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An Efficient Aqua-Based Culture Method for the Propagation of High-Quality *Arundo donax* Seedlings

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Abstract: *Arundo donax* (family: Poaceae) is a perennial rhizomatous plant with considerable economic and ecological benefits. The plant is characterized by its fast growth, high yield, wide adaptability, and strong disease resistance. However, widespread cultivation is difficult because *A. donax* can only be asexually propagated. This study tested the efficacy of an aqua-based culture method with short duration and high rooting rate. The aqua-based culture protocol involved treating cane fragments with the commercial broad-spectrum rooting powder ABT-1 (No. 1 of the ABT series) at 100 mg/L for 24 h, followed by culturing in tap water for 19 days. We found that plant materials sampled from September to February of the following year were more suitable for aqua-based culturing than materials sampled from other months. Nodes on different parts of the cane did not vary significantly in the ability to form seedlings. The rooting percentage reached >95% after only 20 days, while the sprouting percentage was >93%. The seedlings obtained were strong, with high survival rates during cultivation and acclimation. No strict requirements for the growing media were necessary to achieve these results. In conclusion, this study provides an empirical foundation for a technique that could facilitate the factory production of high-quality *A. donax* seedlings.

Keywords: giant reed; aqua-based culture; stem cutting; rooting powder; growth regulator

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1. Introduction

Arundo donax, commonly known as giant reed, is a perennial rhizomatous plant belonging to the Poaceae family [1,2]. While endemic to Asia, *A. donax* has migrated into almost all temperate and tropical zones because it can grow under various conditions [3–5]. Giant reeds have thick and upright canes that can reach 8–10 m in height and 3–4 cm in diameter [6,7]. The flat and broad leaves are 5–8 cm wide and 30–60 cm long [8,9]. The morphology of *A. donax* is very similar to reed [10] and bamboo [11], but they belong to different genera. *A. donax* has an extremely fast growth rate and yields a large amount of biomass [12–14]. Under common growth conditions, aboveground dry mass can reach 20–50 tons per hectare [15,16]. In horizontal subsurface flow wetlands, this number increases to over 140 tons per hectare [17]. Once planted, *A. donax* can maintain sustainably high and stable production for more than 25 years [18].

In addition to a short growth cycle, high biomass yield, and easy field management, *A. donax* has considerable economic value. The plant is widely used in various applications, such as vibrating reeds for woodwind instruments [19], forage production [20], fiber extraction [21,22], biomass energy production [23,24], edible and medicinal fungus cultivation [25–27], wind prevention [28], soil conservation [29], and soil remediation [30,31]. Correspondingly, the demand for *A. donax* seedlings has increased, but scaling up production is difficult because the species is sterile and can only be raised asexually [32–34].

Currently, the main propagation methods for *A. donax* include root-splitting, stem cutting, and tissue culture. Root-splitting propagation involves planting rhizomes after dividing them into clusters of 4–5 buds. This method has a high survival rate but a poor propagation coefficient; moreover, it is costly and labor-intensive and suffers from a long production cycle [35–37]. For instance, only approximately 20 vital rhizome fragments can be obtained per square meter of mature culture (after three years) [38]. Such inefficient use of land severely limits widespread application.

Tissue culture propagation has the advantages of a high reproduction coefficient, short production cycle, and virus-free seedlings [39,40]. However, the intense requirements for production equipment and operation technology result in a prohibitive reproduction cost [33,41]. In contrast, stem-cutting propagation is straightforward, with relatively low labor intensity and equipment cost [42,43]. The process involves cutting canes into segments with complete axillary buds, from which seedlings are grown. These stems are easier to transport than rhizomes and seedlings. These combined qualities make stem cutting the main propagation method for *A. donax*.

The conventional approach for stem-cutting propagation in *A. donax* involves inserting stem segments directly into the soil. However, the segments can only be planted into an open field during certain months because plantlets must derive from canes in specific seasons, and development requires proper temperature and light. For example, in the lower Arno River plain of Italy, only spring cuttings are suitable for on-farm propagation [33]. Furthermore, planting stems during inappropriate seasons results in rooting difficulty and a low survival rate [43–45]. Therefore, industrial *A. donax* cultivation requires an easier and more efficient technique that can obtain plantlets with high survival rates.

Aqua-based culture (culturing cuttings in water until they take root and then planting) has the advantages of fast root growth and high rooting rate, along with the production of plantlets already adapted for transplantation into open fields [37,46]. Like stem-cutting propagation, aqua-based cultivation also uses cane fragments as materials. With 20–30 nodes per cane, hundreds of propagules can be harvested from a single square meter without losing rhizomes [37,47]. In addition to being simple, the method is also rapid. Plantlets can be obtained in 20–30 days after aqua-based culture of cane fragments, and transplantation to the open field is possible 30–40 days later [7,37]. Hence, aqua-based cultivation appears to be the best technique for large-scale applications.

