

Naseem Ahmad · Mohammad Faisal  
*Editors*

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# Thidiazuron: From Urea Derivative to Plant Growth Regulator

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## Preface

Plant biotechnology is a most interesting field for academicians, researchers, and students, and nowadays it becomes a very useful tool in agriculture and medicine and regarded as a popular area of research especially in biological sciences because of its integral use in biochemistry, molecular biology, and engineering sciences in order to achieve technological application of cultured tissues, cells, and microbes. Plant tissue culture (PTC), a technique refers to cultivation of plant cells and other parts on artificial nutrient medium in controlled environment under aseptic conditions, is essential to all plant biotechnologies and is an exciting area of research for basic and applied sciences. PTC requires various nutrients, pH, carbon source, gelling agent, temperature, photoperiod, humidity, etc., and most importantly the judicious use of plant growth regulators. Various natural, adenine and phenyl urea derivatives were employed for the induction and proliferation of different types of explants. Several phenyl urea derivatives were evaluated, and it was observed that thidiazuron (n-phenyl-N'-1,2,3-thidiazol-5-urea) was found to be the most effective among the plant growth regulators. Thidiazuron (TDZ) was initially developed as a cotton defoliant and showed high cytokinin like activity. In some examples, its activity was 100 times more than BA in tobacco callus assay and produces more number of shoots in cultures than Zeatin and 2iP. TDZ also showed major breakthrough in tissue culture of various recalcitrant legumes and woody plant species. For the last two decades, a number of laboratories have been working on TDZ with different aspect, and a number of publications have come out. To the best of our knowledge, there is no comprehensive edited volume on this particular topic. Hence, the edited volume is a deed to consolidate the scattered information on the role of TDZ in plant tissue culture and genetic manipulations that would hopefully prove informative to various researches.

**Thidiazuron: From Urea Derivative to Plant Growth Regulator** is a compilation of review and research papers dealing with various aspects of TDZ in plant tissue culture with profitable implications.

The book will provide basic and applied information for academicians, scientists, and researchers who want to initiate work in this fascinating area of research. The book contains 28 chapters compiled by international dignitaries, which give

much weightage to this edited volume. All the chapters have been organized in a way to provide a crisp information on role of TDZ in plant tissue culture. Special attention has been given to explore the mechanism and mode of actions of TDZ which can lead to organogenesis in vitro.

We are extremely thankful to all the contributors who agreed to our proposal, thus helped in this endeavor.

Aligarh, India  
September 17, 2017

Naseem Ahmad  
Mohammad Faisal

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Dr. Naseem Ahmad  
Dr. Mohammad Faisal

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# Thidiazuron: Modulator of Morphogenesis In Vitro

1

Elham Tavakouli Dinani, Mukund R. Shukla,  
Christina E. Turi, J. A. Sullivan, and Praveen K. Saxena

## Abstract

Thidiazuron (TDZ) is a substituted phenylurea first synthesized in 1967 by the Schering Corporation in Germany. Compared to other plant growth regulators, TDZ is a powerful and potent synthetic growth regulator, leading to a wide array of in vitro and in vivo applications in plants including prevention of leaf yellowing, enhanced photosynthetic activity, breaking of bud dormancy, fruit ripening, as well as proliferation of adventitious shoots, callus production, and induction of somatic embryogenesis. Despite the diversity of effects attributed to TDZ, its application and mode of action for induction of in vitro morphogenesis in plants are not well understood. Thus, this review aims to summarize current understandings for TDZ during in vitro morphogenesis in order to better understand the potential applications of TDZ for induction of in vitro morphogenesis and organogenesis.

## Keywords

Thidiazuron · TDZ · Morphogenesis · Auxin · Cytokinin · Plant growth regulation

## 1.1 Introduction

Thidiazuron (TDZ) is a substituted phenylurea first synthesized in 1967 by the Schering Corporation in Germany, originally being used as a cotton defoliant and eventually becoming registered in the USA in 1982 (Arndt et al. 1976; Pavlista and

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**Fig. 1.1** Summary of the physiological effect of TDZ on apple plant organs which include the stem, root, leaf, flower, and fruit

Gall 2011). Compared to other plant growth regulators (PGRs), TDZ is a powerful and potent synthetic growth regulator exhibiting both auxin- and cytokinin (CK)-like effects in plants, leading to a wide array of *in vitro* and *in vivo* applications including prevention of leaf yellowing, enhanced photosynthetic activity, breaking of bud dormancy, fruit ripening, as well as proliferation of adventitious shoots, callus production, and induction of somatic embryogenesis (Fig. 1.1). Despite this unique and dual effect, TDZ's action is often overgeneralized and referred to as a cytokinin. It is therefore important to note that although TDZ can mimic the effects of auxins and CKs, structurally it differs from both of these PGR groups, possessing both phenyl and thiadiazole functional groups, with both groups required for biological activity (Mok et al. 1987).

Compared with other PGRs, TDZ can be used for regeneration at much lower concentrations (10–1000 times lower) making it a valuable commercial agrochemical (Fig. 1.1; Guo et al. 2011). For instance, TDZ's ability to inhibit leaf yellowing, delay leaf senescence, maintain chlorophyll (Chl) concentrations, inhibit carotenoid degradation, inhibit abscisic acid (ABA) biosynthesis, and decrease ethylene sensitivity in cut flowers (Uthairatanakij et al. 2007; Ferrante et al. 2004) has led to its application in the horticultural industry for the purpose of increasing the longevity of cut flowers such as alstroemeria (*Alstroemeria aurea* Graham), lilies (*Lilium* spp.), tulips (*Tulipa* spp.), and chrysanthemum (*Chrysanthemum* spp.) (Ferrante et al. 2002a, b; Sankhla et al. 2003). In addition to the above, TDZ's ability to increase fruit size without affecting seed number, through the promotion of cell division in the cortex layer of fruits (Stern et al. 2003), has led to its application for improving fruit size in a number of crops including pear (*Pyrus communis* L.), grape (*Vitis vinifera* L.), persimmon (*Diospyros virginiana* L.), cucumber (*Cucumis sativus* L.)

and kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson) (Amarante et al. 2003; Stern et al. 2003). In stone fruits and cut flowers, TDZ has also been used to stimulate bud growth and opening and to accelerate bud breaking (Erez et al. 2006; Wang et al. 1986).

