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The Response of Vegetable Sweet Potato (*Ipomoea batatas* Lam) Nodes to Different Concentrations of Encapsulation Agent and MS Salts

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Abstract: As an emerging technology, shoot encapsulation has been employed in germplasm conservation, distribution, and micropropagation of elite plant species. However, the production of synthetic seeds of sweet potato via non-zygotic embryogenesis requires a large number of embryos per cultured callus suspension and is labour-intensive. Here, we reported a simple method of encapsulating in vitro derived vegetable sweet potato nodal segments with sodium alginate, calcium chloride ($CaCl_2$), and Murashige and Skoog (MS) salts. The nodes encapsulated with 4% sodium alginate (w/v) and 100 mM $CaCl_2$ were the most suitable for propagation. They had uniform spherical beads and took the least number of days to shoot and root emergence. These plantlets produced more leaves, roots, and long shoots. Further evaluation of the MS salts concentration revealed that the plantlets encapsulated and grown with 1/2 MS salts had the least days to shoot and root emergence. They also had a longer shoot, the highest conversion rate (99%), and the least leaf abscission (17%). Thus, the sweet potato nodal segments encapsulated with 4% sodium alginate, 100 mM $CaCl_2$, and 1/2 MS salts could be used as excellent material for micropropagation, germplasm conservation, and exchange of sweet potato planting materials.

Keywords: tissue culture; micropropagation; non-zygotic embryo; germplasm conservation; encapsulated nodes

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1. Introduction

Murashige [1] was the first scientist to propose the concept of encapsulation of in vitro-derived non-zygotic embryos to make synthetic seeds. They have been used in place of somatic embryos in micropropagation and germplasm conservation [2–5]. Plant regeneration and micropropagation from artificial seeds were later reported by several scientists in various plant species [5–10]. The artificial seeds obtained through encapsulation of non-zygotic embryos, shoots, nodes, and meristematic tissues could grow and develop a whole plant under in vitro or in vivo environmental conditions [11–13]. The technology is helpful in germplasm conservation of commercially important and endangered plants using the appropriate storage and encapsulation technique [2,3,14,15]. Several encapsulated plant parts were used in micro-propagation, in vitro conservation, and germplasm storage as a means of reducing frequent transfer and sub-culturing of in vitro plantlets [9,10,16]. After encapsulation with sodium alginate, the ability of the encapsulated explants to remain viable and re-grow is its most appealing characteristic [17,18]. Improving artificial

seed production could help in conserving and propagating elite species and other commercially important crops [9,19,20]. In recent times, the encapsulation of non-zygotic plant parts has gained considerable attention, as the capsules are easy to handle due to their small size, reduced storage cost, and preservation of the genetic uniformity of the propagules [21,22]. Sodium alginate and calcium chloride ($CaCl_2$) are frequently used for encapsulation [9,19,23] because the sodium alginate matrix protects the explants from damages and drying during storage and handling [19,24,25]. The capsule formed also serves as an artificial endosperm that provides nutrients and growth regulators (PGRs) needed for the growth and development of the plantlets [8,26]. Furthermore, sodium alginate coated non-zygotic micro-propagules are comparatively inexpensive and easy to produce [9,27–30].

Sweet potato (*Ipomoea batatas* L.) is a tuberous plant from the Convolvulaceae (Morning glory) [9,19,20,31]. Many people depend on the crop as a staple food [9,10,16]. It was the 15th most valuable crop in 2017 and ranked the 8th most important staple food [32]. Sweet potato leaves are rich in radical scavenging natural antioxidants with anticancer, antimutagenic, and antibacterial activities [29,33,34]. The production and conservation of sweet potato via vine cuttings, shoot tips, or tubers is declining primarily due to its susceptibility to biotic and abiotic stress factors [22,35]. Besides, these methods are costly, labour-intensive, and require large nurseries and storage systems [36,37]. Moreover, sweet potato germplasm has been preserved in situ and in vitro with varying degrees of success [22,38]. Farmers in developing countries have to travel long distances to buy planting materials [22], which often delays its planting and limits the sweet potato planting area and yield [39]. In addition, sweet potato farmers need a constant supply of disease-free planting materials to sustain steady production [21,22]. Therefore, there is a need for a simple alternative method for sweet potato germplasm conservation, exchange, and plantlet production [22,40].

