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Encapsulation of Micro-Cuttings of Acmella Oleracea from Micropropagated Plantlets in Different Concentrations of 6-Benzylaminopurine

Encapsulamento de Microestacas de Acmella Oleracea Obtidas de Plântulas Micropropagadas Sob Diferentes Concentrações de 6-Benzilaminopurina

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Abstract

Acmella oleracea, a leafy vegetable native to the Amazon region, has significant potential for cosmetic, pharmaceutical, and gastronomic industries. This study evaluated biometric characteristics, stomatal morphology, and epicuticular wax deposition in plantlets obtained from the in vitro conversion of encapsulated micro-cuttings. Micro-cuttings derived from plantlets multiplied under five concentrations of 6-benzylaminopurine (BAP; 0.000–0.200 mg L^{-1}) were encapsulated in an alginate matrix and cultured on Murashige and Skoog medium for 30 days, using 11 units per treatment. BAP concentration significantly affected conversion frequency (Fisher's exact test, p = 0.0036; Cramér's V = 0.55) and all biometric traits (ANOVA, $p < 0.001$; $\eta^2 = 0.385–0.532$). Compared with the BAP-free control, material previously exposed to BAP generally showed higher conversion frequency, numbers of leaves and shoots, plant height, and root dry mass. Shoot dry mass was 54.5% higher at 0.100 mg L^{-1} BAP than in the control, although it did not differ significantly from that obtained at 0.200 mg L^{-1}. Conversely, stem diameter was highest in the control. Anomocytic and ellipsoid stomata were observed, whereas evident epicuticular wax deposition and qualitative differences in leaf epidermal micromorphology among treatments were not detected. Therefore, exposure of donor cultures to low BAP concentrations improved the subsequent conversion and vegetative development of encapsulated micro-cuttings, representing a promising strategy for the in vitro propagation of *A. oleracea*.

Keywords: Asteraceae. Encapsulation. Jambu. Micropropagation. Nodal Segment.

Resumo

Acmella oleracea, hortaliça folhosa nativa da região Amazônica, apresenta elevado potencial para as indústrias cosmética, farmacêutica e gastronômica. Este estudo avaliou características biométricas, morfologia estomática e deposição de cera epicuticular em plântulas obtidas pela conversão in vitro de microestacas encapsuladas. Microestacas provenientes de plântulas multiplicadas sob cinco concentrações de 6-benzilaminopurina (BAP; 0,000–0,200 mg L^{-1}) foram encapsuladas em matriz de alginato e cultivadas em meio Murashige e Skoog durante 30 dias, utilizando-se 11 unidades por tratamento. A concentração de BAP afetou significativamente a frequência de conversão (teste exato de Fisher, $p = 0{,}0036$; V de Cramér = 0,55) e todas as características biométricas (ANOVA, $p < 0{,}001$; $\eta^2 = 0{,}385–0{,}532$). Em comparação com o controle sem BAP, o material previamente exposto ao regulador apresentou, em geral, maiores frequência de conversão, números de folhas e brotos, altura das plântulas e massa seca radicular. A massa seca da parte aérea foi 54,5% superior com 0,100 mg L^{-1} de BAP em relação ao controle, embora não tenha diferido significativamente daquela obtida com 0,200 mg L^{-1}. Em contrapartida, o maior diâmetro do caule foi registrado no controle. Foram observados estômatos anomocíticos e elipsoides, mas não foram detectadas deposição evidente de cera epicuticular nem diferenças qualitativas na micromorfologia da epiderme foliar entre os tratamentos. Portanto, a exposição das culturas doadoras a baixas concentrações de BAP melhorou a conversão subsequente e o desenvolvimento vegetativo das microestacas encapsuladas, constituindo uma estratégia promissora para a propagação in vitro de *A. oleracea*.

Palavras-chave: Asteraceae. Encapsulamento. Jambu. Micropropagação. Segmento Nodal.

1 Introduction

Jambu, also known as watercress from Pará state, Brazil, *Acmella oleracea* (R. K. Jansen) (Asteraceae), is cultivated for culinary, medicinal, and insecticidal purposes. The leaves of this species present relevant nutritional composition, including 24.01% proteins, 1.54% lipids, and 63.38% carbohydrates, in addition to important mineral elements such as calcium, magnesium, and copper (Benelli *et al*., 2019; Neves *et al*., 2019; Seal *et al*., 2013).

