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Full Length Article

An Efficient Method for Direct Shoot Regeneration from Leaf Explants of *Solanum nigrum* Induced by Thidiazuron

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Abstract

Direct shoot regeneration was established from leaf, petiole and nodal explants from *in vitro* and field grown plants of *Solanum nigrum* on Murashige and Skoog (MS), and Gamborg's (B_5) media containing different levels of plant growth regulator, Thidiazuron (TDZ). Comparison of the individual effect of basal media, levels of TDZ, explant type or source of explants on shoot regeneration was tested. Percent shoot regeneration from leaf explant was highest (86.66%) followed by *in vitro* source of explant (83.65%), B_5 basal medium (83.20%) or 2.27 µ*M* TDZ (73.70%) after 60 days of initial culture. The number of shoots was highest (35.10) with 9.13 cm longest shoot at 2.27 µ*M* TDZ. There was a little correlation between predictors and dependent variables was found; whereas, GLM analysis demonstrated significant correlation between the factors. *In vitro* shoots were transferred to a half-strength MS medium containing different concentrations of NAA or IBA in MS or B_5 medium. MS medium produced 100% roots supplemented with 0.5 µ*M* NAA after 28 days. However, stout and hardy rooted shoots were obtained at 0.5 μ IBA with 10.19 mean number and 5.57 cm long roots. Rooted shoots were shifted to *ex vitro* conditions in polyethylene-cups filled with peat moss: sand: garden soil (1:1:1) with 85.50% survival with true-to-type morphology under the natural conditions. The present investigation demonstrated that TDZ may be used for high frequency direct shoot regeneration from leaf explants of *S. nigrum* on B_5 basal medium. This protocol may be useful for subsequent high yielding metabolites producing plants. © 2017 Friends Science Publishers

Keywords: Direct shoot regeneration; General Linear Model; *Solanum nigrum*; Thidiazuron (TDZ)

Introduction

Solanum nigrum, a member of the Solanaceae family, commonly known as black nightshade, or hound's berry, is a small-fruited black nightshade of Eurasia (Bhat *et al*., 2010). It is fairly a common weed that grows on undisturbed soil and wastelands. It contains numerous secondary metabolites of medicinal importance including steroidal lactones and alkaloids (Sridhar and Naidu, 2011) which are used for the preparation of traditional folk medicines to cure various ailments (Ding *et al*., 2012). *S. nigrum* has been used as tonic, diuretic, antiseptic, analgesic, anti-dysentric, anti-narcotic, anti-cancerous, emollient, and as a laxative (Mohy-ud-din *et al*., 2010). Tender leaves of *S. nigrum* are used as leafy vegetable for the preparation of dishes and soups. Moreover, root bark is used in liver inflammation (Perez *et al*., 1998). Fruits of this plant are useful for heart ailments and plant juice is used to cure chronic enlargement of liver, piles, and fever (Kumar and Kumar, 1995).

Clonal propagation through tissue culture offers an alternative approach to vegetative propagation. It has the potential to provide high multiplication rates of uniform stocks with high genetic stability (Beck and Dunlop, 2001). Many factors including stresses like drought, nutrient deficiency and diseases ultimately down regulate the accumulation of secondary metabolites in plants. In this regard *In vitro* shoot multiplication is worthy and practical for the production of healthy stock plants for enhanced secondary metabolite production (Jain and Saxena, 2009; Kirakosyan *et al*., 2009).

In vitro shoot proliferation and multiplication is largely based on media formulations with cytokinins as a major plant growth regulator (Hoque, 2010). In tissue culture, cytokinins play an important role as they promote cell division and develop meristematic centers leading to the formation of organs, mainly shoots (Peeters *et al*., 1991). Rooting of *In vitro* regenerated shoots can be initiated by transferring them to a medium containing only auxins.

Thiadizuron (TDZ), a synthetic phenylurea-type cytokinin, has been applied for micropropagation in *Solanum tuberosum* (Sajid and Aftab, 2009) and in *Stevia rebaudiana* (Ghauri *et al.*, 2013), shoot regeneration in *Heterophragma* (Akram and Aftab, 2011), and in *Populus ciliata* (Aggarwal *et al*., 2012), shoot multiplication in *Bryophyllum* (Naz *et al*., 2009), somatic embryogenesis in woody plants (Huetteman and Preece, 1993) and for regeneration and enhancement of flavonoids in *Silybum marianum* (Khan *et al*., 2014). However, reports of the

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effects of TDZ on *In vitro* propagation of *S. nigrum* in literature are limited. The aim of the present investigation is to study the efficacy of TDZ on direct shoot regeneration from field, as well as *In vitro* grown plants of *S. nigrum* on different basal media.