Conservation and propagation by tissue culture ensures a healthy and consistent source of suitable planting materials [41]. Cantliffe et al. [23] first reported the production of sweet potato artificial seeds for clonal propagation through non-zygotic embryogenesis. Subsequently, modified and improved procedures of sweet potato propagation have been reported [36,37,42]. The production of synthetic seeds of sweet potato via non-zygotic embryogenesis requires many embryos in cultured callus suspension. However, the formation of sweet potato embryos occurs through several cycles of growth and fragmentation [37,43]. Certain factors must be considered, i.e., plant growth regulators, medium constituents, medium pH, incubation conditions, the vessel type and size, illumination, agitation, and aeration [36]. Early studies of sweet potato embryogenesis reported malformed and abnormal embryos [44]. Besides, sweet potato regeneration and recovery from non-zygotic embryos was genotype-dependent and highly recalcitrant [45–47]. Thus, sweet potato micropropagation using node cuttings is simple and considered genetically stable [22,48]. Despite the advantages of sweet potato micropropagation using nodal cuttings and shoot tips, subculturing is among the most time-consuming factors in conserving sweet potato germplasm [40]. As such, the encapsulation of non-zygotic micro propagules could replace these methods and is relatively inexpensive and easy [26,49]. Studies have shown that encapsulated nodes and other non-zygotic embryos have been used in the germplasm preservation of several plants [50–52]. However, the research on encapsulation of sweet potato nodal segments using sodium alginate remains limited. It is unclear how the concentrations of these encapsulating agents and Murashige and Skoog (MS) salts concentration could affect the conversion and growth of the plantlets. This study aims to study the effect of sodium alginate, $CaCl_2$ and MS salt concentration in the encapsulation of sweet potato nodal segments, which could facilitate the conservation of sweet potato's genetic materials and the exchange of axenic materials.

2. Materials and Methods

2.1. Sterilization and Establishment of Axenic Plantlets

The experiments were conducted in the Key Laboratory of Crop Biotechnology, Fujian Agriculture and Forestry University, China. 'Fushu-18′ is a sweet potato cultivar used solely for its succulent leaves and stems as a vegetable crop in China. To obtain explants used in this study, young and healthy greenhouse-grown vines of vegetable sweet potato ('Fushu 18') were excised and surface sterilized following the procedure of Namanda et al. [53], with slight modifications. Briefly, the leaves were removed, and the shoots were cut into smaller pieces (2.5 to 3.0 cm) containing two or three buds using a sterilized surgical blade, leaving small fragments of petioles. After rinsing three times with tap water, explants were kept in water with three drops of Tween-20 (BBI Sangon Biotech Co., Ltd., Shanghai, China). An hour later, the explants were transferred to a laminar hood and placed into a sterile container, rinsed with double-distilled water three times, and disinfected with a plant preservative mixture (PPM^{TM} solution; Plant Cell Technology, Inc., Connecticut, Washington, DC, USA) [54], sodium hypochlorite (Sangon Biotech Co., Ltd. Shanghai, China) solution (10% [v/v]), and 75% (v/v) ethanol (Shanghai Macklin Biochemical Co., Ltd., Shanghai, China). The explants were maintained in 5% (v/v) PPM solution supplemented with 3x MS salts (PhytoTech Labs, Inc., Lenexa, KS, USA) [55] for 5 h. The PPM solution was discarded, and each explant was immediately inoculated into 40 mL autoclaved MS medium in a culture vessel fortified with 0.1% (v/v) PPM, 3% (w/v) sucrose (BBI Sangon Biotech Co., Ltd., Shanghai, China), and 0.8% (w/v) agar (PhytoTech Labs, Inc., Lenexa, KS, USA). All media were adjusted to a pH of 5.8 ± 0.1 and autoclaved at 121 °C (105 kPa pressure) for 20 min. All cultured materials were kept under cool fluorescent (white) lamps (100 µmol m^{-2} s^{-1} photon flux), 25 ± 2 °C, and a 16/8 h cycle of light and darkness.

2.2. Sub-Culturing of Plantlets

The in vitro plantlets were sub-cultured every four weeks in 40 mL sterile MS medium supplemented with 0.1% (v/v) PPM, 3% (w/v) sucrose, the pH was adjusted to 5.8 ± 0.1 before autoclaving and solidified using 0.8% (w/v) agar. The cultures were maintained under white fluorescent lamps (100 µmol m^{-2} s^{-1} photon flux), 25 ± 2 °C, and a 16/8 h cycle of light and darkness to produce enough plantlets.

2.3. Preparation of Sodium Alginate and $CaCl_2$

One hundred millilitres of sodium alginate (Shanghai Macklin Biochemical Co., Ltd., Shanghai, China) matrix (3, 4, and 5% [w/v]) and $CaCl_2$ (Shanghai Macklin Biochemical Co., Ltd., Shanghai, China) solution (80, 100, and 120 mM) were prepared following the procedures of Arumugam et al. [34] with slight modification. The sodium alginate was supplemented with MS salts, 3% (w/v) sucrose, and 0.05% (v/v) PPM. The matrix's pH was adjusted to 5.8 ± 0.1 before autoclaving.

2.4. Encapsulation of Sweet Potato Nodal Segments with Sodium Alginate and $CaCl_2$

The sweet potato nodal shoot segments (4 ± 0.2 mm in length) were neatly removed from four-week-old plantlets and suspended in autoclaved 100 mL MS medium supplemented with different concentrations of sodium alginate, 3% (w/v) sucrose, and 0.05% (v/v) PPM without any plant growth regulator. Aliquots of the alginate solutions with a single node were individually taken up using a modified sterile (5 mm) plastic Pasteur pipette and gently dropped one-by-one into 100 mL of the sterile $CaCl_2$ solutions. The beads (4.5 ± 0.2 mm) were kept for 30 min to polymerize completely. They were decanted and washed three times with sterile distilled water before drying on a sterile filter paper under a laminar flow hood.

