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RESEARCH ARTICLE

Nutrition, age of explant and plant growth regulators affect the multiple shoot induction in *lantana camara* l.

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Abstract
We undertook the present study to investigate the factors affecting multiple shoot induction in *Lantana camara* L. Among all the explants used only shoot-tip explants derived from 1- and 2-week-old seedlings could form multiple shoots. BAP (6-Benzylaminopurine) was the most effective PGR (plant growth regulator) in inducing multiple shoots. Similarly, IBA (Indole-3-butyric acid) efficiently induced root in the microshoots. In addition, salts and sugars also affected regeneration efficiency. MS medium supported induction of multiple shoots the most followed by B5, SH and WPM medium in that order. Likewise, among sugars sucrose and glucose were the best for the induction of microshoots. Our findings show that the MS medium containing 3% sucrose and 4.4 µM BAP induce maximum multiple shoots in 2-week-old shoot-tip explant. The shoots root over MS medium containing either 4.9 µM IBA or 5.5 µM IAA (Indole-3-acetic acid).

Keywords: essential oil, micropropagation, ornamental plant, shoot-tip explant, Verbenaceae

Introducation

Lantana camara L. (Family- Verbenaceae) is a native of tropical regions of the world. It has several varieties with perceptible diversity. British introduced *L. camara* in India as an ornamental and hedge plant (Khan *et al.* 2002). Although, *L. camara* is perceived as weed because of its belligerent growth; it has several uses also. Different parts of the plant are used for medicinal and non-medicinal purposes (Jawonisi and Adoga 2013). *L. camara* appears in the list of useful plants of West Tropical Africa (Burkill 2000). The leaves of *L. camara* are used in the folk medicine of Nigeria as an antimicrobial, febrifuge and also for treating chest diseases and high blood pressure (Egharevba *et al.* 2016). Apart from medicinal uses now-a-days *L. camara* is also used in phytoremediation too (Waoo *et al.* 2014). Commercially *L. camara* is known for its essential oil which is reported to possess insecticidal (Patil *et al.* 1997) and anti-feedent (Attri and Singh 1978) activities. The leaf oil exhibits ovipositional and antimicrobial activities (Saxena and Sharma 1999) and is also used in cosmetics (Jawonisi and Adoga 2013).

The collection of plants from natural habitat for commercial use has depleted the plant population in the wild. In extreme cases, such practice has posed a threat to the survival of plant species. Thus, Kumar and Jnanesha (2016) have proposed the cultivation of plants for commercial utilisation. In addition to this, the cultivation of plants ensures an ample supply of genetically homogenous stock. Seed propagation is not advisable as it creates heterozygosity due to open pollination. Although, vegetative propagation guarantees genetic homogeneity; it has some limitations. Production of root suckers is age-dependent and propagation through cuttings is a slow process. Under such conditions, micropropagation is an acceptable option as it ensures uniformity and scheduled year-round production of disease-free or pathogen-free plants (Kozai *et al.* 2000). A few recent reports on micropropagation of *L. camara* are available (Waoo *et al.* 2013, Veraplakorn 2016, 2017).

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However, the effect of factors like nutrition, PGR (plant growth regulator) and source and age of explant on regeneration potential of *L. camara* has not been investigated.

The process of micropropagation is affected by several factors including media composition, carbon source and PGR (Zulfiquar *et al.* 2009). Cytokinin induces shoot regeneration from explant. However, its effect is modified by auxin in many cases. In general, high cytokinin-auxin ratio favours shoot regeneration. Induction of callus and regeneration in wheat is influenced by the chemical nature of auxin. Moreover, the type of auxin also modifies the effect of sugar on the explant (Mendoza and Kaeppler 2002). Explant being cultured over the medium has heterotrophic mode of nutrition. Therefore, exogenous supply of carbon and energy, in form of sugar, is needed. In addition, sugar affects the physiology, differentiation of tissues, growth of tissues and induction and differentiation of organ (Gauchan 2012). Sucrose is the molecule of choice to serve as carbon and energy source in the plant tissue culture medium. However, apart from this several other sugars and sugar alcohols like glucose, fructose, maltose, sorbitol and mannitol have also been used in the medium. Depending up on the species concerned each one of these have proven to be efficient (Mendoza and Kaeppler 2002, Preethi *et al.* 2011, Amiri and Kazemitabar 2011, Gauchan 2012, Praveena and Veeresham 2014, Panathula *et al.* 2014, Rihan *et al.* 2015).

Therefore, we designed a study to investigate the effect of PGRs, salts, carbon source, source of explant and age of explant on multiple shoot induction in *L. camara*, which has several promising attributes, as mentioned above, for commercial exploitation. The standardised protocol for micropropagation of *L. camara* would be helpful mass multiplication of the plant for commercial plantation. In addition, the protocol would also aid the crop improvement of *L. camara* through metabolic engineering using rDNA technology and in vitro mutagenesis.

