DENDROBIOLOGY

2026, vol. 96, 127–144

https://doi.org/10.12657/denbio.096.010

Virginia Sarropoulou, Kortessa Dimassi-Theriou, Ioannis Therios*

Triacontanol enhances cytokinin-mediated *in vitro* shoot proliferation and biomass accumulation in two cherry rootstocks

Received: 31 May 2026; Accepted: 19 October 2026

Abstract: Background: Efficient micropropagation of cherry rootstocks is frequently constrained by low shoot regeneration and limited biomass accumulation. This study evaluated the synergistic effects of the biostimulant triacontanol (TRIA) and the cytokinin 6-benzyladenine (BA) on *in vitro* shoot proliferation, biomass production, and growth behavior in two commercial cherry rootstocks, CAB-6P and Gisela 6.

Shoot-tip explants were cultured on Murashige and Skoog (MS) medium supplemented with varying concentrations of TRIA (2, 4, and 6 μg L^{-1}) and BA (0.5, 1, and 2 mg L^{-1}), alongside plant growth regulator (PGR)-free controls (0 μg L^{-1} TRIA + 0 mg L^{-1} BA). Growth parameters including shoot number, length, multiplication percentage, fresh weight (FW), dry weight (DW), and dry matter content (DW%) were assessed after six weeks.

Significant genotype-dependent TRIA × BA interactions were observed across all evaluated traits. Supplementation with optimized TRIA–BA combinations markedly improved shoot proliferation, multiplication efficiency (100%), and biomass accumulation compared to PGR-free controls. For CAB-6P, maximum shoot proliferation (20.1–23.0 shoots/explant) peaked under intermediate-to-high BA (1 mg L^{-1}) alone and combined with targeted TRIA concentrations (4–6 μg L^{-1}), whereas Gisela 6 responded most strongly (16.0 shoots/explant) to lower BA combined with higher TRIA (0.5 mg L^{-1} BA + 6 μg L^{-1} TRIA). Biomass accumulation (FW and DW) improved under combined treatments: CAB-6P peaked at 6 μg L^{-1} TRIA + 2 mg L^{-1} BA, while Gisela 6 peaked at 4 μg L^{-1} TRIA + 1 mg L^{-1} BA. Across both rootstocks, increasing cytokinin levels enhanced shoot multiplication and biomass while concurrently suppressing excessive shoot elongation, which was greatest in the PGR-free control treatments (CAB-6P: 17.83 mm, Gisela 6: 20.19 mm). Further analysis confirmed strong positive associations among shoot proliferation and biomass accumulation (number, multiplication %, FW, and DW), alongside an inverse physiological relationship between intensive shoot multiplication and elongation.

TRIA acts as an effective growth-promoting supplement that synergistically enhances BA-mediated shoot proliferation and biomass accumulation in a genotype-dependent manner. Optimizing TRIA–BA combinations successfully improves *in vitro* shoot regeneration efficiency, providing a reliable strategy for the commercial micropropagation of cherry rootstocks.

Keywords: 6-Benzyladenine, CAB-6P/Gisela 6, long-chain primary alcohol /triacontanol, *In vitro* shoot regeneration, genotype-dependent morphogenetic growth responses

Address: V. Sarropoulou, K. Dimassi-Theriou, I. Therios, Aristotle University of Thessaloniki, School of Agriculture, Department of Horticulture, Laboratory of Pomology, Thessaloniki 54124, Greece;
VS https://orcid.org/0000-0001-7534-5247, e-mail: vsarrop@gmail.com;
KD-T e-mail: dimassik@agro.auth.gr; IT e-mail: therios@agro.auth.gr
*corresponding author

Introduction

Exogenous application of plant growth regulators (PGRs) – specifically triacontanol (TRIA), a naturally occurring long-chain primary alcohol – is widely recognized for significantly enhancing vegetative parameters and overall growth in horticultural crops like strawberry (Baba et al., 2017). Beyond standard growth promotion, long-chain aliphatic alcohols improve morphogenetic responses and promote direct somatic embryogenesis in woody plant tissue cultures (Gatica et al., 2008), while also boosting micropropagation efficiency, shoot proliferation, biomass accumulation, and the synthesis of valuable antioxidant compounds in medicinal species such as *Salvia officinalis* (Grzegorczyk & Wysokinska, 2008). TRIA ($C_{30}H_{61}OH$) is a long-chain primary alcohol naturally found in plant epicuticular waxes and bee waxes (Ries & Houtz,1983; Karam & Keramat, 2017; Digruber et al., 2018). It acts as a potent endogenous growth promoter, effective at very low concentrations, and is valued for its low toxicity, environmental safety, and low cost (Shil, 2021). Naturally occurring in species such as *Medicago sativa*, *Vaccinium ashei*, and *Oryza sativa* (Freeman et al., 1979; Uchiyama & Ogasawara, 1981; Ries & Houtz, 1983), TRIA is widely reported to significantly enhance plant growth and yield upon exogenous application (Ries & Wert, 1982; Misra & Srivastava, 1991).

TRIA acts via a lysolipid-inositol signaling cascade (Morre, 1990; Knight & Mitchell, 1987) to enhance vegetative growth (Baba et al., 2017), morphogenesis (Gatica et al., 2008), micropropagation and antioxidants in *Salvia officinalis* (Grzegorczyk & Wysokinska, 2008), photosynthesis in microalgae (Haugstad et al., 1983), secondary metabolism in *Catharanthus roseus* (Idrees et al., 2010), and crop yields in lemongrass (Khan et al., 2014), while functioning as a versatile biostimulant that regulates active constituents in *Mentha arvensis* (Naeem et al., 2011) and *Acmella oleracea* (Sut et al., 2020), modulates strawberry fruit development (Pang et al., 2020), improves field pea morphology (Segura & Joaquin, 2021), and mitigates salt, heavy metal, and heat stresses across diverse species (Muthuchelian et al., 1990, 1996, 2003; Kissimon et al., 1999; Virdi et al., 2015; Waqas et al., 2016; Khanan & Mohammad, 2018; Zaid et al., 2019; Khan et al., 2020; Verma et al., 2022; Mudassar et al., 2024).

By enhancing photosynthesis, carbon metabolism, enzyme (e.g., RuBisCO) activity, and respiration (Eriksen et al., 1981; Houtz et al., 1985a,b; Savithiry et al., 1992), modulating membrane processes (activation of ATPase and NADH oxidase), signaling molecules like 9-β-L(+)adenosine (Lesniak et al., 1986, 1989; Ries et al., 1990; Morre et al., 1991), and fortifying stress-response mechanisms (photosynthesis, nutrient uptake, water-use efficiency, antioxidant defense, key metabolic activities) (Naeem et al., 2009, 2012, 2019; Islam & Mohammad, 2020; Chettri et al., 2021), TRIA drives robust plant growth and abiotic stress tolerance.

Although TRIA is effective under field and greenhouse conditions, its use in plant tissue culture is still limited. It is stable during autoclaving and has shown variable effects depending on species, ranging from no response in rice to strong growth stimulation in apple (Yun & Kim, 1986; Ma et al., 1990). *In vitro* studies have reported that TRIA increased shoot proliferation, biomass, and secondary metabolite production in several medicinal-woody plants (Fraternale et al., 2003; Grzegorczyk et al., 2006), including apple, cherry, pine, coffee, and tobacco systems (Hangarter et al., 1978; Tantos et al., 2001; Giridhar et al., 2004; Malabadi & Nataraja, 2007).

Efficient *in vitro* propagation protocols are essential for cherry rootstocks (*Prunus* spp.) to produce uniform, disease-free planting material for intensive cultivation (Doric et al., 2014; Eliwa et al., 2025; Manzoor et al., 2025). Optimizing tissue culture performance during the *in vitro* shoot proliferation stage in CAB-6P and Gisela 6 requires fine-tuning various biochemical and physical factors previously investigated for these specific rootstocks, including dikegulac (Sarropoulou et al., 2014), sodium nitroprusside (Sarropoulou et al., 2015), ethylene inhibitors (Sarropoulou et al., 2016a), medium strength and carbohydrates (Sarropoulou et al., 2016b), and polyamines (Sarropoulou et al., 2017), alongside specific LED lighting (Sarropoulou et al., 2023). Additionally, complex organic extracts – such as walnut, hazelnut, kiwi, and peach water pollen extracts – serve as sustainable, eco-friendly biostimulants that significantly enhance *in vitro* growth, rooting, and metabolic partitioning (Sarropoulou et al., 2026a, 2026b).

Although TRIA is effective in various plant systems, its interaction with 6-benzyladenine (BA) for cherry rootstock micropropagation remains poorly understood. While BA is a standard cytokinin used to stimulate axillary shoot proliferation, combining it with the unique lipid-signaling and growth-promoting properties of TRIA offers a novel approach to overcome conventional multiplication bottlenecks. Thus, based on preliminary trials and established effective concentration ranges for woody plant micropropagation (Ma et al., 1990; Tantos et al., 1999, 2001), this study evaluated the combined effects of TRIA (2–6 μg L^{-1}) and BA (0.5–2 mg L^{-1}) on the proliferation of CAB-6P (*Prunus cerasus* L.) and Gisela 6 (*Prunus cerasus* L. × *Prunus canescens* Spach) rootstocks using growth measurements alongside multivariate analyses including Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and Pearson correlation. As one of the first

comprehensive studies on TRIA–BA interactions in cherry rootstocks, this work highlights TRIA's potential as a biostimulant to improve *in vitro* propagation efficiency and identify optimal hormonal combinations for enhanced proliferation and biomass.

Materials and Methods

Plant material

To initiate axenic cultures, 2–2.5 cm long shoot tips were harvested from *Prunus* cherry rootstocks – specifically CAB-6P and Gisela 6 – during mid-spring. The explants underwent an initial 30-min wash under running tap water to remove surface debris. Under a laminar flow hood, surface sterilization was performed by dipping the explants in 70% (v/v) ethanol for 30 sec, followed by immersion in a 15% (v/v) commercial sodium hypochlorite solution (3% active NaOCl supplemented with 0.1% v/v Tween-20) for 10 min with continuous gentle agitation. Afterward, the explants were rinsed three consecutive times with sterile double-distilled water to eliminate all disinfectant residues prior to trimming and inoculation onto the basal medium.

Virus-free *in vitro* cultures of CAB-6P and Gisela 6 cherry rootstocks were supplied by Dr. Angelos Xylogiannis and kept at the Laboratory of Pomology, School of Agriculture, Aristotle University of Thessaloniki, Greece. These initial stock materials originated from certified pre-basic and basic propagation stock supplied by the Xylogiannis Bros Un.Co. «Fitotechniki» Tissue Culture Laboratory located in Filothei, Arta, Greece. Donor cultures were sustained through routine subculturing every four weeks on full-strength Murashige and Skoog (MS) basal medium (Murashige & Skoog, 1962) containing 30 g L^{-1} sucrose, 0.5 mg L^{-1} BA, 0.05 mg L^{-1} indole-3-butyric acid (IBA), 0.1 mg L^{-1} gibberellic acid (GA_3), and 6 g L^{-1} agar.

To eliminate residual hormonal carryover, shoot-tip stock cultures (at the 3rd to 4th subculture stage) were transferred four weeks prior to the experiment onto PGRs-free MS medium supplemented solely with 30 g L^{-1} sucrose and 6 g L^{-1} agar. For the experimental treatments, uniform, healthy, and actively growing shoot tips measuring 1 cm from this final subculture cycle were exclusively chosen to ensure a standardized physiological condition free of dormancy, senescence, or hyperhydricity.

