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Brief Report

Shoots Regeneration in Brigitta and Duke Blueberry Cultivars from Different Encapsulated Vegetative Propagules

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Abstract: Synthetic seed technology, an advanced *in vitro* propagation method, combines the benefits of micropropagation with the practicality of zygotic seeds, offering an efficient solution for the handling, storage, and transportation of plant propagules. This study represents the first investigation of the role of explant type in blueberry encapsulation. In particular, three non-embryogenic propagules (basal node, median node, and shoot tip) were evaluated in the Brigitta and Duke blueberry cultivars. The artificial endosperm consists of Woody Plant Medium (WPM) macro- and micro-nutrients and Murashige and Skoog vitamins at half concentration, inositol (5 g L^{-1}), Indole-3-butyric acid (IBA) (0.005 mg L^{-1}), zeatin (0.25 mg L^{-1}), and sucrose (50 g L^{-1}). Forty-five days after sowing in *in vitro* conditions, the encapsulated shoot tips showed better results than basal and median nodes in several parameters, including viability, shoot length, and fresh shoot weight, in both cultivars. In both cultivars, none of the encapsulated propagule types developed roots. These results underscore the advantages of using shoot tips in encapsulation technology for blueberries and provide valuable insights for optimizing encapsulation protocols to improve propagation efficiency.

Keywords: *Vaccinium corymbosum* L.; micropropagation; *in vitro* culture; alginate beads

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1. Introduction

Blueberries belong to the genus *Vaccinium* spp., which includes about 450 species [1], and they have been domesticated for a relatively short period, with a history spanning approximately 120 years [2]. Specifically, highbush blueberries (*Vaccinium corymbosum* L.), which originate from Canada and North America, have undergone selective breeding to develop northern highbush cultivars [3]. The hybridization of *V. corymbosum* with *V. darrowii*, an evergreen blueberry species, extended the growth potential of northern highbush blueberries into subtropical and tropical regions, leading to the development of southern highbush varieties [4]. Additionally, further crossbreeding between *V. corymbosum* and other species within the *Cyanococcus* section has contributed to enhanced adaptation (i.e., tolerance to alkaline soils) and improved fruit quality (berry firmness, size, flavor) and yield [5,6].

Blueberry plants are commonly found in forested and woodland regions across the Northern Hemisphere, with a significant presence in North America, Europe, and Asia [7]. Globally recognized as a leading health-promoting food, blueberries have emerged as one of the crops with the highest market value and commercial potential, often referred to as the "king of fruits" due to their wide range of health benefits, including anti-inflammatory, antioxidant, neuroprotective, anticancer, and vision-enhancing properties [7,8]. They

also hold a prominent position in the market for their prebiotic qualities. Among these, the highbush blueberry (*Vaccinium corymbosum* L.) has become the principal species of *Vaccinium* fruit cultivated during the 20th century [1]. The most recent data are for 2022 and indicate that the total cultivated area amounts to approximately 173,924 hectares, with an annual production volume of 1,228,595.98 tons. Between 2020 and 2022, the global area dedicated to blueberry cultivation increased by 8.53%, while worldwide blueberry production saw a 19.01% rise during the same period [9].

Conventional blueberry propagation techniques commonly applied include the use of semi-woody cuttings [10]. However, these conventional methods have limitations, including low rooting success, labor intensity, and slow progress [10,11]. Additionally, their dependence on seasonal and climatic factors complicates continuous seedling production throughout the year and limits their scalability [10,12].

Micropropagation, an *in vitro* culture technique, provides several advantages compared to traditional agamic propagation methods [13,14]. It ensures high genetic and sanitary quality of the propagated material while enabling the production of a large number of plants within a limited space and a short timeframe [11,12,15].

Additionally, micropropagation allows for the use of plant material with juvenile characteristics, which facilitates rapid growth and enables quicker responses to various treatments, making it particularly valuable.

However, managing, storing, and transporting micropropagated plants is difficult due to the risk of deterioration and damage, which can limit their commercial potential when compared to zygotic or gamic seeds [16].

To address these limitations, synthetic seed technology, an *in vitro* culture technique, has emerged as a promising alternative [17]. This method involves encapsulating explants, such as shoot apices, axillary buds, or somatic embryos, in a nutritive and protective coating, combining the advantages of micropropagation with the practicality of zygotic seeds [18–20]. The products of encapsulation are capsules that may evolve in shoots and synthetic or artificial seeds that evolve in plantlets [18].

