Biodegradable hydrogel microspheres from paper waste as a substrate for *ex vitro* adventitious rooting of *Eucalyptus grandis* x *E. urophylla* clonal plants

Cínthia Aparecida Silva
cinthianep1@yahoo.com.br
Federal University of Lavras: Universidade Federal de Lavras https://orcid.org/0000-0001-5021-1840
Evelize Aparecida Amaral Sashiki
Federal University of Lavras: Universidade Federal de Lavras
Rafael Carvalho do Lago
Federal University of Lavras: Universidade Federal de Lavras
Júlia Naves Teixeira
Federal University of Lavras: Universidade Federal de Lavras
Douglas Machado Leite
Federal University of Lavras: Universidade Federal de Lavras
Gilvano Ebling Brondani
Federal University of Lavras: Universidade Federal de Lavras
Gustavo Henrique Denzin Tonoli
Federal University of Lavras: Universidade Federal de Lavras
Lourival Marin Mendes
Federal University of Lavras: Universidade Federal de Lavras

Research Article

Keywords: hydroretenter polymer, cellulose nanofibrils, sustainability, acclimatisation, clonal propagation

Posted Date: September 17th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7546903/v1

Version of Record: A version of this preprint was published at Plant Cell, Tissue and Organ Culture (PCTOC) on November 26th, 2025. See the published version at https://doi.org/10.1007/s11240-025-03308-8.

Abstract

The use of hydrogels as support for plant rooting has been extensively studied. However, mineral substrates remain the most common choice despite their limitations in availability, cost, and environmental impact. In the context of plant biotechnology and sustainable clonal propagation systems, this study evaluated biodegradable hydrogel microspheres composed of cellulose microfibrils (CMF) and nanofibrils (CNF), derived from waste paper, as an alternative substrate for the *ex vitro* adventitious rooting and acclimatisation of *Eucalyptus urophylla* x *E. grandis* (urograndis eucalypt) clonal plants. The microspheres were subjected to alkaline pre-treatments: (1) sodium hydroxide (NaOH); (2) NaOH + hydrogen peroxide (Bleached); (3) calcium silicate ($CaSiO_3$); (4) magnesium silicate ($MgSiO_3$), and characterised using Fourier transform infrared spectroscopy (FTIR). Clonal plants' performance was assessed through morphological traits and the Dickson Quality Index (DQI). Following 30 days of observation, the Bleached, $CaSiO_3$, and $MgSiO_3$ treatments performed similarly to those of the vermiculite control in terms of rooting, vigour, and the absence of contamination. Following 90 days, favourable outcomes were maintained concerning height, stem diameter, and DQI. Notably, $MgSiO_3$-treated microspheres promoted greater leaf and shoot development, while Bleached microspheres enhanced leaf area. In contrast, NaOH-treated samples led to contamination and reduced performance. These findings demonstrate that CMF/CNF-based hydrogel microspheres, particularly those treated with $MgSiO_3$, represent a sustainable biotechnological innovation and effective alternative substrate for the large-scale clonal propagation of urograndis eucalypt.

Introduction

Eucalyptus urophylla × *E. grandis* (urograndis eucalypt), one of the most widely propagated hybrids, stands out due to its rapid growth, adaptability to different environments, and superior wood quality (Souza et al. 2022; Griebeler et al. 2024a,b). This makes it the predominant choice for commercial plantations in tropical and subtropical regions. The large-scale production of this hybrid's clonal plants relies on efficient micropropagation and rooting protocols (Esquivel et al. 2024; Wang et al. 2025). In the *ex vitro* rooting method, acclimatisation of the clonal plants occurs alongside root formation. This reduces the time and cost of micropropagation (Oakes et al. 2020; Nawandish et al. 2024; Souza et al. 2024).

Adventitious rooting represents a critical stage in the micropropagation of woody species, particularly in Eucalyptus (de Oliveira et al. 2024; Griebeler et al. 2024a). The success of this stage depends not only on the physiological competence of the explants but also on the use of efficient and economically sustainable substrates that support rooting, acclimatisation, and subsequent field establishment (Brondani et al. 2018; Sarma et al. 2024; Nawandish et al. 2024).

Although mineral substrates are commonly used at this stage, they may be limited in terms of availability, cost, and environmental impact, as is the case with peat extraction. (Pirata et al. 2022; Madrid-Aispuro et al. 2020). In this context, biodegradable hydrogels emerge as a promising alternative.

They provide physical support, as well as water, nutrients, and oxygen, to the roots. They also allow the study of root architecture (Patra et al. 2022).

Numerous studies have demonstrated that integrating hydrogels into substrates, or utilising them as substitutes for conventional substrates, can enhance the rooting process of Eucalyptus clonal plants and other species (Azevedo et al. 2015; Neres et al. 2019; Ma et al. 2019; Kernosenko et al. 2023). Therefore, improving rooting efficiency through alternative substrates, such as hydrogels, is a key strategy for supporting the sustainable clonal micropropagation systems and expansion of urograndis eucalypt plantations.

Hydrogels are three-dimensional polymers that possess a high capacity for water absorption and retention (Abdel-Raouf et al. 2018). Its absorption properties depend on factors such as composition, degree of cross-linking, pH, and the presence of mineral salts (Shaghaleh et al. 2022; Xu et al. 2019). Hydrogels based on natural polymers, particularly cellulose, have garnered increasing attention due to their biocompatibility, biodegradability, and renewable origin (Ahmed 2015; Nascimento et al. 2018; Saruchi et al. 2019; Akter et al. 2021). In addition to being sustainable, CMF/CNF-based hydrogels have good mechanical properties and are inexpensive (Le et al. 2023).

Specific pre-treatments can be employed to isolate and modify cellulosic fibers for use in hydrogel formulations, promoting their dispersion and interaction within the polymeric matrix (Abe and Yano 2011). Among these, alkaline pre-treatments stand out, as they remove lignin and hemicellulose. This process increases the porosity and reactivity of the fibers, thereby enhancing the hydrogel's water absorption and mechanical resistance (Zhang et al. 2023; Nascimento et al. 2018).

An innovative application of hydrogels involves the development of 'transparent soils' for root phenotyping. This medium enables the imaging of root systems *in vivo*, providing a more accurate representation of root development than traditional hydroponic systems. The transparent soil is made of hydrogel spheres that support root growth by providing air, water, and nutrients. This closely mimics real soil conditions without causing hypoxia in the roots (Ma et al. 2019).

The hydrogel microspheres provide a favourable microenvironment for plant growth and root development (Karipçin 2023). However, it is essential to adjust hydrogel mechanical properties, including resistance and impedance, as they can negatively impact root growth (Wu et al. 2024a,b). Therefore, this study evaluated the use of biodegradable hydrogel microspheres based on CMF/CNF, which had undergone different alkaline pre-treatments. The aim was to analyse the effect of these microspheres on the *ex vitro* rooting and acclimatisation of urograndis eucalypt clonal plants.