Culture conditions

In this experiment, the synergistic effect of TRIA at concentrations of 2, 4, and 6 μg L^{-1} in combination with BA (0.5, 1, and 2 mg L^{-1}) on shoot proliferation of the CAB-6P and Gisela 6 rootstocks was investigated, alongside PGR-free control treatments (devoid of both TRIA and BA at 0 μg L^{-1} TRIA + 0 mg L^{-1} BA) to serve as a baseline for comparison. BA stock solutions (20 mg dissolved in 1 mL of 1 M NaOH, brought to 100 mL with distilled water) and an aqueous 0.1% TRIA solution were used for appropriate dilutions.

The nutrient medium employed was full-strength MS medium, the specific composition of which is detailed in Table S1 (Supplementary Material). The medium was supplemented with 30 g L^{-1} sucrose and 6 g L^{-1} agar, adjusted to pH 5.8, and autoclaved at 121 °C for 20 min. The explants (one per tube) were aseptically transferred into test tubes (25 × 100 mm) containing 10 mL of culture medium. Each treatment consisted of 10 tubes (replicates).

The microshoots were incubated for 6 weeks in a plant growth chamber at 22 ± 2 °C under a 16 h photoperiod and a light intensity of 90 μmol m^{-2} s^{-1}, provided by cool white fluorescent lamps. These lamps delivered a standard broad-spectrum emission across the photosynthetically active radiation (PAR) region (400–700 nm), characterized by primary peaks in the blue (around 435–440 nm) and green-yellow-red wavebands, ensuring balanced lighting conditions conducive to normal *in vitro* shoot proliferation and subsequent developmental processes.

Growth and multiplication assessment

After 6 weeks of culture for both CAB-6P and Gisela 6, measurements were taken for shoot number/explant, shoot length, and shoot multiplication percentage (%). Harvested shoots were gently cleaned of residual medium, and fresh weight (FW) was immediately recorded using a precision analytical balance. To determine dry weight (DW), samples were placed in an oven at 65 °C for 24 h until a constant weight was reached, and DW was recorded on the following day. The dry matter content (DW%) was subsequently calculated using the following equation:

$$\text{DW (\%)} = [(\text{Dry weight (DW)} / \text{Fresh weight (FW)}] \times 100$$

Statistical analysis

All experiments followed a completely randomized design with 10 explants per treatment. Data were analyzed using ANOVA after testing for normality and homogeneity of variance. Treatment means were compared using Duncan's multiple range test at $p \leq 0.05$. A General Linear Model (GLM) and 2-way ANOVA were used to assess the effects of TRIA, BA,

and their interaction (TRIA × BA) for each cherry rootstock separately, with significance set at $p \leq 0.05$, 0.01, and 0.001. Additionally, a GLM and 3-way ANOVA were employed to determine the main and interactive effects of rootstock type, TRIA concentration, and BA concentration. Pearson correlation analysis was used to examine relationships among treatments and shoot proliferation traits, with significance at $p < 0.05$ and $p < 0.01$; results were visualized using heatmaps. PCA on Z-score–standardized data was performed to assess multivariate trait variation, and variable contributions were visualized via loading plots. HCA using Euclidean distance and average linkage was applied to standardized data to group treatments and traits, with results presented as dendrograms. All analyses and visualizations were performed using SPSS v21.0 (IBM Corp., USA).

Results

Morphological Response of CAB-6P and Gisela 6 to TRIA and BA

For CAB-6P, shoot proliferation varied significantly across TRIA–BA combinations (Figs 1 and 2), peaking with 1 mg L^{-1} BA alone (23.0 shoots/explant), followed closely by 4–6 µg L^{-1} TRIA combined with 1–2 mg L^{-1} BA (19.90–20.90) (Fig. 1a). Conversely, shoot length peaked in the PGRs-free control (17.83 mm) (Fig. 1b), whereas multiplication efficiency increased from 40% in controls to 90–100% under supplemented treatments (Fig. 1c). Biomass accumulation peaked with 6 µg L^{-1} TRIA + 2 mg L^{-1} BA, yielding 2.915 g FW and 0.16 g DW compared to controls (Fig. 1d and e), while dry matter content was highest in the control (14.77%) versus 5.60 to 11.31% in supplemented treatments (Fig. 1f). GLM

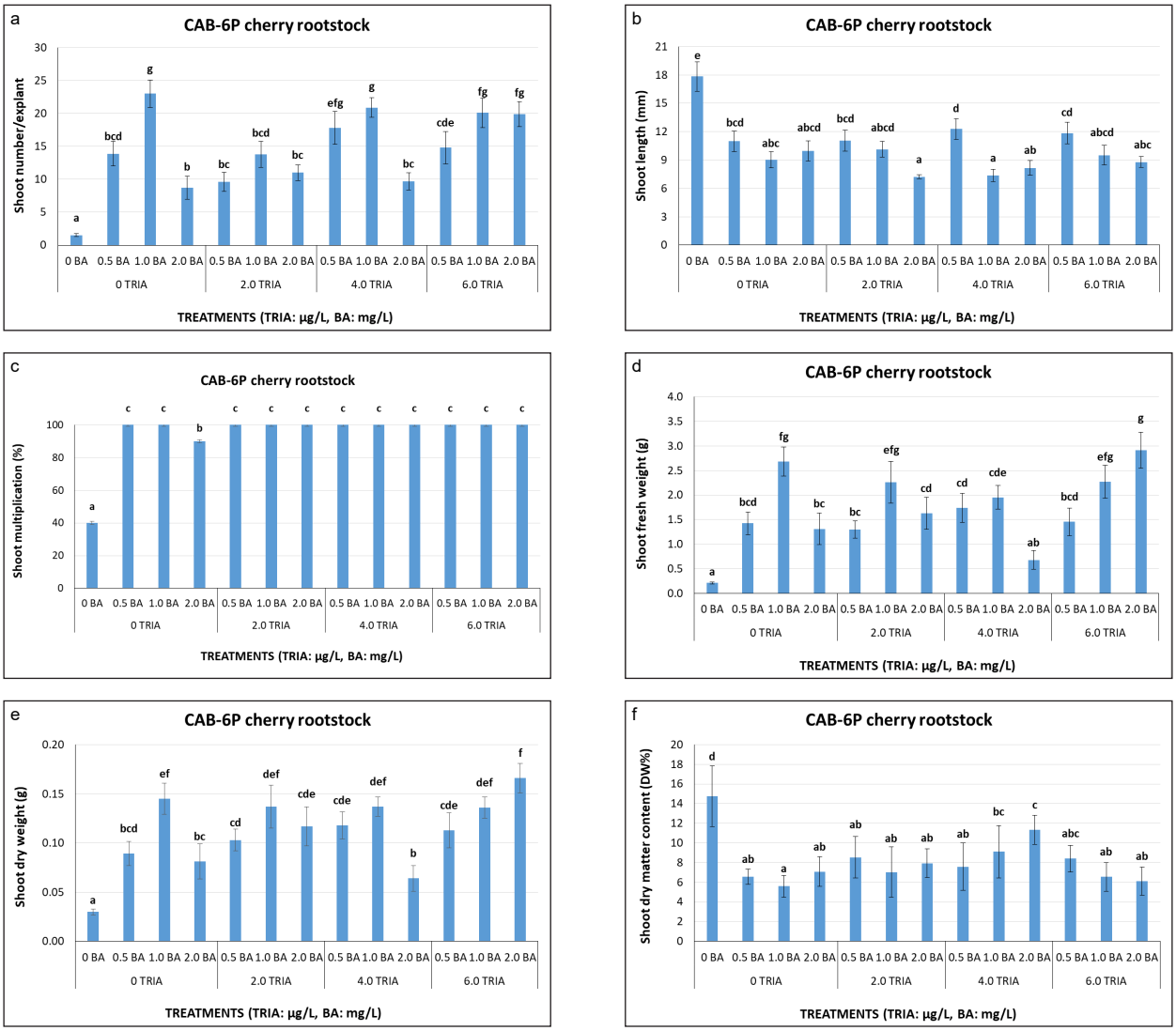


Fig. 1. Effects of TRIA and BA combinations on *in vitro* shoot proliferation of CAB-6P rootstock: (a) shoot number; (b) length; (c) multiplication %; (d) FW; (e) DW; (f) DW%. Means (n=10) ± SE. Different letters denote significant differences ($p \leq 0.05$; Duncan's test)

analysis (Table S2) demonstrated that TRIA, BA, and their interaction significantly influenced shoot number, FW, and DW ($p < 0.001$), whereas shoot length was affected solely by BA ($p < 0.001$).

For Gisela 6 (Figs 3 and 4), the highest shoot proliferation (16.00 shoots per explant) occurred with 6 μg L^{-1} TRIA + 0.5 mg L^{-1} BA, whereas BA alone produced only 5.75–5.88 shoots per explant (Fig. 3a). Shoot length was greatest in the control (20.19 mm) and declined with increasing BA concentration, reaching 8.68 mm at 6 μg L^{-1} TRIA + 2 mg L^{-1} BA (Fig. 3b). Multiplication efficiency increased from 22.22% in the control to 100% under all TRIA–BA combinations (Fig. 3c). Maximum FW (2.388 g) and DW (0.148 g) were obtained with 4 μg L^{-1} TRIA + 1 mg L^{-1} BA (Fig. 3d and e), while dry matter content was highest in the control (12.24%) (Fig. 3f). GLM analysis (Table S2) indicated that TRIA, BA, and their interaction significantly influenced shoot number ($p \leq 0.023$), whereas length was primarily affected by BA ($p < 0.001$), with FW and DW governed predominantly by TRIA and relatively limited effects from BA.