Initially, synthetic seeds were defined solely as encapsulated somatic embryos. However, since some plant species exhibit recalcitrance in generating somatic embryos, the concept of artificial seeds was later broadened to include the encapsulation of various *in vitro*-derived propagules [17,21]. The latter can be represented by propagules like shoot buds (apical or axillary), nodal segments, etc., that can be used for encapsulation and can convert into a plantlet (in *in vitro* or *ex vitro* conditions) and that can retain this ability even after storage [21]. The main advantages related to the use of non-embryogenic propagules for encapsulation are not only related to the overcoming of the abovementioned recalcitrance of some plant species in the production of somatic embryos, but they are also useful for the species that do not produce uniform quality embryos [21].

The encapsulation technology guarantees genetic uniformity of plants and facilitates exchange between laboratories or direct delivery and sowing to the field or greenhouse [21]. The encapsulated propagules can be sown like regular seeds and can develop into plants under both *in vitro* and *ex vitro* conditions [22]. Synthetic seeds should also be able to keep high conversion/regrowth rates for a medium–long period [16]. Moreover, if artificial seeds were directly sown, the acclimation steps could be eliminated, and so breeders could have greater flexibility [17].

Encapsulation technology provides efficient year-round mass propagation, offering benefits such as easy handling, long-term storage, and use in germplasm preservation and exchange of sterile plant material between laboratories [16,23].

This study aims to determine, to the best of our knowledge for the first time, the optimal explant type for the application of encapsulation technology in blueberries by

assessing three types of non-embryogenic propagules (basal node, median node, and shoot tip) in the cultivars Brigitta and Duke that are largely cultivated.

2. Materials and Methods

2.1. Plant Material

The experiment was conducted on the blueberry cultivars 'Brigitta' and 'Duke' in the 'Micropropagation and *in vitro* Biotechnology Laboratory' of the Research Unit 'Tree Science' of the Department of Agricultural, Food and Environmental Sciences of the University of Perugia.

2.2. In Vitro Culture Conditions

The Brigitta and Duke blueberry cultivars were grown *in vitro* on a medium consisting of Woody Plant Medium (WPM) nutrients [24] mixed with Murashige and Skoog (MS) vitamins [25], inositol (10 g L^{-1}), and sucrose (30 g L^{-1}). Indole-3-butyric acid (IBA) (0.01 mg L^{-1}) and zeatin (0.5 mg L^{-1}) were used as plant growth regulators, agar (7 g L^{-1}) as a solidifying agent, and the pH was adjusted at 5.6. Glass vessels (500 m L^{-1} capacity) were used, each containing fifteen single shoots and 100 mL of the substrate described above. The substrates and vessels were autoclaved at 115 °C for 20 min before being used under a horizontal laminar flow cabinet in aseptic conditions. The vessels were maintained in a growth chamber at a 22 ± 2 °C temperature and a 16 h photoperiod with a light intensity of 40 μE m^{-2} s^{-1}.

2.3. Encapsulating Procedure and Growing Conditions

After a proliferation subculture from the *in vitro* proliferated shoots, three types of propagules (basal node, median node, and shoot tip) were selected and subjected to encapsulation to verify which was the most suitable. Encapsulation was performed according to the procedure described by Regni et al. [23]. Specifically, the explants were first immersed for a few minutes in an encapsulating solution consisting of the nutrient substrate (growth regulator included) described above at half concentration and added with sucrose (50 g L^{-1}), and sodium alginate (25 g L^{-1}) of medium viscosity (Merck KGaA, Darmstadt, Germany) and deprived of calcium chloride, pH 5.6 for the coating phase.

The explants covered with an encapsulating matrix were then immersed in the complexing solution, which differed from the previous one for the addition of anhydrous calcium chloride (11 g L^{-1}) (Merck KGaA, Darmstadt, Germany) instead of sodium alginate, pH 5.6. The encapsulated explants were then rinsed three times for 15 min each in a sterile washing solution (artificial endosperm solution) consisting of the nutrient substrate (growth regulator included) described above at half concentration and added with sucrose (50 g L^{-1}), pH 5.6.

