then, several studies in Vietnam have demonstrated the potential of the microponic system for *Chrysanthemum* propagation. Nhut *et al*.[4] found that pretreatment with NAA combined with the microponic system significantly promoted shoot and root formation, resulting in higher plantlet survival rates. Building upon these findings, Tung *et al*.[5] reported that microponic culture under LED illumination enhanced shoot elongation, fresh biomass accumulation and chlorophyll content compared with conventional micropropagation. In a subsequent study by Tung *et al*.[6], further demonstrated that *Chrysanthemum* plantlets derived from the microponic system exhibited superior vegetative growth and earlier flowering under both greenhouse and field conditions.

Beyond *Chrysanthemum*, the advantages of the microponic system have also been demonstrated in other species. For instance, Vo and Ngo[7] showed that combining microponics with auxin pretreatment enhanced root development, leaf expansion and overall plant vigor in *Hibiscus sagittifolius*, while significantly improving survival rates during *ex vitro* acclimatization. Additionally, Tung *et al*.[8] expanded the application of the microponic system to other crops, including carnation, strawberry, potato and gloxinia. Collectively, these findings highlight the broad applicability and effectiveness of the microponic system for large-scale propagation of various economically important ornamental and medicinal plants.

Despite these promising results, comparative studies assessing the efficiency of microponic and micropropagation systems for *Chrysanthemum* production remain limited. Therefore, this study was conducted to evaluate the effectiveness of these two asexual propagation systems in the red cultivar of *Chrysanthemum*×*morifolium*.

MATERIALS AND METHODS

Plant material: *In vitro* shoots of the red cultivar of *Chrysanthemum*×*morifolium*, approximately 10 cm in height, were subcultured at the Faculty of Agronomy, Vietnam National University of Agriculture (VNUA), Ngo Xuan Quang, 12400, Hanoi, Vietnam, from January, 2023 to June, 2024.

Medium and culture systems

Microponic system: Polypropylene tubes with a 1.2 cm diameter and 24 cm length were cut into 1 cm segments. The microponic nutrient solution consisted of MS (Murashige and Skoog)[9] medium without sugar, adjusted to pH 5.8 and used without autoclaving.

Micropropagation medium: The micropropagation medium consisted of MS medium supplemented with 30 g/L sucrose and 6.5 g/L agar, adjusted to pH 5.8 and autoclaved.

Methods: The experiments were designed and modified based on previously established protocols by Nhut *et al*.[5] and Tung *et al*.[8], as well as on practical conditions of the laboratory.

In vitro shoots of *Chrysanthemum* were excised into cuttings, each containing two nodes (1.2-1.5 cm in length). Leaves at the basal parts of the cuttings were removed. Other factors were adjusted according to each experiment. All experiments were arranged in a completely randomized design with five replicates.

Growth characteristics of *Chrysanthemum* plantlets in a microponic system: Effect of NAA pre-treatment or treatment on the growth of *Chrysanthemum* cultured in a microponic system: The basal parts of the cuttings were pretreated by dipping them in a 500 ppm NAA solution for 20 min. Cuttings treated with water served as the control. Other treatments were supplemented directly with NAA at different concentrations (0.1, 0.3 and 0.5 ppm). Six *in vitro* shoots were placed in each vessel, with 20 mL of MS culture medium per vessel. The best treatment was used for the subsequent experiments.

Effect of nutrient solutions on the growth of *Chrysanthemum* cultured in a microponic system: Four types of nutrient media were used to evaluate the growth of *Chrysanthemum*: 1/2MS (macronutrients reduced by half), MS, MS1/4 and MS1/2 (both macro- and micronutrients reduced proportionally). All media were supplemented with 0.1 ppm NAA. Six *in vitro* shoots were placed in each vessel containing 20 mL of medium. The best-performing medium was used for subsequent experiments.

Effect of medium volume on the growth of *Chrysanthemum* cultured in a microponic system: Shoots were cultured in the selected medium with four different volumes (10, 15, 20 and 30 mL). Six *in vitro* shoots were placed in each culture vessel. The optimal medium volume was used for the subsequent experiments.

Effect of culture density on the growth of *Chrysanthemum* cultured in a microponic system: *In vitro* shoots were cultured in the selected medium at four different densities (6, 9, 12 and 15 shoots per vessel). The optimal culture density was used for the subsequent experiments.

Growth characteristics of *Chrysanthemum* plantlets in a micropropagation system: Effect of NAA or AC on the growth of *Chrysanthemum* in a micropropagation system: Six *in vitro* shoots were cultured in vessels containing 30 mL of MS solid medium supplemented with different concentrations of NAA (0.1, 0.3, 0.5 and 0.7 ppm) or different concentrations of AC (0.1, 0.3, 0.5 and 1 g/L). The MS medium without plant growth regulators served as the control. The optimal NAA or AC concentration was used for subsequent experiments.

Effect of NAA and AC combination on the growth of *Chrysanthemum* in a micropropagation system: The MS medium was used to culture *in vitro* shoots at a density of six shoots per vessel. The experiment included six treatments, each containing a unique combination of NAA and AC concentrations. Control treatments contained either 0.3 ppm NAA or 0.5 g/L AC, while the other four treatments contained 0.3 ppm NAA combined with 0.1, 0.3, 0.5 or 1 g/L AC, respectively.

Effect of nutrient medium on the growth of *Chrysanthemum* in a micropropagation system: Six *in vitro* shoots were cultured in vessels containing 30 mL of four different solid nutrient media: 1/2MS, MS, MS1/4 and MS1/2. The best-performing nutrient medium was used for subsequent experiments.

Effect of culture density on the growth of *Chrysanthemum* in a micropropagation system: *In vitro* shoots were cultured in the selected medium at four different densities (6, 9, 12 and 15 shoots per vessel). The optimal culture density was used for subsequent experiments.

Growth of *Chrysanthemum* in microponic versus micropropagation systems

Microponic system: *In vitro* shoots at the selected culture density were grown in the optimized nutrient solution supplemented with 0.1 ppm NAA, pH 5.8.

Micropropagation system: *In vitro* shoots at the selected culture density were grown in the optimized solid medium supplemented with 30 g/L sucrose, 6.5 g/L agar, 0.3 ppm NAA and 0.5 g/L AC, pH 5.8.

Culture conditions

***In vitro*:** Cultures were maintained at 25±2°C, 75-80% relative humidity, under a Photosynthetic Photon Flux Density (PPFD) of 40-45 µmol/m^2/sec with a 16 hrs photoperiod.

In the nursery: The experiment was conducted in a net house at temperatures ranging from 12-20°C and relative humidity of 70-80% under natural light. Plantlets were acclimatized in the nursery of VNUA. The growing substrate was peat moss. After 30 days, plantlets were transferred to pots measuring 15×13 cm.

Data collection

***In vitro* experiments:** Data were recorded after two weeks of culture, including shoot height, fresh mass, root initiation, number of roots per explant, root length, number of leaves, leaf length and leaf width.

***Ex vitro* experiments:** Data were recorded after 30, 60 and 90 days of nursery growth, including survival rate, shoot height, shoot diameter, canopy diameter, number of leaves, leaf length, leaf width and number of flower buds.