Despite the diversity of effects attributed to TDZ, its application and mode of action for induction of in vitro morphogenesis in plants is not well understood. This notion largely stems from TDZ's ability to display both CK- and auxin-like activities individually or simultaneously during in vitro regeneration. To complicate matters further, TDZ's ability to induce a defensive response in plant tissues can also initiate the up- or downregulation of other PGRs (i.e., ABA, ethylene, melatonin, serotonin) and secondary metabolites (i.e., polyamines) while also modulating the influx/efflux of specific cations (i.e., calcium) across biological membranes (Murch et al. 1997; Murch and Saxena 1997; Murthy et al. 1995; Proctor et al. 1996). In order to better understand potential applications of TDZ for induction of in vitro morphogenesis and organogenesis, the current review aims to summarize the current uses of this multipurpose synthetic PGR in plant tissue culture processes.

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## 1.2 Application of TDZ During Plant Morphogenesis

Although shoot production and plant development reportedly vary in response to TDZ concentration, plant material, and species (Liu et al. 1998), generally, TDZ is more biologically active than BAP, kinetin, or zeatin (Capelle et al. 1983). For example, Lu (1993) observed that TDZ is more effective at lower concentrations compared to classical CKs during shoot regeneration of woody species. In addition to the above, TDZ's ability to exhibit its effects in explants well after the initial treatment (subsequently transferred to media without TDZ) indicates that some explants only require limited exposure (Matand and Prakash 2007). Short exposure time and low concentrations of TDZ have, in fact, been found to be highly effective in stimulation of shoot regeneration across diverse species (Mihaljević and Vršek 2009). TDZ's unique property of high efficacy at low doses and/or short exposure times may be explained by TDZ's ability to resist enzymatic degradation in vivo (Murthy et al. 1998; Kumar and Reddy 2012) which in turn enables TDZ levels to remain stable over time (Dey et al. 2012). For example, in bean callus incubated with radiolabeled TDZ for 33 days, TDZ was found to remain largely intact, with only a small fraction being glycosylated (Mok and Mok 1985). Tracer studies by Benezet and Knowles (1982) have also observed limited degradation (oxidation) of the TDZ molecule within etiolated hypocotyls by 13 species of microorganisms, as evidenced by limited evolution of  $^{14}\text{CO}_2$ , which is one of the principle degradation products of TDZ. This indicates TDZ molecules were not undergoing significant degradation and likely remained within plant tissues over the duration of the experiment, up to a 28-day incubation period. Furthermore, through the use of  $^{14}\text{C}$ -TDZ and fractionation experiments, Murch and Saxena (2001) noted that TDZ may in fact exist in several forms, i.e., TDZ-free molecules, sequestered TDZ molecules, and conjugated forms associated with proteins or cell wall components within plant tissues.

### 1.3 Shoot Bud Induction

In plants, the induction of shoot buds is dependent upon a balance between auxin and CK levels, whereby an increased presence of auxin and CKs can inhibit or initiate bud formation, respectively (Wang et al. 1986). TDZ appears to promote shoot bud initiation by stimulating cell division and multiplication in the apical meristem while also reprogramming cells to the appropriate developmental stage for initiation of shoot differentiation (Dey et al. 2012; Vu et al. 2006). As in other processes affected by TDZ, diverse factors may affect the ability of TDZ to induce shoot bud initiation and growth including: concentration of TDZ, type and source of explant, age or phase of growth, cultivar, presence of other PGRs, particularly auxin, in the medium, balance of endogenous growth regulators, and presence of light (Sanikhani et al. 2006; Visser et al. 1992; Table 1.1).

In general, low concentrations ( $\geq 2.5 \mu\text{M}$ ) of TDZ enhance axillary bud formation on cultured shoot tip meristems, while moderate concentrations of TDZ (5–10  $\mu\text{M}$ ) can result in somatic embryo formation. At higher concentrations, morphological abnormalities like hyperhydricity have been reported (Lu 1993; Mithila et al. 2003). Not surprisingly, TDZ is typically applied at low concentrations to a wide range of explant types in order to induce bud growth (Murashige 1974; Jiang et al. 2008); however, the concentration required varies with explant type. For instance, direct shoot bud formation occurred only on cotyledonary nodes when TDZ was applied at rates of 0.9–5.4  $\mu\text{M}$  during in vitro regeneration of soybean (*Glycine max* (L.) Merr.) seeds. On the other hand, 10  $\mu\text{M}$  TDZ was optimal for induction of shoot buds in leaf explants of apple (*Malus domestica* Borkh.) (Fasolo et al. 1989), while low concentrations of TDZ (0.02–0.56  $\mu\text{M}$ ) induced bud/shoot regeneration in excised roots (*Albizia julibrissin* Durazz.) (Sankhla et al. 1996). TDZ (10  $\mu\text{M}$ ) has also been found to induce bud formation and regeneration in thin cell layer (TCL) system from the common bean *Phaseolus vulgaris* L., where pretreatment significantly increased bud regeneration. Optimal bud induction and further development of the formed buds were observed in 2-week cultures of TCLs on 10  $\mu\text{M}$  TDZ later reduced to 1  $\mu\text{M}$  TDZ (Cruz De Carvalho et al. 2000). The length of time the explants are exposed to TDZ can also impact the ability of TDZ to induce bud formation. In *Curculigo orchioides* Gaertn., pretreatment with 15  $\mu\text{M}$  TDZ for 24 h significantly stimulated adventitious shoot regeneration from leaves, while in *Tecomella undulata* (Sm.) Seem., exposure to a concentration of 0.7  $\mu\text{M}$  for a duration of 1–3 weeks was most efficient for shoot regeneration (Varshney and Anis 2012). Interestingly, duration and level of exposure of explants to light during TDZ treatment can also influence shoot organogenesis. For example, de novo shoot bud formation in strawberry (*Fragaria x ananassa* Duch) was achieved using leaf disks cultured in the dark and on MS medium containing 9.08  $\mu\text{M}$  TDZ (Husaini and Abidin 2007). Although it is not yet fully understood how light affects TDZ action, it is believed that TDZ's ability to induce shoot bud production in the dark is triggered by calcium stress, which in turn affects the production of ethylene (Mundhara and Rashid

