

REGULAR ARTICLE

Micropropagation of endemic *Scutellaria orientalis* L. subsp. *bicolor* using modified MS medium & TDZ

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ABSTRACT

Scutellaria orientalis L. subsp. *bicolor* (HOCHST.) Edmondson is a perennial endemic shrub bearing pale grey-white flowers that grows on marginal lands. They have high value as rock garden plants and in managed arid land scaping. They are also important medicinally for valuable secondary metabolites of pharmaceutical importance. The study compared effects of 1 and 2 × KH₂PO₄ concentrated MS medium for micropropagation of the plant using cotyledon leaf, cotyledon petiole, cotyledon node and hypocotyl explants. Variable number of healthy green shoots were regenerated on above mentioned explants variants of TDZ in 2 × KH₂PO₄ concentrated MS medium with maximum shoot regeneration on cotyledon node explants on 0.04 µg/ml TDZ. These were rooted on 1 × KH₂PO₄ concentrated MS medium containing 0.50 µg/ml NAA. All developing plants were acclimatized in greenhouse to flower and set seeds. The study serve to provide basic information on role 2 × KH₂PO₄ for *Scutellaria* mass propagation and will help in breeding and genetic transformation studies in future.

Keywords: KH₂PO₄; Mass propagation; MS medium; Skullcaps; Thidiazuron

INTRODUCTION

The genus *Scutellaria* (*Lamiaceae* family) has more than 350 species spread in tropical mountainous and temperate regions (Shu et al., 1994). *Scutellaria orientalis* L. is wide spread in Turkey with 18 subspecies, many of which are endemic. *Scutellaria orientalis* L. subsp. *bicolor* (HOCHST.) Edmondson is a perennial endemic shrub 10 - 40 cm high that flowers in June - July and grows on poor and arid soil slopes of high altitudes, volcanic rocks and barren lands extending from Mediterranean coasts to Central parts of Eastern Anatolia (Edmondson, 1982, Anonymous 2015) with average 1000 seed weight of 0.6454 g. They have antis leafy spikes that bear pale grey white or pale grey white violet flowers (Duman, 2000). The plant has high value as rock garden plant in arid land scaping, as the plant is drought tolerant and needs low amount of water for maintenance.

All subspecies contain anti fungal compounds that repel insects due to specific odour (Rodriguez et al., 1993, Bruno et al., 2002, Rosselli, et al., 2007).

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Plant parts like herbs, leaves, fruits and seeds are used to obtain various secondary metabolites of pharmaceutical and pharmacological importance. Aerial parts of *S. orientalis* are collected (Anonymous 2015) in Turkey and their extracts are used for treatment stress and many disorders in folk medicine. *Scutellaria* species contain sesquiterpenoids, monoterpenoids and phenylpropane derivatives iridoid glycosides (Hernandez, 1999) and glucuronide or aglucuronide type phenolic compounds (Nishikawa et al., 1999). All species of *Scutellaria* are rich in scutellarin, that is known to have anti cancer properties and induce apoptosis of breast and ovarian tumor cells *in vitro* and is long time used in treatment of cardiovascular diseases. Plants grown through tissue culture are excellent source of obtaining secondary metabolites in alternative way (Cui et al. 2010).

In vitro plant propagation techniques help to standardize production of biochemically and medicinally active secondary products synthesis, and select clones of superior individual genotypes (Li et al. 2000).

Tascan (2007) studied *S. baicalensis* in liquid culture medium to evaluate flavonoid contents. The researcher

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also evaluated effects of TDZ treatments for different durations of time in MS medium (containing MS salts, B5 vitamin and 3% sucrose), to induce regeneration. Similarly, shoot and callus culture techniques have also been used to observe phenolic compound in *S. orientalis*, *S. baicalensis*, *S. iyoensis*, *S. taurica*, *S. lateriflora*, *S. ventenatii*, *S. pontica*, *S. taurica*, *S. galericulata*, *S. integrifolia* and *S. alpina* (Nishikawa et al. 1999, Hirotsu et al. 1998, Zobayed et al. 2004 and Joshee et al. 2007).

No data regarding *in vitro* tissue cultures for *S. orientalis* sub sp. *bicolor* is available. The present study evaluated four different explants of *S. orientalis* sub sp. *bicolor* for their ability to regenerate under *in vitro* conditions.

MATERIALS AND METHODS

The experimental material (seeds) was obtained from the Department of Biology, Faculty of Art and Science, Bitlis Eren University, Bitlis, Turkey. Voucher specimens of plants are deposited at Herbarium of the Faculty of Science, Firat University, Elazig, Turkey.