Acmella oleracea is preferably cultivated under hot and humid environmental conditions, typical of the Amazon region, with temperatures above 25 °C, annual rainfall exceeding 2,500 mm, and relative humidity around 80% (Spinozzi *et al*., 2022). Water availability is one of the main factors influencing seed germination in this species, since rehydration intensifies respiration and promotes the degradation of reserve tissues, providing energy and nutrients required for embryonic axis growth (Sarmento *et al*., 2019). Conversely, environments characterized by high temperatures associated with low relative humidity reduce water potential and negatively affect the speed, percentage, and vigor of germination of *A. oleracea* seeds (Ávila *et al*., 2007; Botelho; Perez, 2001).

In vitro cultivation using plant tissue culture techniques includes different vegetative propagation strategies, such as the production of encapsulable units (EU). This technique facilitates clonal propagation for commercial purposes, short-term conservation, in vitro germplasm exchange and transport, as well as staggered plantlet production both in vitro and ex vitro. Additionally, it contributes to reducing production time and increasing uniformity, while maintaining high phytosanitary quality and productive potential (Behera *et al*., 2020; Bellumori *et al.,* 2022; Singh;

Singh, 2021).

Protocols for EU production using micro-cuttings (MC), especially nodal segments, have been established for species of the Asteraceae family, such as *Artemisia vulgaris*, *Spilanthes mauritiana*, and *Stevia rebaudiana* (Kundu *et al.,* 2018; Sharma; Shahzad, 2014; Sudarshana *et al*., 2013). In these species, higher conversion rates of MC into plantlets have been associated with the use of the growth regulator 6-benzylaminopurine (BAP) (Lalthafamkimi *et al.,* 2022).

However, studies evaluating the biometric performance and anatomical characteristics of *A. oleracea* plantlets obtained from encapsulated micro-cuttings under different BAP concentrations are still limited. Previous studies have demonstrated that *A. oleracea* responds to relatively low concentrations of BAP during in vitro multiplication. Almeida *et al.* (2020), for example, evaluated BAP concentrations ranging from 0 to 0.750 mg L^{-1} and reported the best multiplication response at 0.125 mg L^{-1}. These findings support further investigation of the response of *A. oleracea* within the lower BAP concentration range.

Therefore, the aim of this study was to evaluate biometric characteristics (conversion frequency, number of leaves and shoots, stem diameter, plant height, shoot and root dry mass), describe stomatal morphology, and verify the presence or absence of epicuticular wax on leaves of *A. oleracea* plantlets obtained from the in vitro conversion of encapsulable units prepared from micro-cuttings derived from micropropagation under different BAP concentrations.

2 Material and Methods

2.1 In vitro establishment of A. oleracea

The experiment was conducted at the Plant Tissue Culture Laboratory of the Department of Plant Science at the Federal University of Ceará (UFC), in Fortaleza, Ceará state, Brazil. *Acmella oleracea* (R.K. Jansen) seeds, obtained from the main producing region of the state of Pará (1°27' 31" S and 48° 26' 04.5" W), were surface sterilised for 15 min in a 2% sodium hypochlorite solution with three drops of Tween 20 per 100 mL and then rinsed three times with sterile distilled water.

Seedlings were established in vitro from seeds inoculated on hormone-free medium in test tubes. The culture medium consisted of MS salts and vitamins, 30 g L^{-1} sucrose, and 5.8 g L^{-1} agar (HIMEDIA®). The pH was adjusted to 5.8 ± 0.2, and the medium was autoclaved at 121 °C and 1.2 atm for 15 min. Cultures were maintained for two weeks in a growth room at 25 ± 2 °C, with a 16 h photoperiod and light intensity of 35.1 µmol m^{-2} s^{-1} (Murashige; Skoog, 1962).

All explants subsequently used in the experiment were derived from a single clonal source of *Acmella oleracea*. Thus, the experimental material represented the same genotype throughout the study.

2.2 Shoots multiplication

Plantlets derived from the same clonal source were used for the multiplication phase. Nodal segments approximately 2.0 cm long, containing two axillary buds, were excised and inoculated into culture flasks containing the different BAP treatments. The medium contained MS salts and vitamins, 30 g L^{-1} sucrose, 5.8 g L^{-1} agar (HIMEDIA®), and 6-benzylaminopurine (BAP) at concentrations of 0.000, 0.025, 0.050, 0.100, and 0.200 mg L^{-1}.