The capsules were sown respecting polarity in glass jars (500 mL volume) containing 100 mL of the growth medium used for the proliferation of the two blueberry cultivars but without growth regulators and with sucrose added (30 g L^{-1}). In each jar, 10 capsules were sown, and 4 replicates for each treatment were foreseen. The jars containing the encapsulated propagules were placed in a growth chamber at a constant temperature of 22 ± 2 °C, and a 16 h photoperiod of light with an intensity of 40 μE m^{-2} s^{-1}. All the plant's manipulations were carried out in sterile conditions under a horizontal laminar flow cabinet.

2.4. Data Collection

The evaluation of the following parameters was made 45 days after sowing:

- Viability (%): incidence of green propagules without browning or necrosis;

- Regrowth (%): incidence of propagules that sprouted or rooted;
- Conversion (%): incidence of propagules that sprouted and rooted at the same time);
- Shoots produced (n) and shoot length (mm): average number of shoots produced per vital explant and their length;
- Roots produced (n) and root length (mm): average roots produced per vital explant and their length;
- Callus (%) and callus fresh weight (mg): incidence of propagules with callus and callus fresh weight;
- Green fresh weight (mg): average fresh weight of vegetative organs (leaves, stems, buds) per vital explant;
- Total dry weight per explant (mg) obtained by keeping the plant material in an oven for three days at 105 °C.

2.5. Statistical Analysis

The trial was organized according to a completely randomized design; for each cultivar, three types of propagules (basal node, median node, and shoot tip) were considered with 40 encapsulated explants for each treatment. Data on percentages were arcsine-transformed before performing statistical analysis. Moreover, an analysis of the variance was also conducted according to a two-way ANOVA considering cultivar and explant type as factors. The obtained results are expressed as mean values ± standard error (SE). The statistical analysis was performed using the EXCEL® add-in macro DSAASTAT.

3. Results

In the cultivar Brigitta, the viability of the capsules obtained from the median and basal nodes was lower than that obtained from the shoot tips, which was equal to 85% and higher according to the Tukey HSD test ($p \leq 0.05$) (Table 1) compared to median and basal encapsulated nodes. In particular, a 64.7% and 52.91% reduction in viability was observed in capsules from median nodes and basal nodes, respectively, compared to those obtained from apices (Table 1). All encapsulated explants, irrespective of the type of propagule, gave rise to shoots. Therefore, the regrowth data coincides with the viability data (Table 1). In contrast, none of the three encapsulated propagule types produced roots.

Table 1. Vitality, regrowth, shoot number and shoot length, shoots with basal callus, callus fresh weight, shoot fresh weight, and total dry weight of Brigitta cultivar's encapsulated explants (basal node, median node, and shoot tip). Data are expressed as means ± SE from 40 encapsulated explants for each explant type. Different letters indicate statistically significant differences according to the Tukey HSD test ($p \leq 0.05$).

Explant's Type	Vitality (%)	Regrowth (%)	Shoot Number (n)	Shoot Length (mm)	Shoots with Basal Callus (%)	Callus Weight (mg)	Shoot Fresh Weight (mg)	Total Dry Weight (mg)
Shoot tips	85 ± 5 a	85 ± 5 a	1.1 ± 0.05 a	11.66 ± 1.03 a	85 ± 5 a	9.28 ± 2.03 a	5.89 ± 0.42 a	4.44 ± 0.62 a
Medium node	30 ± 6 b	30 ± 6 b	1.1± 0.1 a	5.28 ± 1.10 b	27 ± 6 b	9.25 ± 2.50 a	2.49 ± 0.68 b	2.44 ± 0.87 a
Basal node	40 ± 9 b	40 ± 9 b	1.2 ±0.1 a	5.15 ± 0.68 b	40 ± 9 b	9.98 ± 2.67 a	2.64 ± 0.42 b	2.93 ± 0.46 a

The type of propagule used did not influence the number of shoots obtained per capsule but influenced their length (Table 1). The length of shoots obtained from encapsulated shoot tips was longer than those obtained from encapsulated median and basal nodes (Table 1). In contrast, no differences were found in the length of shoots obtained from encapsulated median and basal nodes (Table 1).

The incidence of shoots with basal callus was higher in shoots obtained from encapsulated shoot tips than in those obtained from encapsulated median and basal nodes, which showed no significant differences between them (Table 1).