The seeds are 1.8 mm \pm 0.2 mm long and 1.2 mm \pm 0.2 mm wide. They were initially tested with tetrazolium for vitality. Thereafter, they were treated with 100% commercial bleach (5% NaOCl Ace, Turkey) for 20 min. Surface sterilized seeds were rinsed 3 \times 3 min with sterilized distilled water. Thereafter, they were cultured on agar solidified MS medium (Murashig and Skoog, 1962) containing 1 \times concentration of KH_2PO_4 + 3% (w/v) sucrose in Petri dishes. These were sprouted under 16 h light photoperiod (35 μMol photons m^{-2} s^{-1}) in Sony versatile growth chamber at 24 \pm 1°C. (MS medium contain 170 $\mu\text{g}/\text{ml}$ KH_2PO_4 , therefore, it was called 1 \times KH_2PO_4 concentrated MS medium and when the concentration of KH_2PO_4 was doubled to 340 $\mu\text{g}/\text{ml}$ it was called 2 \times KH_2PO_4 concentrated MS medium. This terminology was coined to understand the differences in experimental results easily). Cotyledon leaf, hypocotyl, cotyledon petiole and cotyledon node explants were obtained from 7 - 8 days old young seedlings. These explants were cultured on MS medium containing both 1 and 2 \times concentrated KH_2PO_4 + 0.00 (control), 0.04, 0.08, 0.12, 0.16, 0.20, 0.24, 0.28, 0.32, 0.36, and 0.40 $\mu\text{g}/\text{ml}$ TDZ + 3% (w/v) sucrose.

Regenerating shoots were cultured on MS medium containing 0.50 $\mu\text{g}/\text{ml}$ NAA for rooting. *In vitro* regenerated plants were potted in peat moss and transferred to greenhouse under 16 h light photoperiod for 20 day.

Statistical analysis

All experimental datas were generated from the means of 60 explants per treatment, each of which was divided into 12 replications containing 5 explants per replication. Statistical analysis was carried out using one way ANOVA of IBM SPSS 20 for Windows. Probability of $P < 0.05$ was considered significant. Care was taken to arcsine transform all datas taken in percentages according to Snedecor and Cochran (1967) before subjecting them to statistical analysis.

RESULTS

Tetrazolium test showed that all seeds had 100% vitality. This confirmed that the seed material was safe to proceed for tissue culture studies.

Effects of various TDZ concentrations on shoot regeneration from cotyledon leaf explant of *S. orientalis* subsp. *bicolor*

The four type of explants were compared and evaluated for shoot regeneration on variants of TDZ in MS medium concentrated with 1 and 2 \times concentrated KH_2PO_4 .

All 11 concentrations were inhibitive or non regenerative and the explants induced severe hyperhydricity when they were cultured on MS medium containing 1 \times concentrated KH_2PO_4 + any concentration of TDZ (Fig. 1a). However, all explants on 2 \times KH_2PO_4 concentrated MS medium + 0.20 $\mu\text{g}/\text{ml}$ TDZ induced 40.33% callus regeneration only. These calli were partially regenerative and induced shoot buds on respective explants. The shoot buds showed severe dormancy and were difficult to elongate. About 20% explants cultured on 0.08 $\mu\text{g}/\text{ml}$ TDZ + 2 \times KH_2PO_4 concentrated MS medium induced direct shoot regeneration with 3 - 4 shoots per explant (Fig. 1b). Rest of the explants failed to induce any callus or shoot irrespective of the concentration of TDZ in the culture.

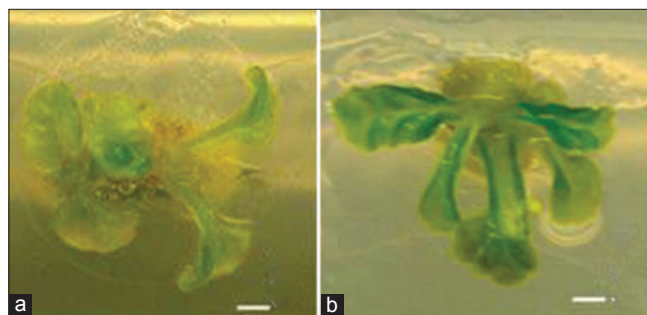


Fig 1. Shoot regeneration from cotyledon leaf explant of *S. orientalis* subsp. *bicolor* (a) inhibitive and hyperhydric shoots regenerated on MS medium containing 1 \times concentrated KH_2PO_4 + 0.20 $\mu\text{g}/\text{ml}$ TDZ (b) Induction of shoots on 0.08 $\mu\text{g}/\text{ml}$ TDZ + 2 \times KH_2PO_4 concentrated MS medium. Bar Fig 1a, b= bar=0.75 cm.