Materials and Methods

Plant Material and Culture Environment

Shoots of *S. nigrum* were collected from the field of Punjab University Botanical Garden, University of the Punjab, New Campus, Lahore, Pakistan. Young 4–5 cm long shoots with leaves were excised to 1.5 cm^2 leaf, 1.0 cm long petiole, and nodal explants. The explants were washed with tap water followed by detergent. They were then immersed in a 10% (v/v) solution of bleach (NaOCl) and few drops of Tween-20 (Merck) for another 10 min and then rinsed 3–4 times with sterile distilled water and inoculated in culture vessels (25 × 150 mm,) containing 10 mL specified culture medium set with pH 5.8 and then autoclaved at 121°C of 104 kPa pressure for 15 min. The cultures were placed in growth room at 25 ± 2°C under 16 h photoperiod (40 µmoL $m^{-1} s^{-1}$) provided with white fluorescent tube lights (Phillips, Pakistan).

Establishment of Axenic Shoot Cultures

To obtain axenic shoots, 1.0 cm long sterile nodal field explants were inoculated on MS (Murashige and Skoog, 1962) medium supplemented with 1.0 µ*M* BA for 7 days under the same culture conditions as mentioned above, and maintained for further experimentation.

Direct Shoot Regeneration (DSR)

One-centimeter-long explants of leaves, petioles and nodes from field as well as *in vitro* shoots of *S. nigrum* were cultured on MS or B_5 (Gamborg *et al*., 1968) basal media. Each basal medium was solidified with 0.8% agar, (Agar Technical, OXOID, England) supplemented with various concentrations (0.45, 0.91, 1.36, 1.82 or 2.27 µ*M*) of TDZ (Sigma Aldrich). The data for DSR, number and length of shoots were recorded after 60 days of initial culture.

Shoot Proliferation

The regenerated shoots were shifted on liquid MS basal medium in glass baby jars (50 × 130 mm) for further growth and development.

in vitro Rooting

About 2.0 cm long shoot tips were cultured on half strength MS or B_5 media supplemented with 0.5, 1.0, 1.5 or 2.0 µ*M* of either α-Naphthalene acetic acid (NAA) or indole-3-butyric acid (IBA). The data were recorded for rooting percentage, number and length of roots after 28 days of culture.

Hardening and Acclimatization

Rooting zone was washed with water to remove traces of the agar and treated with 0.1% solution (w/v) of fungicide Dithane (Dove Agro, France) for one min, and transferred to polyethylene-cups containing soil mixture (substrate). Different rooting substrates were tested for plant survival in hardening and acclimatization process.

The plants were covered with polythene bags for maintaining moisture content under the same culture room conditions. The plantlets were irrigated 2 times a day with ¼ MS macro salts for 30 days. Plantlets were shifted in the field and recorded the data for survival or for any morphological abnormality.

Histological Analysis

Cultures of regenerating shoot buds were placed in distilled water and very thin sections were prepared with a sharp blade by placing the plant material in potato blocks. Sections were then placed on glass slide and covered with cover slip and observed under compound microscope (Optika, B-130, Italy) fitted with digital camera and then photographs were taken.

Experimental Design and Data Analysis:

Completely randomized design was used with 4 factorial (2 × 5 × 3 × 2) (Medium: *MS* × B_5, TDZ 5 *concentrations* × Explants: *petiole, leaf, node* × Source of explants: *field, in vitro*) shoot regeneration experiment. Data were subjected to Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) to check most significant treatment for shoot regeneration and rooting percentage. Multiple linear regression was performed to calculate R^2 and adjusted-R^2 to check correlation between the variables. General Linear Model (GLM) was used to comprehend interactive effect of predictors on dependent variables by SPSS v. 16.0 at $P \leq 0.05$.