Materials and methods

Induction of microshoots

Seeds of *L. camara* were collected from plants growing in wild from Nagpur (India). The seeds were mixed in a polythene bag with germination mixture (Seedcel, Excel Crops Sciences, Mumbai) purchased from the local market and kept in the refrigerator for one week. Subsequently, the mixture containing seeds was spread over the sterile mixture of sand, cocopeat and vermicompost (1:1:1) in a tray. The tray was incubated at room temperature for seeds to germinate. The explants viz. hypocotyl, cotyledonary leaf and shoot-tip were harvested from the seedlings after 1, 2 and 3 weeks of germination. The seedlings were washed with Extran™ (Merck, Germany) for 2 minutes before harvesting the explants. Subsequently, the explants were harvested under aseptic conditions in a laminar airflow cabinet and surface sterilized by washing them sequentially with 0.1% $HgCl_2$, distilled water and 70% ethanol for 1min. each. Twenty-five surface-sterilized explants were then inoculated over agar-gelled MS medium (Murashige and Skoog 1962) containing, various concentrations of BAP, kinetin, NAA (1-Napthaleneacetic acid) and 2,4-D (2,4-Dichlorophenoxyacetic acid) either alone or in combination. The cultures were incubated at 25 ± 2^{o} C and16 h photoperiod for four weeks. The explants were observed on daily basis to score induction of microshoots. The explants which regenerated the microshoot were scored as responding explants, while those which either formed callus or showed no response were scored as non-responding explants. The number of microshoots induced by an explant was counted up to 4 weeks after inoculation.

Rooting of shoots

Fifty microshoots were harvested on attaining the height of 4–5 cm and then transferred to full-strength MS medium containing various concentrations of IBA, NAA, IAA and TCA (2,4,5-Trichloroacetic acid). The shoots were incubated under the same conditions as mentioned above for three weeks. The root induction was scored by visual observation of the shoots transferred over rooting medium. The shoots which formed roots were scored as regenerated plantlets.

Acclimatization

Fifty fully regenerated plantlets with well-developed roots were removed from the culture

