https://doi.org/10.48048/tis.2023.5729

In Vitro Propagation and Histochemical Analysis of *Launaea sarmentosa* (Willd.) Kuntze

Yurachat Meksuwan[1,*] and Pornsawan Sutthinon[2]

[1]*Faculty of Technology and Environment, Prince of Songkla University, Phuket Campus, Phuket 83120, Thailand*
[2]*Department of Botany, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand*

([*]Corresponding author's e-mail: yurachat.y@phuket.psu.ac.th)

Received: 24 August 2022, Revised: 21 September 2022, Accepted: 28 September 2022, Published: 9 March 2023

Abstract

Launaea sarmentosa (Willd.) Kuntze., an edible perennial herb, is critically considered an endangered species due to its habitat destruction. *In vitro L. sarmentosa* propagation was therefore conducted to preserve this plant. Two types of explants including leaf and stolon were applied to induce the multiple shoots by culturing on Murashige and Skoog (MS) medium supplemented with BA (0, 1, and 2 mg/L) and 2,4-D (0, 1, 2, and 4 mg/L). The optimal shoot induction medium for both explants was MS medium containing 2 mg/l BA (presented 100 % shoot induction). Roots were induced by various types and concentrations of auxins [1-naphthalene acetic acid (NAA), indole-3-acetic acid (IAA), and indole-3-butyric acid (IBA)]. The highest percentage of rooting (70.00 ± 11.05 %) was obtained from stolon-derived shoots on MS medium supplemented with 0.1 mg/l IAA. One-hundred percent survivability was presented after transforming plantlets into a mixture of soil, sand, and peat moss (1:1:1). Starch, protein, and lipid were accumulated in leaves detected by histochemical technique. The obtained information will be a useful tool for mass propagation of *L. sarmentosa* and to obtain basic knowledge for plant accumulating substances.

Keywords: Tissue culture, Plant histochemistry, Micropropagation, Medicinal plant, Coastal plant, Linharn

Introduction

L. sarmentosa (Willd.) Kuntze, locally known as "Linharn" in Phuket, Thailand, belongs to the family Compositae (Asteraceae). It is an endemic plant species and is mainly distributed on the sandy beaches in southwest Thailand including Prachuap Khiri Khan, Ranong, Phangnga, Phuket, and Krabi. The genus *Launaea* contains about 54 species. Most of them can grow and adapt to the dry, saline, and sandy habitats [1]. Additionally, some species of this genus (*Launaea cervicornis*) exhibit great ecological importance as a pioneer and keystone species of the plant communities in the Mediterranean Basin [2]. Thus, the adaptation of this plant to literal sand dunes was interesting. In addition to its ecological importance, several studies have demonstrated that the *Launaea* species is useful as a folk medicine [3,4]. In India, Vietnam, and the Maldives, several diseases (e.g., gonorrhea, syphilis, sore throats, coughs, typhus, nasal-pharyngeal infections, measles, swollen testicles, pain in the spleen, earaches, hookworm eradication, as well as fever) were cured by this plant species. Due to the various possibilities for its pharmaceutical profit, numerous bioactive compounds including sesquiterpenoids, terpenoids, and flavonoids in aerial parts have been explored [5].

L. sarmentosa in Phuket is also considered to be rare and at risk of extinction due to natural disasters such as tsunamis and storms and human disturbance (e.g., coastal construction,). Since the Indian Ocean earthquake and tsunami in Thailand occurred in 2004, the number of coastal sand dune species, especially stoloniferous plants, including *L. sarmentosa*, significantly decreased [6]. Therefore, this medicinal plant has become a high-value vegetable in the local market. Currently, attempts to plant the *L. sarmentosa* in the growing media are being done. However, some problems (e.g., restriction of planting area around coastal sand) and the quality of producing plants (e.g., bitterness) have occurred [7]. This obstacle retarded the commercial exploitation of *L. sarmentosa*. In this case, plant tissue culture techniques, a tool for rapid plant propagation, can assist in the large-scale production of valuable clones in a very short time. This is not only for commercial purposes but also for its conservation.

The *in vitro* propagation of *Launaea cornuta*, an important indigenous vegetable and medicinal plant, using axillary buds as explants has been conducted [8]. In addition, direct regeneration of *L. sarmentosa* from the leaf was discovered by adding 6-benzylaminopurine (BAP) and NAA to the MS medium [9]. However, no report was noted on the morphogenic response of different hormones having an advantage for plant regeneration potential. Moreover, the effect of BA and 2,4-D on shoot and root induction of this plant was unclear. Thus, this study aimed to investigate the suitable medium for producing *in vitro* propagation *L. sarmentosa*, localize the important macromolecule, which may be related to the medicinal potential, and observe some anatomical characteristics associated with adaptation to littoral sand dunes.

