Contents lists available at ScienceDirect

Asian Pacific Journal of Reproduction

Journal homepage: www.elsevier.com/locate/apjr

Document heading 10.1016/S2305-0500(13)60045-7

Micropropagation of *Tigridia pavonia* (L.f) DC-a potential floricultural plant from twin scale explants

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ARTICLE INFO

Article history:
Received 15 February 2012
Received in revised form 16 February 2012
Accepted 15 March 2012
Available online 20 March 2012

Keywords:
Tigridia pavonia
Twin scale explant
Murashige and Skoog medium
Growth regulator

ABSTRACT

Objective: The present study was performed to standardize an effective protocol for micropropagation of *Tigridia pavonia* using tissue culture. **Methods:** The explants were cultured on Murashige and Skoog (MS) medium supplemented with cytokinins like thidiazuron (TDZ), zeatin, kinetin and auxins such as indole-3-acetic acid (IAA), 1-naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA), individually at different concentrations. **Results:** Multiple shoots were obtained on MS medium containing either 2.0 mg/L TDZ or 2.0 mg/L IAA or 0.5 mg/L IBA and in the same medium for a long period (120 d) produced tiny bulbs at the base of the senescent leaves. TDZ favored only multiple shoots without roots, whereas IAA or IBA individually or in combination with TDZ produced rooted shoots. Shoots developed on MS medium supplemented with TDZ were rooted on MS medium containing either IBA or NAA at 0.5 mg/L. The plantlets were acclimatized in pots containing garden soil. Regenerated plantlets developed into normal plants. The plants showed 99% survival. **Conclusions:** The highest number of bulblets obtained in the present study represents an effective alternative to the conventional method.

1. Introduction

The world floriculture market is estimated to be worth US $ 60 billion, and constituent increase in demand for cut and potted flower has made floriculture one of the important commercial trades in Indian agriculture.

The Iridaceae is a large family with over 2 000 species many of which are endemic to South Africa and produce attractive flowers and foliage. Approximately 40 species and hybrids from 12 genera have been successfully micropropagated by tissue culture[1]. *Tigridia pavonia* (L.f) DC (*T. pavonia*) commonly known as 'Mexican shell flower' or 'Peacock flower' or 'Tiger lily' plant is a native of Mexico. The name alludes to coloration and spotting of flowers. The bulb of the plant is edible and the plant is known to promote fertility.

Tissue culture technique is a powerful tool which can be employed as an alternative to the conventional method of vegetative propagation with the objective of enhancing the rate of multiplication of desired genotypes[2,3]. Tissue culture procedures allow the rapid production of superior disease free clonal material for nurseries and growers. Additionally production of storage organs (Corms, tubers or bulbs) during micropropagation can reduce the losses incurred during acclimatization and decrease the time to flower[4].

Successful micropropagation in Iridaceae has been achieved using explants such as anthers, bulbs, corms, flowers, hypocotyl, leaves, ovaries, roots, shoots and twin scales[1]. Many bulbous species successfully produce adventitious shoots from the tissue at the base of bulb scales[5,6], and from the junction of the scales on the basal plate[7], so the present study was conducted to develop a feasible and reliable method for multiple shoot regeneration using twin bulb scale explants in *T. pavonia*.

2. Materials and methods

2.1. Surface sterilization, explants preparation and medium preparation

The bulbs of *T. pavonia* were collected from Government Botanical Garden, Udhagamandalam, Tamilnadu, India. The root, leaves and the brown coating of the bulbs were removed. Twin scales with basal meristem was used as an explant. The explant was washed several times with tap water along with teepol for 30 min to remove the dirt and debris.

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The explant was then treated with a systemic fungicide Bavistin (2%, v/v) for 30 min and washed thoroughly. An additional treatment of 20 min soaking in antibiotic gentamycin sulphate (0.01%, v/v) was essential to eliminate contamination. The explants were then thoroughly washed and surface sterilized with one or two drops of Tween 80 in ethanol (70%, v/v) for 20 min and then treated with mercuric chloride (0.02%, v/v) and washed repeatedly prior to inoculation.

Explants were cultured on Murashige and Skoog medium (MS medium) containing various growth regulators. Autoclaving was done at 121 °C for 20 min. All the cultures were maintained at a temperature of (25±2) °C and at relative humidity of 65%-70%. The cultures were kept under white light at intensity of 2 000 lux provided from white fluorescent lamps (PHILIPS, India) with 12 h photoperiodic duration.

