Agr. Nat. Resour. 55 (2021) 431–439

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Chitosan elicitor stimulation of *in vitro* growth and *ex vitro* acclimatization of *Lantana camara* L.

Varaporn Veraplakorn†, Sanya Kudan*,†

Department of Biotechnology, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand

Article Info

Article history:
Received 8 February 2021
Revised 16 May 2021
Accepted 3 June 2021
Available online 30 June 2021

Keywords:
Chitosan,
Lantana,
Natural elicitor,
Pathogen,
Transplantation

Abstract

The efficiency of chitosan on *in vitro* growth and *ex vitro* acclimatization of lantana (*Lantana camara* L.) was compared to thidiazuron (TDZ) or indole-3-butyric acid (IBA). It was found that chitosan promoted *in vitro* shoot regeneration. Lantana shoots cultured on Murashige and Skoog (MS) medium supplemented with 2.8 mg/L TDZ and 0.1 mg/L high molecular weight chitosan (HMC) produced the highest shoot number of 14.2 ± 6.1 shoots with long lengths of the main shoot and axillary shoot of 3.95 ± 0.96 cm and 0.29 ± 0.12 cm, respectively. Plantlets cultured on MS medium supplemented with 0.1–0.5 mg/L low molecular weight chitosan (LMC) or HMC had root induction of 73.7–94.7% and axillary shoot proliferation of 50.0–61.6%. After transplanting, the plantlets were treated with foliar and soil applications of 0.6 mg/L HMC of 5 mL three times weekly. The chitosan treatment increased the survival percentage and growth of plantlets *ex vitro*. Plantlets obtained from the MS medium supplemented with 1.5 mg/L IBA and 0.3 mg/L HMC or with only 0.3 mg/L HMC had high survival rates of 82.0% and 81.0%, respectively. The highest fresh and dry weights were for the plantlets derived from the MS medium supplemented with 1.5 mg/L IBA and 0.3 mg/L HMC. Chitosan treatment after transplanting could increase the survival percentage. In addition, the highest biomass resulted from applying both *in vitro* and *ex vitro* treatments. This suggested chitosan could be a stimulator for lantana shoot growth *in vitro* and for acclimatization *ex vitro*.

Introduction

Chitosan is a natural elicitor and is a polysaccharide derived from the shells of crustaceans and other sources such as insects, fish scales and fungi. Chitosan can be made soluble through alkali or enzymatic deacetylation (Kaczmarek et al., 2019). Chitosan has been reported regarding numerous biological responses in plants depending on it's structure and concentration, the plant species and the developmental stage (Asghari-Zakaria et al., 2009; Baque et al., 2012;

† Equal contribution.

* Corresponding author.
E-mail address: kudansan54@gmail.com (S. Kudan)

https://doi.org/10.34044/j.anres.2021.55.3.13

Malerba and Cerana, 2016). Chitosan plays an essential role in plant resistance to pathogens and in defense mechanisms (Hadrami et al., 2010; Pichyangkura and Chadchawan, 2015; Malerba and Cerana, 2016). Under stress conditions, chitosan enhances many plant defense mechanisms such as lignification, ion flux variations, cytoplasmic acidification, membrane depolarization and protein phosphorylation, chitinase and glucanase activation and phytoalexin biosynthesis (Hadrami et al., 2010). There have been many reports that chitosan showed positive effects on the growth and yields of many plant species, such as *Dendrobium phalaenopsis* (Nge et al., 2006), *Solanum tuberosum* (Kowalski et al., 2006; Asghari-Zakaria et al., 2009), *Vaccinium corymbosum* (Cabrera et al., 2010) and freesia (Salachna and Zawadzińska, 2014). Applying chitosan increased stomatal closure and abscisic acid content in treated leaves, which could reduce the transpiration rate which increases the survival rate of plantlets and increases biomass production or yield (Hadrami et al., 2010).