**Table 1.1** Summary of TDZ used during induction of shoot buds

Plant response	Optimal TDZ concentration	Explant used	Species	Family	Exposure time	Other PGRs used	Exposure to light/dark	References
Adventitious bud regeneration	0.01, 0.03, 0.1, 0.3, 1.0, 3.0 $\mu\text{M}$	Normal and hairy root cultures	<i>Centaurium erythraea</i> Rafn	Gentianaceae	30 days	Kinetin, BA, 6- $\gamma$ , $\gamma$ -dimethylallylaminopurine (2iP), N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU), 6-[4-hydroxy-3-methyl-but-2-enylamino]purine (ZEA)	16-h photoperiod	Subotić et al. (2009)
Shoot differentiation	0.1, 1, 10 $\mu\text{M}$	Leaf explants	<i>Rhododendron</i> spp.	Ericaceae	2 weeks	IBA, 2iP	16-h photoperiod	Prece and Imel (1991)
Plantlet regeneration	50 $\mu\text{M}$	Cell suspensions derived from leaf callus	<i>Actinidia chinensis</i> Planch	Actinidiaceae	1 month	Zeatin, NAA, BA, 2,4-D	Dark/16-h photoperiod	Suezawa et al. (1988)
Plant regeneration	1–50 $\mu\text{M}$	Protoplast-derived pea callus	<i>Pisum sativum</i> L.	Fabaceae	16 weeks	–	16-h photoperiod	Böhmer et al. (1995)
Adventitious bud formation	0.01–0.1 mg dm <sup>-3</sup>	Cotyledonary nodes	<i>Medicago sativa</i> L.	Fabaceae	28 days	NAA	14-h photoperiod	Li et al. (2009)
Bud induction	22.7 $\mu\text{M}$	Cotyledon explants	<i>Capsicum annum</i> L.	Solanaceae	2–4 weeks	BA, IAA	16-h photoperiod	Hyde and Phillips (1996)
Shoot regeneration	5 $\mu\text{M}$	Root explant	<i>Hypericum perforatum</i> L.	Hypericaceae	28 days	–	16-h photoperiod	Zobayed and Saxena (2003)
Bud regeneration	1, 2.5, and 5 $\mu\text{M}$	Leaves	<i>Pyrus communis</i> L.	Rosaceae	20 days	BA, IBA	Dark	Leblay et al. (1991)

(continued)



Table 1.1 (continued)

Plant response	Optimal TDZ concentration	Explant used	Species	Family	Exposure time	Other PGRs used	Exposure to light/dark	References
Bud/shoot regeneration	0.02, 0.11, 0.56, 2.8, 14.1 $\mu\text{M}$	Root segment	<i>Populus alba</i> L.	Salicaceae	7 weeks	–	Dark/16-h photoperiod	Tsvetkov et al. (2007)
Shoot bud development	0.01–1 mg/l	Seed-derived rhizomes	<i>Cymbidium sinense</i> (Jacks.) Willd	Orchidaceae	4 months	NAA	16-h photoperiod	Chang and Chang (2000)
Shoot bud differentiation	0.90–22.72 $\mu\text{M}$	Leaf explants	<i>Jatropha curcas</i> L.	Euphorbiaceae	6 weeks	BAP, IBA	16-h photoperiod	Khurana-Kaul et al. (2010)
Shoot bud formation	0.1, 1.0 $\mu\text{M}$	Root segments, entire roots	<i>Albizia julibrissin</i> Durazz.	Fabaceae	30 days	6-BAP, TDZ	Light/dark	Hosseini-Nasr and Rashid (2002)
Shoot bud formation	0.05–0.2 mg/l	Flower clusters	<i>Vitis vinifera</i> L.	Vitaceae	3–6 weeks	2,4-D	16-h photoperiod	Oláh et al. (2003)
Shoot bud formation	4.54, 9.08, 13.12, 18.16 $\mu\text{M}$	Seedling	<i>Tamarindus indica</i> L.	Fabaceae	6 weeks	–	Dark/light	Mehta et al. (2004)
Shoot bud formation	0, 0.45, 2.27, 4.54 $\mu\text{M}$	Roots of 15-day-old seedlings	<i>Solanum melongena</i> L.	Solanaceae	28 days	BA, NAA	16-h photoperiod	Franklin et al. (2004)
Shoot bud induction	0.05, 0.1, 0.2, 0.4 $\mu\text{M}$	Leaves and cotyledons	<i>Solanum melongena</i> L.	Solanaceae	30 days	–	16-h photoperiod	Magrioli et al. (1998)
Shoot bud induction	0.5–1 $\mu\text{M}$	Nodal and internodal segment	<i>Ephedra gerardiana</i> Wall. ex Stapf	Ephedraceae	10–15 days	–	Light	Sharma et al. (2013)
Shoot bud induction	0.01–0.1.0 mg/l	Axillary buds	<i>Prunus dulcis</i> (Mill.) D.A. Webb	Rosaceae	30 days	BAP, IAA, IBA, NAA	16-h photoperiod	Choudhary et al. (2015)

Shoot bud differentiation	0.1, 1, 10 $\mu$ M	Leaf explants	<i>Rhododendron</i> spp.	Ericaceae	2 weeks	IBA, 2iP	16-h photoperiod	Preece and Imel (1991)
Plantlet regeneration	50 $\mu$ M	Cell suspensions derived from leaf callus	<i>Actinidia chinensis</i> Planch	Actinidiaceae	1 month	Zeatin, NAA, BA, 2,4-D	Dark/16-h photoperiod	Suezawa et al. (1988)
Shoot bud regeneration	0.04, 0.45, 4.54, 13.62 $\mu$ M	1-month-old root seedling	<i>Melia azedarach</i> L.	Meliaceae	3 weeks	BA, kinetin, adenine sulfate (AD)	14-h photoperiod or darkness	Vila et al. (2005)
Shoot regeneration	0.5-0.1 mg/l	Nodal explants	<i>Medicago scutellata</i> (L.) Mill., <i>M. rigidula</i> (L.) All.	Fabaceae	4 weeks	NAA, BAP	16-h photoperiod	Band et al. (2011)

2002). Given the above, future research is greatly needed to explore the interaction between light and TDZ as it will open new avenues for discovery in terms of its mechanism of action.