2.5. Growth Media and Plantlet Retrieval from the Beads

The encapsulated nodes were inoculated into plant growth regulator-free MS medium fortified with 3% (w/v) sucrose, 0.05% (v/v) PPM, and solidified with a 0.8% (w/v) agar in

plastic Petri dishes. After three weeks of culture, the plantlets were transferred to vessels with the same medium combination. The Petri dishes and vessels were kept under cool fluorescent lamps with a 100 µmol m^{-2} s^{-1} photon flux, 25 ± 2 °C, and a 16/8 h cycle of light and darkness. Data were collected at three and five weeks after inoculation.

2.6. Evaluation of MS Salts Strength on the Conversion of Sweet Potato Plantlets

The treatments consist of three levels of MS salts in the capsule and the growth medium (full, 1/2, and 1/4 MS salts). Sodium alginate (4% [*w*/*v*]) and $CaCl_2$ (100 mM) were used as the encapsulation matrix and complexation, respectively. The beads were cultured into vessels containing 30 mL of the different MS medium having 0.8% (*w*/*v*) agar. The sodium alginate matrix and the MS medium were fortified with 3% (*w*/*v*) sucrose and 0.05% (*v*/*v*) PPM. The medium pH was maintained at 5.8 ± 0.1 before autoclaving. The vessels were kept under fluorescent lamps with a 100 µmol m^{-2} s^{-1} photon flux, 25 ± 2 °C, and a 16/8 h cycle of light and darkness. Data were collected at four weeks after inoculation.

2.7. Plantlets Growth Evaluation

The plantlet growth was evaluated visually. Shoot and root lengths (cm) were measured with a plastic ruler at the end of the culture. The conversion frequency was calculated following Rai et al. [2]. Briefly, the number of encapsulated nodes having well-developed plantlets out of the number of encapsulated nodes was calculated as the percentage. The ratio of leaf abscission and the total number of leaves were calculated following Sakamoto and Suzuki [56].

2.8. Acclimatization and Transplanting

Well-developed plantlets were collected from the media, thoroughly washed with distilled water, removing agar and other media components. Before transplanting, the plantlets were kept in distilled water and covered in a transparent plastic container. The plantlets were transplanted to plastic pots filled with sterilized sand, coco peat, and soil mixture (2:1:2). They were covered with transparent polythene bags to retain high relative humidity. The plantlets were kept under fluorescent lamps with a 100 µmol m^{-2} s^{-1} photon flux, 25 ± 2 °C, and a 16/8 h cycle of light and darkness for two weeks before exposure to field condition for further evaluation.

2.9. Statistical Analysis

All experimental treatments were factorially combined and laid out in a completely randomized design with six replicates. The experiment was repeated three times. Each treatment had six vessels inoculated with five encapsulated nodes. The data generated were subjected to analysis of variance using statistical analysis system software [57] and graphs plotted with GraphPad prism [58]. Results with significant interactions were presented in tables, and significantly different means were separated with Fishers' least significant difference (LSD) test. All analyses were performed at a 5% probability level.

3. Results

3.1. Effect of Sodium Alginate and $CaCl_2$ Concentration on the Encapsulation of Vegetable Sweet Potato Nodes

The concentration of the sodium alginate significantly ($p \leq 0.05$) affected the number of days to root and shoots emergence. The nodes encapsulated with 4% sodium alginate were the first to break the capsule (9 days) and produced roots. In contrast, root emergence was delayed in the nodes treated with 3% sodium alginate (11 days). However, varying the sodium alginate concentration did not affect the days to shoot emergence. On the other hand, the roots and shoots emergence occurred significantly earlier in the beads treated with 80 mM $CaCl_2$ than those treated with a higher concentration (120 mM) (Figure 1A). These beads are softer and thus allow the shoots to break out of the matrix easily. At

three weeks after inoculation, the plantlets obtained from 4% sodium alginate had more leaves (53%) than those encapsulated with 5% and 3%, while the number of roots was not significantly affected. The beads polymerized with 100 mM $CaCl_2$ produced plantlets with the highest leaves at three weeks after inoculation, while the number of roots was not affected by the concentration of $CaCl_2$ (Figure 1B). After the plantlets were transferred to the culture vessel, the nodes encapsulated with 5% sodium alginate produced more leaves (9). The plantlets obtained from 4% sodium alginate had more roots (4 roots) than those encapsulated with 5% and 3% sodium alginate. Five weeks after inoculation, the use of 100 mM $CaCl_2$ significantly enhanced the production of more leaves and roots (Figure 1C). The nodes encapsulated with 5% sodium alginate had the longest root (8.6 cm), whereas the longest shoot (1.4 cm) was obtained in those encapsulated with 4% sodium alginate. However, the encapsulated nodes treated with 120 mM $CaCl_2$ had the longest root. In contrast, the plantlets treated with 100 mM $CaCl_2$ produced the longest shoots (1.4 cm) (Figure 1D).