The combined three-way ANOVA revealed significant rootstock-dependent responses to TRIA and BA treatments (Table S3). Main factors and their interactions significantly affected shoot number, multiplication efficiency (%), and FW (Fig. 5a,c,d; Table S3). Shoot length was influenced by rootstock, BA, and

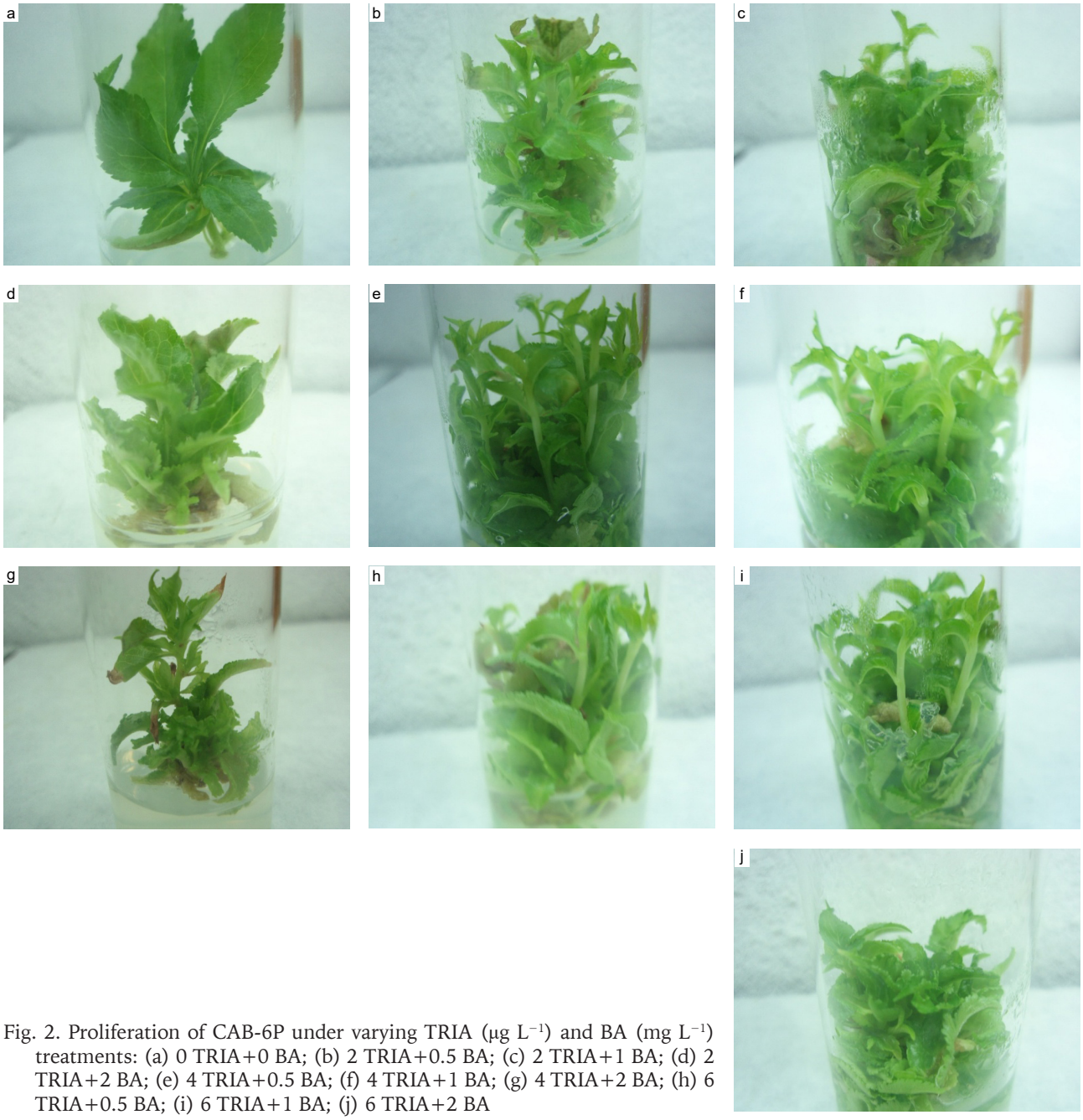


Fig. 2. Proliferation of CAB-6P under varying TRIA (μg L^{-1}) and BA (mg L^{-1}) treatments: (a) 0 TRIA+0 BA; (b) 2 TRIA+0.5 BA; (c) 2 TRIA+1 BA; (d) 2 TRIA+2 BA; (e) 4 TRIA+0.5 BA; (f) 4 TRIA+1 BA; (g) 4 TRIA+2 BA; (h) 6 TRIA+0.5 BA; (i) 6 TRIA+1 BA; (j) 6 TRIA+2 BA

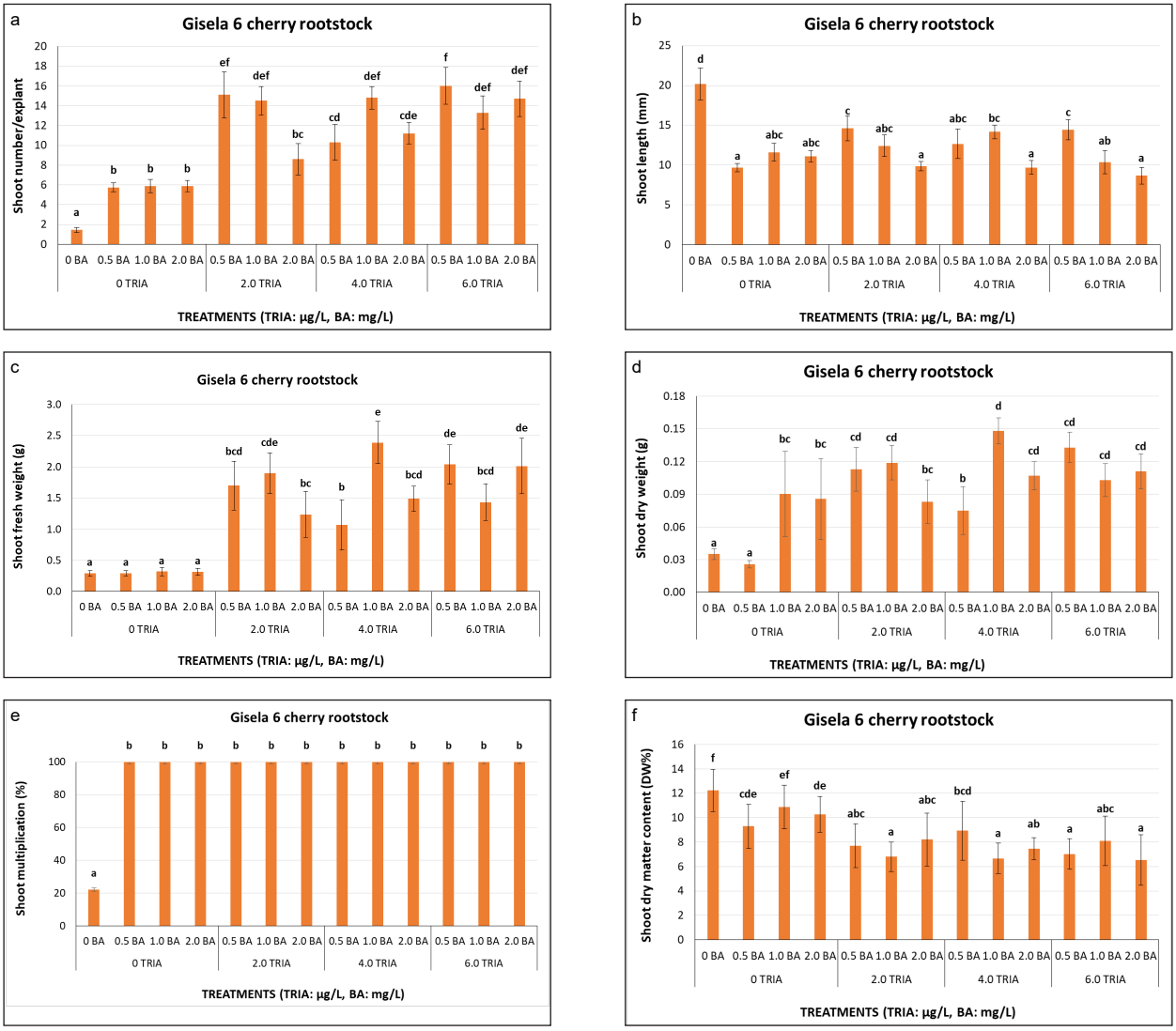


Fig. 3. Effects of TRIA and BA combinations on *in vitro* shoot proliferation of Gisela 6 rootstock: (a) shoot number; (b) length; (c) multiplication %; (d) FW; (e) DW; (f) DW%. Means (n = 10) ± SE. Different letters denote significant differences ($p \leq 0.05$; Duncan's test)

their interaction (Fig. 5b; Table S3), while DW and dry matter content responded to TRIA, BA, and interactive effects without direct main rootstock effects (Fig. 5e and f; Table S3). Overall, CAB-6P demonstrated superior proliferation and biomass accumulation, whereas Gisela 6 exhibited enhanced elongation, emphasizing strong interactive regulation by rootstock, TRIA, and BA (Fig. 5a–f; Table S3).

Treatments Pearson Correlation Analysis of CAB-6P and Gisela 6

In the CAB-6P rootstock (Fig. 6a), shoot number was negatively correlated with shoot length and dry matter content, but showed strong positive correlations with FW, DW, and multiplication percentage. Shoot length itself was negatively correlated with FW, DW, and multiplication percentage, yet positively correlated with dry matter content. Additionally, shoot FW and DW strongly correlated with each other and positively with multiplication percentage, while showing a strong negative correlation with dry matter content.

For the Gisela 6 rootstock (Fig. 6b), shoot number similarly exhibited strong positive correlations with FW, DW, and multiplication percentage, alongside a strong negative correlation with dry matter content, though its correlation with shoot length was not significant. Shoot length correlated significantly and negatively only with multiplication percentage.

Across both rootstocks (Fig. 6a and b), shoot multiplication percentage consistently showed strong negative correlations with shoot dry matter content. Overall, these patterns indicate robust associations between shoot number, biomass accumulation, and multiplication performance – particularly in CAB-6P – while shoot dry matter content consistently

maintained inverse relationships with shoot number and biomass-related traits in both rootstocks.

Principal Component Analysis of CAB-6P and Gisela 6

PCA revealed high data adequacy and a robust two-component structure explaining 89.90% and 92.53% of the total variance for CAB-6P (Figs S1a–c) and Gisela 6 (Figs S2a–c), respectively. For both rootstocks, PC1 was primarily driven by biomass and quantity traits (FW, DW, and shoot number) alongside dry matter content, accounting for 77.67% of the variance in CAB-6P and 69.08% in Gisela 6 (Figs S1d–f, S2d–f; Fig. 7a and b). PC2 captured the remaining variance (12.22% for CAB-6P and 23.45% for Gisela 6) and was consistently governed by shoot length and multiplication percentage across both genotypes (Figs S1f and g, S2f and g). While both rootstocks shared this fundamental two-component architecture – where growth is dictated primarily by biomass (PC1) and secondarily by elongation traits (PC2) – they exhibited distinct physiological patterns (Fig. 7a and b). CAB-6P concentrated variance more heavily in PC1, whereas Gisela 6 displayed a