The effect of the aqua-based culture method for *A. donax* propagation might be influenced by many factors, such as sampling months, cane sections, and pretreatment approaches [42,43]. Our study aimed to provide an aqua-based culture method with a short rooting time and high rooting rate for the propagation of *A. donax*. The findings should benefit efforts to produce high-quality *A. donax* seedlings on a large scale.

2. Materials and Methods

2.1. Plant Materials

A. donax plants used in this study were stored in the Henan International Joint Laboratory of Crop Gene Resources and Improvements, and they were planted at the experimental field of the School of Agricultural Sciences, Zhengzhou University (Henan, China; 34°81′ N, 113°52′ E). All propagules came from the same clone to avoid genetic differences. In March 2022, rhizomes were transplanted into the soil and placed approximately 80 cm apart in 20 m rows separated by 100 cm. Irrigation and weeding were performed in accordance with normal field management. In February 2023, the aboveground plants were harvested. The new canes grown from rhizomes were used as aqua-based culture materials and were sampled from June 2023 to April 2024.

2.2. Aqua-Based Culture

Healthy *A. donax* plants of equal size were selected. After removing leaves, canes were cut into fragments, each with one node and one axillary bud. Fragments were approximately 2–3 cm long below the node and 6–8 cm long above the node. After rinsing

with tap water, fragments were cleaned under running water for 60 min. Cuttings were then split into groups of 10 and bundled in a 100 mL centrifuge tube filled with sand, which could help the cane fragments stay upright in the water because the sand's density is greater than that of water. In this step, the axillary buds of the stem cuttings were adjusted outward. Next, cuttings were placed in boxes (40 cm × 26 cm × 14 cm) containing a corresponding concentration of ABT-1 rooting powder solution prepared with tap water. ABT-1 rooting powder contained 20% naphthylacetic acid (NAA) and 30% 3-Indoleacetic acid (IAA) and was obtained from Beijing EbTY Biotechnology Co., Ltd., Beijing, China. The liquid level was about 4 cm to submerge axillary buds. Boxes were maintained in a plant-growth room for aqua-based cultivation. After 24 h, the ABT-1 rooting powder solution was replaced with tap water for 19 days of aqua-based culturing. During this entire culture period, the temperature was maintained at 25 ± 2 °C, with a 16–8 h day–night cycle and 8000–10,000 Lx light intensity. Clean water was renewed every two days throughout the experiment to avoid algal proliferation. The flowchart of our aqua-based culture method is shown in Figure 1.

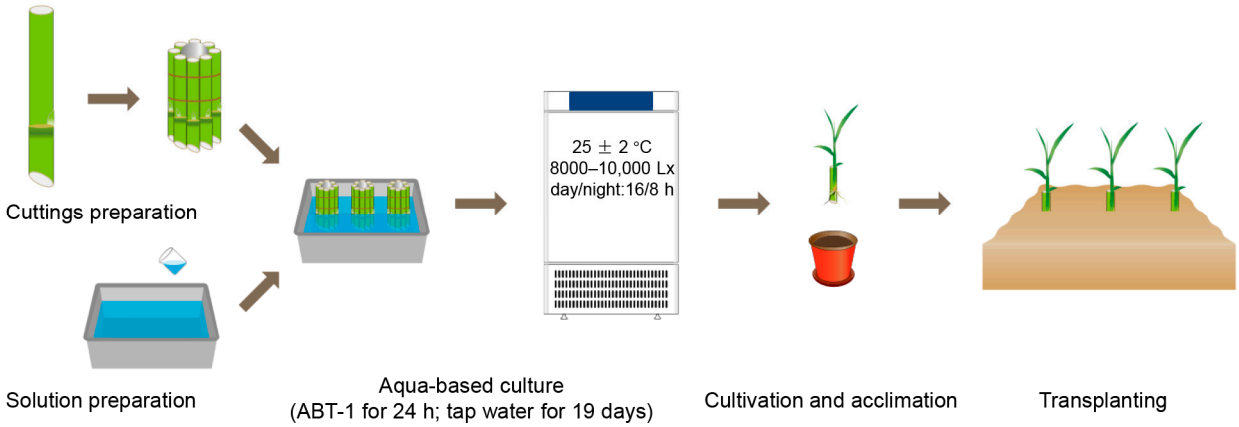


Figure 1. Experimental flowchart of the aqua-based culture method.