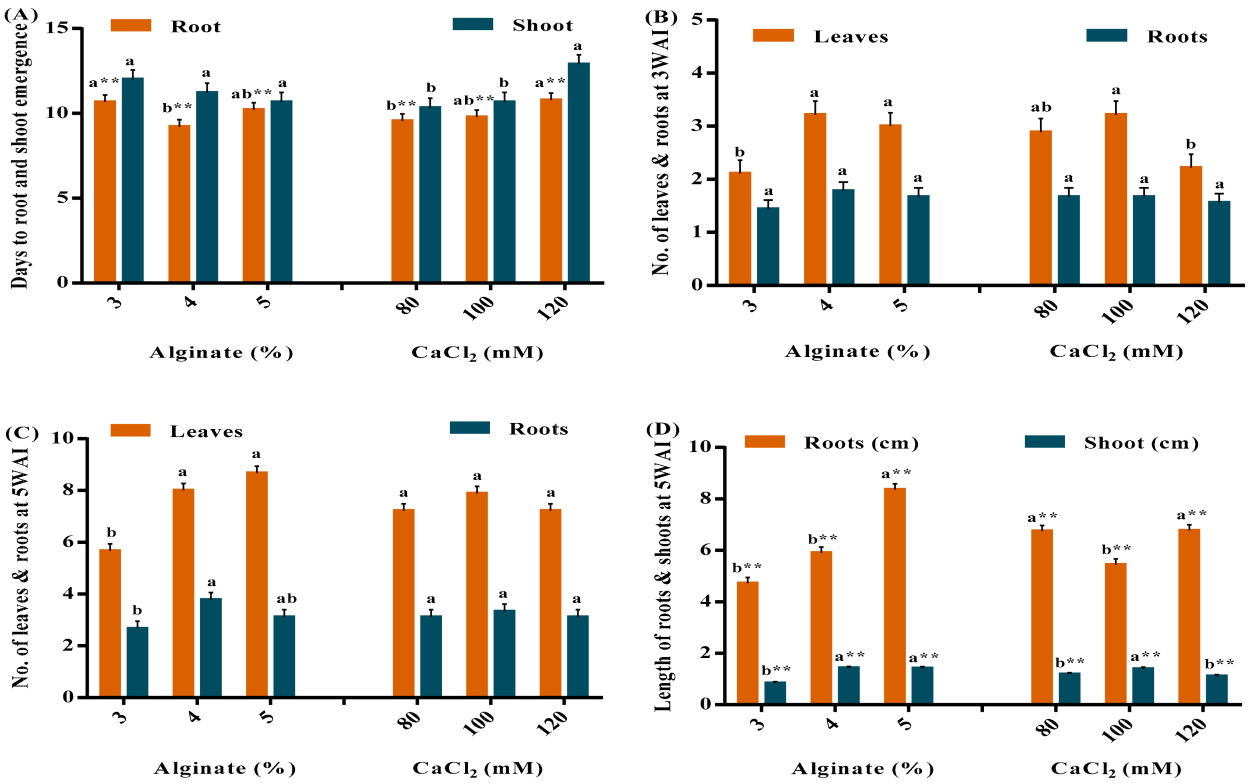

Figure 1. Effect of sodium alginate and $CaCl_2$ concentration on encapsulated vegetable sweet potato (*Ipomoea batatas* L) nodes, i.e., number of days to root and shoot emergence (**A**), number of leaves and roots at three weeks after inoculation into Murashige and Skoog (MS) medium (**B**), number of leaves and roots at five weeks after inoculation into MS medium (**C**), and length of roots and shoot at five weeks after inoculation into MS medium (**D**); (**) indicates significant interaction between the evaluated parameter at 1% probability levels. Means with a different letter(s) within the bars differ significantly at a 5% probability level using LSD.

Significant interactions ($p \leq 0.01$) between $CaCl_2$ and sodium alginate were observed on the days for root emergence, length of roots, and shoot at five weeks after inoculation (Table 1). The minimum days from inoculation to root emergence (9 days) were recorded in the nodes encapsulated with 3% sodium alginate in 100 mM $CaCl_2$. However, root emergence was delayed in the nodes gelled using 3% sodium alginate and 120 mM $CaCl_2$. The sweet potato nodes encapsulated with 5% and 80 mM $CaCl_2$ had the longest roots (10.8). In contrast, the nodal segments encapsulated in 4% sodium alginate and 100 mM $CaCl_2$ produced the longest shoots (1.7 cm) at five weeks after inoculation. The sweet potato

nodes encapsulated with 4% sodium alginate were firm and hard enough to allow shoot and root emergence (Figure 2).

Table 1. Interaction of sodium alginate and $CaCl_2$ concentration on days to root emergence, roots, and shoot length of vegetable sweet potato (*Ipomoea batatas* L.) at five weeks after inoculation into MS medium.