Materials and Methods

Source of plant material and study site

The genetic material employed in this study to obtain explants was *in vitro* established from a small sample of the A211 hybrid clone of *Eucalyptus grandis* Hill ex Maiden x *Eucalyptus urophylla* S.T. Blake. The ministumps were established in a clonal minigarden under an automated semi-hydroponic system in a sand bed. They received a nutrient solution by means of dripping, which was distributed in four daily applications totalling 4 L m^{-2}, according to Souza et al. (2022). The collection of shoots occurred 20 days after the pruning of the apex of the ministump. Shoots were maintained on Murashige and Skoog (MS) medium supplemented with indole-3-butyric acid (IBA), adjusted to pH 5.8 before autoclaving (121°C, 20 min), as described by Souza et al. (2024).

The microcuttings obtained were standardised and allocated to different experimental treatments. The adventitious rooting and acclimatisation tests were conducted in the Laboratory of *in vitro* Culture of Forest Species and the Forest Nursery at the Department of Forestry Sciences, Federal University of Lavras (UFLA), Lavras, Minas Gerais, Brazil.

Adventitious rooting and acclimatisation ex vitro

From tufts of elongated shoots in vitro in medium MS supplemented with 2.68 µM α-naphthaleneacetic acid and 0.22 µM 6-benzylaminopurine (Molinari et al. 2021), microcuttings measuring 3 cm in length, were transplanted into microtubes and packaged in a mini-stufim system inside 250 ml bottles. They were kept under a photoperiod of 16 hours, with a luminous intensity of 40 µmol m^{-2} s^{-1}. Irrigation was performed manually with a pipette every five days for 30 days (Brondani et al. 2018). At the end of this period, the following parameters were evaluated: shoot and leaf number, root presence and length, height (cm), vigour, and the occurrence of contamination. The rooted plants were then transferred to the nursery, where they remained for 90 days under an alternating irrigation system. After this period, their survival rate, height, stem diameter, leaf area, and Dickson Quality Index (DQI) were evaluated. The DQI is an integrative parameter that reflects the robustness and morphological balance of the clonal plants. It is therefore a good indicator of the overall quality of the plant material (Dickson et al. 1960).

The substrates tested in the acclimatisation were vermiculite, used as a control, and hydrogel microspheres formulated from paper waste, which had undergone various types of pre-treatments.

Pre-treatment of waste paper

The hydrogel microspheres were formulated from waste kraft paper tubes, which had an average grammage of 400 g/m². These tubes were supplied by Tubominas Indústria e Comércio LTDA. The waste material was shredded in a knife mill and then immersed in four distinct pre-treatment solutions: (1) NaOH 5% (w/w); (2) NaOH 5% + H_2O_2 24% (w/w) - Bleached; (3) $CaSiO_3$ 10% (w/w); (4) $MgSiO_3$ 10% (w/w). The suspensions were maintained at 80 ± 2°C under constant stirring (400 rpm) for 2 hours. Following the administration of the treatments, the waste was subjected to a series of washes with deionised water until it reached a neutral pH level (Dias et al. 2019; Mascarenhas et al. 2022).

The formulation of the microspheres included sodium alginate (CAS 9005-38-3) and calcium dihydrate chloride (CAS 10035-04-8).

Obtaining cellulose micro/nanofibrils (CMF/CNF)

The pre-treated fibers were suspended in deionised water at a concentration of 2% (w/w) and stirred at 500 rpm for 30 minutes to promote hydration and homogenisation. Fibrillation was performed using a Masuko Sangyo MKGA-80 Supermass-colloid defibrillator (Kawaguchi, Japan) operating at 1500 rpm with ten passes. The distance between the discs was initially set at 10 µm and increased to 100 µm as the viscosity of the suspension increased (Mascarenhas et al. 2022).

Obtaining the hydrogel microspheres

CMF/CNF suspensions were produced from waste paper tubes that underwent different alkaline pre-treatments, as mentioned above. The solids content of all suspensions was adjusted to 1% (w/w).

In synthesizing the hydrogels, a 100:1 (w/w) ratio was employed, using a mixture of CMF/CNF suspension and sodium alginate. Subsequently, the mixtures were placed in an ultrasonic bath at 60°C for 30 minutes to ensure complete and uniform homogenisation of the solutions.

The microspheres were formed by ionic gelification through dripping the suspensions into a calcium chloride ($CaCl_2$) solution using 1 mL Pasteur pipettes. The size of the obtained spheres was directly influenced by the diameter of the pipette nozzle. The hydrogel structure is formed through the interaction between alginate and the divalent calcium ions present in the solution. The formed microspheres were kept in a $CaCl_2$ solution for 24 hours to stabilise them. They were then washed with deionised water and left to soak in it until they were analyzed.

Characterisation of hydrogel microspheres using Fourier transform infrared spectroscopy (FTIR)

The pre-treated hydrogel microspheres were analysed using a Varian 600-IR FTIR spectrometer to examine their infrared vibrational spectroscopy. This device was coupled with a GladiATR accessory from Pike Technologies to enable attenuated total reflectance (ATR) measurements at a 45° angle using a zinc selenite crystal. The analysed spectral range was from 400 to 4000 cm^{-1}, with a total of 32 scans and a resolution of 2 cm^{-1}.

Statistical analysis

Ten repetitions were performed for each of the treatments that were tested. Data collection took place after 30 days of *ex vitro* culture in the laboratory and subsequently after 90 days in the nursery. The data for the response variables were verified for homoscedasticity and normal distribution using the Hartley and Shapiro–Wilk tests, respectively, with a 5% probability level. The results were evaluated using analysis of variance (ANOVA) ($p < 0.05$), followed by a Tukey's test ($p < 0.05$) to compare the means of the treatments.

Results and Discussion

The use of biodegradable hydrogel microspheres based on CMF/CNF as a substrate for the *ex vitro* adventitious rooting and acclimatisation of urograndis eucalypt clonal plants was evaluated. Figure 1 shows the effects of pre-treated hydrogel microspheres on the initial development of urograndis eucalypt clonal plants. The following variables were analysed after 30 days: leaf number (A), shoot number (B), shoot length (C), root presence (D), root length (E), vigour (F), and contamination (G).

Figure 1A shows that the leaf number was significantly higher in the $MgSiO_3$ treatment than in the vermiculite treatment. This increase may be related to the action of silicates as substrate conditioners, which improves the microsphere's physical and chemical properties, such as porosity, aeration, and water retention (Krahl et al. 2022). The greater number of leaves suggests an increase in photosynthetic area, which could encourage biomass accumulation in the initial growth stage, even without an external nutritional supply.

Regarding shoot number (Fig. 1B), only the $MgSiO_3$ treatment differed significantly from vermiculite. This trend was reinforced by the shoot length data (Fig. 1C), which showed that the NaOH, bleached, and $CaSiO_3$ treatments performed better than vermiculite alone, although there were no significant differences between them. These results suggest that these treatments favour the formation and growth of shoots. This can be attributed to an improvement in the substrate's physical and chemical conditions, and a possible reduction in osmotic stress (Manning et al. 2017).

