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In Vitro Shoot Culture of *Sesuvium portulacastrum*: An Important Plant for Phytoremediation

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Abstract: *Sesuvium portulacastrum* L., a member of the family *Aizoaceae*, is an important coastal halophyte. Due to its adaptability to salinity and heavy metals, *S. portulacastrum* has now been widely used for the phytoremediation of saline soils and wastewater and the protection of the coast from erosion. The increasing use of this plant requires a large number of propagules. Stem cutting propagation and seed germination cannot meet this demand, and such propagations can initiate and spread diseases. A recent occurrence of *Bipolaris sesuvii* J.Z. Zhang and *Gibbago trianthemae* E.G. Simmons in *S. portulacastrum* resulted in the substantial loss of the plants during the remediation of aquaculture wastewater. Thus, there is an urgent need for establishing efficient methods of propagating disease-free starting materials. In the present study, we evaluated different growth regulators in the induction of axillary shoots from nodal explants cultured on Murashige and Skoog medium and identified that zeatin (ZT) and α-naphthaleneacetic acid (NAA) was an appropriate combination for inducing high numbers of axillary shoots. The nodal explants were then cultured on MS medium supplemented with different concentrations of ZT and NAA, and the combination of ZT at 1.0 mg L^{-1} and NAA at 0.3 mg L^{-1} induced more than 12 axillary shoots per explant. The axillary shoots were excised to produce microcuttings or microshoots, which were rooted on half-strength MS medium supplemented with different concentrations of indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA). The results showed that IBA at 0.6 mg L^{-1} induced 91.7% of the microcuttings to root with root numbers of over 36 per cutting. The rooted plantlets were healthy and true-to-type and grew vigorously in plug trays or plastic containers with a 100% survey rate in a greenhouse. Thus, this established protocol could be used for the rapid propagation of genetically identical and disease-free plants of *S. portulacastrum* for phytoremediation and the protection of shoreline soils from erosion.

Keywords: axillary shoots; disease-free plants; in vitro rooting; micropropagation; phytoremediation; *Sesuvium portulacastrum* L.; shoot culture

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1. Introduction

Sesuvium portulacastrum L., a member of the family Aizoaceae, is an important halophyte with a chromosome number of $2n = 48$. It is a perennial creeping succulent herb grown in coastal sandy or saline soils in the subtropical and tropical regions; thus, it is commonly known as sea purslane [1–3]. Sea purslane has a thick taproot and many fibrous secondary roots and also produces adventitious roots from the node. Its leaves are evergreen, simple, and succulent, and stems are creeping or prostrate. Sea purslane plants produce small, star-shaped flowers that are white, pinkish purple, or pendent and subsequent capsule fruit containing about 50 seeds. *S. portulacastrum* is a fast-growing plant and can quickly cover the ground or grow in water with trailing vines or internodes that overlap to create dense evergreen floating plant clusters.

A distinct characteristic of *S. portulacastrum* is its adaptability to high saline environments [4]. Sea purslane plants grow well under a sodium (Na) concentration ranging from 100 to 400 mM [5]. In a study conducted regarding the effects of Na and potassium (K) on the growth of *S. portulacastrum*, stem cuttings grown in a modified Hoagland solution [6] supplemented with 200 mM NaCl produced significantly higher biomass than the control, Na content in the leaves reached up to 100 mg g^{-1} dry weight, and the authors concluded that Na was more effective than K for cell expansion, shoot development, and leaf succulence [7].

Sea purslane can also take up a large amount of nitrogen (N) and phosphorus (P) and tolerate heavy metals. A study showed that *S. portulacastrum* plants grown in eutropic marine water on floating beds (60 rooted cuttings per m^2) absorbed 377 g N m^{-2} and 22.9 g P m^{-2} in eight months [8]. Other researchers have reported that *S. portulacastrum* plants tolerate heavy metals [9,10]. *S. portulacastrum* can be used as a phytostablizer in heavy-metal-contaminated coastal environments [11]. Studies have shown that *S. portulacastrum* is highly tolerant to Cd, Cu, Ni, and Zn [11,12]. *S. portulacastrum* plants have been reported to grow in hydroponic solutions containing 50 μM Ni [13], 90 mg L^{-1} Zn [14], or 50 μM to 600 μM Cd [15,16] without a significant reduction in dry weight.

Soil salinization is an important problem that seriously affects plant growth and crop productivity [17]. Environmental pollution, such as eutrophication and soil and water contamination by heavy metals, poses a great threat to the health of living things [18–21]. Phytoremediation is considered the most effective way to minimize these pollutants [22–25]. Due to its tolerance and accumulation of Na and heavy metals and its absorption of N and P, *S. portulacastrum* has become a valuable plant for phytoremediation [1]. It has also been used for stabilizing coastal erosion and the restoration of shorelines [26]. Additionally, organic pollution, such as pesticides and antibiotics, is another ecological and environmental problem in freshwater and coastal water, which represents a challenge to the aquaculture industry. China is a major country in aquaculture and has a total aquaculture area over 7.83 million hectares [27]. This large-scale aquaculture has caused severe water pollution problems including enriched N, P, pesticides, and antibiotics in the water. There is an urgent need for the remediation of the wastewater from aquaculture [28].

Today, biological floating-bed technology has been increasingly applied to aquaculture ponds and waterbodies [29] for phytoremediation. *S. portulacastrum* has been used to construct floating beds for improving water quality in the aquaculture and mariculture industry (Figure 1A). Due to the expansion of aquaculture and the increased use of *S. portulacastrum* for stabilizing shoreline erosion (Figure 1B), there is a great demand for *S. portulacastrum* plants. Sea purslane can be propagated by seeds, but seed production is limited [1]; furthermore, there is no commercial supply of seeds. Additionally, the plants propagated from seeds may not be true-to-type, as sea purslane is an open-pollinated plant. As a result, sea purslane is commonly propagated by stem cuttings. Cutting propagation, however, requires a large number of stock plants and can often initiate and spread disease, resulting in large-scale infections and deaths (Figure 1C,D). A new species of *Bipolaris* known as *P. sesuvii* has been reported to infect sea purslane plants in Guangdong Province [30], and it has recently been identified that *Gibbago trianthemae* can heavily infect sea purslane in Fujian Province, China (data not shown).