The five BAP levels were structured as a low-concentration gradient, including a BAP-free control and concentrations below, close to, and above 0.125 mg L^{-1}, a concentration previously reported to promote efficient in vitro multiplication of *A. oleracea* (Almeida *et al*., 2020). This narrower concentration range was used to characterize the response of the clonal material to relatively small changes in BAP availability during the multiplication phase.

2.3 Production of micro-cuttings

Encapsulable units (EU) of *A. oleracea* were produced from micro-cuttings (MC) (nodal segments) approximately 0.3 cm long obtained from nodal segments of micropropagated plantlets cultivated under BAP concentrations of 0.000, 0.025, 0.050, 0.100, and 0.200 mg L^{-1} (Figure 1A).

2.4 Production of encapsulable units

Micro-cuttings of *A. oleracea* were mixed with a sodium alginate matrix (2% w/v) (NEON®) dissolved in hormone-free medium. Individual micro-cuttings were collected using a 3 mL Pasteur pipette sterilised in 99% alcohol and transferred into $CaCl_2 \cdot 2H_2O$ solution (75 mM) (NEON®), where they remained for 20 min for complexation and encapsulable unit formation.

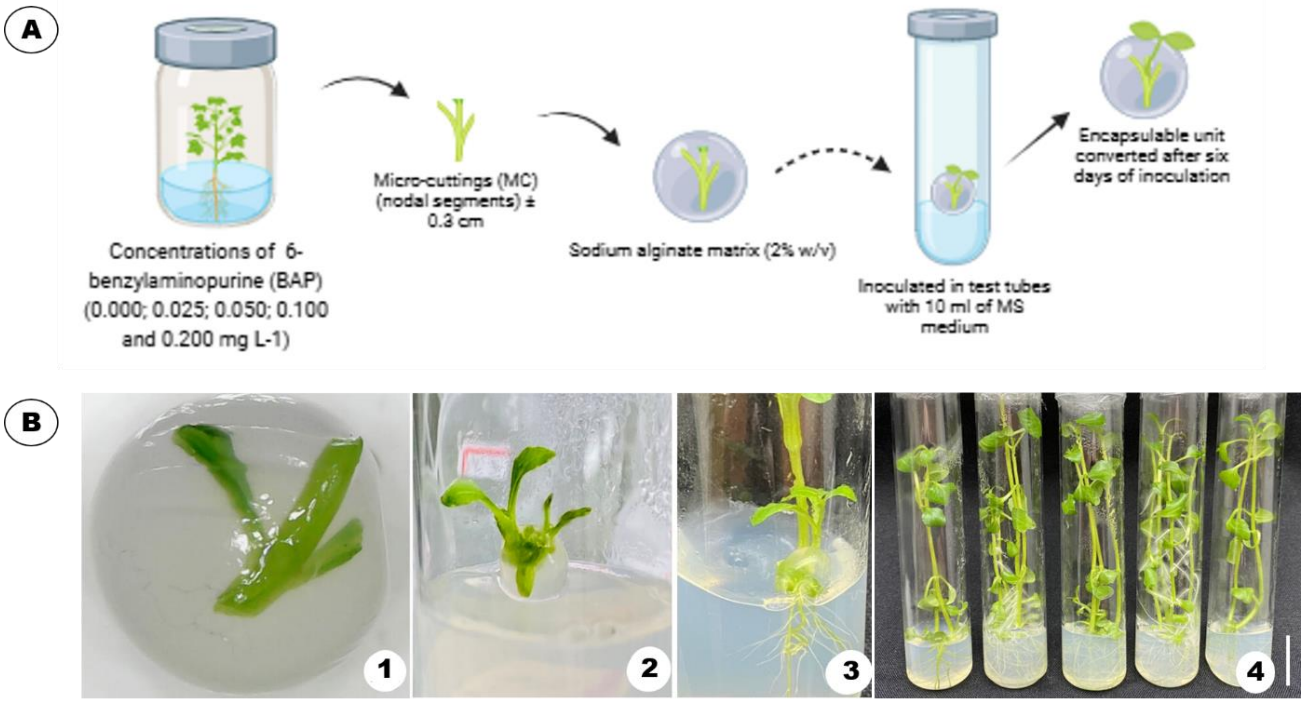
Encapsulable units were washed three times with distilled and autoclaved water to remove excess $CaCl_2 \cdot 2H_2O$ and immersed in KNO_3 solution (200 mM) (EXODO CIENTÍFICA®) for 15 min for decomplexation. The medium contained MS salts and vitamins, and the pH was adjusted to 5.8 ± 0.2. The medium and all solutions were autoclaved at 121 °C and 1.2 atm for 15 min.