The aqua-based culture experiment was designed to evaluate the effects of different ABT-1 concentrations (0, 50, 100, 300, and 500 mg/L), different sampling months (June, September, the following February, and the following April), and different cane sections (apical, median, and basal) on the ability to develop *A. donax* seedlings. For the test of different ABT-1 concentrations, the cane fragments were sampled from September to the following February and were not distinguished between sections. For the test of different sampling months, the cane fragments were also not distinguished between sections and were first treated with ABT-1 rooting powder solution for 24 h at 100 mg/L. Furthermore, for the test of different cane sections, the fragments were sampled from September to the following February and were first treated with ABT-1 rooting powder solution for 24 h at 100 mg/L. In addition, the node fragments were excised from the cane's basal, median, and apical sections. Two nodes at each section's distal part were excluded to achieve better differentiation between sections.

The experiment was conducted in a random complete block design. Each treatment contained 20 randomly assigned cane fragments and was performed in a single box. Different treatments were replicated in triplicate and randomly assigned to boxes. Sprouting percentage, rooting percentage, bud length, root length, and root number were recorded at the end of culturing. The sprouting and rooting percentages were equal to the number of sprouting (or rooting) fragments divided by the total number of cane fragments in each treatment. The sprouting fragment was evaluated visually by the axillary bud's development, with the bud's length greater than 3 cm or the first leaf extension occurring normally. Meanwhile, a cane fragment with a taproot length of more than 1 cm was recorded as a rooting fragment. Trait values were averaged across the three replications.

2.3. Cultivation and Acclimation

After 20 days of aqua-based culture, healthy seedlings of equal size were selected and cultivated in flowerpots (diameter: 17 cm; height: 14 cm). Growth media in these pots comprised different combinations of field soil, nutritional soil, and vermiculite. The field soil was obtained directly from the experimental field of the School of Agricultural Sciences, Zhengzhou University, where *A. donax* plants were cultivated. The nutritional soil was commercial Pindstrup Substrate, which was obtained from Pindstrup Horticulture Co., Ltd., Shanghai, China [48]. The type of vermiculite used in this study was 2–4 mm. Flowerpots were placed in a plant growth room under the same conditions as those for aqua-based culture. Survival rates per treatment were measured after cultivation and acclimation for 30 days. Each treatment consisted of 20 seedlings and was repeated three times to obtain mean trait values.

2.4. Statistical Analyses

Data were processed in IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA) and Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA). Between-group differences were determined using analysis of variance and the least significant difference (LSD) test for multiple comparisons. Significance was set at $p < 0.05$.

3. Results

3.1. Effects of Rooting Powder Concentrations on Aqua-Based Culture

When the culture solution did not contain rooting powder, the sprouting percentage reached 100% after 20 days of aqua-based culture (Figure 2). However, the rooting percentage was only 38.33%. As the rooting powder concentration increased, the sprouting percentage decreased (Figure 2f), and the rooting percentage increased (Figure 2g). Sprouting percentages were 98.33%, 93.33%, and 91.67% under 50 mg/L, 100 mg/L, and 300 mg/L of ABT-1, respectively. Although the 100 mg/L and 300 mg/L treatments yielded significantly lower results than the 50 mg/L ones, the lowest was still as high as 91.67%. However, at 500 mg/L ABT-1, the sprouting percentage dropped significantly to 66.67%.