Micropropagation is the most effective method for achieving a rapid increase in propagules on a year-round basis [31]. It includes the culture of preexisting meristems and regeneration of plants through organogenesis or somatic embryogenesis. Indirect organogenesis and indirect somatic embryogenesis through the callus phase may produce plantlets with somaclonal variation [31,32]. As a consequence, propagation from preexisting meristems, such as shoots or nodes is the most reliable method, as it produces genetically identical plants without somaclonal variation [33,34]. Thus far, there has only been one report on the micropropagation of *S. Portulacastrum* [35], where nodal explants were cultured on Murashige and Skoog (MS) medium [36] supplemented with different concentrations of BA (6-benzylaminopurine), Kin (kinetin), TDZ or thidiazuron (N-phenyl-

N′-1,2,3-thidiazol-5-ylurea), and 2iP (6-(γ, γ-dimethylallylamino)purine). BA at 20 µM and 2iP at 40 µM induced an average of 4.95 and 5.2 axillary shoots, respectively, which were considered the highest induction rates compared to the other growth regulators. However, callus and extensive callus were induced at the aforementioned 2iP and BA concentrations, respectively. As a result, there were two complications with this reported protocol: one being the low induction rates of the axillary shoots and the other being the formation of callus. Callus may lead to the regeneration of adventitious shoots, which may produce somaclonal variants, resulting in genetic infidelity [34].

The objective of the present study was to establish an efficient and rapid protocol for the micropropagation of *S. Portulacastrum* through existing meristems (node cultures) to provide the aquaculture industry with disease-free, true-to-type, and high-quality plants for phytoremediation.

Figure 1. *Sesuvium portulacastrum* plants were used for remediation of pollutants in aquaculture (**A**) and stabilization of shoreline soil (**B**) in Fujian, China. The occurrence of disease caused by *Gibbago trianthemae* in *S. portulacastrum* grown in coastal soil (**C**) and during cutting propagation (**D**). All the photos were taken by Xiangying Wei.

2. Materials and Methods

2.1. Plant Materials

Fresh mature seeds were collected from coastal-grown *S. portulacastrum* plants in Fujian Province, China. The seeds were washed in sterile distilled water three times and surface-disinfected with 75% (*v*/*v*) alcohol solution for 1 min, followed by immersion in 2% sodium hypochlorite solution for 20 min with occasional agitation, and finally were washed 5 times with sterile distilled water. MS medium [36] (PhytoTechnology, Shawnee Mission, KS, USA) was supplemented with 30 g L^{-1} sucrose (Morebetter, Hangzhou, China) and 8 g L^{-1} agar (Solarbio Life Sci., Beijing, China). After the pH was adjusted to 5.8, the medium was autoclaved at 121 °C for 25 min. Autoclaved medium was poured into 250 mL glass culture vessels (Shanghai Zeshine Equipment Co., Shanghai, China), 20 mL each. The disinfected seeds were inoculated on the MS medium in the glass culture vessels, which were placed on the shelves of a growth chamber (PGX, Saifu Experimental Apparatus Tech. Co., Ltd., Ningbo, China) where the temperature was set at 25 °C, relative humidity was adjusted to 70%, and the photoperiod was programmed to 16 h of light, provided by fluorescent lamps at a light level of 25 µmol m^{-2} $s^{-1,}$ and 8 h of dark. The seeds began to germinate and produced two leaves in two weeks. Germinated seedlings were transferred

to culture vessels containing the same MS medium for continuous growth in the same environment for about four months.

2.2. Evaluation of Growth Regulator Combinations for Axillary Shoot Induction

To identify appropriate combinations of cytokinin and auxin for inducing axillary shoots, the nodal explants were cultured on the aforementioned MS medium containing nine growth regulator combinations with no growth regulator as a control (Table 1). The MS medium, after supplementation with 30 g L^{-1} sucrose and 8 g L^{-1} agar and adjustment of the pH to 5.8, was aliquoted into 10 bottles, 100 mL each, which were autoclaved at 121 °C for 25 min. Three cytokinins, BA, zeatin or ZT (6-(4-hydroxy-3-methylbut-2-enylamino) purine, and TDZ, and four auxins, 2,4-D (2,4-dichlorophenoxyacetic acid), IBA (indole-3-butyric acid), IAA (indole-3-acetic acid), and NAA (1-naphthaleneacetic acid), were sterilized by filtration through 0.2 µm membranes (Thermo Fisher Scientific, Waltham, MA, USA), respectively. After the medium was cooled to about 50 °C, the sterilized auxins and cytokinins were added to the respective bottles, resulting in nine growth regulator combinations plus a control listed in Table 1. Nodal explants (about 15 mm in length with three nodes and no leaves) derived from the abovementioned seedlings were cultured on the media, four per culture vessel. The experiment was arranged as a completely randomized design with three replications. Axillary bud number per explant was recorded after 56 days of culture, and plants were photographed.

Table 1. Axillary shoots induced from nodal explants of *S. portulacastrum* after culturing on MS medium supplemented with different growth regulators for eight weeks.

Media No.	Growth Regulator Concentrations	Number of Axillary Shoots [z]
MS 1	2.0 mg L^{-1} BA + 0.3 mg L^{-1} IBA	5.27 ± 0.42 bc
MS 2	1.0 mg L^{-1} BA + 0.5 mg L^{-1} IAA	5.60 ± 1.20 b
MS 3	2.0 mg L^{-1} 6-BA + 0.5 mg L^{-1} NAA	3.79 ± 0.27 cd
MS 4	0.2 mg L^{-1} 6-BA + 1.0 mg L^{-1} 2,4-D	3.00 ± 0.44 d
MS 5	0.4 mg L^{-1} ZT + 1.0 mg L^{-1} IAA	4.31 ± 0.74 bcd
MS 6	2.0 mg L^{-1} ZT + 1.0 mg L^{-1} IBA	5.12 ± 0.29 bc
MS 7	2.0 mg L^{-1} ZT + 0.2 mg L^{-1} NAA	7.41 ± 0.53 a
MS 8	0.1 mg L^{-1} NAA + 3.0 mg L^{-1} BA + 1.0 mg L^{-1} ZT	4.19 ± 0.43 bcd
MS 9	2.0 mg L^{-1} TDZ + 0.1 mg L^{-1} NAA	4.12 ± 0.31 bcd
MS 10	No growth regulators	1.21 ± 0.24 e

[z] Different letters within the column indicate significant differences among treatments based on LSD analysis at $p < 0.05$ level.

2.3. Optimization of Axillary Shoot Induction

The above experiment showed that ZT in combination with NAA induced the highest number of axillary shoots without callus formation. In order to optimize axillary shoot induction, the same MS medium was used to formulate 20 combinations of ZT with NAA at different concentrations, which were used for evaluation of the rate of axillary shoot induction. Additional nodal explants were cultured on MS medium supplemented with 2 mg L^{-1} ZT and 0.2 mg L^{-1} NAA for about 60 days to produce a large number of axillary shoots. Nodal explants (three nodes of about 15 mm) were then cultured on the 20 media. There were four nodal explants per culture vessel. The experiment had a completely randomized design with three replications. The culture was maintained in the abovementioned growth chamber. The culture was monitored weekly and photographed, and the number of axillary shoots per explant was scored after 90 days.