Data analysis: The recorded data were obtained from 15 randomly selected samples for each treatment. Data were subjected to Analysis of Variance (ANOVA) using IRRISTAT 5.0, SPSS 20.0 and Microsoft Excel. Treatment means were compared using Duncan's Multiple Range Test (DMRT) at the 5% significance level.

RESULTS AND DISCUSSION

Growth and development characteristics of *Chrysanthemum* plantlets in a microponic system

Effect of NAA pre-treatment or treatment on the growth of *Chrysanthemum* cultured in a microponic system: After two weeks of culture, *in vitro* shoots of the red cultivar of *Chrysanthemum*× *morifolium* grown on medium supplemented with 0.1 ppm NAA exhibited superior rooting efficiency and growth compared to explants pretreated with distilled water or 500 ppm NAA solution.

Root initiation was generally high across all treatments. As shown in Table 1, the highest rooting percentage was obtained in the microponic medium supplemented with 0.1 ppm NAA (100%), with 5.13 roots per explant. In contrast, explants pretreated with 500 ppm NAA solution showed the lowest response, with 88.67% rooting and 3.11 roots per explant. These differences were statistically significant.

A significant effect of NAA concentration on root number was observed. As the NAA concentration in the culture medium increased, a linear decrease in rooting performance was detected, indicating that higher concentrations (0.3 and 0.5 ppm) inhibited root formation and growth of *Chrysanthemum* plantlets.

Compared with the other treatments, shoots pretreated with distilled water produced the longest roots (1.25 cm), followed closely by explants cultured on medium containing 0.1 ppm NAA (1.24 cm). Both results were significantly different from those of plantlets pretreated with 500 ppm NAA and those cultured on media supplemented with 0.3 and 0.5 ppm NAA. The poorest response was observed in explants pretreated with 500 ppm NAA solution, which produced roots averaging only 0.15 cm in length (Table 1).

Data presented in Table 1 and Fig. 1 also showed that explants pretreated with distilled water produced the tallest shoots (2.00 cm), whereas those cultured on medium supplemented with 0.5 ppm NAA exhibited the shortest shoot height (1.41 cm). These differences were statistically significant ($p<0.05$). Similarly, explants pretreated with distilled water developed the greatest number of leaves (3.57 leaves per shoot) and the largest leaf size (1.49 cm in length and 1.43 cm in width), while those grown on medium supplemented with 0.5 ppm NAA produced the smallest leaves (1.18 cm in length and 1.19 cm in width). The differences among treatments were statistically significant.

Interestingly, in the treatment where plantlets were pretreated with distilled water and cultured in hormone-free microponic medium, root formation was still observed (Table 1 and Fig. 1). This indicated that the endogenous auxin level in *in vitro* shoots was sufficient to induce root initiation. Therefore, only a low concentration of NAA is required to optimize root formation in the microponic system. Increasing the NAA concentration in the microponic medium or pre-treatment with high NAA concentration, however, resulted in the inhibition of root formation and a reduction in shoot height and leaf size (Table 1).

These findings suggest that low NAA concentrations promote root induction and shoot elongation, whereas higher concentrations inhibit root initiation and suppress shoot and leaf development. Similar results have been reported by Srilakshmi *et al.*[1], Daffalla *et al.*[10] and Trang and Ha[11], in micropropagation systems. This phenomenon can be explained by the hormonal balance theory, in which auxin at optimal levels stimulates cell differentiation and elongation and acts synergistically with cytokinins to regulate growth. However, excessive exogenous auxin disrupts the endogenous auxin-to-cytokinin ratio and alters signal transduction pathways, thereby inhibiting cell division and elongation in shoots and leaves and may even induce tissue toxicity or oxidative stress[2,12].

Effect of nutrient solution on the growth of *Chrysanthemum* cultured in a microponic system: The culture medium should contain only the minimum amount of nutrients necessary to reduce water and chemical stress on plantlets, particularly during the early stages of culture, while minimizing nutrient costs and potential environmental pollution caused by medium waste[13]. Therefore, controlling the nutrient concentration in the culture medium is an important method for regulating plantlets' growth.

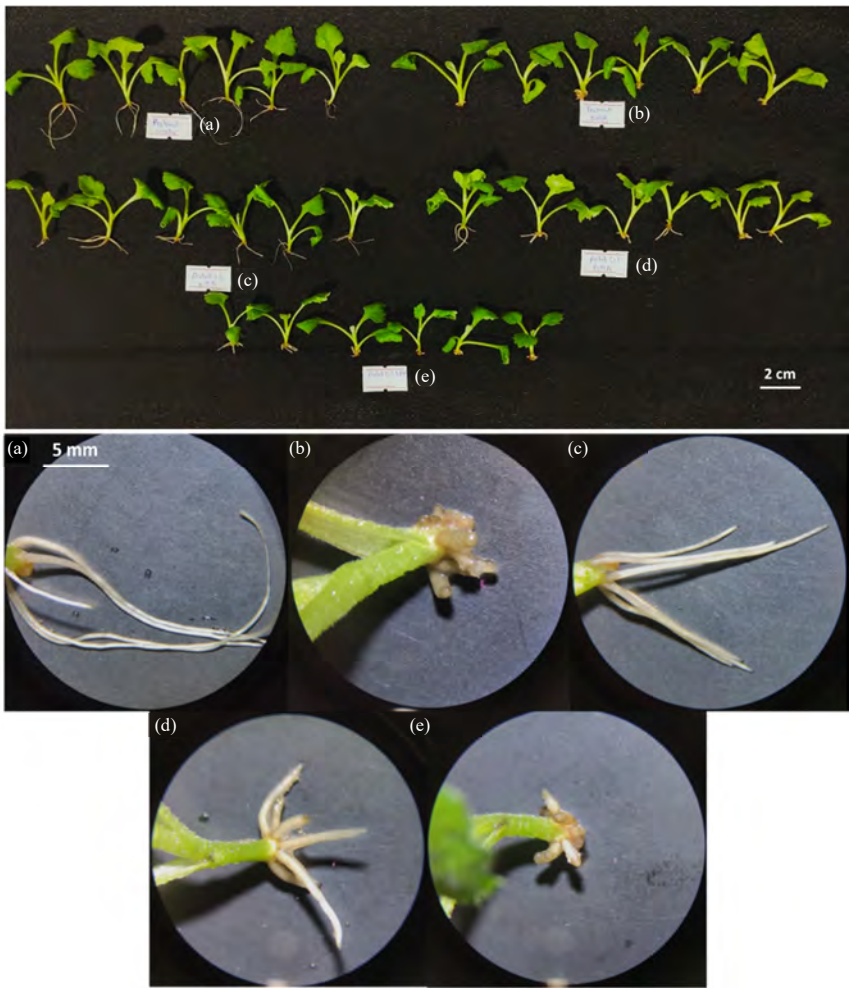

Fig. 1(a-e): Shoots and roots of *Chrysanthemum* plantlets cultured in a microponic system with NAA pre-treatment or treatment after two weeks of culture, (a) Pre-treated with distilled water, (b) Pre-treated with 500 ppm NAA, (c) 0.1 ppm NAA, (d) 0.3 ppm NAA and (e) 0.5 ppm NAA

Table 1: Growth responses of *Chrysanthemum* plantlets to NAA pre-treatment and treatment in a microponic system after two weeks of culture