Materials and methods

Plant materials

L. sarmentosa (Willd.) Kuntze leaves and stolons were collected from coastal areas in Phangnga province, Thailand. The eighth leaf from the apex and the fourth to sixth stolon counted from terminal bud explants were surface-sterilized by soaking in 70 % ethanol for 2 min followed by dipping in 1.2 % sodium hypochlorite (1.14 % (*v*/*v*) active chlorine) with 2 drops of Tween 20 for 20 min. Samples were then rinsed with sterile distilled water thrice.

Shoot induction

Leaf (0.5×0.5 cm^2), each containing a midrib vein and stolon (0.5 cm long), containing an internode were cut using sterilized scalpels. The explants were inoculated on Murashige and Skoog (MS) medium [10], 3 % sucrose, and various BA (Sigma-Aldrich Co.) concentrations (0, 1, and 2 mg/L) in combination with 2,4-D (Fluka Chemie GmbH) (0, 1, 2, and 4 mg/L). The media were adjusted to pH 5.7 with 1 N NaOH (EMSURE®) or 1 N HCl (EMSURE®) and solidified with 0.2 % phytagel (Sigma-Aldrich Co.) before autoclaving at 121 °C for 20 min. The cultures were subsequently maintained under illumination at 23 µmol m^{-2} s^{-1} by cool daylight fluorescent lamps with a photoperiod of 16-h light and 8-h darkness at 25 ± 2 °C. The experiment was performed based on a factorial experiment in a completely randomized design with 5 replications for each treatment. Each replication included 2 pieces of explants. After 4 weeks of culture, the percentages of the shoot, callus, and root formation as well as the number of shoots/explant were calculated.

Root induction

One-month-old (2.0 cm in length) individual shoots derived from stolon and leaf explants (cultured on the best shoot induction medium) were isolated from the shoot clumps and inoculated on an MS medium containing indole-3-butyric acid (IBA), 1-naphthaleneacetic acid (NAA), and indole-3-acetic acid (IAA) at different concentrations (0, 0.5, and 1 mg/L). The pH was adjusted to 5.7 before autoclaving at 121 °C for 20 min. IAA or IBA was added by filter sterilization to the autoclaved medium (60 °C). The inoculated media were maintained under a similar condition as described above for shoot induction. Each treatment consisted of 10 replications with three shoots each. After 4 weeks of culture, the percentage of root formation, number of roots per shoot, and root length were evaluated.

Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA), and the means were compared by Duncan's multiple range test with a significance level of 0.05 using the Statistical Package for the Social Sciences, version 20.

Acclimatization and transplantation

Thirty plantlets (4 weeks old) were gently washed to remove the phytagel using 1,000 mL sterilized distilled water with 2 to 3 drops of antiseptic solution (Betadine®). The acclimatized plantlets were transferred to 30 g transplant substrates (a sterilized mixture of vermiculite-peat moss-sand (1:1:1)) and planted under a controlled temperature of 25 ± 2 °C for 4 weeks. The rate of survivability was then determined. The survival acclimatized plantlets were transferred to grow in local sand. The 4-week-old acclimatized plantlets were used for histological and histochemical testing.

Histochemical observation

The eighth leaf having the central midvein (midrib)of 1-month-old *in vitro* plantlets was sectioned transversely at 5-mm thick and fixed in cryostat embedding medium. The sections were then cut (20 - 30 µm thick) with a cryostat (Leica Camera AG, Wetzlar, Germany), tested with periodic acid-Schiff (PAS)

reaction for insoluble carbohydrate distribution analysis [11], Sudan IV for lipid observation [12], and ninhydrin for protein analysis [13]. For PAS reaction, the sections were tested stained with 1 % periodic acid for 15 min, then soaked in Schiff's reagent for 15 min. Slide sections were stained with Harris Haematoxylin for 10 min. Carbohydrates were pink to purple. For lipid accumulation, samples were stained with Sudan IV for 5 - 10 min, lipid droplets were reddish brown. For protein observation, samples were stained with 0.5 % ninhydrin. Proteins were pink or red. Three plantlets were tested for each reaction. Semipermanent slides were mounted using glycerin-gelatin [12] and photographed under light microscopy (Optika B-380, Bergamo, Italy).