In addition, the molecular weight of chitosan was the most critical factor affecting its biological activity (Kulikov et al., 2006; Li et al., 2011). High molecular weight chitosan (HMC) is readily soluble in dilute acids but insoluble in water since it contains a considerably high molecular weight (500–1,000 kDa). Increasing water solubility occurred when HMC was degraded by enzymatic depolymerization into low molecular weight compounds in the range 1–20 kDa (Shadia et al., 2008; Yao et al., 2008; Zhang et al., 2010; Xie et al., 2011). However, chitosan has considered in plant growth stimulant efficiency regarding the antagonistic effect between HMC and low molecular weight chitosan (LMC), as HMC induced higher plant growth and freesia weight than for the LMC treatment (Salachna and Zawadzińska, 2014). On the other hand, LMC could increase the *in vitro* fresh weight, radicle length and total carbon and nitrogen contents of *Arabidopsis*, while HMC produced contrary responses (Winkler et al., 2017).

Lantana camara L. is a flowering ornamental plant belonging to the family Verbinaceae that has long been quoted as a traditional medicinal plant containing highly potent allelopathic effects (Reddy, 2013; Mishra, 2015). Lantana has become famous in the development of modern drugs and as an unusual natural source for the development of natural herbicides (Kalita et al., 2012; Reddy, 2013; Veraplakorn, 2017). Interestingly, extracts of lantana in vitro tissue have an allelopathic effect on many plant species, such as *Salvinia molesta* Mitchell (Saxena et al., 2013) and *Brassica campestris, Ipomoea aquatic* Forsk, *Sorghum bicolor* L. and *Zea mays* L. (Veraplakorn, 2017, 2018). High efficiency of lantana shoot production was recommended by using Murashige and Skoog (MS) medium supplemented with cytokinins such as N6-benzyl adenine (BA), kinetin, zeatin and thidiazuron (TDZ) (Affonso et al., 2007; Waoo et al., 2013; Samani et al., 2014; Veraplakorn, 2016, 2017). For root induction, a lantana rooted plantlet was induced using MS medium supplemented with indole-3-butyric acid (IBA), which was a better supplement than 1-naphthalene acetic acid (NAA) (Veraplakorn, 2016, 2017). Many plant species need some specific acclimatization before transferring to the greenhouse or field environment, as a substantial number of *in vitro* plantlets have not survived under *ex vitro* conditions (Hazarika, 2003). Interestingly, chitosan was indicated to improve the acclimatization of some species such as *Solanum tuberosum* L. (Kowalski et al., 2006; Asghari-Zakaria et al., 2009) and *Grammatophyllum scriptum* (Pitoyo et al., 2015).

Tissue culture is a classical technique in industrial plant propagation, especially for bioactive compound production (Ramirez-Estrada et al., 2016). It has been shown that elicitation in the cell culture system increased the production of secondary metabolites such as taxanes, ginsenosides, aryltetralin lignans and other types of polyphenols (Ramirez-Estrada et al., 2016). Applying chitosan as an elicitor in plant cell culture was reported to increase podophyllotoxin production in *Lilium album*, paclitaxel production in *Taxus chinensis* and phenylethanoid glycoside accumulation in *Cistanche deserticola* (Ramirez-Estrada et al., 2016).

However, rooted shoots of lantana without chitosan treatment cannot be successfully transferred to natural condition according to the result presenting in this report. This may have been due to the poor quality of the lantana plantlets (thin leaves and stem) as well as their sensitivity to microbial activity. Using chitosan as a natural elicitor in plant production is beneficial for a sustainable future because of its quality and it is safe and eco-friendly (Kurita, 2006). Lantana plantlets from *in vitro* culture would be appropriate as a natural resource for bioactive compounds and allelochemicals production. Chitosan may help to increase the amounts of valuable active ingredients. Notably, there has been no reporting of success regarding lantana transplantation to *ex vitro* conditions. Hence, the current research aimed to investigate the effect of HMC and LMC on *in vitro* growth stimulation and *ex vitro* acclimatization on lantana plant production.

Materials and Methods

Explant preparation

In vitro shoots of lantana were proliferated by culturing on MS medium supplemented with 1.0 mg/L BA and transferring onto the fresh medium regularly at four-weekly intervals for 3 mth. Each single shoot tip was subcultured onto MS medium without plant growth regulator for 7 wk to prepare the explants for the subsequent experiments. The incubation conditions were a 16 hr photoperiod (40.0 μmol/m^2/s) at 25±2°C.

Chitosan preparation

HMC prepared from shrimp shell with an approximately 97% degree of deacetylation (DD) was purchased from Ta Ming Enterprises Ltd, Thailand. LMC was prepared by using 10 milliunits (mU) of chitinase to hydrolyze 10 mg of chitosan and incubating overnight at 50°C. The chitinase was sourced from *Bacillus licheniformis* SK-1, with an activity of 2.0 U/mg (Kudan and Pichyangkura, 2009).