Treatments	Days to Root Emergence			Root Length (cm)			Shoot Length (cm)		
	80 mM $CaCl_2$	100 mM $CaCl_2$	120 mM $CaCl_2$	80 mM $CaCl_2$	100 mM $CaCl_2$	120 mM $CaCl_2$	80 mM $CaCl_2$	100 mM $CaCl_2$	120 mM $CaCl_2$
3% Alginate	10.0 ± 0.6^{bc}	8.6 ± 0.3^{c}	13.3 ± 0.3^{a}	3.3 ± 0.3^{e}	5.8 ± 0.3^{cd}	5.0 ± 0.4^{cd}	0.7 ± 0.1^{c}	1.2 ± 0.0^{b}	0.5 ± 0.0^{c}
4% Alginate	9.0 ± 0.6^{c}	8.6 ± 0.9^{c}	10 ± 1.2^{bc}	6.1 ± 0.4^{c}	5.6 ± 0.1^{cd}	5.9 ± 1.0^{cd}	1.2 ± 0.2^{b}	1.6 ± 0.0^{a}	1.4 ± 0.1^{ab}
5% Alginate	9.6 ± 0.3^{c}	12.0 ± 1.2^{ab}	9.0 ± 0.6^{c}	10.8 ± 0.1^{a}	4.9 ± 0.3^{d}	9.3 ± 0.2^{b}	1.6 ± 0.1^{a}	1.2 ± 0.2^{bc}	1.4 ± 0.1^{ab}

Means with a different letter(s) differ significantly at a 5% probability level using LSD.

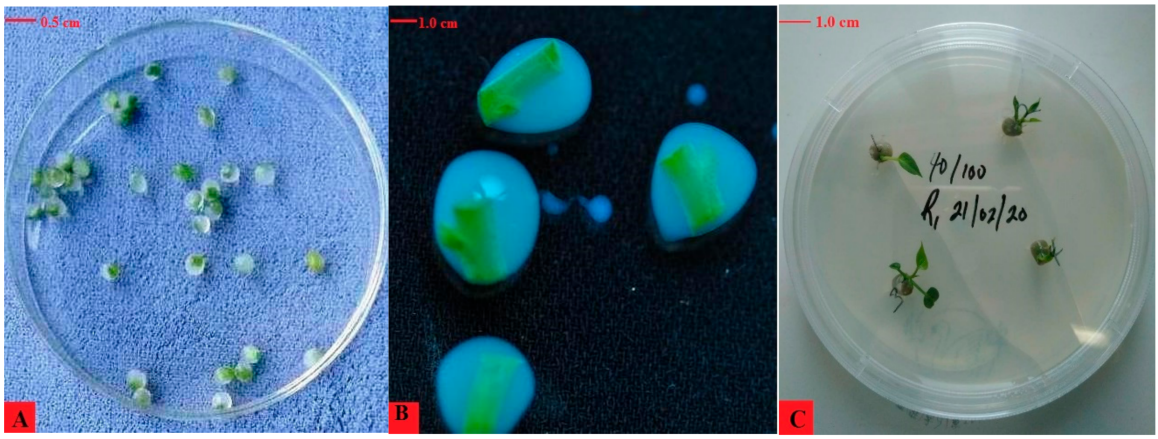

Figure 2. Encapsulated nodes of vegetable sweet potato (*Ipomoea batatas* L.) at different development stages. Single nodal segments of sweet potato encapsulated with sodium alginate and $CaCl_2$ (**A**,**B**), growing sweet potato plantlets at three weeks after inoculation into MS medium before transferring to a new MS medium (**C**).

3.2. Effect of MS Salts Strength on the Conversion of Sweet Potato Plantlets

The application of 1/2 MS salts in the beads significantly ($p \leq 0.05$) reduced the plantlet's days to root emergence (4.6 days). The root's emergence was extended by two days when full MS salts were used in the beads. Similarly, the nodes encapsulated with 1/2 MS salts in the beads were the first to break the capsule. The plantlets cultured on 1/4 MS medium were rooted earlier (4.5) than those cultured in full MS medium (Figure 3A). The nodes encapsulated with 1/2 MS salts produced more leaves and roots at two weeks after inoculation. Moreover, the nodes cultured on 1/2 MS medium had more roots and shoots (Figure 3B). However, the concentration of MS did not affect the number of leaves at four weeks after inoculation, and both concentrations were at par. Moreover, the plantlets treated with 1/2 MS salts had the highest number of leaves at four weeks after inoculation (Figure 3C). The use of 1/2 MS in the encapsulation matrix and MS medium was ideal. The plantlets produced long shoots and roots, although they are at par with those treated with 1/4 MS salts (Figure 3D). The beads treated with 1/2 MS salts had the highest conversion percentage and leaf abscission. The highest conversion was observed in the nodes treated with 1/2 MS salts. Leaf abscission is usually accompanied due to nutrient deficiency and other factors. In this study, 25% of the leaves abscised when the MS salts were reduced to 1/4 in the medium (Figure 4).