The fresh callus weight per explant was not different in the three types of encapsulated propagules (Table 1). As a consequence of the greater shoot length found in shoots obtained from encapsulated shoot tips, there was a higher fresh weight of the shoots compared to shoots obtained from encapsulated median and basal nodes (Table 1 and Figure 1). On the other hand, the total dry weight was not influenced by the type of propagule used for encapsulation.

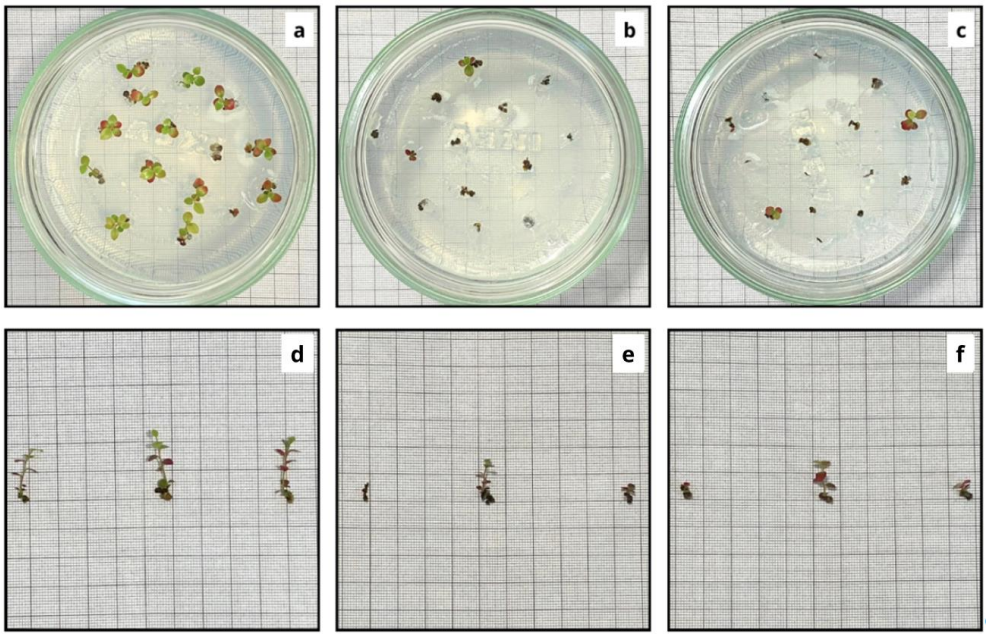


Figure 1. Shoots obtained from the encapsulated shoot tip (**a**,**d**), median node (**b**,**e**), and basal node (**c**,**f**) of the Brigitta cultivar.

For the cultivar Duke, the highest viability values were recorded in the encapsulated shoot tips, followed by the median node and the encapsulated basal node (Table 2). All viable encapsulated explants gave rise to shoots and/or calluses, so the regrowth data coincide with the viability data (Table 2). As already observed for 'Brigitta', none of the three encapsulated propagule types produced roots.

Table 2. Vitality, regrowth, shoot number and shoot length, shoots with basal callus, callus fresh weight, shoot fresh weight, and total dry weight of Duke cultivar's encapsulated explants (basal node, median node, and shoot tip). Data are expressed as means ± SE from 40 encapsulated explants for each explant type. Different letters indicate statistically significant differences according to the Tukey HSD test ($p \leq 0.05$).

Explant's Type	Vitality (%)	Regrowth (%)	Shoot Number (n)	Shoot Length (mm)	Shoots with Basal Callus (%)	Callus Fresh Weight (mg)	Shoot Fresh Weight (mg)	Total Dry Weight (mg)
Shoot tips	85 ± 3 a	85 ± 3 a	1.0 a	6.73 ± 0.60 a	82 ± 6 a	6.08 ± 1.72 a	5.33 ± 0.91 a	2.72 ± 0.62 a
Medium node	38 ± 10 b	38 ± 10 b	1.0 a	2.13 ± 0.31 b	15 ± 8 b	1.09 ± 0.29 b	2.25 ± 0.47 b	0.84 ± 0.17 b
Basal node	12 ± 3 c	12 ± 3 c	1.2 ± 0.17 a	2.50 ± 0.29 b	0 c	0 c	3.83 ± 0.50 ab	1.23 ± 0.11 b

For the number of shoots produced from encapsulated shoot tips, median and basal nodes did not differ (Table 2). The length of the shoots produced was longer in the encapsulated shoot tips than in the median and basal nodes (Table 2). No differences

were found in the length of shoots produced by the encapsulated median and basal nodes (Table 2).

