Thidiazuron Influenced Morphogenesis in Some Medicinal Plants

11

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Abstract

Thidiazuron (*N*-phenyl-*N′*-1,2,3-thiadiazol-5-ylurea; TDZ) is an artificial plant growth regulator that is widely used in plant tissue culture. Due to its dynamic role in plant tissue culture, it has gained ample attention for several workers since the past decades. Wide array of TDZ-influenced physiological responses are reported in different medicinal plant species. TDZ has shown both auxin- and cytokinin-like effects, although, chemically, it is totally different from commonly used auxins and cytokinins. A number of physiological and biochemical events in cells are induced or enhanced by TDZ, but the mode of action of TDZ is yet to explore. However, varieties of underlying mechanisms have been revealed in several reports to defend the morphogenic events induced by the application of TDZ. Some reports emphasized that TDZ may modify endogenous plant growth regulators, either directly or indirectly, and produce reactions in cell/tissue, necessary for its division/regeneration. Other possibilities include modification in cell membrane, fluidity, nutrient uptake, transport and assimilation, etc. In this review, recent advancements in TDZ application in plant sciences are discussed.

Keywords

Thidiazuron · Plant growth regulators · Morphogenesis · Somatic embryogenesis

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11.1 Introduction

Thidiazuron (*N*-phenyl-*N′*-1,2,3-thidiazol-5-yl-urea; TDZ) a multitask plant growth regulator has played a vital role to trigger differential physiological response in plant cell and tissue culture. Its peculiar feature is the capacity to act as the substitute for the both auxins and cytokinin (Casanova et al. 2004). The TDZ is a substituted phenylurea compound known to act as cotton defoliant (Arndt et al. 1976) but later was found to mimic the cytokinin-like activity (Wang et al. 1986). The response of TDZ alone in plant tissue culture has become more advanced and continued to increase over the decades. The action of TDZ directly depends upon its concentration, exposure time, and cultured explants. According to Murthy et al. (1998), the effect of TDZ is 20 times more advanced as compared to other cytokinins, and hence, the comparison of TDZ and purine-based cytokine is complicated. The supremacy of the TDZ among other phytohormone is might be due to nutrients uptake capacity of the cell with the alteration in cell membrane and enhanced purine and cytokinin metabolism in the cell (Capelle et al. 1983). The production and accumulation of phenols and enzymes like peroxidase and catalase is one of the major effects of TDZ activity in to the cell (Wang et al. 1991a). Moreover alteration in several enzyme concentrations such as ribulose diphosphate, carboxylase oxidase, and pentose enzymes is also an aftereffect of TDZ action (Mok et al. 1987). Wang et al. (1991a, b) reported that most of the TDZ-influenced enzymes are related to the cell wall, cell membrane, and its fluidity. They found that TDZ-influenced organogenesis leads a metabolic cascade which affects directly or indirectly to the other endogenous plant hormone. TDZ has been proved to be an effective plant growth regulator for shoot proliferation and adventitious shoot organogenesis in various plant species (Table 11.1).

Several factors including genotype, type of culture medium and explants, plant growth hormones, their concentration and exposure time, and environmental condition affect the adventitious shoot induction in vitro (Casanova et al. 2008; Casas et al. 2010). The action of TDZ has been found to promote both the organogenesis and somatic embryogenesis in vitro.

The concentration and duration of exposure of TDZ to the explants is well documented by several plant biotechnologists. The short time exposure of TDZ with low concentration has been effective for morphogenesis, while higher levels, on the other hand, promote callus and somatic embryo formation (Rida et al. 2001; Fengyen and Han 2002; Tulac et al. 2002). The abnormal morphogenesis, stunted growth of shoot, hyperhydricity, and fasciculation to the cell were the consequences of TDZ when the exposure was extended beyond the optimum level (Huetteman and Preece 1993; Faisal et al. 2005; Ahmad and Anis 2007). Shirani et al. (2009) also reported the deleterious effect of higher concentration of TDZ in regenerated shoots of banana and plantain (*Musa* spp.) after in vitro multiplication with TDZ and BAP from excised shoot tips. Additionally, continuous or more than optimal exposure of TDZ resulted in the inhibition of shoot elongation and formation of fasciated/distorted shoot development.

