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EFFECT OF THIDIAZURON (TDZ) ON *IN VITRO* MICROPROPAGATION OF *SOLANUM TUBEROSUM* L. CVS. DESIREE AND CARDINAL

ZAHOOR AHMAD SAJID AND FAHEEM AFTAB*

Department of Botany,
University of the Punjab, Q. A. Campus Lahore-54590, Pakistan.

Abstract

Thidiazuron (1-phenyl-3- (1,2,3- Thiadiazol-5-yl) urea; TDZ) is one of the several substituted ureas that have been investigated recently for their cytokinin-like activity. TDZ is known to be more active than zeatin for stimulating the growth when added to a tissue culture medium at a low concentration. In this study, effect of TDZ on several *In vitro* growth parameters in *Solanum tuberosum* cvs. Desiree and Cardinal were observed. Shoot apices (1.0cm each) from both the cultivars were separately inoculated on full strength MS basal media as well as on MS full strength supplemented with different concentrations of TDZ (10^{-10}, 10^{-9} or 10^{-8} M) thus forming four combinations. Results were recorded for shoot length, shoot number, root length, root number, number of nodes, fresh and dry weight of plants after 30 days of explant inoculation. MS full strength medium was found to be the best for *In vitro* micropropagation. However, TDZ did have an influence on the studied growth parameters, which have been discussed and interpreted herein.

Introduction

TDZ a substituted phenylurea (N-phenyl-N-1,2,3-thiadiazol-5-ylurea) is used as a synthetic herbicide and a plant growth regulator to stimulate high rate of axillary shoot proliferation in many woody plant species (Malik & Saxena, 1992). It has been considered to be more potent than most of the commonly used cytokinins (Huetteman & Preece, 1993). Thidiazuron releases the lateral bud dormancy and stimulates shoot formation in wide variety of plant species (Fiola *et al*., 1990; Malik & Saxena, 1992). According to Capelle *et al*., (1983), TDZ directly promotes growth due to its own biological activities in a fashion similar to that of an N- substituted cytokinin or it may induce the synthesis and accumulation of an endogenous cytokinin. In woody plant species, low levels of TDZ induce the axillary shoot proliferation but higher levels may inhibit it. Higher levels, on the other hand, promote callus and somatic embryo formation (Huetteman & Preece, 1993).

Potato is one of the most important widely-grown crops and is an integral part of diet in the entire world. It produces more protein (524 kg/ha) as compared to wheat (254kg/ha). It also supplies at least 12 essential minerals including Vitamin C (Irfan, 1992). Apart from woody plant species, TDZ has shown promise in many other plants belonging to diverse groups or families such as *Camellia sinensis* (Mondal *et al*., 1998), *Hordeum vulgare* (Ganeshan *et al*., 2003), *Oryza sativa* (Gairi & Rashid, 2004), *Hyoscyamus niger* (Uranbey 2005). To our knowledge, it has never been tested for *In vitro* clonal propagation of potato. It is important that the effect of TDZ be tested on important crops like potato. The goal of this research work was to study the effect of TDZ on growth of *Solanum tuberosum* L., using shoot apices as explant from *In vitro* raised plants.

*E-mail: faheem@botany.pu.edu.pk

Materials and Methods

Apical shoot explants of *Solanum tuberosum* L. cvs. Desiree and Cardinal were grown on MS (Murashige & Skoog, 1962) full strength or MS medium containing three different concentrations of TDZ (10^{-8}, 10^{-9} or 10^{-10} M). All media were solidified with 0.7% Agar- agar (Merck) and adjusted to pH 5.8. Apical shoot explants (ca. 1.0 cm) from both the cultivars were grown in each medium. Ten culture vessels were used for each treatment per experiment and each experiment was repeated thrice for the two varieties. The cultures were incubated under 16-h photoperiod (35 μmol m^{-2} s^{-1} from cool fluorescent lights) at 25 ± 2°C.

Results were recorded for shoot length, shoot number, root length, root number, number of nodes, fresh and dry weight of plants after 30 days of explant inoculation. For this purpose, the plants were harvested and shoot length was recorded with the help of a suitable scale from the top of the medium to the tip of shoot minus 1.0 cm (size of the original explant). The root length was measured from the tip of the root up to the basal end of the shoot. Fresh weights of the plantlets were determined. Afterwards, the plant material was placed in small brown envelopes and dried in hot air oven at 60°C for up to 48 hours to estimate dry weight.

Results and Discussion

In vitro establishment of potato shoot apices

a. Shoot and root length: The effect of TDZ, a substituted phenyl-urea as a plant growth regulator has been reported for many plant species including several recalcitrant woody species like *Quercus robur* L., (Chalupa, 1988) and *Pinus strobes* L., (Pijut *et al*., 1991). In potato (cv. Cardinal), highest shoot length (5.74 cm) and root length (5.34 cm) was observed in MS full strength medium after 30 days of initial culture (Table 1). This was followed by MS + TDZ (10^{-10}) where the shoot and root length was 5.51 cm and 5.24 cm, respectively. Lowest shoot and root length (5.30 and 4.08 cm respectively) was observed at 10^{-8} M TDZ. In cv. Desiree, highest shoot and root length was 5.48 cm and 5.32 cm respectively on MS full strength medium (Table 2). A somewhat similar pattern for shoot and root data was observed since the lowest values for the two parameters were observed on MS medium supplemented with 10^{-8} M TDZ.