The shoots produced by the encapsulated basal nodes did not show calluses By contrast, the highest percentage of shoots with calluses was found in those obtained from encapsulated shoot tips (Table 2).

The callus produced by the encapsulated shoot tips was heavier than that of the encapsulated basal nodes (Table 2). The fresh weight of shoots produced by shoot tips was higher than that produced by median nodes (Table 2 and Figure 2). The highest total dry weight was recorded for encapsulated shoot tips (Table 2).

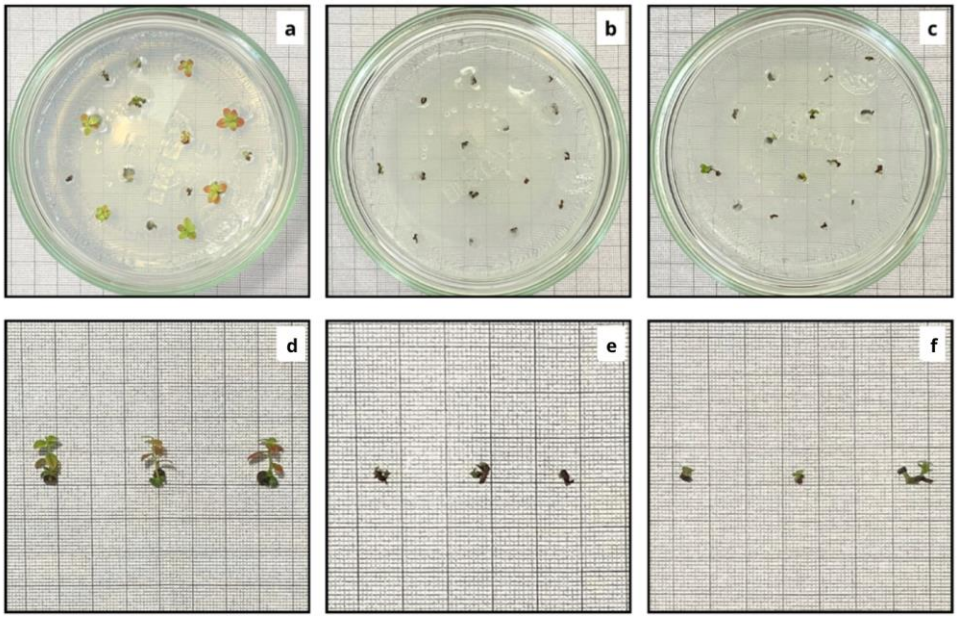


Figure 2. Shoots obtained from encapsulated shoot tip (**a**,**d**), median node (**b**,**e**), and basal node (**c**,**f**) of the Duke cultivar.

Two-way ANOVA revealed that the explant type factor was statistically significant for the vitality and regrowth rates, and the highest value was recorded for encapsulated shoot tips (Table 3). Both factors (cultivar and explant type) significantly affected shoot length (Table 3). In particular, shoot length was higher in Brigitta than in Duke (Table 3). Regarding the explant type, the highest value for shoot length was found for encapsulated shoot tips. At the same time, there was no difference between median and basal encapsulated nodes in shoot length (Table 3). A similar behavior was found for the percentage of shoots with calluses and green fresh weight. Again, the highest values were found for the cultivar Brigitta and encapsulated shoot tips as a type of explant (Table 3). For total dry weight, the highest values were observed for the cultivar Brigitta and shoot tips as explant type (Table 3). Details are included in Table S1.

Table 3. Results of two-way ANOVA analysis considering cultivar and explant type as factors.

	Vitality (%)	Regrowth (%)	Shoot Number (n)	Shoot Length (mm)	Callus (%)	Callus FW (mg)	Green FW (mg)	Total DW (mg)
Propagule type (A)	**	**	ns	**	**	*	**	*
Cultivar (B)	ns	ns	ns	**	**	*	ns	**
A × B	ns	ns	ns	ns	ns	ns	ns	ns

ns not significant; * $p \leq 0.05$; ** $p \leq 0.01$.

