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EFFICIENT PLANT REGENERATION OF YELLOW LOOSESTRIFE (*LYSIMACHIA VULGARIS* L.), A MEDICINAL PLANT

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Lysimachia vulgaris L. (yellow loosestrife) is a medicinal plant that has been used in the treatment of fever, ulcer, diarrhea and wounds in traditional medicine. A reliable *in vitro* culture protocol for yellow loosestrife was established. Explants (leaf lamina, stem internode and root segments) were cultured on Murashige and Skoog minimal organics (MSMO) medium supplemented with various plant growth regulator combinations. Of the tested combinations, those involving benzyladenine (BA) with either indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) were the most effective for all used explants in shoot production. Best shoot proliferation was obtained from leaf lamina explant cultured on media containing 0.5 mg/l BA and 0.1, 0.5 or 1 mg/l IBA, from stem internode explant cultured on media containing 1 mg/l BA and 0.5 mg/l IBA or 0.01 mg/l thidiazuron (TDZ) and 0.5 mg/l IAA, and from root explant cultured on media containing 0.5 mg/l BA and 0.5 mg/l IAA. Regenerated shoots were rooted on MSMO medium containing different concentrations of IAA, IBA, 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthalene acetic acid (NAA). IBA was determined as the most effective auxin for rooting. Most shoots developed roots on medium with 0.5 mg/l IBA.

Keywords: Growth regulators – *in vitro* culture – *Lysimachia vulgaris* – micropropagation – yellow loosestrife

INTRODUCTION

Lysimachia vulgaris L. (yellow loosestrife) is a rhizomatous perennial herb that is often cultivated as a garden plant for its beauty [2, 4, 5]. Genus *Lysimachia* that has been traditionally placed in the Primulaceae family was moved to family Myrsinaceae upon review of phylogenetic researches [6, 13]. Yellow loosestrife is native to Europe and grows along roadsides, marshes and riverbanks [2–4]. Because of the astringent property, it has been used for the treatment of gastrointestinal conditions such as diarrhaea and dysentery, to stop internal and external bleeding, and to cleanse wounds in traditional medicine [2, 4, 5]. Moreover, *L. vulgaris* has expectorant, demulcent, febrifuge, analgesic, vulnerary and anti-inflammatory activities [2, 4, 5]. It makes a

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serviceable mouthwash for treating sore gums and mouth ulcers [2]. Yellow loosestrife contains benzoquinones (embelin and rapanone), saponins, flavonoids, and tannins [2, 8, 14, 15, 17, 22].

Although *L. vulgaris* or some other medicinal plants can easily be found in the wild easily, they are generally subjected to some herbicides and attacked by some insects and pathogens. *In vitro* micropropagation of medicinal plants provides pesticide or disease free plants and produces large numbers of vegetative planting stock easily. In addition, with an *in vitro* propagation method, medicinal plants can be obtainable all year around and genetically uniform plant materials (less genetic diversity) can be produced easily that will be higher with seed germination.

Yellow loosestrife is a valuable medicinal herb, but there are no reports on an *in vitro* culture protocol of this species. The present work reports an *in vitro* culture procedure for rapid clonal propagation of *L. vulgaris*.

MATERIALS AND METHODS

Seeds of *L. vulgaris* were collected from Abant Lake, Bolu/Turkey in September of 2008. Identification of the species was made by using "Flora of Turkey and The East Aegean Islands" [3] and voucher specimens (AUT-2008) were deposited at the Abant Izzet Baysal University (AIBU) Herbarium, Bolu/Turkey.

Seeds were washed with anti-bacterial soap, rinsed with distilled water and surface disinfected by shaking for 20 min in 20% Ethanol (EtOH), for 10 min in 20% Domestos® (<5% sodium hypochloride), and then rinsed with sterile distilled water three times. Seeds were placed in sterile, disposable Petri dishes (80 × 15 mm) containing 15 ml of Murashige and Skoog minimal organics medium (4.43 g/l, MSMO, Sigma Chemical Co., St. Louis, MO, USA) [11] with 30 g/l sucrose, 8 g/l Difco Bacto-agar (pH 5.7, autoclaved for 20 min at 121 °C and 105 kPa). After two weeks incubation on this medium, seedlings were transferred to Magenta containers (GA-7 Vessel, Sigma Chemical Co.) containing the same medium for an additional three weeks. For shoot regeneration, leaf lamina (36 mm^2), stem internode (4–5 mm of internode segments) and root explants (6–7 mm segments) were excised from four weeks old sterile seedlings and placed in sterile disposable petri plates containing 4.43 g/l MSMO with different combinations and concentrations of plant growth regulators; thidiazuron (TDZ; 0.01, 0.05 and 0.1 mg/l)+indole-3-acetic acid (IAA; 0.05, 0.1 and 0.5 mg/l); benzyladenine (BA; 0.1, 0.5, 1.0, 2.0 and 3 mg/l)+IAA (0.1, 0.5, 1.0 and 2.0 mg/l); BA (0.1, 0.5, 1.0, 2.0 and 3 mg/l)+naphthalene acetic acid (NAA; 0.1, 0.5, 1.0 and 2.0 mg/l); kinetin (KIN; 0.1, 0.5, 1.0 and 3.0 mg/l)+IAA (0.1, 0.5 and 1 mg/l); KIN (0.5, 1.0 and 2.0 mg/l)+2,4-dichlorophenoxyacetic acid (2,4-D; 0.01, 0.1, 0.5 and 1.0 mg/l); BA (0.1, 0.5, 1.0 and 2.0)+2,4-D (0.01, 0.1, 0.5 and 1.0); BA (0.1, 0.5 and 1.0)+indole-3-butyric acid (IBA; 0.1, 0.5 and 1.0); zeatin (ZEA; 0.01, 0.1, 0.5 and 1.0)+IBA (0.5, 1.0 and 3.0). All cultures were incubated at 22 °C under a 16-h photoperiod (cool-white fluorescent lights, 22–28 μmol m^{-2} s^{-1}). The shoot number and percentage of explants producing shoots were recorded after 4–5