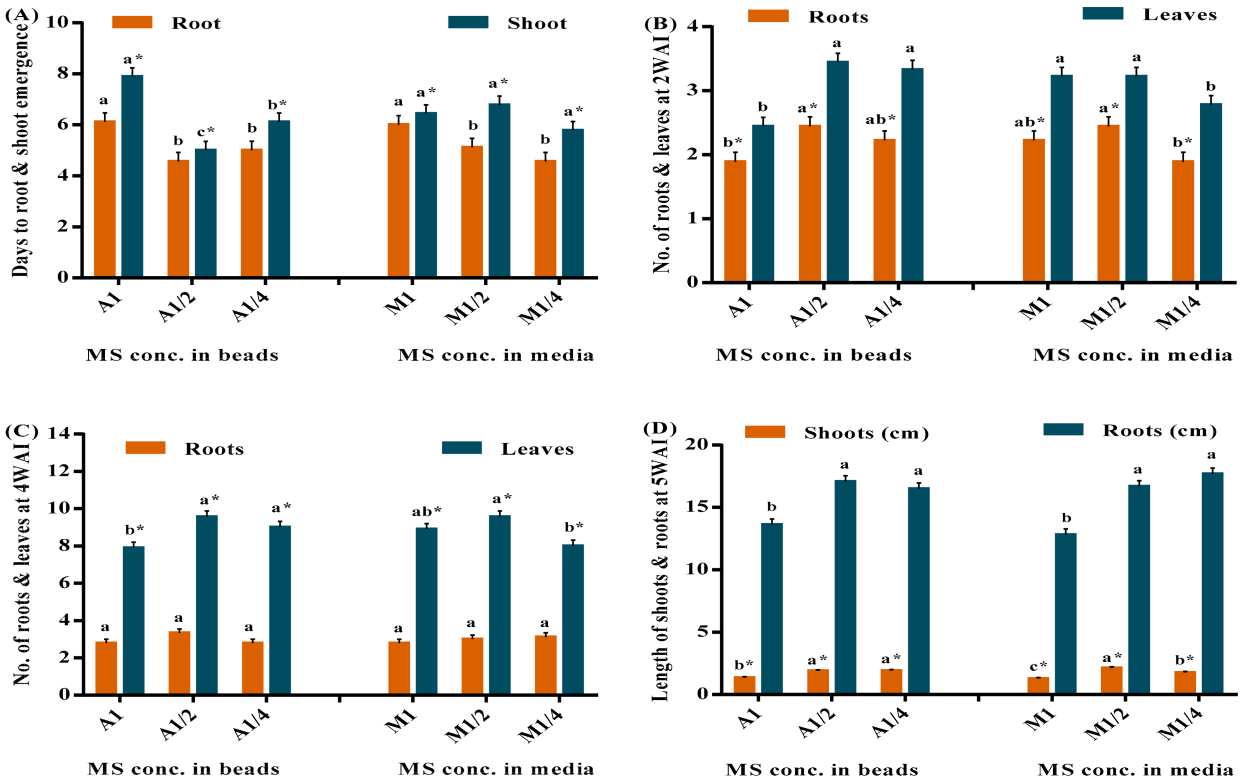

Figure 3. Effect of MS salts concentration on encapsulated vegetable sweet potato (*Ipomoea batatas* L.) nodes, i.e., number of days to root and shoot production (**A**), number of roots and leaves at two weeks after inoculation into MS medium (**B**), number of roots and leaves at four weeks after inoculation into MS medium (**C**), and length of shoot and roots at four weeks after inoculation into MS medium (**D**). Full MS salts in the capsule (A1), 1/2 MS in the capsule (A1/2), 1/4 MS in the capsule (A1/4). Full MS salts in the growth media (M1), 1/2 MS salts in the growth media (M1/2), 1/4 MS salts in the growth media (M1/4). (*) indicate a significant interaction between the evaluated parameter at 5% probability levels. Means with a different letter (s) within the bars differ significantly at a 5% probability level using LSD.

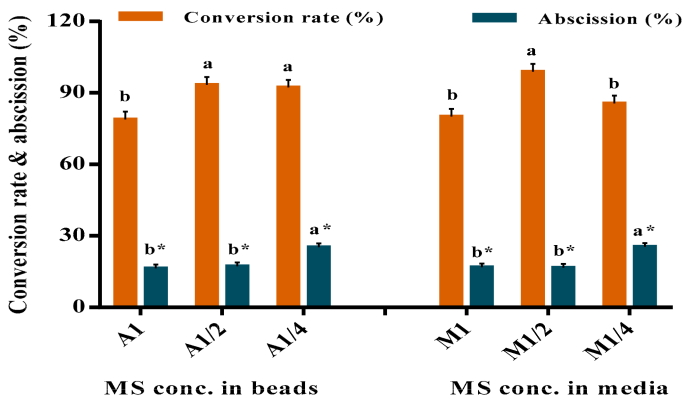

Figure 4. Effect of MS salts concentration on encapsulated vegetable sweet potato (*Ipomoea batatas* L.) nodes on plantlets conversion rate and leaf abscission. Full MS in the capsule (A1), 1/2 MS in the capsule (A1/2), 1/4 MS in the capsule (A1/4). Full MS salts in growth media (M1), 1/2 MS salts in growth media (M1/2), 1/4 MS salts in growth media (M1/4). (*) indicate a significant interaction between the evaluated parameter at a 5% probability level. Means with a different letter within the bars differ significantly at a 5% probability level using LSD.