2.5 Encapsulable unit inoculation and growth conditions

Each encapsulable unit was individually inoculated into a separate test tube containing 10 mL of MS medium and maintained in a growth room at 25 ± 2 °C, with a 16 h photoperiod and light intensity of 35.1 μmol m^{-2} s^{-1}.

At 30 days after inoculation, biometric and leaf epidermal characteristics of plantlets obtained from encapsulable unit conversion were evaluated.

Figure 1 – Encapsulable unit of *Acmella oleracea* (1), A- Production of encapsulable units, B- converted after six days of inoculation (2) rooted after 10 days of inoculation (3) and after 30 days (4). Scale bar ≈ 1cm



Source: the authors.

2.6 Biometric characteristics evaluated

At 30 days after encapsulable unit inoculation, conversion of each unit was recorded as converted or non-converted, and conversion frequency was subsequently calculated for each treatment. The number of leaves and number of shoots per plant were also recorded. Stem diameter and plant height were measured using a digital caliper (MTX®). Shoot and root dry mass were determined using a precision balance after drying plantlets in a forced-air drying oven (VITCHLAB®) at 65 °C for 72 h.

2.7 Leaf preparation for analysis of the leaf epidermis

Leaf samples of *A. oleracea* plantlets were prepared according to Dias *et al*. (2017), dried in a critical point dryer (K850 Critical Point Drier®) using liquid CO_2, mounted on stubs for silver metallization, and analysed using scanning electron microscopy (Quanta 450-FEG – FEI).

2.8 Statistical analysis

The experiment was conducted in a completely randomized design with five BAP concentrations (0.000, 0.025, 0.050, 0.100, and 0.200 mg L^{-1}) and 11 experimental replicates per treatment.

Each experimental replicate consisted of one encapsulable unit (synthetic seed) inoculated

individually into one test tube containing 10 mL of MS medium, totaling 55 experimental units. All explants belonged to the same clonal lineage; therefore, replication refers to independent culture units rather than to genetically independent plant sources.

No a priori power analysis was performed when the experiment was established. The experiment comprised 11 experimental units per treatment and five BAP treatments, resulting in a total of 55 experimental units. A design-sensitivity analysis was subsequently performed using the pwr.anova.test function of the *pwr* package in R, considering five groups, 11 observations per group, $\alpha = 0.05$, and 80% statistical power. The minimum detectable standardized effect was approximately Cohen's $f = 0.49$ (Champely, 2020).

Biometric data were analyzed considering the individual encapsulable unit cultured in a separate test tube as the experimental unit. Normality of residuals of the continuous biometric variables was assessed using the Shapiro-Wilk test (Shapiro; Wilk, 1965).

The number of shoots did not meet the normality assumption and was transformed using the Box-Cox procedure with the bestNormalize package (Peterson, 2021). Continuous biometric variables were subjected to one-way analysis of variance, and treatment means were compared using Tukey's test at $p < 0.05$ using the HSD.test function of the *agricolae* package (Mendiburu, 2023).

For number of shoots, statistical inference was based on the transformed data, whereas descriptive values are presented on the original scale. Treatment effect magnitude was expressed as eta squared (η^2).

Conversion was recorded for each encapsulable unit as a binary response (converted or non-converted) and was therefore analyzed separately using Fisher's exact test for the 2 × 5 contingency table. Conversion frequencies are presented as the number of converted units/total units, percentage, and 95% Wilson confidence interval. Cramér's V was calculated as a measure of effect magnitude. Welch's one-way ANOVA was additionally performed on the original scale as a sensitivity analysis for the continuous biometric variables. All statistical analyses were performed in R software (R Core Team, 2021), adopting a significance level of 5%.

3 Results and Discussion

The biometric performance of *Acmella oleracea* plantlets obtained from encapsulable units was significantly influenced by the BAP concentration used during the multiplication phase of the donor material. Significant treatment effects were observed for number of leaves, number of shoots, stem diameter, plant height, shoot dry mass, and root dry mass (*p* < 0.001). The magnitude of these effects was substantial, with η^2 values ranging from 0.385 for shoot dry mass to 0.532 for plant height, indicating that BAP concentration accounted for an important proportion of the variability observed in the evaluated biometric traits (Table 1).