weeks for all explants. Tests had 10 replications for each explant and the experiment was repeated three times.

After three weeks, regenerated explants were transferred to Magenta containers (GA-7 Vessel, Sigma Chemical Co.) containing MSMO medium with 0.5 mg/l gibberellin (GA_3) for shoot elongation for an additional two weeks. Shoots were then separated individually and placed in rooting medium containing MSMO and varying concentrations of different auxins; IAA (0.5, 1 and 3 mg/l), IBA (0.5, 1 and 3 mg/l), 2,4-D (0.1, 0.5 and 1 mg/l) and NAA (0.5, 1 and 3 mg/l). After three weeks, the number of roots and percentage of explants producing roots were recorded. There were 10 replications and experiment was replicated three times. Rooted explants were transferred to vermiculite (Agrekal®) in Magenta containers for acclimatization and after 2 weeks they were transferred to plastic pots containing potting soil.

All data were analyzed by analysis of variance (ANOVA) and mean values were compared with Duncan's Multiple Range Tests using SPSS vers. 15 (SPSS Inc, Chicago, IL, USA).

RESULTS

Although *L. vulgaris* is a very valuable medicinal and horticultural plant, there is no study whatsoever about *in vitro* propagation of this plant. We therefore aimed to develop an *in vitro* culture protocol for high frequency regeneration of loosestrife plants by adventitious organogenesis.

Three different explants (leaf lamina, stem internode and root) were excised from 4- or 5-week-old sterile (*in vitro* germinated) seedlings and cultured on MSMO medium containing BA in combination with IAA, IBA, NAA and 2-4-D (Table 1); TDZ in combination with IAA (Table 2); ZEA in combination with IBA (Table 3); KIN in combination with IAA or 2,4-D (Table 4). Of these plant growth regulator (PGR) combinations, BA and 2,4-D or KIN and 2,4-D were not found effective for shoot multiplication, producing very few, if any, shoots (data not provided). Control treatments involving no plant growth regulators produced no shoots in all 3 explants (Tables 1, 2, 3 and 4). Overall, all explants tested showed high regeneration capacity on media containing BA in combination with IAA or IBA (Table 1). Number of shoots per explants was lower for all explants on media with BA plus NAA combinations when compared to BA plus IAA or IBA combinations (Table 1). When cultured on media containing combinations of different TDZ and IAA concentrations, stem internode explants were much more productive for shoot formation than leaf and root explants (Table 2). The regeneration efficiency was found to be higher in stem internode and root explants than leaf explants with regard to both the mean number of shoots per explant and the percentages of explants forming shoots with ZEA in combination with IBA (Table 3). With regard to the effects of the combinations of KIN with IAA, shoot proliferation was observed when KIN was used alone with stem internode (30.2 shoots per explant with 1 mg/l KIN) and root explants (30 shoots per explant with 3 mg/l KIN) (Table 4).

Regarding the leaf explants, best shoot proliferation was obtained on media containing 0.5 mg/l BA in combination with 0.1, 0.5 or 1.0 mg/l IBA (56.9, 50.7 and 50.3 shoots per explant, respectively; 100% explants formed shoots in all 3 combinations) (Table 1; Fig. 1a). With regard to the stem internode explants, highest number of shoots per explant was observed on media with 0.01 mg/l TDZ plus 0.5 mg/l IAA (63.8 shoots per explant; 100% explants formed shoots) (Table 2; Fig. 1b). Media containing 1.0 mg/l BA plus 0.5 mg/l IBA and 1.0 mg/l BA plus 2.0 mg/l IAA, 0.1 mg/l TDZ plus 0.5 mg/l IAA and 1.0 BA plus 1.0 mg/l IBA were also effective for shoot formation with stem internode explants (59.1, 52.9, 47.4 and 46.9 shoots per explant, respectively; 100% explants formed shoots in all 4 combinations) (Tables 1 and 2). With the root explants, the greatest number of shoots per explant was recorded on media containing 0.5 mg/l BA plus 0.5 mg/l IAA (51.2 shoots per explant; 100% explants formed shoots) (Table 1; Fig. 1c). Media containing 2.0 mg/l BA plus 0.1 or 0.5 mg/l IAA and 0.5 mg/l BA plus 0.1 mg/l IAA were also effective for shoot formation (44.2, 46.2 and 45.8 shoots per explant; 67%, 78% and 97% explants formed shoots, respectively) (Table 1). Observing callus formation before shoot development from all tested explants for all used PGR combinations suggested that adventitious shoots were of indirect origin (data not shown).