Results and discussion

Shoot induction

Multiple shoots were directly regenerated at the cut surface of stolon and leaf explants after inoculation in MS medium adding various concentrations of BA and 2,4-D. The percentage of shoot induction was recorded after 4 weeks of culture. Moreover, 100 % of multiple shoot formation from stolon was found in MS medium adding BA alone (**Table 1**). Shoots appeared 2 - 3 weeks after explant showing a swelling surface of stolon around the cutting region and presenting a large number of shoots at 4 weeks (**Figure 1(A)**, arrow). The highest number of shoots was presented in the MS medium supplemented with 2 mg/L BA (11.00 ± 2.91 shoots/explant). MS medium adding 1 mg/l BA and MS free plant growth regulators (PGRs) were the second and third best medium-producing shoots with 7.00 ± 2.42 and 3.80 ± 0.80 shoots/explant, respectively. Regarding leaf explants, 100 % of multiple shoot induction was found in the MS medium added with 1 and 2 mg/L of BA (**Table 1**). Several shoots were regenerated from the cutting edge of the leaf after culturing for 2 weeks (**Figure 1(B)**, arrow). The highest shoot number (24.20 ± 6.02 shoots/explant) was obtained on MS medium added with 2 mg/L, which was significantly higher than the other treatments (**Table 1**). The interaction between 2,4-D and BA in multiple shoot induction from stolon and leaf explants was tested by two-way ANOVA. Results show that BA was significant for shoot induction of stolon and leaf explants, while 2,4-D and BA interaction influence shoot induction only from leaves (**Table 2**).

The present study confirmed that only BA, one of the cytokinin types, had a significant effect on shoot induction from stolon and leaf explants of *L. sarmentosa*, and the satisfactory BA concentration was 2 mg/l. This finding was similar to the study of shoot induction in strawberry cultivars, Praratchatan No. 80, which were generated in an MS medium containing 2.0 mg/L BA [14]. The results were also in agreement with Mahesh and coworkers [9] who reported that shoot regeneration of *L. sarmentosa* from the leaf required a high concentration of cytokinin (0.5 mg/L BAP) and low concentration of auxin (0.2 mg/L NAA). However, the best concentrations and types of cytokinin were different, probably due to the size and age of the leaves used. Generally, BA was commonly applied to induce multiple shoots of various plants, e.g., chickpea (*Cicer arietinum* L.) and cotton (*Gossypium hirsutum*) [15]. In soybean (*Glycine max* L.), the combination of BA and kinetin was the most effective for multiple shoot induction [16].

The number of shoots produced from leaf explants (24.20 ± 6.01 shoots/explant) was about twice higher than stolon explants (11.00 ± 2.91 shoots/explant), indicating that the number of shoots produced may depend on the types of explants, probably due to both explants responding differently to BA. The type of organ used was one of the factors affecting shoot induction by exogenous PGRs. Leaf and stem explants of *Labisia pumila* var. *Alata.* had significantly different shoot formations [17]. Besides explant type, other factors such as endogenous PGRs and age of explants have been frequently claimed to result in specific organs [18]. In the current study, higher shoot numbers were performed by leaf explants experiment which was consistent with Tsai and To [19] who induced plant regeneration from *Wedelia chinensis* leaf. They found that younger leaves provide a higher regeneration potential than others. According to the previous study, the molecular mechanism of shoot formation was depended on multiple factors. After explants uptake of cytokinin from the media, cytokinin signaling pathway was induced. Epidermal and hypodermal cells of explants were activated to form a primordial. However, the ability of each organ responsive to signal molecule was the most importance factors [20]. Leaf developments were associated with endogenous cytokinin in several functions such as cell division, apical dominance, shoot initiation and growth, phyllotaxis, vascular bundles, leaf senescence, branching, nodulation, stress response and stimulating shoot apical meristem [21]. Thus, leaf explants might have a higher amount of endogenous cytokinin and exhibited higher number of shoot formation than stolon.