2.4. In Vitro Rooting and Acclimatization

Axillary shoots were excised from explants cultured on the medium containing 1 mg L^{-1} ZT and 0.3 mg L^{-1} NAA, and the resultant microcuttings were cultured on half-strength MS medium supplemented with either IBA or IAA, four concentrations each.

There were four microcuttings per culture vessel, and the experiment had a completely randomized design with three replications. After 24 days of culture, the number of microcuttings with roots, root numbers, and root lengths were recorded. The percentages of rooting per culture vessel and mean root length per microcutting were calculated.

Plantlets from the in vitro rooting were washed to remove agar and transferred to plug trays containing a substrate comprised of 40% peat, 20% pine bark, 20% perlite, and 20% sand based on volume (Zhonghe Agriculture, Huaian, China). The plug trays were covered with transparent plastic film to reduce evaporation and placed on the benches of a shaded and evaporative-pad-cooling greenhouse with a temperature ranging from 20 °C to 28 °C, a relative humidity varying from 50% to 80%, and a light intensity of about 400 µmol m^{-2} s^{-1}. One month after the plantlets became adapted to the environment, the plastic film was removed. The plants in the plug trays were watered with half-strength Hoagland nutrient solution every 15 days for two months, and then transplanted into 10 cm square plastic pots containing the same substrate for an additional two months. The survey rate of ex vitro grown plants was recorded each month for up to four months.

2.5. DNA Flow Cytometry Analysis of Plants Derived from Shoot Culture

Young leaves were collected from three randomly selected plants derived from the shoot culture or three original plants initially used for the shoot culture and analyzed for possible occurrence of somaclonal variants at ploidy levels [37]. Tomato leaves were used as an internal reference standard. The analysis was conducted by the Molecular Biology Experiment Center, Germplasm Bank of Wild Species in Southwest China (Kunming, China). The DNA histograms of nuclei from parental plants and shoot-cultured plants were compared.

2.6. Data Collection and Statistics Analysis

Data were examined for normality and homogeneity of variance using SPPS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). The data were subjected to analysis of variance (ANOVA) using SPPS. If significance occurred, mean differences among treatments were separated by the least significant difference (LSD) test at $p < 0.05$ level. All data were presented as means ± standard errors with $n = 3$.

3. Results and Discussion

3.1. Effect of Growth Regulator Combinations on Axillary Shoot Induction

Axillary shoots were induced from the nodes of *S. portulacastrum* explants cultured on MS medium supplemented with different growth regulator combinations (Figure 2). The average number of axillary shoots per explant induced by the growth regulators ranged from 3.0 to 7.4 but was only 1.21 without the growth regulators (Table 1). When the explants were cultured on MS medium containing BA with either IBA, IAA, or NAA, the average number of axillary shoots differed from 3.79 to 5.60, and callus was observed on MS 2-6 and MS 8-9. BA with 2,4-D (MS 4) induced abundant callus at the base of the stem segment, resulting in the lowest number of axillary shoots. The axillary shoots induced on MS 1 exhibited a reddish color (Figure 2). TDZ, as a synthetic phenyl urea derivative possessing strong cytokinin-like activity [38], only induced 4.12 axillary shoots. When the nodal explants were cultured on MS medium supplemented with ZT and IBA, IAA, or NAA, the average number of axillary shoots ranged from 4.31 to 7.41. ZT at 2 mg L^{-1} with 0.2 mg L^{-1} NAA induced the highest number of axillary shoots (7.4), which was 42.5% higher than the number of 5.2 induced by 40 µM 2iP, reported by Lokhande et al. [35]. Furthermore, no callus appeared at the bases of the explants.

Our results showed that among the growth regulators tested, ZT with NAA was the most effective combination for inducing axillary shoots, with shoot lengths ranging from 10 to 20 mm. Although we did not test 2iP, which was reported by Lokhande et al. [35], we did evaluate BA and TDZ; the number of axillary shoots induced by ZT and NAA was much higher than the 5.2 induced by 2iP. As a result, ZT with NAA was considered to

be the optimal cytokinin and auxin combination for the induction of axillary shoots from *S. portulacastrum*.

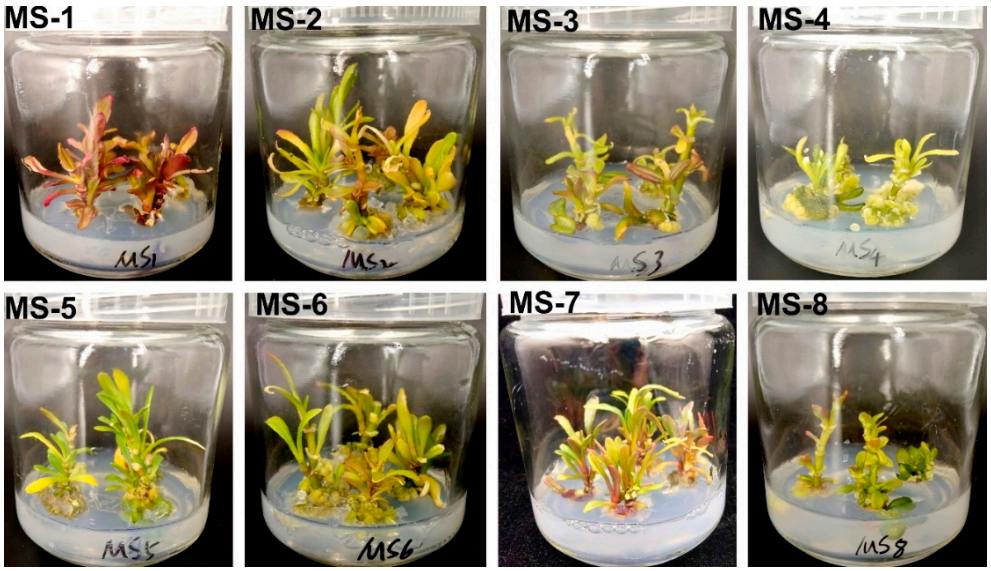

Figure 2. Effect of different growth regulator combinations (see Table 1) on induction of axillary shoots from nodal explants of *S. portulacastrum* cultured on MS medium. The photos were taken after eight weeks of culture (photos for MS 9 and MS 10 are not shown).

3.2. Optimization of Shoot Multiplication

To identify the optimal concentration for inducing axillary shoots, the nodal explants were cultured on 20 combinations of ZT and NAA. Axillary shoots appeared from the node of the explants after two weeks of culture (Figure 3). With the continuation of the culture, more axillary shoots were induced. The number of axillary shoots ranged from 6.44 to 12.11 per explant (Table 2). At the ZT concentration of 0.5 mg L^{-1}, increased concentrations of NAA did not significantly change the number of axillary shoots. However, at the ZT concentration of 1.0 mg L^{-1}, increased NAA concentrations from 0.05 mg L^{-1} to 0.3 mg L^{-1} resulted in a significant increase in axillary shoots from 8.89 to 12.11, but a further increase of NAA to 0.4 mg L^{-1} caused a reduction in axillary shoot numbers. At the ZT concentration of 2.0 mg L^{-1}, the number of axillary shoots induced by 0.2 mg L^{-1} NAA L^{-1} was significantly higher than that induced by the NAA concentrations of 0.3 mg L^{-1} to 0.4 mg L^{-1}. There was no significant difference in the axillary shoot numbers when the explants were induced by 3.0 mg L^{-1} ZT with either concentration of NAA (Table 2).

