In vitro Propagation of *Ornithogalum saundersiae*: potential of Thidiazuronas a chemical of choice.

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ABSTRACT

The objective of the study was to evaluate the efficacy of Thidiazuron (TDZ) as a plant growth regulator in evoking morphogenesis, whole plant regeneration and subsequent rooting and bulbing from shoot tip cultures of *Ornithogalum saundersiae* L. Shoot tip explants maintained on MSO (basal medium devoid of any plant growth regulators), formed only one shoot over the 10-week period of culture, which also failed to root or form bulbs. Inclusion of TDZ at various concentrations (0.1-5.0 μM) promoted direct adventitious shoot proliferation. Most of the shoots subsequently formed roots and bulbs when transferred to MSO. Addition of naphthalene acetic acid (NAA) to the culture medium inhibited shoot formation causing the shoot tip explants to brown and die after about 7 days of culture without further growth, development or callus formation. Benzylaminopurine (BAP), on the other hand, slightly improved shoot formation above the controls, although subsequent rooting and bulbing were low. Of significance was the synergistic effects of BAP and NAA which, in combination, improved shoot proliferation, as well as subsequent root and bulb formation from the shoot tip explants to levels comparable to those of explants cultured in TDZ at 0.1-0.4 μM concentration range. In summary, TDZ at 5 μM concentration was more effective, but at lower concentrations (0.1-1.0 μM) was just as effective as combined NAA + BAP in eliciting morphogenic responses from shoot tip explants of *Ornithogalum saundersiae* L.

KEY WORDS: *Ornithogalum saundersiae*, Thidiazuron (TDZ), propagation

1.0 INTRODUCTION

Ornithogalum saundersiae, belonging to the Liliaceae family (Van Scheepen, 1991) has gained importance as an export cut flower crop in Kenya (Horticultural Crops Development Authority, HCDA, 2000). *Ornithogalum* grows easily under outdoor

conditions under high light intensities, is not attacked seriously by insect pests and requires low levels of management (Wabule *et al.* 1991). The crop however has been reported to be susceptible to fungi, bacteria and viruses (De Hertogh and Le Nard, 1993), a challenge especially since propagation is mainly through bulbing. Commercial production of *Ornithogalum* is therefore hampered by the rapid spread of infection from parental to propagule materials through such vegetative propagation methods. In addition, the system is slow with a multiplication rate of only 6-12 bulblets formed per bulb per year (Wabule *et al.* 1991). Tissue culture has been used as a system of rapid multiplication of clean planting materials in many systems (Hartmann *et al.* 1990).

There are a few reports of attempts to develop *in vitro* propagation protocols of *Ornithogalum* mainly *Ornithogalum umbellatum* and *O. thyroides* (De Hertogh and Le Nard, 1993). Shoot tip explants provide a better chance of regenerating 'clean' propagules than other parts of the plant connected by vascular tissues e.g. stems, roots, bulbs, leaves etc (Hartmann *et al.* 1990). The traditional auxin and cytokinin (Skoog and Miller, 1957) have been used to regenerate plants from bulb scales and shoot tip explants. Nayak and Sen (1995) demonstrated rapid and stable propagation of *O. umbellatum* L. in long-term callus cultures using a combination of 0.5 mg/L BAP and 2 mg/L NAA.

Thidiazuron, (N'-phenyl-N'-1,2,3-thidiazol-5-ylurea, TDZ), is a phenyl urea that has been reported to be just as effective or better on its own, in evoking morphogenic responses *in vitro* in several plant systems (Fiola *et al.* 1990; Visser *et al.* 1992; Murthy *et al.* 1995; Hutchinson *et al.* 1996a; b; Huetterman and Preece, 1993). Despite its high efficacy, there are no reports of TDZ-mediated plant regeneration from *O. saundersiae* L. shoot tip cultures. The overall objective of the current study was to evaluate the efficacy of TDZ as a plant growth regulator (PGR) in evoking morphogenesis, whole plant regeneration and bulbing from shoot tip explants of *O. saundersiae*.