Treatments	Shoot height (cm)	Fresh mass (g)	Root initiation (%)	Number of roots/ explant (roots)	Root length (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)
Pre-treated with distilled water	1.65^{b}	0.14^{a}	100.00^{a}	4.20^{ab}	1.25^{a}	3.57^{a}	1.49^{a}	1.43^{a}
Pre-treated with 500 ppm NAA	1.55^{c}	0.13^{ab}	88.67^{a}	3.11^{b}	0.15^{b}	3.35^{a}	1.18^{b}	1.19^{c}
0.1 ppm NAA	2.00^{a}	0.11^{bc}	100.00^{a}	5.13^{a}	1.24^{a}	3.27^{ab}	1.41^{a}	1.38^{ab}
0.3 ppm NAA	1.57^{c}	0.11^{c}	100.00^{a}	4.93^{a}	0.54^{b}	3.27^{ab}	1.27^{b}	1.29^{bc}
0.5 ppm NAA	1.41^{d}	0.09^{c}	90.00^{a}	4.50^{ab}	0.22^{b}	2.97^{b}	1.18^{b}	1.19^{c}
CV (%)	3.10	12.70	11.50	20.30	46.00	6.10	5.80	7.40
$LSD_{0.05}$	0.07	0.02	14.71	1.19	0.42	0.27	0.10	0.13

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's test

According to Table 2 and Fig. 2, there were no statistically significant differences in shoot height among the four treatments. However, significant differences were observed in root formation. Explants cultured in 1/2MS medium produced the highest number of roots per explant (5.12 roots) and the longest roots (1.21 cm), which were statistically different from the other treatments. In contrast, explants grown in full-strength MS medium showed the lowest response, with only 3.27 roots per explant and the shortest roots (0.31 cm). These findings are consistent with the results of Nhut *et al.*[4], who reported that a high concentration of mineral nutrients was not suitable for the growth and development of *Chrysanthemum* cuttings. For some plant species, the mineral concentration in full-strength MS medium may be excessive and even detrimental to cultured tissues.

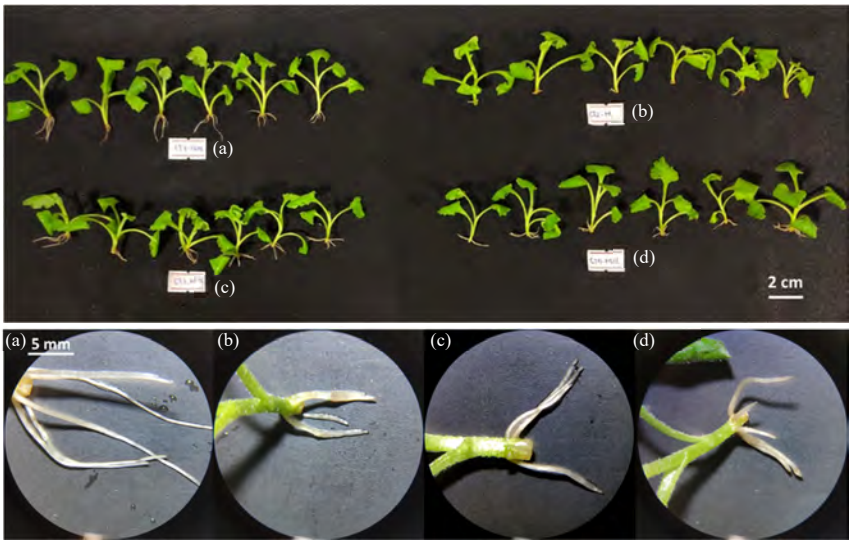

Fig. 2(a-d): Shoots and roots of *Chrysanthemum* cultured in a microponic system under different nutrient solutions after two weeks of culture, (a) 1/2MS, (b) MS (c) MS1/4 and (d) MS1/2

Table 2: Effect of nutrient solutions on the growth of *Chrysanthemum* cultured in a microponic system after two weeks of culture

Nutrient solutions	Shoot height (cm)	Fresh mass (g)	Root initiation (%)	Number of roots/ explant (roots)	Root length (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)
1/2MS	1.91^{a}	0.17^{a}	100.00^{a}	5.12^{a}	1.21^{a}	3.90^{ab}	1.61^{ab}	1.54^{b}
MS	1.76^{a}	0.17^{a}	100.00^{a}	3.27^{c}	0.31^{d}	4.77^{a}	1.70^{a}	1.60^{a}
MS1/4	1.79^{a}	0.15^{b}	100.00^{a}	4.63^{b}	0.94^{b}	3.73^{ab}	1.54^{b}	1.48^{b}
MS1/2	1.89^{a}	0.15^{b}	100.00^{a}	4.80^{ab}	0.75^{c}	3.83^{b}	1.58^{b}	1.51^{b}
CV (%)	5.60	7.80	0.00	6.30	11.10	4.40	4.50	3.20
$LSD_{0.05}$	0.14	0.02	0.00	0.12	0.23	0.10	0.07	0.02

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's test

Data presented in Table 2 also showed significant differences in fresh mass between plantlets grown on 1/2MS and MS media (0.17 g) compared with those cultured on MS1/4 and MS1/2 media (0.15 g). This indicates that decreasing the nutrient concentration in the MS medium led to slower growth of *Chrysanthemum* explants.

Based on all evaluated parameters, the 1/2MS medium was identified as the most appropriate for achieving 100% root initiation, optimal shoot height (1.91 cm), the highest number of roots per explant (5.12 roots) and the longest roots (1.21 cm). These results are consistent with those of Nhut *et al.*[4], who reported that in *in vitro* rooting of *Chrysanthemum*, the best response was obtained when microshoots were cultured on 1/2MS medium.

Effect of medium volume on the growth of *Chrysanthemum* cultured in a microponic system: As part of the evaluation of *Chrysanthemum* growth, different culture medium volumes (10, 15, 20 and 30 mL) were tested to determine the optimal conditions for plantlet development. The results presented in Table 3 and Fig. 3 indicate that plantlets cultured in 20 mL of medium exhibited the best growth performance. This treatment was significantly superior to the others in terms of shoot height (2.12 cm), fresh mass (0.17 g), number of roots (4.10 roots per plant), number of leaves (4.27 leaves per shoot), leaf length (1.69 cm) and leaf width (1.56 cm).

Data presented in Table 3 revealed that explants cultured in 10 mL of 1/2MS medium exhibited the lowest rooting efficiency (96.67%) and their growth parameters-including shoot height, fresh mass and the number of roots and leaves were significantly lower than those of other treatments. This reduction in growth may be attributed to the limited nutrient availability associated with the smaller medium volume, which restricted nutrient uptake and consequently decreased overall growth performance. Similar results have been reported in previous studies on *Chrysanthemum* spp.[14].