Table 11.1 TDZ-influenced morphogenesis in some medicinal plant

	Plant name	Family	Explants	Treatment	References
1	*Acacia mangium*	Mimosacea	Cotyledons	MS + 4.55 μM TDZ + 1.43 μM IAA	Xie and Hong (2001)
2	*Acacia sinuate*	Mimosacea	Nodal segment	MS + 0.6 μM TDZ + 0.1 I μM AA	Shahzad et al. (2006)
3	*Acanthophyllum sordidum*	Caryophyllaceae	Leaf segment	MS + 2.69 μM NAA + 4.54 μM TDZ + 2.46 μM IBA	Meratan et al. (2009)
4	*Aerva lanata*	Amaranthaceae	Leaf segment	MS + 2.0 mg L^{-1} TDZ	Varutharaju et al. (2014)
5	*Arachis correntina*	Leguminosae	Leaf segment	MS + 5.0 μM TDZ	Mroginski et al. (2004)
6	*Bactris gasipaes*	Arecaceae	Shoot	MS + 0.36 μM TDZ	Graner et al. (2013)
7	*Bauhinia tomentosa*	Fabaceae	Cotyledons/nodal segment	MS + 0.8 μM TDZ	Naaz et al. (2012)
8	*Beta vulgaris*	Amaranthaceae	Petiole	MS + 4.6 μM TDZ	Zhang et al. (2001)
9	*Cannabis sativa*	Cannabaceae	Nodal segments	MS + 0.5 μM TDZ	Lata et al. (2009)
10	*Cassia sophera*	Fabaceae	Cotyledonary node	MS + 2.5 μM TDZ	Parveen and Shahzad (2010)
11	*Ceropegia ensifolia*	Apocynaceae	Nodal (in vitro derived)	MS + 20 μM TDZ	Reddy et al. (2015)
12	*Cicer arietinum*	Fabaceae	Cotyledons	MS + 10.0 μM TDZ	Murthy et al. (1996)
13	*Citrus sinensis*	Apocynaceae	Epicotyl/hypocotyl	MS + 5.0 μM TDZ	Kumari et al. (2008)
14	*Chirita swinglei*	Gesneriaceae	Leaf	MS + 2.0 μM TDZ + 2.5 μM BA	Chen et al. (2016)
15	*Cotoneaster wilsonii*	Rosaceae	Nodal segment	MS + 2.1 μM TDZ + 0.4 μM NAA	Sivanesan et al. (2011)
16	*Echinacea purpurea*	Asteraceae	Petiole	MS + 2.5 μM BA + 0.5 μM TDZ	Choffe et al. (2000)
17	*Embelia ribes*	Primulaceae	In vitro derived leaf	MS + 0.27 μM TDZ	Raghu et al. (2006)
18	*Fraxinus americana*	Oleaceae	Cotyledon/hypocotyl	MS + 10.0 μM TDZ	Bates et al. (1992)
20	*Glycine max*	Fabaceae	Cotyledonary nodes	MS + 5.4 μM TDZ	Radhakrishnan et al. (2009)
21	*Hypericum perforatum*	Hypericaceae	Hypocotyl	MS + 5.0 μM TDZ	Murch et al. (2000)
22	*Jatropha curcas*	Euphorbeacea	Leaf	MS + 2.27 μM TDZ + 2.22 μM BA + 0.49 μM IBA	Deore and Johnson (2008)
23	*Kigelia pinnata*	Bignoniaceae	Nodal segment	MS + 3.0 μM TDZ + 0.5 μM NAA	Thomas and Puthur (2004a, b)

(continued)

Table 11.1 (continued)