4. Discussion

Encapsulation technology has emerged as a promising tool for propagating and conserving plant material, particularly in fruit crops [21]. While the technology shows potential for commercial applications in nurseries and tissue culture laboratories, challenges remain, including the need for automation to reduce labor-intensive steps and improve economic viability [18].

In the present study, the possibility of using *in vitro*-derived vegetative propagules for blueberry encapsulation was investigated for the first time. The performance of synthetic seeds/capsules can be significantly impacted by the type of propagule, the composition of the artificial endosperm, the sowing substrate, and the cultivation conditions [21]. Shoot tips and axillary nodes with buds, commonly referred to as microcuttings, are extensively utilized for encapsulation due to their ease of availability and higher genetic stability compared to somatic embryos [17,26,27]. One possible limitation, as also found in the present study, of using microcuttings for encapsulation is the lack of root primordia, which can hinder the spontaneous development of adventitious roots [23].

Among the evaluated propagules, only shoot tips provided positive results in terms of viability and regrowth and showed greater shoot growth. Similar results were found for the grapevine rootstock 'Kober 5BB'. Indeed, encapsulated 'Kober 5BB' shoot tip explants showed higher regrowth ability than encapsulated nodal explants [28]. The regrowth rates observed for both blueberry cultivars for encapsulated shoot tips were similar to those found in other small-fruit species, *Rubus* spp. in particular, for other encapsulated vegetative propagules [23]. Indeed, even in *Rubus* spp., the encapsulated clump's bases showed regrowth rates over 80% [23]. On the contrary, microcuttings of *Rubus* spp. containing single nodes performed lower than those of the encapsulated clump's bases [23]. However, blackberry encapsulated microcuttings performed betetr than encapsulated basal and median nodes of the blueberry. The high regeneration rates achieved with encapsulated shoot tips are particularly noteworthy, considering the widely recognized importance of this parameter [29]. Among various non-embryogenic materials, shoot tip explants are the most responsive due to the mitotic activity in their meristem [30]. For this reason, shoot types were used for the encapsulation in different species: eucalypt [31], medicinal plants [32], *Camellia japonica* L. and *Camellia reticulata* [30], 'Kober 5BB' grapevine rootstock [28], myrtle [33], and sugarcane [34] and for the conservation of rare and endangered species [35].

5. Conclusions

In conclusion, the viability and shoot growth of encapsulated propagules in Brigitta and Duke cultivars were strongly influenced by the type of propagule used. Encapsulated shoot tips showed higher viability and produced longer shoots compared to the median and basal encapsulated nodes. Indeed, while the type of propagule did not affect the number of shoots produced, shoot length was significantly greater in shoot tips for both cultivars. These results revealed for the first time for blueberry the superior performance of encapsulated shoot tips in terms of viability and shoot elongation compared to median and basal encapsulated nodes. If further studies confirm the advantages of encapsulated shoot tips over other types of encapsulated propagules, standardized protocols could be developed for the large-scale application of encapsulation technology with this type of propagule in the nursery sector. Furthermore, encapsulated blueberry shoot tips could be validly employed for the medium- to long-term conservation of blueberry germplasm.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11030259/s1.