The optimization experiment showed that ZT at 1.0 mg L^{-1} and 0.3 mg L^{-1} NAA were the most effective concentrations for inducing axillary shoots from nodal explants of *S. portulacastrum*, since more than 12 shoots were produced (Table 2) and the shoot lengths varied from 15 to 32 mm. As reported by Fay and Gratton [39], there was little information available on the micropropagation of plants in the family Aizoaceae. Thus far, there has been only one report on the in vitro culture of *S. portulacastrum* [35]. In said study, ZT was not used, and the authors found that 2iP, not BA and TDZ, was more effective in axillary shoot induction. The present study showed that ZT at 1.0 mg L^{-1} with 0.3 mg L^{-1} NAA induced 12.11 axillary shoots per nodal explant compared to 5.2 induced by 2iP. It is known that plant species, growth regulators, and even genotypes and explant sources significantly affect in vitro culture [35,40,41]. Lokhande et al. [35] showed that clones of *S. portulacastrum* collected from different geographical regions in India responded differently to axillary shoot induction. Nevertheless, our results are consistent with those reported by Fan et al. [42] on the shoot culture of blueberry and Wei et al. [33] on the shoot culture of *Rhododendron fortune*, where ZT was more effective in the induction of axillary shoots than BA. Similarly, ZT was reported to induce more shoots from the explants of

raspberry [43] and olive [44] than BA. The effectiveness of ZT may imply that ZT, as a native plant growth hormone in the cytokinin family, is more compatible than the synthetic cytokinins with the other native growth hormones in *S. portulacastrum* for multiplication. Further studies are needed to test this proposition.

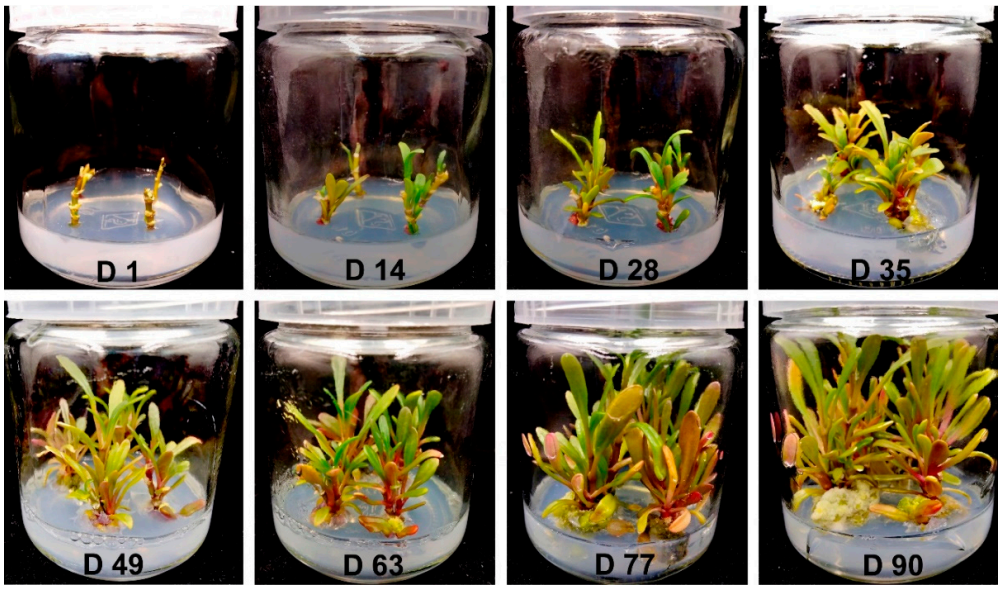

Figure 3. Axillary shoots induced from nodal explants of *S. portulacastrum* cultured on MS medium supplemented with 1.0 mg L^{-1} ZT and 0.3 mg L^{-1} NAA from day one (D 1) to day 90 (D 90).

Table 2. Effect of different concentrations of ZT and NAA on induction of axillary shoots from nodal explants of *S. portulacastrum* cultured on 20 shoot induction media.

Media No.	ZT (mg L^{-1})	NAA (mg L^{-1})	Number of Axillary Shoots [z]
SMS 1	0.5	0.05	8.89 ± 1.05 cdef
SMS 2	0.5	0.1	9.11 ± 0.92 bcdef
SMS 3	0.5	0.2	8.11 ± 0.70 defg
SMS 4	0.5	0.3	8.00 ± 0.76 defg
SMS 5	0.5	0.4	8.56 ± 0.67 cdef
SMS 6	1.0	0.05	8.89 ± 0.48 cdef
SMS 7	1.0	0.1	9.44 ± 0.82 bcde
SMS 8	1.0	0.2	10.07 ± 0.96 abc
SMS 9	1.0	0.3	12.11 ± 0.59 a
SMS 10	1.0	0.4	9.56 ± 0.38 bcd
SMS 11	2.0	0.05	10.00 ± 1.18 abc
SMS 12	2.0	0.1	9.11 ± 0.56 bcdef
SMS 13	2.0	0.2	9.00 ± 0.55 cdef
SMS 14	2.0	0.3	7.44 ± 0.53 efg
SMS 15	2.0	0.4	7.67 ± 0.55 efg
SMS 16	3.0	0.05	7.89 ± 0.90 defg
SMS 17	3.0	0.1	7.33 ± 0.81 fg
SMS 18	3.0	0.2	7.11 ± 0.39 fg
SMS 19	3.0	0.3	8.11 ± 0.48 defg
SMS 20	3.0	0.4	6.44 ± 0.34 g

[z] Different letters within the column indicate significant differences among treatments based on LSD analysis at $p < 0.05$ level.

It is noteworthy that callus occurred in the bases of the nodal explants on day 77 (Figure 3, D 77) and more callus appeared on day 90 (Figure 3, D 90) when nodal explants were induced by 1.0 mg L^{-1} ZT and 0.3 mg L^{-1} NAA. Since there is a possibility of adventitious shoot regeneration from the callus [45], and shoots derived from indirect

organogenesis have the potential to produce somaclonal variants [32,34], it is strongly advised that axillary shoots induced by 1.0 mg L^{-1} ZT and 0.3 mg L^{-1} NAA should be excised before day 77 to avoid any adventitious shoots regenerated from the callus.