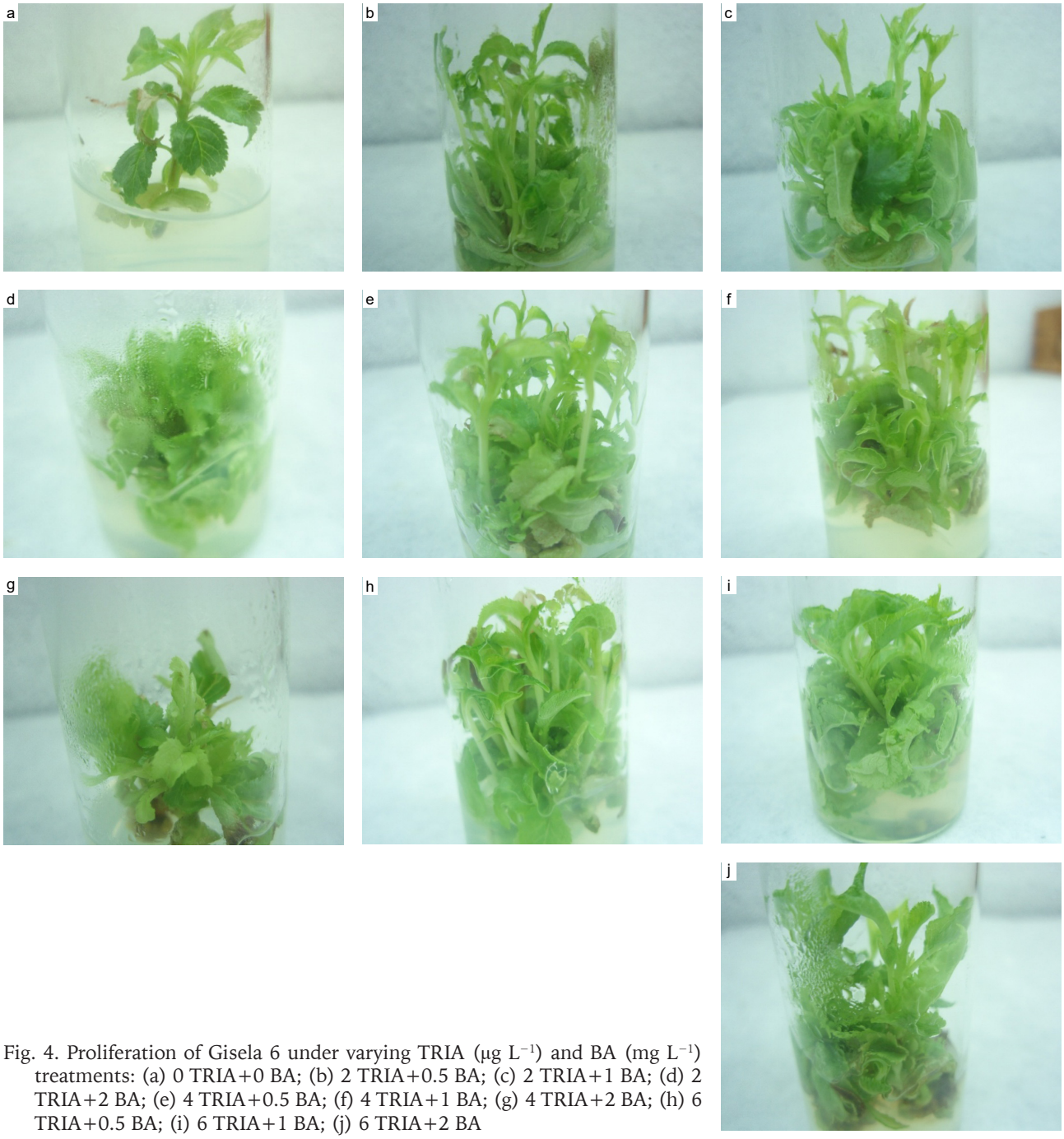


Fig. 4. Proliferation of Gisela 6 under varying TRIA (µg L^{-1}) and BA (mg L^{-1}) treatments: (a) 0 TRIA+0 BA; (b) 2 TRIA+0.5 BA; (c) 2 TRIA+1 BA; (d) 2 TRIA+2 BA; (e) 4 TRIA+0.5 BA; (f) 4 TRIA+1 BA; (g) 4 TRIA+2 BA; (h) 6 TRIA+0.5 BA; (i) 6 TRIA+1 BA; (j) 6 TRIA+2 BA

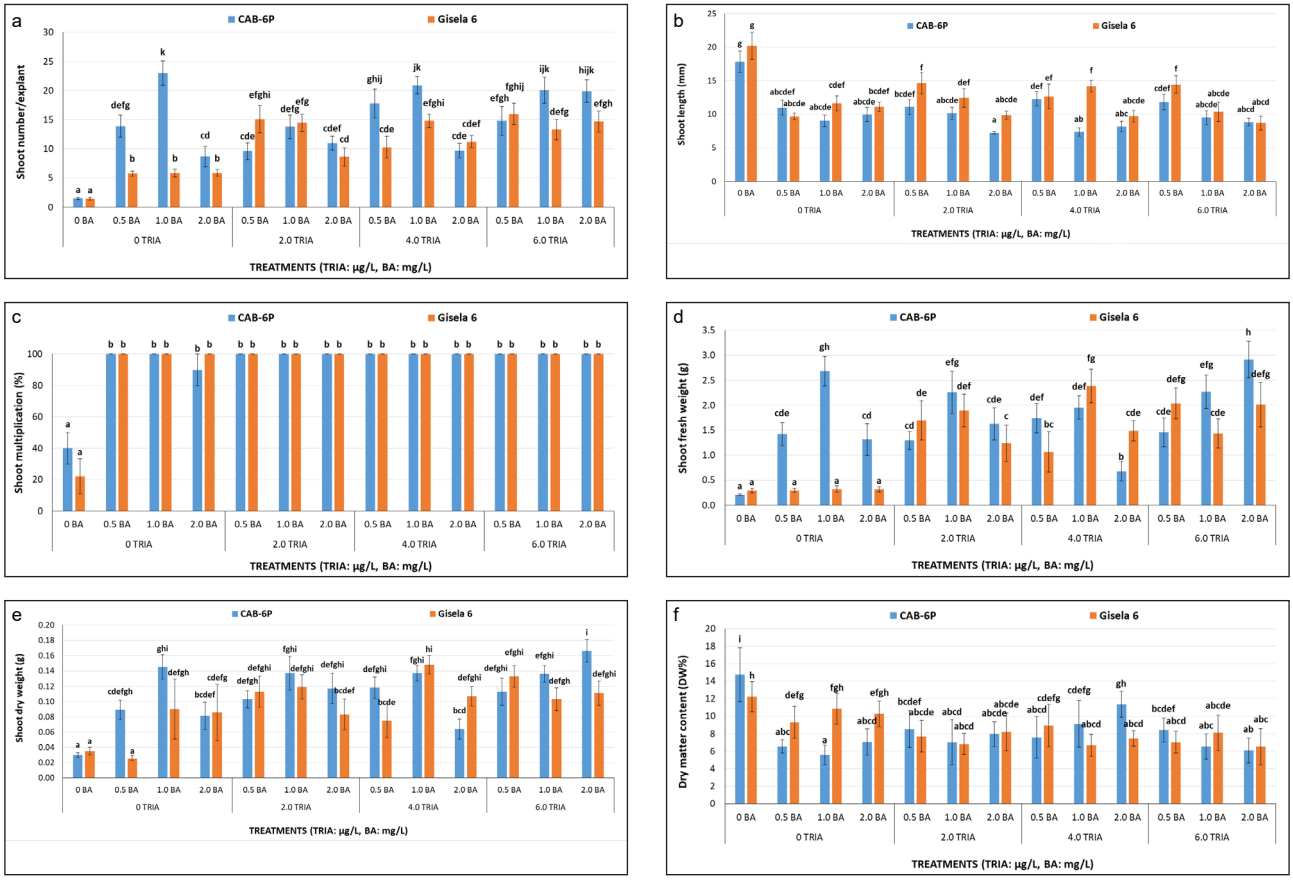


Fig. 5 Comparative effects of TRIA and BA on *in vitro* shoot proliferation of CAB-6P and Gisela 6: (a) shoot number; (b) length; (c) multiplication %; (d) FW; (e) DW; (f) DW%. Data ± SE (n = 10). Different letters denote significant differences ($p \leq 0.05$; Duncan's test)

more balanced distribution between components, with contrasting score coefficients highlighting genotype-specific trait adaptations under TRIA and BA treatments (Figs S1g, S2g; Fig. 7a and b).

Hierarchical Cluster Analysis of CAB-6P and Gisela 6

For CAB-6P, the treatment clustering (Fig. 8a) revealed that the PGRs-free control formed a distinct group separate from all combined TRIA and BA treatments. The variable clustering (Fig. 8b) grouped shoot FW, shoot DW, and shoot number into a primary cluster closely linked at a low rescaled distance, while shoot multiplication percentage joined them at a slightly higher distance, and shoot length and shoot dry matter content formed a separate independent cluster.

For Gisela 6, the treatment dendrogram (Fig. 8c) similarly isolated the PGRs-free control from the BA + TRIA treatments. The variable clustering (Fig. 8d) showed that shoot number closely clustered with shoot FW and DW, followed by shoot multiplication percentage, whereas shoot length and shoot dry matter content formed a separate secondary cluster.

In the comparative analysis of treatments across both cherry rootstocks (Fig. 8e), the dendrogram successfully categorized the combined treatments and highlighted that the control treatments for both CAB-6P and Gisela 6 clustered distinctly apart from the fortified TRIA and BA combinations, reflecting their unique morphological and physiological profiles.

Discussion

The results of the present investigation clearly demonstrate that the optimal combined use of TRIA and BA significantly enhances the *in vitro* propagation performance of both CAB-6P and Gisela 6 cherry rootstocks, whereas inappropriate or excessive combinations yielded comparatively lower responses. PGRs-supplemented media enhanced shoot formation, multiplication efficiency, and biomass accumulation in cherry rootstocks compared to untreated controls, demonstrating that exogenous growth regulators are essential for maximizing micropropagation success. Comparable stimulatory effects of TRIA on shoot multiplication have been documented across a diverse range of plant species. These include

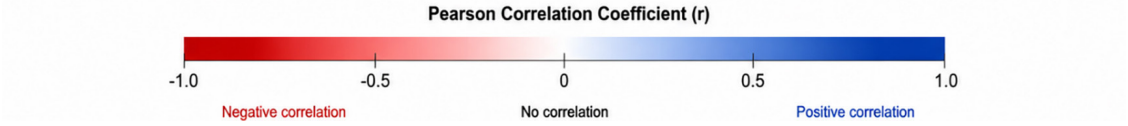
a

Pearson Correlation Heatmap (CAB-6P)

		Shoot_number	Shoot_length	Shoot_FW	Shoot_DW	Shoot_multiplication_ percentage	Shoot_dry_matter_ content
Shoot_number	Pearson Correlation	1	-.565*	.872**	.878**	.671*	-.726**
	Sig. (2-tailed)		.044	.000	.000	.012	.005
Shoot_length	Pearson Correlation	-.565*	1	-.553*	-.611*	-.811**	.577*
	Sig. (2-tailed)	.044		.050	.027	.001	.039
Shoot_FW	Pearson Correlation	.872**	-.553*	1	.966**	.608*	-.835**
	Sig. (2-tailed)	.000	.050		.000	.027	.000
Shoot_DW	Pearson Correlation	.878**	-.611*	.966**	1	.693**	-.791**
	Sig. (2-tailed)	.000	.027	.000		.009	.001
Shoot_multiplication_ percentage	Pearson Correlation	.671*	-.811**	.608*	.693**	1	-.772**
	Sig. (2-tailed)	.012	.001	.027	.009		.002
Shoot_dry_matter_ content	Pearson Correlation	-.726**	.577*	-.835**	-.791**	-.772**	1
	Sig. (2-tailed)	.005	.039	.000	.001	.002	

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).



b

Pearson Correlation Heatmap (Gisela 6 rootstock)

		Shoot_number	Shoot_length	Shoot_FW	Shoot_DW	Shoot_multiplication_ percentage	Shoot_dry_matter_ content
Shoot_number	Pearson Correlation	1	-.214	.937**	.855**	.586*	-.932**
	Sig. (2-tailed)		.483	.000	.000	.035	.000
Shoot_length	Pearson Correlation	-.214	1	-.076	-.104	-.771**	.402
	Sig. (2-tailed)	.483		.806	.736	.002	.173
Shoot_FW	Pearson Correlation	.937**	-.076	1	.858**	.390	-.922**
	Sig. (2-tailed)	.000	.806		.000	.188	.000
Shoot_DW	Pearson Correlation	.855**	-.104	.858**	1	.512	-.762**
	Sig. (2-tailed)	.000	.736	.000		.074	.002
Shoot_multiplication_ percentage	Pearson Correlation	.586*	-.771**	.390	.512	1	-.638*
	Sig. (2-tailed)	.035	.002	.188	.074		.019
Shoot_dry_matter_ content	Pearson Correlation	-.932**	.402	-.922**	-.762**	-.638*	1
	Sig. (2-tailed)	.000	.173	.000	.002	.019	