Gel permeation chromatography (GPC; Waters 600E; USA) was used to determine the molecular weight of the HMC and LMC. An ultrahydrogel 1 column (flow rate of 0.6 mL/min) was used at 30°C with pullulans standard molecular weight 5.9–708 kDa. Each chitosan sample (2 mg/mL) was dissolved in 0.5 M acetic acid and 0.5 M sodium acetate(eluent). The eluent and samples were filtered using a nylon membrane with a pore size of 0.45 μm.

Investigation for chitosan concentration as lantana elicitor

Single shoots of lantana (each 1.0 cm long) were cultured on MS medium supplemented with HMC or LMC at concentrations of 1.0 mg/L, 2.0 mg/L, 3.0 mg/L, 4.0 mg/L, 5.0 mg/L, 10.0 mg/L, 15.0 mg/L and 20.0 mg/L for 4 wk. The characteristics of the shoots were recorded. The range in chitosan concentration identified as suitable for examining shoot production and root induction was then used in the next experiments.

Effect of chitosan on lantana shoot growth compared to thidiazuron

Single shoots of 1.0 cm in length were cultured on MS medium supplemented with only 2.8 mg/L TDZ and 2.8 mg/L TDZ combined with HMC or LMC at different concentrations of 0.1 mg/L, 0.5 mg/L and 1.0 mg/L. The shoot number, shoot length and shoot characteristics were recorded after 4 wk.

Effect of chitosan on lantana root induction compared to indole-3-butyric acid

Single shoots of 1.0 cm in length were cultured on hormone-free MS medium as a control treatment. Shoots were also cultured on the MS medium supplemented with 1.5 mg/L IBA and the MS medium supplemented with HMC or LMC at different concentrations of 0.1 mg/L, 0.3 mg/L or 0.5 mg/L. The percentage of shoots produced root, numbers of roots, and their length and shoot height were recorded after 4 wk, while the percentage of shoot proliferation and the fresh weight and dry weight of plantlets were recorded after 6 wk.

Chitosan application for acclimatization ex vitro

The *in vitro* healthy plantlets with expanded green leaves and long roots were collected from three media: 1) the MS medium supplemented with 1.5 mg/L IBA, 2) the MS medium supplemented with 1.5 mg/L IBA and 0.3 mg/L HMC and 3) the MS medium supplemented with 0.3 mg/L HMC. A sample of 100 plantlets from each of the three media were examined for the effect of chitosan on acclimatization *ex vitro*. Another 100 plantlets from the first medium were used as a control treatment which was grown without any foliar and soil application. The plantlets were exposed to greenhouse condition by loosening the bottle caps for 1 wk and subsequently, they were transferred to bags containing sterilized perlite and vermiculite (1:1 weight per weight). foliar and soil applications were conducted for the chitosan treatment with 0.6 mg/L HMC of 5 mL in the morning (0800–0900 hours) three times a week. All plantlets were kept for 2 wk in a misting chamber with intermittent mist (70–80% relative humidity). After that, all plants were grown in 15 cm × 30 cm growing bags containing soil, coconut husk and rice husk ash (1:1:1 weight per weight per weight) and transferred to the open bench. The survival percentage was recorded after 4 wk.

In all experiments, the treatments were assigned to experimental units at random with 10 replicates.

Statistical analysis

Data were analyzed using analysis of variance. Whenever F-values were significant ($p< 0.05$), the significant differences between means were tested using Tukey's b multiple range test. All data were analyzed using the PASW Statistics 18 software (SPSS Inc.; Quarry Bay, Hong Kong). Results were presented as mean ± SD.

Results

Chitosan characterization

The molecular weight of the HMC and the LMC were determined using GPC, with values of 604 kDa (604,000 g/mol) with 97% DD and 29.8 kDa (29,800 g/mol) with >97% DD, respectively.