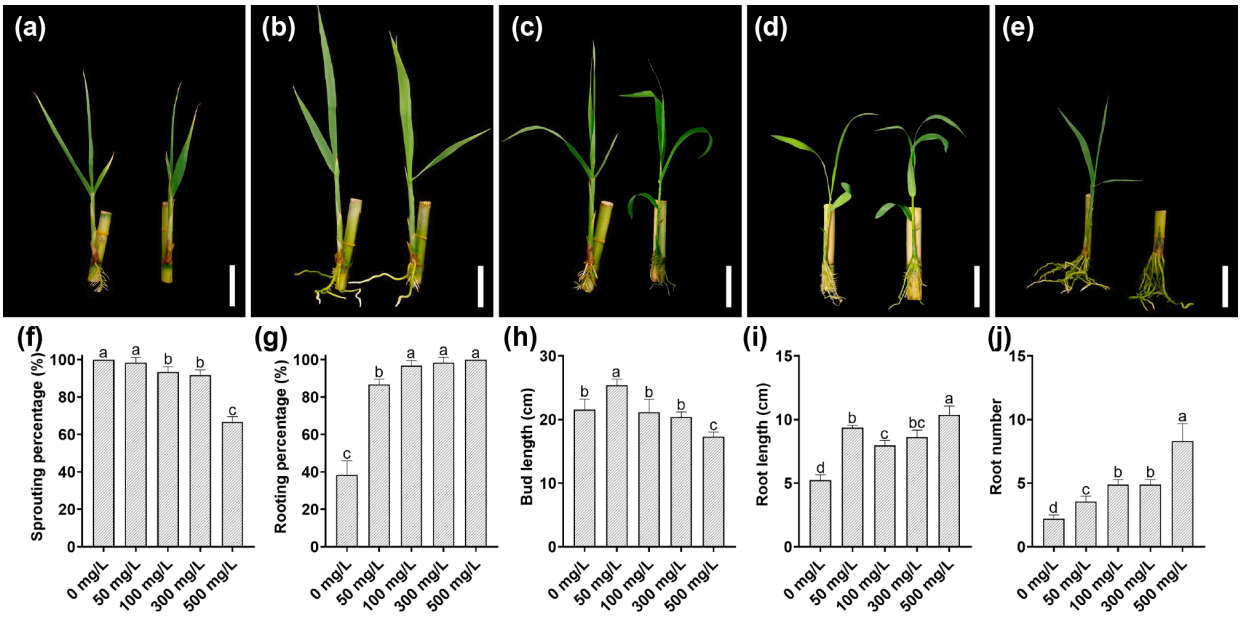


Figure 2. Effects of ABT-1 concentrations on obtaining good seedlings. (**a**–**e**) Morphological characteristics of seedlings obtained from cane fragments treated with 0, 50, 100, 300, and 500 mg/L ABT-1, respectively; (**f**) sprouting percentage; (**g**) rooting percentage; (**h**) bud length; (**i**) root length; (**j**) root number. Bars: 5 cm in (**a**–**e**). The means labeled with the same letter are not significantly different according to the LSD test at $p = 0.05$ in (**f**–**j**).

The rooting percentage increased rapidly from 38.33% (0 mg/L ABT-1) to 86.67% with 50 mg/L rooting powder (Figure 2g). At 100 mg/L ABT-1, the rooting percentage increased significantly to 96.67%. However, there were no significant differences among 100 mg/L, 300 mg/L, and 500 mg/L ABT-1 (rooting percentages were 96.67%, 98.33%, and 100%, respectively).

Without ABT-1, bud lengths of cane fragments averaged 21.53 cm (Figure 2h). Under 50 mg/L ABT-1, bud length significantly increased to 25.40 cm, then decreased with greater ABT-1 concentrations. Bud lengths at 100, 300, and 500 mg/L were 21.16 cm, 20.34 cm, and 17.31 cm, respectively. Lengths did not differ significantly between 100 and 300 mg/L ABT-1, but both were significantly higher than lengths at 500 mg/L and significantly lower than lengths at 50 mg/L. Bud lengths did not vary significantly between the 0, 100, and 300 mg/L ABT-1 conditions.

Untreated cane fragments averaged 2.2 in root number and had a mean root length of 5.23 cm (Figure 2i,j). When ABT-1 was added to the growth solution, both values increased significantly. At 500 mg/L ABT-1, roots averaged 10.37 cm in length and numbered 8.30, significantly higher than values under other treatments. At 50 mg/L, 100 mg/L, and 300 mg/L ABT-1, root lengths were 9.35 cm, 7.96 cm, and 8.62 cm, respectively, while root numbers were 3.56, 4.88, and 4.89. When comparing between pairs of treatments, root length differed significantly only between 50 mg/L and 100 mg/L, while no significant difference was found either between 50 and 300 mg/L or between 100 and 300 mg/L (Figure 2i). Additionally, 50 mg/L ABT-1 yielded significantly fewer roots than both 100 mg/L and 300 mg/L ABT-1 (Figure 2j).

Hence, a growth solution containing 100–300 mg/L rooting powder was optimal for *A. donax* aqua-based culture.

3.2. Effects of Sampling Months on Aqua-Based Culture

Stem morphology varied greatly from month to month in the fast-growing *A. donax*. In order to determine the most suitable stem for aqua-based culture, different materials sampled from various months were aqua-based cultured, and the effects were investigated (Figure 3). When cane fragments were obtained in June, only 25% of the axillary buds sprouted normally and developed into seedlings (Figure 3e). When *A. donax* grew until September, the sprouting percentage significantly increased to 93.33%. We did not observe significant differences in sprouting between September and the following February, but by the following April, the sprouting percentage decreased significantly to 51.67%.