In relation to adventitious rooting, there were no significant differences in root presence (Fig. 1D) or length (Fig. 1E) between treatments. These results indicate that the hydrogels performed as well as vermiculite, thus validating their effectiveness as a means of providing physical support for the root system during the initial stage of acclimatisation outside of the soil environment.

Vigor (Fig. 1F) was evaluated based on morphological parameters and was found to be significantly higher in the silicate treatments than in the NaOH treatment. This performance may be related to the balance between the growth of the aerial part and the root system, which highlights the importance of silicates in forming robust and well-structured clonal plants (Etesami and Jeong 2018).

Regarding contamination (Fig. 1G), there was a significant increase in the NaOH treatment, which negatively affected the overall performance of the plants in this group. This result can be attributed either to the residual presence of alkaline compounds in the material or to the incomplete degradation of organic components, which favour microbial proliferation. By contrast, treatments involving silicates and bleached produced low levels of contamination similar to those of vermiculite. This indicates that these substrates are non-toxic and safe for *ex vitro* cultivation.

The effects of different pre-treatments on the morphology of urograndis eucalypt hybrid clonal plants transferred to the nursery after 90 days of acclimatisation are shown in Fig. 2 (A–H). The results showed that hydrogel microsphere substrates performed equally or better than vermiculite, particularly in silicate treatments. Regarding leaf area (Fig. 2A), the Bleached treatment showed higher average values than vermiculite. This may be due to an improvement in the substrate's physical and chemical properties, such as roughness, water retention, and aeration, resulting in greater leaf expansion and consequently a larger photosynthetic surface area (de Paula et al. 2024). Tomaszewska-Sowa (2020) also observed an increased number of leaves and leaf area under the influence of hydrogel during the acclimatisation of *Lippia dulcis* Trev. clonal plants.

There were no statistically significant differences in plant height (Fig. 2B) or stem diameter (Fig. 2C) between treatments. However, the survival rate (Fig. 2D) was significantly lower only in the NaOH treatment ($p < 0.05$). The other treatments had higher values that were statistically similar to each other. This indicates that the modified substrates (Bleached, $CaSiO_3$, and $MgSiO_3$) contributed to the establishment and viability of the clonal plants as much as the vermiculite.

In terms of root dry mass (Fig. 2E), there was no statistically significant difference between the treatments and the vermiculite treatment. However, the NaOH treatment was significantly lower. A similar trend was observed for the shoot dry mass (Fig. 2F), although there was no statistical significance between treatments. The same pattern was observed for total dry mass (Fig. 2G). These results suggest that the silicates, particularly $MgSiO_3$, promoted biomass accumulation by mitigating the effects of water stress, such as improved water retention and aeration. This benefited root development and consequently shoot growth. In addition, silicon (Si) is classified as a beneficial plant nutrient. It is commonly applied in the form of silicate and has been shown to induce tolerance to adverse conditions and contribute to plant growth under abiotic stress (Aras et al. 2020; Meng et al. 2021).

Healthy clonal plants with a good stem diameter, well-formed roots, and an adequate root-to-aerial part ratio, as well as balanced nutrition, have a higher survival rate when planted, as well as greater resistance to environmental stresses and better growth (Nicoletti et al. 2014; Silva et al. 2015). The evaluated DQI values were good for all the hydrogel treatments and were similar to those for vermiculite. Treatment with $MgSiO_3$ produced a higher DQI value than treatment with NaOH (Fig. 2H), demonstrating the positive impact of silicates on the formation of more uniform and robust clonal plants.

According to Tomaszewska-Sowa (2020), hydrogels are effective in micropropagation and acclimatisation of plants. Using them can increase the efficiency of rooting and clonal plant growth during the transition to *ex vitro* conditions. Figure 3 (A–E) shows the acclimatisation and rooting stages *ex vitro* of urograndis eucalypt clonal plants.

The hydrogels promote rooting by providing physical support to plants and maintaining moisture levels, which relates to the hydrogel's physical properties of porosity, roughness, and water retention capacity. These properties are closely linked and have a critical influence on the performance of the hydrogel. Good crosslinking in the matrix contributes to mechanical and biological compatibility between the root system and the hydrogel, optimising the substrate's water-air balance (Qin et al. 2025). Conversely, in substrates with low porosity, water accumulation can hinder aeration, resulting in hypoxic conditions that are detrimental to rhizogenesis (Schafer and Lerner 2022).

Porosity directly influences the swelling and biological performance of hydrogels, which vary according to cellulose concentration and crosslinking agent type (Akalin and Pulat 2018). The degree of crosslinking and the synthesis method can modulate the surface roughness, which can increase the swelling rate (Kabiri et al. 2003; Xu et al. 2016; Womack et al. 2022). Increasing the amount of crosslinker intensifies this roughness, providing greater structural stability and resistance to degradation. (Wanat et al. 2025).

Thus, it is possible that the CMF/CNF hydrogel microspheres, cross-linked with $CaCl_2$, became more stable and humid, favouring the extension and formation of the urograndis eucalypt root system branches. Figure 4 shows the FTIR spectra of hydrogels with different pre-treatments, confirming the presence of functional groups that are characteristic of interactions between MFC/NFCs, alginate, and $CaCl_2$.

Bleached and NaOH treatments showed an increase in the intensity of the bands at ~ 3335 and ~ 3313 cm^{-1}, respectively. This is attributed to axial stretching of the hydroxyl groups (-OH), which indicates greater exposure of free hydroxyls and favours interaction with alginate through hydrogen bonds. (Goncharuk et al. 2024; Shen et al. 2017). In addition, it increases water retention capacity, a property that is relevant for plant rooting applications. The absence of bands at ~ 1730 cm^{-1}, which are associated with the C = O stretching of oxidised carboxyl and carbonyl groups, suggests low surface functionalisation (Rzayev et al. 2016). This condition may be associated with the maintenance of a more alkaline residual pH and a lower antimicrobial barrier capacity, thereby corroborating the increased incidence of contamination observed in treatment with NaOH, which in turn compromises the performance of the clonal plants.

The presence of the band at ~ 1602 cm^{-1} was observed in all treatments analysed, which corresponds to the asymmetric elongation of the carboxylate group ($-COO^-$) (Bajestani et al. 2025). The different intensities are attributed to the complexation between the carboxylate groups ($-COO^-$) and the calcium íons (Ca^{2+}), which are derived from crosslinking with $CaCl_2$. (Samchenko et al. 2024). This complexation reduces the vibrational mobility of the carboxylate groups, resulting in a decrease in band intensity, as observed in silicate treatments. In the Bleached treatment, the intensity of the 1602 cm^{-1} band was higher, suggesting a greater formation of carboxylate groups resulting from the oxidative process (Huang et al. 2025).

The band at ~ 1419 cm^{-1} corresponds to the symmetric stretching of the carboxylate group ($-COO^-$), which was evident in all treatments. This corroborates the formation of ionic bonds between Ca^{2+} and the functional groups present in alginate and CMF/CNF (Bajestani et al. 2025; Goncharuk et al. 2024). This ionic crosslinking was fundamental to forming a stable three-dimensional network. It also allowed for the controlled release of Ca^{2+} ions, which are essential for root development due to their role in regulating cell division, root elongation, and signalling responses to stress.