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

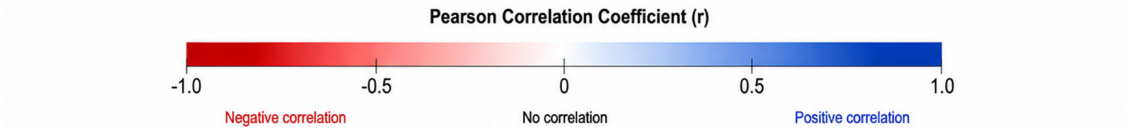
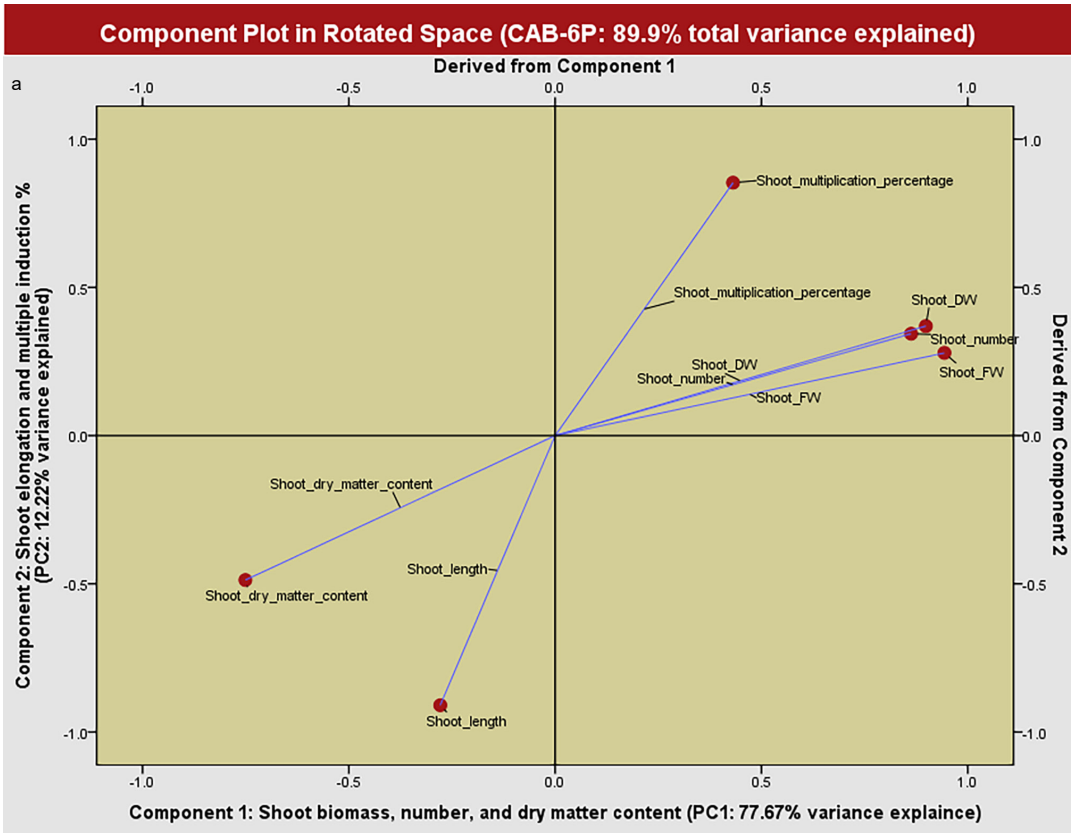


Fig. 6 Pearson correlation heatmaps for traits in: (a) CAB-6P; (b) Gisela 6 (r from −1.0 to 1.0)



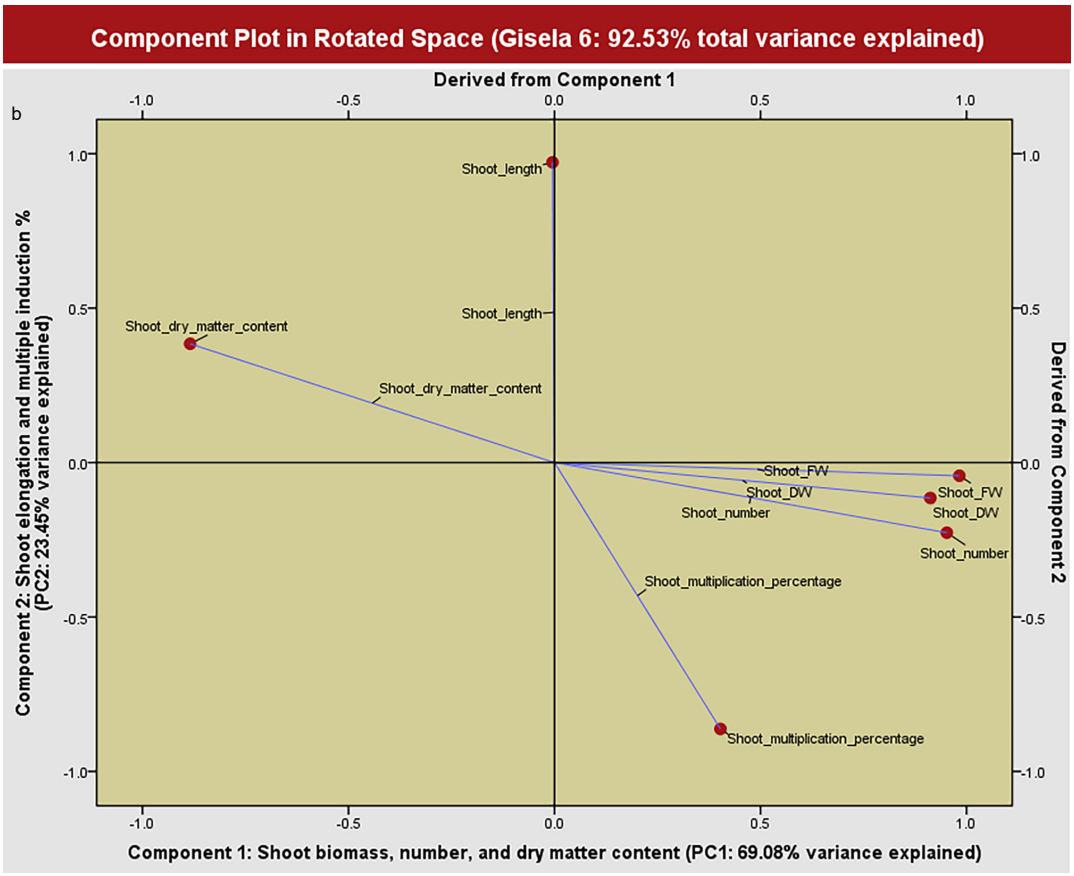


Fig. 7 Rotated PCA biplots for: (a) CAB-6P; (b) Gisela 6. Red points at blue vector tips denote traits; vector lengths/angles indicate correlations, grouping variables into biomass versus elongation/multiplication clusters (acute/obtuse angles = positive/negative correlations)

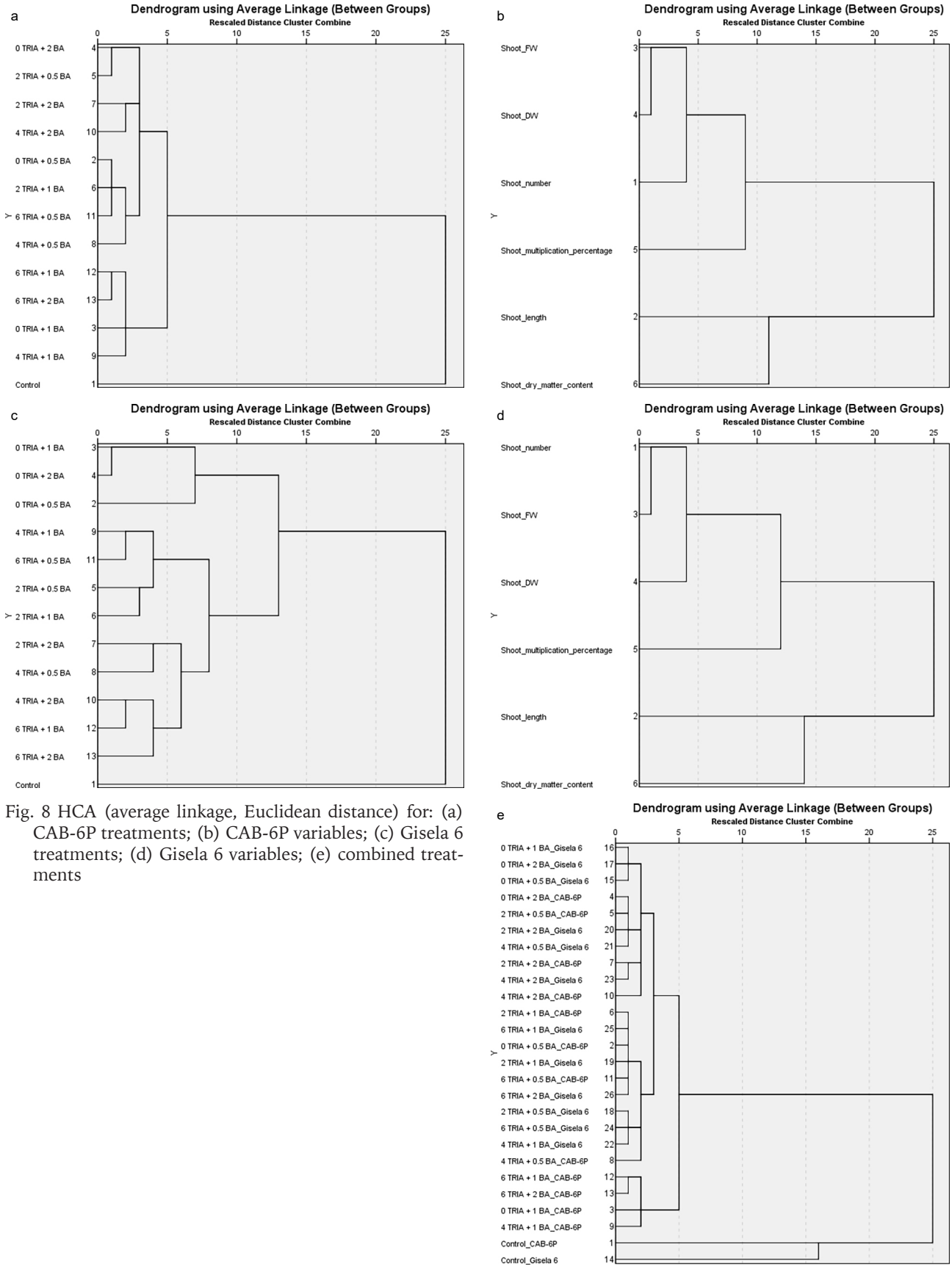


Fig. 8 HCA (average linkage, Euclidean distance) for: (a) CAB-6P treatments; (b) CAB-6P variables; (c) Gisela 6 treatments; (d) Gisela 6 variables; (e) combined treatments

woody fruit and forest species such as *Malus domestica*, *Cerasus fruticosa*, and date palm (Tantos et al., 2001; Madi & Al-Mayahi, 2024), numerous medicinal and aromatic herbs like *Melissa officinalis*, *Salvia officinalis*, *Thymus mastichina*, and *Dracocephalum forrestii* (Tantos et al., 1999; Fraternale et al., 2003; Grzegorczyk et al., 2006; Weremczuk-Jezyna et al., 2022), as well as various other horticultural and economic crops (Fraternale et al., 2002; Reddy et al., 2002; Mishra et al., 2001; Malabadi et al., 2005; Parimalan et al., 2009).