Rooting percentages from plants harvested in June, September, and the following February were 90%, 98.33%, and 95%, respectively (Figure 3f). Percentages did not differ significantly between September and February or between June and February, but the difference between September and June was significant. When growth was allowed until the following April, the rooting percentage decreased to 65%, a significant change from the other three months.

To determine the obtained seedling quality, we measured their bud length, root length, and root number across treatments (Figure 3g–i). Bud and root lengths from September (22.20 cm, 5.63 cm) and the following February (21.16 cm, 6.27 cm) did not differ significantly from each other, but both were significantly higher than the corresponding values in June (12.39 cm, 3.54 cm) and the following April (3.56 cm, 3.06 cm). Root numbers did not change significantly between June (4.92), September (5.22), February (5.19), and April (5.08).

3.3. Effects of Cane Sections on Aqua-Based Culture

The cane of *A. donax* can grow to 6–8 m tall, and different parts of the cane may have different abilities for aqua-based culture. So, we investigated whether the cane's basal, median, and apical parts differed in suitability for aqua-based culture (Figure 4). For the morphology, we found that stem diameter gradually increased from the apical to basal parts (Figure 4a–f). In contrast, axillary buds did not exhibit the same trend. Axillary buds

from the median portion were the largest and fullest (Figure 4d). Basal axillary buds were the smallest but fuller than the moderately sized apical axillary buds.

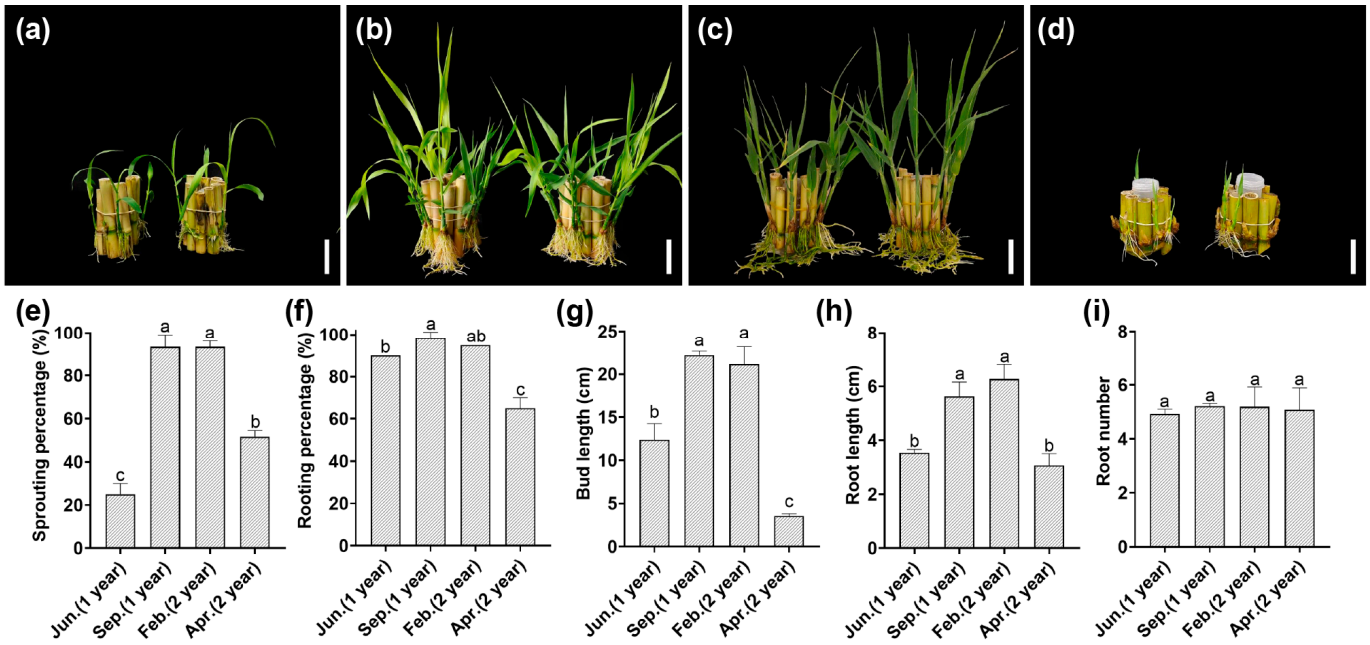


Figure 3. Effects of sampling months on obtaining good seedlings. (**a**–**d**) Morphological characteristics of seedlings from cane fragments sampled in June, September, the following February, and the following April, respectively; (**e**) sprouting percentage; (**f**) rooting percentage; (**g**) bud length; (**h**) root length; (**i**) root number. Bars: 5 cm in (**a**–**d**). The means labeled with the same letter are not significantly different according to the LSD test at $p = 0.05$ in (**e**–**i**).