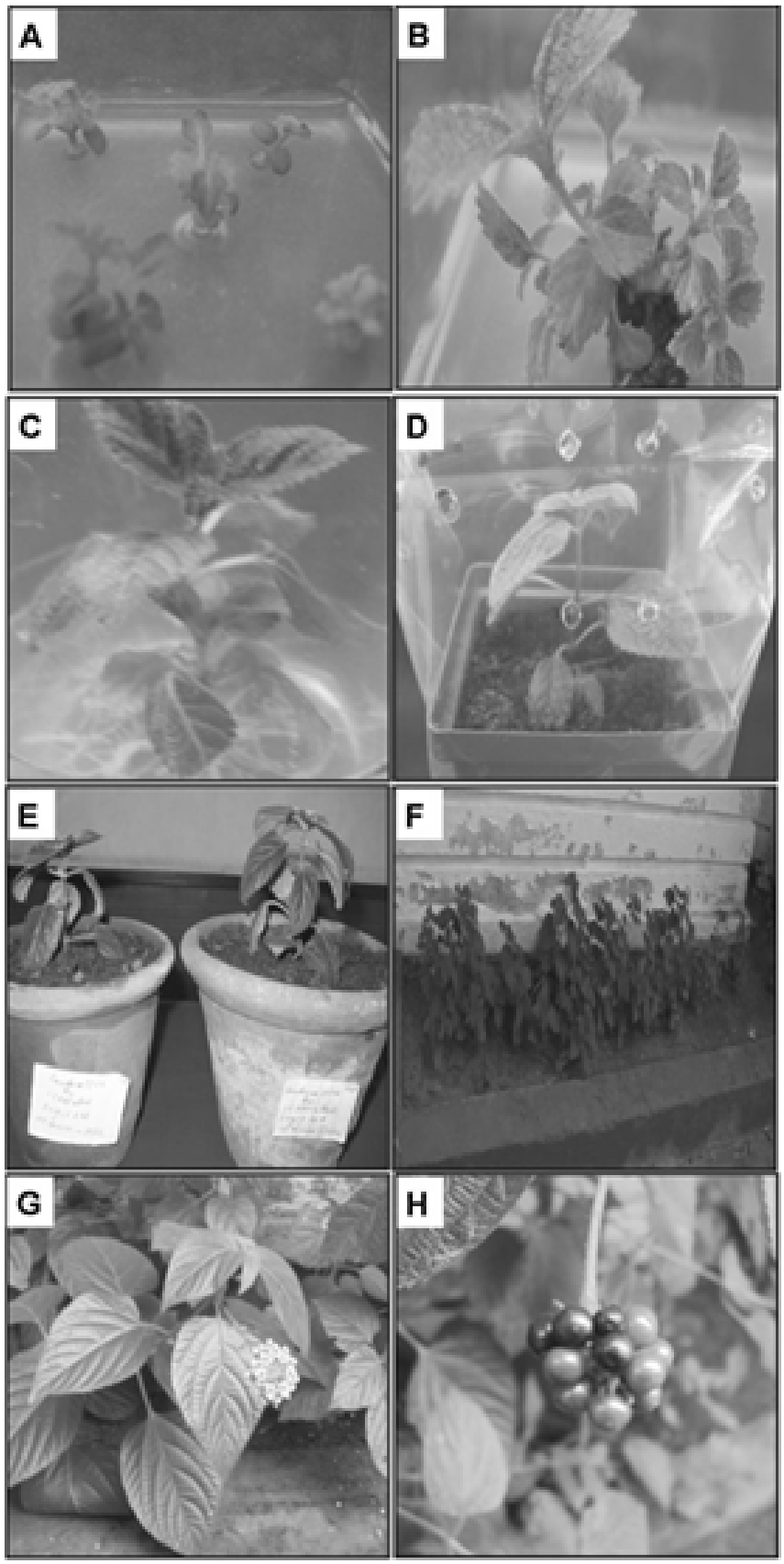

Fig. I. Regeneration in *L. camara*. **A.** Early stage of shoot regeneration. **B.** Multiple shoot induction. **C.** Root induction. D-Acclimatization of re-generated plant. **E.** Regenerated plant transferred to pot. **F.** Regenerated plant transferred to field. **G.** Flowering in regenerated plant. **H.** Fruit setting in regenerated plant.

medium and washed gently with distilled water to remove agar. These plantlets were transferred to pots containing a sterile mixture of sand, coco-peat and vermicompost (1:1:1). The pots with plantlets were covered with a transparent polythene bag to ensure sufficient humidity for plantlets to grow. These plantlets were maintained at 25±2° C under 16 h photoperiod and irrigated with 1/8th strength MS medium containing macronutrients, $CaCl_2$ and Fe-EDTA. After one month, the plantlets were transferred to a bigger pot in the nursery under natural conditions for two months and then transferred to the field.

Effect of salt composition and sugars on multiple shoot induction

The source of explant, its age and PGR concentration were standardized for multiple shoot induction in the preliminary study. Later, the effect of salts, vitamins and carbon source on multiple shoot induction was studied. For this, three different media viz., B5 (Gamborg *et al.* 1968), SH (Schenk and Hilderbrandt 1972) and WPM (Lloyd and McCown 1980) in addition to MS medium were used. These media differ in their salt and vitamin content. The media were fortified with 2–4% of either sucrose or dextrose or maltose. The explant inoculated over these media and the PGR concentration and combination added to the medium were those, which gave the best results in preliminary experiments. For this, 1- and 2-week-old shoot-tip explant (50 each) from the seedlings of *L. camara* were harvested and inoculated over the medium containing 2.2 and 4.4 µM BAP, respectively.

Statistical analysis

The statistical analysis was performed by computing mean, standard error, ANOVA and Duncan's multiple range test (DMRT) (p = 5%), using MS-Excel and XLSTAT.

Results

The initial phase of study was aimed at identifying suitable explant and PGR requirement for multiple shoot induction. Later, studies attempted to test the regeneration protocol by studying the effect of inorganic and organic requirement on regeneration efficiency of the explant. Regeneration efficiency is the product of shoot per explant and percent frequency of response. This metric represents the number of shoots expected to induce from 100 explants cultured over the medium, which aids comparing the response of explant over several media which variously affect the shoots per explant and frequency of response. The explants which could induce at least a single shoot was counted as responding explant. On such a parameter, only shoot-tip explant qualified of being a responding explant (Fig. 1A–B). BAP and kinetin induced microshoots at all the concentrations in combination with NAA. BAP was more effective in inducing multiple shoots compared to kinetin. The age of explant also affected the shoot regeneration from explant in terms of number of shoots induced per explant and frequency of regeneration. BAP alone invoked maximum response in 1-, 2- and 3-week-old explants. Addition of NAA lowered the effectiveness of BAP in inducing the multiple shoots. BAP, at the 2.2 µM concentration, induced the most significant effect in 1-week old explant both in terms of shoots per explant and frequency of response (Table 1). In the 2-week-old explant, however, 4.4 µM BAP induced maximum shoots per explant and regeneration efficiency (Table 1). The 3-week-old explant, although, induced higher number of shoots per explant but the frequency of response was lower *i.e.*, between 16 and 24%. The lower frequency of response reduced the regeneration efficiency of explant (Table 1). Kinetin induced 1 shoot per explant irrespective of its age. Addition of 0.53 or 1.06 µM NAA sporadically enhanced the shoots per explant to 2.0 (Table 1).

BAP in combination with 2,4-D induced callusing in the shoot-tip explant independent of age with frequency ranging from 16 to 48% (Table 2). In contrast 2,4-D when combined with kinetin induced a single microshoot per explant with frequency varying from 12 to 40% (Table 3).