3.3. Rooting of Microcuttings

The induced axillary shoots were excised from the nodal explants, and the resultant microcuttings were rooted on half-strength MS medium supplemented with four concentrations of IBA or IAA. The percentages of adventitious root occurrence varied with the IAA or IBA concentrations (Figure 4). IBA at 0.6 mg L^{-1} induced 91.7% of the microcuttings to root, and each cutting produced 36 roots with a mean root length of 1.0 cm (Table 3). On the other hand, the highest rooting percentage induced by IAA was 66.7%, and the root numbers were only 15, which were significantly lower than those induced by 0.6 mg L^{-1} IBA. In the report of Lokhande et al. [35], microcuttings of *S. portulacastrum* were rooted in four concentrations of NAA or IAA. NAA at 10 µM induced 95% of the microcuttings to form adventitious roots compared to 85% induced by 5.0 µM IAA [35].

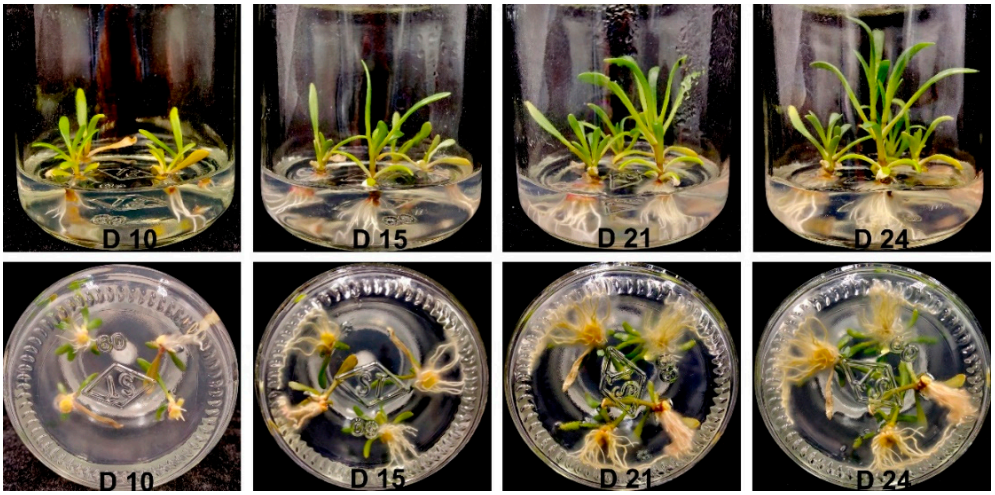

Figure 4. Microcuttings of *S. portulacastrum* rooted on half-strength MS medium supplemented with 0.6 mg L^{-1} IBA from day 10 (D 10) to day 24 (D 24) (upper panel). The lower panel shows the increase in root numbers viewed from the bottom of culture vessels.

Table 3. Effect of half-strength MS supplemented with different concentrations of IBA or IAA on in vitro rooting of *S. portulacastrum* over 24 days.

Media No.	IBA (mg L^{-1})	IAA (mgL^{-1})	% of Microcuttings Rooted [z]	Number of Roots	Mean Root Length (cm)
RMS 1	0.2	-	75.0 ± 4.43 abc	18 ± 2.33 cd	1.1 ± 0.13 a
RMS 2	0.4	-	83.3 ± 8.33 ab	17 ± 0.33 c	1.0 ± 0.14 a
RMS 3	0.6	-	91.7 ± 8.03 a	36 ± 3.79 a	1.0 ± 0.10 ab
RMS 4	0.8	-	33.3 ± 8.12 de	25 ± 2.08 b	0.7 ± 0.08 bc
RMS 5	-	0.2	66.7 ± 8.04 abc	15 ± 0.88 c	1.0 ± 0.16 a
RMS 6	-	0.4	58.7 ± 4.40 bcd	9 ± 2.40 de	0.9 ± 0.14 abc
RMS 7	-	0.6	50.0 ± 3.86 cd	4 ± 1.76 e	0.8 ± 0.13 abc
RMS 8	-	0.8	16.7 ± 4.05 e	3 ± 0.88 e	0.6 ± 0.05 c

[z] Different letters within the column indicate significant differences among means based on LSD analysis at $p < 0.05$ level.

The auxins of IAA, IBA, and NAA are commonly used for inducing adventitious roots. IAA, as the native hormone of plants, was the first to be used to promote the rooting of cuttings, and IBA and NAA are synthesized forms of auxins. IBA was found to be more effective than IAA in the induction of adventitious roots [46]. Our data, along with those

of Fan et al. [42] and Wei et al. [33], support the claim that IBA is generally more effective than IAA in promoting the rooting of microcuttings. Furthermore, the application of IBA substantially increases the root numbers of microcuttings.

3.4. Ex Vitro Rooting and Acclimatization

The in vitro rooted plants (Figure 4), after being transplanted into cells of plastic plug tray filled with the aforementioned substrate, were established in one month (Figure 5 upper panel). The plants in the plug trays had a survey rate of 100% after two months of acclimatization (Figure 5, middle panel). The liners (individual young plants derived from tissue culture or seed germination grown in cell plug trays with well-developed root systems) were transplanted into 10-cm square plastic pots filled with the same substrate for further acclimatization in the greenhouse. After two months of growth in the square pots, the plants were healthy, disease-free, and grew vigorously (Figure 5, bottom panel). These plants were either allowed to grow into stock plants for producing cuttings or transplanted to seashore soils for beach restoration, floating beds for the phytoremediation of wastewater, or saline soils for reducing salinization.