Effects of various TDZ concentrations on shoot regeneration from hypocotyl explant of *S. orientalis* subsp. *bicolor*

All explants cultured on $1 \times \text{KH}_2\text{PO}_4$ concentrated MS medium + variants of TDZ induced swelling followed by hyperhydricity and growth of abnormal structures on explants that died after 10 days due to high necrosis. All these observations were counted as no regeneration (Table 1).

No callus regeneration was noted on any hypocotyl explant cultured on $2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium (control - Table 1). All hypocotyl explants cultured on variants of TDZ (except $0.40 \mu\text{g/ml}$ TDZ) in $2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium promoted callus regeneration (Table 1) variably. Increase in concentration of TDZ promoted callus regeneration with maximum callus regeneration percentage of 100.00% on $2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium containing $0.24 \mu\text{g/ml}$ TDZ. It was followed by 76.60% callusing on $2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium containing 0.16 and $0.20 \mu\text{g/ml}$ TDZ each (Table 1).

Although shoot buds were very visible on explants, all of them were stunted and showed no shoot regeneration. Negligible shoot regeneration was noted on $2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium containing 0.16 and $0.36 \mu\text{g/ml}$ TDZ with 1.20 and 2.60 shoots per explant respectively.

Effects of various TDZ concentrations on shoot regeneration from cotyledon petiole explant of *S. orientalis*

Regeneration on all cotyledon petiole explants cultured on all concentrations of TDZ on $1 \times \text{KH}_2\text{PO}_4$ concentrated MS medium was proceeded by swelling of explants followed by growth of shoot meristems on a very few explants and treatments. The developing shoots were hyperhydric with abnormal translucent leaves. These were

counted nil at the time of final collection of data (Table 2). The experiment was terminated here.

Second experiment on $2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium containing variants of TDZ showed partial recovery of hyperhydric shoots. Cotyledon petiole explant was moderately regenerative in terms of callus and shoot regeneration percentage. No callus or shoot regeneration was noted on $2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium containing $0.04 \mu\text{g/ml}$ TDZ or 0.32 , 0.36 and $0.40 \mu\text{g/ml}$ TDZ. Callus regeneration percentage on rest of the cultures ranged 6.60 to 66.60% (Table 2). Maximum percentage of callusing explants was noted on $2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium containing 0.24 and $0.28 \mu\text{g/ml}$ TDZ.

Shoot regeneration percentage was very moderate with narrow range of 16.60 to 30.00%. Highest shoot regeneration percentage was noted on $2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium containing $0.08 \mu\text{g/ml}$ TDZ. Increasing concentrations of TDZ were inhibitive resulting in decreased or no shoot regeneration on respective treatments. Visible shoots had difficulty in attaining length and were stunted.

Number of shoots per explant excluding non regenerative cultures decreased from 8.00 to 2.20 shoots per explant. Increasing concentrations of TDZ had negative effects on number of shoots per explant. Highest number of shoots were noted on $2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium containing $0.08 \mu\text{g/ml}$ TDZ. It was followed by 6.60 shoots per explant that differed significantly from the number of shoots on rest of the treatments.

Effects of various TDZ concentrations on shoot regeneration from cotyledon node explant of *S. orientalis*

All developing shoots had partial hyperhydricity after 3 - 4 weeks of culture and showed translucent abnormal

Table 1: Effects of various TDZ concentrations on 1 and $2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium on shoot regeneration from hypocotyl explant of *S. orientalis* subsp. *bicolor*