Moderate-to-high BA concentrations enhanced shoot proliferation by stimulating cell division and meristem activity (Gao, 2020). However, optimal hormone combinations varied by genotype, with CAB-6P maximizing proliferation at 1 mg L^{-1} BA (± TRIA) and Gisela 6 performing best at 6 μg L^{-1} TRIA combined with 0.5 mg L^{-1} BA. Similar synergistic interactions between TRIA and BA have been documented in various other tissue culture systems, including *Tinospora cordifolia* (Gururaj et al., 2007), *Arachis hypogaea* (Verma et al., 2011), *Dendrocalamus strictus* (Mishra et al., 2001), *Bixa orellana* (Parimalan et al., 2009), *Dracocephalum forrestii* (Weremczuk-Jeżyna et al., 2022), and *Artemisia dracunculus* (Kadhim et al., 2025).

TRIA significantly enhanced shoot regeneration, multiplication, and biomass in both rootstocks, aligning with similar findings in *Malus domestica* and *Cerasus fruticosa* (Tantos et al., 2001), *Salvia officinalis* (Grzegorczyk et al., 2006), *Thymus mastichina* (Fraternale et al., 2003), *Capsicum frutescens* and *Decalepis hamiltonii* (Reddy et al., 2002), and *Dracocephalum forrestii* (Weremczuk-Jeżyna et al., 2022).

Several physiological mechanisms may explain the beneficial effects of TRIA. Early studies indicate that TRIA promotes growth primarily through stimulated cell division rather than cell enlargement (Hangarter et al., 1978). By triggering the synthesis of signaling molecules like 9-β-L(+) adenosine, TRIA exhibits cytokinin-like properties that can act synergistically with BA to enhance axillary shoot multiplication (Ries & Houtz, 1983; Ries & Stutte, 1985). Furthermore, TRIA is associated with improved photosynthetic efficiency, carbon assimilation, and nutrient acquisition (Borowski et al., 2000; Chen et al., 2002; Krishnan & Kumari, 2008; Alharbi et al., 2021), alongside broader metabolic and PGRs regulation (Ries et al., 1993; Perveen et al., 2017; Islam & Mohammad, 2020; Sarwar et al., 2021). While the precise biochemical and molecular pathways were not directly measured in this study, the growth-promoting effects of TRIA observed here – expressed as significant improvements in shoot proliferation rates, shoot length, and biomass accumulation – align with its established capacity to stimulate cell division rather than simple cell elongation (Hangarter et al., 1978; Ries & Houtz, 1983). By exhibiting cytokinin-like properties (Ries & Stutte, 1985), TRIA likely provides synergistic hormonal support when combined with BA, helping to overcome traditional *in vitro* propagation bottlenecks and directly translating into the superior morphological performance recorded for both cherry rootstocks.

Elevated BA and TRIA concentrations promoted dense shoot clusters while inversely reducing shoot length. This trade-off – where enhanced meristematic activity and multiple shoot formation occur at the expense of internode elongation – is typical of cytokinin-rich culture systems and is supported by significant negative correlations with multiplication percentages. While this pattern mirrors findings in other species (Tantos et al., 2001, 1999), contrasting reports of TRIA-stimulated elongation exist (Reddy et al., 2002; Borowski et al., 2009), likely reflecting differences in species-specific PGRs sensitivity and endogenous balance.

Combined TRIA and BA treatments maximized biomass production (FW, DW), with optimal yields occurring at 6 μg L^{-1} TRIA + 2 mg L^{-1} BA for CAB-6P and 4 μg L^{-1} TRIA + 1 mg L^{-1} BA for Gisela 6. Strong positive correlations and PCA confirmed that increased shoot proliferation (number, FW, and DW) drove greater tissue accumulation. These findings align with documented TRIA-induced biomass gains in *Salvia officinalis* (Grzegorczyk et al., 2006), *Capsicum frutescens* (Reddy et al., 2002), *Thymus mastichina* (Fraternale et al., 2003); *Dracocephalum forrestii* (Weremczuk-Jeżyna et al., 2022), and rice (Alharbi et al., 2021), typically attributed to enhanced photosynthesis, nutrient assimilation, and cellular growth.

Multivariate analyses revealed a clear separation between proliferation/biomass traits and shoot elongation, reflecting the physiological trade-off between cell division and expansion. Gisela 6 displayed a more integrated response pattern than CAB-6P, and HCA grouped treatments by PGRs composition, separating PGR-free controls from active TRIA and BA combinations. Across all metrics, shoot number closely tracked biomass while shoot length varied independently. Ultimately, while excessive cytokinins reduced elongation, combining moderate-to-high TRIA concentrations with intermediate BA levels optimized the balance between multiplication efficiency and biomass production, highlighting TRIA as a promising supplement for cherry rootstock micropropagation.

CAB-6P and Gisela 6 exhibited distinct, rootstock-dependent responses to TRIA and BA treatments. While Gisela 6 generally showed a higher overall shoot proliferation rate and greater mean shoot length than CAB-6P across most corresponding TRIA and BA combinations, CAB-6P excelled in specific biomass accumulation, whereas Gisela 6 displayed a more buffered response. Across both

rootstocks, PGRs increased multiplication success to 90–100%. An inverse relationship between shoot number and elongation reflected the expected trade-off from axillary bud activation to proliferation (Shimizu-Sato et al., 2009; Šmeringai et al., 2023), mirrored by concentration-dependent molecular regulation in *Prunus* (Testone et al., 2023). Furthermore, rapid BA-induced proliferation reduced dry matter content – dropping from 14.8% to 5.6% in CAB-6P controls versus high-BA treatments – largely due to increased tissue hydration and hyperhydricity risks (Polivanova & Bedarev, 2022; Ersali, 2024). Because TRIA influences growth, photosynthesis, and stress metabolism in a concentration- and genotype-dependent manner (Islam & Mohammad, 2020; Weremczuk-Jeżyna et al., 2022), strategic supplementation helped mitigate these hydration effects. In CAB-6P, adding 4 μg L^{-1} TRIA + 2 mg L^{-1} BA restored dry matter to 11.3%, whereas Gisela 6 maintained stable dry matter levels (7.7% to 12.2%), underscoring inherent physiological differences (Sarropoulou et al., 2014, 2016a, 2016b). Ultimately, multiplication rates alone should not dictate protocol design. Because CAB-6P benefits from targeted TRIA-BA balancing to counteract tissue hydration while Gisela 6 favors elongation and stable dry matter retention, rootstock-specific optimization is essential to balance overall efficiency, shoot extension, and tissue quality. Despite these quantitative performance differences indicating genotype-specific PGR requirements and variation in endogenous regulatory mechanisms, both rootstocks responded positively to the synergistic application of the growth regulators.

Conclusions

Combined application of TRIA and BA significantly enhanced *in vitro* shoot proliferation, biomass accumulation, and regeneration in CAB-6P and Gisela 6 cherry rootstocks compared with the control, confirming TRIA as an effective biostimulant. Optimal responses were genotype-dependent, with CAB-6P performing best under higher BA combined with TRIA, whereas Gisela 6 responded more strongly to lower BA and higher TRIA. Although cytokinin-rich media promoted proliferation and biomass, they reduced shoot elongation, highlighting a clear growth trade-off. Pearson correlation analysis, PCA, and HCA showed strong positive associations among shoot number, FW, and DW, while shoot length behaved independently and was negatively related to intensive proliferation. Overall, TRIA–BA combinations are promising for improving micropropagation efficiency in cherry rootstocks and other woody species. Future work should investigate the physiological, biochemical, and molecular mechanisms underlying TRIA action – including PGRs regulation, antioxidant defense, and gene expression via omics-based approaches – while also evaluating plantlet stability, rooting, acclimatization, and field performance. Optimization of PGRs balance is needed to overcome reduced shoot elongation under high cytokinin levels, and broader testing across other *Prunus* and woody species could expand its commercial application.

Acknowledgements

We would like to express our sincere gratitude to Angelos Xylogiannis, co-owner of the Tissue Culture Laboratory Xylogiannis Bros Un.Co. "Fitotechniki" (Filothei, Arta, Greece), for kindly providing the virus-free *in vitro* cultures of the CAB-6P and Gisela 6 cherry rootstocks used in this study. Also our thanks to Sofia Kuti and Vasiliki Tsakiridou for technical assistance. The authors gratefully acknowledge the financial support of the Aristotle University of Thessaloniki.

Author contributions

Conceptualization: KDT and IT; Methodology: VS; Formal analysis and investigation: VS; Writing - original draft preparation: VS; Writing - review and editing: VS, KDT, and IT; Funding acquisition: VS; Resources: KDT and IT; Supervision: KDT. All authors have read and approved the final version of the manuscript.

Funding

This research was funded by the 'Aristotle University of Thessaloniki (AUTH)', Greece as a grant of scholarship excellence for post-doctoral research, year 2013. Research program entitled "Scholarships of Excellence - Awards of Excellence and Innovation of the Aristotle University of Thessaloniki", No. 46389/2012.

Data Availability

The authors declare that the data supporting the findings of this study are available within the paper. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

Declarations

Ethical approval

Not applicable. This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing Interests
The authors have no competing interests to declare that are relevant to the content of this article.

References

Alharbi BM, Abdulmajeed AM & Hassan H (2021) Biochemical and molecular effects induced by triacontanol in acquired tolerance of rice to drought stress. Genes 12: 1119. doi:10.3390/genes12081119.

Baba TR, Ali A, Javid R, Kumar A, Qayoom S, Wani AW & Ali MT (2017) Periodic response of vegetative growth of strawberry to salicylic acid and triacontanol. International Journal of Chemical Studies 5: 2414–2417.

Borowski E, Blamowski ZK & Michałek W (2000) Effects of Tomatex/Triacontanol/on chlorophyll fluorescence and tomato (*Lycopersicon esculentum* Mill.) yields. Acta Physiologiae Plantarum 22: 271–274. doi:10.1007/s11738-000-0030-5.

Borowski E & Blamowski ZK (2009) The effects of triacontanol 'TRIA' and Asahi SL on the development and metabolic activity of sweet basil (*Ocimum basilicum* L.) plants treated with chilling. Folia Horticulturae 21: 39–48. doi:10.2478/fhort-2013-0124.

Chen X, Yuan H, Chen R, Zhu L, Du B, Weng Q & He G (2002) Isolation and characterization of triacontanol-regulated genes in rice (*Oryza sativa* L.): Possible role of triacontanol as a plant growth stimulator. Plant and Cell Physiology 43: 869–876. doi:10.1093/pcp/pcf100.