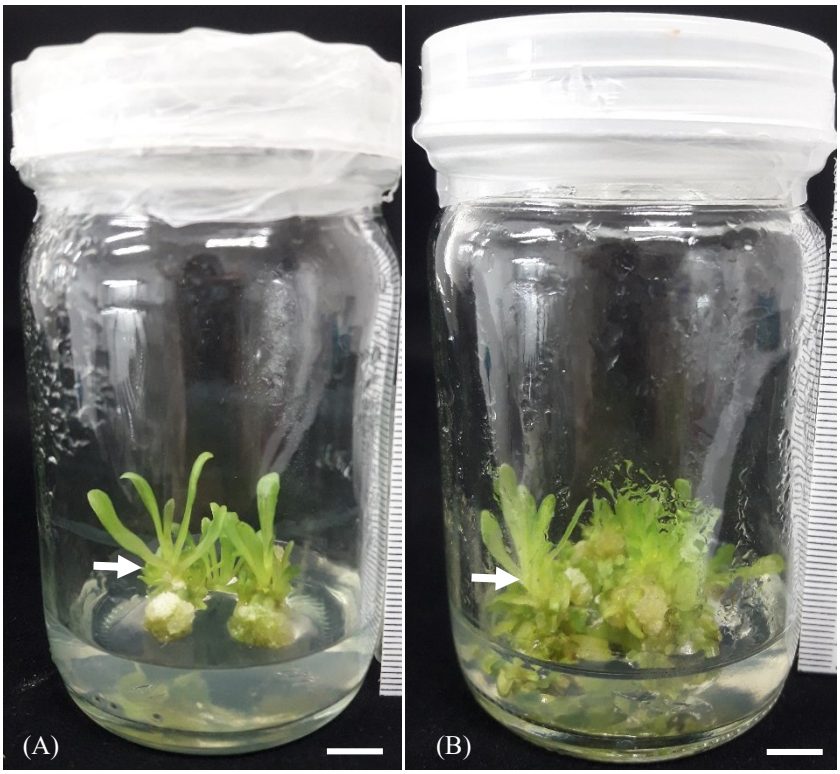

Figure 1 Multiple shoots (*arrow*) induced from (A) stolon and (B) leaf explants of *L. sarmentosa* after culturing on MS medium containing 2 mg/lL BA for 4 weeks.

Table 1 Effect of various concentrations of BA and 2,4-D on multiple shoot induction and number of shoots per explant from stolon and leaf explants of *L. sarmentosa* after culture for 4 weeks.

PGR (mg/l)		Stolon explants		Leaf explants	
BA	2,4-D	Multiple shoot induction (%)	Number of shoots/explant	Multiple shoot induction (%)	Number of shoots/explant
0	0	100.00 ± 0.00^{a}	3.80 ± 0.80^{c}	20.00 ± 12.24^{b}	2.80 ± 0.49^{b}
0	1	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}
0	2	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}
0	4	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}
1	0	100.00 ± 0.00^{a}	7.00 ± 2.42^{b}	100.00 ± 0.00^{a}	9.40 ± 4.73^{b}
1	1	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}
1	2	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}
1	4	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}
2	0	100.00 ± 0.00^{a}	11.00 ± 2.91^{a}	100.00 ± 0.00^{a}	24.20 ± 6.01^{a}
2	1	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}
2	2	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}
2	4	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}

Values shown above represent the mean ± standard error (S.E.).
Comparison of the mean values was analyzed using DMRT.
Values followed by different letters (a-d in column) indicate significant differences at $p \leq 0.05$.

Table 2 Assessment of 2,4-D and BA and their interactions on percentage of shoot produced from stolon and leaf.

Factors	Stolon				Leaf			
	df	MS	F	*P*	df	MS	F	*p*
2,4-D	2	0.00	0.00	ns	2	2666	42.667	*
BA	3	37500	6.616	*	3	20166	322.667	*
Interaction 2,4-D x BA	6	0.00	0.00	ns	6	2666	42.667	*
Error	48				48			
Total	60				60			

Df: Degree of freedom, MS: Mean square, F: F-ratio
*Indicated significant difference at $p \leq 0.05$, ns: Non-significant difference

Root induction

One-month-old shoots derived from leaf and stolon were maintained on the best medium for 4 weeks. The single shoots were separated from the shoot clumps and transferred to the root induction medium. In stolon-derived shoots, roots appeared after culture for 2 weeks. Among the three types of auxins used, IAA was the most successful PGRs in inducing root. The highest percentage of root formation (70.00 ± 11.05 %) was shown in the MS medium supplemented with 0.1 mg/L IAA (**Table 3**). The adventitious roots were also observed from each shoot, sunken in the phytagel (**Figures 2(A)** and **2(B)**). In leaf-derived shoots, the percentage of root formation and the number of roots per shoot were not significantly different among treatments (**Table 3**). However, the percentage of callus formation in all treatments added with auxin (IAA, IBA, and NAA) were significantly different in controlling the MS medium alone (**Table 3**). Callus was presented in all treatments except in the control MS medium. Two treatments including MS medium containing 0.5 and 1 mg/L NAA presented callus clumps that covered the producing roots (**Figures 2(C)** and **2(D)**). The number of roots per shoot and root length could not be evaluated from these treatments. The result confirmed that stolon- and leaf- derived shoots have the potential to produce roots by themselves. The presented study probably due to the endogenous levels of auxin in stolon- and leaf- derived shoots may be enough for root formation. Consequently, related with the study was found in some plants including oil palm (*Elaeis quineensis* Jacq), root induction from excised shoots was generated without adding exogenous auxin [22]. However, IAA and NAA adding medium showed high percentage of callus clamp, while IBA affects callus formation moderately. This result probably due to the potential of IBA. IBA has a low potential of auxin activity compared to IAA (main auxin in most plants), so it could induce only several adventitious roots and produce strong shoot growth [23]. Moreover, the callus formation was probably inhibited by IBA in the early period of root development [24]. Thus, callus clumps were lower amount comparing to other auxins.