TDZ ($\mu\text{g/ml}$)	Callus regeneration percentage		Shoot regeneration percentage		Number of shoots per explant	
	$1 \times \text{KH}_2\text{PO}_4$ concentrated MS medium	$2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium	$1 \times \text{KH}_2\text{PO}_4$ concentrated MS medium	$2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium	$1 \times \text{KH}_2\text{PO}_4$ concentrated MS medium	$2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium
0.04	0.00	66.60c	0.00	0.00	0.00	0.00
0.08	0.00	60.00c	0.00	0.00	0.00	0.00
0.12	0.00	66.60c	0.00	0.00	0.00	0.00
0.16	0.00	76.60b	0.00	6.60	0.00	1.20b
0.20	0.00	76.60b	0.00	0.00	0.00	0.00
0.24	0.00	100.00a	0.00	0.00	0.00	0.00
0.28	0.00	56.60d	0.00	0.00	0.00	0.00
0.32	0.00	56.60d	0.00	0.00	0.00	0.00
0.36	0.00	56.60d	0.00	6.60	0.00	2.60a
0.40	0.00	0.00	0.00	0.00	0.00	0.00
MS medium (control)	0.00		0.00		0.00	

All values in a single column showed by different small letters are significantly different at 0.05 level of significance using Tukeys test

Table 2: Effects of 1 and 2×KH₂PO₄ concentrated MS medium containing variants of TDZ on shoot regeneration from cotyledon petiole explant of *S. orientalis*

TDZ (µg/ml)	Callus regeneration percentage		Shoot regeneration percentage		Number of shoots per explant	
	1×KH ₂ PO ₄ concentrated MS medium	2×KH ₂ PO ₄ concentrated MS medium	1×KH ₂ PO ₄ concentrated MS medium	2×KH ₂ PO ₄ concentrated MS medium	1×KH ₂ PO ₄ concentrated MS medium	2×KH ₂ PO ₄ concentrated MS medium
0.04	0.00	0.00	0.00	0.00	0.00	0.00
0.08	0.00	33.30b	0.00	30.00a	0.00	8.00a
0.12	0.00	13.30d	0.00	26.60b	0.00	6.60b
0.16	0.00	6.60e	0.00	26.60b	0.00	2.20c
0.20	0.00	20.00c	0.00	20.00c	0.00	2.60c
0.24	0.00	66.60a	0.00	26.60b	0.00	3.20c
0.28	0.00	66.60a	0.00	16.60d	0.00	3.20c
0.32	0.00	0.00	0.00	0.00	0.00	0.00
0.36	0.00	6.60e	0.00	0.00	0.00	0.00
0.40	0.00	0.00	0.00	0.00	0.00	0.00
MS medium (control)	0.00		0.00		0.00	

All values in a single column showed by different small letters are significantly different at 0.05 level of significance using Tukeys test

shaped structures on explants cultured on 1 × KH₂PO₄ concentrated MS medium containing variants of TDZ (Fig. 2a). No callus regeneration was noted on MS medium.

Regeneration on cotyledon node explants proceeded after 9 to 11 days of callus induction on 2 × KH₂PO₄ concentrated MS medium containing variants of TDZ. It was followed by growth of shoot meristems that converted to shoots within 20 - 21 days. Callus regeneration percentage on rest of the cultures remained 20.00 to 66.60% (Table 3). 2 × KH₂PO₄ concentrated MS medium containing variants of TDZ induced inconsistent callusing. Maximum callus regeneration was noted on 2 × KH₂PO₄ concentrated MS medium containing 0.16, 0.28 and 0.32 µg/ml TDZ (66.60%).

No shoot regeneration was noted on 2 × KH₂PO₄ concentrated MS medium containing 0.40 µg/ml TDZ. Shoot regeneration percentage had range of 26.60% to 100.00% (Table 3). Maximum shoot regeneration was noted on 2 × KH₂PO₄ concentrated MS medium containing 0.04 µg/ml TDZ. Shoot regeneration percentage decreased drastically with each increasing concentrations of TDZ in 2 × KH₂PO₄ concentrated MS medium.

Number of shoots per explant decreased consistently on 2 × KH₂PO₄ concentrated MS medium containing 0.04 to 0.40 µg/ml TDZ with range of 3.50 - 9.00 shoots/explant (Fig. 2b) (excluding no shoot regeneration on 0.40 µg/ml TDZ). Highest number of 9.00 shoots per explant was noted on 2 × KH₂PO₄ concentrated MS medium containing 0.04 µg/ml TDZ.