Results

Effect of Basal Medium

Generally, explants on B_5 showed early response with high frequency (83.20%) of DSR than on MS medium (32.10%). The number (17.91) as well as length of the shoot (5.96 cm) was however highest on MS medium during the same culture period of 60 days (Table 1). Significant results were obtained of DSR percentage (p=0.000) and shoot length (SL) (p=0.010) with media having fair correlation (R^2 = 0.158, R^2 = 0.074), as compared to other predictors and shoot number (SN), respectively as analyzed by multiple regression analysis (Table 2). Fig. 1 shows the tendency of increased or decreased predictor's coefficient values. Full factorial analysis, general linear model (GLM) showed the significant effect of media on dependent variables (Table 3).

Table 1: Effect of different basal media, concentrations of TDZ, and type of explants on i*n vitro* DSR and multiplication of *S. nigrum* after 60 days of culture

Experiment	Variables	Shoot regeneration (%)	Number of shoot	Shoot length (cm)
Basal medium	MS	32.10 ± 3.32^{bc}	17.91 ± 2.36^{a}	05.96 ± 1.26^{a}
N = 96	B_5	83.20 ± 5.66^{a}	05.60 ± 1.45^{b}	05.08 ± 1.10^{ab}
TDZ concentrations (μM)	0.45	03.33 ± 1.25^{de}	08.00 ± 2.36^{cd}	02.66 ± 1.00^{de}
	0.91	16.83 ± 2.54^{cd}	31.83 ± 3.21^{ab}	03.00 ± 1.14^{d}
	1.36	53.50 ± 6.56^{bc}	06.86 ± 2.56^{cd}	05.25 ± 1.32^{cd}
N = 135	1.82	18.50 ± 4.55^{cd}	07.76 ± 2.45^{cd}	07.05 ± 1.45^{bc}
	2.27	73.70 ± 6.54^{a}	35.10 ± 3.24^{a}	09.13 ± 2.45^{a}
Explants	Petiole	57.65 ± 2.75^{b}	03.25 ± 1.25^{bcd}	02.46 ± 2.33^{bc}
	Leaf	86.66 ± 6.56^{a}	29.63 ± 3.25^{a}	12.77 ± 3.24^{a}
N = 288	node	12.70 ± 2.34^{cde}	02.36 ± 1.14^{bcd}	01.30 ± 0.98^{c}
Source of explant	Field	52.37 ± 5.65^{b}	11.76 ± 2.14^{b}	01.51 ± 0.78^{b}
N = 576	*in vitro*	83.65 ± 4.25^{a}	28.80 ± 3.65^{a}	05.31 ± 1.25^{a}

Differed small alphabetical letters with the means indicated significantly different results according to DMR Test at $P < 0.05$

Table 2: Effect of different factors on shoot regeneration percentage (SR), number of shoots (SN) and shoot length (SL) compared by regression analysis at p<0.05 of *S. nigrum*

Regression analysis	Predictors								
	Basal medium			TDZ			Explant		
	Dependent variables								
	SR%	SN	SL	SR%	SN	SL	SR%	SN	SL
Coefficients									
B	-32.6	4.889	-0.031	0.000	-0.001	0.018	-0.007	-0.003	-0.035
SE of B	7.579	4.521	0.012	0.002	0.003	0.016	0.002	0.004	0.020
Beta	-0.397	0.115	-0.272	0.018	-0.042	0.121	-0.329	-0.067	-0.187
t	-4.301	1.081	-2.648	0.169	-0.397	1.148	-3.268	-0.629	-1.783
Model summary									
R	0.397	0.115	0.272	0.018	0.042	0.121	0.329	0.067	0.187
R^2	0.158	0.013	0.074	0.000	0.002	0.015	0.108	0.004	0.035
Adjusted R^2	0.148	0.002	0.063	-0.011	-0.010	0.004	0.098	-0.007	0.024
SE estimate	38.082	21.447	0.486	0.650	0.650	0.645	0.780	0.823	0.811
Sig.	0.000*	0.283^{NS}	0.010*	0.867^{NS}	0.693^{NS}	0.254^{NS}	0.002*	0.531^{NS}	0.078^{NS}

Table 3: General Linear Model (GLM) Multivariate analysis for estimation of the effects of medium, levels of TDZ and explants on direct shoot regeneration of *S. nigrum*