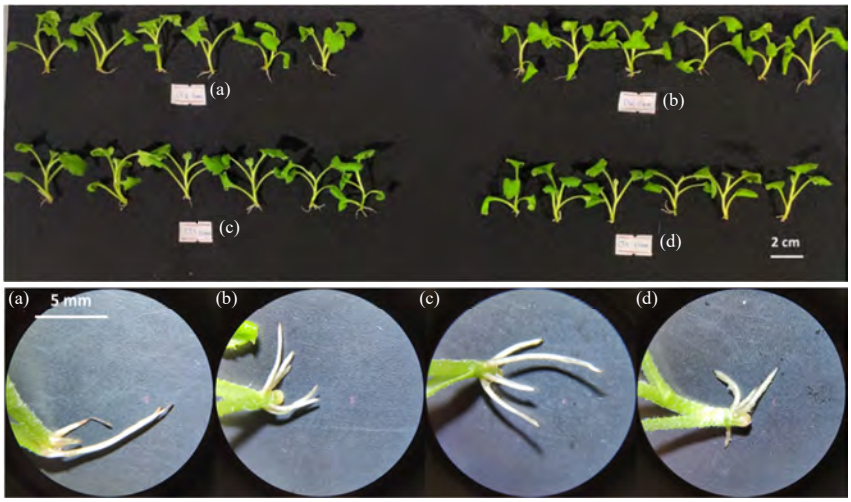

Fig. 3(a-d): Shoots and roots of *Chrysanthemum* cultured in a microponic system under different medium volumes after two weeks of culture, (a) 10 mL, (b) 15 mL, (c) 20 mL and (d) 30 mL

Table 3: Effect of medium volume on the growth of *Chrysanthemum* cultured in a microponic system after two weeks of culture

Medium volume (mL)	Shoot height (cm)	Fresh mass (g)	Root initiation (%)	Number of roots/ explant (roots)	Root length (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)
10	1.91^{c}	0.13^{b}	96.67^{a}	3.67^{a}	0.66^{a}	3.60^{c}	1.38^{b}	1.40^{b}
15	1.96^{bc}	0.14^{ab}	100.00^{a}	3.83^{a}	0.43^{b}	4.16^{ab}	1.43^{b}	1.46^{ab}
20	2.12^{a}	0.17^{a}	100.00^{a}	4.10^{a}	0.53^{ab}	4.27^{a}	1.69^{a}	1.56^{a}
30	2.07^{ab}	0.15^{ab}	100.00^{a}	3.30^{a}	0.37^{b}	3.93^{b}	1.45^{b}	1.42^{b}
CV (%)	10.90	5.10	2.80	17.20	35.70	5.80	6.80	6.10
$LSD_{0.05}$	0.14	5.14	0.88	0.24	0.32	0.14	0.12	0.02

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's test

However, increasing the medium volume to 30 mL resulted in reduced plant growth (Table 3 and Fig. 3). This decline could be explained by the partial submersion of shoots in the medium, which impeded gas exchange and reduced root respiration, ultimately leading to a lower number of roots and poor root system development. Similar effects of vessel ventilation, dissolved oxygen and gas-exchange rates on plantlet morphology and vigor have been reported in recent studies[15,16].

Effect of culture density on the growth of *Chrysanthemum* cultured in a microponic system: Culture density plays a crucial role in the growth of plantlets cultured in a microponic system. When an excessive number of shoots are cultured in the same vessel, plantlets tend to become stunted and exhibit slow growth, requiring more care and effort during the acclimatization stage. Conversely, culturing explants too sparsely may lead to inefficient use of the culture medium and reduced economic effectiveness.

In this experiment, different culture densities (6, 9, 12 and 15 shoots per vessel) were tested in 1/2MS medium supplemented with 0.1 ppm NAA at pH 5.8. Observations were recorded after two weeks of culture.

As shown in Table 4 and Fig. 4, *Chrysanthemum* growth varied significantly among the four culture densities. In terms of root initiation, treatments with 6 and 9 shoots per vessel achieved the highest rooting rates (100%), whereas the highest density treatment (15 shoots per vessel) showed the lowest value (89.33%), with statistically significant differences. The greatest number of roots was obtained in the 6 shoots per vessel treatment, with an average of 4.47 roots per plantlet, while the 15 shoots per vessel treatment produced the fewest roots (2.93 roots per plantlet).

An increase in culture density from 6 to 9 shoots per vessel improved shoot height and root length, increasing from 2.01 to 2.03 cm and from 0.56 to 0.64 cm, respectively. The treatment with 9 shoots per vessel produced the tallest shoots, differing significantly from the other densities. However, further increases in culture density (12-15 shoots per vessel) resulted in decreased growth parameters, including shoot height, fresh mass, root number and length and leaf size.

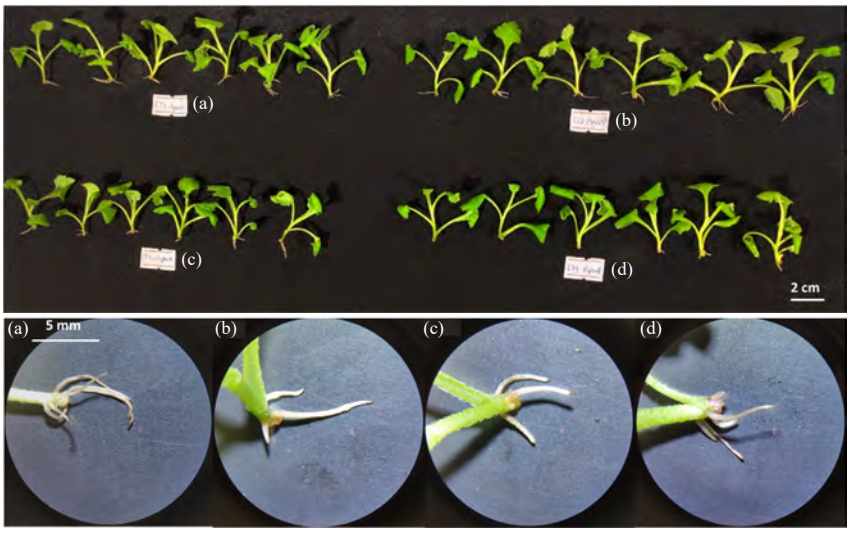

Fig. 4(a-d): Shoots and roots of *Chrysanthemum* cultured in a microponic system under different culture densities after two weeks of culture, (a) 6 shoots/vessel, (b) 9 shoots/vessel, (c) 12 shoots/vessel and (d) 15 shoots/vessel

Table 4: Effect of culture densities on the growth of *Chrysanthemum* cultured in a microponic system after two weeks of culture

Culture densities (shoots/vessel)	Shoot height (cm)	Fresh mass (g)	Root initiation (%)	Number of roots/ plantlet (roots)	Root length (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)
6	2.01^{ab}	0.17^{a}	100.00^{a}	4.47^{a}	0.56^{a}	4.70^{a}	1.42^{a}	1.45^{ab}
9	2.03^{a}	0.17^{a}	100.00^{a}	3.89^{ab}	0.64^{a}	4.26^{b}	1.44^{a}	1.49^{a}
12	2.00^{ab}	0.16^{a}	95.00^{ab}	3.40^{b}	0.60^{a}	3.93^{bc}	1.38^{a}	1.38^{bc}
15	1.92^{b}	0.16^{a}	89.33^{b}	2.93^{c}	0.48^{a}	3.87^{c}	1.34^{a}	1.32^{c}
CV (%)	3.20	16.90	5.40	9.20	23.50	6.10	5.30	4.60
$LSD_{0.05}$	0.09	0.04	7.17	0.46	0.18	0.35	0.10	0.09

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's test

As shown in Table 4, poor performance was observed in the treatment with 15 shoots per vessel, which produced an average shoot height of 1.92 cm, 2.93 roots, a root length of 0.48 cm and 3.87 leaves per plantlet. This reduction in growth may be attributed to excessive culture density, which likely caused competition for nutrients and light among the shoots, leading to uneven growth and reduced rooting ability. These findings are consistent with those reported by Hamad[14].