## 1.4 Shoot Growth, Elongation, and Multiplication

TDZ's CK-like activity has also shown to be useful for the development of shoot buds and shoot proliferation/multiplication in plants (Table 1.2) (Mok et al. 1982; Thomas and Katterman 1986; Fiola et al. 1990; Malik and Saxena 1992; Huetteman and Preece 1993; Murch et al. 1997; Faisal et al. 2014; Singh and Dwivedi 2014; Parveen and Shahzad 2011; Jones et al. 2015). TDZ's CK-like activity is believed to be largely responsible for its ability to release lateral buds from dormancy or induce bud regeneration in vitro (Mok et al. 2005; Singh and Dwivedi 2014). Still it is important to note that TDZ likely modulates levels of other PGRs, including auxin, to achieve shoot bud regeneration by evoking regenerative responses, i.e., dedifferentiation and redifferentiation of tissue cells (Malik and Saxena 1992; Guo et al. 2011; Visser et al. 1992). For example, treatment of geranium hypocotyl explants with TDZ in combination with auxin increased shoot regeneration (Hutchinson et al. 1996). With respect to shoot proliferation, a wide spectrum of factors can influence TDZ's effects in vitro including: plant PGR perception and transduction, dedifferentiation and subsequent redifferentiation of cells, genotype, wounding of explants, donor plant condition (e.g. explant age), and duration of exposure to TDZ (Lazzeri and Dunwell 1984; Kumar and Reddy 2012; Magyar-Tábori et al. 2010; Sharifi et al. 2010). Furthermore, TDZ's ability to influence shoot proliferation has shown to be concentration and species specific. At low concentrations, between 1 and 10  $\mu\text{M}$  TDZ can be used to enhance axillary shoot proliferation (Husain et al. 2007), while at much higher concentrations, shoot elongation can be either inhibited (Kumar and Reddy 2012) or stimulated to produce adventitious shoots (Feng et al. 2012; Guo et al. 2012). This trend has been observed for several spp. including "Gala" apples (*M. domestica*), where shoot production was found to decrease with increasing concentrations of TDZ (from 1 to 10  $\mu\text{M}$ ) (Liu et al. 1998), while TDZ concentrations greater than 22.7  $\mu\text{M}$  inhibited shoot regeneration (Montecelli et al. 1999).

In addition to concentration, other factors can impact shoot organogenesis including the presence of other PGRs. For example, in vitro shoot multiplication of *Capsicum annuum* L. from cotyledonary node explants excised from seedlings was optimized on MS medium supplemented with 1.5  $\mu\text{M}$  TDZ and 0.5  $\mu\text{M}$  IAA. Compared to purine-type CKs, TDZ is superior at inducing shoot proliferation (Lu 1993) while also working synergistically with other PGRs to induce a response. The synergistic effect of TDZ with other CKs may be due to differences in uptake, recognition by the cells, or mechanisms of action of these different compounds (Huetteman and Preece 1993). For instance, the effect of TDZ on axillary meristem and shoot production was found to be 5–10 times greater compared to CKs (i.e., BA) in species such as soybean (*G. max*), peanut (*Arachis hypogaea* L.), and saffron

**Table 1.2** Summary of TDZ used during shoot proliferation

Plant response	Optimal TDZ concentration	Explant used	Species	Family	Exposure time	Other PGRs used	Exposure to light/dark	References
Adventitious shoot regeneration	1, 2, 4, 8, 16 $\mu\text{M}$	Hypocotyl, cotyledon, and stem	<i>Hyoscyamus niger</i> L.	Solanaceae	3 weeks	BAP	16-h photoperiod	Uranbey (2005)
Adventitious shoot formation	2.0 mg/L	Leaves	<i>Malus domestica</i> Borkh.	Rosaceae	8 weeks	NAA, BAP, 2,4-D	16-h photoperiod	Bacha et al. (2009)
Adventitious shoot formation	10 $\mu\text{M}$	Leaves	<i>Malus domestica</i> Borkh.	Rosaceae	3 months	BA	Dark/light	Fasiola et al. (1989)
Adventitious shoot induction	22.7 or 27.3 $\mu\text{M}$	Leaf	<i>Paulownia tomentosa</i> Steud.	Paulowniaceae	2 weeks	IAA, BA	16-h photoperiod	Corredoira et al. (2008)
Adventitious shoot regeneration	0–20 mg/L	Root explants	<i>Gentiana triflora</i> Pall.	Gentianaceae	8 weeks	NAA	16-h photoperiod	Hosokawa et al. (1996)
Axillary shoot formation	2.2 $\mu\text{M}$	Seeds	<i>Malus domestica</i> Borkh.	Rosaceae	Not mentioned	BA	16-h photoperiod	Sharma et al. (2004)
Callus formation, shoot regeneration	0.05 $\mu\text{M}$	Leaf	<i>Populus tremula</i> L., <i>Populus alba</i> L.	Salicaceae	6 weeks	2,4-D	16-h photoperiod	Chupeau et al. (1993)
Direct organogenesis	0, 0.2, 0.5, 1, 2 mg/L	Shoot meristem	<i>Oryza sativa</i> L.	Poaceae	3–4 weeks	2,4-D, NAA, BA	16-h photoperiod	Chakrabarty et al. (2010)
Direct organogenesis	0.1 mg/L	Stem node	<i>Teucrium polium</i> L.	Lamiaceae	4 weeks	BAP, kinetin, NAA	16-h photoperiod	Rad et al. (2014)
Direct shoot regeneration	0.0, 0.005, 0.01, 0.02, 0.04, 0.08 mM	Excised root tips	<i>Populus alba</i> L., <i>P. tremula</i> L., <i>P. tremuloides</i> Michx.	Salicaceae	8 weeks	–	16-h photoperiod	Sherif and Khattab (2011)

(continued)

Table 1.2 (continued)