Source	Dependent Variable	Mean Square	F	Sig.
Medium	shoot regeneration %	23912.100	335.060	0.000
	number of shoots	537.778	6.041	0.017
	shoot length	124.844	34.308	0.000
TDZ	shoot regeneration %	365.622	5.123	0.001
	number of shoots	843.906	9.480	0.000
	shoot length	15.410	4.235	0.004
Explant	shoot regeneration %	43325.911	607.089	0.000
	number of shoots	7922.544	88.995	0.000
	shoot length	347.786	95.575	0.000
Medium × TDZ	shoot regeneration %	558.489	7.826	0.000
	number of shoots	500.639	5.624	0.001
	shoot length	9.101	2.501	0.052
Medium × explant	shoot regeneration %	10680.133	149.652	0.000
	number of shoots	2049.478	23.022	0.000
	shoot length	31.603	8.685	0.000
TDZ × explant	shoot regeneration %	721.564	10.111	0.000
	number of shoots	639.156	7.180	0.000
	shoot length	50.928	13.996	0.000
Medium × TDZ × explant	shoot regeneration %	730.981	10.243	0.000
	number of shoots	587.672	6.601	0.000
	shoot length	10.599	2.913	0.008
Error	shoot regeneration %	71.367		
	number of shoots	89.022		
	shoot length	3.639		

The model is based on full factorial descriptive analysis at $P > 0.05$

Effect of TDZ Concentrations

Within 5 days, DSR was observed on all levels of TDZ with subsequent production of shoot buds up to 60 days. Generally, by increasing the amount of TDZ, all DSR was significantly improved. Highest DSR (73.70%) with 35.10 mean number and 9.13 cm long shoots was obtained on B_5 + 2.27 µ*M* TDZ after 60 days (Table 1). The number of shoot buds was initially high (31.83) at 0.91 µ*M* TDZ which subsequently reduced due to high death rate. Regression analysis demonstrated the non-significant effect of different levels of TDZ on the parameters of DSR. There was no correlation (R^2 = 0.000) with percent DSR, R^2 = 0.002 correlation in case of SN or R^2 = 0.015 correlation with SL was observed. Fig. 1 indicated the coefficient variation with high 't' value in terms of shoot length. On the other hand, GLM demonstrated the significant effect of TDZ on all shoot parameters (Table 3). Shoot length was non-significant when medium × TDZ was compared. The interactive effect of TDZ × explant showed highly significant results by GLM.

Effect of Explant/Source of Explant

When the individual effect of explant and source of explant was compared, it was found that highest 86.66% DSR with 29.63 SN and 12.77 cm SL was obtained from leaf explant, as compared to *in vitro* source DSR (83.65%) after 60 days of culture (Table 1). The *in vitro* derived leaf explant produced 28.80 SN and 5.31 cm SL after the same culture period. Table 2 demonstrated that there was significant effect (p = 0.002) of explants with fair correlation (R^2 = 0.108) on percent DSR followed by non-significant effect of explants on SL (p = 0.078). The effect of explant on dependent variables clearly demonstrated the trend analysis of different coefficients (Fig. 1). GLM showed that highly significant results conflicted with regression analysis. Individual effect of explant, interaction with basal medium or TDZ or basal medium and TDZ showed highly significant (p = 0.000) results for DSR, SN and SL (Table 3).

Morphology of Shoot Cultures

The morphology of shoot buds was different on different media. Green shoot buds were observed from cut margins of leaf explants (Fig. 2A) after 60 days of culture at 2.27 µ*M* TDZ in MS medium. Such buds proliferated to culture-able shoots on the same medium for another 20 days of culture (Fig. 2B). Regeneration frequency of shoots from *in vitro* leaf explant was quite efficient originating after forming a little callus mass on B_5 basal medium supplemented with 2.27 µ*M* TDZ after 60 days (Fig. 2C). On the other hand, shoot buds initiated from midrib of the field grown leaf explants on MS + 0.91 µ*M* TDZ after 10 days of culture (Fig. 2D). Subsequently, the whole surface of leaf became totipotent and formed numerous shoot buds after 60 days (Fig. 2E) that further developed and elongated (Fig. 2F).