Growth characteristics of *Chrysanthemum* plantlets in a micropropagation system

Effect of NAA or AC on the growth of *Chrysanthemum* cultured in a micropropagation system: In this experiment, NAA was tested at concentrations ranging from 0 to 0.7 ppm and AC was used at concentrations from 0 to 1 g/L to evaluate the growth of *Chrysanthemum*.

As shown in Table 5 and Fig. 5, plantlets cultured in the medium without NAA and AC produced an average of 4.43 roots per plantlet, with a maximum root length of 2.93 cm. However, these roots were thin, fragile and pale white, indicating poor structural quality. Root lengths across all treatments ranged from 1.12 to 3.20 cm.

Media containing either NAA or AC generally promoted greater root formation than the other treatments, except for the medium supplemented with 0.1 g/L AC. Root quality in these treatments was also superior. Supplementation with 0.3 ppm NAA or 0.5 g/L AC yielded the best results, producing 5.23 and 5.27 roots per plantlet, respectively.

As the NAA concentration increased, root initiation, root length and shoot height gradually declined. Plantlets cultured in the medium supplemented with 0.3 ppm NAA exhibited the greatest shoot height (3.27 cm) and the highest number of leaves (5.27 leaves per plantlet). In contrast, higher NAA concentrations (0.7 ppm) resulted in significantly poorer growth performance (Table 5). Plantlets in the 0.7 ppm NAA treatment displayed a shoot height of 2.82 cm, a root initiation rate of 93.33% and only 3.53 roots with an average root length of 1.12 cm, all of which were significantly lower than those of the other treatments.

The results presented in Table 1 and 5, together with the findings from previous publications[1,10,11], indicate that the physiological response to NAA varies depending on the plant genotype, tissue type and culture system.

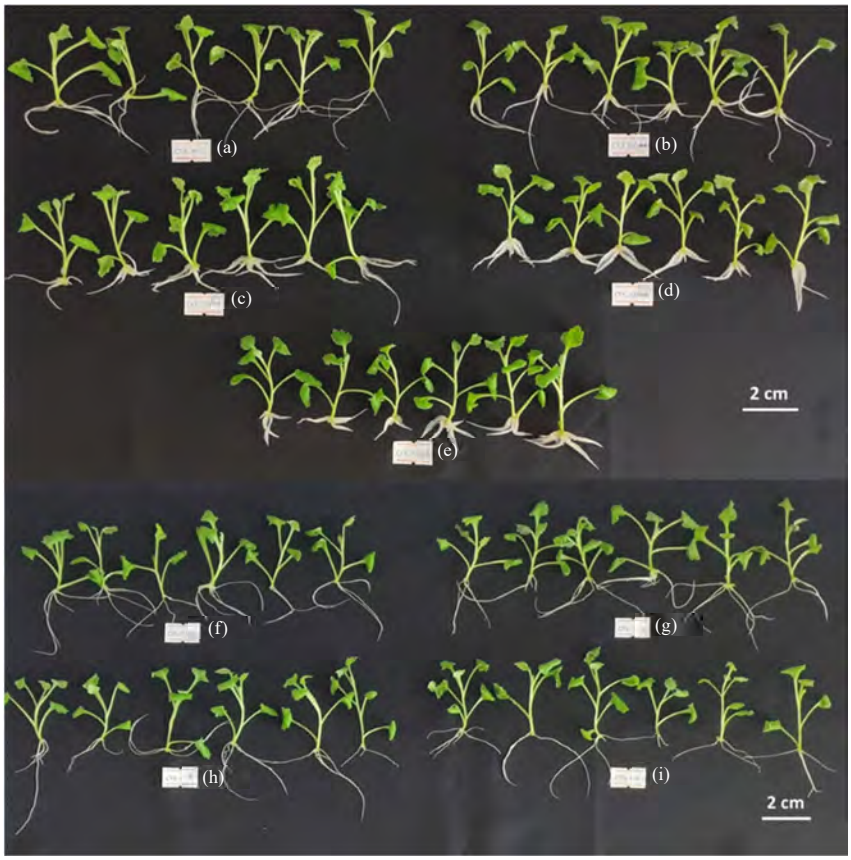

Fig. 5(a-i): Plantlets of *Chrysanthemum* in a micropropagation system with NAA or AC treatments after 2 weeks of culture, (a) MS, (b) MS+0.1 ppm NAA, (c) MS+0.3 ppm NAA, (d) MS+0.5 ppm NAA, (e) MS+0.7 ppm NAA, (f) MS+0.1 g/L AC, (g) MS+0.3 g/L AC, (h) MS+0.5 g/L AC and (i) MS+1.0 g/L AC

Table 5: Effect of NAA or AC on the growth of *Chrysanthemum* cultured in a micropropagation system after two weeks of culture

Treatments	Shoot height (cm)	Fresh mass (g)	Root initiation (%)	Number of roots/ explant (root)	Root length (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)
0 ppm NAA+0 g/L AC	2.40^{c}	0.19^{bc}	100.00^{a}	4.43^{ab}	2.93^{a}	4.50^{c}	1.05^{abc}	1.03^{abc}
0.1 ppm NAA	2.34^{c}	0.25^{a}	100.00^{a}	5.03^{a}	2.96^{a}	3.93^{d}	1.11^{abc}	1.12^{ab}
0.3 ppm NAA	3.27^{a}	0.26^{a}	100.00^{a}	5.23^{a}	2.02^{b}	5.27^{a}	1.20^{a}	1.14^{ab}
0.5 ppm NAA	2.84^{b}	0.26^{a}	96.67^{ab}	4.27^{ab}	1.45^{bc}	4.97^{abc}	0.99^{bc}	0.98^{bc}
0.7 ppm NAA	2.82^{b}	0.24^{ab}	93.33^{b}	3.53^{b}	1.12^{c}	4.87^{abc}	0.94^{c}	0.93^{c}
0.1 g/L AC	2.25^{c}	0.15^{c}	100.00^{a}	4.97^{a}	3.11^{a}	4.57^{bc}	1.13^{abc}	1.07^{abc}
0.3 g/L AC	2.79^{b}	0.15^{c}	100.00^{a}	5.10^{a}	3.20^{a}	5.17^{a}	1.13^{ab}	1.16^{ab}
0.5 g/L AC	2.99^{b}	0.15c	100.00^{a}	5.27^{a}	3.13^{a}	5.33^{a}	1.17^{ab}	1.20^{a}
1 g/L AC	2.83^{b}	0.15c	100.00^{a}	4.65^{a}	2.83^{a}	5.09^{ab}	1.07^{abc}	1.03^{abc}
CV (%)	9.40	18.90	4.10	14.70	19.80	8.20	12.40	11.80
$LSD_{0.05}$	0.33	0.05	5.19	0.89	0.64	0.51	0.17	0.16

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's test

Data in Table 5 also revealed that explants grown in media containing AC showed enhanced root initiation, root number and root length, confirming that AC has a strong positive effect on rooting in *Chrysanthemum*. The best root formation was obtained when 0.5 g/L of AC was added to the medium. However, further increasing the AC concentration to 1.0 g/L led to a decline in plant growth (Table 5 and Fig. 5). These findings are consistent with previous studies[11,17-19]. Accoding to Thomas[20] and Buckseth *et al.*[21], activated charcoal can alter the light environment by darkening the medium, thereby promoting root initiation and growth. Moreover, AC can adsorb inhibitory compounds and toxic metabolites, reducing phenolic oxidation and browning in the medium.