It is evident from these results that TDZ does have an influence on micro propagation of potato even at very low concentrations as the second highest shoot and root length was recorded using MS medium supplemented with 10^{-10} TDZ.

b. Number of shoots, roots and nodes: Maximum number of shoots (2.66 and 2.96) was obtained on MS + TDZ (10^{-8}) in cvs. Cardinal and Desiree, respectively (Tables 1 and 2). It might be due to the high concentration of TDZ as it modifies the endogenous cytokinin metabolism (Capelle *et al*., 1983; Hare & Van Staden, 1994; Murthy *et al*., 1995; Hutchinson & Sexena, 1996). Alternatively, it has been suggested that TDZ may mimic an auxin response (Visser *et al*., 1992) or modifies endogenous auxin metabolism (Murthy *et al*., 1995; Hutchinson *et al*., 1996). However, highest number of roots (12.60 and 14.90) and nodes (7.90 and 7.20) was observed on MS full strength medium in the two cultivars respectively. The lowest number of shoot (2.0 and 1.5), root (7.3 and 4.9) and node (6.3 and 6.3 respectively) was obtained on MS + TDZ 10^{-9} in both the cultivars.

Table 1. Effect of three different TDZ levels supplemented to MS media on *In vitro* establishment of shoot apices of potato (*Solanum tuberosum* L. cv. Cardinal) at day 30 of the initial culture.

Media composition	Shoot length (cm)	Root length (cm)	Number of shoots	Number of roots	Number of nodes	Fresh weight of plantlets (g)	Dry weight of plantlets (g)
MS full strength (S1)	5.74 ± 0.664	5.34 ± 0.449	2.30 ± 0.246	12.60 ± 1.861	7.90 ± 0.780	0.4460 ± 0.0620	0.0347 ± 0.008
MS + 10^{-10} TDZ (S2)	5.51 ± 0.556	5.24 ± 0.650	2.28 ± 0.389	8.42 ± 1.45	6.71 ± 0.629	0.4066 ± 0.110	0.0320 ± 0.006
MS + 10^{-9} TDZ (S3)	5.36 ± 0.536	4.09 ± 0.314	2.00 ± 0.244	7.30 ± 1.20	6.30 ± 0.0813	0.5430 ± 0.093	0.0524 ± 0.008
MS + 10^{-8} TDZ (S4)	5.30 ± 0.622	4.08 ± 0.541	2.66 ± 0.222	10.50 ± 1.611	7.55 ± 0.686	0.3633 ± 0.137	0.0430 ± 0.010

*Results are mean ± S.E. from thirty replicate cultures.

Table 2. Effect of three different TDZ levels supplemented to MS media on *In vitro* establishment of shoot apices of potato (*Solanum tuberosum* L., cv. Desiree).

Media composition	Shoot length (cm)	Root length (cm)	Number of shoots	Number of roots	Number of nodes	Fresh weight of plantlets (g)	Dry weight of plantlets (g)
MS full strength (S1)	5.48 ± 0.664	5.32 ± 0.399	1.90 ± 0.223	14.90 ± 1.151	7.20 ± 0.394	0.6040 ± 0.0720	0.0747 ± 0.008
MS + 10^{-10} TDZ (S2)	5.27 ± 0.436	5.12 ± 0.309	2.90 ± 0.220	14.72 ± 0.670	6.81 ± 0.347	1.0560 ± 0.091	0.0965 ± 0.068
MS + 10^{-9} TDZ (S3)	5.43 ± 0.436	4.73 ± 0.284	1.50 ± 0.259	4.90 ± 0.538	6.30 ± 0.691	0.1750 ± 0.043	0.0898 ± 0.070
MS + 10^{-8} TDZ (S4)	5.16 ± 0.594	4.20 ± 0.254	2.96 ± 0.386	13.65 ± 2.081	6.80 ± 0.796	0.9620 ± 0.130	0.0570 ± 0.090

*Results are mean ± S.E. from thirty replicate cultures.

c. Fresh and dry weight of plantlets: The maximum fresh and dry weight of the plantlets (0.543 g and 0.0524 g) in cv. Cardinal was obtained on MS medium containing 10^{-9} M TDZ. In Desiree, the highest fresh and dry weights (1.0560 and 0.0965 g, respectively) were observed on MS medium containing 10^{-10} M TDZ (Tables 1 and 2). Although the highest shoot and root length (in both the cultivars) was obtained on MS full strength medium but highest fresh and dry weights in Desiree and Cardinal were observed using MS medium with either 10^{-10} or 10^{-9} TDZ, respectively.

The results from the present work demonstrate the possibility to micropropagate potato using TDZ that to our knowledge has not been tested before in potato for the purpose of *In vitro* clonal propagation. Its superiority over general basal MS medium, however, could not be established in this study. It also demonstrates that TDZ might be used at very low concentration (less than a nM level) for potato micropropagation as compared to other cytokinins (such as BAP) that are usually used at relatively higher (μM) levels (Rida *et al*., 2001: Fengyen & Han 2002). Although TDZ is relatively costly, a very low concentration of TDZ used in our studies circumvents its price consideration and seems to be quite cost-effective. Moreover, its influence on all the growth parameters under study necessitates further work using TDZ as a growth regulator to better understand its role in potato tissue culture.

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