The regenerated shoots were inoculated over MS medium fortified with different concentrations of IBA, NAA, IAA and TCA at 2-node stage to induce roots in them. Microshoots rooted between 7 to 10 days after transfer to rooting medium (Fig. 1C). This is true for all the media except the one containing TCA, which induced the roots between 10 to 12 days after transfer (Table 4).

Table 1: Response of shoot-tip explant over MS medium fortified with NAA and BAP.

PGR Conc. (µM)		Age of explant								
		1 Week			2 Week			3 Week		
		S/E	F (%)	Effi.	S/E	F (%)	Effi.	S/E	F (%)	Effi.
NAA	**BAP**									
0.00	2.2	$4.0^{a}\pm 1.72$	28	112	$2.7^{ab}\pm 2.40$	36	97	$4.0^{a}\pm 1.35$	24	96
	4.4	$3.0^{b}\pm 0.67$	16	48	$4.8^{a}\pm 1.65$	24	115	$3.5^{abc}\pm 1.20$	16	56
	6.6	$2.8^{b}\pm 0.96$	20	56	$2.5^{bc}\pm 0.83$	16	40	$4.6^{a}\pm 1.98$	24	96
	8.8	$3.0^{b}\pm 1.12$	16	48	$2.6^{bc}\pm 0.74$	12	31	$2.6^{bc}\pm 0.74$	16	41
0.53	2.2	$1.0^{b}\pm 0.48$	24	24	$1.0^{bc}\pm 0.40$	32	32	$1.0^{bc}\pm 0.50$	32	32
	4.4	$1.0^{b}\pm 0.31$	20	20	$1.0^{bc}\pm 0.67$	32	32	$1.0^{bc}\pm 0.50$	28	28
	6.6	$1.0^{b}\pm 0.44$	20	20	$1.0^{c}\pm 0.48$	16	16	$1.0^{bc}\pm 0.50$	32	32
	8.8	$1.0^{b}\pm 0.58$	36	36	$1.0^{bc}\pm 0.58$	36	36	$1.0^{abc}\pm 0.31$	20	20
1.06	2.2	$1.0^{b}\pm 0.54$	40	40	$2.0^{abc}\pm 1.69$	32	64	$2.0^{ab}\pm 1.09$	40	80
	4.4	$2.2^{ab}\pm 1.89$	36	79	$2.0^{bc}\pm 0.74$	16	32	$2.0^{abc}\pm 0.74$	24	48
	6.6	$1.0^{b}\pm 0.63$	20	20	$2.0^{bc}\pm 1.16$	24	48	$1.0^{c}\pm 0.48$	16	16
	8.8	$2.0^{b}\pm 0.97$	24	48	$2.0^{abc}\pm 1.20$	32	64	$1.0^{bc}\pm 0.50$	32	32
1.59	2.2	$1.0^{b}\pm 0.70$	40	40	$1.0^{bc}\pm 0.50$	32	32	$1.0^{abc}\pm 0.63$	40	40
	4.4	$1.0^{b}\pm 0.50$	28	28	$1.0^{bc}\pm 0.31$	40	40	$1.0^{c}\pm 0.44$	20	20
	6.6	$1.0^{b}\pm 0.31$	40	40	$1.0^{bc}\pm 0.67$	48	48	$1.0^{bc}\pm 0.73$	44	44
	8.8	$1.0^{b}\pm 0.50$	32	32	$1.0^{bc}\pm 0.31$	40	40	$1.0^{bc}\pm 0.60$	32	32
NAA	**Kinetin**									
0.00	2.3	$1.0^{z}\pm 0.48$	20	20	$1.0^{z}\pm 0.31$	20	20	$1.0^{z}\pm 0.58$	24	24
	4.6	$1.0^{z}\pm 0.48$	16	16	$1.0^{z}\pm 0.44$	16	16	$1.0^{z}\pm 0.48$	16	16
	6.9	$1.0^{z}\pm 0.50$	20	20	$1.0^{z}\pm 0.20$	24	24	$1.0^{z}\pm 0.50$	28	28
	9.2	$1.0^{z}\pm 0.37$	16	16	$1.0^{z}\pm 0.37$	16	16	$1.0^{z}\pm 0.48$	24	24
0.53	2.3	$1.0^{z}\pm 0.31$	40	40	$1.0^{z}\pm 0.48$	16	16	$1.0^{z}\pm 0.58$	36	36
	4.6	$1.0^{z}\pm 0.58$	16	16	$1.0^{z}\pm 0.31$	20	20	$1.0^{z}\pm 0.66$	36	36
	6.9	$1.0^{z}\pm 0.54$	40	40	$2.0^{z}\pm 1.16$	24	48	$2.0^{z}\pm 0.89$	20	40
	9.2	$1.0^{z}\pm 0.40$	12	12	$1.0^{z}\pm 0.48$	16	16	$1.0^{z}\pm 0.37$	16	16
1.06	2.3	$1.0^{z}\pm 0.48$	16	16	$2.0^{z}\pm 1.16$	24	48	$2.0^{z}\pm 1.26$	20	40
	4.6	$1.0^{z}\pm 0.48$	24	24	$1.0^{z}\pm 0.58$	24	24	$1.0^{z}\pm 0.48$	16	16
	6.9	$1.0^{z}\pm 0.31$	40	40	$1.0^{z}\pm 0.58$	36	36	$1.0^{z}\pm 0.58$	24	24
	9.2	$1.0^{z}\pm 0.63$	20	20	$1.2^{z}\pm 0.48$	20	20	$1.0^{z}\pm 0.48$	20	20
1.59	2.3	$1.0^{z}\pm 0.44$	20	20	$1.0^{z}\pm 0.58$	24	24	$1.0^{z}\pm 0.48$	16	16
	4.6	$1.0^{z}\pm 0.40$	12	12	$1.0^{z}\pm 0.37$	16	16	$1.0^{z}\pm 0.58$	24	24
	6.9	$1.0^{z}\pm 0.48$	24	24	$1.0^{z}\pm 0.48$	28	28	$1.0^{z}\pm 0.63$	20	20
	9.2	$1.0^{z}\pm 0.58$	24	24	$1.0^{z}\pm 0.63$	20	20	$1.0^{z}\pm 0.40$	12	12