The $CaSiO_3$ and $MgSiO_3$ treatments exhibited spectral patterns that were similar to those of the other treatments, but with more pronounced bands in the region of ~ 1021 e ~ 999 cm^{-1}. These bands are attributed to asymmetric stretching of Si–O–Si bonds (Chen et al. 2021; Han et al. 2022), as well as possible contributions from C–O–C bonds in glycoside bridges of CMF/CNFs. The presence of these bands indicates that there are surface interactions between the ions released by the silicates (Ca^{2+}, Mg^{2+}, and Si) and the functional groups of the polymer matrix. This results in structural alterations to the hydrophilic network. These modifications result in the gradual release of ions, which may have been beneficial for rooting since silicon is associated with increased resistance to abiotic stress, such as drought and salinity, as well as cell wall strengthening and root growth stimulation (Santos et al. 2021).

Root penetration and elongation are directly affected by hydrogel impedance. Different gel substrate compositions promote phenotypic variations in root development. When gel hardness exceeds a certain threshold, root penetration is significantly hindered (Wu et al. 2024a,b). Making adjustments to the pre-treatment of cellulose fibers and the reticulation solutions of the hydrogel matrix can effectively meet the needs of plant roots (Ma et al. 2023). The results showed that the hydrogel microspheres formulated with 1% alginate provided adequate physical structure, allowing penetration and root development, regardless of the pre-treatment applied. In addition to the growth of the main root, significant formation of lateral roots was observed (Fig. 3E). These lateral roots play a fundamental role in the absorption of water and nutrients, making the plant more efficient at acquiring resources from the medium.

Previous studies on the optimisation of sodium alginate concentrations added to MFC/NFC showed that a concentration of 1% produced greater hardness, resilience, and water retention. These mechanical properties are favourable for rooting, since the microspheres need to be strong enough not to collapse under their own weight (data not shown). In addition, alginate-based hydrogels are particularly promising due to their ability to promote plant growth. Sodium alginate, a polysaccharide derived from brown algae, is composed of β-D-manuronic and α-L-guluronic acids. The enzymatic degradation of these monomers produces oligosaccharides that improve germination, shoot elongation, and root growth (Pettinelli et al. 2024).

Hydrogels have the potential to enhance the rooting process in vegetative propagation through cuttings, particularly for forest species such as Eucalyptus, by providing the necessary moisture and structural conditions for the development of adventitious roots. When choosing a hydrogel, it is important to consider not only its water retention function but also how it interacts directly with the roots, influencing the supply of water, nutrients, and aeration (Agbna and Zaidi 2025).

Conclusion

This study demonstrated that CMF/CNF-based hydrogel microspheres, particularly those pre-treated with $MgSiO_3$, improved rooting, leaf development, and overall plant quality more effectively than vermiculite. Treatments with Bleached, $CaSiO_3$, and $MgSiO_3$ maintained low contamination levels, thereby reinforcing their suitability for use in controlled micropropagation systems. These biodegradable hydrogel microspheres, derived from paper waste, represent a sustainable biotechnological alternative to non-renewable mineral substrates. They contribute to the efficient clonal propagation of urograndis eucalypt and support more environmentally responsible forestry practices.

Declarations

Founding sources

This work was supported by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) (Code Project RED 180/2023 and Code Project RED-00225-23).

Declaration of Competing Interest

All the authors of this manuscript state that they have no conflicts of interest.

Authors Contribution Statement

Cínthia Aparecida Silva: conceptualisation, data curation, formal analysis, investigation, methodology, writing – original draft, writing – review and editing. Evelize Aparecida Amaral Sashiki: Conceptualisation, data curation, formal analysis, investigation, writing – original draft. Rafael Carvalho do Lago: conceptualisation, data curation, formal analysis, project administration. Júlia Naves Teixeira: Conceptualisation, investigation, methodology. Douglas Machado Leite: Conceptualisation, data curation, formal analysis, investigation, methodology, writing – original draft. Gilvano Ebling Brondani: conceptualisation, funding acquisition, supervision. Gustavo Henrique Denzin Tonoli: conceptualisation, funding acquisition, supervision. Lourival Marin Mendes: conceptualisation, funding acquisition, supervision, project administration.

Acknowledgments

The authors gratefully acknowledge the financial support provided by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

Data Availability

All data generated or analyzed during this study are included in this published article or can be obtained from the corresponding author on request.

References

1. Abdel-Raouf ME, El-Saeed SM, Zaki EG, Al-Sabagh AM (2018) Green chemistry approach for preparation of hydrogels for agriculture applications through modification of natural polymers and investigating their swelling properties. Egyptian J Petroleum 27:1345–1355. https://doi.org/10.1016/j.ejpe.2018.09.002
2. Abe K, Yano H (2011) Formation of hydrogels from cellulose nanofibers. Carbohydr Polym 85:733–737. https://doi.org/10.1016/j.carbpol.2011.03.028
3. Agbna GHD, Zaidi SJ (2025) Hydrogel Performance in Boosting Plant Resilience to Water Stress—A. Rev Gels 11(4):276. https://doi.org/10.3390/gels11040276
4. Ahmed EM (2015) Hydrogel: Preparation, characterization, and applications: A review. J Adv Res 6:105–121. https://doi.org/10.1016/j.jare.2013.07.006
5. Akalin GO, Pulat M (2018) Preparation and Characterization of Nanoporous Sodium Carboxymethyl Cellulose Hydrogel Beads. Journal of Nanomaterials 2018: 9676949. https://doi.org/10.1155/2018/9676949
6. Akter M, Bhattacharjee M, Dhar AK, Rahman FBA, Haque S, Rashid TU, Kabir SMF (2021) Cellulose-Based Hydrogels for Wastewater Treatment: A Concise Review. 1–28
7. Aras S, Keles H, Eşitken A (2020) Silicon nutrition counteracts salt-induced damage associated with changes in biochemical responses in apple. Bragantia 79:1–7. https://doi.org/10.1590/1678-4499.20190153
8. Azevedo GT, de OS et al (2015) Enraizamento de miniestacas de eucalipto com diferentes doses de polímero hidroretentor incorporado ao substrato Minicutting rooting of eucalyptus with different doses of the hydrophylic polymer incorporated into the substrate. Scientia Forestalis 43:773–780
9. Bajestani MS, Kiani F, Ebrahimi S et al (2025) Effect of bentonite/alginate/nanocellulose composites on soil and water loss: An response surface methodology (RSM)-based optimization approach. Int J Biol Macromol 304:140815. https://doi.org/10.1016/j.ijbiomac.2025.140815
10. Brondani GE, De Oliveira LS, Konzen ER et al (2018) Mini-incubators improve the adventitious rooting performance of corymbia and eucalyptus microcuttings according to the environment in which they are conditioned. An Acad Bras Cienc 90:2409–2423. https://doi.org/10.1590/0001-3765201720170284
11. Chen YX, Sepahvand S, Gauvin F et al (2021) One-pot synthesis of monolithic silica-cellulose aerogel applying a sustainable sodium silicate precursor. Constr Build Mater 293:123289. https://doi.org/10.1016/j.conbuildmat.2021.123289