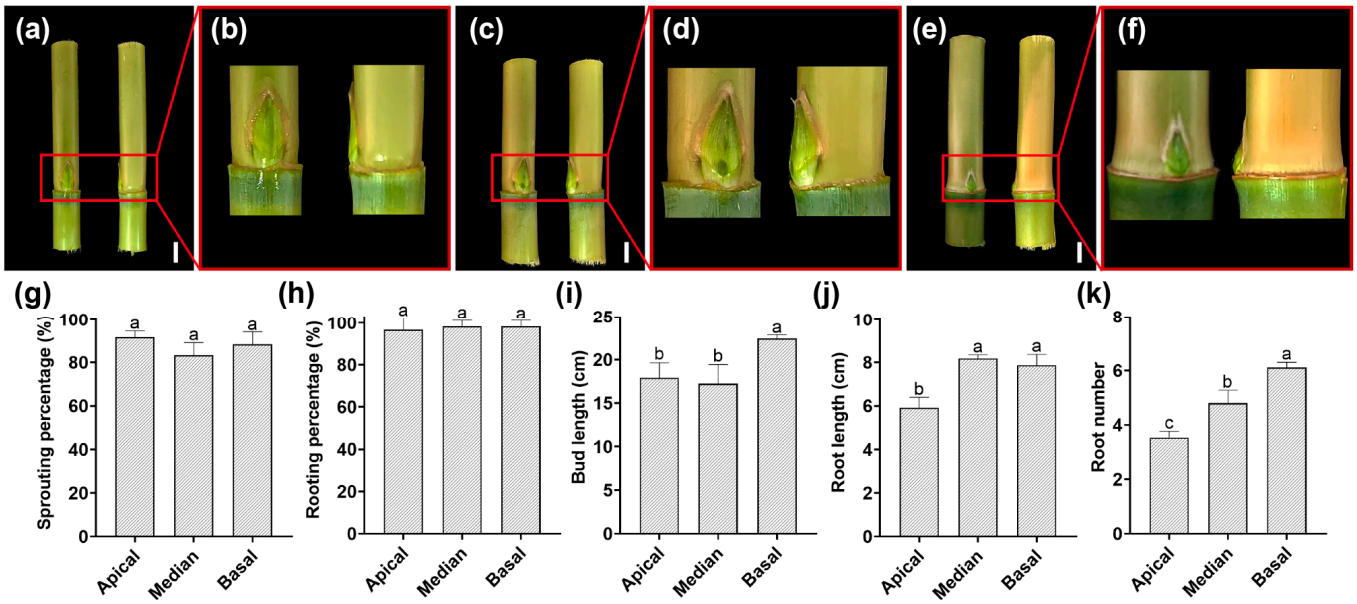


Figure 4. Effects of cane sections on obtaining good seedlings. (**a**,**b**) Cane fragments from the apical section; (**c**,**d**) cane fragments from the median section; (**e**,**f**) cane fragments from the basal section; (**g**) sprouting percentage; (**h**) rooting percentage; (**i**) bud length; (**j**) root length; (**k**) root number. Bars: 1 cm in (**a**,**c**,**e**). The means labeled with the same letter are not significantly different according to the LSD test at $p = 0.05$ in (**g**–**k**).

Sprouting and rooting percentages (average: 87.78% and 97.78%, respectively) did not differ across the three different cane sections (Figure 4g,h). However, bud length, root length, and root number exhibited variations (Figure 4i–k). Apical and median bud lengths averaged 17.91 cm and 17.23 cm, significantly shorter than the average basal bud length (22.44 cm). Median and basal root lengths were not significantly different (8.18 cm and 7.87 cm), but both were significantly greater than apical root length (5.91 cm). Finally, apical, median, and basal root numbers were significantly different (3.53, 4.80, and 6.11, respectively).

3.4. Effects of Growing Media on Cultivation and Acclimation

Aqua-based cultured seedlings were cultivated and acclimated in the growing media before transplantation. We measured the survival rates of seedlings across five different growing media and found that field soil yielded the lowest survival (81.48%) (Table 1). The other four growing media resulted in significantly higher survival (>92.50%) without significant differences. The highest survival rate (98.15%) occurred when using equal amounts of field soil and vermiculite. Overall, aqua-based cultured *A. donax* seedlings did not require a specific growing medium for cultivation and acclimation.

Table 1. Survival rate of seedlings acclimated in different growing media.