Mean with same letter(s) in the same column are not significantly different at 5% level of confidence using Duncan's multiple range test.

Abbreviations: S/E- Number of shoots/explants, Freq- % Frequency of regeneration, Effi.- Regeneration efficiency (= S/E × % Frequency).

Note: Same for Tables 2 and 3.

The frequency of rooting was 100% with IBA and IAA at all the concentrations. However, with NAA and TCA, the rooting frequency was lesser *i.e.*, from 72 to 88%. It was noteworthy that rooting frequency with NAA and TCA was inversely proportional to their concentration (Table 4). The regenerated plantlets were transferred to the field after hardening with the success rate of 88% (Fig. 1D–F). All the plants which survived in the field flowered and set fruits (Fig. 1G–H).

We studied the effect of salts, vitamins and carbon source on multiple shoot induction in the present investigation to optimise the conditions for regeneration of *L. camara*. The composition of MS, B5, SH and WPM basal media differ regarding the concentration of salts and vitamins. We supplemented these basal media with sugars like sucrose, glucose and maltose as a carbon source at the concentration of 2, 3 or 4%. Our preliminary studies showed that shoot-tip explant harvested

Table 2: Response of shoot-tip explant over MS medium fortified with BAP and 2,4-D

PGR Conc. (µM)		Age of explant					
		1 Week		2 Week		3 Week	
2,4-D	BAP	Response	F (%)	Response	F (%)	Response	F (%)
0.45	2.2	Callus	32	Callus	24	Callus	40
	4.4	Callus	40	Callus	28	Callus	16
	6.6	Callus	44	Callus	48	Callus	28
	8.8	Callus	44	Callus	44	Callus	24
0.95	2.2	Callus	36	Callus	48	Callus	36
	4.4	Callus	40	Callus	40	Callus	36
	6.6	Callus	48	Callus	40	Callus	36
	8.8	Callus	48	Callus	40	Callus	32
1.35	2.2	Callus	40	Callus	44	Callus	36
	4.4	Callus	24	Callus	44	Callus	40
	6.6	Callus	44	Callus	28	Callus	32
	8.8	Callus	44	Callus	40	Callus	40

Table 3: Response of shoot-tip explant over MS medium fortified with 2,4-D and kinetin.

PGR Conc. (µM)		Age of explant								
		1 Week			2 Week			3 Week		
2,4-D	Kinetin	S/E	F (%)	Effi.	S/E	F (%)	Effi.	S/E	F (%)	Effi.
0.45	2.3	$1.0^a \pm 0.37$	24	24	$1.0^a \pm 0.81$	32	32	$1.0^a \pm 0.70$	40	40
	4.6	$1.0^a \pm 0.48$	40	40	$1.0^a \pm 0.58$	36	36	$1.0^a \pm 0.31$	28	28
	6.9	$1.0^a \pm 0.63$	20	20	$1.0^a \pm 0.60$	28	28	$1.0^a \pm 0.48$	24	24
	9.2	$1.0^a \pm 0.67$	28	28	$1.0^a \pm 0.37$	16	16	$1.0^a \pm 0.40$	12	12
0.90	2.3	$1.0^a \pm 0.63$	20	20	$1.0^a \pm 0.60$	28	28	$1.0^a \pm 0.50$	32	32
	4.6	$1.0^a \pm 0.44$	20	20	$1.0^a \pm 0.48$	24	24	$1.0^a \pm 0.37$	16	16
	6.9	$1.0^a \pm 0.48$	24	24	$1.0^a \pm 0.60$	28	28	$1.0^a \pm 0.80$	36	36
	9.2	$1.0^a \pm 0.50$	28	28	$1.0^a \pm 0.37$	16	16	$1.0^a \pm 0.37$	16	16
1.35	2.3	$1.0^a \pm 0.63$	20	20	$1.0^a \pm 0.48$	16	16	$1.0^a \pm 0.37$	24	24
	4.6	$1.0^a \pm 0.63$	20	20	$1.0^a \pm 0.48$	24	24	$1.0^a \pm 0.63$	20	20
	6.9	$1.0^a \pm 0.44$	20	20	$1.0^a \pm 0.48$	24	24	$1.0^a \pm 0.63$	20	20
	9.2	$1.0^a \pm 0.44$	24	24	$1.0^a \pm 0.31$	20	20	$1.0^a \pm 0.37$	20	20