2.0 MATERIALS AND METHODS

2.1 Site and source of stock plants

Ornithogalum saundersiae stock plants, grown under recommended cultural conditions (HCDA, 1997), were obtained were obtained from Cianda Flowers Ltd, a commercial export flower grower in Kiambu district of Kenya, which is situated at 2300 m above sea level and around 10° South of the Equator.

2.2 Preparation of explant and sterilization

Ornithogalum saundersiae bulbs were obtained from 3-month old plants. The bulbs were cleaned with dilute detergent (Bioagent) to remove excess soil particles, and rinsed in running tap water for 15 minutes. Excised shoot tips (1-2cm long) were immersed in 95% alcohol for 5 minutes and then rinsed in sterile distilled water for 5 minutes. The tips were then placed in 0.5% NaOcl solution containing 'Tween 20'(2 drops/100mL solution), for 20 minutes, washed in 3 changes of distilled water. Shoot tips (0.5 – 1mm long), consisting of an apical dome and one to two leaf primodia were excised under a dissecting microscope and used as explants.

2.3 Culture of shoot-tip explants

Each explant was cultured in a universal bottle containing 10mL of culture medium. The medium consisted of MS (Murashige and Skoog, 1962) salts, B5 (Gamborg *et al.* 1968) vitamins, 30g/L sucrose, 8% agar and supplemented with various plant growth regulators as outlined below:

- TDZ (0.1, 0.4, 1 and 5 μM)
- 2 mg/L NAA+ 0.5 mg/L BAP
- NAA (2 mg/L)
- BAP (0.5 mg/L)
- Basal medium devoid of any plant growth regulators (MSO), acted as a control in all experiments.

The levels of NAA and BAP used were determined from preliminary studies and those reported to be optimum for other *Ornithogalum* spp. (Nayak and Sen, 1995). The pH of the media were adjusted to 5.7± 0.1 before autoclaving at 121^0C for 20 mins. The cultures were placed on growth shelves set at 25 ± 2^0 C and illuminated (16 h photoperiod 70 – 78 μmol/m^2/s) by cool white fluorescent tubes. Explants cultured in NAA- and BAP-supplemented media were maintained on the medium for 4 weeks and subsequently sub-cultured every month. Those held in TDZ-supplemented media were held for 10 days before transferring to basal medium devoid of any plant growth regulators (MSO) and subsequently sub-cultured in MSO every month.

3.0 RESULTS

3.1 Shoot proliferation

The addition of various plant growth regulators to the culture media significantly influenced morphogenic responses from shoot tip cultures of *O. saundersiae* L. Shoot tip explants maintained on basal medium devoid of any plant growth regulators (MSO, control) formed only 1 shoot over the 10-week period of culture (Table 1). Thidiazuron at various concentrations (0.1-5.0 μM) promoted direct adventitious shoot proliferation from shoot tip explants, passing the callus phase (Figure 1). The number of shoots formed increased with increasing concentration of TDZ incorporated in the culture media, 1-5 μM forming the highest number (28-34) after 10 weeks in culture (Table 1). Media supplemented with 2 mg/L NAA did not promote any shoot growth and the explants turned brown and died after 7 days in culture (data not shown). On the other hand, BAP alone, slightly improved shoot proliferation with 10 week-old explants forming an average of 7 shoots. Surprisingly, a combination of BAP and NAA at the same concentrations of 0.5 and 2 mg/L, respectively, improved shoot proliferation to similar levels to those of explants cultured on media supplemented with low levels (0.1-0.4 μM) of TDZ (Table 1).

Table 1. Effect of plant growth regulators on number of shoots formed from shoot tip explants of *Ornithogalum saundersiae* L.