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References

1. Song, G.-Q. Blueberry (*Vaccinium corymbosum* L.). In *Agrobacterium Protocols: Volume 2*; Springer: New York, NY, USA, 2015; pp. 121–131.
2. Ehlenfeldt, M. The Delightful Domesticated American Blueberry: Some Research Challenges for Its Next 100 Years. *Agric. Res.* **2011**, *59*, 2.
3. Yu, X.; Yuan, H.; Jin, Y.; Xia, C.; Zhu, J.; Che, J.; Yang, J.; Wang, X.; Zheng, B.; Yang, S.; et al. Establishment of a Breeding Approach Combined with Gamma Ray Irradiation and Tissue Regeneration for Highbush Blueberry. *Agronomy* **2025**, *15*, 217. [CrossRef]
4. Cui, F.; Ye, X.; Li, X.; Yang, Y.; Hu, Z.; Overmyer, K.; Brosché, M.; Yu, H.; Salojärvi, J. Chromosome-Level Genome Assembly of the Diploid Blueberry *Vaccinium Darrowii* Provides Insights into Its Subtropical Adaptation and Cuticle Synthesis. *Plant Commun.* **2022**, *3*, 100307. [CrossRef]
5. Edger, P.P.; Iorizzo, M.; Bassil, N.V.; Benevenuto, J.; Ferrão, L.F.V.; Giongo, L.; Hummer, K.; Lawas, L.M.F.; Leisner, C.P.; Zalapa, J.; et al. There and back again; historical perspective and future directions for Vaccinium breeding and research studies. *Hortic. Res.* **2022**, *9*, uhac083.
6. Brevis, P.A.; Bassil, N.V.; Ballington, J.R.; Hancock, J.F. Impact of Wide Hybridization on Highbush Blueberry Breeding. *J. Am. Soc. Hortic. Sci.* **2008**, *133*, 427–437. [CrossRef]
7. Ashique, S.; Mukherjee, T.; Mohanty, S.; Garg, A.; Mishra, N.; Kaushik, M.; Bhowmick, M.; Chattaraj, B.; Mohanto, S.; Srivastava, S.; et al. Blueberries in Focus: Exploring the Phytochemical Potentials and Therapeutic Applications. *J. Agric. Food Res.* **2024**, *18*, 101300. [CrossRef]
8. Lobos, G.A.; Hancock, J.F. Breeding Blueberries for a Changing Global Environment: A Review. *Front. Plant Sci.* **2015**, *6*, 782. [CrossRef]
9. FAOSTAT. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 17 January 2025).
10. Mazurek, M.; Siekierzyńska, A.; Piechowiak, T.; Spinardi, A.; Litwińczuk, W. Comprehensive Analysis of Highbush Blueberry Plants Propagated In Vitro and Conventionally. *Int. J. Mol. Sci.* **2024**, *25*, 544. [CrossRef]
11. Mohamed, G.R.A.; Khusnetdinova, L.Z.; Timofeeva, O.A. Elaboration of micropropagation protocol for Vaccinium corymbosum cv." Sunt Blue Giant. *Asian J. Plant Sci. Res.* **2018**, *8*, 1–11.
12. Correia, S.; Matos, M.; Leal, F. Advances in Blueberry (*Vaccinium* spp.) In Vitro Culture: A Review. *Horticulturae* **2024**, *10*, 533. [CrossRef]
13. Regni, L.; Micheli, M.; Del Pino, A.M.; Palmerini, C.A.; D'Amato, R.; Facchin, S.L.; Famiani, F.; Peruzzi, A.; Mairech, H.; Proietti, P. The First Evidence of the Beneficial Effects of Se-Supplementation on In Vitro Cultivated Olive Tree Explants. *Plants* **2021**, *10*, 1630. [CrossRef]
14. Dobránszki, J.; da Silva, J.A.T. Micropropagation of Apple—A Review. *Biotechnol. Adv.* **2010**, *28*, 462–488. [CrossRef]
15. Kavand, S.; Kermani, M.J.; Haghnazari, A.; Khosravi, P.; Azimi, M.R. Micropropagation and Medium-Term Conservation of Rosa Pulverulenta. *Acta Sci. Agron.* **2011**, *33*, 297–301. [CrossRef]
16. Regni, L.; Micheli, M.; Pino, A.M.D.; Facchin, S.L.; Rabica, E.; Camilloni, L.; Cesarini, A.; Proietti, P. Blackberry Synthetic Seeds Storage: Effects of Temperature, Time, and Sowing Substrate. *Plant Cell Tissue Organ Cult.* **2024**, *158*, 17. [CrossRef]
17. Rihan, H.Z.; Kareem, F.; El-Mahrouk, M.E.; Fuller, M.P. Artificial Seeds (Principle, Aspects and Applications). *Agronomy* **2017**, *7*, 71. [CrossRef]
18. Standardi, A.; Micheli, M. Encapsulation of in Vitro-Derived Explants: An Innovative Tool for Nurseries. *Methods Mol. Biol.* **2013**, *11013*, 397–418. [CrossRef]
19. Ahmad, N.; Shahid, A.; Javed, S.B.; Khan, M.I.; Anis, M. Micropropagation of Vitex spp. through in Vitro Manipulation: Current Status and Future Prospectives. *J. Appl. Res. Med. Aromat. Plants* **2015**, *2*, 114–123. [CrossRef]
20. Asmah, N.H.; Hasnida, N.H.; Zaimah, N.A.N.; Noraliza, A.; Salmi, N.N. Synthetic Seed Technology for Encapsulation and Regrowth of in Vitro-Derived Acacia Hyrid Shoot and Axillary Buds. *Afr. J. Biotechnol.* **2011**, *10*, 7820–7824. [CrossRef]