The sweet potato nodes encapsulated with $\frac{1}{2}$ MS salts were the first to produce shoots when cultured in $\frac{1}{4}$ MS medium (4 days) than those treated with full MS salts. The nodes treated with full MS salts had few roots (Table 2). Moreover, the plantlets treated with $\frac{1}{2}$ MS salts in the capsule and the growth medium produced more leaves, while fewer were recorded in plantlets treated with $\frac{1}{2}$ MS salts. Furthermore, the nodes treated with $\frac{1}{2}$ MS salts had longer shoots (Table 3). In addition, the application of $\frac{1}{2}$ MS salts in both the capsule and the growth medium decreased the leaf abscission to 17%. In contrast, plantlets treated with $\frac{1}{4}$ MS salts recorded the highest leaf abscission (36%) (Table 4). For further assessment, well-established plantlets were successfully acclimatized and transplanted to plastic pots (Figure 5).

Table 2. Interaction of MS salts concentration in encapsulated sweet potato (*Ipomoea batatas* L.) nodes (A) and growth media (M) on number of days to shoot emergence and number of roots at two weeks after inoculation.

Treatments MS Conc. in Media (M) g/L	Number of Days to Shoot Emergence			Number of Roots at Two Weeks after Inoculation		
	MS Concentration in Encapsulated Nodes (A) g/L					
	Full MS (A1)	1/2 MS (A1/2)	1/4 MS (A1/4)	Full MS (A1)	1/2 MS (A1/2)	1/4 MS (A1/4)
Full MS in media (M1)	8.6 ± 0.9 ^{a}	5.6 ± 0.3 ^{bc}	5.0 ± 0.6 ^{cd}	1.3 ± 0.3 ^{b}	2.6 ± 0.3 ^{a}	2.6 ± 0.3 ^{a}
1/2 MS in media (M1/2)	7.0 ± 1.2 ^{ab}	5.6 ± 0.3 ^{bc}	7.6 ± 0.3 ^{a}	2.6 ± 0.3 ^{a}	2.6 ± 0.3 ^{a}	2.0 ± 0.0 ^{ab}
1/4 MS in media (M1/4)	8.0 ± 0.6 ^{a}	3.6 ± 0.3 ^{d}	5.6 ± 0.7 ^{bc}	1.6 ± 0.3 ^{b}	2.0 ± 0.6 ^{ab}	2.0 ± 0.0 ^{ab}

Means with a different letter(s) differ significantly at a 5% probability level using LSD.

Table 3. Interaction of MS salts concentration in encapsulated sweet potato (*Ipomoea batatas* L.) nodes (A) and growth media (M) on number of leaves and shoot length at four weeks after inoculation.

Treatments MS Conc. in Media (M) g/L	Number of Leaves			Length of Shoots (cm)		
	MS Concentration in Encapsulated Nodes (A) g/L					
	Full MS (A1)	$\frac{1}{2}$ MS (A1/2)	$\frac{1}{4}$ MS (A1/4)	Full MS (A1)	$\frac{1}{2}$ MS (A1/2)	$\frac{1}{4}$ MS (A1/4)
Full MS in media (M1)	7.0 ± 0.6 ^{d}	9.3 ± 0.3 ^{ab}	10.3 ± 0.8 ^{a}	0.9 ± 0.1 ^{c}	1.3 ± 0.3 ^{bc}	1.4 ± 0.1 ^{bc}
$\frac{1}{2}$ MS in media (M1/2)	8.6 ± 0.7 ^{bc}	10.6 ± 0.3 ^{a}	9.3 ± 0.3 ^{ab}	1.3 ± 0.1 ^{bc}	2.6 ± 0.1 ^{a}	2.4 ± 0.4 ^{a}
$\frac{1}{4}$ MS in media (M1/4)	8.0 ± 0.6 ^{bcd}	8.6 ± 0.3 ^{bc}	7.3 ± 0.3 ^{cd}	1.7 ± 0.2 ^{b}	1.7 ± 0.3 ^{b}	1.8 ± 0.1 ^{b}

Means with a different letter(s) differ significantly at a 5% probability level using LSD.

Table 4. Interaction of MS salts concentration in encapsulated sweet potato (*Ipomoea batatas* L.) nodes (A) and growth media (M) on plantlets leaf abscission.

Treatments MS Conc. in Media (M) g/L	Leaf Abscission (%)		
	MS Concentration in encapsulated nodes(A) g/L		
	Full MS (A1)	1/2 MS (A1/)	1/4 MS (A1/4)
Full MS in media (M1)	17 ± 1.6 ^{bc}	17 ± 3.2 ^{bc}	17 ± 0.0 ^{bc}
1/2 MS in media (M1/2)	15 ± 1.2 ^{c}	12 ± 1.1 ^{c}	23 ± 1.9 ^{b}
1/4 MS in media (M1/4)	17 ± 2.8 ^{bc}	23 ± 5.3 ^{b}	36 ± 4.1 ^{a}

Means with a different letter(s) differ significantly at a 5% probability level using LSD.

Figure 5. Grown vegetable sweet potato (*Ipomoea batatas* L.) plantlets from nodes encapsulated with sodium alginate (4%) and $CaCl_2$ (100 mM) matrices at five weeks after inoculation (**A**), grown vegetable sweet potato plantlets after acclimatization just before transplanting to pots (**B**), and transplanted vegetable sweet potato plantlets from the encapsulated nodes, two weeks after transplanting (**C**).