Regarding the leaf lamina explant, in the absence of auxins (IAA, IBA or NAA), 0.5 mg/l BA alone was not effective for shoot regeneration (2.6 shoots at 40% shoot formation frequency). When different concentrations of IBA (0.1, 0.5 and 1 mg/l) were combined with 0.5 mg/l BA, much higher frequencies of shoot formation and mean number of shoots per explant were achieved (56.9, 50.7 and 50.3 shoots, respectively, at 100% frequency) and increasing concentration of IBA did not change the shoot number significantly (Table 1). Similarly, combination of 0.5 mg/l BA with different concentrations of IAA (0.1, 0.5, 1 and 2 mg/l) enhanced the number of regenerated shoots (12.7, 17.1, 15.5 and 19.2 shoots, respectively) and increasing concentration of IAA increased the frequency of shoot formation (63%, 68%, 76% and 83%, respectively) in leaf lamina explants. Combination of 0.5 mg/l BA with 0.1 mg/l NAA also increased the shoot regeneration rate to 38 shoots per leaf lamina explant at 100% shoot formation frequency (Table 1). When 1 mg/l BA was used alone on the stem internode explant, low level of shoot formation number (4.4 shoots) and frequency (17%) were observed. The addition of different concentrations of IAA (0.1, 1 and 2 mg/l) or IBA (0.1, 0.5 and 1 mg/l IBA) enhanced the multiple shoot formation. For example, the addition of 0.5 mg/ l IBA to 1 mg/l BA increased the shoot number per stem internode explant to 59.1 and shoot formation frequency to 100% (Table 1). When root explants were cultured on media containing 0.5 mg/l BA alone, 32.7 shoots were produced with 74% shoot frequency formation. The addition of 0.1 or 0.5 mg/l IAA to 0.5 mg/l BA increased the shoot number and % root explants forming shoots (45.8 and 51.2 shoots at 97% and 100% frequency, respectively). But, higher concentration of IAA (1 or 2 mg/l) severely inhibited shoot development and decrease mean number of shoots to 22.9 and 10.6, respectively. In general it is evident that multiple shoot formation and increased shoot formation frequency induced by BA were closely related to the auxin supply for all explants (Table 1). On the other

Table 1

Shoot regeneration from leaf lamina, stem internode and root segment explants cultured on MSMO medium containing BA in combination with IAA, IBA and NAA