	Plant name	Family	Explants	Treatment	References
24	*Kigelia pinnata*	Bignoniaceae	Nodal segment	MS + 3 μM TDZ + 0.5 μM NAA	Thomas et al. (2004)
25	*Metabriggsia ovalifolia*	Gesneriaceae	Leaf	MS + 2.5 μM BA + 5.0 μM TDZ	Ouyang et al. (2016)
26	*Morus alba*	Moraceae	Leaf	MS + 18.17 μM TDZ	Chitra and Padmaja (2005)
27	*Nothapodytes foetida*	Icacinaceae	Shoot/hypocotyl	L2 + TDZ 0.44 μM + BAP 2.22 μM + L- glutamine 0.03 μM	Tejavathi et al. (2011)
28	*Ochna integerrima*	Ochnaceae	Leaf/shoot	MS + 10.0 μM TDZ	Ma et al. (2011)
29	*Pterocarpus marsupium*	Fabaceae	Cotyledonary nodes	MS + 0.4 μM TDZ	Husain et al. (2007)
30	*Psoralea corylifolia*	Fabaceae	Nodal segment	MS + 2.0 μM TDZ	Faisal and Anis (2006)
31	*Rauvolfia tetraphylla*	Apocynaceae	Nodal segment	MS + 5.0 μM TDZ	Faisal et al. (2005)
32	*Ricinus communis*	Euphorbeacea	Cotyledon	MS + 2.5 mg dm^{-3} TDZ + 0.4 mg dm^{-3} NAA + 15 mg dm^{-3} glutamine	Kumari et al. (2008)
33	*Saintpaulia ionantha*	Gesneriaceae	Leaf/petiole	MS + 2.5 μM TDZ	Mithila et al. (2003)
				MS + 5.0 μM TDZ	
34	*Santalum album*	Santalaceae	Nodal	WPM + 0.6 mg/l TDZ + 1.5 mg/l 2,4-D	Singh et al. (2016)
35	*Saussurea involucrata*	Asteraceae	Leaf	MS + 0.5 μM TDZ	Guo et al. (2017)
36	*Selenicereus megalanthus*	Cactaceae	Cotyledons	MS + 200 μM TDZ	Pelah et al. (2002)
37	*Stevia rebaudiana*	Asteraceae	Nodal segments	MS + 1.0 μM TDZ	Lata et al. (2013)
38	*Tylophora indica*	Apocynaceae	Leaf callus	MS + 8.0 μM TDZ	Thomas and Philip (2005)
39	*Vitex trifolia*	Verbenaceae	Nodal explants	MS + 5.0 μM TDZ + 1.0 μM BA + 0.5 μM NAA	Ahmed and Anis (2012)
40	*Vitex negundo*	Verbenaceae	Nodal segment	MS + 1.0 μM TDZ + 1.0 μM BA + 0.5 μM NAA	Ahmad and Anis (2007)

A highest number of shoot were produced in *Artemisia judaica* when TDZ concentration was used at 1 μM for 20 days; when the exposure time was stretched, further differentiation of shoot was restricted coupled with abnormality in the shoot (Liu et al. 2003). Besides the magnificent response of TDZ in plant tissue culture, its deleterious responses were also known as the days advance. The deleterious effect of the continued presence of TDZ on the growth and multiplication has been earlier reported from time to time for several species. However the investigators have found a solution to overcome the harmful effect of TDZ by transferring the regenerated shoot to TDZ-free medium after the optimum exposure. The technique brings a balanced morphogenesis to the in vitro plant, and it is used by various workers including Huetteman and Preece (1993), Shahzad et al. (2006), Siddique and Anis (2007a, b), Faisal et al. (2008), Jahan and Anis (2009), Makara et al. (2010), and Jahan et al. (2011), Saeed and Shahzad (2015). The present chapter documents the detailed account of physiological and morphological effects of TDZ in several plant species.

11.2 Mechanism of Action of TDZ

There are several reports available dealing the physiological role of TDZ in different medicinal plant species. However, the mechanism of action of TDZ is not well documented, and only preliminary reports are available. The pioneer work of Hare and Cress (1997) for the mechanism of action of TDZ established that proline itself being as a stress marker was involve in the activity of TDZ (Fig. 11.1). The higher proline accumulation in the cell controls the NAD(P)′/NAD(P)H ratios as a consequence of plant undergoing stress which favors the oxidative pentose phosphate pathway leading to the production of precursor for auxin and cytokinin biosynthesis. In this way, the accumulations of the plant growth regulators occur as a result of the cascade of biochemical reactions initiated by TDZ. Murthy et al. (1996a) have reported high level of accumulation of proline during TDZ-induced regeneration via embryogenesis. In another study of Casanova et al. (2004), they found that the application of TDZ at a very low concentration (0.0–0.005 μM) leads to the formation of zeatin (ZT) while at higher concentration (0.5 μM) induces isopentyl adenine (IP) production in carnation petals. In the recent study of Jones et al. (2007) on the regeneration of *Echinacea purpurea*, they concluded the probable role of auxin, indolamines, and ion signaling in the morphogenesis. They found that the level of endogenous indoleamines is potentially influenced by the exposure of TDZ and enhanced level of the calcium and sodium transport in the cell was also found by the TDZ activity, and hence a positive effect was shown in regeneration.