Fig. 6(a-f): Plantlets of *Chrysanthemum* cultured in a micropropagation system with combined NAA and AC treatments after two weeks of culture, (a) 0.3 ppm NAA, (b) 0.5 g AC, (c) 0.3 ppm NAA+0.1 g/L AC, (d) 0.3 ppm NAA+0.3 g/L AC, (e) 0.3 ppm NAA+0.5 g/L AC and (f) 0.3 ppm NAA+1.0 g/L AC

Table 6: Effect of the combination of NAA and AC on the growth of *Chrysanthemum* cultured in a micropropagation system after two weeks of culture

Treatments	Shoot height (cm)	Fresh mass (g)	Root initiation (%)	Number of roots/ plantlet (roots)	Root length (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)
0.3 ppm NAA	2.64^{a}	0.32^{a}	100.00^{a}	3.73^{c}	2.67^{c}	4.14^{b}	1.28^{a}	1.21^{a}
0.5 g/L AC	2.32^{a}	0.18^{a}	100.00^{a}	4.80^{ab}	3.49^{b}	4.37^{ab}	1.29^{a}	1.17^{ab}
0.3 ppm NAA+0.1 g/L AC	2.30^{a}	0.21^{a}	100.00^{a}	4.43^{ab}	3.65^{ab}	4.13^{b}	1.21^{a}	1.14^{ab}
0.3 ppm NAA+0.3 g/L AC	2.35^{a}	0.17^{a}	100.00^{a}	4.57^{ab}	3.88^{ab}	4.57^{ab}	1.20^{a}	1.13^{ab}
0.3 ppm NAA+0.5 g/L AC	2.68^{a}	0.39^{a}	100.00^{a}	5.00^{a}	4.15^{a}	4.77^{a}	1.22^{a}	1.14^{ab}
0.3 ppm NAA+1.0 g/L AC	2.37^{a}	0.16^{a}	100.00^{a}	4.33^{b}	2.83^{c}	4.45^{ab}	1.14^{a}	1.04^{b}
CV (%)	11.30	71.80	0.00	9.80	12.20	8.30	10.40	9.40
$LSD_{0.05}$	0.36	0.23	0.00	0.58	0.55	0.48	0.17	0.14

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's test

Effect of NAA and AC combination on the growth of *Chrysanthemum* cultured in a micropropagation system: In this experiment, AC was used at concentrations ranging from 0.1 to 1.0 g/L in combination with 0.3 ppm NAA to evaluate the growth and development of *Chrysanthemum*. Two additional MS media, one supplemented with 0.5 g/L AC and another with 0.3 ppm NAA, were used as controls. The results are presented in Table 6 and Fig. 6.

The data indicated that both NAA and AC positively influenced root formation in *in vitro Chrysanthemum* shoot explants. The root initiation rate reached 100% when either NAA or AC was added to the culture medium. Plantlets treated with a combination of NAA and AC produced a greater number of roots than those treated with NAA alone (Table 6, Fig. 6). Specifically, MS medium containing 0.3 ppm NAA produced an average of 3.73 roots per plantlet, whereas the addition of 0.5 g/L AC resulted in 4.80 roots per plantlet. The best performance was observed in plantlets cultured in MS medium supplemented with both 0.3 ppm NAA and 0.5 g/L AC, which produced an average of 5.00 roots per plantlet and the longest roots (4.15 cm). These differences were statistically significant compared with the other treatments.

All treatments produced shoots ranging from 2.30 to 2.68 cm in height, with no statistically significant differences among them. However, plantlets cultured in media supplemented with 0.3 ppm NAA+0.5 g/L AC developed the highest number of leaves (4.77 leaves per plantlet), followed by those grown in media containing 0.3 ppm NAA+0.3 g/L AC (4.57 leaves) and 0.3 ppm NAA+1.0 g/L AC (4.45 leaves). Shoots cultured in medium supplemented with 0.3 ppm NAA+0.1 g/L AC produced the fewest leaves (4.13 leaves per plantlet) and this difference was statistically significant compared with the aforementioned treatments.

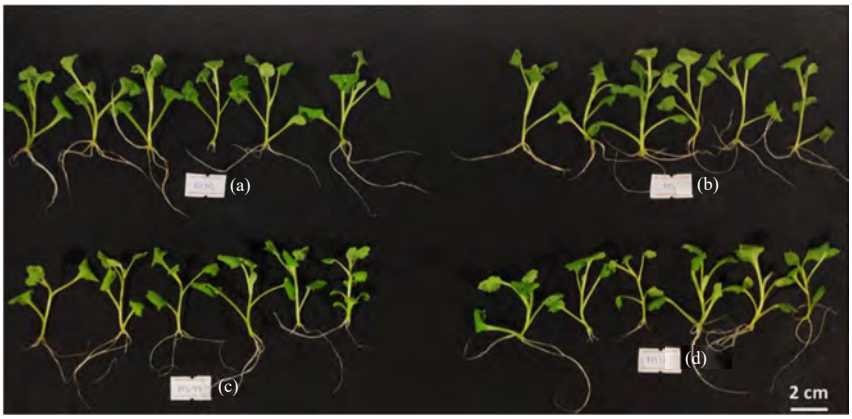

Fig. 7(a-d): Plantlets of *Chrysanthemum* cultured in a micropropagation system with different nutrient media after two weeks of culture, (a) 1/2MS, (b) MS, (c) MS1/4 and (d) MS1/2

Table 7: Effect of nutrient medium on the growth of *Chrysanthemum* cultured in a micropropagation system after two weeks of culture

Nutrition solution	Shoot height (cm)	Fresh mass (g)	Root initiation (%)	Number of roots/ plantlet (roots)	Root length (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)
1/2MS	3.04^{ab}	0.22^{a}	100.00^{a}	4.76^{ab}	3.85^{ab}	5.07^{a}	1.42^{ab}	1.38^{a}
MS	3.39^{a}	0.24^{a}	100.00^{a}	4.97^{a}	4.21^{a}	5.20^{a}	1.48^{a}	1.41^{a}
MS1/4	2.80^{b}	0.17^{b}	100.00^{a}	4.07^{b}	3.22^{b}	4.37^{b}	1.29^{b}	1.30^{a}
MS1/2	2.99^{ab}	0.20^{ab}	100.00^{a}	4.30^{ab}	3.43^{ab}	4.76^{ab}	1.36^{ab}	1.32^{a}
CV (%)	13.00	14.30	0.00	12.10	14.80	6.60	7.80	7.00
$LSD_{0.05}$	0.55	0.04	0.00	0.75	0.75	0.44	0.15	0.13

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's test

Thus, it can be concluded that NAA, AC and their combination have distinct effects on *in vitro* root formation of *Chrysanthemum* plantlets. The combination of 0.3 ppm NAA and 0.5 g/L AC proved to be superior to using either NAA or AC alone at this stage of culture. These results are consistent with the findings reported by Lee and Chang[22].