Plant growth regulators		Explants					
		Leaf lamina		Stem internode		Root segment	
		Mean number of shoots per explant (±SE)	% explants forming shoots	Mean number of shoots per explant (±SE)	% explants forming shoots	Mean number of shoots per explant (±SE)	% explants forming shoots
BA (mg/l)	IAA (mg/l)						
0.0	0.0	–	–	–	–	–	–
0.1	0.0	–	–	6.6 ± 2.3^{ijk}	32	17.6 ± 3.6^{cdefg}	63
0.1	0.1	1.0 ± 0.5^{j}	18	2.9 ± 1.3^{jk}	31	12.8 ± 3.4^{defg}	47
0.1	0.5	0.3 ± 0.2^{j}	13	5.8 ± 1.7^{jk}	41	4.3 ± 0.9^{efg}	58
0.1	1.0	1.7 ± 0.7^{ij}	24	4.9 ± 1.4^{jk}	44	11.2 ± 2.1^{defg}	79
0.1	2.0	0.1 ± 0.1^{j}	6	0.5 ± 0.3^{k}	25	3.5 ± 0.8^{efg}	72
0.5	0.0	2.6 ± 0.9^{ij}	40	15.1 ± 4.1^{fghijk}	44	32.7 ± 4.4^{bc}	74
0.5	0.1	12.7 ± 2.2^{defghij}	63	5.2 ± 1.9^{jk}	32	45.8 ± 5.2^{ab}	97
0.5	0.5	17.1 ± 4.4^{cdefg}	68	18.0 ± 3.8^{efghijk}	76	51.2 ± 6.4^{a}	100
0.5	1.0	15.5 ± 2.7^{cdefghi}	76	28.8 ± 5.0^{defg}	91	22.9 ± 3.4^{cd}	95
0.5	2.0	19.2 ± 4.1^{cde}	83	25.9 ± 8.8^{defghi}	50	10.6 ± 1.8^{defg}	83
1.0	0.0	6.7 ± 2.2^{efghij}	50	4.4 ± 2.1^{jk}	17	24.6 ± 4.7^{cd}	66
1.0	0.1	17.6 ± 3.4^{cdef}	74	10.5 ± 3.2^{ghijk}	58	33.4 ± 4.9^{bc}	76
1.0	0.5	25.0 ± 4.9^{cd}	84	35.5 ± 6.1^{bcde}	82	19.6 ± 3.9^{cdef}	74
1.0	1.0	37.6 ± 4.9^{b}	82	23.0 ± 4.3^{defghij}	89	8.6 ± 1.3^{defg}	84
1.0	2.0	27.6 ± 8.2^{bc}	78	52.9 ± 9.9^{ab}	100	23.8 ± 5.3^{cd}	89
2.0	0.0	9.8 ± 4.6^{efghij}	56	3.4 ± 2.0^{jk}	21	21.6 ± 6.5^{cde}	72
2.0	0.1	11.1 ± 3.1^{efghij}	67	13.1 ± 6.1^{ghijk}	36	44.2 ± 11.0^{ab}	67
2.0	0.5	4.3 ± 2.1^{ghij}	44	11.4 ± 5.0^{ghijk}	47	46.2 ± 11.3^{ab}	78
2.0	1.0	1.1 ± 0.4^{ij}	39	21.9 ± 6.1^{defghij}	93	1.2 ± 0.5^{fg}	33
2.0	2.0	8.3 ± 2.7^{efghij}	61	19.9 ± 4.5^{efghijk}	93	1.2 ± 0.8^{fg}	17
3.0	0.0	–	–	7.6 ± 2.8^{ijk}	43	1.1 ± 0.4^{fg}	39
3.0	0.1	0.3 ± 0.2^{j}	11	7.8 ± 3.5^{ijk}	38	3.9 ± 1.5^{efg}	61
3.0	0.5	0.3 ± 0.2^{j}	11	18.2 ± 7.2^{efghijk}	56	8.9 ± 2.4^{defg}	67
3.0	1.0	3.6 ± 1.7^{ghij}	28	28.6 ± 10.0^{defg}	50	2.8 ± 1.2^{fg}	50
3.0	2.0	2.1 ± 0.8^{ij}	50	22.6 ± 7.5^{defghij}	44	4.0 ± 1.6^{efg}	50

BA (mg/l)	IBA (mg/l)						
0.1	0.1	3.7 ± 3.3^{ghij}	30	39.9 ± 9.1^{bcd}	100	1.36 ± 0.5^{fg}	64
0.1	0.5	6.1 ± 2.8^{efghij}	60	19.7 ± 2.4^{efghijk}	100	2.3 ± 0.8^{fg}	80
0.1	1.0	12.9 ± 2.2^{defghij}	100	8.6 ± 2.3^{hijk}	90	1.1 ± 0.5^{fg}	50
0.5	0.1	56.9 ± 12.3^{a}	100	7.8 ± 4.4^{ijk}	40	30.9 ± 8.3^{bc}	91
0.5	0.5	50.7 ± 6.4^{a}	100	27.7 ± 6.6^{defgh}	90	12.9 ± 4.6^{defg}	80
0.5	1.0	50.3 ± 5.8^{a}	100	33.5 ± 5.9^{cdef}	100	2.0 ± 0.8^{fg}	60
1.0	0.1	11.0 ± 4.5^{efghij}	60	40.0 ± 11.3^{bcd}	90	12.8 ± 7.5^{defg}	80
1.0	0.5	12.2 ± 4.2^{defghij}	80	59.1 ± 5.2^{a}	100	2.8 ± 1.7^{fg}	40
1.0	1.0	16.1 ± 2.9^{cdefgh}	100	46.9 ± 7.5^{abc}	100	2.8 ± 1.5^{fg}	60
BA (mg/l)	NAA (mg/l)						
0.1	0.1	5.6 ± 0.8^{efghij}	100	1.4 ± 0.5^{k}	88	3.1 ± 1.3^{efg}	50
0.1	0.5	–	–	–	–	3.9 ± 1.1^{efg}	90
0.1	2.0	–	–	–	–	0.4 ± 0.3^{fg}	20
0.5	0.1	38.0 ± 7.8^{b}	100	6.3 ± 3.1^{ijk}	63	0.4 ± 0.2^{fg}	30
0.5	0.5	–	–	–	–	0.3 ± 0.1^{g}	30
0.5	1.0	–	–	–	–	0.4 ± 0.2^{fg}	40
1.0	0.1	4.0 ± 1.2^{fghij}	70	17.5 ± 3.8^{efghijk}	100	–	–
1.0	0.5	–	–	–	–	0.5 ± 0.3^{fg}	30
2.0	0.1	8.4 ± 3.6^{efghij}	80	27.8 ± 9.2^{defgh}	100	–	–
2.0	0.5	7.8 ± 1.9^{efghij}	100	3.0 ± 1.1^{ijk}	63	–	–
2.0	1.0	2.3 ± 0.7^{ij}	70	0.5 ± 0.3^{k}	25	–	–
2.0	2.0	0.3 ± 0.3^{j}	10	–	–	–	–
3.0	0.1	12.3 ± 2.3^{defghij}	100	15.8 ± 3.7^{fghijk}	88	–	–
3.0	0.5	0.3 ± 0.2^{j}	20	7.9 ± 2.1^{ijk}	100	0.3 ± 0.3^{g}	10
3.0	1.0	0.1 ± 0.1^{j}	10	0.9 ± 0.4^{k}	50	–	–
3.0	2.0	0.4 ± 0.3^{j}	20	–	–	–	–

Means with the same letter within columns are not significantly different at $P > 0.05$.