Determination of chitosan concentrations as lantana elicitor

All the shoots cultured on the media containing chitosan (HMC or LMC) in the range 1.0–20.0 mg/L were retarded when the period was longer than 2 wk. A concentration of chitosan over 1.0 mg/L inhibited shoot growth and produced necrotic leaves. At 4 wk, the shoot was completely inhibited and displayed browning leaves and stem (Fig. 1). Consequently, the maximum concentration of 1.0 mg/L chitosan was applied to determine lantana shoot proliferation in the next experiment.

Fig.1 Stunted shoot of lantana which was cultured on Murashige and Skoog medium supplemented with chitosan 1.0 mg/L for 2 wk

Effect of chitosan on shoot promotion

The highest shoot number (14.2 ± 6.1) was derived from MS medium supplemented with 2.8 mg/L TDZ and 0.1 mg/L HMC which was not significantly different from the shoots (11.4 ± 2.7) produced from the medium supplemented with only 2.8 mg/L TDZ. The media supplemented with only 2.8 mg/L TDZ or 2.8 mg/L TDZ and 0.1 mg/L HMC or LMC could induce no significant difference in the shoot length (Table 1). Shoots derived from the TDZ medium produced a long main shoot (4.90 ± 0.73 cm) with a short axillary shoot (0.18 ± 0.08 cm) while shoots from 2.8 mg/L TDZ and 0.1 mg/L HMC produced a long length for both the main shoot (3.95 ± 0.96 cm) and axillary shoot (0.29 ± 0.12 cm). In addition, the medium supplemented with 2.8 mg/L TDZ and 0.1 mg/L LMC also produced a long main shoot (3.70 ± 1.14 cm) with a long axillary shoot (0.23 ± 0.14 cm), as shown in Table 1 and Figs. 2A and 2B. Regardless of molecular weight, the medium supplemented with a high concentration of chitosan produced low shoot numbers and short shoot lengths. Adding 1.0 mg/L HMC produced the lowest shoot number (4.4 ± 1.3) with the lowest length of main shoots (1.74 ± 0.02 cm) and the shortest axillary shoot (0.11 ± 0.03 cm), as shown in Table 1 and Fig. 2C. These results suggested using a concentration of chitosan lower than 1.0 mg/L for root induction of lantana in the next experiment.

Table 1 Chitosan in combination with thidiazuron (TDZ) compared to TDZ for shoot multiplication of *Lantana camara* L. after 4 wk

Medium	Shoot number	Shoot length (cm)	
		Main shoot	Axillary shoot
2.8 mg/L TDZ	11.4 ± 2.7^{ab}	4.90 ± 0.73^{a}	0.18 ± 0.08^{ab}
2.8 mg/L TDZ + 0.1 mg/L LMC	8.8 ± 5.3^{bc}	3.70 ± 1.14^{ab}	0.23 ± 0.14^{ab}
2.8 mg/L TDZ + 0.5 mg/L LMC	5.8 ± 4.6^{c}	3.30 ± 1.67^{b}	0.17 ± 0.07^{ab}
2.8 mg/L TDZ + 1.0 mg/L LMC	6.1 ± 1.8^{bc}	1.95 ± 0.55^{c}	0.23 ± 0.11^{ab}
2.8 mg/L TDZ + 0.1 mg/L HMC	14.2 ± 6.1^{a}	3.95 ± 0.96^{ab}	0.29 ± 0.12^{a}
2.8 mg/L TDZ + 0.5 mg/L HMC	8.8 ± 4.9^{bc}	3.85 ± 0.88^{ab}	0.26 ± 0.21^{ab}
2.8 mg/L TDZ + 1.0 mg/L HMC	4.4 ± 1.3^{c}	1.74 ± 0.02^{c}	0.11 ± 0.03^{b}

TDZ = thidiazuron; LMC = low molecular weight chitosan; HMC = high molecular weight chitosan
Mean ± SD in same column with different lowercase superscripts are significantly (p< 0.05) different.