Media Composition	Survival Rate (%)
Field soil:Nutritional soil:Vermiculite = 1:0:0	81.48 ± 1.85 [b]
Field soil:Nutritional soil:Vermiculite = 0:1:0	92.59 ± 1.85 [a]
Field soil:Nutritional soil:Vermiculite = 1:1:0	92.59 ± 3.70 [a]
Field soil:Nutritional soil:Vermiculite = 1:0:1	98.15 ± 1.85 [a]
Field soil:Nutritional soil:Vermiculite = 1:1:1	96.29 ± 1.85 [a]

Data are presented as mean ± SD; the means labeled with the same letter are not significantly different according to the LSD test at $p = 0.05$.

4. Discussion

A. donax is a sterile plant, so it can be propagated only agamically by transplanting rhizome fragments or plantlets already endowed with leaves and roots [33,37]. In this study, we developed an extremely rapid and simple aqua-based culture method for harvesting *A. donax* plantlets.

The key to aqua-based cultivation is the successful sprouting and rooting of axillary buds [43–45]. Our experiments revealed that aqua-based cultivation in water only led to an almost 100% sprouting percentage but a 38.33% rooting percentage. The latter is too low for large-scale utilization. However, plant growth regulators can promote rooting [49–51]. In particular, the commercial broad-spectrum rooting powder ABT-1 has been successfully used on cuttings from multiple species, such as *Catalpa bignonioides* [52], *Tamarix chinensis* [53], *Morus alba* [54], and *Podocarpus nagi* [55]. In line with these previous data [51–53], we observed a significant increase in rooting percentage when cane fragments were treated with ABT-1. Even at 50 mg/L of ABT-1, the rooting percentage reached 86.67%. Furthermore, the rise in rooting percentage led to a corresponding increase in root length and number. We can conclude that ABT-1 is an excellent rooting promoter in aqua-based *A. donax* cultivation.

However, ABT-1 had an adverse effect on axillary bud sprouting. At concentrations <300 mg/L, the impact was minimal, but at 500 mg/L, ABT-1 decreased sprouting percentage to 66.67% and bud length to 17.31 cm, significantly lower than values under the no-ABT-1 condition. A similar inhibiting effect of ABT-1 on bud development was also found in the study [51,53]. The possible reason for this phenomenon was that a high concentration of ABT-1 influenced the content and distribution of plant hormones in the axillary bud, as ABT-1 contained 20% NAA and 30% IAA. Thus, when we combined the promoting effect on rooting and the inhibiting effect on budding, we concluded that the best ABT-1 concentration for aqua-based culture was 100–300 mg/L. Since we found no

difference in the seedlings within this range, 100 mg/L was the ideal concentration when considering economic factors.

Aqua-based culture exerted distinct effects on cane fragments depending on the growth month. When plant materials were sampled in September and the following February, sprouting and rooting percentages exceeded 93%, a significant improvement over plants sampled in June and the following April. Seedling quality (bud length, root length, and root number) was also higher in September and February, revealing that cane fragments sampled from September to the following February were more suitable for aqua-based culture. The reason for this outcome is related to the *A. donax* growth cycle (Figure 5). First, March to August is the vegetative growth stage, when axillary buds on underground rhizomes and aboveground stems start to develop. During this stage, canes grow rapidly to several meters in height but have low lignification and relatively less full axillary buds. Next, the reproductive growth stage occurs from September to November. In this period, inflorescences form, and the plants turn from green to yellow, while lignification increases and nutrients accumulate. Because *A. donax* is sterile, the nutrients are not used to form seeds but are instead transferred to axillary buds or rhizomes for development. Lignification and nutrient accumulation peak during the dormancy period (December to February), when temperatures may fall below 0 °C and leaves gradually senesce. In summary, axillary buds had more nutrients during the reproductive growth and dormancy periods, consistent with September to February being the best time to harvest cane fragments for aqua-based culture. The results were consistent with the previous report that nutrient availability of the propagation material played a key role in the rooting development of single-node stem cuttings in *A. donax* [42].