There is another report on role of IAA published by Chhabra et al. (2008). They proposed that the involvement of the phytohormone is closely related to the biosynthesis and transportation of IAA. These reports indicate that TDZ-influenced morphogenesis is the demonstration of metabolic cross talk that includes a primary signaling, accumulation, and transport of endogenous plant signals such as auxin and cytokinin and enhanced transport of secondary messengers.

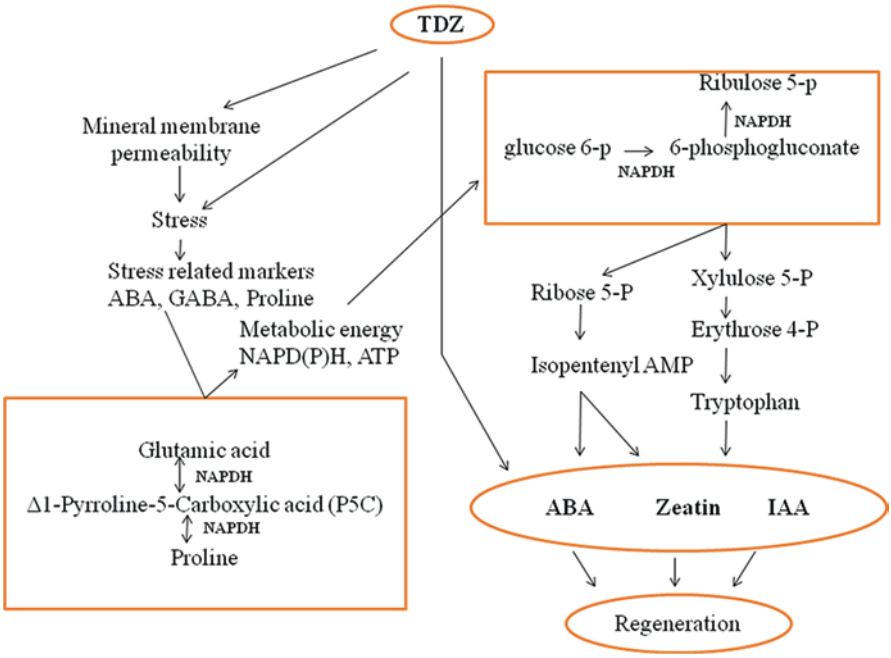

Fig. 11.1 Diagrammatic representation of proposed mechanism of action of TDZ

11.3 Effect of TDZ on Organogenesis

TDZ supposes to be less susceptible to enzymatic degradation in vivo than other naturally occurring amino purine cytokinins and has proved to be effective at lower concentrations (0.0091–3.99 μM) for the micropropagation of several plant species (Lu 1993). It has been shown to induce high bud regeneration rates than purine-based cytokinins and also has capability of fulfilling both the cytokinin and auxin requirements of regeneration responses in a number of woody plants (Jones et al. 2007). However, there was another report of Augustine and D'Souza in (1997) for the in vitro propagation of *Zanthoxylum rhetsa* using TDZ at higher concentration (2.27–145.41 μM). The use of TDZ for shoot regeneration from different explants has been widely reported at a great extent for a number of woody plant species such as *Hydrangea quercifolia* (Ledbetter and Preece 2004), *Cassia angustifolia* (Siddique and Anis 2007a, b), *Pterocarpus marsupium* (Husain et al. 2007), and *Vitex negundo* (Ahmad and Anis 2007).

Ahmed and Anis (2014) investigated the prompt response of TDZ and developed a rapid and commercially applicable regeneration protocol for *Cassia alata.* They tried various concentrations of TDZ with different duration of exposure; however, harmful effect was also shown by the in vitro culture when exposure time stretches

beyond the optimum period. The highest number of shoots 17.9 ± 0.3 with shoot length of 4.6 ± 0.1 cm was achieved when the explants were exposed to TDZ (5.0 μM) for 4 weeks. To avoid the deleterious effect of TDZ, they were transferred to TDZ-free medium. Likewise in another species of *Cassia*, Parveen and Shahzad (2010) found that MS medium fortified with TDZ (2.5 μM) was optimum for the production of 6.7 ± 0.2 shoot per explants. To avoid the aftereffect of TDZ, the microshoot was consequently transferred to TDZ-free medium containing BA for proper multiplication, proliferation, and shoot elongation.