Rooting and acclimatisation

S. orientalis sub-sp. *bicolor* shoot regenerated on cotyledon node explant rooted readily on 2 × KH₂PO₄ concentrated

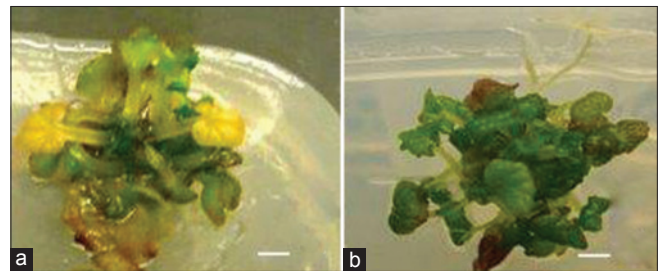


Fig 2. Shoot regeneration from cotyledon node explant of *S. orientalis* subsp. *bicolor* (a) Partial hyperhydric shoots after 3 - 4 weeks of culture with translucent abnormal shaped structures on explants cultured on 1 × KH₂PO₄ concentrated MS medium containing variants of TDZ. (b) shoot regeneration on 2 × KH₂PO₄ concentrated MS medium containing 0.40 µg/ml TDZ. Bar Fig 2a, b= bar=0.85 cm.

MS medium containing containing 0.5 µg/ml NAA. The plants were transferred to pots containing peat moss on attaining 4 - 5 cm length. The rooting percentage after four weeks of culture was 80.40%; whereas, no rooting was noted on MS medium (control). The rooted plants were acclimatized in Sanyo versatile environmental test chamber at 24 ± 2°C under 3000 lux light and 16 h light photoperiod. Rooted plantlets were hardened and acclimatized under greenhouse conditions; where, they continued to grow without signs of water stress.

DISCUSSION

TDZ is an important cytokinin that has been used by numerous researchers for inducing shoot regeneration in many plant species. The results showed that 1 × KH₂PO₄ concentrated MS medium containing variants of TDZ was inhibitory and tended to induce hyperhydricity followed by necrosis. However, 2 × KH₂PO₄ concentrated MS medium containing any concentration of TDZ showed variable regeneration on cotyledon leaf, hypocotyl, cotyledon petiole and cotyledon node of *S. orientalis*

Table 3: Effects of 1 and 2×KH₂PO₄ concentrated MS medium containing variants of TDZ on shoot regeneration on cotyledon node explant of *S. orientalis*

TDZ (µg/ml)	Callus regeneration percentage		Shoot regeneration percentage		Number of shoots per explant	
	1×KH ₂ PO ₄ concentrated MS medium	2×KH ₂ PO ₄ concentrated MS medium	1×KH ₂ PO ₄ concentrated MS medium	2×KH ₂ PO ₄ concentrated MS medium	1×KH ₂ PO ₄ concentrated MS medium	2×KH ₂ PO ₄ concentrated MS medium
0.04	0.00	0.00	0.00	100.00a	0.00	9.00a
0.08	0.00	46.60c	0.00	80.00b	0.00	8.50b
0.12	0.00	30.00d	0.00	46.60c	0.00	7.80c
0.16	0.00	66.60a	0.00	40.00d	0.00	7.00d
0.20	0.00	53.00b	0.00	33.30de	0.00	7.00d
0.24	0.00	20.00e	0.00	30.00e	0.00	6.00e
0.28	0.00	66.60a	0.00	36.60d	0.00	6.60de
0.32	0.00	66.60a	0.00	33.30de	0.00	5.50f
0.36	0.00	53.60b	0.00	26.60e	0.00	3.50g
0.40	0.00	0.00	0.00	0.00	0.00	0.00
MS medium (control)	0.00		0.00		0.00	

All values in a single column showed by different small letters are significantly different at 0.05 level of significance using Tukeys test

sub sp. Bicolor. It was found that each explant behaved variably on 1 and 2 × KH₂PO₄ concentrated MS medium containing variants of TDZ in concentration dependent manner. The best shoot regeneration in terms of callus regeneration percentage and shoot regeneration was noted on 2 × KH₂PO₄ concentrated MS medium containing variants of TDZ using cotyledon node explants. Similarly, Aasim et al. (2009) compared the effects 0.15, 0.25, 0.35 mg/l (µg/ml) thidiazuron (TDZ), on regeneration of cowpea and found maximum number of shoots per explant on MS medium containing 0.25 mg/l (µg/ml) TDZ. Hyperhydricity was recorded on some regenerated shoots. Composition of regeneration medium play important role on callusing and shoot regeneration. Physiological disorders like hyperhydricity are very difficult challenges in tissue culture of almost all plant species. Variable factors are known to cause growth disorders including hyperhydricity and shoot tip necrosis. The results of the experiment clearly shows that TDZ based hyperhydricity could be reduced significantly, when the concentration of KH₂PO₄ in MS medium was doubled (or 2 × KH₂PO₄ concentrated MS medium was used in the experiment). Similarly, Reed et al. (2011) noted effect of mineral stock solutions on development of hyperhydricity and shoot tip necrosis etc. They tested effects of MS macro and minor elements over a range of concentrations in a 5 dimensional experimental design. Hyperhydricity was more prominent with low concentration of macro elements. They noted that hypertrophy was either due to interactions between macro elements and KNO₃ or to low NH₄NO₃. They noted that callus induction was very common on MS medium and positively affected by low concentrations of NH₄NO₃.