Table 4: Effect of auxin on rooting of microshoots

Auxin	Concentration (µM)	Days required for root induction	Frequency of rooting (%)
IBA	4.9	7-10	100
	9.8	7-10	100
	14.7	7-10	100
NAA	5.4	7-10	80
	10.8	7-10	76
	16.2	7-10	72
IAA	5.5	7-10	100
	11.0	7-10	100
	16.5	7-10	100
TCA	3.9	10-12	88
	7.8	10-12	80
	11.7	10-12	76

from 1- and 2-week-old seedling expressed maximum regeneration efficiency over MS medium fortified with 2.2 and 4.4 µM BAP, respectively (Table 1). Hence, we tested the effect of nutrition on regeneration efficiency of 1- and 2-week-old explant over medium containing 2.2 and 4.4 µM BAP, respectively. MS medium proved to be the best medium supporting multiple shoot induction in 1-week old shoot-tip explant compared to other media (Table 5). Similarly, in terms of carbon source sucrose and glucose had a similar effect on regeneration efficiency. Maltose,

Table 5: Effect of nutrition along with 2.2µM BAP on multiple shoot induction from one week old shoot-tip explant.

Salts	Conc. of sugar (%)	Carbon source (sugar)								
		Sucrose			Glucose			Maltose		
		S/E	F (%)	Effi.	S/E	F (%)	Effi.	S/E	F (%)	Effi.
MS	2	3.0±1.64	20	60	3.3±1.09	24	79	2.0±0.63	20	40
	3	4.0±1.15	28	112	3.8±1.80	20	76	2.0±0.74	24	48
	4	2.5±0.63	16	40	3.0±0.94	20	60	2.0±0.00	16	32
B5	2	3.0±1.46	24	72	3.4±2.03	28	95	2.0±0.63	20	40
	3	3.8±2.35	28	106	4.2±2.33	24	100	2.0±0.63	20	40
	4	2.0±0.74	16	32	2.8±1.46	20	48	2.0±0.48	12	24
SH	2	1.0±0.63	20	20	1.0±0.37	24	24	1.0±0.37	16	16
	3	1.0±0.48	24	24	1.0±0.37	28	28	1.0±0.48	24	24
	4	1.0±0.40	12	12	1.0±0.48	16	16	1.0±0.24	12	12
WPM	2	2.6±1.77	20	52	2.8±1.46	24	67	2.2±1.37	20	40
	3	3.0±2.22	24	72	3.5±2.24	24	84	2.0±0.89	20	40
	4	2.0±0.48	12	24	2.0±0.80	12	24	2.0±0.97	16	32

Table 6: Two way ANOVA to study the effect of sugar and salts on multiple shoot induction from 1 week old shoot-tip explant inoculated over media containing 2.2µM BAP

Source of Variation	SS	df	MS	F	P-value	F crit
Sugar	22.35556	8	2.794444	2.3922	0.04702	2.355081
Medium	27.35444	3	9.118148	7.805644	0.000831	3.008787
Error	28.03556	24	1.168148			
Total	77.74556	35				

however, was lesser effective as a carbon source to induce microshoots (Table 5). A critical analysis revealed that the media and carbon source affected the regeneration efficiency of explant by affecting shoot per explant rather than the frequency of response (Table 5). Irrespective of carbon source, 3% concentration of sugar was better compared to 2 and 4% concentration. The two-way ANOVA analysis revealed that salts and vitamins affected regeneration efficiency more compared to sugar (Table 6).

In contrast to 1-week old explant; the composition of media and sugar with respect to regeneration efficiency variously affected 2-week-old explant. In any case, 3% concentration of sugar was most effective and resulted in the highest regeneration efficiency except for 2% maltose with B5 basal medium (Table 7). The combination of MS medium with sucrose was far better for multiple shoot induction compared to B5, SH and WPM media (Table 7). On the other hand, combination of glucose with B5 medium was best for shoot regeneration. It was followed by MS, WPM and SH medium in that order (Table 7). In contrast, the combination of maltose was better with MS or B5 medium regarding regeneration efficiency compared to SH and WPM media (Table 7). The two-way ANOVA performed using medium and sugar as factors, revealed that both these factors affect regeneration efficiency, although, the medium has a greater effect than the sugar (Table 8).