Plant response	Optimal TDZ concentration	Explant used	Species	Family	Exposure time	Other PGRs used	Exposure to light/dark	References
Direct shoot regeneration and callus-mediated protocorm-like body (PLB)	0.5–9.0 $\mu\text{M}$	Root tips	<i>x Doritaenopsis</i>	Orchidaceae	8 weeks	BA, zeatin	16-h photoperiod	Park et al. (2003)
Shoot formation	0.22 $\mu\text{M}$	Nodal explants	<i>Manihot esculenta</i> Crantz	Euphorbiaceae	6–8 weeks	BA, GA3	16-h photoperiod	Bhagwat et al. (1996)
Shoot formation	0.22 and 2.20 ppm	Root	<i>Panax quinquefolius</i> L.	Araliaceae	20 weeks	–	78% shade	Proctor et al. (1996)
Shoot formation	0.05 $\mu\text{M}$	Root segments from 15- to 20-day-old seedlings	<i>Albizia julibrissin</i> Durazz.	Fabaceae	15–30 days	IAA, IBA, NAA, BA, zeatin, 2iP	16-h photoperiod	Sankhla et al. (1996)
Shoot formation	0.1–30 $\mu\text{M}$ TDZ	Root	<i>Pelargonium domesticum</i> L.H. Bailey	Geraniaceae	18 days	–	16-h photoperiod	Murch et al. (1997)
Shoot formation	1pM–100 nM	Node	<i>Camellia sinensis</i> (L.) Kuntze	Myrtaceae	2 months	BAP, NAA, IBA	16-h photoperiod	Mondal et al. (1998)
Shoot formation	0.046–4.6 $\mu\text{M}$	Cotyledonary nodes	<i>Dalbergia sissoo</i> DC.	Fabaceae	30 days	BA, kinetin, 2iP	16-h photoperiod	Pradhan et al. (1998)
Shoot formation	0, 5, 10, 15, 20 mM	Hypocotyls	<i>Hypericum perforatum</i> L.	Clusiaceae	3, 6, 9, and 12 days	BAP, NAA, IAA, and 2,4-D	16-h photoperiod	Murch et al. (2000)

Shoot formation	0.05, 0.1, 0.3 mg/L	Leaf, cotyledons, and stem	<i>Oenothera</i> spp.	Agavaceae	3 weeks	IBA, IAA, 2,4-D	16-h photoperiod	De Gyves et al. (2001)
Shoot formation	0.1 µM	Hypocotyl	<i>Linum usitatissimum</i> L.	Linaceae	5–7 days	NAA	Continues light	Jain and Rashid (2001)
Shoot formation	0.001–1 mg/L	Hypocotyl	<i>Cuminum cyminum</i> L.	Apiaceae	30 days	–	15-h photoperiod	Gupta and Bhargava (2001)
Shoot formation	50–10,000 nM	Nodal shoot segments	<i>Wrightia arborea</i> (Dennst.) Mabb.	Apocynaceae	3 weeks	BAP, GA3, kinetin	16-h photoperiod	Purohit et al. (2004)
Shoot formation	0.5 and 5.0 µM	Petal, leaf	<i>Dianthus</i> spp.	Caryophyllaceae	30 days	IAA, NAA	16-h photoperiod	Casanova et al. (2004)
Shoot formation	0.05, 0.1, 0.5, 1.0, 5.0 µM	Leaf	<i>Hydrangea quercifolia</i> W. Bartram	Hydrangeaceae	16 weeks	IBA	16-h photoperiod	Ledbetter and Preece (2004)
Shoot formation	4.5 µM	Cotyledon, hypocotyls	<i>Arnebia euchroma</i> (Royle)	Boraginaceae	12 days	–	16-h photoperiod	Jiang et al. (2005)
Shoot formation	2–10 µM	Leaf	<i>Tylophora indica</i> (Burm. f.) Merr.	Apocynaceae	45 days	2,4-D, BA, kinetin	16-h photoperiod	Thomas and Philip (2005)
Shoot formation	18.16–72.64 µM	Bud	<i>Curcuma longa</i> L.	Zingiberaceae	1 week	BA	16-h photoperiod	Prathanturug et al. (2005)
Shoot formation	0, 0.4, 1, 2.5 µM	Shoot tip	<i>Musa</i> spp.	Musaceae	3 weeks	BAP, IAA	16-h photoperiod	Gubbuk and Pekmezci (2006)
Shoot formation	0.05, 0.10, 0.25, 0.50, 1.0 mg/L	Cotyledonary leaves	<i>Astragalus cicer</i> L.	Fabaceae	3–5 weeks	–	16-h photoperiod	Basalma et al. (2008)
Shoot formation	1.0–15 µM	Seedling	<i>Firmiana simplex</i> (L.) W. Wight	Malvaceae	8 weeks	BA, 2-iP, zeatin, kinetin, GA3	16-h photoperiod	Hussain et al. (2008)

(continued)

Table 1.2 (continued)

Plant response	Optimal TDZ concentration	Explant used	Species	Family	Exposure time	Other PGRs used	Exposure to light/dark	References
Shoot formation	0.5, 0.75, 1, 1.25 mg/L	Seedling	<i>Hypericum triquetrifolium</i> Turra	Hypericaceae	8 weeks	IAA, BAP	Dark	Oluk and Orhan (2009)
Shoot formation	0.5–9.0 µM	Nodal	<i>Cannabis sativa</i> L.	Cannabaceae	28 days	-	16-h photoperiod	Lata et al. (2009)
Shoot formation	20, 40, 80 µM	Immature seed	<i>Epimedium alpinum</i> L.	Berberidaceae	7 days	CPPU, 2,4-D	Dark	Mihaljević and Vršek (2009)
Shoot formation	0.22–1.8 µM	Shoot tip	<i>Hoya wightii</i> Hook.f.	Apocynaceae	8 weeks	KN, BA, 2-IP, IBA, IAA, NAA	16-h photoperiod	Lakshmi et al. (2010)
Shoot formation	0, 0.5, 1.0, 3 mg/L	Immature embryos	<i>Sandersonia aurantiaca</i> Hook	Colchicaceae	4–6 weeks	NAA	16-h photoperiod	Deroles et al. (2010)
Shoot formation	1 mg/L	Male inflorescence	<i>Musa</i> sp.	Musaceae	4 weeks	BAP, kinetin, 2iP, zeatin	16-h photoperiod	Darvari et al. (2010)
Shoot formation	0.5, 1, 2, 3 µM	Hypocotyls and cotyledon	<i>Lycopersicon esculentum</i> Mill.	Solanaceae	2–8 weeks	BAP	16-h photoperiod	Osman et al. (2010)
Shoot formation	5–100 µM	Nodal segments	<i>Nyctanthes arbor-tristis</i> L.	Oleaceae	4, 8, 12, and 16 days	IBA	16-h photoperiod	Jahan et al. (2011)
Shoot formation	0.5, 1.0, 2.5, 5.0, 7.5, or 10.0 µM	Nodal explants	<i>Vitex trifolia</i> L.	Lamiaceae	4 weeks	BA, NAA	16-h photoperiod	Ahmed and Anis (2012)
Shoot formation	2.0 mg/L	Meristem-derived callus of leaf protoplasts	<i>Malus domestica</i> Borkh.	Rosaceae	2–3 months	2,4-D, BA, IAA, ABA	16-h photoperiod	Saito and Suzuki (1999)