Effect of nutrient medium on the growth of *Chrysanthemum* cultured in a micropropagation system: In this experiment, direct root initiation and development were observed on different media (1/2MS, MS, MS1/4 and MS1/2) supplemented with 0.3 ppm NAA and 0.5 g/L AC after two weeks of culture. As shown in Table 7, the root initiation rate for all treatments was consistently high (100%). The highest number of roots per explant (4.97) and the greatest root length (4.21 cm) were recorded on MS medium supplemented with 0.3 ppm NAA and 0.5 g/L AC (Table 7 and Fig. 7). The weakest response across all parameters was observed in plantlets grown on MS1/4 medium, which produced an average of 4.07 roots per plantlet and root length of 3.22 cm.

The results in Table 7 indicated that plantlets grown on media with reduced macro- or/and micronutrient concentrations exhibited shorter shoot height and root length compared with those cultured on the full-strength MS medium. The greater the reduction in mineral concentration, the more pronounced the decline in growth parameters. (Table 7).

These results indicate that the composition and concentration of the nutrient medium have a significant effect on root quality and overall plant growth, with the MS nutrient medium being the most effective for root formation in the red cultivar of *Chrysanthemum* × *morifolium*. In contrast, for most plant species, the optimal basal medium for the rooting stage is one with reduced salt concentration compared to full-strength MS medium. According to previous studies[23-25], reducing the total salt concentration can alleviate the inhibitory effects of excessive nitrogen and other ions, thereby creating more favorable physiological conditions for root induction and development.

Effect of culture density on the growth of *Chrysanthemum* cultured in a micropropagation system: Four culture densities were used in this experiment, including 6, 9, 12 and 15 shoots per vessel. The explants were cultured on MS medium supplemented with 0.3 ppm NAA and 0.5 g/L AC for two weeks. The shoot and root growth in the micropropagation system appeared to depend on plant density. It was evident that as plantlet density increased from 9 to 15 shoots per vessel, shoot length, number of roots, root length and number of leaves decreased (Table 8).

Fig. 8(a-d): Plantlets of *Chrysanthemum* cultured in a micropropagation system with different culture densities after two weeks of culture, (a) 6 shoots/vessel, (b) 9 shoots/vessel, (c) 12 shoots/vessel and (d) 15 shoots/vessel

Table 8: Effect of culture density on the growth of *Chrysanthemum* cultured in a micropropagation system after two weeks of culture

Culture densities (shoots/vessel)	Shoot height (cm)	Fresh mass (g)	Root initiation (%)	Number of roots/ plantlet (roots)	Root length (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)
6	3.07^{a}	0.19^{a}	100.00^{a}	5.07^{a}	3.32^{a}	5.30^{a}	1.25^{a}	1.24^{a}
9	3.14^{a}	0.20^{a}	100.00^{a}	4.87^{ab}	3.44^{a}	5.00^{a}	1.15^{a}	1.19^{a}
12	3.09^{a}	0.18^{a}	100.00^{a}	4.67^{ab}	3.08^{a}	4.77^{a}	1.17^{a}	1.15^{a}
15	2.78^{b}	0.15^{b}	100.00^{a}	4.17^{b}	2.78^{a}	4.50^{a}	1.13^{a}	1.14^{a}
CV (%)	5.50	9.60	0.00	11.60	16.50	13.80	7.70	7.90
$LSD_{0.05}$	0.23	0.02	0.00	0.75	0.72	0.93	0.13	0.13

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's test

Data presented in Table 8 and Fig. 8 revealed that increasing the plantlet density from 6 to 9 shoots per vessel resulted in an increase in shoot height, fresh mass and root length, whereas the number of roots decreased slightly from 5.07 to 4.87 per plantlet. However, these differences were not statistically significant. Overall, the results indicated that higher explant density led to slower growth and development of *Chrysanthemum* plantlets.

Comparison of *Chrysanthemum* growth in microponic and micropropagation systems: In this experiment, two optimized culture conditions were used to compare the growth of *Chrysanthemum* in the microponic and micropropagation systems. The microponic system consisted of liquid 1/2MS medium supplemented with 0.1 ppm NAA, a plantlet density of 9 shoots per vessel and a medium volume of 20 mL. The micropropagation system consisted of solid MS medium supplemented with 30 g/L sucrose, 6.5 g/L agar and a combination of 0.3 ppm NAA and 0.5 g/L AC, using the same plant density as in the microponic system.

After two weeks of culture, the results indicated that *Chrysanthemum* plantlets grew better in the micropropagation system than in the microponic system. Growth parameters such as shoot height (3.17 cm), root number (5.20), root length (3.63 cm), fresh mass (0.18 g) and number of leaves (5.33) were superior in plantlets cultured in the micropropagation system. (Table 9 and Fig. 9).

To evaluate the adaptability of plantlets to *ex vitro* conditions, explants from the previous experiment were acclimatized in a nursery. After 30 days, the young plants were transplanted into pots (15×13 cm) for further growth and development.

Results presented in Table 10 showed that the survival rate of both plantlet groups reached 100% after 90 days of cultivation and no significant difference was observed in leaf size after 30 and 60 days of planting. The number of leaves in micropropagated plantlets was also significantly higher after 30, 60 and 90 days. In contrast, microponic plantlets developed larger shoot diameters after 60 and 90 days, as well as greater leaf size and canopy diameter after 90 days, with statistically significant differences. Micropropagated plantlets also initiated flowering earlier than microponic plantlets, which remained in the vegetative stage after 90 days of planting (Table 10).

This result contrasts with the findings of Tung *et al.*[6], who reported the superiority of *Chrysanthemum* shoots grown in microponic culture. It can be explained by the fact that the initial explants used for both the microponic and micropropagation systems were *in vitro* shoots of similar height and number of leaves. In the micropropagation system, plantlets were supplied with a full complement of mineral nutrients, sucrose and optimal sterile conditions, which promoted favorable growth. In contrast, in the microponic system-where the nutrient solution does not contain

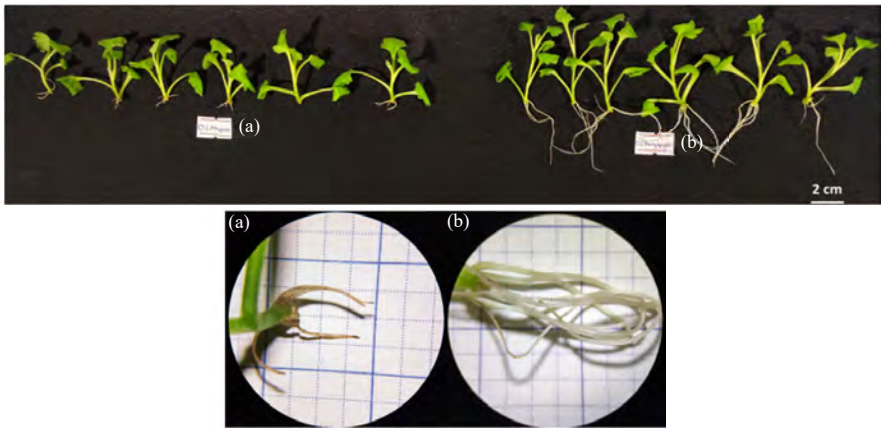

Fig. 9(a-b): Plantlets and roots of *Chrysanthemum* cultured in microponic and micropropagation systems after two weeks of culture (1 small square equals 1 mm^2), (a) Microponics and (b) Micropropagation

Table 9: Comparison of the growth of *Chrysanthemum* cultured in a microponic system and a micropropagation system after two weeks of culture