4. Discussion

Sweet potato is conventionally propagated asexually using vines or tubers [59]. However, using vines or tubers is costly, labour-intensive, and requires large nurseries and storage facilities [36]. The use of tissue culture techniques allows the mass production of disease-free planting materials via artificial seeds [19,60]. An encapsulation substance such as sodium alginate enables the handling of artificial seeds without breakage and often allows the emergence of explants from the beads upon re-growth [28,29,61,62]. In this study, 4% sodium alginate was optimal, and it easily allowed the emergence of shoots, roots, and plantlets with more leaves. However, Ahmed et al. [63] observed that 3% sodium alginate gave the best gel complexation in artificial seeds of *Vitex trifolia*. Moreover, an efficient encapsulation of micro cuttings of *Withania coagulans* was achieved with 3% sodium alginate [64]. Soft beads are produced when a lower concentration of sodium alginate is used, making them difficult to handle and store [20,29,50,65]. Higher concentrations of sodium alginate produced hard iso-diametrical beads and caused a considerable delay in the shoot and root emergence in *Cannabis sativa* [20]. Similar results were reported in kiwi fruit (*Actinidia deliciosa*) artificial seeds [66], olive (*Olea europaea*) [52], and jojoba (*Simmondsia chinensis*) [51]. A higher sodium concentration from sodium alginate changes the balance of water potential, leading to less water for root growth in *Hibiscus moscheutos* explants [16].

The consistency and firmness of artificial seeds largely depend on the amount of sodium and calcium ions exchanged in the process of encapsulation [62,67,68]. Encapsulation with 100 mM $CaCl_2$ was optimal and more effective than using lower or higher concentrations. The concentration of $CaCl_2$ significantly affected the texture, quality, and germination of *Capparis decidua* and *Withania coagulans* artificial seeds [17,64]. Kumar et al. [51] reported that 100 mM $CaCl_2$ was the ideal concentration in the artificial seeds of *Simmondsia chinensis*. In this study, $CaCl_2$ at a higher concentration (120 mM) increased the days to shoot and root emergence from the capsule. Similar results were reported by Shipha et al. in *Solanum trilobatum* [69], Saha et al. in *Ocimum* species. [70], Siddique and Bukhari in *Capparis decidua* [17], and Ahmed et al. in *Vitex trifolia* [63]. In addition, the beads made with 100 mM $CaCl_2$ had more leaves, roots, and long shoots.

The MS salts strength affected the number of days to shoot and root emergence, where the plantlets encapsulated with 1/2 MS salts were the first to break the bead. The sodium alginate matrix served as an artificial endosperm and provided the nutrients for the explants, enhancing their re-growth ability and conversion [9,31]. The plantlets obtained from 1/2 MS salts in beads and full MS salts in the growth media produced more leaves because the nutrients supplied to the explants in the capsule are optimum for their growth [8,71]. In comparison, the nodes treated with full MS in the beads produced fewer

leaves. Similarly, Rezali et al. [72] reported that plantlets of *Typhonium flagelliforme* grown from artificial seeds in 1/2 MS medium had the longest shoots. The highest conversion rate was recorded in plantlets treated with 1/2 MS salts in the media. Our result agreed with the findings of Rezali et al. [72] on *T. flagelliforme*, Wan Nurul Hidayah et al. [73] on *P. cablin*, and Bukhari et al. [74] on *C. angustifolia*. Additionally, 1/2 MS salts in the growth media produced more shoots and roots in *Mentha spicata* [75]. Rai et al. [2] also reported that 1/2 MS salts were favourable for the growth of guava encapsulated shoot tips. A high in vitro conversion percentage of artificial seeds of *Cassia angustifolia* was also observed on 1/2 MS growth media [74].

The MS salts concentration significantly affects the development of the plantlets. Explants grown with 1/4 MS salts showed more leaf abscission during the culture. It was observed that the nutrient requirements of sweet potatoes vary with developmental stages [56]. Bouwkamp [76] reported that sweet potato leaves were abscised at a later stage of development when the supply of nitrogen and phosphorus was low. Poor nutrient supply triggered leaf abscission in sweet potatoes [56]. The nodes encapsulated and grown with 1/2 MS were more active and produced ideal sweet potato plantlets that were acclimatized and transferred to pots. The plantlets assumed normal growth and development.

5. Conclusions

The sweet potato nodal segments encapsulated with 4% sodium alginate and 100 mM $CaCl_2$ were the most suitable for encapsulation. Plantlets grown with 1/2 MS salts in the capsule and growth medium had the least days to shoot and root emergence, and produced more roots, leaves, longer shoots, and had the highest plantlet conversion with minimal leaf abscission. This encapsulation could be used as an important approach in micropropagation, transportation of germplasm, conservation, and exchange of sweet potato planting materials. However, further investigation is needed to extend the conservation period of sweet potato beads using slow growth or low-temperature procedures. An attempt to store the beads beyond seven days failed (data not shown).

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