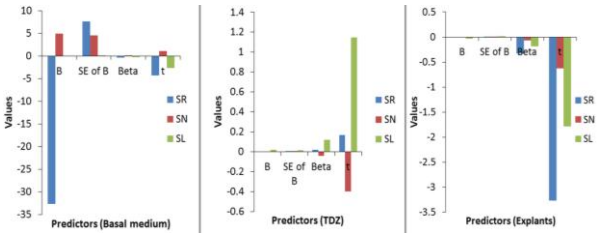

Fig. 1: Graphical representation of the effect of Basal medium, TDZ, Explant on percent shoot regeneration (SR), number of shoot (SN) and shoot length (SL) compared model summary of regression analysis at p<0.05

Fig. 2: Effect of different factors affecting on DSR from different explants of *S. nigrum*. A): Direct shoot regeneration from *in vitro* grown leaf explant on MS + 2.27 µ*M* TDZ after 60 days of culture, *Bar* = 8 mm. B): Proliferation of the same regenerating shoots on liquid MS medium in glass jars, *Bar* = 22 mm. C): Shoot regeneration from cut margins from *in vitro* leaf explants on B_5 basal medium + 2.27 µ*M* TDZ after 60 days of initial culture, *Bar* = 5 mm. D: Growth of DSR from field grown leaf explants on MS + 0.91µ*M* TDZ after 10 days of culture, *Bar* = 5 mm. E-F: Huge number of micro shoot buds on leaf explant on the same medium (MS + 0.91 µ*M* TDZ) after 90 days of culture, *Bar* = 7 mm. G-H): 30- day old pigmented and fascinating shoot buds were observed at B_5 + 2.27 µ*M* TDZ on field grown leaf explants, *Bar* = 5 mm. I-J-K): Rooting of regenerated shoots on half strength MS + 0.5, 1 or 2 µ*M* IBA after 28 days of initial culture, *Bars* = 13, 15 or 20 mm, respectively. L): Acclimatization of plantlet in poly - cup filled with peat moss + garden soil + sand, *Bar* = 35 mm. LE = leaf explant; DSB = direct shoot bud

Table 4: Effect of different basal media and concentrations of IBA and NAA on different rooting parameters of *S. nigrum*

Experiment	Variable	Rooting (%)	Number of roots	Root length (cm)	R^2
Basal medium	MS	100[a]	09.55 ± 2.88[a]	02.72 ±1.21[a]	0.8024
N = 80	B_5	45.32 ± 4.24[b]	06.47 ± 2.65[b]	01.93 ± 0.87[b]	0.8275
NAA concentrations (µ*M*)	0.5	100[a]	05.20 ± 1.74[b]	01.35 ± 0.98[b]	0.7214
	1	75.20 ± 5.55[b]	05.30 ± 1.32[b]	00.55 ± 0.01[c]	
N = 120	1.5	50.00 ± 4.32[c]	11.40 ± 2.33[a]	01.55 ± 0.87[a]	
	2	55.10 ± 5.35[c]	08.30 ± 3.22[ab]	01.20 ± 0.85[b]	
IBA concentrations (µ*M*)	0.5	75.30 ± 6.45[a]	10.19 ± 3.33[b]	05.57 ± 1.32[a]	0.7523
	1	75.20 ± 6.45[ab]	8.71 ± 2.55[c]	04.25 ± 1.36[c]	
N = 120	1.5	75.21 ± 6.14[ab]	06.15 ± 2.87[c]	01.40 ± 0.98[d]	
	2	75.21 ± 5.55[ab]	09.50 ± 3.14[a]	04.15 ± 1.36[b]	

Differed small alphabetical letters with the means indicated significantly different results according to DMR Test at P <0.05. Reading was taken after 28 days

Table 5: Effect of different soil mixtures on the growth of *S. nigrum* plantlets under glasshouse conditions after 30 days of transfer

Soil mixtures	Ratio (v/v)	Plant's survival (%)
Peat moss : sand : garden soil*	01:01:01	55.5 ± 2.51
Peat moss : sand : clayey soil **	02:01:01	42.1 ± 3.22
Peat moss : garden soil	01:01	43.5 ± 4.55
Garden soil : sand	01:02	35.2 ± 6.25
Peat moss : clayey soil	02:01	23.2 ± 4.25

*garden soil contained 50% leaf manure; **clayey soil contained 50% clay, ± SE

Fascinating violet-blue colored various shoot primordia were observed from field grown leaf explant on B_5 basal medium supplemented with 2.27 µ*M* TDZ after 30 days of culture (Fig. 2G, H).