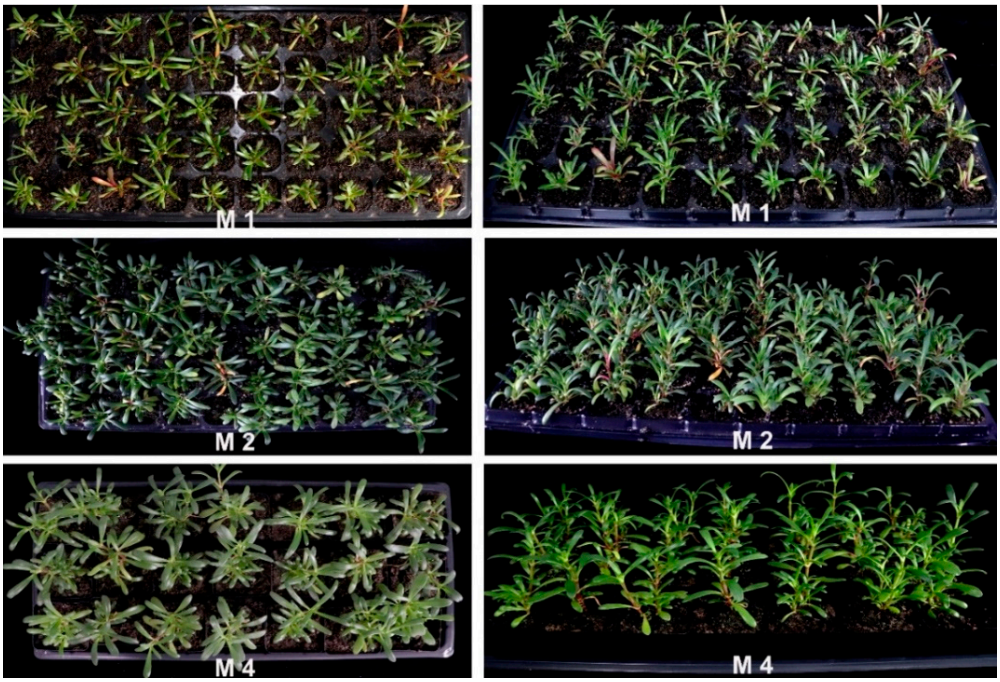

Figure 5. Acclimatization of plants in a greenhouse where in vitro rooted plants were transplanted into a cell plug tray filled with a substrate and grown in the greenhouse for one month (**upper** panel, M1 top view and side view, respectively). **Middle** panel: a top view and side view of plants after two months of growth in a plug tray. **Bottom** panel: a top view and side view of plants grown in 10 cm square pots at month four in the greenhouse.

3.5. DNA Flow Cytometry Analysis of Plants Derived from Shoot Culture

Flow cytometry analysis showed that the plants derived from the shoot culture (Figure 6A) had the same peaks as the original *S. portulacastrum* plants initially used for establishing the shoot culture protocol (Figure 6B). The first peak was the internal reference of tomato plants, and the next peak with the count over 120 was derived from *S. portulacastrum*. There were two additional small peaks, which were probably related to the chromosome characteristics, as type A, C, and D chromosomes were common in *S. portulacastrum* [47]. Regardless, the number of peaks and their positions in the histograms between the original and the shoot-cultured plants were identical, suggesting that there was no ploidy variation in the plants derived from the nodal culture. Additionally, no morphologically different plants were observed among the plants derived from the shoot culture. These

results were as expected, as in vitro culture using existing meristems generally produces true-to-type plantlets [34,38]. Our results were also consistent with the report of Thiem and Sliwinska [48], where the shoot culture of *Rubus chamaemorus* produced plantlets without chromosome variation.

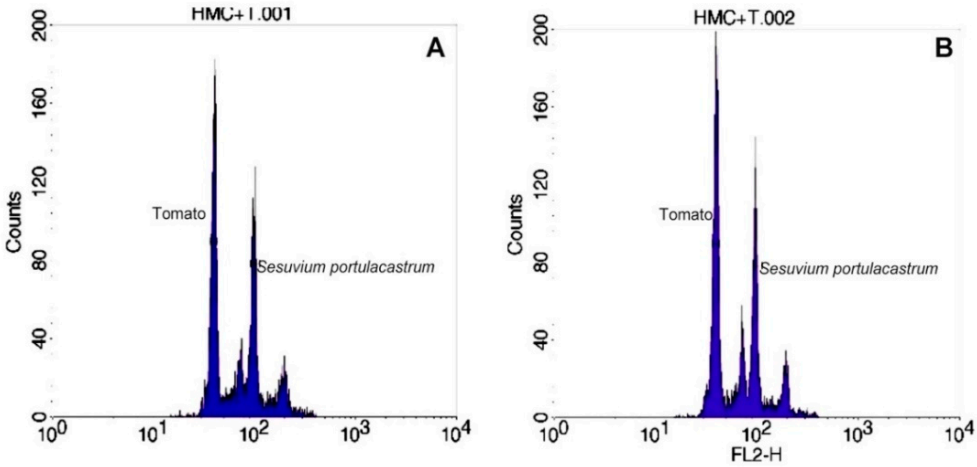

Figure 6. Histograms of relative fluorescence intensity of plants derived from the shoot culture (**A**) and the original *S. portulacastrum* plants (**B**).

4. Conclusions

Sesuvium portulacastrum has been used for the restoration of shorelines [49] and the desalination of saline soils [50]. Recently, it has been recognized as an important plant for the remediation of N, P, and antibiotics in aquaculture ponds or waterbodies and of heavy metals in wastewater [1,51]. As a result, there has been an increasing demand for propagules of *S. portulacastrum* used for phytoremediation. This study developed an efficient and simple protocol for propagating *S. portulacastrum* through nodal culture. The in vitro culture of a nodal explant on MS medium supplemented 1.0 mg L^{-1} ZT and 0.3 mg L^{-1} NAA induced an average of 12.11 axillary shoots. The microcuttings derived from the axillary shoots were easily rooted in vitro in 24 days, and the in vitro rooted plants were acclimatized in a greenhouse with a 100% survival rate. Furthermore, the plants were stable, without variation in ploidy levels. As the axillary shoots were induced in vitro under aseptic conditions, they were disease-free. The healthy and uniform plants could be readily transplanted to seashore soils for beach restoration, floating beds for the phytoremediation of wastewater, or saline soils for reducing salinization.