Chettri P, Thapa U & Tamang T (2021) Triacontanol: A promising plant growth regulator. Krishi Udyan Darpan 1: 105–107.

Digruber T, Sass L, Cseri A, Paul K, Nagy AV, Remenyik J, Molnár I, Vass I, Toldi O, Gyuricza C & Dudits D (2018) Stimulation of energy willow biomass with triacontanol and seaweed extract. Industrial Crops and Products 120: 104–112. doi:10.1016/j.indcrop.2018.04.047.

Doric D, Ognjanov V, Ljubojevic M, Barac G, Dulic J, Pranjic A & Dugalic K (2014) Rapid propagation of sweet and sour cherry rootstocks. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 42: 488–494. doi:10.15835/nbha4229671.

Eliwa GI, El Dengawy ERF, Gawish MS & Yamany MM (2025) Comprehensive study on *in vitro* propagation of some imported peach rootstocks b. *In vitro* rooting and acclimatization. Scientific Reports 15: 17905. doi:10.1038/s41598-025-00848-z.

Eriksen AB, Sellden G, Skagen D & Nilsen S (1981) Comparative analysis of the effect of triacontanol on photosynthesis, photorespiration and growth of tomato (C3-plant), and maize (C4-plant). Planta 152: 44–49. doi:10.1007/BF00384983.

Ersali Y (2024) Control of hyperhydricity of *Pistacia khinjuk* stocks *in vitro* shoots. BMC Biotechnology 24: 97. doi:10.1186/s12896-024-00929-3.

Fraternale D, Giamperi L, Ricci D & Rocchi MBL (2002) Micropropagation of *Bupleurum fruticosum*: The effect of triacontanol. Plant Cell, Tissue and Organ Culture 69: 135–140. doi:10.1023/A:1015245810053.

Fraternale D, Giomper L, Ricci D, Rocchi MBL, Guidi L & Epifano F (2003) The effects of triacontanol on micropropagation and secretory system of *Thymus mastichina* L. Plant Cell, Tissue and Organ Culture 74: 87–97. doi:10.1023/A:1023321024040.

Freeman B, Albrigo LG & Biggs RH (1979) Cuticular waxes of developing leaves and fruit of blueberry, *Vaccinium ashei* Reade cv. Bluegem. Journal of the American Society for Horticultural Science 104: 398–403. doi:10.21273/JASHS.104.3.398.

Gao S (2020) Function and mechanism study of plant cytokinins. Proceedings of the 2020 10th International Conference on Biomedical Engineering and Technology (ICBET '20), Association for Computing Machinery, New York, NY, USA, pp. 80–84. doi:10.1145/3397391.3397395.

Gatica AM, Arrieta G & Espinosa AM (2008) Direct somatic embryogenesis in *Coffea arabica* L. cvs Catura and Catuai: Effect of triacontanol, light condition, and medium consistence. Agronomía Costarricense 32: 139–147.

Giridhar P, Indu EP, Ravishankar GA & Candrasekar A (2004) Influence of triacontanol on somatic embryogenesis in *Coffea arabica* L. and *Coffea canephora* P. ex FR. In Vitro Cellular and Developmental Biology –Plant 40: 200–203. doi:10.1079/IVP2003519.

Grzegorczyk I, Bilichowski I, Mikiciuk-Olasik E & Wysokińska H (2006) The effect of triacontanol on shoot multiplication and production of antioxidant compounds in shoot cultures of *Salvia officinalis* L. Acta Societatis Botanicorum Poloniae 75: 11–15. doi:10.5586/asbp.2006.002.

Grzegorczyk I & Wysokinskam H (2008) Liquid shoot culture of *Salvia officinalis* L. for micropropagation and production of antioxidant compounds: Effect of triacontanol. Acta Societatis Botanicorum Poloniae 77: 99–104. doi:10.5586/asbp.2008.013.

Gururaj HB, Giridhar P & Ravishankar GA (2007) Micropropagation of *Tinospora cordifolia* (Willd.)

Miers ex Hook. F & Thoms – A multipurpose medicinal plant. Current Science 92: 23–26.

Hangarter R, Ries SK & Carlson P (1978) Effect of triacontanol on plant cell cultures *in vitro*. Plant Physiology 61: 855–857. doi:10.1104/pp.61.5.855.

Haugstad M, Ulsaker LK, Ruppel A & Nilsen S (1983) The effect of triacontanol on growth, photosynthesis and photo respiration in *Chlamidomonas reinhardii* and *Anacystis nidulans*. Physiologia Plantarum 58: 451–456. doi:10.1111/j.1399-3054.1983.tb05726.x.

Houtz RL, Ries SK & Tolbert NE (1985a) Effect of triacontanol on *Chlamidomonas* I. Stimulation of growth and photosynthetic CO_2 assimilation. Plant Physiology 79: 357–364. doi:10.1104/pp.79.2.357.

Houtz RL, Ries SK & Tolbert NE (1985b) Effect of triacontanol on *Chlamidomonas* II. Specific activity of ribulose-bisphosphate carboxylase/oxygenase, ribulose-bisphosphate concentration and characteristics of photorespiration. Plant Physiology 79: 365–370. doi:10.1104/pp.79.2.365.

Idrees M, Khan MMA, Aftab T & Naeem M (2010) Synergistic effects of gibberellic acid and triacontanol on growth, physiology, enzyme activities and essential oil content of *Coriandrum sativum* L. Asian-Australasian Journal of Bioscience and Biotechnology 4: 24–29.

Islam S & Mohammad F (2020) Triacontanol a dynamic growth regulator for plants under diverse environmental conditions. Physiology and Molecular Biology of Plants 26: 871–883. doi:10.1007/s12298-020-00815-0.

Kadhim JS, Kahdim ZK & Almukhtar SA (2025) Investigation of the impact of illumination type and triacontanol on the development and chemical compound production of the tarragon plant, *Artemisia dracunculus* L. *in vitro*. IOP Conference Series: Earth and Environmental Science 1538: 012052. doi:10.1088/1755-1315/1538/1/012052.

Karam EA & Keramat B (2017) Foliar spray of triacontanol improves growth by alleviating oxidative damage in coriander under salinity. Indian Journal of Plant Physiology 22: 120–124. doi:10.1007/s40502-017- 0286-z.

Khan ZH, Mohammad F & Khan MMA (2014) Enhancing the growth, yield and production of essential oil and citral in lemongrass by the application of triacontanol. International Journal of Agricultural Research 4: 113–122.

Khan A, Hayat F, Nawaz H, Javed S & Zafar S (2020) Application of triacontanol to increase salinity tolerance in sunflower (*Helianthus annuus* L.). Journal of Plant Sciences 2: 24–32.

Khanam D & Mohammad F (2018) Plant growth regulators ameliorate the ill effect of salt stress through improved growth, photosynthesis, antioxidant system, yield and quality attributes in *Mentha piperita* L. Acta Physiologiae Plantarum 40: 188. doi:10.1007/s11738-018-2769-6.

Kissimon J, Tantos Á, Mészáros A, Jámbor-Benczúr E & Horváth G (1999) Stress alterations in growth parameters, pigment content and photosynthetic functions of *in vitro* cultured plants. Zeitschrift für Naturforschung C 54: 834–839. doi:10.1515/znc-1999-9-1033.

Knight SL & Mitchell CA (1987) Stimulation productivity of hydroponic lettuce in controlled environments with triacontanol. HortScience 22: 1307–1309. doi:10.21273/HORTSCI.22.6.1307.

Krishnan RR & Kumari BD (2008) Effect of N-triacontanol on the growth of salt stressed soybean plants. Journal of Biosciences 19: 53–62.

Lesniak AP, Haug A & Ries SK (1986) Stimulation of ATP-ase activity in barley (*Hordeum vulgare*) root plasma membrane after treatment of intact tissue and cell-free extracts with triacontanol. Physiologia Plantarum 68: 20–26. doi:10.1111/j.1399-3054.1986.tb06590.x.

Lesniak AP, Haug A & Ries SK (1989) Stimulation of ATPase activity in barley (*Hordeum vulgare*) root plasma membranes after treatment with triacontanol and calmodulin. Physiologia Plantarum 75: 75–80. doi:10.1111/j.1399-3054.1989.tb02066.x.

Ma FW, Wang JC & Rong W (1990) Effects of plant growth regulators on *in vitro* propagation of apple cultivar Fuji. Journal of Fruit Science 7: 201–206.

Madi A & Al-Mayahi W (2024) Triacontanol 'TRIA' application to mitigate the adverse effects of drought and salinity stress under *in vitro* culture of date palm plants. Folia Oecologica 51: 250–262. doi:10.2478/foecol-2024-0023.

Malabadi RB, Mulgund GS & Nataraja K (2005) Effect of triacontanol on the micropropagation of *Costus speciosus* (Koen.) Sm. using rhizome thin. In Vitro Cellular and Developmental Biology - Plant 41: 129–132. doi:10.1079/IVP2004586.

Malabadi RB & Nataraja K (2007) Influence of triacontanol on somatic embryogenesis of *Pinus roxburghii* Sarg. Baltic Forestry 13: 39–44.

Manzoor I, Bhat KM, Mir MA, Nair NM, Pandit AH, Kulsum U, Quadri S, Deshmukh S & Pasternak T (2025) The optimization of *in vitro* culture establishment and shoot proliferation of "GiSelA 17" (*Prunus canescens* × *Prunus avium*): a novel cherry rootstock. International Journal of Plant Biology 16: 33. doi:10.3390/ijpb16010033.

Misra A & Srivastava NK (1991) Effects of the triacontanol formulations "Miraculan" on photosynthesis, growth, nutrient uptake, and essential oil yield of lemongrass (*Cymbopogon flexuonses*) Stend. *Watts*. Plant Growth Regulation 10: 57–63. doi:10.1007/BF00035131.

Mishra Y, Rana PK, Shirin F & Ansari SA (2001) Augmenting *in vitro* shoot multiplication by vipul (triacontanol) and adventitious rhizogenesis by rice bran extract in *Dendrocalamus strictus*. Indian Journal of Experimental Biology 39: 165–169.

Morre DJ (1990) A lysolipid cascade in signal transduction cycle of growth control: Inositol metabolism in plants (ed. by DJ Morre & FA Loewus) Wiley-Liss, New York, pp. 227–257.

Morre DJ, Sellden G, Zhu XZ & Brightman A (1991) Triacontanol stimulates NADH oxidase of soybean hypocotyl plasma membranes. Plant Science 79: 31–36. doi:10.1016/0168-9452(91)90065-G.

Mudassar S, Ahmed S, Sardar R, Yasin NA, Jabbar M & Lackner M (2024) Exogenously applied triacontanol mitigates cadmium toxicity in *Vigna radiata* L. by optimizing growth, nutritional orchestration, and metal accumulation. Toxics 12: 911. doi:10.3390/toxics12120911.

Murashige T & Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum 15: 473–497. doi:10.1111/j.1399-3054.1962.tb08052.x.

Muthuchelian K, Paliwal K, Guanam A & Mitchel RK (1990) The effect of three long-chain aliphatic alcohols on photosynthesis and growth of *Pennisetum plystochyoen* Schult. Photosynthetica 24: 257–260.

Muthuchelian K, Murugan C & Harigovindan R (1996) Ameliorating effect of triacontanol on salt stressed *Erythrina variegata* seedlings. Changes in growth, biomass, pigments and solute accumulation. Biologia Plantarum 38: 133–136. doi:10.1007/BF02879647.