Histological Study

Histology of regenerating shoots (Fig. 3) clearly showed the pattern of DSR development from leaf explant. Regenerants confirmed the formation of an individual shoot buds (Fig. 3A, B) that later formed more shoots with the growth of vascular development (Fig. 3C). Shoot buds along with vascular system was quite prominent on MS (Fig. 3D) as compared to B_5 basal medium (Fig. 3E, F) at either TDZ level after 60 days of culture.

in vitro Rooting and Acclimatization

There were three factors affecting *in vitro* rooting of shoots. Basal medium, NAA or IBA concentrations affected differently at different levels. MS basal medium was quite effective with 100% root induction with 9.55 highest number and 2.72 cm long shoots were obtained after 28 days (Table 3). When the individual level of either NAA or IBA were tested and compared, a 100% rooting was obtained at 0.5 µ*M* NAA (Table 4). Higher NAA levels were detrimental for the same rooting response. On the other hand, similar rooting response was observed at all levels of IBA. Number (10.19, 8.71) and root length (5.57 cm, 4.25 cm) were higher at 0.5 or 1 µ*M* IBA, respectively (Fig. 2I, J). Such parameters were decreased at 1.5 or 2 µ*M* IBA (Fig. 2K).

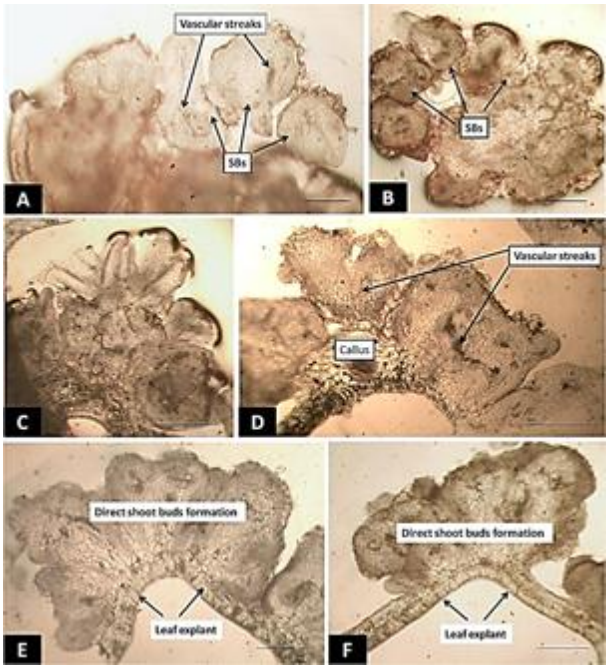

Fig. 3: Histological observations of regenerating shoot buds of *S. nigrum*. **A-B)** Arrows showed the initiated shoot buds of 60 days old at 0.91 µ*M* TDZ on MS and B_5 medium, respectively. **C)** Ten days later (70 days old) shoot buds further developed. **D-E-F)** 60 day sold DSR on top of leaf at 0.45 µ*M* TDZ on MS or B_5 medium. *Bar* = 3 mm. SBs = shoot buds

Rooted shoots were shifted in soil mixtures for hardening and acclimatization. Highest (85.5%) plantlet's survival was obtained in soil mixture of peat moss: sand: garden soil containing 50% leaf manure after 30 days of transplantation in glasshouse as compared to other soil types (Table 5).

Discussion

Plants have been an important source of secondary metabolites for use in medicines since thousands of years (Tripathi and Tripathi, 2003). As the demand for medicinal

plants is increasing day by day, consequently some of them are being threatened in their natural habitats (Muthukumar *et al.*, 2004). Therefore, alternative source to circumvent this problem lies in cell culture technologies for providing reliable and renewable source of plant products for the production of secondary metabolites (Vanisree *et al*., 2004). *In vitro* grown plants retain the capacity to synthesize alkaloids identical to the intact plant (Yoshimatsu and Shimomura, 1999).

Generally, it is considered that shoot regeneration from field grown explants is more difficult than from *In vitro* derived shoots due to contamination and low vitality of adult tissues. During the present study, we developed an efficient method of DSR from both adult and *In vitro* shoots of *S. nigrum*. Both explants were excellent source material for 100% DSR on B_5 medium supplemented with different concentrations of TDZ. In the present study, highest number of shoots, however, was obtained on MS medium from *In vitro* grown leaf explants. Aggarwal *et al.* (2012) reported maximum percent of shoot regeneration from leaf explants of *Populus* on MS medium supplemented with TDZ. Similar results of regeneration from leaf explants using TDZ has been reported in primrose (Gyves *et al.*, 2001), mulberry (Chitra and Padmaja, 2005), *Paulownia tomentosa* (Corredoira *et al*., 2008) and *Jatropha curcus* (Khurana-Kaul *et al*., 2010), which are in agreement with our results. Similarly, *In vitro* shoot regeneration has also been reported in plants of family Solanaceae (Magioli *et al*., 1998; Mallaya and Ravishankar, 2013; Rathore *et al*., 2016). While, Hussain *et al.* (2005) reported that field grown explants of different varieties of *S. tuberosum* had high regeneration potential.