21. Rai, M.K.; Asthana, P.; Singh, S.K.; Jaiswal, V.S.; Jaiswal, U. The Encapsulation Technology in Fruit Plants—A Review. *Biotechnol. Adv.* **2009**, *27*, 671–679. [CrossRef]
22. Magray, M.M.; Wani, K.P.; Chatto, M.A.; Ummyiah, H.M. Synthetic Seed Technology. *Int. J. Curr. Microbiol. Appl. Sci.* **2017**, *6*, 662–674. [CrossRef]
23. Regni, L.; Micheli, M.; Facchin, S.L.; Del Pino, A.M.; Silvestri, C.; Proietti, P. The Influence of the Explant's Type on the Performance of Synthetic Seeds of Blackberry (*Rubus* spp.). *Plants* **2024**, *13*, 32. [CrossRef]
24. Lloyd, G.; McCown, B.H. Commercially-Feasible Micropropagation of Mountain Laurel, Kalmia Latifolia, by Use of Shoot-Tip Culture. *Comb. Proc.-Int. Plant Propagator's Soc.* **1980**, *30*, 421–427.
25. Murashige, T.; Skoog, F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Plant Physiol.* **1962**, *15*, 473–497. [CrossRef]
26. Piccioni, E.; Standardi, A. Encapsulation of Micropropagated Buds of Six Woody Species. *Plant Cell Tissue Organ Cult.* **1995**, *42*, 221–226. [CrossRef]
27. Sharma, S.; Shahzad, A.; Teixeira da Silva, J.A. Synseed Technology—A Complete Synthesis. *Biotechnol. Adv.* **2013**, *31*, 186–207. [CrossRef]
28. Benelli, C. Encapsulation of Shoot Tips and Nodal Segments for in Vitro Storage of 'Kober 5BB' Grapevine Rootstock. *Horticulturae* **2016**, *2*, 10. [CrossRef]
29. Micheli, M.; Standardi, A.; Fernandes da Silva, D. Encapsulation and Synthetic Seeds of Olive (*Olea europaea* L.): Experiences and Overview. In *Synthetic Seeds: Germplasm Regeneration, Preservation and Prospects*; Faisal, M., Alatar, A.A., Eds.; Springer International Publishing: Cham, Switzerland, 2019; pp. 347–361. ISBN 978-3-030-24631-0.
30. Ballester, A.; Janeiro, L.V.; Vieitez, A.M. Cold Storage of Shoot Cultures and Alginate Encapsulation of Shoot Tips of *Camellia japonica* L. and *Camellia reticulata* Lindley. *Sci. Hortic.* **1997**, *71*, 67–78. [CrossRef]
31. Cao, H.; Trueman, S. Alginate Encapsulation of Shoot Tips and Nodal Segments for Short-Term Storage and Distribution of the Eucalypt *Corymbia torelliana* × *C. citriodora*. *Acta Physiol. Plant.* **2012**, *34*, 117–128. [CrossRef]
32. Kikowska, M.; Thiem, B. Alginate-Encapsulated Shoot Tips and Nodal Segments in Mictopropagation of Medicinal Plands. A Review. *Herba Pol.* **2011**, *57*, 45–57.
33. Dönmez, D. Regeneration of Plants from Alginate-Encapsulated Shoot Tips of Myrtle (*Myrtus communis* L.). *Erwerbs-Obstbau* **2022**, *64*, 307–314. [CrossRef]
34. Barraco, G.; Sylvestre, I.; Engelmann, F. Comparing Encapsulation-Dehydration and Droplet-Vitrification for Cryopreservation of Sugarcane (*Saccharum* spp.) shoot tips. *Sci. Hortic.* **2011**, *130*, 320–324. [CrossRef]
35. Coelho, N.; Gonçalves, S.; Romano, A. Endemic Plant Species Conservation: Biotechnological Approaches. *Plants* **2020**, *9*, 345. [CrossRef] [PubMed]

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