Table 3 Effects of auxin types and concentrations on root induction from stolon and leaf-derived shoots of *L. sarmentosa.*

PGR (mg/L)			Stolon-derived shoots				Leaf-derived shoots			
IBA	IAA	NAA	%Root formation	Number of roots/shoot^{ns}	Root length	%Callus formation	%Root formation^{ns}	Number of roots/shoot^{ns}	Root length^{ns}	% Callus formation
0	0	0	57.50 ± 11.21^{ab}	6.00 ±1.81	56.51 ± 15.05^{ab}	0.00 ± 0.00^{d}	67.50 ± 11.21	8.70 ± 1.78	75.86 ± 16.09	0.00 ± 0.00^{d}
0.1	0	0	57.50 ± 8.37^{ab}	7.00 ± 2.25	58.70 ± 19.15^{ab}	2.50 ± 2.50^{d}	72.50 ± 10.17	5.40 ± 2.37	111 ± 29.82	0.00 ± 0.00^{d}
0.5	0	0	60.00 ± 9.27^{ab}	8.70 ± 2.33	66.40 ± 13.04^{ab}	20.00 ± 7.26^{cd}	55.00 ± 11.67	10.80 ± 3.03	72.2 ± 25.62	5.00 ± 3.33^{cd}
1	0	0	65.00 ± 10.67^{ab}	13.10 ± 3.84	89.10 ± 20.18^{ab}	47.50 ± 10.83^{b}	77.50 ± 10.17	8.60 ± 2.02	60.6 ± 19.51	25.00 ± 6.45^{bcd}
0	0.1	0	70.00 ± 11.05^{a}	13.00 ± 2.82	117.80 ± 24.82^{a}	17.50 ± 9.16^{cd}	75.00 ± 11.18	8.80 ± 1.86	94.35 ± 16.47	0.00 ± 0.00^{d}
0	0.5	0	57.50 ± 11.81^{ab}	12.60 ± 3.43	80.50 ± 15.17^{ab}	25.00 ± 9.86^{cd}	67.50 ± 8.37	4.60 ± 0.61	51.6 ± 15.66	32.50 ± 7.50^{abcd}
0	1	0	32.50 ± 11.21^{b}	7.60 ± 2.78	67.00 ± 21.80^{ab}	42.50 ± 13.96^{b}	77.50 ± 9.46	5.30 ± 1.13	74.6 ± 20.89	47.50 ± 10.83^{ab}
0	0	0.1	45.00 ± 11.67^{ab}	6.30 ± 1.66	52.00 ± 14.04^{b}	52.50 ± 12.61^{b}	70.00± 11.67	4.70 ± 1.92	121.01 ± 31.17	42.50 ± 12.93^{abcd}
0	0	0.5	35.00 ± 12.47^{ab}	7.20 ± 1.89	66.40 ± 25.25^{ab}	100.00 ± 0.00^{a}	62.50 ± 11.93	-	-	62.50 ± 15.47^{ab}
0	0	1	32.50 ± 12.93^{b}	6.50 ± 1.95	36.80 ± 18.78^{b}	100.00 ± 0.00^{a}	52.50 ± 13.67	-	-	70.00 ± 15.27^{a}

Data indicates mean ± standard error and treatments denoted by the same letter in a column.
ns: Non significance. Ten replicates were used per treatment.
- : Callus clump was observed.