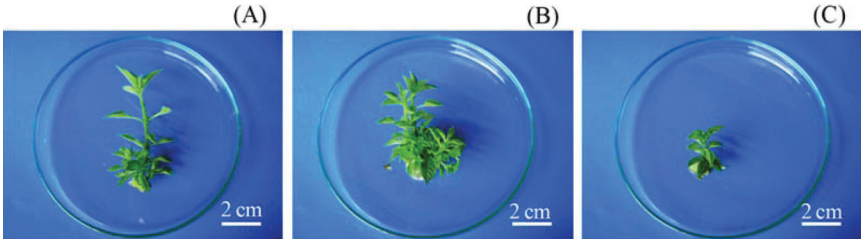

Fig. 2 *Lantana camara* L. shoot production on Murashige and Skoog medium supplemented with: (A) 2.8 mg/L thidiazuron (TDZ); (B) 2.8 mg/L TDZ and 0.1 mg/L high molecular weight chitosan (HMC); (C) 2.8 mg/L TDZ and 1.0 mg/L HMC

Effect of chitosan on root induction

The MS medium alone produced 80.0% of root induction (Table 2 and Fig. 3A). The highest percentage (100.0%) of root induction was for MS medium supplemented with 1.5 mg/L IBA, which produced the highest root number (9.6 ± 3.6), as shown in Table 2 and Fig. 3B. The media supplemented with chitosan had low percentages of root induction (73.7–94.7%) and low root numbers (3.9 ± 1.1–4.9 ± 1.4) which were not significantly different from the MS alone treatment (Table 2 and Fig. 3C). The root length was not significantly different in all media. The plantlets cultured on the 1.5 mg/L IBA medium had a greater shoot height but this was not significantly different from the other treatments except for the medium supplemented with 0.5 mg/L LMC (Table 2).

The MS medium or the MS medium supplemented with IBA produced only a single shoot with a long internode (Figs. 4A and 4B). On the contrary, plantlets of lantana cultured on the media supplemented at any of the chitosan concentrations had axillary shoot regeneration (Fig. 4C). After prolonged culturing for 6 wk, the axillary shoot proliferation was high (50.0–61.6%) in all chitosan treatments (Table 3). All treatments produced no significant differences in shoot fresh and dry weight for the growth of plantlets. Notably, the root weight of the plantlets derived from the MS medium supplemented with 1.5 mg/L IBA significantly differed from all chitosan treatments (Table 3).

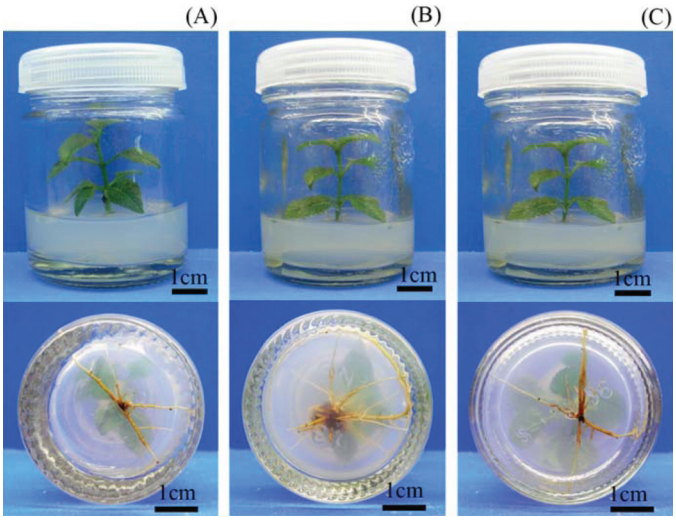

Fig. 3 *Lantana camara* L. root induction on Murashige and Skoog medium: (A) without supplement; (B) supplemented with 1.5 mg/L indole-3-butyric acid; (C) supplemented with 0.3 mg/L high molecular weight chitosan

Table 2 Chitosan treatments compared to indole-3-butyric acid (IBA) on shoot and root parameters of *Lantana camara* L. after 4 wk

Medium	Shoot-produced root (%)	Root number (root/shoot)	Root length (cm)	Shoot height (cm)
MS	80.0	3.7 ± 2.9^{b}	1.1 ± 0.7^{a}	2.2 ± 1.0^{ab}
1.5 mg/L IBA	100.0	9.6 ± 3.6^{a}	0.7 ± 0.5^{a}	2.8 ± 1.0^{a}
0.1 mg/L LMC	81.3	4.9 ± 1.4^{b}	1.6 ± 1.6^{a}	2.5 ± 0.7^{ab}
0.3 mg/L LMC	94.7	4.1 ± 0.7^{b}	1.3 ± 0.5^{a}	2.1 ± 0.7^{ab}
0.5 mg/L LMC	75.0	4.0 ± 1.2^{b}	1.0 ± 0.5^{a}	1.8 ± 0.5^{b}
0.1 mg/L HMC	84.2	4.5 ± 1.0^{b}	1.6 ± 1.3^{a}	2.5 ± 0.6^{ab}
0.3 mg/L HMC	73.7	3.9 ± 1.1^{b}	1.4 ± 0.9^{a}	2.2 ± 0.7^{ab}
0.5 mg/L HMC	94.4	4.4 ± 1.8^{b}	1.1 ± 0.6^{a}	1.9 ± 0.7^{ab}