Sharma and Shahzad (2008) reported TDZ-induced organogenesis in *Abelmoschus moschatus* using cotyledonary explants. TDZ alone proved to be superior in comparison to the combination of BA and NAA. MS medium augmented with low concentration of TDZ (0.01 mg L^{-1}) was optimum for the multiple shoot induction in A. moschatus, and a maximum of 16.8 ± 1.46 shoot per explants were achieved. Faisal and Anis (2006) studied the effect of TDZ on in vitro axillary shoot proliferation from nodal explant of *Psoralea corylifolia*, an endangered medicinal plant. Proliferation of shoots was achieved on MS medium supplemented with different concentration of 0.5, 1, 2, 3, 4, and 5 μM TDZ. The maximum number (13.6 ± 1.4) of shoots per explants was obtained from nodal segments on TDZ (2 μM) after 4 weeks of culture and followed by the transfer to hormone-free MS medium wherein the shoot differentiation significantly induced to 29.7 ± 2.1 after 8 weeks. In another study on *Cassia siamea* by Parveen et al. (2010), it was found that TDZ could not be able to evoke a significant response in the terms of shoot multiplication. They applied distinct concentration of cytokinin, viz., BA, Kn, and TDZ, alone or in combination singly or in combination with auxins for regeneration from excised codeledonary nodal explants, and MS + BA (1.0 μM) found to be best for direct shoot regeneration as it induced an average of 8.20 ± 0.66 shoots per explant. The regeneration frequency further improved with synergistic response of BA with auxin. In the highest frequency for shoot regeneration (90%), the maximum number of shoots per explants (12.20 ± 0.73) was obtained on the medium which consisted of MS + BA (1.0 μM) + NAA (0.5 μM) in C. siamea.

Shahzad et al. (2006) established a protocol for the organogenesis in *Acacia sinuata* using cotyledon. All the concentration of TDZ with MS was able to generate callusing to the explants, and MS + TDZ (0.6 μM) was found to be better in the terms of maximum callus formation in *A. sinuata*. However, the callus was further transferred to the shooting medium augmented with BA (3.0 μM) for optimum shoot induction wherein 6.60 ± 0.54 shoots were produced. Cocu et al. (2004) recorded highest frequency of adventitious shoot regeneration in *Calendula officinalis* in MS medium containing TDZ (0.75 mg dm^{-3}). Likewise, Phippen and Simon (2000) reported both callus and shoot induction with TDZ (16.8 μM) alone in *Ocimum basilicum* via using leaf explants. Murthy et al. (1996) observed direct organogenesis and somatic embryogenesis in *Cicer arietinum* when cotyledonary explants were inoculated on BA- and TDZ-amended MS medium. Multiple shoots formed de novo without an intermediary callus phase at the cotyledonary notch of the seedlings within 2–3 weeks of culture initiation. TDZ was found to be more

effective as compared to BA as an inductive signal of regeneration. The TDZ induced multiple shoot formation at all the concentrations tested (1.0–10.0 μM), although maximum morphogenic response was observed at 10.0 μM of TDZ.

De novo shoot organogenesis was reported in *Artemisia judaica* using TDZ (1.0 μM) by Liu et al. (2003). The role of TDZ has also been reported in several herbs and shrub like, *Bacopa monnieri* (Tiwari et al. 2001), *Artemisia judaica* (Liu et al. 2003), *Hordeum vulgare* (Ganeshan et al. 2003), *Cineraria maritime* (Banerjee et al. 2004), *Hyoscyamus niger* (Uranbey 2005), *Psoralea corylifolia* (Faisal and Anis 2006), *Rauvolfia tetraphylla* (Faisal et al. 2005), *Ricinus communis* (Kumari et al. 2008), *Hypericum perforatum* (Murch et al. 2000), *Embelia ribes* (Raghu et al. 2006), *Ochna integerrima* (Ma et al. 2011), Morus alba (Chitra and Padmaja 2005), *Bauhinia tomentosa* (Naaz et al. 2012), *Bactris gasipaes* (Graner et al. 2013), *Ceropegia ensifolia* (Reddy et al. 2015), and *Cassia sophera* (Parveen and Shahzad 2010).