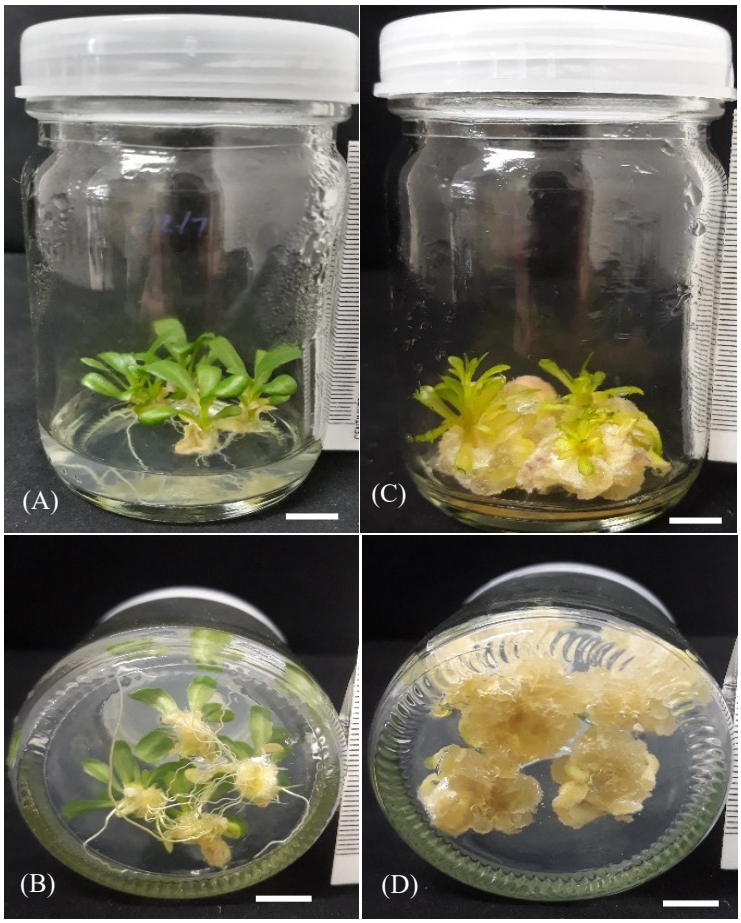

Figure 2 Roots induced from stolon-derived shoot culturing on the best root induction medium (MS medium added with 0.1 mg/L IAA) for 4 weeks (A) and (B), while dwarf shoot and callus clumps present at the base of shoots after culturing leaf-derived shoots on MS added 1 mg/L NAA (C) and (D). (A) and (C) side view, (B) and (D) bottom view; *bar* = 1 cm.

Acclimatization and transplantation

Eight-week-old *in vitro* rooted plants (from the leaf- (n = 30) and stolon- (n = 30) derived shoots) were applied in this experiment (**Figure 3(A)**). Plantlets were successfully acclimatized under controlled growth conditions. After 1 month of cultivation, both stolon- and leaf-derived plantlets were healthy and possessed 100 % survivability (**Figure 3(B**)). Plantlets grown in the greenhouse for 2 months appeared normal and did not exhibit any morphological abnormalities (**Figure 3(C**)). The present results provide the successful method for *in vitro L. sarmentosa* propagation, which will be useful to further apply both its commercial propagation and conservation approach.

(A) (B) (C)

Figure 3 *In vitro L. sarmentosa* plantlet (A), after transplantation in a mixture of sand-soil-peat moss (1:1:1) (B), and after growing in sand from primary habitats for 1 month (C). Bar = 1 cm.

Histochemical analysis

Red droplets of oil body reacted with oil red O (**Figure 4(A)**, arrow), and brown oil droplets reacted with Sudan IV (**Figure 4B**, arrow), were detected in *in vitro* leaves. Pink protein bodies (**Figure 4(C)**, arrow) and starch granules positive to the PAS reaction presented purple to blue particles (**Figure 4(D)**, arrow) were detected at the leaf mesophyll. From these results, primary substances including fat, protein, and carbohydrates were accumulated in leaves of *in vitro* plantlets. Large amounts of oil droplets were found in mesophyll cells similarly described in several species such as *Hesperozygis ringens* (Benth.) Eplinh. belonging to Family Lamiiacea [21]. Oil bodies in leaf mesophyll cells were also found in many species of the Family Asteracea, which are a significant cellular component and provide sources of commercial oils [25]. Lipids stored in the vegetative organs do not function for growth only but may produce for chemical protection against herbivores and pathogens [26]. Thus, oil droplets in *L. sarmentosa* leaves may be important in defense mechanisms that need to investigate the type and amount of lipid in future studies. However, *L. sarmentosa* habitats are coastal environments, and the presence of oil bodies in leaf mesophyll cells may be related to the adaptation of this plant. In the case of the relationship between oil bodies and environmental stresses, most triacylglycerols are combined with other lipids to form a barrier complex to protect plant organs from dehydration and UV radiation [27].

Starch accumulation was found on leaf mesophyll of *L. sarmentosa* plantlets. Generally, starch was the main photosynthesis product, providing an energy source and functioning on vegetative growth and development. Starch is also used pharmaceutical excipients due to it is one of the natural products [28]. However, the potential as pharmaceutical excipients differ in their morphology and types of starch [28]. Thus, amount and type need to identify further.

Protein droplets were also investigated in leaves of *in vitro* plantlets. Leaf protein was the precursor of medicinal substances. A large number of proteins isolated from medicinal plants presented antimicrobial and antioxidant [29]. Thus, *L. sarmentosa* leaf may be a precursor for medicinal properties, which need to be investigated further