MS = Murashige and Skoog; LMC = low molecular weight chitosan; HMC = high molecular weight chitosan
Mean ± SD in same column with different lowercase superscripts are significantly (p< 0.05) different.

Table 3 Shoot proliferation of *Lantana camara* L. under chitosan treatments compared to indole-3-butyric acid (IBA) after 6 wk

Medium	Axillary shoot proliferation (%)	Fresh weight (mg)		Dry weight (mg)	
		Shoot	Root	Shoot	Root
MS	0.0	289.4 ± 108.7[a]	52.3 ± 20.3[b]	53.9 ± 11.7[a]	6.8 ± 2.7[b]
1.5 mg/L IBA	0.0	368.1 ± 121.4[a]	104.0 ± 49.2[a]	51.6 ± 15.9[a]	13.1 ± 5.6[a]
0.1 mg/L LMC	50.0	274.5 ± 139.4[a]	36.3 ± 23.9[b]	42.5 ± 16.4[a]	4.3 ± 2.6[b]
0.3 mg/L LMC	50.0	253.4 ± 58.9[a]	43.1 ± 27.0[b]	42.1 ± 16.4[a]	4.9 ± 2.7[b]
0.5 mg/L LMC	61.6	293.6 ± 60.5[a]	41.4 ± 14.4[b]	46.4 ± 9.0[a]	7.0 ± 1.8[b]
0.1 mg/L HMC	50.0	298.7 ± 72.0[a]	48.1 ± 45.3[b]	48.6 ± 10.6[a]	7.8 ± 3.8[b]
0.3 mg/L HMC	52.6	299.4 ± 103.9[a]	40.4 ± 23.9[b]	49.4 ± 13.8[a]	7.2 ± 3.1[b]
0.5 mg/L HMC	50.0	324.2 ± 96.1[a]	40.8 ± 23.2[b]	49.9 ± 12.3[a]	6.5 ± 3.6[b]

MS = Murashige and Skoog; IBA = indole-3-butyric acid; LMC = low molecular weight chitosan; HMC = high molecular weight chitosan
Mean ± SD in same column with different lowercase superscripts are significantly ($p< 0.05$) different.

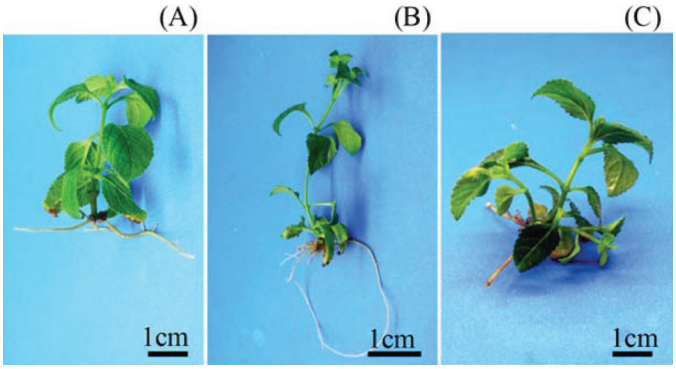

Fig. 4 Plantlets of *Lantana camara* L. after culturing for 4 wk: (A) healthy plantlet cultured on Murashige and Skoog (MS) medium; (B) single shoot with high number of roots cultured on MS medium supplemented with 1.5 mg/L indole-3-butyric acid; (C) axillary shoot proliferation cultured on 0.3 mg/L high molecular weight chitosan

Ex vitro survival

Plantlets of lantana without a foliar and soil application of chitosan could not survive (Table 4). Plantlets derived from the MS medium supplemented with 1.5 mg/L IBA and 0.3 mg/L HMC or only 0.3 mg/L HMC, which were treated with foliar and soil applications consisting of 5 mL of 0.6 mg/L HMC after transplanting, had high survival rates of 82.0% and 81.0%, respectively. Plantlets obtained from 1.5 mg/L IBA alone had the lowest survival percentage of 38.0%. The shoot and root fresh weights of plantlets from the medium supplemented with only 0.3 mg/L HMC were not significantly different from plantlets cultured on the medium supplemented with only 1.5 mg/L IBA (Table 4). Regarding plantlet growth, the highest shoot and root weights (which were nearly two times higher than the other treatments) were for the plantlets cultured on the medium supplemented with 1.5 mg/L IBA and 0.3 HMC. These plantlets were healthy after growing under natural conditions for 8 wk (Fig.5).