Shoot formation and development	0.36 mM	Peach palm plants	<i>Bactris gasipaes</i> Kunth	Areaceae	84 days	NAA, BAP	16-h photoperiod	Grner et al. (2013)
Shoot formation and somatic embryogenesis	0.01, 0.1, 1.0, 10.0 $\mu$ M	Immature, mature nonstratified seed	<i>Fraxinus americana</i> L.	Oleaceae	4 weeks	BA, 2iP, 2,4-D	16-h photoperiod	Bates et al. (1992)
Shoot formation and somatic embryogenesis	0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, 10 $\mu$ M	Leaf and petiole	<i>Saintpaulia ionantha</i> H.Wendl.	Gesneriaceae	3, 6, or 9 days	CPPU, 2,4-D, NAA, BA	16-h photoperiod	Mithila et al. (2003)
Shoot induction	0.1–10 $\mu$ M	Cotyledonary nodes	<i>Pterocarpus marsupium</i> Roxb.	Fabaceae	6 weeks	BA	16-h photoperiod	Husain et al. (2007)
Shoot organogenesis	0, 0.25, 0.5, 0.75, 1.0, 2.5, 5.0 $\mu$ mol/L	Leaf	<i>Artemisia judaica</i> L.	Asteraceae	20 days	–	Dark/light	Liu et al. (2003)
Shoot organogenesis	9.08, 13.62, 18.17, 22.7 $\mu$ M	Leaf	<i>Morus</i> spp.	Moraceae	30 days	2,4-D, BAP, kinetin	16-h photoperiod	Chitra and Padmaja (2005)
Shoot organogenesis	0, 2.5, 5.0, 7.5, 10.0, 20.0 $\mu$ M	Intact seedlings, etiolated hypocotyl, sterile stem	<i>Scutellaria baicalensis</i> Georgi	Lamiaceae	14–20 days	–	16-h photoperiod	Li et al. (2000)
Shoot organogenesis	0.5–9 mM	Nodal segment	<i>Kigelia africana</i> (Lam.) Benth.	Bignoniaceae	4 weeks	2,4-D, NAA	16-h photoperiod	Thomas and Puthur (2004)
Shoot organogenesis	0.45, 1.20, 2.30, 4.54, 22.70 $\mu$ M	Leaf explants	<i>Rhododendron</i> spp.	Ericaceae	12 weeks	IAA	16-h photoperiod	Pavingerová (2009)
Shoot production	0.5–40 mg/L	Leaf, petiole, hypocotyl, internode, cotyledon, root	<i>Arachis hypogaea</i>	Legumes	15 days	BA	16-h photoperiod	Kanyand et al. (1994)

(continued)



Table 1.2 (continued)

Plant response	Optimal TDZ concentration	Explant used	Species	Family	Exposure time	Other PGRs used	Exposure to light/dark	References
Shoot regeneration	0.88–3.3 mg/L	Excised cotyledons and rooted hypocotyls	<i> Capsicum annuum </i> L.	Solanaceae	3 weeks	BAP, IAA	16-h photoperiod	Szász et al. (1995)
Shoot regeneration	1.0 or 2.0 mg/L	Cotyledons, leaves, cotyledonary nodes and shoot tip	<i> Capsicum annuum </i> L.	Solanaceae	30 days	–	16-h photoperiod	Dabauza and Pena (2001)
Shoot regeneration	0.1, 1, 5, and 10 $\mu$ M	Untransformed and transformed roots	<i> Malus domestica </i> Borkh.	Rosaceae	–	NAA	16-h photoperiod	Pawlicki-Julian et al. (2002)
Shoot regeneration	2–9 $\mu$ M	Cotyledonary explants	<i> Morus alba </i> L.	Moraceae	7, 14, 21 days	BAP	16-h photoperiod	Thomas (2003)
Shoot regeneration	0.5, 1.0 $\mu$ M	Root	<i> Fragaria × ananassa </i> (Duchesne ex Weston) Duchesne ex Rozier	Rosaceae	3–4 weeks	BA, 2,4-D, NAA	16-h photoperiod	Passey et al. (2003)
Shoot regeneration	0–12 $\mu$ M	Cotyledon, hypocotyls	<i> Pinus strobus </i> L.	Pinaceae	6 weeks	2,4-D, NAA, IAA, BA, 2iP	16-h photoperiod	Tang and Newton (2005)
Shoot regeneration	0, 0.5, 2, 4 $\mu$ M	Young expanding sepals	<i> Fragaria × ananassa </i> (Duchesne ex Weston) Duchesne ex Rozier	Rosaceae	4–5 weeks	Zeatin	Dark/light	Debnath (2005)