Discussion

The present study was aimed to derive amicable conditions for the efficient regeneration of *L. camara* for commercial exploitation and crop improvement. Therefore, we first selected suitable explant which is amenable for micropropagation and/or transgenic production. Seedling-derived explants have several advantages over those derived from mature plants. The juvenility of seedling-derived explants makes them acquiescent to *in vitro* manipulations (Rathore *et al.* 2008). Moreover, since juvenile explants have a higher proportion of meristematic cells, it eases regeneration by stimulating the proliferation of cells by PGRs (Madke *et al.* 2014). However, even though juvenile, only shoot-tip explant responded over media by regenerating microshoot(s). Waoo *et al.* (2014) have also reported induction of shoots from shoot-tip explant besides nodal explant. The

Table 7: Effect of nutrition along with 6.6µM BAP on multiple shoot induction from 2 week old shoot-tip explant

Salts	Conc. of sugar (%)	Carbon source (sugar)								
		Sucrose			Glucose			Maltose		
		S/E	F (%)	Effi.	S/E	F (%)	Effi.	S/E	F (%)	Effi.
MS	2	2.0±1.26	20	40	3.3±1.81	24	79	2.0±0.74	16	32
	3	4.8±1.65	24	115	4.0±2.05	24	96	2.0±0.97	24	48
	4	2.4±0.97	20	48	2.4±0.96	20	48	1.4±0.87	16	22
B5	2	2.1±0.83	16	34	3.4±1.20	28	95	2.5±1.48	24	60
	3	3.0±1.26	20	60	4.2±2.03	24	100	2.0±0.97	24	48
	4	2.3±0.97	12	28	2.8±0.97	20	56	2.0±0.74	16	32
SH	2	1.0±0.50	28	28	1.0±0.48	24	24	1.0±0.63	20	20
	3	1.0±0.63	20	20	1.0±0.63	24	24	1.0±0.44	20	20
	4	1.0±0.37	16	16	1.0±0.63	20	20	1.0±0.48	16	16
WPM	2	2.1±1.49	24	50	2.6±1.66	20	52	2.0±1.16	24	48
	3	2.0±1.16	28	56	2.4±1.31	28	95	2.0±0.97	16	32
	4	2.0±0.97	16	32	2.0±0.97	16	32	1.3±0.58	12	16

Table 8: Two way ANOVA to study the effect of sugar and salts on multiple shoot induction from 2 week old shoot-tip explant inoculated over media containing 4.4µM BAP

Source of Variation	SS	df	MS	F	P-value	F crit
Sugar	21.52889	8	2.691111	4.021029	0.003772	2.355081
Medium	20.09778	3	6.699259	10.00996	0.000183	3.008787
Error	16.06222	24	0.669259			
Total	57.68889	35				

regeneration of microshoot from shoot-tip shows that the media composition (wherever the microshoot was induced in the present study) was suitable for the proliferation of axillary bud rather than adventitious shoot induction (Warhade and Badere 2015). Moreover, the advantage shoot-tip explants have is that they ensure genetic stability of the regenerated plants and are also acquiescent for genetic transformation (Orlikowska *et al.* 2000, Warhade and Badere 2015). This finding, therefore, fulfils the aim of present investigation to develop an efficient protocol for micropropagation of *L. camara* for commercial exploitation. Whatsoever, the physiological state of explant and its endogenous PGR levels dictate the fate of explant over the medium (Badere *et al.* 2002). In addition, every tissue has a specific nutritional demand (Narayanswamy 1994). Badere *et al.* (2002), Madke *et al.* (2014) and Warhade and Badere (2015, 2017) have agreed that the response of explant towards culture medium and condition is the resultant of endogenous PGR levels of explant and PGRs supplied exogenously through the medium. Therefore, the success of shoot-tip explant in regenerating microshoots might be because of its physiological state and endogenous PGR levels conducive for shoot induction in combination with exogenous PGR levels supplied through culture medium.

The regeneration efficiency of shoot-tip in the present investigation was best with BAP, which lowered in combination with auxin. Similar observation was made by Veraplakorn (2016) in *L. camara* where BAP induced maximum number of shoots per explant. In the same report Veraplakorn (2016) has mentioned zeatin to support elongation of regenerated shoots rather than their induction. In addition, Veraplakorn (2017) also found TDZ to be efficient in induction of microshoots in *L. camara*. Although, auxin apparently supports shoot induction by promoting cell division (Warhade and Badere 2015), in our case it was inhibitory. Gana (2010) reported that BAP promotes shoot formation by decreasing the apical dominance. Tyub *et al.* (2007) and Zarki and Elmtili (2012) have also reported induction of multiple shoots in *Mentha pulergium* and *Lavandula officinalis*, respectively by BAP alone. However, in contrast to our findings, Waoo *et al.* (2014) have found BAP

along with NAA and kinetin to induce multiple shoots in *L. camara*. In addition, earlier we have reported that NAA improved the effect of BAP on the regeneration efficiency of explant in *Gmelina arborea* (Madke *et al.* 2014).