2.2. Shoot multiplication

For culture initiation, twin scales with the basal meristem were cultured in MS basal medium containing various concentrations of thidiazuron (TDZ, 0.5-3.0 mg/L), zeatin (0.5-1.0 mg/L), kinetin (0.5-1.0 mg/L), 1-naphthalene acetic acid (NAA, 0.5-1.0 mg/L), indole-3-acetic acid (IAA, 0.5-3.0 mg/l) and indole-3-butyric acid (IBA, 0.5-1.0 mg/L) individually with 30 g/L sucrose, activated charcoal (2 mg/L) solidified with 0.8% agar. The pH was adjusted to 5.8.

2.3. Root formation

The multiple shoots were cultured in MS medium supplemented with NAA (0.5 mg/L) and IBA (0.5 mg/L). MS medium containing TDZ (2.0 mg/L) in combination of IAA and IBA was also employed to induce rooting.

2.4. Acclimatization

The bulblets were washed thoroughly in distilled water to remove the traces of medium and then acclimatized in pots containing garden soil for hardening.

2.5. Statistical analysis

Each treatment consisted of five replicates and the experiment was repeated thrice. After 2 weeks of culture, data was recorded for shoot initiation and thereafter periodically for shoot development and rooting. Analysis of variance (ANOVA) was performed on all the data to compare concentration effects of growth regulators on multiple shoot regeneration. Means were separated using Duncan's multiple range test.

3. Results

3.1. Effect of NAA, kinetin, zeatin and IBA on multiple shoot formation

The explants did not respond when cultured on MS medium devoid of plant growth regulators (control). Shoots were initiated on the twin scales on all the media irrespective of the growth regulators tested individually. Multiple shoots were formed between the twin scales containing the basal tissue and single scale explants were not suitable for shoot initiation. In the present study, IBA at 0.5 mg/L regenerated maximum number of shoots (3.6/twin scale) (Table 1). NAA failed to induce multiple shoots (Table 1). The effect of kinetin and zeatin in bulblet initiation was negligible. Also, the development of the shoots was very slow. Significant variation existed between concentrations ($F_{0.01(1,16)}$=48.19; P<0.01), among hormones ($F_{0.01(3,16)}$=9.06; P<0.01) and hormone concentration interactions ($F_{0.01(3,16)}$=17.51; P<0.01) in number of shoots. Maximum bulblet diameter (1.43 cm) and bulblet length (1.43 cm) was recorded in IBA rather than NAA at 0.5 mg/L. Significant variation also existed between concentrations ($F_{0.01(1,16)}$=14.58; P<0.01), among hormones ($F_{0.01(3,16)}$=5.18; P<0.01) and hormone concentration interactions ($F_{0.01(3,16)}$=10.01; P<0.01) in shoot length.

3.2. Effect of IAA and TDZ on multiple shoot formation

IAA at 2 mg/L regenerated maximum number of shoots (4.67/twin scale) (Table 2). The highest number of shoots per explant was recorded as 6.4 in MS medium containing TDZ at 2 mg/L (Table 2). Tiny bulbs appeared at the base of the developing shoots after 2 month period. One bulb/shoot was observed. The maximum bulb diameter (1.90 cm) was recorded on MS medium with TDZ at 3 mg/L. The maximum bulb length (2.87 cm) was obtained on MS with TDZ at 2 mg/L. Significant variations existed between hormone ($F_{0.01(1,24)}$=27.97; P<0.01), hormone concentration interaction ($F_{0.01(5,24)}$=8.09; P<0.01) and no variation in concentration ($F_{0.01(5,24)}$=1.00) (Table 2).

3.3. Effect of growth regulators on rooting

Twin scales when cultured on MS medium supplemented with auxins such as IAA or IBA individually or in combination with a cytokinin like TDZ produced shoots and roots in the same medium. Maximum number of roots (4.34) was produced from medium containing IBA at 0.5 mg/L. The highest root length (12.50 cm) was recorded on medium containing TDZ (2.0 mg/L) and IBA (0.5 mg/L). MS medium

Table 1
Effect of growth regulators on the formation of multiple shoots and lengths.