Table 1 – Summary of the statistical analysis and effect sizes for biometric characteristics of *Acmella oleracea* plantlets obtained from encapsulable units produced from micro-cuttings exposed to different BAP concentrations during the multiplication phase

Trait	F	*p*	η²	CV (%)
Number of leaves	8.88	<0.001	0.415	30.85
Number of shoots[1]	8.05	<0.001	0.392	27.66
Stem diameter	10.75	<0.001	0.462	29.10
Plant height	14.19	<0.001	0.532	26.12
Shoot dry mass	7.84	<0.001	0.385	20.68
Root dry mass	11.14	<0.001	0.471	46.96

Note: η² = eta squared; CV = coefficient of variation. *F* and *p* values refer to the overall effect of BAP concentration assessed by one-way analysis of variance (ANOVA).
Source: the authors.

Conversion frequency differed significantly among BAP treatments (Fisher's exact test, $p = 0.0036$), with a Cramér's V of 0.55, indicating a substantial association between the BAP concentration used during the multiplication phase and subsequent conversion of the encapsulable units (Table 2). The BAP-free control showed the lowest conversion frequency, with 5 of 11 units converted (45.5%; 95% CI: 21.3–72.0%), whereas conversion in material previously exposed to BAP ranged from 81.8% to 100%. Complete conversion (11/11 units) was observed for the 0.050 and 0.200 mg L^{-1} treatments. However, because the overall Fisher's exact test evaluates the association across all treatments, these numerical differences should not be interpreted as evidence that each individual BAP concentration differed significantly from the control.

Table 2 – Biometric characteristics of *Acmella oleracea* plantlets obtained from encapsulable units produced from micro-cuttings exposed to different concentrations of 6-benzylaminopurine (BAP) during the multiplication phase

Variable	BAP Concentration (mg L^{-1})				
	0.000	**0.025**	**0.050**	**0.100**	**0.200**
Conversion, *n*/N (%)	5/11 (45.5)	9/11 (81.8)	11/11 (100)	10/11 (90.9)	11/11 (100)
95% CI	21.3–72.0	52.3–94.9	74.1–100	62.3–98.4	74.1–100
NL	7.55 ± 6.72^{b}	14.18 ± 2.14^{a}	14.64 ± 1.86^{a}	18.00 ± 3.10^{a}	15.18 ± 5.42^{a}
SN[1]	0.93 ± 0.67^{b}	1.55 ± 0.17^{a}	1.64 ± 0.24^{a}	1.90 ± 0.26^{a}	1.49 ± 0.51^{a}
SD (mm)	2.86 ± 1.14^{a}	1.71 ± 0.20^{bc}	1.43 ± 0.09^{c}	1.67 ± 0.11^{bc}	2.14 ± 0.51^{b}
H (cm)	3.41 ± 2.83^{b}	8.13 ± 1.12^{a}	8.26 ± 1.16^{a}	8.45 ± 0.73^{a}	7.87 ± 2.58^{a}
SDM (g)	0.022 ± 0.006^{c}	0.027 ± 0.004^{bc}	0.027 ± 0.005^{abc}	0.034 ± 0.007^{a}	0.032 ± 0.007^{ab}
RDM (g)	0.001 ± 0.001^{b}	0.007 ± 0.002^{a}	0.006 ± 0.002^{a}	0.008 ± 0.003^{a}	0.008 ± 0.005^{a}

Note: Eleven encapsulable units were evaluated per treatment. Conversion is expressed as the number of converted units relative to the total number evaluated (*n*/N), followed by the percentage and its 95% Wilson confidence interval (95% CI). Conversion differed among BAP concentrations according to Fisher's exact test ($p = 0.0036$; Cramér's V = 0.55). The remaining variables are presented as mean ± standard deviation. Within each row, means followed by the same superscript letter do not differ according to Tukey's test ($p > 0.05$). [1]The values presented are on the original scale, whereas the number of shoots was statistically analyzed after the transformation described in the Statistical Analysis section. NL = number of leaves; SN = number of shoots; SD = stem diameter; H = plant height; SDM = shoot dry mass; RDM = root dry mass.
Source: the authors.