12. de Oliveira LS, Leite DM, Mendes FM et al (2024) Micropropagation and in vitro rejuvenation of *Eucalyptus cloeziana* F. Muell. 3 Biotech 14:292–303. https://doi.org/10.1007/s13205-024-04131-2
13. de Paula EHL, Paulino LR, Santos CC et al (2024) Potassium silicate and light availability affect photochemical efficiency and morphology of *Alibertia edulis*. Scientia Agricola 81:e20230006. https://doi.org/10.1590/1678-992X-2023-0006
14. Dias MC, Mendonça MC, Damásio RAP et al (2019) Influence of hemicellulose content of Eucalyptus and Pinus fibers on the grinding process for obtaining cellulose micro/nanofibrils. Holzforschung 73:1035–1046. https://doi.org/10.1515/hf-2018-0230
15. Dickson A, Leaf AL, Hosner JF (1960) Quality Appraisal of White Spruce and White Pine Seedling Stock in Nurseries. Forestry Chron 36:10–13. https://doi.org/10.5558/tfc36010-1
16. Esquivel F, Castillo A, Bentancor M et al (2024) Potential of metatopoline in the in vitro multiplication and rooting of *Eucalyptus globulus* Labill. Clones Cerne 30(1):e–103413. https://doi.org/10.1590/01047760202330013413
17. Etesami H, Jeong BR (2018) Silicon (Si): Review and future prospects on the action mechanisms in alleviating biotic and abiotic stresses in plants. Ecotoxicol Environ Saf 147:881–896. https://doi.org/10.1016/J.ECOENV.2017.09.063
18. Goncharuk O, Siryk O, Frąc M et al (2024) Synthesis, characterization and biocompatibility of hybrid hydrogels based on alginate, κ-carrageenan, and chitosan filled with montmorillonite clay. Int J Biol Macromol 278:134703. https://doi.org/10.1016/j.ijbiomac.2024.134703
19. Griebeler AM, Araujo MM, Turchetto F et al (2024a) Influence of mini-tunnel and different rooting environments on the production of rooted mini-cuttings of subtropical Eucalyptus. New Forest 55:459–476. https://doi.org/10.1007/s11056-023-09988-4
20. Griebeler AM, Araujo MM, Turchetto F et al (2024b) *Acacia mearnsii* pyroligneous acid as a promoter of rooting and quality of rooted cuttings of subtropical Eucalyptus. Trees - Struct Function 38:1063–1077. https://doi.org/10.1007/s00468-024-02536-3
21. Han X, Liang J, Fukuda S et al (2022) Sodium alginate–silica composite aerogels from rice husk ash for efficient absorption of organic pollutants. Biomass Bioenergy 159:1–7. https://doi.org/10.1016/j.biombioe.2022.106424
22. Huang Y, Wu L, Chang L et al (2025) Sodium alginate-crosslinked montmorillonite nanosheets hydrogel for efficient gallium recovery. Int J Biol Macromol 295:139474. https://doi.org/10.1016/j.ijbiomac.2025.139474
23. Kabiri K, Omidian H, Hashemi SA, Zohuriaan-Mehr MJ (2003) Synthesis of fast-swelling superabsorbent hydrogels: Effect of crosslinker type and concentration on porosity and absorption rate. Eur Polymer J 39:1341–1348. https://doi.org/10.1016/S0014-3057(02)00391-9
24. Karipçin MZ (2023) Hydrogels improved parsley (*Petroselinium crispum* (Mill. Nyman) growth and development under water deficit stress. PeerJ 11:e15105. https://doi.org/10.7717/peerj.15105
25. Kernosenko L, Samchenko K, Goncharuk O et al (2023) Polyacrylamide Hydrogel Enriched with Amber for In Vitro Plant Rooting. Plants 12:1–12. https://doi.org/10.3390/plants12051196

26. Krahl LL, Valadares LF, Sousa-Silva JC et al (2022) Dissolution of silicate minerals and nutrient availability for corn grown successively. Pesquisa Agropecuária Brasileira 57:e01467. https://doi.org/10.1590/S1678-3921.PAB2022.V57.01467
27. Le VT, Joo SW, Berkani M et al (2023) Sustainable cellulose-based hydrogels for water treatment and purification. Ind Crops Prod 205:117525. https://doi.org/10.1016/j.indcrop.2023.117525
28. Ma L, Shi Y, Siemianowski O et al (2019) Hydrogel-based transparent soils for root phenotyping in vivo. Proc Natl Acad Sci USA 166:11063–11068. https://doi.org/10.1073/pnas.1820334116
29. Ma X, Qu K, Zhao X et al (2023) Oxidized sodium alginate/polyacrylamide hydrogels adhesive for promoting wheat growth. Int J Biol Macromol 253:127450. https://doi.org/10.1016/j.ijbiomac.2023.127450
30. Madrid-Aispuro RE, Prieto-Ruíz JÁ, Aldrete A et al (2020) Alternative substrates and fertilization doses in the production of *Pinus cembroides* Zucc. in nursery. Forests 11(1):71. https://doi.org/10.3390/f11010071
31. Manning DAC, Baptista J, Sanchez Limon M, Brandt K (2017) Testing the ability of plants to access potassium from framework silicate minerals. Sci Total Environ 574:476–481. https://doi.org/10.1016/j.scitotenv.2016.09.086
32. Mascarenhas ARP, Scatolino MV, Dias MC et al (2022) Fibers pre-treatments with sodium silicate affect the properties of suspensions, films, and quality index of cellulose micro/nanofibrils Effect of Na2SiO3. Nordic Pulp Paper Res J 37:534–552. https://doi.org/10.1515/npprj-2022-0037
33. Meng X, Luo S, Dawuda MM et al (2021) Exogenous silicon enhances the systemic defense of cucumber leaves and roots against CA-induced autotoxicity stress by regulating the ascorbate-glutathione cycle and photosystem II. Ecotoxicol Environ Saf 227:112879. https://doi.org/10.1016/j.ecoenv.2021.112879
34. Molinari LV, Souza DMSC, Avelar MLM, e al (2021) Effects of chemical sterilization of the culture media, porous membranes and luminosity on in vitro culture of *Eucalyptus grandis × Eucalyptus urophylla*. J Forestry Res 32:1587–1598. https://doi.org/10.1007/s11676-020-01240-5
35. Nascimento DM, Nunes YL, Figueirêdo MCB et al (2018) Nanocellulose nanocomposite hydrogels: Technological and environmental issues. Green Chem 20:2428–2448. https://doi.org/10.1039/c8gc00205c
36. Nawandish F, Dumanoğlu H, Sarıkamış G (2024) Novel approaches to improve rooting of microshoots, acclimatization and plant growth of Pyrodwarf pear rootstock. Planr Cell Tissue Organ Cult 157(3):58. https://doi.org/10.1007/s11240-024-02781-x
37. Neres YXC, De Azevedo GTOS, De Souza AM et al (2019) Effect of the hydrogel incorporation on different substrates on the rooting and quality of clonal Eucalyptus seedlings. Scientia Forestalis/Forest Sci 47:336–345. https://doi.org/10.18671/scifor.v47n122.16
38. Nicoletti MF, Navroski MC, Andriollo K, Pereira MO, Frigotto T (2014) Efeito do hidrogel no enraizamento e crescimento inicial de miniestaca do híbrido Eucalyptus urograndis. Cultivando o saber 7:1–203