	Systems	
Characteristics	Microponics	Micropropagation
Shoot height (cm)	2.00±0.16	3.17±0.86
Fresh mass (g)	0.16±0.01	0.18±0.01
Root initiation (%)	100.00±0.00	100.00±0.00
Number of roots/plantlet (roots)	4.17±0.05	5.20±0.21
Root length (cm)	0.93±0.08	3.63±0.14
Number of leaves (leaves)	4.07±0.14	5.33±0.14
Leaf length (cm)	1.44±0.01	1.29±0.02
Leaf width (cm)	1.27±0.03	1.25±0.03

Values representing the Average±Standard Error (SE)

Table 10: Growth of *Chrysanthemum* cultured in microponic and micropropagation systems after 30, 60 and 90 days of planting

	Systems					
	After 30 days of planting		After 60 days of planting		After 90 days of planting	
Characteristics	Microponics	Micropropagation	Microponics	Micropropagation	Microponics	Micropropagation
Survival rate (%)	100.00±0.00	100.00±0.00	100.00	100.00	100.00	100.00
Shoot height (cm)	2.62±0.09	4.72±0.13	4.83±0.16	9.41±0.28	9.22±0.15	16.10±2.05
Shoot diameter (mm)	2.83±0.14	2.23±0.15	4.53±0.41	3.47±0.17	5.73±0.38	4.53±0.34
Canopy diameter (cm)	7.03±0.15	6.89±0.22	16.65±0.32	16.36±0.30	45.09±1.23	25.59±1.60
Number of leaves (leaves)	7.53±0.17	7.87±0.19	10.73±0.33	13.00±0.40	15.69±0.22	16.68±0.55
Leaf length (cm)	2.62±0.12	2.56±0.09	5.98±0.13	5.78±0.16	11.03±0.10	10.26±0.26
Leaf width (cm)	2.27±0.19	2.52±0.09	5.29±0.14	5.97±0.11	7.28±0.10	6.87±0.20
Number of flower bud	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.07±0.91

DAP: Days after planting and values representing the Average±Standard Error (SE)

sucrose-plantlets must rely on photosynthesis to produce their own carbon source and gradually adapt to new growth conditions. This physiological adjustment often results in slower initial growth, reflected in lower shoot height and fewer leaves compared with plants cultured through conventional micropropagation. However, plantlets grown in the microponic system generally exhibited more vigorous morphological characteristics, including thicker leaves and sturdier stems (Fig. 9). When transferred to the nursery, although microponic plantlets may be shorter and have fewer leaves, their thicker and bigger leaves, larger stems and wider shoot diameters and canopy diameters indicate superior physiological adaptation compared with micropropagated plantlets (Table 10 and Fig. 10). The enhanced shoot elongation observed in microponic plantlets after transfer to the nursery, as reported by Tung *et al.*[6], may be explained by the relatively uniform initial plantlet size between the two systems and the better acclimatization capacity developed

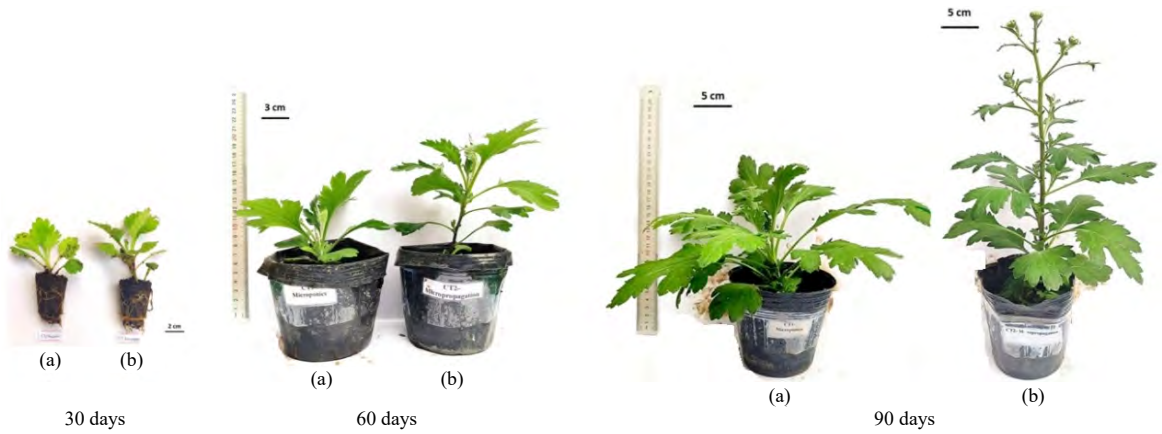

Fig. 10(a-b): *Chrysanthemum* plantlets derived from, (a) Microponic and (b) Micropropagation systems after 30, 60 and 90 days of planting

under microponic conditions. Using longer *in vitro* explants as the starting material for the microponic system may yield similar results to those reported by Tung *et al.*[6]. Previous studies on *Chrysanthemum*[4-6,14] and other plant species[7,8], have primarily focused on developing and optimizing the microponic system; however, detailed comparative analyses between the two *in vitro* culture systems have not yet been conducted. So, using longer *in vitro* explants as the starting material for the microponic system may yield similar results to those reported by Tung *et al.*[6].

The significantly higher number of leaves observed at 30, 60 and 90 days after planting in this study may also explain the earlier flowering of micropropagated plantlets compared with microponic ones, which contrasts with the findings reported by Tung *et al.*[6].

Overall, the present study indicates that the micropropagation system is more effective than the microponic system for the growth and development of red cultivar *Chrysanthemum* plantlets during the first two weeks of the *in vitro* stage and that micropropagated plantlets exhibited superior performance in plant height and number of leaves after 90 days in the nursery and earlier flowering. However, microponic plantlets developed larger shoot diameters, greater leaf size and wider canopy diameters, suggesting better adaptability under nursery conditions.

CONCLUSION

The present study successfully identified the optimal culture conditions for *in vitro* propagation of red *Chrysanthemum*× *morifolium* using both microponic and micropropagation systems. The micropropagation system, based on solid MS medium supplemented with 0.3 ppm NAA and 0.5 g/L activated charcoal, at a culture density of nine shoots per vessel, provided the best results for shoot growth, root formation, shoot height, number of leaves and early flowering. In contrast, the microponic system using liquid 1/2MS medium supplemented with 0.1 ppm NAA, with a medium volume of 20 mL and a culture density of nine shoots per vessel, supported better acclimatization performance, producing plantlets with larger shoot diameters, broader leaves and wider canopy diameters. These findings highlight that the two systems can complement each other micropropagation for vigorous *in vitro* growth and microponics for improved acclimatization - thereby offering an efficient approach for large-scale production of high-quality *Chrysanthemum* plantlets.

SIGNIFICANCE STATEMENT

This study provides comparative insights into the efficiency of microponic and micropropagation systems for *in vitro* growth and acclimatization of red *Chrysanthemum*. The findings demonstrate that the micropropagation system offers superior shoot development and earlier flowering, while the microponic system enhances acclimatization through improved shoot and leaf morphology. These results contribute to optimizing *in vitro* culture protocols for *Chrysanthemum* and other ornamental crops, offering a practical basis for large-scale, high-quality plantlet production. The outcomes of this study can assist researchers and commercial growers in selecting the most suitable system depending on their propagation objectives.

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