Importantly, BAP was supplied during the preceding multiplication phase rather than during

conversion of the encapsulable units. Therefore, the higher conversion frequencies observed in material derived from BAP-treated cultures are more appropriately interpreted as a response associated with the physiological condition acquired during multiplication. Cytokinins such as BAP regulate cell division, shoot development, and organogenic competence, which may influence the subsequent regenerative performance of nodal tissues (Sosnowski *et al*., 2023). High conversion efficiency of encapsulated non-embryogenic explants has also been reported in other plant species and has been associated with the regenerative competence of the explant and its developmental state at the time of encapsulation (Bertsouklis; Tsopela, 2023; Bustillo-Avendaño *et al*., 2018; Priyadharshini *et al.*, 2020; Rodrigues *et al.,* 2020; Santika; Fang, 2024).

BAP treatment significantly affected the biometric performance of the converted encapsulable units. The magnitude of the treatment effect was substantial for the evaluated traits, with η^2 ranging from 0.385 for shoot dry mass to 0.532 for plant height. The concentration of 0.100 mg L^{-1} BAP produced the highest observed mean number of leaves and shoot dry mass. For shoot dry mass, the estimated difference between 0.100 mg L^{-1} BAP and the BAP-free control was approximately 0.0125 g (Tukey-adjusted 95% CI: 0.0055–0.0196 g). However, shoot dry mass did not differ significantly between 0.100 and 0.200 mg L^{-1} BAP, indicating that increasing the concentration beyond 0.100 mg L^{-1} did not result in an additional statistically detectable gain in this trait. This pattern is more appropriately interpreted as a plateau in the response within the evaluated concentration range rather than as evidence of inhibition at 0.200 mg L^{-1}. Cytokinin responses depend not only on exogenous concentration but also on endogenous hormonal balance, tissue competence, receptor-mediated responses, and resource allocation. Thus, increasing BAP availability does not necessarily produce a proportional increase in shoot growth or biomass accumulation.

These results agree with reports for in vitro culture of *Artemisia laciniata* and *Lactuca indica* using similar concentrations of BAP, which intensify organogenic processes in Asteraceae species (Kodym *et al*., 2018; Oliya *et al*., 2022). BAP acts in the regulation of plant growth, cell division, regeneration, organ formation and induction of multiplication and development of shoots in EU, as reported for the in vitro cultivation of *Achillea fragrantissima* and *Ansellia africana* (Asghar *et al*., 2023; Bhattacharyya *et al.*, 2018; Goda *et al*., 2023). In addition, cytokinins such as BAP increase nutrient translocation, photosynthesis, and production and transport of photoassimilates, promoting shoot and root development (Sosnowski *et al*., 2023).

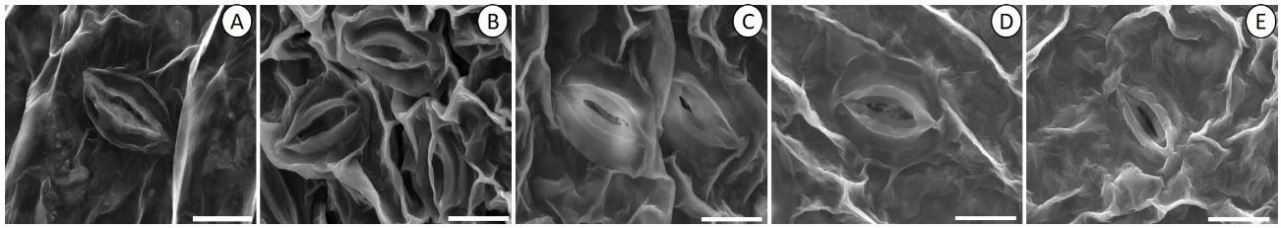
Stem diameter showed a different response pattern from the other biometric traits, with the highest mean observed in the BAP-free control (2.86 ± 1.14 mm), whereas lower values were recorded in BAP-treated material (Table 2). One possible explanation is that the lower shoot proliferation observed in the control may have favored biomass allocation to fewer growing points, whereas the greater shoot production associated with BAP may have increased competition among

developing organs for available resources. Similar relationships between shoot proliferation and biomass allocation have been reported in nodal-segment cultures of other species (Alves et al., 2018; Feyisa, 2023). However, because photoassimilate partitioning and nutrient allocation were not directly measured in the present study, this interpretation should be regarded as a physiological hypothesis rather than a demonstrated mechanism.