39. Oakes A, Pilkey H, Powell W (2020) Improving *Ex Vitro* Rooting and Acclimatization Techniques for Micropropagated American Chestnut1. J Environ Hortic 38:149–157. https://doi.org/10.24266/0738-2898-38.4.149
40. Patra SK, Poddar R, Brestic M et al (2022) Prospects of Hydrogels in Agriculture for Enhancing Crop and Water Productivity under Water Deficit Condition. Int J Polym Sci 2022:9484. https://doi.org/10.1155/2022/4914836
41. Pettinelli N, Sabando C, Rodríguez-Llamazares S et al (2024) Sodium alginate-g-polyacrylamide hydrogel for water retention and plant growth promotion in water-deficient soils. Ind Crops Prod 222:119759. https://doi.org/10.1016/j.indcrop.2024.119759
42. Pirata MS, Correia S, Canhoto J (2022) Ex Vitro Simultaneous Acclimatization and Rooting of In Vitro Propagated Tamarillo Plants (*Solanum betaceum* Cav.): Effect of the Substrate and Mineral Nutrition. Agronomy 12(2):1082. https://doi.org/10.3390/agronomy12051082
43. Qin C, Kan X, Xu D et al (2025) Adjustable P(AM-co-NIPAM)/gelatin hydrogel soilless cultivation substrates for soybean seedling and root growth. Ind Crops Prod 231:121189. https://doi.org/10.1016/j.indcrop.2025.121189
44. Rzayev ZMO, Salimi K, Bunyatova U et al (2016) Fabrication and characterization of PVA/ODA-MMT-poly(MA-alt-1-octadecene)-g-graphene oxide e-spun nanofiber electrolytes and their response to bone cancer cells. Mater Sci Eng C 61:257–268. https://doi.org/10.1016/j.msec.2015.12.045
45. Samchenko Y, Terpilowski K, Samchenko K et al (2024) Calcium Alginate/Laponite Nanocomposite Hydrogels: Synthesis, Swelling, and Sorption Properties. Coatings 14(12):1519. https://doi.org/10.3390/coatings14121519
46. dos Santos LC, Silva GAM da, Abranches M, de O et al (2021) O papel do silício nas plantas. Research, Society and Development 10:e3810716247. https://doi.org/10.33448/rsd-v10i7.16247
47. Sarma U, Verma P, Sharma NC et al (2024) Adventitious Root Induction in Apple (*Malus* × domestica Borkh.) Clonal Rootstocks Through Mound Layering Under Shade Net Conditions. Appl Fruit Sci 66:1187–1198. https://doi.org/10.1007/s10341-024-01144-2
48. Saruchi KV, Mittal H, Alhassan SM (2019) Biodegradable hydrogels of tragacanth gum polysaccharide to improve water retention capacity of soil and environment-friendly controlled release of agrochemicals. Int J Biol Macromol 132:1252–1261. https://doi.org/10.1016/j.ijbiomac.2019.04.023
49. Schafer G, Lerner BL (2022) Physical and chemical characteristics and analysis of plant substrate. Ornam Hortic 28:181–192. https://doi.org/10.1590/2447-536X.v28i2.2496
50. Shaghaleh H, Alhaj Hamoud Y, Xu X et al (2022) A pH-responsive/sustained release nitrogen fertilizer hydrogel based on aminated cellulose nanofiber/cationic copolymer for application in irrigated neutral soils. J Clean Prod 368:133098. https://doi.org/10.1016/j.jclepro.2022.133098
51. Shen X-J, Huang P-L, Chen J-H et al (2017) Comparison of Acid-hydrolyzed and TEMPO-oxidized Nanocellulose for Reinforcing Alginate Fibers. BioResources 12:8180–8198

52. Silva CRA, Ribeiro A, de Oliveira AS et al (2015) Desenvolvimento biométrico de mudas de eucalipto sob diferentes lâminas de irrigação na fase de crescimento. Pesquisa Florestal Brasileira 35:381–390. https://doi.org/10.4336/2015.pfb.35.84.897
53. Souza DMCS, Martins AR, Fernandes SB et al (2022) In Vitro Multiplication of *Eucalyptus pilularis* and *Eucalyptus grandis* x *E. urophylla* (Urograndis Eucalypt): Effect of Light Quality in Temporary Immersion Bioreactor. Mindanao J Sci Technol 20:72–86. https://mjst.ustp.edu.ph/index.php/mjst/article/view/1193
54. Souza DMSC, Fernandes SB, Duarte VP et al (2024) Effect of light intensity and seal type on the in vitro elongation and adventitious rooting of *Eucalyptus grandis* × E. *urophylla*. New Zealand Journal of Forestry Science 54:1–17. https://doi.org/10.33494/nzjfs542024x284x
55. Tomaszewska-Sowa M (2020) In vitro clonal propagation and efficient acclimatization with use of hydrogel of intensively sweet medicinal plant *Lippia dulcis* Trev. Herba Pol 66:25–31. https://doi.org/10.2478/hepo-2020-0019
56. Wanat D, Garbowska C, Wrzesińska W et al (2025) Evaluation of Physicochemical Properties of Polymeric Systems for Potential Applications in Cartilage Tissue Engineering. Int J Mol Sci 26:1–19. https://doi.org/10.3390/ijms26052057
57. Wang X, Wei H, Zhang S et al (2025) Transgenic *Eucalyptus urophylla* × *Eucalyptus grandis* superior clone DH3229: achieving glyphosate resistance through genetic modification. Planr Cell Tissue Organ Cult 160:1–11. https://doi.org/10.1007/s11240-025-02967-x
58. Womack NC, Piccoli I, Camarotto C et al (2022) Hydrogel application for improving soil pore network in agroecosystems. Preliminary results on three different soils. CATENA 208:105759. https://doi.org/10.1016/j.catena.2021.105759
59. Wu Q, Xie J, Li J et al (2024a) Engineering Rapeseed Germination and Root Growth with Mechanical Strength of Polysaccharide Hydrogel. ACS Appl Bio Mater 7:3496–3505. https://doi.org/10.1021/acsabm.4c00416
60. Wu Q, Xie J, Li J, Men Y (2024b) Engineering living root with mechanical stimulation derived from reciprocating compression in a double network hydrogel as elastic soil. Adv Agrochem 1–9. https://doi.org/10.1016/j.aac.2024.10.001
61. Xu H, Liu Y, Xie Y et al (2019) Doubly cross-linked nanocellulose hydrogels with excellent mechanical properties. Cellulose 26:8645–8654. https://doi.org/10.1007/s10570-019-02689-2
62. Xu Z, Li J, Zhou H et al (2016) Morphological and swelling behavior of cellulose nanofiber (CNF)/poly(vinyl alcohol) (PVA) hydrogels: Poly(ethylene glycol) (PEG) as porogen. RSC Adv 6:43626–43633. https://doi.org/10.1039/c6ra03620a
63. Zhang Z, Abidi N, Lucia L et al (2023) Cellulose/nanocellulose superabsorbent hydrogels as a sustainable platform for materials applications: A mini-review and perspective. Carbohydr Polym 299:120140. https://doi.org/10.1016/j.carbpol.2022.120140