Fig. 5 Healthy plantlets of *Lantana camara* L. under natural conditions after transplanting for 8 wk

Table 4 Plantlet of *Lantana camara* L. from *in vitro* grown with or without the foliar treatment under greenhouse conditions for 8 wk

Treatment	Survival rate (%)	Fresh weight (mg)		Dry weight (mg)	
		Shoot	Root	Shoot	Root
1.5 mg/L IBA	0.0	-	-	-	-
1.5 mg/L IBA + Foliar	38.0	362.1 ± 116.5[b]	114.6 ± 40.5[b]	33.9 ± 12.4[b]	8.3 ± 2.8[b]
1.5 mg/L IBA + 0.3mg/L HMC +Foliar	82.0	621.9 ± 143.6[a]	182.8 ± 61.4[a]	60.1 ± 13.5[a]	12.1 ± 4.0[a]
0.3 mg/L HMC + Foliar	81.0	376.7 ± 97.6[b]	75.5 ± 16.3[b]	33.6 ± 5.9[b]	5.0 ± 1.1[c]

IBA = indole-3-butyric acid; HMC = high molecular weight chitosan
Mean ± SD in same column with different lowercase superscripts are significantly ($p< 0.05$) different.

Discussion

The application of chitosan has been reported to promote the growth of many plant species including *Solanum tuberosum* (Asghari-Zakaria et al., 2009), *Vaccinium corymbosum* (Cabrera et al., 2010), freesia (Salachna and Zawadzińska, 2014), *Ipomoea purpurea* (Acemi et al., 2018) and *Serapiasvomeracea* (Burm.f.) Briq. (Acemi, 2020). However, the appropriate conditionsfor chitosan application depended on the chitosan structure and concentration, as well as the plant species and the developmental stage (Asghari-Zakaria et al., 2009; Baque et al., 2012; Malerba and Cerana, 2016; Acemi, 2020). Mehrabi et al. (2018) classified chitosan into three groups based on molecular weight, LMC (<150 kDa), medium molecular weight chitosan (MMC) (150–700 kDa) and HMC (>700 kDa). In contrast, Vila et al. (2004) mentioned chitosan with 23 k Daand 38 kDa as LMC and with 70 kDa as HMC. In the current study, 604 kDa chitosan was classified as HMC. Comparing LMC and HMC, HMC had a greater effect on lantana growth than LMC. The MS medium supplemented with 0.1 mg/L HMC produced a significantly higher shoot number of shoots (14.2 ± 6.1) with 48.1 mg root fresh weight while the MS medium supplemented with 0.1 mg/L LMC produced a significantly lower shoot number of shoots (8.8 ± 5.3) with 36.3 mg root fresh weight. LMC had an inversely proportional effect compared to HMC for the growth of *Serapias vomeracea* (Acemi, 2020). Increased lignification was reported as one reason for promoting shoots (Bayraktar et al., 2016). Furthermore, the seedling growth of soybean increased when the seeds had been soaked in HMC(>1,000 kDa) according to Lee et al. (2005). Chitosan plays an important role in enhancing growth and development by auxin biosynthesis (Ahmadi and Shariatpanahi, 2015; Lopez-Moya et al., 2017; Acemi, 2020). Plant height and the numbers of shoots and leaves increased following HMC treatment on freesia corms (Salachna and Zawadzińska, 2014). HMC may induce growth by increasing the chlorophyll content, resulting from enhanced nutrients uptake (Salachna and Zawadzińska, 2014). Van et al. (2013) suggested that applying chitosan with a high molecular weight (600 kDa) could cause effects on coffee seedlings, with treated seedling shaving increased contents of nitrate, phosphorus and potassium in the leaves as evidence that net photosynthesis had increased.