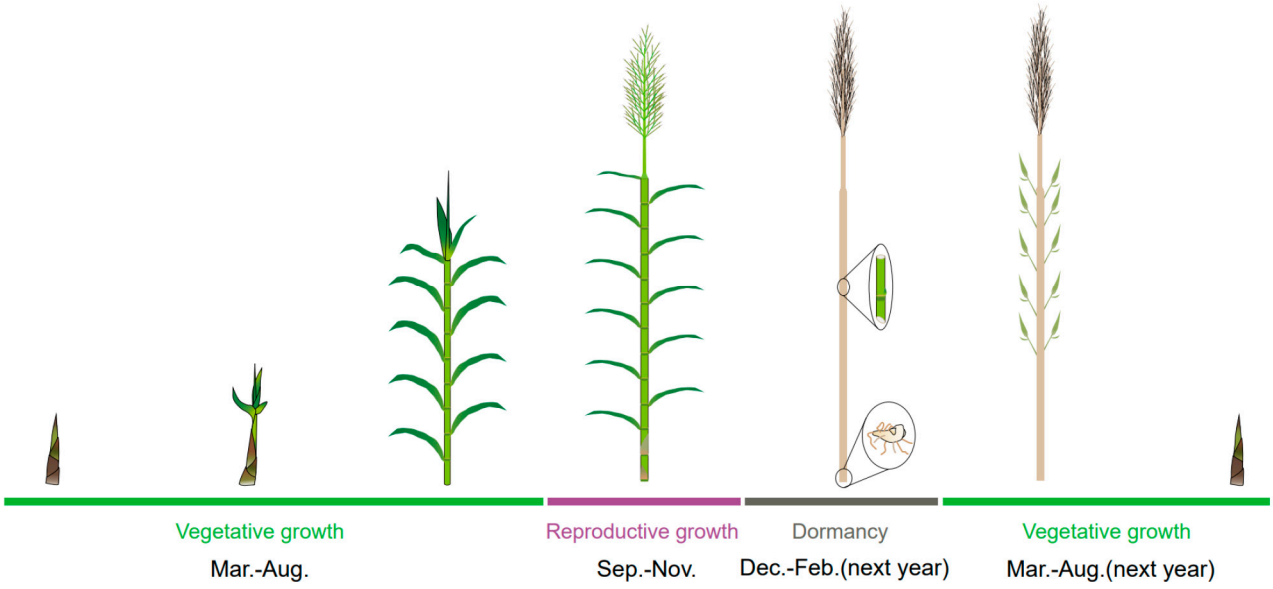


Figure 5. Subsequent growth stages of *Arundo donax*.

When choosing cuttings, we did not find a difference in sprouting and rooting percentages between cane sections, indicating that axillary buds had the same ability to form seedlings regardless of their location on the cane; this was inconsistent with previous results showing that the nodes of basal sections exhibited a better ability to sprout and root when those stem segments were inserted directly into the soil [43,56]. This might be explained by the fact that the rooting ability of axillary buds was improved by the ABT-1 treatment, and the aqua-based culture provided an easier growing environment than traditional stem-cutting. However, seedlings from the basal section were of higher quality than those from the median or apical sections. This outcome aligns with our observation that the basal section was thicker and exhibited more lignification. Such traits reflect the presence of more nutrients for seedling development.

Plants must acclimate before transplantation into a new growing environment [57,58]. In this study, we tested the suitability of five media for acclimation and found that all media except field soil led to a >92.50% survival rate. The presence of vermiculite may explain this outcome, as its inclusion improves soil breathability. Given these data, we determined that *A. donax* seedlings derived from our aqua-based culture method did not have particularly strict soil requirements for cultivation and acclimation.

In this study, each treatment contained 20 cane fragments; further research should use a larger sample size to obtain a more accurate result. In addition, the growth condition and yield of seedlings transplanted into an open field were not analyzed. In a previous study, the biomass yield of stem-cutting propagated plants was significantly lower than that of rhizome-propagated plants in the first harvest year, but the differences were largely mitigated during the following years [43,56].

5. Conclusions

In conclusion, this study established an aqua-based culture method to rapidly develop *A. donax* seedlings. Briefly, using the canes from September to February of the next year as materials, the canes were cut into fragments containing one node with one axillary bud. Subsequently, the cane fragments were first treated with ABT-1 rooting powder at 100 mg/L for 24 h, then cultured in tap water for 19 days. During this entire culture period, the temperature was maintained at 25 ± 2 °C, with a 16–8 h day–night cycle and 8000–10,000 Lx light intensity. This aqua-based method yielded >95% rooting percentage and >93% sprouting percentage within only 20 days. The seedlings obtained were healthy and had high survival rates during cultivation and acclimation with no strict requirement for the growing media. This study provided a theoretical foundation for the large-scale production of high-quality seedlings. We recommend that future studies test the viability of scaling up this aqua-based culturing of *A. donax* and verify its industry application.

Author Contributions: Conceptualization, methodology, and formal analysis: J.G., W.L., F.W., and B.T.; investigation and software: W.L.; resources, data curation, and project administration: G.C., L.Z., Z.X., W.C., and G.S.; visualization and writing—original draft preparation: J.G. and W.L.; writing—review and editing: J.G. All authors have read and agreed to the published version of the manuscript.

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