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References

1. Lokhande, V.H.; Gor, B.K.; Desai, N.S.; Nikam, T.D.; Suprasanna, P. *Sesuvium portulacastrum*, a plant for drought, salt stress, sand fixation, food, and phytoremediation. A review. *Agron. Sustain. Dev.* **2013**, *33*, 329–348. [CrossRef]
2. Peng, C.; Chang, L.; Yang, Q.; Tong, Z.; Wang, D.; Tan, Y.; Sun, Y.; Yi, X.; Ding, G.; Xiao, J.; et al. Comparative physiological and proteomic analyses of the chloroplasts in halophyte *Sesuvium portulacastrum* under differential salt conditions. *J. Plant Physiol.* **2019**, *232*, 141–150. [CrossRef] [PubMed]
3. Li, R.; Wei, X.; Wang, Y.; Zhang, Y. The complete mitochondrial genome of a mangrove associated plant: *Sesuvium portulacastrum* and its phylogenetic implications. *Mitochondrial DNA Part B* **2020**, *5*, 3112–3113. [CrossRef] [PubMed]
4. Ghnaya, T.; Nouairi, I.; Slama, I.; Messedi, D.; Grignon, C.; Abdelly, C.; Ghorbel, M.H. Cadmium effects on growth and mineral nutrition of two halophytes: *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum*. *J. Plant Physiol.* **2005**, *162*, 1133–1140. [CrossRef] [PubMed]
5. Messedi, D.; Labidi, N.; Grignon, C.; Abdelly, C. Limits imposed by salt to the growth of the halophyte *Sesuvium portulacastrum*. *J. Plant Nutr. Soil Sci.* **2004**, *167*, 720–725. [CrossRef]
6. Hoagland, D.R.; Snyder, W.C. "Nutrition of strawberry plant under controlled conditions. (a) Effects of deficiencies of boron and certain other elements, (b) susceptibility to injury from sodium salts". *Proc. Amer. Soc. Hort. Sci.* **1933**, *30*, 288–294.
7. Wang, D.; Wang, H.; Han, B.; Wang, B.; Guo, A.; Zheng, D.; Liu, C.; Chang, L.; Peng, M.; Wang, X. Sodium instead of potassium and chloride is an important macronutrient to improve leaf succulence and shoot development for halophyte *Sesuvium portulacastrum*. *Plant Physiol. Biochem.* **2012**, *51*, 53–62. [CrossRef] [PubMed]
8. Liu, X. Model assessment of nutrient removal via planting *Sesuvium portulacastrum* in floating beds in eutrophic marine waters: The case of aquaculture areas of Dongshan Bay. *Acta Oceanol. Sin.* **2019**, *38*, 91–100. [CrossRef]
9. Zaier, H.; Ghnaya, T.; Lakhdar, A.; Baioui, R.; Ghabriche, R.; Mnasri, M.; Sghair, S.; Lutts, S.; Abdelly, C. Comparative study of Pb-phytoextraction potential in *Sesuvium portulacastrum* and *Brassica juncea*: Tolerance and accumulation. *J. Hazard Mater.* **2010**, *183*, 609–615. [CrossRef] [PubMed]
10. Fourati, E.; Vogel-Mikuš, K.; Bettaieb, T.; Kavčič, A.; Kelemen, M.; Vavpetič, P.; Pelicon, P.; Abdelly, C.; Ghnaya, T. Physiological response and mineral elements accumulation pattern in *Sesuvium portulacastrum* L. subjected in vitro to nickel. *Chemosphere* **2018**, *219*, 463–471. [CrossRef] [PubMed]
11. Feng, J.; Lin, Y.; Yang, Y.; Shen, Q.; Li, Z. Tolerance and bioaccumulation of Cd and Cu in *Sesuvium portulacastrum*. *Ecotoxicol Environ. Saf.* **2017**, *147*, 306–312. [CrossRef]
12. Feng, J.; Lin, Y.; Yang, Y.; Shen, Q.; Huang, J.; Wang, S.; Zhu, X.; Li, Z. Tolerance and bioaccumulation of combined copper, zinc, and cadmium in *Sesuvium portulacastrum*. *Mar. Pollut. Bull.* **2018**, *131*, 416–421. [CrossRef] [PubMed]
13. Fourati, E.; Vogel-Mikuš, K.; Wali, M.; Kavčič, A.; Gomilšek, J.P.; Kodre, A.; Kelemen, M.; Vavpetič, P.; Pelicon, P.; Abdelly, C.; et al. Nickel tolerance and toxicity mechanisms in the halophyte *Sesuvium portulacastrum* L. as revealed by Ni localization and ligand environment studies. *Environ. Sci. Pollut. Res. Int.* **2019**, *27*, 23402–23410. [CrossRef]
14. Lin, Y.; Gao, S.; Chen, J.; Zhai, Z.; Yuan, X.; Huang, J.; Liu, W.; Li, Z. Tolerance and its zinc bioaccumulation characteristic of *Sesuvium portulacastrum* to zinc (In Chinese). *Wetl. Sci.* **2016**, *14*, 561–567.
15. Uddin, M.M.; Chen, Z.; Huang, L. Cadmium accumulation, subcellular distribution and chemical fractionation in hydroponically grown *Sesuvium portulacastrum* [*Aizoaceae*]. *PLoS ONE* **2020**, *15*, e0244085. [CrossRef] [PubMed]
16. Wali, M.; Gunsè, B.; Llugany, M.; Corrales, I.; Abdelly, C.; Poschenrieder, C.; Ghnaya, T. High salinity helps the halophyte *Sesuvium portulacastrum* in defense against Cd toxicity by maintaining redox balance and photosynthesis. *Planta* **2016**, *244*, 333–346. [CrossRef] [PubMed]
17. Zelm, E.V.; Zhang, Y.; Testerink, C. Salt tolerance mechanisms of plants. *Annu. Rev. Plant Biol.* **2020**, *71*, 403–433. [CrossRef]
18. Lee, K.; Han, C.; Hong, S.B.; Jun, S.J.; Hong, S. A 300-year high-resolution greenland ice record of large-scale atmospheric pollution by arsenic in the northern hemisphere. *Environ. Sci. Technol.* **2019**, *53*, 12999–13008. [CrossRef]
19. Zhou, J.F.; Guo, W.D.; Deng, X.; Zhang, Z.Y.; Xu, J.; Haung, L.F. Fluorescence excitation-emission matrix spectroscopy of cdom from yundang lagoon and its indication for organic pollution. *Spectrosc. Spect. Anal.* **2010**, *30*, 1539–1544. [CrossRef]
20. Han, P.; Kumar, P.; Ong, B.L. Remediation of nutrient-rich waters using the terrestrial plant, *Pandanus amaryllifolius* Roxb. *J. Environ. Sci.* **2014**, *26*, 404–414. [CrossRef]
21. Rasheed, T.; Bilal, M.; Nabeel, F.; Adeel, M.; Lqbal, H.M.N. Environmentally-related contaminants of high concern: Potential sources and analytical modalities for detection, quantification, and treatment. *Environ Int.* **2019**, *122*, 52–66. [CrossRef] [PubMed]
22. Ashraf, S.; Ali, Q.; Zahir, Z.A.; Ashraf, S.; Asghar, H.N. Phytoremediation: Environmentally sustainable way for reclamation of heavy metal polluted soils. *Ecotoxicol. Environ. Saf.* **2019**, *174*, 714–727. [CrossRef] [PubMed]
23. Feng, N.-X.; Yu, J.; Zhao, H.-M.; Cheng, Y.-T.; Mo, C.-H.; Cai, Q.-Y.; Li, Y.-W.; Li, H.; Wong, M.-H. Efficient phytoremediation of organic contaminants in soils using plant–endophyte partnerships. *Sci. Total Environ.* **2017**, *583*, 352–368. [CrossRef]
24. Buono, D.D.; Terzano, R.; Panfili, I.; Bartucca, M.L. Phytoremediation and detoxification of xenobiotics in plants: Herbicide-safeners as a tool to improve plant efficiency in the remediation of polluted environments. A mini-review. *Int. J. Phytoremed.* **2020**, *22*, 789–803. [CrossRef]
25. Huang, J.W.; Chen, J.; Berti, W.R.; Cunningham, S.D. Phytoremediation of lead-contaminated soils: Role of synthetic chelates in lead phytoextraction. *Environ. Sci. Technol.* **1997**, *31*, 800–805. [CrossRef]