MS basal medium is a common and wide spread salt formulation used in plant tissue culture experiments. In addition to this, B_5 has also been used in various *In vitro* plant experiments for secondary metabolites production (Gaurav *et al*., 2016). In the present study, both MS and B_5 media produced high frequency shoot regeneration, while B_5 medium was comparatively more effective than MS for direct shoot regeneration. Our results are in agreement with the previous report in which B_5 medium produced more shoots as compared to MS and SH basal media in *S. nigrum* (Hassanein and Soltan, 2000). In contrast, Bhat *et al*. (2010) achieved high regeneration potential of *S. nigrum* on MS medium. Similarly, Gaurav *et al*. (2016) also reported the efficacy of MS than B_5 basal media in shoot regeneration of *Withania somnifera*.

Previous reports demonstrated that shoot regeneration of *S. nigrum* can be achieved with maximum 20 mean number of shoots from *In vitro* leaf cultures on MS medium supplemented with 2.8 µ*M* Zeatin riboside (O'Connor-Sánchez *et al*., 2010). Moreover, 21 number of shoots on B_5 medium supplemented with 0.5 µ*M* BA in *S. nigram* has also been reported (Hassanein and Soltan, 2000). Kinetin was reported to be less effective as compared to BA (2.0 µ*M*) for direct shoot regeneration (84%) in *Physalis minima* (Sheeba *et al*., 2010). Besides, MS medium supplemented with BA and coconut water also responded well with 95% regeneration response and 32 mean number of shoots in *P. minima*. These reports suggest that shoot regeneration using purine based cytokinins alone or in combination with auxins gave better results. However, no published report is available in the contemporary literature regarding *In vitro* use of TDZ for direct shoot regeneration in *S. nigrum*. Our results demonstrated that TDZ had high correlation with shoot regeneration than basal media and explant. Results of the present study are quite promising with TDZ as we have achieved high regeneration response with five-fold production of *In vitro* shoots as compared to other reports. The efficacy of TDZ has been well documented for multidimensional response in different plants (Murthy *et al*., 1995) and in few species of family Solanaceae (Sajid and Aftab, 2009).

MS medium was better than B_5 augmented with IBA or NAA for producing more number of roots. Various reports demonstrated the usefulness of MS medium for *In vitro* rooting of regenerated shoots (Singh *et al*., 2016). Gour *et al.* (2007) concluded B_5 medium better than MS for successful rooting of *Balanites* shoot cultures. In the present study, IBA formed more number and longest roots on MS medium, whereas NAA produced fewer roots in B_5 medium. Similarly, Bhat *et al.* (2010) reported 100% rooting with 15.2 number and average root length of 4.5 cm at 0.25 mg/L IBA in *S. nigrum*. Our results are different from these reports in terms of early root induction and vigorous growth under the variable conditions. In the present work, it was observed that the addition of auxins promoted vigorous and stout root formation. Generally, auxins increase the survival ability in the rooted shoots under stress conditions. IBA is more stable and less toxic for improved growth of roots (Kurepin *et al*., 2011).

Soil types were also tested for *In vitro* rooting in the present study. We failed to harden and acclimatize the plants in a single soil type. For this purpose, different potting mixtures were tested. Highest rate of plant survival (85.5%) was achieved in mixture of peat moss: sand: garden soil than other soil types. It seems that *S. nigrum* plantlets need high organic matter and porosity for growth in the green house which are characteristics of garden soil and sand, respectively. The plants were then shifted to the field where they showed true-to-type morphology.

Conclusion

In conclusion, the present investigation demonstrated that TDZ may be used instead of other adenine-based cytokinins for high frequency DSR from leaf explants derived from *In vitro* grown plants of *S. nigrum* on B_5 basal medium. This protocol with multiple number of shoots may be useful for rapid multiplication, conservation as well as production of high yielding metabolites.

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