The experiment showed that cotyledon node regenerated shoots had no problem in shoot regeneration on 2 × KH₂PO₄ concentrated MS medium containing variants

of TDZ. The results are in agreement with Mohamed (2011), who found that *Dianthus caryophyllus* explant growth and morphogenesis were positively affected by increasing phosphate level in MS medium.

Shoot regeneration percentage was maximum on 2 × KH₂PO₄ concentrated MS medium containing any concentration of TDZ on cotyledon node explant. Cotyledon leaf explant was extremely recalcitrant and induced regeneration on negligibly reduced number of explants. Although hypocotyl explant induced variable callus but shoot regeneration was almost negligible that was noted on only two regeneration treatments. Cotyledon petiole was partially regenerative and did not induce more than 30.00% regeneration. This confirmed that these explants (Cotyledon leaf, hypocotyl, cotyledon petiole and cotyledon node explants) had variable regeneration capacity and they behaved variably on reaction with 2 × KH₂PO₄ concentrated MS medium containing different concentrations of TDZ.

Aasim et al. (2009) suggested that TDZ could be effectively used to induce high number of shoots. 2 × KH₂PO₄ concentrated MS medium containing higher TDZ concentrations reduced shoot regeneration and resulted in stunted growth on shoots in partial agreement with Malik and Saxena (1992). It was assumed that inhibition in regeneration on 3 out of 4 explants was mostly due to excretion of phenolics that inhibited growth of tissues in agreement with Khawar et al (2002, 2004). Ivanova and Van Staden (2011) observed liquid media containing TDZ induced hyperhydricity and lost results in their ability of explants to regenerate. The use 1 × KH₂PO₄ concentrated MS medium containing any concentration of TDZ resulted in no or very low shoot regeneration and high hyperhydricity. Shoot regeneration from *S. orientalis* has never been reported

earlier. *S. orientalis* is highly important medicinal plant that could be multiplied easily under laboratory conditions in the presence of TDZ on $2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium with correct selection of explants and has not been reported previously. Similar results were noted in *Hibiscus rosa-sinensis* by Preece et al. (1991); who confirmed use of TDZ for shoot regeneration. In general terms, $2 \times \text{KH}_2\text{PO}_4$ concentrated MS medium containing lower doses of TDZ were more effective compared to higher doses of TDZ in shoot regeneration in agreement with Khawar et al. (2004), who obtained shoot regeneration from nodal explants cultures of lentil on media supplemented with 0.05 mg/l ($\mu\text{g}/\text{ml}$) TDZ. Similarly, Khawar et al. (2005); found that *Plantago lanceolata* could be effectively micropropagated on various concentrations of TDZ + IBA.

The developing shoots were rooted on $1 \times \text{KH}_2\text{PO}_4$ concentrated MS medium containing 0.50 $\mu\text{g}/\text{ml}$ (mg/l) NAA. The results are not in agreement with Fratini and Ruiz (2002), who found that TDZ inhibits rooting.

The plants were easily rooted and acclimatised using peat moss without any problem in growth chamber and established in the greenhouse. This practice helped in chlorophyll autobiosynthesis and led to easy establishment of plants under outside conditions.

Previous studies by Kendir et al. (2009) emphasize that an increase in root length and number is very important for acclimation under *ex vitro* conditions. Yildirim (2013) and Ozdemir et al. (2014) also emphasize that number of roots and their length helps in easy uptake of water, and nutrients under *in vitro* conditions during rooting of *Origanum acutidens* and *Lallemantia iberica* respectively.

CONCLUSION

Present study contribute positively to objective of the study and underlines importance of TDZ + KH_2PO_4 concentration in the culture medium. The results would be of enormous economic importance in efficient mass propagation, breeding and genetic transformation studies in future.

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Author contribution

Fethi Ahmet Ozdemir & Mehmet Ugur Yildirim planned the project, collected the plant material and executed the

experiments, collection of experimental data, scientific literature and writing of the experiment. Parisa Pourali Kahriz helped in execution of experiment, statistical analysis, with proper commentary to the results describing the results.

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