Growth regulator	Number of shoots		Shoot length (cm)	
	0.5 mg/L regulator	1.0 mg/L regulator	0.5 mg/L regulator	1.0 mg/L regulator
NAA	1.40^{c}	1.00^{a}	5.40^{b}	6.30^{a}
Kinetin	2.30^{b}	0.00^{b}	5.70^{b}	0.00^{b}
Zeatin	0.30^{d}	0.67^{ab}	1.37^{c}	2.77^{ab}
IBA	3.60^{a}	0.30^{ab}	10.87^{a}	0.80^{b}

IAA, indole-3-acetic acid; IBA, indole-3-butyric acid. Means in a column followed by different letters are significantly (P<0.05) different according to Duncan's multiple range test.

Table 2
Morphometric parameters obtained from the twin scales of *Tigridia pavonia*.

Growth regulator	Concentrations (mg/L)	Number of shoots	Shoot length (cm)	Bulblet diameter (cm)	Bulblet length (cm)
IAA	0.5	1.40^{c}	6.50^{b}	0.80^{a}	1.23^{b}
	1.0	2.67^{b}	6.76^{ab}	0.47^{a}	2.50^{a}
	1.5	2.30^{bc}	7.26^{a}	0.80^{a}	2.23^{a}
	2.0	4.67^{a}	8.06^{a}	0.84^{a}	2.00^{ab}
	2.5	3.30^{b}	6.13^{b}	0.67^{a}	1.57^{ab}
	3.0	2.67^{b}	3.83^{c}	0.74^{a}	1.67^{ab}
TDZ	0.5	1.40^{cd}	2.06^{c}	1.54^{ab}	1.60^{b}
	1.0	1.30^{cd}	2.87^{c}	1.57^{ab}	1.24^{b}
	1.5	4.40^{b}	5.86^{a}	1.27^{ab}	1.24^{b}
	2.0	6.40^{a}	5.60^{ab}	1.03^{b}	2.87^{a}
	2.5	2.70^{c}	4.46^{b}	1.40^{ab}	1.94^{b}
	3.0	0.67^{d}	2.97^{c}	1.90^{a}	1.87^{b}
Hormone (H) $_{1, 24}$		27.97^{**}	1.23^{ns}	52.75^{**}	<1.00
Concentration (C) $_{5, 24}$		<1.00	6.32^{*}	1.04^{ns}	2.45^{ns}
H × C $_{5, 24}$		8.09^{**}	<1.00	1.86^{ns}	3.99^{**}

NAA, 1-naphthalene acetic acid; TDZ, thidiazuron. Means in a column followed by different letters are significantly (P<0.05) different according to Duncan's multiple range test. The data in the last three lines represents F values. The superscript ** indicates significant difference (P<0.01) between the treatments, while the superscript ns indicates no significant difference.

supplemented with TDZ (2.0 mg/L) failed to produce roots, so the multiple shoots were separated and cultured in MS medium containing either IBA or NAA at 0.5 mg/L to induce rooting (Table 3).

3.4. Acclimatization

Tiny bulbs with shoots and roots were separated, rinsed with distilled water to remove the medium and planted in pots containing garden soil. A survival rate of 99% was observed when the plantlets were transferred to pots.

Table 3
Effect of growth regulators on root length and number.

Growth regulator	Root length (cm)	Root number
0.5 mg/L NAA	0.87^{c}	1.67^{c}
0.5 mg/L IBA	11.30^{a}	4.34^{a}
0.5 mg/L IAA + 2.0 mg/L TDZ	6.20^{b}	2.67^{bc}
1.0 mg/L + 2.0 mg/L TDZ	0.84^{c}	2.67^{bc}
0.5 mg/L IBA + 2.0 mg/L TDZ	12.50^{a}	3.67^{ab}
1.0 mg/L IBA + 2.0 mg/L TDZ	3.00^{c}	2.34^{c}

NAA, 1-naphthalene acetic acid; IBA, indole-3-butyric acid; IAA, indole-3-acetic acid; TDZ, thidiazuron. Means in a column followed by different letters are significantly (P<0.05) different according to Duncan's multiple range test. Significant variation existed between concentrations in root length ($F_{0.01(5,12)}$=35.18; P<0.01) and in root number ($F_{0.01(5,12)}$=8.27; P<0.01).