Table 2
Shoot regeneration from leaf lamina, stem internode and root segment explants cultured on MSMO medium containing different concentrations of TDZ and IAA

Plant growth regulators		Explants					
		Leaf lamina		Stem internode		Root segment	
TDZ (mg/l)	IAA (mg/l)	Mean number of shoots per explant (±SE)	% explants forming shoots	Mean number of shoots per explant (±SE)	% explants forming shoots	Mean number of shoots per explant (±SE)	% explants forming shoots
0.00	0.00	–	–	–	–	–	–
0.01	0.00	5.9 ± 4.3^{b}	30	22.8 ± 5.5^{cd}	80	–	–
0.01	0.05	3.6 ± 0.7^{bc}	90	27.3 ± 6.6^{c}	100	–	–
0.01	0.10	2.1 ± 0.9^{bc}	60	13.4 ± 3.4^{cdef}	70	0.7 ± 0.3^{cd}	40
0.01	0.50	10.1 ± 1.2^{a}	100	63.8 ± 9.8^{a}	100	–	–
0.05	0.00	–	–	24.6 ± 6.5^{cd}	90	1.2 ± 0.6^{bcd}	40
0.05	0.05	5.8 ± 1.7^{b}	80	9.6 ± 2.5^{def}	80	0.3 ± 0.2^{d}	20
0.05	0.10	0.7 ± 0.5^{c}	30	11.9 ± 4.7^{cdef}	70	2.1 ± 1.1^{abc}	40
0.05	0.50	2.3 ± 0.8^{bc}	50	19.7 ± 5.9^{cde}	100	–	–
0.10	0.00	–	–	5.2 ± 2.4^{ef}	60	2.4 ± 0.9^{abc}	70
0.10	0.05	–	–	10.3 ± 2.1^{def}	80	2.8 ± 1.4^{ab}	30
0.10	0.10	2.1 ± 1.2^{bc}	30	21.3 ± 4.0^{cde}	100	1.5 ± 0.4^{bcd}	70
0.10	0.50	0.5 ± 0.3^{c}	20	47.4 ± 9.6^{b}	100	3.7 ± 0.9^{a}	80

Means with the same letter within columns are not significantly different at $P > 0.05$.

hand, 1 mg/l ZEA alone gave rise to best results in terms of the mean number of shoots per stem explant. Combination of 1 mg/l ZEA with different concentrations of IBA (0.5, 1 and 3 mg/l) significantly decreased shoot number (Table 3).

Regenerated shoots were cultured on shoot elongation medium containing 0.5 mg/l GA_3 for additional 2 weeks (Fig. 1d). After 2 weeks, regenerated shoots were separated individually and cultured on MSMO medium containing IAA, IBA, 2.4-D or NAA. Of the different auxins investigated for rooting, IBA was superior, with the greatest number of roots per explant (38.35 roots produced per regenerated shoot with a 100% frequency of root formation) observed in media supplemented with 0.5 mg/l IBA (Table 5; Fig. 1e). They formed roots in 4–5 weeks. Medium containing 1 mg/l IBA and 0.1 mg/l 2,4-D were also effective for root formation (28.45 and 22.90 roots per regenerated shoot, respectively, with a 100% frequency of root formation). Increasing concentration of IBA or 2,4-D caused significant reductions in terms of mean number of roots per regenerated shoot (Table 5). Root induction occurred on the control treatment to which no auxin was added to the media. However, adding 0.5 or 1 mg/l IBA to the medium not only increased the mean number of roots per shoot

Table 3
Shoot regeneration from leaf lamina, stem internode and root segment explants cultured on MSMO medium containing different concentrations of ZEA and IBA