Shoot regeneration	0.1, 0.5, 1.0, 2.5, 5.0 $\mu\text{M}$	Root explant from 30-day-old seedlings	<i>Senna alexandrina</i> Mill.	Fabaceae	6 weeks	BA, kinetin, NAA	16-h photoperiod	Parveen and Shanzad (2011)
Shoot regeneration	0.22, 0.90, 2.27, 4.54, 9.08 $\mu\text{M}$	Cotyledonary petiole	<i>Jatropha curcas</i> L.	Euphorbiaceae	6 weeks	Kinetin, BAP, NAA, IAA	16-h photoperiod	Kumar and Reddy (2012)
Shoot regeneration	15 mg/L	Young leaf	<i>Prunus persica</i> (L.) Batsch	Rosaceae	5 weeks	NAA, IBA	Dark/light	Soliman (2013)
Shoot regeneration	0, 2.5, 5, 7.5 $\mu\text{M}$	Nodal explants, leaf	<i>Pyrus communis</i> L.	Rosaceae	4 weeks	NAA, BAP	Dark/16-h photoperiod	Yousefiara et al. (2014)
Shoot regeneration	0.45–22.7 $\mu\text{M}$	Apical and axillary buds, leaf, and internode	<i>Guzotia abyssinica</i> (L.f.) Cass	Asteraceae	3–4 months	–	16-h photoperiod	Baghel and Bansal (2014)
Shoot regeneration	4.54, 6.81, 9.08 $\mu\text{M}$	Leaf explant	<i>Prunus persica</i> (L.) Batsch	Rosaceae	4–6 weeks	NAA	Dark/16-h photoperiod	San et al. (2015)
Shoot regeneration	1.0 $\mu\text{M}$	Leaf disks	<i>Mentha x piperita</i> L.	Lamiaceae	6 weeks	NAA, BA	16-h photoperiod	Faure et al. (1998)
Shoot and root regeneration	1.0 mg/L	Aerial stem explants	<i>Zingiber officinale</i> Roscoe	Zingiberaceae	3–4 months	IBA, BAP, NAA	16-h photoperiod	Lincy and Sasikumar (2010)
Shoot induction	4.5, 9.1 $\mu\text{M}$	Mature embryos		Poaceae	4 weeks	BAP	16-h photoperiod	Ganeshan et al. (2006)
Shoot multiplication	0.5, 1, 2, 3, 4, 5 $\mu\text{M}$	Nodal	<i>Cullen corylifolium</i> (L.) Medik.	Fabaceae	4 weeks	–	16-h photoperiod	Faisal et al. (2006)
Shoot regeneration	1.0 $\mu\text{M}$	Leaf disks	<i>Mentha x piperita</i> L.	Lamiaceae	6 weeks	NAA, BA	16-h photoperiod	Faure et al. (1998)

(continued)

Table 1.2 (continued)

Plant response	Optimal TDZ concentration	Explant used	Species	Family	Exposure time	Other PGRs used	Exposure to light/dark	References
Shoot regeneration	0.45–45.41 $\mu$ M	Rhizome sections	<i>Cheilocosmus speciosus</i> (J.Koenig) C.D.Specht	Costaceae	6 weeks	–	16-h photoperiod	Malabadi et al. (2004)
Shoot regeneration	0.045, 0.23, 1.14, 2.27, 4.54 $\mu$ M	Cotyledons excised	<i>Boehmeria nivea</i> (L.) Gaudich.	Urticaceae	4 days	2,4-D, IAA, IBA, NAA	16-h photoperiod	Wang et al. (2007)
Shoot regeneration	2 mg/L	Root, cotyledon, hypocotyl, and intact seedlings	<i>Brassica oleracea</i> L.	Brassicaceae	6 weeks	BA, zeatin	16-h photoperiod	Ćosić et al. (2015)

(*Crocus sativus* L.) (Victor et al. 1999; Radhakrishnan et al. 2009; Sharifi et al. 2010). Furthermore, TDZ alone or in combination with other auxins/CKs (e.g., BA) can induce shoot bud formation and multiplication especially after transfer of shoots to TDZ-free medium (Singh and Dwivedi 2014). Consequently, the transfer of explants from enriched TDZ medium to a secondary medium without growth regulators has been successfully applied in plant regeneration systems for a variety of species (Malik and Saxena 1992; Victor et al. 1999).

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## 1.5 Somatic Embryogenesis

TDZ is a substitute for the auxin/CK requirement that is needed during somatic embryogenesis, thereby increasing the number of formed somatic embryos (Visser et al. 1992). Somatic embryogenesis changes somatic cells to embryonic cells in a physiological sequence that is tightly regulated by a delicate balance of PGRs (Murthy et al. 1998). Induction and development of somatic embryogenesis are associated with endogenous PGRs including auxins and CKs; not surprisingly, TDZ promotes somatic embryogenesis, alone or in combination with other PGRs, for a wide range of recalcitrant species (Durkovic and Misalova 2008; Nhut et al. 2006) as well as a variety of commercial crops including tobacco (*Nicotiana tabacum* L.), peanut (*A. hypogaea*), geranium (*Pelargonium* spp.), African violet (*Saintpaulia* spp.; Mithila et al. 2003; Shukla et al. 2013), and chickpea (*Cicer arietinum* L.) (Visser et al. 1992; Saxena et al. 1992; Gill and Saxena 1993; Murthy et al. 1995) (Table 1.3).

Different types of tissues can be selected for induction, for instance, TDZ has been described to induce somatic embryos on hypocotyl, epicotyl, cotyledonary node, cotyledon, and leaves of intact seedlings of *Azadirachta indica* A. Juss. (Gairi and Rashid 2004; Saxena et al. 1992; Iantcheva et al. 1999). In peanut, induction of direct somatic embryogenesis occurs by culturing mature intact seeds on a medium supplemented with 0.5–10  $\mu\text{M}$  TDZ or N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU). Explants with no cotyledons, and thus no embryogenic potential, did not respond to increasing levels of TDZ. In contrast, retention of one or both cotyledons resulted in increased response to TDZ (Saxena et al. 1992; Murthy and Saxena 1994; Murthy et al. 1995). Exposure time can impact the effectiveness of TDZ. For example, application of TDZ on plant tissues alone or in combination with other PGRs for short periods of time at low concentration (10  $\mu\text{M}$ ) has been found to induce embryogenic responses (Hutchinson et al. 1997; Malik and Saxena 1992; Murthy et al. 1998), while exposure to TDZ for longer than 3–4 weeks (10  $\mu\text{M}$ ) led to a reduced induction of roots (Malik et al. 1993). This is interesting, as it reflects patterns also observed in induction of somatic embryogenesis by the synthetic auxin and pesticide, 2,4-dichlorophenoxyacetic acid (2,4-D). Similar to TDZ, short exposure to 2,4-D followed by explant transfer to growth regulator-free medium allows for first an accumulation of 2,4-D in tissues followed by a gradual decrease over time with somatic embryos developing with these falling concentrations (Zee 1981; Fujimura and Komamine 1980; Feher et al. 2002). The similarity between this