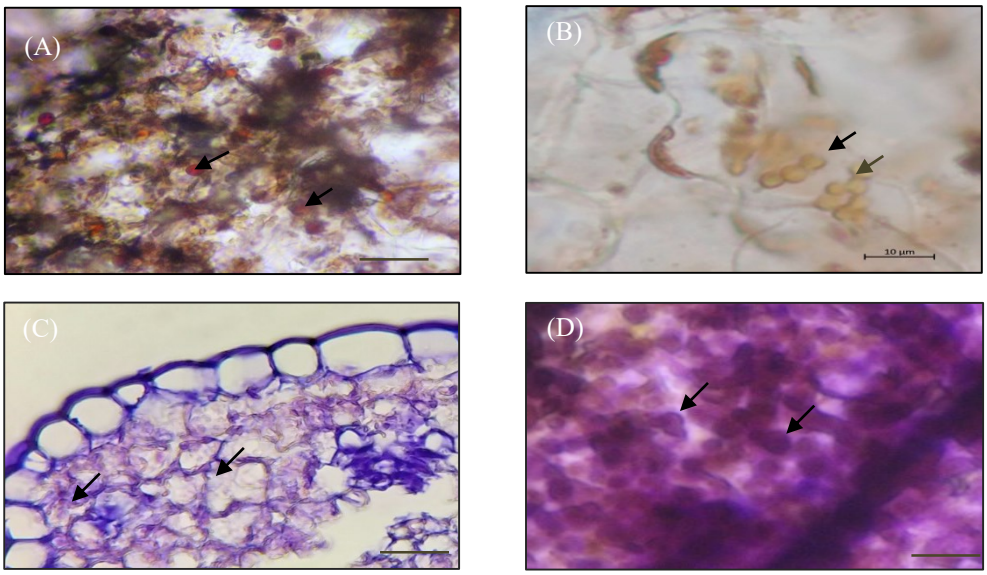

Figure 4 Red oil droplets ((A) arrow) with a positive reaction to oil red O and brown oil droplets ((B) arrows) with a positive reaction to Sudan IV were found in leaf mesophyll cells. Positive protein reaction tested by ninhydrin staining in the mesophyll cell ((C) arrows). Starch grains ((D) arrows) on the leaf were detected by PAS reactions. *Scale bar* A and D = 20 µm, *scale bar* C = 100 µm.

Conclusions

The *in vitro* propagation for *L. sarmentosa* can be summarized as follows: Shoot induction by MS medium supplemented with 2 mg/L BA in both stolon and leaf explants. Among the 3 types of auxins used, IAA was the most successful PGRs inducing root from the stolon-derived shoot, while percentage of root formation and the number of roots per shoot from the leaf-derived shoot were not significantly different among treatments. Both leaf- and stolon-derived plantlets were successfully transferred and grown in the greenhouse. The accumulation of the important primary substances (i.e., lipid, protein, and carbohydrate) were found in the leaves of in vitro plantlets can serve as a precursor for food and pharmaceutical supply in the future. This research may be beneficial for large-scale production of *L. sarmentosa* serving the demand of the pharmaceutical industry

Acknowledgements

This work was supported by the Faculty of Technology and Environment, Prince of Songkla University, Phuket Campus, Thailand under Grant (number TAE6304132S). We would like to thank Assist. Prof. Dr. Charan Leeratiwong, Division of Biological Science (Biology), Faculty of Science, Prince of Songkla University for plant identification. Plant collection was carried out by Assist. Prof. Dr. Phuripong Meksuwan, Phuket Rajabhat University, and plant microtechnique by assistant Mr. Seree Niyomdecha.

References

[1] H Koyama, S Bunwong, P Pornpongrungrueng and DJN Hind. *Flora of Thailand*. Vol 13. Part 2. The Forest Herbarium, Royal Forest Department, Bangkok, 2016, p. 349-350.
[2] L Llorens, L Gil and H Boira. Reproductive biology of *Launaea cervicornis*: A key stone species of the Balearic coastal shrublands. *Flora Morphol. Distribution Funct. Ecol. Plants* 2009; **204**, 456-62.
[3] AB Cheriti, M Belboukhari, N Belboukhari and H Djeradi. Phytochemical and biological studies on *Launaea* Cass. Genus (Asteraceae) from Algerian Sahara. *Curr. Top. Phytochem*. 2012; **11**, 67-80.
[4] GS Raju, MR Moghal, MS Hossain, M Hassan, M Billan, SK Ahamed and M Rana. Assessment of pharmacological activities of two medicinal plant of Bangladesh: *Launeae sarmentosa* and *Aegialitis rotundifolia* roxb in the management of pain, pyrexia and inflammation. *Biol. Res.* 2014; **47**, 55.
[5] Y Salih, CR Harisha, VJ Shukla and R Acharya. Pharmacognostical evaluation of *Launaea sarmentosa* (Willd.) Schultz-bip. ex Kuntze root. *Ayu* 2014; **34**, 90-4.