Plant Growth Regulator	Number of shoots formed after	
	4 weeks of culture	10 weeks of culture
MSO	1^{c}	1^{c}
0.1 μM TDZ	6^{d}	21.8^{b}
0.4 μM TDZ	9.5^{bc}	25.0^{b}
1.0 μM TDZ	10.8^{b}	27.8^{ab}
5.0 μM TDZ	15.3^{a}	34.3^{a}
0.5 mg/L BAP + 2 mg/L NAA	7^{cd}	22.5^{b}
0.5 mg/L BAP	2.5^{c}	6.8^{c}
2 mg/L NAA	0^{e}	0^{c}
SE	1.315	3.6
W (Tukeys')	3.1	8.4

Figure 1. Direct shoot morphogenesis from shoot tip explants of *Ornithogalum Saundersiae* cultured in media supplemented with 5 µM Thidiazuron after 12 weeks in culture

3.2 Rooting and bulbing

The inclusion of various plant growth regulators in the culture medium had a significant effect on subsequent root and bulb formation from shoot tip cultures of *O. saundersiae* L. (Table 2). Shoots from cultures previously held on MSO failed to root and also failed to form any bulbs. A high percentage (>70%) of shoots previously cultured on various concentrations of TDZ rooted and formed bulbs. Similar percentages of rooting and bulbing were obtained from shoots formed from explants cultured on media

supplemented with combined NAA and BAP. Shoots previously held on media supplemented with BAP alone had comparably lower rates of rooting and bulbing. It was noted that similar numbers of shoots that rooted also formed bulbs.

Table 2: Effect of plant growth regulators on subsequent percent rooting and bulbing of shoots formed from shoot tip explants of *Ornithogalum saundersiae* L.

Plant Growth Regulator	% Rooting	% Bulbing
MSO	0^{d}	0^{d}
0.1 µM TDZ	71.3^{b}	42.5^{bc}
0.4 µM TDZ	70.9^{b}	47.7^{bc}
1.0 µM TDZ	85.8^{ab}	75.8^{a}
5.0 µM TDZ	100^{a}	86^{a}
0.5 mg/L BAP + 2 mg/L NAA	95.9^{a}	55.9^{b}
0.5 mg/L BAP	33.8^{c}	33.8^{c}
2 mg/L NAA	0^{d}	0^{d}
SE	0.139	0.086
W (Tukeys')	0.327	0.201

4.0 Discussion and Conclusion

Development of *in vitro* propagation protocols for several crops continues to require empirical studies. All morphogenic responses *in vitro* are thought to be regulated, primarily, by an intricate ratio of plant growth regulators, namely auxin and cytokinin, in the culture medium (Skoog and Miller, 1957). In the present system, TDZ, a phenyl urea, is reported to have had a strong intrinsic cytokinin activity (Mok *et al.* 1982), induced direct shoot regeneration from *O. saundersiae* L. shoot tip explants, without an intervening callus phase. Thidiazuron was more potent than combined auxin (NAA) and cytokinin (BAP), over the tested concentrations, in evoking shoot proliferation. Thidiazuron has been reported to evoke similar morphogenic responses in geranium (Visser *et al.* 1992), peanut (Malik and Saxena, 1992; Murthy *et al.* 1995) and other systems (Lu, 1993). Shoots raised on TDZ-supplemented media when transferred to basal medium exhibited high rooting as well as bulbing, an attribute beneficial for whole plant

regeneration. Shoots maintained on basal medium, however, failed to root and may required auxin-supplemented rooting media for whole plant regeneration.

Several hypotheses have been put forward on the mode of action of Thidiazuron. Despite structural differences between adenine cytokinins and phenyl ureas (Iwamura *et al.* 1979), the latter have exhibited strong cytokinin activity in tissue culture systems (Mok *et al.* 1982), leading to both groups forming the cytokinin group of phytohormones (Mok *et al.* 1982; 1987). Recent findings however indicate that TDZ modulates endogenous levels of, not only, cytokinins, but also auxins, gibberellic acids and ethylene in geranium (Hutchinson and Saxena, 1996; Hutchinson *et al.* 1996 a; b) and peanut (Murthy *et al.* 1995) plant systems.

In conclusion, the results of this study indicate that TDZ at 1-5 μM was more potent than combined auxin (NAA) and cytokinin (BAP) but elicited similar responses at lower levels (<1 μM concentrations). The above results suggest that TDZ could have modulated elevated levels of auxins, cytokinins and ethylene as reported in other plant systems e.g. geranium (Hutchinson and Saxena, 1996; Hutchinson *et al.* 1996a; b), peanut (Murthy *et al.* 1995), which evoked responses from *O. saundersiae* L. shoot tip explants.

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