Figures

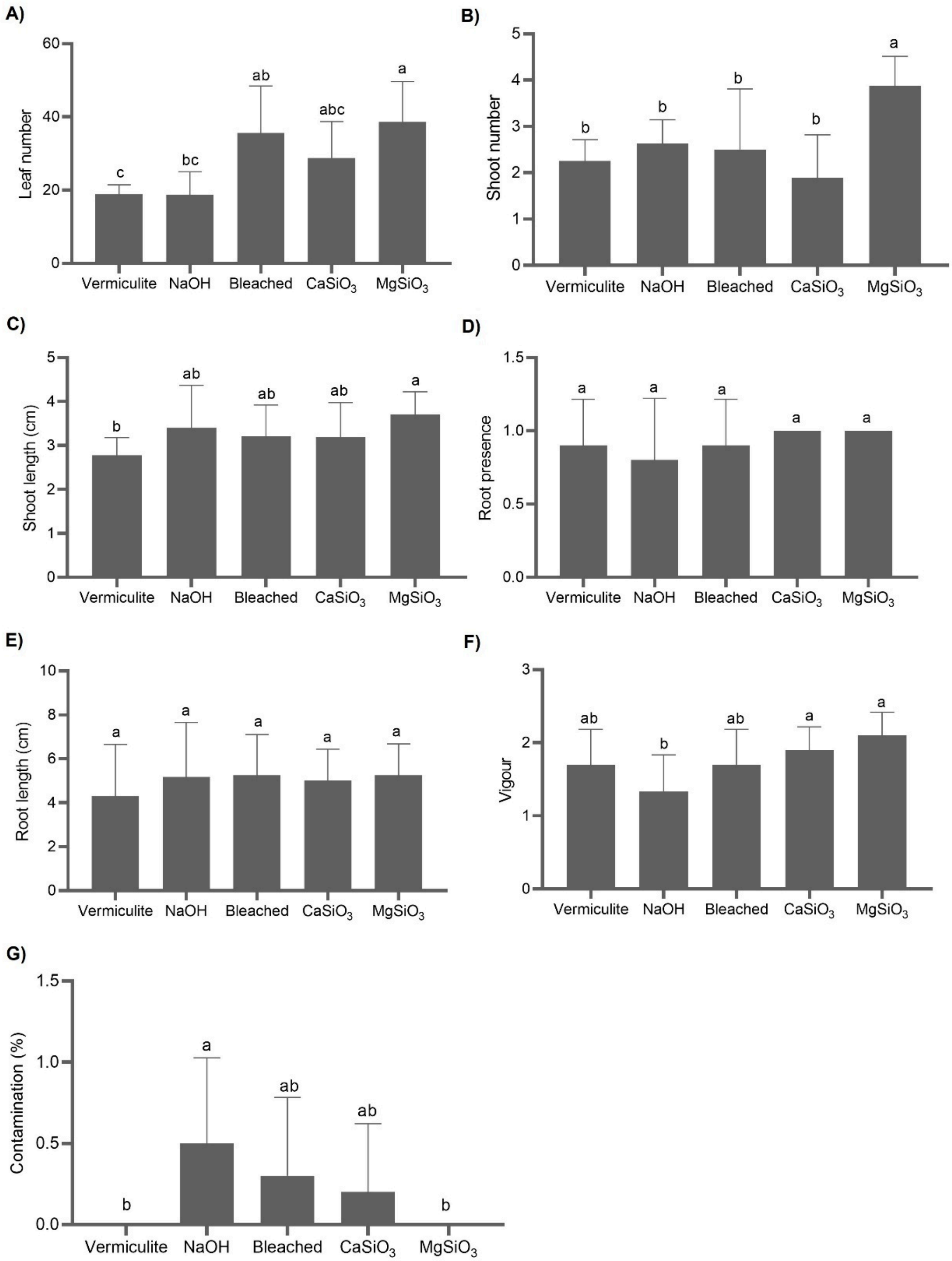

Figure 1

Variables were evaluated during acclimatisation of urograndis eucalypt microcuttings after 30 days of *ex vitro* culture: A) Leaf number; B) Shoot number; C) Shoot length; D) Root presence; E) Root length; F) Vigour; G) Contamination. Different letters indicate a significant difference between the treatments ($p < 0.05$).