[6] D Hayasaka, K Goka, W Thawatchai and K Fujiwara. Ecological impacts of the 2004 Indian Ocean tsunami on coastal sand-dune species on Phuket Island, Thailand. *Biodivers. Conserv.* 2012; **21**, 1971-85.
[7] C Suksamran, P Somnak and S Samakkit. Effect of calcium oxide on growth and sensory quality of Linharn (*Lanaea sarmentosa*). *Thai Sci. Tech. J.* 2020; **29**, 148-56.
[8] F Ambajo and JM Matheka. Micropropagation of *Launaea cornuta* - an important indigenous vegetable and medicinal plant. *Afr. J. Biotechnol*. 2016; **15**, 1726-30.
[9] A Mahesh, D Thangadurai and G Melchias. Rapid *in vitro* plant regeneration from leaf explants of *Launaea sarmentosa* (Willd.) Schultz-bip. ex Kuntze. *Biol. Res*. 2012; **45**, 131-6.
[10] T Murashige and F Skoog. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plantarum.* 1962; **15**, 473-97.
[11] E Strasburger. *Handbook of practical botany*. George Allen and Company, London, 1913, p. 16-25.
[12] WA Jensen. *Botanical histochemistry: Principles and practice*. WH Freeman and Co, San Francisco, 1962, p. 175-256.
[13] DA Johansen. *Plant microtechnique*. McGraw-Hill Publishing, London, 1940, p. 68-94.
[14] M Sirijan, M Pipattanawong and P Chaiprasart. Effect of 1-naphthalene acetic acid and 6-benzyladenine on micropropagation of strawberry cultivar 'Praratchatan No.80'. *Agr. Nat. Resour*. 2019; **53**, 355-63.
[15] JL Morre, HR Permingeat, MV Romagnoli, CM Heisterborg and RH Vallejos. Multiple shoot induction and plant regeneration from embryonic axes of cotton. *Plant Cell Tissue Org.* 1998; **54**, 131-6.
[16] P Mangene, PW Mokwala and RV Nikolova. *In vitro* multiple shoot induction in Soybean. *Int. J. Agr. Biol*. 2015; **17**, 838-42.
[17] APK Ling, KP Tan and S Hussein. Comparative effects of plant growth regulators on leaf and stem explants of *Labisia pumila* var. alata. *J Zhejiang Univ. Sci. B* 2013; **14**, 621-31.
[18] SS Bhojwani and MK Razdan. *Plant tissue culture: Theory and practice*. Elsevier, Amsterdam, The Netherlands, 1996, p. 95-125.
[19] YT Tsai and KY To. Plant regeneration from leaf explants of the medicinal herb *Wedelia chinensis. Horticulture* 2021; **7**, 407.
[20] H Motte, D Vereecke, D Geelen and S Werbrouck. The molecular path to in vitro shoot regeneration. *Biotechnol Adv*. 2014; **32**, 107-21.
[21] W Wu, K kang and H Wei. The diverse roles of cytokinins in regulating leaf development. *Horticult. Res*. 2021; **8**, 118.
[22] A Hilae and S Te-chato. Effects of carbon sources and strength of MS medium on germination of somatic embryos of oil palm (*Elaeis quineensis* Jacq.). *Songklanakarin J. Sci. Tech.* 2005; **27**, 629-35.
[23] E Epstein and J Ludwig-Müller. Indole-3-butyric acid in plants: Occurrence, synthesis, metabolism and transport. *Physiol. Plantarum*. 1993; **88**, 382-9.
[24] M Štefančič, F Štampar and G Osterc. Influence of IAA and IBA on root development and quality of Prunus 'GiSelA 5' leafy cuttings. *Hortscience* 2005; **40**, 2052-2055.
[25] CG Pinheiro, JMS Oliveira and BM Heinzmann. Histological and histochemical characterization of leaves and petals of the endangered native Brazilian species *Hesperozygis ringens* (Benth.) Epling. *Flora* 2018; **239**, 1-10.
[26] NR Lersten, AR Czlapinski, JD Curtis, R Freckmann and HT Horner. Oil bodies in leaf mesophyll cells of angiosperms: overview and a selected survey. *Am. J. Bot*. 2006; **93**, 1731-9.
[27] TL Shimada, Y Takano, T Shimada, M Fujiwara, Y Fukao, M Mori, Y Okazaki, K Saito, R Sasaki, K Aoki and I Hara-Nishimura. Leaf oil body functions as a subcellular factory for the production of a phytoalexin in Arabidopsis. *Plant Physiol*. 2014; **164**, 105-18.
[28] KD Chapman, JM Dyer and RT Mullen. Biogenesis and functions of lipid droplets in plants. *J. Lipid Res.* 2012; **53**, 215-26.
[29] OO Kunle. *Starch source and its impact on pharmaceutical applications*. *In*: M Emeje (Ed.). Chemical properties of starch. IntechOpen, London, 2019.