4. Discussion

In the present investigation, twin scales were used as explants. The use of bulb scales as primary explants for the micropropagation of *Narcissus* has been reviewed by Seabrook[8]. The *invitro* micropropagation of *Lilium* species using bulb scales as an explants source for the production of bulblets has been reported[9].

In *Urginea maritime*, *invitro* bulblet regeneration was achieved both on single and double scale explants and double scales were more potent in producing bulblets than single scale[10]. For Amaryllidaceae, twin scale explants with two adjacent scales connected by a piece of basal plate tissue have been successfully used for *invitro* regeneration[7]. *Crinum macowanii* bulblets were regenerated *in vitro* using twin scales[11]. In *T. pavonia*, plantlet regeneration was achieved using twin scales[12].

In our study, NAA was less potent in inducing multiple shoots and the same effect of the hormone on the growth of bulblets was reported in *Narcissus bulbocodium* by Chow *et al*.[13] and in *Narcissus tazetta* by Steinitz & Yahel[14]. This is in contrast with the reports of Ascough *et al*.[15] where NAA promoted bulb induction in *Albuca bracteata*, *Albuca nelsonii* and oriental lily. The effect of kinetin and zeatin in the formation of bulblets was negligible. IBA produced a maximum of 3.6 shoots. IBA is employed for rooting in bulbous plants. For the first time, we reported the role of IBA in shoot induction. IAA was equally favoured for shoot induction and was found to be superior to other auxins in *Hyacinthus orientalis* where the greatest height and diameter of the bulblet was obtained with 1.5 mg/L IAA in modified Hellers medium[16].

In our study, a maximum of 6.4 shoots/explant were recorded using TDZ (2.0 mg/L). This was found to be higher than the shoot numbers reported in *Eucumis autumnalis* (1.0-3.7 shoots/explant)[17], *Eucrosia stricklandii* (0.7-2.2 shoots/explant)[18], *Urginea maritime* (1.17-3.58 shoots/explant)[10] and *Neotchihatchewia isatidea* (3.73)[19]. Previous report on regeneration of *T. pavonia* recorded the maximum number of four shoots[12]. Cytokinin like compound TDZ has an important regulatory role in plant growth and development[20-22]. The role of TDZ in shoot initiation in our study was obvious. This is in accordance with study in *Lilium* where the role of TDZ in shoot multiplication was well documented[23,24] and also in *Urginea maritima* where TDZ increased bulblet regeneration[10].

In the present study, activated charcoal at 2 mg/L accelerated shoot growth. Activated charcoal is often used in plant tissue culture to improve cell growth and development[25]. The inclusion of activated charcoal in the growth medium stimulated bulblet formation in *Crinum moorei*[26], *Cyranthus*[27], *Eucrosia* Ker Gawl.[28], *Eucrosia stricklandii*[18], *Lilium*[29], *Lilium longiflorum*[30], *Narcissus*[28,31] and *Nerine*[32].

MS medium supplemented with IAA and IBA produced rooted shoots. In *Urginea maritima*, adventitious bulblets rooted on MS medium complemented with IBA (1 mg/L) [10] and in *Neotchihatchewia isatidea* where rooting was achieved on half strength MS medium containing IBA (0.25 mg/L)[19]. Multiple shoots derived from TDZ at 2.0 mg/L were subjected to rooting on NAA and IBA. NAA at 1.0 mg/L was found to be favorable for root induction in other bulbous plants like *Eucomis autumnalis*, *Eucomis comosa* and *Eucomis zambesiaca*[17].

The diameter of the tiny bulbs developed by our method was found to be higher than that of *Urginea maritime*[10], *Lilium* spp.[33] and *Albuca* spp.[15]. Under natural conditions, the plant produces an average of 5 bulblets per year. *Invitro* regeneration of *T. pavonia* supplied 48 plantlets in 5 month period as recorded by Jose *et al*.,[12] but using the present protocol, we obtained an average of 60 plantlets in 4 month period which was a 20% increase in plantlets.

The present investigation investigated the influence of growth regulators on multiple shoot formation in *T. pavonia* derived from twin bulb scales. The number of regenerated shoots was highest on MS medium supplemented with 2.0 mg/L TDZ; the number of roots was highest on MS medium containing 0.5 mg/L IBA individually. The highest number of bulblets showed that the present method is an effective alternative to the conventional method.

Conflict of interest statement

We declare that we have no conflict of interest.

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