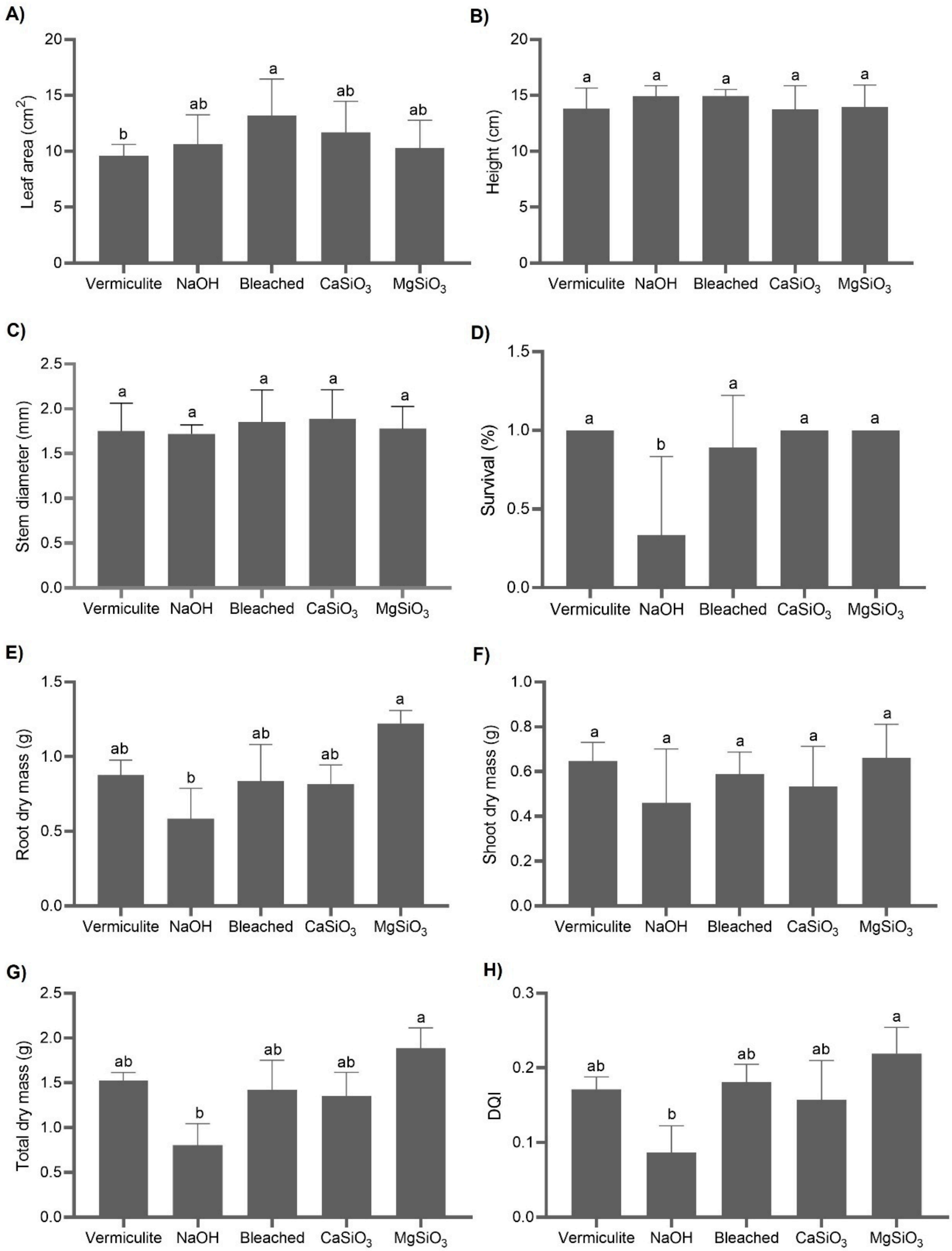

Figure 2

Variables evaluated during acclimatisation of urograndis eucalypt microcuttings in the nursery, after 90 days: A) leaf area; B) height, C) stem diameter; D) survival; E) root dry mass, F) shoot dry mass, G) total dry mass; H) DQI. Different letters indicate a significant difference between the treatments ($p < 0.05$).

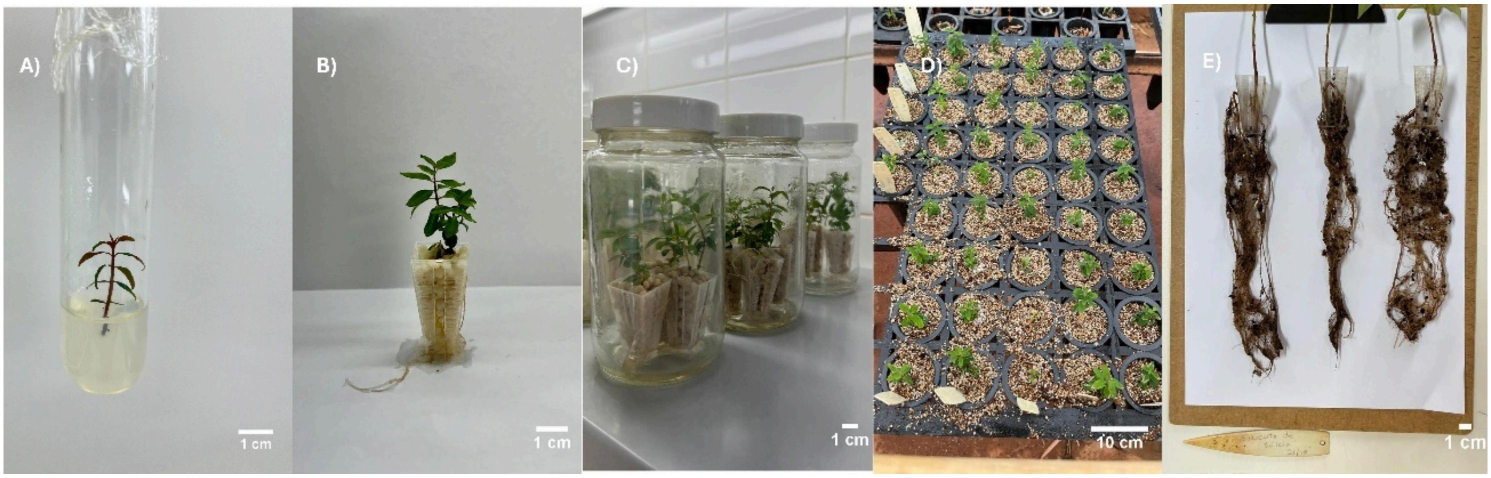

Figure 3

Elongation, acclimatisation and adventitious rooting of urograndis eucalypt microcuttings: A) Microcuttings in the elongation stage in the culture laboratory; B) Clonal plants transferred to hydrogel microspheres in microtubes; C) Clonal plants in microtubes in a mini-stufim system with hydrogel microspheres after 30 days; D) Clonal plants transferred to the nursery; E) Roots evaluated after 90 days.

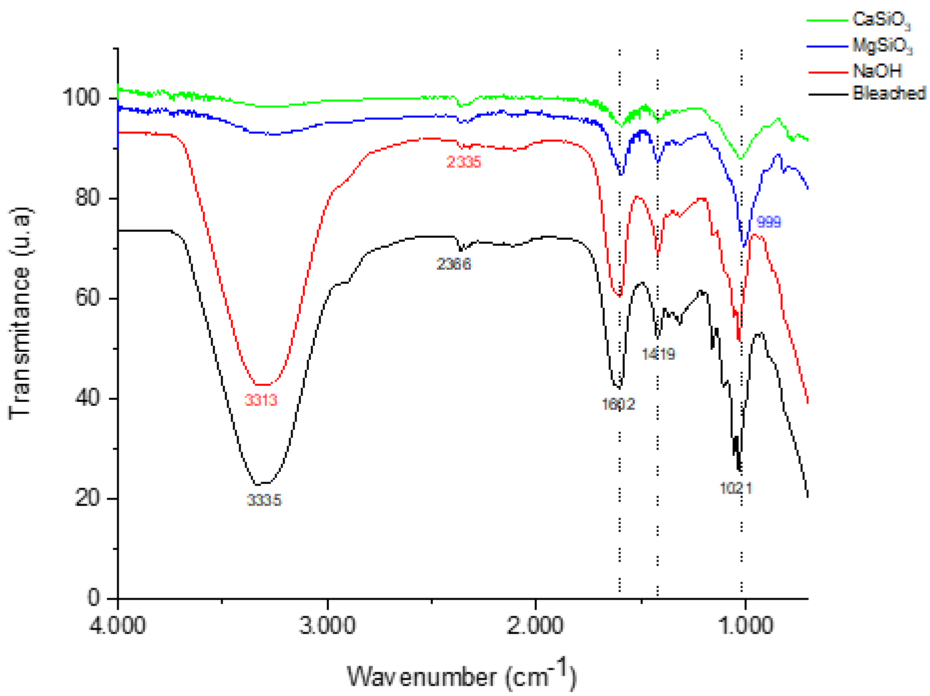

Figure 4

FTIR spectra of hydrogel microspheres subjected to different pre-treatments: NaOH, Bleached; $CasiO_3$; $MgSiO_3$.