In the second phase of study, we tested the effect of MS, B5, SH and WPM media on shoot induction in the present investigation. These media differ with each other in terms of their composition and concentration of salts. MS and B5 media supported shoot regeneration better than SH and WPM media. Madke *et al.* (2014) in *G. arborea* and Warhade and Badere (2015) in *Celosia cristata* recorded the similar observations. Both media *i.e.*, MS and B5 are high-salt media with a similar composition. Therefore, probably, they had a similar effect on explant. The SH is also a high-salt medium but it is poor in sulphur and iron content, which play an important role in cellular metabolism. Moreover, the SH medium contains an unusually high concentration of myo-inositol, which may lead to poor regeneration in many species (Narayanswamy 1994). It is possible that elemental deficiency might cause poor response of explant towards SH medium. Our earlier study with *G. arborea* also found SH to be a weaker medium to support shoot regeneration (Madke *et al.* 2014). The fourth medium we tested was WPM, which is a low-salt medium. It is poor in nitrogen, potassium and chloride. Moreover, media has a high content of copper, which is toxic to the cell. The paucity of essential elements coupled with the toxic concentration of copper might be responsible for poor performance of shoot-tip explant over WPM.

In plant tissue culture sugar plays various roles in the medium. First, it is the major source of energy and carbon–an important element of life forms. Second, it serves as osmoticum and maintains osmotic potential of the medium (Amiri and Kazemitabar 2011; Gauchan 2012). Apart from this, sugars also modify the effect of auxin (Mendoza and Kaeppler 2002). We tested the effect of sucrose, glucose and maltose on regeneration efficiency of shoot-tip explant of *L. camara*. Although, sucrose is a preferred sugar used in plant tissue culture but each species has a specific requirement of sugar. Amiri and Kazemitabar (2011) have mentioned that type of carbohydrate and its concentration affects morphogenesis and physiological state in explant. Sucrose proved to be the best source of carbon closely followed by glucose. The effect of maltose on regeneration was comparatively poor. Similar findings are reported in *Asparagus racemosus* (Thakur *et al.* 2014), *Toddalia asiatica* (Praveena and Veeresham 2014) and *C. cristata* (Warhade and Badere 2015). However, in *Stevia rebaudiana* and *Centella asiatica* fructose induced a greater number of shoots per explant than sucrose (Preethi *et al.* 2011, Panathula *et al.* 2014) and in *Zea mays* and *Brassica oleracea* var. *botrytis* maltose was equally effective as sucrose in shoot regeneration (Gauchan 2012, Rihan *et al.* 2015). In the present investigation the performance of sucrose would have been because it is generally preferred during catabolism (Amiri and Kazemitabar 2011) and its high solubility in water, presence in phloem sap and almost nil inhibitory action on metabolism (Panathula *et al.* 2014).

In addition to type of sugar, its concentration also plays an important role in shoot regeneration. The limited supply of carbon dioxide to the explant growing in culture vessels necessitates appropriate concentration of sugar for regeneration (Panathula *et al.* 2014). Studies carried out in various plants indicate that the effect of sugar concentration on regeneration increases with an increase in concentration till a certain concentration and then decreases with further increase in concentration (Karim *et al.* 2007, Preethi *et al.* 2011, Amiri and Kazemitabar 2011, Gauchan 2012, Praveena and Veeresham 2014, Panathula *et al.* 2014). These studies found the concentration of sugar around 3% to support regeneration most favourably. In the present investigation also irrespective of the chemical nature of sugar, the explant responded best over media containing 3% sugar. Although 2% sugar also induced regeneration in the explant; but the effect was lesser than 3% sugar. This is probably because of the starvation of explant due to the paucity of sugar supply (Madke *et al.* 2014). In contrast, 4% sugar had an inhibitory effect on shoot induction. The decrease in regeneration frequency, in our case, might be due to the higher osmotic potential of media containing 4% sugar, which affected the explant adversely by interfering with the shoot growth (Karim *et al.* 2007, Madke *et al.* 2014, Praveena and Veeresham 2014).

Conclusions

Based on our findings we propose that *L. camara* can be efficiently micropropagated through shoot regeneration from 2-week-old seedling derived shoot-tip explant cultured over culture medium composed of MS salts and vitamins, 3% sucrose and 4.4 µM BAP. The induced microshoots can be rooted over MS medium containing either 4.9 µM IBA or 5.5 µM IAA. The regenerated plantlets can be transferred to the field after hardening where they flower and set fruits.

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