Plant growth regulators		Explants					
		Leaf lamina		Stem internode		Root segment	
Zeatin (mg/l)	IBA (mg/l)	Mean number of shoots per explant (±SE)	% explants forming shoots	Mean number of shoots per explant (±SE)	% explants forming shoots	Mean number of shoots per explant (±SE)	% explants forming shoots
0.0	0.0	–	–	–	–	–	–
0.01	0.0	–	–	7.8 ± 1.9^{efg}	80	–	–
0.01	0.5	4.9 ± 1.6^{c}	90	17.8 ± 1.7^{bc}	100	0.7 ± 0.3^{c}	40
0.01	1.0	12.9 ± 3.2^{ab}	90	17.7 ± 2.1^{bc}	100	0.1 ± 0.1^{c}	10
0.01	3.0	9.3 ± 3.9^{b}	70	20.7 ± 2.2^{b}	100	0.3 ± 0.2^{c}	20
0.1	0.0	–	–	16.8 ± 5.9^{bcd}	60	1.3 ± 1.3^{c}	10
0.1	0.5	0.1 ± 0.1^{d}	10	13.3 ± 2.6^{bcde}	100	3.4 ± 1.0^{c}	100
0.1	1.0	4.0 ± 1.4^{cd}	80	11.4 ± 2.6^{cdef}	100	10.2 ± 3.5^{b}	80
0.1	3.0	16.9 ± 1.7^{a}	100	13.5 ± 1.6^{bcde}	100	24.5 ± 4.1^{a}	100
0.5	0.0	–	–	8.4 ± 4.6^{defg}	90	–	–
0.5	0.5	0.1 ± 0.1^{d}	10	4.4 ± 0.8^{fg}	90	11.9 ± 2.3^{b}	100
0.5	1.0	1.0 ± 0.5^{cd}	40	3.6 ± 0.3^{fg}	100	13.5 ± 1.7^{b}	100
0.5	3.0	2.8 ± 0.7^{cd}	70	8.3 ± 1.7^{defg}	100	2.4 ± 0.8^{c}	70
1.0	0.0	–	–	36.2 ± 5.9^{a}	100	–	–
1.0	0.5	0.8 ± 0.6^{cd}	30	6.4 ± 2.2^{efd}	100	4.4 ± 1.5^{c}	90
1.0	1.0	4.5 ± 2.2^{cd}	80	14.9 ± 3.0^{bcde}	100	1.3 ± 0.8^{c}	30
1.0	3.0	13.8 ± 0.6^{a}	100	10.8 ± 2.4^{cdef}	100	4.1 ± 1.1^{c}	100

Means with the same letter within columns are not significantly different at $P > 0.05$.

explant (from 6.80 to 38.35 or 28.45, respectively) and the frequency of root formation (from 80% to 100%) significantly but also produced longer, thicker and more robust roots (Fig. 1e).

The rooted plants were transferred to Magenta containers including vermiculite for acclimatization (Fig. 1f). After 3 weeks, they were transferred to soil and kept under growth room conditions. Approximately 85% of the generated plants survived through the hardening off process (Fig. 1g).

Table 4

Shoot regeneration from leaf lamina, stem internode and root segment explants cultured on MSMO medium containing different concentrations of KIN and IAA

Plant growth regulators		Explants					
		Leaf lamina		Stem internode		Root segment	
KIN (mg/l)	IAA (mg/l)	Mean number of shoots per explant (±SE)	% explants forming shoots	Mean number of shoots per explant (±SE)	% explants forming shoots	Mean number of shoots per explant (±SE)	% explants forming shoots
0.0	0.0	–	–	–	–	–	–
0.1	0.0	–	–	2.7 ± 2.2^{b}	20	1.8 ± 0.6^{ef}	70
0.1	0.1	–	–	2.3 ± 0.8^{b}	50	2.5 ± 0.6^{ef}	90
0.1	0.5	0.1 ± 0.1^{c}	10	5.1 ± 1.3^{b}	90	2.9 ± 0.7^{ef}	80
0.1	1.0	3.0 ± 1.1^{b}	60	3.0 ± 0.6^{b}	90	2.4 ± 0.8^{ef}	70
0.5	0.0	–	–	1.2 ± 0.9^{b}	30	13.3 ± 7.3^{bcde}	70
0.5	0.1	–	–	3.6 ± 1.3^{b}	60	4.4 ± 1.0^{def}	100
0.5	0.5	0.9 ± 0.4^{c}	50	0.3 ± 0.3^{b}	10	0.6 ± 0.3^{ef}	30
0.5	1.0	4.3 ± 1.6^{b}	70	2.0 ± 1.8^{b}	20	2.4 ± 1.3^{ef}	50
1.0	0.0	–	–	30.2 ± 12.9^{a}	70	25.1 ± 11.2^{ab}	60
1.0	0.1	–	–	–	–	7.0 ± 2.5^{cdef}	80
1.0	0.5	7.0 ± 1.8^{a}	80	0.5 ± 0.5^{b}	10	9.3 ± 1.8^{cdef}	100
1.0	1.0	4.5 ± 1.4^{b}	70	5.3 ± 2.1^{b}	60	15.6 ± 2.8^{bcd}	100
3.0	0.0	–	–	1.8 ± 1.8^{b}	10	30.0 ± 4.8^{a}	100
3.0	0.1	–	–	–	–	17.2 ± 7.5^{bc}	40
3.0	0.5	–	–	0.9 ± 0.6^{b}	30	2.4 ± 1.2^{ef}	50
3.0	1.0	0.2 ± 0.1^{c}	20	0.9 ± 0.9^{b}	10	6.2 ± 4.7^{cdef}	30

Means with the same letter within columns are not significantly different at $P > 0.05$.