Muthuchelian K, Velayutham M & Nedunchezhian N (2003) Ameliorating effect of triacontanol on acidic mist-treated *Erythrina variegata* seedlings changes in growth and photosynthetic activities. Plant Science 165: 1253–1259. doi:10.1016/S0168-9452(03)00333-9.

Naeem M, Khan MMA & Siddiqui MH (2009) Triacontanol stimulates nitrogen fixation, enzyme activities, photosynthesis, crop productivity and quality of hyacinth bean (*Lablab purpureus* L.). Scientia Horticulturae 121: 389–396. doi:10.1016/j.scienta.2009.02.030.

Naeem M, Khan MM, Moinuddin MMA, Idrees M & Aftab T (2011) Triacontanol-mediated regulation of growth and other physiological attributes, active constituents and yield of *Mentha arvensis* L. Plant Growth Regulation 65: 195–206. doi:10.1007/s10725-011-9588-8.

Naeem M, Khan MMA & Moinuddin (2012) Triacontanol: A potent plant growth regulator in agriculture. Journal of Plant Interactions 7: 129–142. doi:10.1080/17429145.2011.619281.

Naeem M, Ansari AA, Aftab T, Shabbir A, Alam MM, Khan MMA & Uddin M (2019) Application of triacontanol modulates plant growth and physiological activities of *Catharanthus roseus* (L.). International Journal of Botany Studies 4: 131–135.

Pang Q, Chen X, Lv J, Li T, Fang J & Jia J (2020) Triacontanol promotes the fruit development and retards fruit senescence in strawberry: A transcriptome analysis. Plants 9: 488. doi:10.3390/plants9040488.

Parimalan R, Giridhar P, Gururaj HB & Ravishankar GA (2009) Micropropagation of *Bixa orellana* using phytohormones and triacontanol. Biologia Plantarum 53: 347–350. doi:10.1007/s10535-009-0064-5.

Perveen S, Iqbal M, Parveen S, Akram MS, Shahbaz M, Akber S & Mehboob A (2017) Exogenous triacontanol-mediated increase in phenolics, proline, activity of nitrate reductase, and shoot K^+ confers salt tolerance in maize (*Zea mays* L). Brazilian Journal of Botany 40: 1–11. doi:10.1007/s40415-016-0310-y.

Polivanova OB & Bedarev VA (2022) Hyperhydricity in plant tissue culture. Plants 11: 3313. doi:10.3390/plants11233313.

Popova LP, Zheleva DI & Vaklinova SG (1989) Effect of triacontanol on growth, photosynthesis and photorespiration in barley. Comptes Rendus de l'Académie Bulgare des Sciences 42: 95–98.

Reddy BO, Giridhar P & Ravishankar GA (2002) The effect of triacontanol on micropropagation of *Capsicum frutescens* and *Decalepis hamiltonii* W & A. Plant Cell, Tissue and Organ Culture 71: 253–258. doi:10.1023/A:1020342127386.

Ries SK & Wert VF (1982) Rapid effects of triacontanol *in vivo* and *in vitro*. Plant Growth Regulation 1: 117–127.

Ries S & Houtz R (1983) Triacontanol as a plant growth regulator. HortScience 18: 654–662.

Ries SK & Stutte CA (1985) Regulation of plant growth with triacontanol. Critical Reviews in Plant Sciences 2: 239–285. doi:10.1080/07352688509382197.

Ries SK, Wert V, O'Leary NFD & Nair M (1990) 9-β-L(+)adenosine: A new naturally occurring plant growth substrate elicited by triacontanol in rice. Plant Growth Regulation 9: 263–273. doi:10.1007/BF00045289.

Ries S, Savithiry S, Wert V & Widders I (1993) Rapid induction of ion pulses in tomato, cucumber, and maize plants following a foliar application of L (+)-adenosine. Plant Physiology 101: 49–55. doi:10.1104/pp.101.1.49.

Sarropoulou V, Dimassi-Theriou K & Therios I (2014) Dikegulac sodium effect on micropropagation and biochemical parameters in the cherry rootstocks CAB-6P and Gisela 6. Turkish Journal

of Agricultural and Natural Sciences, Special Issue 2: 1713–1726.

Sarropoulou V, Dimassi-Theriou K & Therios I (2015) Effect of sodium nitroprusside on micropropagation and biochemical parameters of CAB-6P and Gisela 6 cherry rootstocks. Turkish Journal of Biology 39: 595–610. doi:10.3906/biy-1409-68.

Sarropoulou V, Dimassi-Theriou K & Therios I (2016a) Effect of the ethylene inhibitors silver nitrate, silver sulphate and cobalt chloride on micropropagation and biochemical parameters in the cherry rootstocks CAB-6P and Gisela 6. Turkish Journal of Biology 40: 670–683. doi:10.3906/biy-1505-92.

Sarropoulou V, Dimassi-Theriou K & Therios I (2016b) Mineral strength, sucrose level and mannitol concentration effects on cherry rootstocks micropropagation. Agriculture and Forestry 62: 7–25. doi:10.17707/AGRICULTFOREST.62.3.01.

Sarropoulou V, Dimassi-Theriou K & Therios I (2017) Effects of the exogenous polyamines on micropropagation of cherry rootstocks. Indian Journal of Plant Physiology 22: 227–239. doi:10.1007/s40502-017-0289-9.

Sarropoulou V, Sperdouli I, Adamakis ID & Grigoriadou K (2023) The use of different LEDs wavelength and light intensities for *in vitro* proliferation of cherry rootstock: influence on photosynthesis and photomorphogenesis. Plant Cell, Tissue and Organ Culture 152: 317–330. doi:10.1007/s11240-022-02408-z.

Sarropoulou V, Sotiropoulos T, Dimassi-Theriou K & Therios I (2026a) Walnut and hazelnut pollen extracts enhance rooting, growth, and biochemical traits of cherry roostocks *in vitro*. Plant Cell, Tissue and Organ Culture 164: 35. doi:10.1007/s11240-025-03298-7.

Sarropoulou V, Dimassi-Theriou K & Therios I (2026b) Natural biostimulants from kiwi and peach pollen extracts improve *in vitro* growth and rooting along with metabolic partitioning in cherry rootstocks. Discover Plants 3: 360. doi:10.1007/s44372-026-00822-7.

Sarwar M, Anjum S, Ali Q, Alam MW, Haider MS & Mehboob W (2021) Triacontanol modulates salt stress tolerance in cucumber by altering the physiological and biochemical status of plant cells. Scientific Reports 11: 24504. doi:10.1038/s41598-021-04174-y.

Savithiry S, Wert V & Ries S (1992) Influence of 9-β-L(+)-adenosine on malate dehydrogenase activity in rice. Physiologia Plantarum 84: 460–466. doi:10.1111/j.1399-3054.1992.tb04691.x.

Segura HC & Joaquin AH (2021) Interaction of triacontanol with other plant growth regulators on morphology and yield of field pea (*Pisum sativum* L.). Agronomía Colombiana 39: 187–195. doi:10.15446/agron.colomb.v39n2.94096.

Shil S (2021) Physio-biochemical and molecular perspectives of TRIA-mediated growth responses in plants. Journal of Innovative Agriculture 8: 6–13. doi:10.37446/jinagri/ra/8.3.2021.6-13.

Shimizu-Sato S, Tanaka M & Mori H (2009) Auxin–cytokinin interactions in the control of shoot branching. Plant Molecular Biology 69: 429–435. doi:10.1007/s11103-008-9416-3.

Šmeringai J, Schrumpfová PP & Pernisová M (2023) Cytokinins – Regulators of *de novo* shoot organogenesis. Frontiers in Plant Science 14: 1239133. doi:10.3389/fpls.2023.123913.

Srivastava NK & Sharma S (1990) Effect of triacontanol on photosynthesis, alkaloid content and growth in (*Papaver somniferum* L.). Plant Growth Regulation 9: 65–71. doi:10.1007/BF00025280.

Sut S, Ferrasse I, Shrestha SS, Kumar G, Salviero A, Sello S, Atissimo A, Pagni L, Gattesco F & Dall'Acqua S (2020) Comparison of biostimulant treatments in *Acmella oleracea* cultivation for alkylamides production. Plants 9: 818. doi:10.3390/plants9070818.

Tantos A, Meszaros A, Kissimon J, Horvath G & Farkas T (1999) The effect of triacontanol on micropropagation of balm, *Melissa officinalis* L. Plant Cell Reports 19: 88–91. doi:10.1007/s002990050715.

Tantos A, Mészáros A, Farkas T, Szalai J & Horváth G (2001) Triacontanol-supported micropropagation of woody plants. Plant Cell Reports 20: 16−21. doi:10.1007/s002990000282.

Testone G, Caboni E, D'Angeli S, Altamura MM & Giannino D (2023) *Prunus knotted*-like genes: Genome-wide analysis, transcriptional response to cytokinin in micropropagation, and rootstock transformation. International Journal of Molecular Sciences 24: 3046. doi:10.3390/ijms24033046.

Uchiyama T & Ogasawara N (1981) Constituents of plant leaf wax contained in rice callus tissues. Agricultural and Biological Chemistry 45: 1261–1263. doi:10.1080/00021369.1981.10864688.

Verma A, Malik CP, Gupta VK & Bajaj BK (2011) Effects of *in vitro* triacontanol on growth, antioxidant enzymes, and photosynthetic characteristics in *Arachis hypogaea* L. Brazilian Journal of Plant Physiology 23: 271–277. doi:10.1590/S1677-04202011000400004.

Verma T, Bhardwaj S, Singh J , Kapoor D & Prasad R (2022) Triacontanol as a versatile plant growth regulator in overcoming negative effects of salt stress. Journal of Agriculture and Food Research 1: 100351. doi:10.1016/j.jafr.2022.100351.

Virdi AS, Singh S & Singh P (2015) Abiotic stress responses in plants: roles of calmodulin-regulated proteins. Frontiers in Plant Science 6: 809. doi:10.3389/fpls.2015.00809.

Waqas M, Shahzad R, Khan AL, Asaf S, Kim YH, Kang SM, Bilal S, Hamayun M & Lee IJ (2016) Salvaging effect of triacontanol on plant growth, thermotolerance, macro-nutrient content, amino acid concentration and modulation of defense hormonal levels under heat stress. Plant Physiology and Biochemistry 99: 118–125. doi:10.1016/j.plaphy.2015.12.012.

Weremczuk-Jeżyna I, Hnatuszko-Konka K, Lebelt L, Piotrowska DG & Grzegorczyk-Karolak I (2022) The effect of the stress-signalling mediator triacontanol on biochemical and physiological modifications in *Dracocephalum forrestii* culture. International Journal of Molecular Sciences 23: 15147. doi:10.3390/ijms232315147.

Yun CH & Kim HI (1986) Differences in callus formation from different explant sources and effect of plant growth regulators in plant regeneration in rice (*Oryza sativa* L). Research Report to Rural Development Administration 1: 113–116.

Zaid A, Mohammad F & Fariduddin Q (2019) Plant growth regulators improve growth, photosynthesis, mineral nutrient and antioxidant system under cadmium stress in menthol mint (*Mentha arvensis* L.). Physiology and Molecular Biology of Plants 26: 25−39. doi:10.1007/s12298-019-00715-y.