11.4 Synergistic Effect of TDZ and Cytokinin

The synergistic effect of TDZ with other cytokinin found to be very useful to trigger organogenesis significantly (Chen et al. 2016). Lee and Pijut (2017) proposed an efficient regeneration system through adventitious shoot organogenesis in black ash (*Fraxinus nigra*), an endangered hardwood. In their study the MS medium augmented with BA (22.2 μM) + TDZ (31.8 μM) was found good with the production of 1.9 ± 0.65 adventitious shoots per leaf explant. Similarly Ouyang et al. (2016) reported the efficiency of combined treatment of TDZ + BA on the improvement of regenerability and somatic embryo formation from the leaf of *Metabriggsia ovalifolia*. Chen et al. (2016) reported a positive effect on shoot bud regeneration in *Chirita swinglei*. A maximum of 23.1 ± 0.20 shoot bud per explants were produced on MS + TDZ (2.0 μM) + BA (2.5 μM). The shoot bud obtained in *C. swinglei* depends upon the exposure and concentration of the TDZ. The first observation they recorded was the swallowing of leaf explants after culture for 15 days at (2.0 μM) TDZ. Some shoot buds were observed after 20 days of culture. Shoot buds were clearly visible as culture period was extended from 35 to 45 days. Callus could also be induced from leaves when α-naphthalene acetic acid (NAA) was used alone or in combination with TDZ and BA.

Parveen and Shahzad (2011) established a protocol for the in vitro propagation of the *Cassia angustifolia*. MS medium supplemented with TDZ (1.0 μM) was used for the production of organogenic calli followed by subsequent transfer to the TDZ-free medium augmented with different cytokinin, viz., BA, Kn, or TDZ for proper regeneration of shoot. They achieved a maximum of 35.63 ± 0.75 shoot per explants on MS + BA (2.5 μM) + NAA (0.6 μM) from the TDZ-induced calli. Zeng et al. (2008) reported an efficient micropropagation system for *Tigridiopalma magnifica* using leaves as explants. Up to 7.6 adventitious buds formed per leaf explant after a 40-day culture on MS + BA (2.0 $mg^{-}1$) + TDZ (0.1 mg^{-1}). To avoid the aftereffect

of TDZ, the culture were transferred to the TDZ-free medium containing other cytokinin-like BA for enhanced proliferation rate of adventitious buds, and it reached to 5.7 on MS medium supplemented with 2.0 mg^{-1} of BA.

11.5 Effect of TDZ with Auxin/Growth Additives

The role of TDZ with different auxin and growth additives is also well documented by several workers. The auxin-like NAA, 2, 4-D, IBA, and IAA at various concentrations with optimum TDZ concentration was reported to play an important role in both direct and indirect organogenesis. In recent study of Baskaran et al. (2016) on developing a regeneration protocol for *Ledebouria ovatifolia* through direct and indirect organogenesis by using leaf explants demonstrated that the adventitious shoot was best produced on MS + TDZ (5 μM) + NAA (2 μM), while organogenic callus was obtained on MS + IAA (2.0 μM) + TDZ (5.0 μM) + glutamine (30 μM). A maximum of 26.8 ± 1.06 and 32.0 ± 1.73 shoot per explants were achieved via direct and indirect organogenesis in *L. ovatifolia*.

A micropropagation protocol was developed by Babaei et al. (2014) for *Curculigo latifolia.* They used distinct concentration of auxin with optimum TDZ concentration for direct and indirect organogenesis using shoot tip explants. MS medium augmented with TDZ (0.5 mg L^{-1}) + IBA (0.25 mg L^{-1}) was found to be best for direct regeneration in terms of percentage of explants producing shoot, shoot number, and shoot length. Prathanturarug et al. (2012) studied the in vitro propagation of *Stemona hutanguriana* via using nodal and intermodal segment as explants. MS medium augmented with TDZ alone or in combination with NAA was able to promote regeneration in the *S. hutanguriana*. A regeneration frequency of 91.67% with shoot regeneration rate of 5.46 shoots/responding explant was observed when nodal segment inoculated on MS + TDZ (18.16 μM) + NAA (0.54 μM) for 8 weeks and followed by transferred to the PGR-free medium to avoid the adverse effect of TDZ.