Relatively high coefficients of variation were observed for some biometric traits, particularly root dry mass (46.96%) and number of leaves (30.85%). This variability indicates considerable heterogeneity among individual encapsulable units, especially for root dry mass, whose absolute values were very low. Therefore, treatment differences for these traits should be interpreted together with their effect sizes and confidence intervals rather than solely on the basis of statistical significance. Nevertheless, Welch's one-way ANOVA confirmed that the treatment effect remained significant for number of leaves ($p = 0.0018$), number of shoots ($p = 0.0006$), stem diameter ($p < 0.0001$), plant height ($p = 0.0004$), shoot dry mass ($p = 0.0012$), and root dry mass ($p < 0.0001$), supporting the robustness of the main treatment-associated responses.

Qualitative analysis of the leaf epidermis by scanning electron microscopy revealed anomocytic stomata with an ellipsoidal outline in all evaluated treatments (Figure 2). Anomocytic stomata have also been previously described in anatomical studies of *A. oleracea* (Matyushin; Evdokimova, 2017). No evident qualitative differences in stomatal morphology were observed among plantlets derived from the different BAP treatments. These observations suggest that the marked biometric responses associated with BAP exposure during the multiplication phase were not accompanied by conspicuous alterations in leaf epidermal micromorphology during the first 30 days of in vitro development. However, because stomatal dimensions, density, and stomatal index were not quantitatively measured, subtle treatment-related anatomical differences cannot be excluded.

Figure 2 – Scanning electron micrographs of the abaxial leaf epidermis of Acmella oleracea plantlets 30 days after inoculation of the encapsulable units, showing anomocytic stomata and the absence of evident epicuticular wax deposition. Plantlets were obtained from micro-cuttings previously exposed during the multiplication phase to 0.000 (A), 0.025 (B), 0.050 (C), 0.100 (D), and 0.200 (E) mg L^{-1} BAP. Scale bar ≈ 20 µm



Source: the authors.

The absence of evident epicuticular wax deposition is consistent with the high-humidity conditions characteristic of in vitro culture. Under these conditions, the low evaporative demand may

reduce the requirement for extensive cuticular development and other structural mechanisms associated with water-loss control (Lando et al., 2016; Vieira et al., 2020; Garvita; Wawangningrum, 2020). Plantlets developed under high relative humidity may be particularly susceptible to increased water loss when suddenly exposed to the lower humidity and greater evaporative demand of the external environment. Therefore, gradual humidity reduction during acclimatization may be important for plantlets produced through this system. However, because acclimatization and ex vitro survival were not evaluated in the present study, the consequences of the observed epidermal characteristics for post-transfer performance remain to be experimentally determined.

Overall, BAP exposure during the multiplication phase was associated with improved subsequent conversion and vegetative development of the encapsulable units, whereas no evident qualitative differences in leaf epidermal micromorphology were observed among treatments. These results indicate that the biometric response to BAP was not accompanied by conspicuous structural changes detectable by the qualitative microscopic assessment employed in this study.

The present study has limitations that should be considered when interpreting the results. Plantlets were evaluated at a single time point, 30 days after inoculation, and all experimental material originated from a single clonal lineage. Therefore, the results represent the response of the evaluated genotype and do not account for possible genotypic variation within *A. oleracea*. In addition, leaf epidermal analysis was qualitative and was not accompanied by quantitative measurements of stomatal dimensions, density, or stomatal index, nor by physiological measurements such as stomatal conductance or transpiration. Finally, acclimatization and ex vitro survival were not evaluated. Further studies incorporating multiple genotypes, quantitative anatomical and physiological measurements, longer evaluation periods, and ex vitro acclimatization are therefore warranted.

4 Conclusion

BAP exposure during the multiplication phase improved the subsequent performance of encapsulated micro-cuttings of *Acmella oleracea*, particularly conversion frequency, shoot development, plant height, and biomass accumulation. Among the concentrations evaluated, 0.100 mg L^{-1} produced the highest observed shoot dry mass, although its response did not differ significantly from 0.200 mg L^{-1}, indicating a plateau rather than a clear optimum within the tested range. Stem diameter showed an opposite response, with the highest value observed in the BAP-free control.

Qualitative scanning electron microscopy revealed anomocytic stomata and no evident epicuticular wax deposition, with no conspicuous differences in leaf epidermal micromorphology among treatments. Further studies should determine whether these epidermal characteristics influence plantlet performance during ex vitro acclimatization.

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