26. Donnelly, M.; Shaffer, M.; Connor, S.; Sacks, P.; Walters, L. Using mangroves to stabilize coastal historic sites: Deployment success versus natural recruitment. *Hydrobiologia* **2017**, *803*, 389–401. [CrossRef]
27. Farquhar, S.D.; Sims, S.M.; Wang, S.J.; Morrill, K.S. A brief answer: Why is China's aquaculture industry so successful? *Environ. Manag. Sustain. Dev.* **2017**, *6*, 234. [CrossRef]
28. Huang, L.; Zhuo, J.; Guo, W.; Spencer, R.G.; Zhang, Z.; Xu, J. Tracing organic matter removal in polluted coastal waters via floating bed phytoremediation. *Mar. Pollut. Bull.* **2013**, *71*, 74–82. [CrossRef]
29. Li, X.L.; Marella, T.K.; Tao, L.; Da, I.L.L.; Peng, L.; Song, C.F.; Li, G. The application of ceramsite ecological floating bed in aquaculture: Its effects on water quality, phytoplankton, bacteria and fish production. *Water Sci. Technol.* **2018**, *77*, 2742–2750. [CrossRef]
30. Zhang, J.Z.; Li, M.J. A new species of *Bipolaris* from the halophyte *Sesuvium portulacastrum* in Guangdong province, China. *Mycotaxon* **2009**, *109*, 289–300. [CrossRef]
31. Chen, J.; Henny, R.J. Role of micropropagation in the development of the foliage plant industry. In *Floriculture, Ornamental and Plant Biotechnology. Vol. V*; Teixeira da Silva, J.A., Ed.; Global Science Books: London, UK, 2008; pp. 206–218.
32. Shen, X.; Chen, J.; Kane, M.; Henny, R.J. Assessment of somaclonal variation in Dieffenbachia plants regenerated through indirect shoot organogenesis. *Plant Cell Tiss. Org. Cult.* **2007**, *91*, 21–27. [CrossRef]
33. Wei, X.; Chen, J.; Zhang, C.; Wang, Z. In vitro shoot culture of *Rhododendron fortunei*: An important plant for bioactive phytochemicals. *Ind. Crop. Prod.* **2018**, *126*, 459–465. [CrossRef]
34. Kane, M.E. Propagation from preexisting meristems. In *Plant Tissue Culture Concepts and Laboratory Exercises*; Trigiano, R.N., Gray, D.J., Eds.; CRC Press: New York, NY, USA, 1996; pp. 61–71.
35. Lokhande, V.H.; Nikam, T.D.; Ghane, S.G.; Suprasanna, P. In vitro culture, plant regeneration and clonal behaviour of *Sesuvium portulacastrum* (L.) L.: A prospective halophyte. *Physiol. Mol. Bio. Plants* **2010**, *16*, 187–193. [CrossRef] [PubMed]
36. Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
37. Cui, J.; Chen, J.; Henny, R.J. Regeneration of *Aeschynanthus radicans* via direct somatic embryogenesis and analysis of regenerants with flow cytometry. *In Vitro Cell Dev. Biol. Plant* **2009**, *45*, 34–43. [CrossRef]
38. Chen, J.; Wei, X. Thidiazuron in micropropagation of aroid plants. In *Thidiazuron: From Urea Derivative to Plant Growth Regulator*; Ahmad, N., Faisal, M., Eds.; Springer: Singapore, 2018. [CrossRef]
39. Fay, M.F.; Gratton, J. Tissue culture of cacti and other succulents: A literature review and a report on micropropagation at Kew. *Bradleya* **1992**, *10*, 33–48. [CrossRef]
40. Debnath, S.C. Micropropagation of lingonberry: Influence of genotype, explant orientation, and overcoming TDZ-induced inhibition of shoot elongation using zeatin. *HortScience* **2005**, *40*, 185–188. [CrossRef]
41. Shen, X.; Kane, M.E.; Chen, J. Effects of genotype, explants source, and plant growth regulators on indirect shoot organogenesis in *Dieffenbachia* cultivars. *In Vitro Cell Dev. Biol. Plant* **2008**, *44*, 282–288. [CrossRef]
42. Fan, S.; Jian, D.; Wei, X.; Chen, J.; Beeson, R.C.; Zhou, Z.; Wang, X. Micropropagation of blueberry 'Bluejay' and 'Pink Lemonade' through in vitro shoot culture. *Sci. Hortic.* **2017**, *226*, 277–284. [CrossRef]
43. Debnath, S.C. Clonal propagation of dwarf raspberry (*Rubus pubescens* Raf.) through in vitro axillary shoot proliferation. *Plant Growth Regul.* **2004**, *43*, 179–186. [CrossRef]
44. Grigoriadou, K.; Vasilakakis, M.; Eleftheriou, E.P. In vitro propagation of the Greek olive cultivar 'Chondrolia Chalkidikis'. *Plant Cell Tiss. Organ Cult.* **2002**, *71*, 47–54. [CrossRef]
45. Shen, X.; Chen, J.; Kane, M.E. Indirect shoot organogenesis from leaves of *Dieffenbachia* cv. Camouflage. *Plant Cell Tiss. Organ* **2007**, *89*, 83–90. [CrossRef]
46. Zimmerman, P.W.; Wilcoxon, F. Several chemical growth substances which cause initiation of roots and other responses in plants. *Contrib. Boyce Thompson Inst.* **1935**, *7*, 209–229.
47. Jena, S.; Sahoo, P.; Das, A.B. New reports of chromosome number and genome size in eight mangroves from coastal Orissa. *Caryologia* **2003**, *56*, 353–358. [CrossRef]
48. Thiem, B.; Sliwinska, E. Flow cytometric analysis of nuclear DNA content in cloudberry (*Rubus chamaemorus* L.) in vitro cultures. *Plant Sci.* **2003**, *164*, 129–134. [CrossRef]
49. Mckee, K.L.; Feller, R.I.C. Mangrove recruitment after forest disturbance is facilitated by herbaceous species in the Caribbean. *Ecol. Appl.* **2007**, *17*, 1678–1693. [CrossRef] [PubMed]
50. Iniyalakshimi, B.R.; Avudainayagam, S.; Shanmugasundaram, R.; Sebastian, S.P.; Thangavel, P. Evaluation of *Sesuvium portulacastrum* for the phytodesalination of soils irrigated over a long-term period with paper mill effluent under non-leaching conditions. *Int. J. Curr. Microbiol. Appl. Sci.* **2019**, *8*, 880–893. [CrossRef]
51. Ayyappan, D.; Sathiyaraj, G.; Ravindran, K. Phytoextraction of heavy metals by *Sesuvium portulacastrum* L. a salt marsh halophyte from tannery effluent. *Int. J. Phytoremed.* **2016**, *18*, 453–459. [CrossRef] [PubMed]