DISCUSSION

Only a few numbers of investigations have been carried out on *in vitro* regeneration of members of the genus *Lysimachia*. Zheng et al. [25] reported the *in vitro* culture protocol of three ornamental species of *Lysimachia* genus (*L. christinae, L. rubinervis* and *L. nummularia* 'Aurea'). They investigated the regenerability of these species using *in vitro* leaf and shoot tip explants on Murashige and Skoog (MS) medium containing only different concentrations of BA and NAA. Best shoot proliferation was obtained with both shoot tip and leaf explant for *L. christinae* with 3 mg/l BA+0.1 mg/l NAA or 5 mg/l BA+0.1 mg/l NAA combinations (around 12.25–14.34 shoots). Only shoot tip explant of *L. rubinervis* was successful for shoot regeneration with 3 mg/l BA+0.1 mg/l NAA or 5 mg/l BA+0.1 mg/l NAA (around 16.87–17.20

Table 5
Effects of the tested auxins on root formation from regenerated shoots

Treatments	Mean number of roots per explant (±SE)	% explants forming roots
Control	6.8±1.2^{fg}	80
IAA (mg/l)		
0.5	11.2±1.8^{ef}	95
1.0	18.2±1.8^{cd}	100
3.0	14.4±2.3^{de}	100
IBA (mg/l)		
0.5	38.3±3.7^{a}	100
1.0	28.4±3.4^{b}	100
3.0	2.2±0.7^{g}	75
2,4-D (mg/l)		
0.1	22.9±3.1^{bc}	100
0.5	7.6±1.1^{fg}	100
1.0	3.9±1.9^{g}	90
NAA (mg/l)		
0.5	6.0±1.4^{fg}	85
1.0	1.8±0.5^{g}	70
3.0	2.4±0.7^{g}	75

Means with the same letter within columns are not significantly different at $P>0.05$.

shoots) and no regeneration was observed with leaf explant of *L. rubinervis*. Controversially, leaf explant of *L. nummularia* 'Aurea' was successful for shoot regeneration with 1 mg/l BA+0.1 mg/l NAA (12.73 shoots) and no regeneration was observed with shoot tip explant. Consistently with the results of Zheng et al. [25] for leaf explants of *L. christinae* cultured with 1 mg/l BA+0.1 mg/l NAA (3.7 shoots) and with 3 mg/l BA+0.1 mg/l NAA (13.01 shoots), leaf explants of *L. vulgaris* regenerated 4 shoots with 1 mg/l BA+0.1 mg/l NAA and 12.3 shoots with 3 mg/l BA+0.1 mg/l NAA in our study (Table 1). Wang et al. [21] reported that MS medium supplemented with 0.5 mg/l BA and 1 mg/l NAA was the preferable propagation medium for the regeneration of *L. christinae* from the leaf explant. In contrast, no shoot formation was obtained with this combination (0.5 mg/l BA plus 1 mg/l NAA) from leaf lamina explant of *L. vulgaris* (Table 1). Generally, BA in combination with NAA was not as effective as BA in combination with IAA or IBA in our study (Table 1).

Similarly to our findings, IBA was reported as the most effective auxin for rooting in case of some medicinal plants including *Filipendula ulmaria* (L.) Maxim [23], *Solanum dulcamara* [12] and *Cassia angustifolia* Vahl [18].

Zheng et al. [25] used full strength or half strength MS medium containing only 0.1 mg/l NAA for *in vitro* rooting of regenerated three ornamental species of *Lysimachia* genus (*L. christinae, L. rubinervis* and *L. nummularia* 'Aurea'). Half