**Table 1.3** Summary of TDZ used during somatic embryo formation

Plant response	Optimal TDZ concentration	Explant used	Species	Family	Exposure time	Other PGRs used	Exposure to light/dark	References
Somatic embryogenesis	10 $\mu\text{M}$	Hypocotyl explants	<i>Pelargonium x hortorum</i> Bailey	Geraniaceae	3 days	Gas	16-h photoperiod	Hutchinson et al. (1997)
Somatic embryogenesis	0.045, 0.45, 4.54 $\mu\text{M}$	Immature embryo		Fagaceae	2, 4, and 8 weeks	-	Dark	Tsvetkov (1999)
Somatic embryogenesis	0.01–5.0 $\mu\text{M}$	Embryonic axes	<i>Quercus robur</i> L.	Fagaceae	4 weeks	BA, NAA	16-h photoperiod	Martínez et al. (2008)
Somatic embryogenesis	2.5, 5, 7.5 mg/L	Nucellar tissue	<i>Mangifera indica</i> L.	Anacardiaceae	1–2 months	BAP, 2iP, IAA	15-h photoperiod	Kidwai et al. (2009)
Somatic embryogenesis	5.0 or 10.0 $\mu\text{M}$	Leaf disk	<i>Vitis vinifera</i> L.	Vitaceae	40 days	2,4-D, BA, KIN, ZEA, 2iP	16-h photoperiod	Matsuta and Hirabayashi (1989)
Somatic embryogenesis	0.455 $\mu\text{M}$	Nodal and intermodal tissues	<i>Bambusa odashimae</i> Hatus. ex D.Z.Li & Stapleton	Poaceae	3–4 weeks	Kinetin, 2,4-D	16-h photoperiod	Lin et al. (2004)
Somatic embryogenesis	0.1–2.5 $\mu\text{M}$	Stem segments and shoot tips	<i>Capsicum annuum</i> L.	Solanaceae	4 weeks	-	16-h photoperiod	Khan et al. (2006)
Somatic embryos	1 $\mu\text{M}$	Leaf	<i>Echinacea purpurea</i> (L.) Moench	Asteraceae	4 weeks	-	Dark	Jones et al. (2007)
Somatic embryos	1.5, 10, 20, 40 $\mu\text{M}$	Seedling	<i>Arachis hypogaea</i> L.	Fabaceae	4–6 weeks	-	16-h photoperiod	Saxena et al. (1992)

well-documented process and the pattern observed in TDZ treatment supports a strong auxin-like role for TDZ in this mechanism. Further, it is likely that the inherent stability of TDZ in living tissues is a strong contributing factor in establishing this function.

## 1.6 Intact Seedling Development

TDZ enhances seed germination via improvement of shoot regeneration, with positive effects being reported in soybean (*G. max*), pea (*Pisum sativum* L.), common bean (*P. vulgaris*), chickpea (*C. arietinum*), and lentil (*Lens culinaris* Medik) (Radhakrishnan et al. 2009; Malik and Saxena 1992) (Table 1.4). In contrast, the intact seedling regeneration system is a unique morphogenetic system which involves the direct development of multiple shoots on the germinating seedling. For the first time, Malik (1993) reported a direct seed culture method for de novo differentiation of shoots from intact seedling without explanting. The number of shoots regenerated from intact seedling of *Lathyrus sativus* L., *L. cicera* L., and *L. ochrus* L. DC. was significantly higher than that observed with explants. These results indicated that excision of explant is not always necessary for induction of morphogenesis and also that the morphological integrity of intact seedlings plays a critical role in the induction of organogenesis/somatic embryogenesis (Malik 1993). TDZ induction of shoot production in the intact seedling system effectively depends on the applied concentration. For example, intact seedlings of silk tree (*A. julibrissin*) grown on MS medium containing 0.1–10  $\mu\text{M}$  TDZ produced shoots indirectly through callus. Interestingly, at higher TDZ concentrations (2.5–10  $\mu\text{M}$ ), shoots were produced, but did not form callus (Mok et al. 1987). Sankhla et al. (1994) also reported high efficiency of TDZ in inducing shoot formation from roots of intact seedling of *A. julibrissin* at 0.1–1.0  $\mu\text{M}$  TDZ. Regeneration of multiple shoots from intact seedlings of switch grass (*Panicum virgatum* L.) was induced on MS medium supplemented with 4.5  $\mu\text{M}$  2,4-D, and 18.2  $\mu\text{M}$  TDZ (Gupta and Conger 1998). An in vitro propagation system for *Artemisia judaica* L., a medicinal plant, induced shoot organogenesis by culturing intact seedlings on medium supplemented with 1  $\mu\text{M}$  TDZ for 20 days (Liu et al. 2003). In a study with seeds of *Firmiana simplex* (L.) W. Wight, induction of shoot proliferation was assessed on MS medium supplemented with 5.0  $\mu\text{M}$  TDZ + 1.5 M  $\text{GA}_3$  + 0.1% ascorbic acid compared to various levels (1.0–15 M) of several different cytokinins (BA, 2-iP, zeatin, and kinetin). Shoots formed within 8 weeks of culture and the shoot-forming capacity of seeds were found to be influenced by the type and concentration of CKs, with TDZ showing up to 13% greater regeneration rates than other cytokinins tested (Hussain et al. 2008). Induction of shoot organogenesis for felty germander (*Teucrium polium* L.), an endangered medicinal plant, was obtained using intact seedlings cultivated in MS medium supplemented with 22.72  $\mu\text{M}$  TDZ (Rad et al. 2014). Regeneration ability of kohlrabi (*Brassica oleracea* var. *gongylodes*) cultivars Vienna Purple (VP) and Vienna White (VW) has also been tested. Intact seedlings were cultivated on MS media