Regarding lantana shoot induction, the MS medium supplemented with 1.0 mg/L chitosan (the highest concentration applied in the current study), produced a low shoot number with the shortest shoot length. There have been other reports of the inhibitory effect of a high concentration chitosan. For example, increasing the chitosan concentration and exposure time reduced the root fresh and dry weight of ginseng (Baque et al., 2012) as well as root elongation of *Serapias vomeracea* (Burm.f.) Briq. (Acemi, 2020). Chitosan could be suggested as a shoot growth stimulator for lantana. This would be consistent with its effect on some other plant species where chitosan has induced *in vitro* shoot growth, such as *Dendrobium phalaenopsis* (Nge et al., 2006), *Vaccinium corymbosum* (Cabrera et al., 2010), and *Solanum tuberosum* (Kowalski et al., 2006; Asghari-Zakaria et al., 2009).

There was 100% root induction on healthy plantlets following the application of the MS medium supplemented with only 1.5 mg/L IBA. This result was consistent with lantana root induction reported by Veraplakorn (2016, 2017). In addition, chitosan has been reported to improve the plantlet quality in many plant species, such as *Dendrobium phalaenopsis* (Nge et al., 2006) and *Solanum tuberosum* (Kowalski et al., 2006; Asghari-Zakaria et al., 2009). Chitosan treatment has been suggested for improving acclimatization *ex vitro* (Hadrami et al., 2010; Pitoyo et al., 2015). In the current study, lantana plantlets rooted on the MS medium supplemented with 1.5 mg/L IBA and 0.3 mg/L HMC or with only HMC treated with 0.6 mg/L HMC of 5 mL three times a week produced high survival percentages of 82.0% and 81.0%, respectively. The fresh and dry weights of shoots and roots and rooted shoots derived from MS medium supplemented with IBA and HMC were the highest among the three treatments. The increase in plant growth and development may have been due to chitosan stimulating hormone accumulation as has been reported in tomatoes (Suarez-Fernandez et al., 2020).

Chitosan treatment after transplanting stimulated plantlet growth in some species, such as *Solanum tuberosum* L. (Asghari-Zakaria et al., 2009) and *Serapias vomeracea* (Burm.f.) Briq (Pitoyo et al., 2015). In addition, chitosan treatment on potato during *in vitro* culture, combined with a foliar application *ex vitro*, could improve plantlet quality and increase mini-tuber seed quality (Kowalski et al., 2006). The application of chitosan increased the survival percentage, reduced water use and protected plantlets from pathogens (Hadrami et al., 2010). Some species, such as pepper (Bittelli et al., 2001) and bean plants (Iriti et al., 2009) were applied with chitosan as a foliar treatment agent, which resulted in partial stomatal closure and transpiration rate reduction (Bittelli et al., 2001; Iriti et al., 2009; Hadrami et al., 2010). In tomato plants treated with chitosan, lipid signaling and defense compounds were found in root exudates, inhibiting soil-borne pathogens such as fungi and nematodes (Suarez-Fernandez et al., 2020).

Furthermore, it has been reported that chitosan can chelate nutrients and minerals such as Fe and Cu, which can reduce fungal spoilage (Tsai and Su,1999; Hadrami et al., 2010). The current results indicated that chitosan may not be necessary for root induction. However, plantlet growth was promoted when cultured on medium added with chitosan. In addition, chitosan increased the survival percentage and promoted the growth rate of lantana plantlet *ex vitro*.

In conclusion, chitosan as an elicitor is suggested for the growth of lantana *in vitro* and *ex vitro*. HMC had higher efficiency than LMC. The results indicated the highest efficiency using 0.1 mg/L HMC combined with 2.8 mg/L TDZ for shoot multiplication. Additive use of 0.1–0.5 mg/L chitosan increased axillary shoot regeneration. All chitosan treatments induced high root induction percentages, but they produced lower root numbers than the 1.5 mg/L IBA treatment. For *ex vitro* acclimatization, plantlets cultured on 1.5 mg/L IBA plus 0.3 mg/L HMC and treated with a foliar and soil application of 0.6 mg/L HMC for 5 mL three times a week produced the highest biomass with increased survival up to 82.0%.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The Department of Biotechnology, Faculty of Science, Ramkhamhaeng University provided supporting facilities.

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