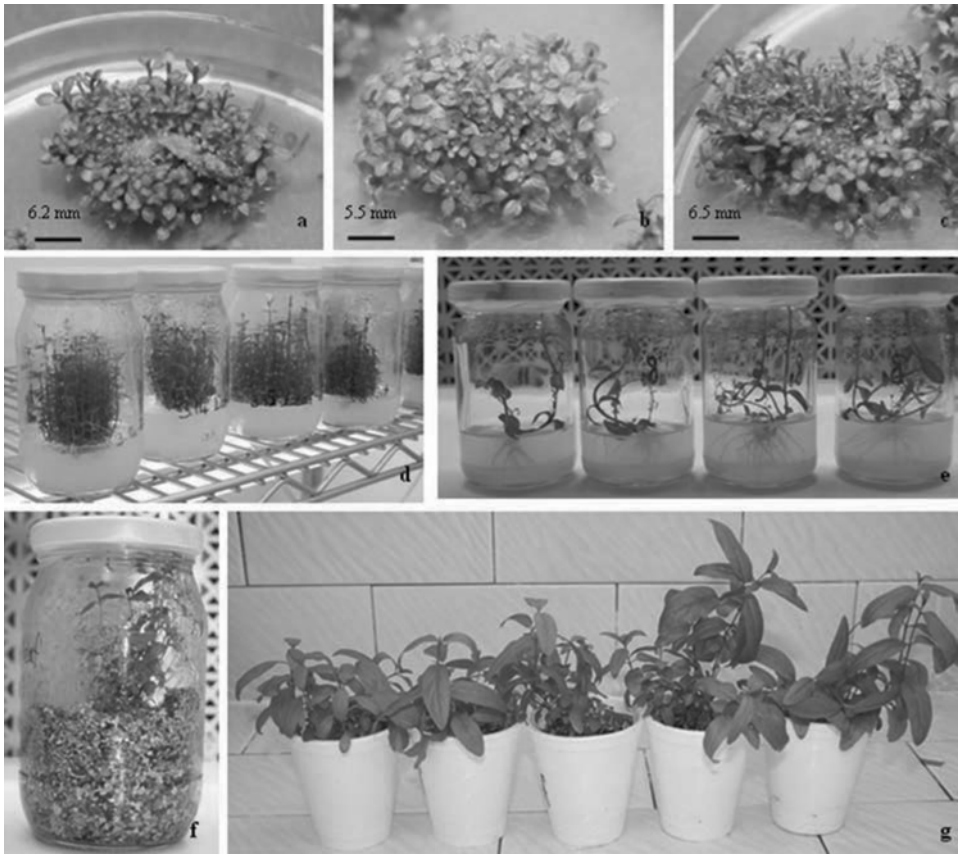

Fig. 1. In vitro regeneration of yellow loosestrife (*Lysimachia vulgaris* L.). (a) Shoot regeneration from leaf lamina explant on medium containing 0.5 mg/l BA+0.1 mg/l IBA, (b) from stem internode explant on medium containing 0.01 mg/l TDZ + 0.5 mg/l IAA, (c) from root explant on medium containing 0.5 mg/l BA+0.5 mg/l IAA. (d) Shoot elongation of regenerated shoots on medium containing 0.5 mg/l GA_3. (e) Rooting of the regenerated shoots on medium containing 0.5 mg/l IBA. (f) Regenerated plant in glass jar including vermiculite for acclimatization. (g) Regenerated plants transferred to foam cups containing sterile potting soil under growth room conditions

strength MS medium containing 0.1 mg/l NAA was the best medium for rooting in these *Lysimachia* species. Generally, NAA concentrations were not as effective as other used auxins (IAA, IBA and 2,4-D) for rooting of regenerated shoots of *L. vulgaris* in our study (Table 5). Three different concentrations of NAA (0.5, 1 and 3 mg/l) were used and more root formation was obtained with 0.5 mg/l NAA (6 roots) in our study (Table 1). Similarly, Zheng et al. [25] obtained 4.26 roots for *L. christinae*, 7.56 roots for *L. rubinervis* and 8.17 roots for *L. nummularia* 'Aurea' on full strength MS medium containing 0.1 mg/l NAA.

Both direct and indirect shoot regeneration require plant cells to undergo dedifferentiation and redifferentiation, both of which are known to be affected by not only exogenous plant growth regulators but also endogenous content of the hormones [19]. Different tissues may have different levels of endogenous hormones and different responses to PGRs, therefore, the type of explant source would have a critical impact on the regeneration success. In our study, when leaf lamina, stem internode and root explants were compared, it was clear that every used explant had different response to the used PGR combinations. For example, leaf lamina explant gave the best response with BA in combination with IBA (Table 1). Root explant gave the best response with BA in combination with IAA (Table 1). Stem internode explant were more productive with TDZ in combination with IAA and BA in combination with IBA. It was noticeable that other used explants (leaf lamina and root segments) were not productive with TDZ and IAA combinations (Table 2). The promoting effect of TDZ on *in vitro* development has been lately reported for many species [7, 10, 20, 23, 24]. Our data showed that, although TDZ produced some amounts of shoots from stem internode explants when used alone (0.01, 0.05 and 0.1 mg/l), its combinations with the auxin IAA were far more effective in shoot induction. Furthermore, combination of 0.01 mg/l TDZ with 0.5 mg/l IAA was more effective than 0.05 or 0.1 mg/l TDZ in combination with 0.5 mg/l IAA. Higher concentrations of TDZ reduced the mean number of shoots per explant regardless of the IAA concentration. Similarly, Huetteman and Preece [7] and Kumar et al. [9] reported that low concentrations of TDZ have been found to be useful for micropropagation and higher concentrations of TDZ increased callus formation in woody plants. They also emphasized the potential use of TDZ in the regulation of adventitious shoot production and synergism existing between TDZ and both endogenous and some exogenous auxins [7].

This paper is the first report to describe highly efficient and rapid regeneration systems for *L. vulgaris* via multiple shoot formation. Plant tissue culture is an alternative method of commercial propagation and is being used widely for the commercial propagation of a large number of plant species, including many medicinal plants [16]. Chemical constituents of medicinal plants and their parts are required to be uniform both qualitatively and quantitatively to have standardized formulations [1]. Micropropagation of *L. vulgaris* can provide a mass production of pesticide, herbicide and disease free plants on a commercial scale and unlimited plant materials can consistently be obtained throughout the whole year. Moreover, this protocol can provide plant material for future pharmacological, physiological and biochemical studies. Future studies will focus on comparison between *in vitro*-grown and field-grown plants in terms of their secondary metabolites and biological activities.

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