In another study by Ma et al. (2011) on *Metabriggsia ovalifolia*, TDZ at higher concentration (5.0 μM) was found to be better for efficient propagation and regeneration of 36.7 shoots per leaf explants; however, the regeneration efficiency was further enhanced when auxin was supplemented with optimum TDZ. Among the various auxins, NAA at 0.5 μM with optimum TDZ concentration was efficient to induce a maximum of 79.1 adventitious shoots from each leaf explants. TDZ-mediated indirect organogenesis was also achieved by Siddique et al. (2010) in *Cassia angustifolia* via using petiole explants excised from 21-day-old axenic seedlings. They used MS medium fortified with 2, 4-D (5.0 μM) and TDZ (2.5 μM) for the organogenic callus induction. TDZ at higher concentration (5.0 μM) was able to induce calli differentiation to the adventitious shoot with the highest of 8.5 ± 0.98 shoots per culture. However, the regeneration efficiency of the explants was significantly improved when combination of TDZ (5 μM) + IAA (1.5 μM) was applied and produces a maximum of 12.5 ± 1.10 shoots per culture.

Sujatha and Dinesh Kumar (2007) compared the efficacy of cytokinin with TDZ for direct organogenesis in the species of *Carthamus*. The MS medium fortified with TDZ (0.2 mg dm^{-3}) + NAA (0.2 mg dm^{-3}) was more efficient for the induction of shoot from the leaf explants of *C. tinctorius*. On the other hand Radhika et al. (2006) found that optimum TDZ (0.2 mg dm^{-3}) with high concentration of NAA (1.0 mg dm^{-3}) was proved to be better for regeneration in *C. arborescens.* Faisal and Anis (2005) has set a protocol for the in vitro propagation *Tylophora indica* using petiole as an explant. They obtained optimum callus from the explants when inoculated on to the MS + 2,4-D (10 μM) + TDZ (2.5 μM). To achieve the shoot induction, TDZ-derived callus was transferred to the shoot induction medium. TDZ alone found to be best for the shoot multiplication in *T. indica* and a highest of 56 ± 3.6 adventitious shoot were obtained from the surface of the callus when MS medium fortified with TDZ (2.5 μM) was used. In another study of Thomas and Puthur (2004a, b) on a multipurpose tree, *Kigelia pinnata*, they used nodal segment and inoculated to the MS medium augmented with 2,4-D (3 μM) for callus induction. The obtained calli were then transferred to the shooting medium fortified with TDZ (3.0 μM) + NAA (0.5 μM) for the proliferation and multiplication of the shoot where 21 ± 0.3 shoots per culture were obtained.

11.6 Effect of TDZ on Somatic Embryogenesis

Somatic embryogenetic systems are of growing interest for medicinal, ornamental, and horticultural plants (Ji et al. 2011). Dedifferentiation of cells, activation of cell division, reprogramming of cell physiology, metabolism, and gene expression patterns occurred during unique developmental pathways of somatic embryogenesis. However, morphological abnormalities such as embryo fusion and lack of suitable apical meristems or loss of bipolarity have occurred resulting in poor yields (Benelli et al. 2010). TDZ-influenced regeneration via somatic embryogenesis is well documented by several workers for different medicinal plant species. In the recent study of Baskaran and Staden (2017), they were able to get friable embryogenic callus (FEC) from the leaf explants of *Lachenalia montana* through suspension culture for the first time. Liquid MS medium (MS_L) supplemented with 2, 4-D (0.5 μM) + TDZ (1 μM) was optimum for the formation of somatic embryos of different stages (globular to cotyledonary stages, respectively). However, the enhanced concentration of 2,4-D and TDZ was needed for the germination of somatic embryos, and liquid MS medium augmented with 2,4-D (1.0 μM) + TDZ (2.0 μM) was proved to be best in terms of enhanced germination frequency.

Naaty et al. (2017) found best response for somatic embryo production in *Schizozygia coffaeoides* on the medium comprises of MS + BA (2.0 mg/l) + Kn (0.8 mg^{-1}) + NAA (0.4 mg^{-1}) + TDZ (0.5 mg^{-1}), which survived to maturity and formed shoot. Baskaran et al. (2016) achieved embryogenic callus induced on liquid MS augmented with sucrose (15 g L^{-1}) + TDZ (0.2 Mm) + picloram (0.1 μM) + glutamine (10 μM) with the highest numbers of somatic embryos, 43.2–35.6

(globular to cotyledonary stages, respectively). Baskaran and Staden (2014) were able to achieve different developmental stages of somatic embryos, globular embryos, partial pear-shaped embryos and club-shaped embryos obtained from leaf explants of *Drimia robusta* on MS + Picloram (10 μM) + TDZ (1 μM) + glutamine (20 μM). Sahai et al. (2010) developed a protocol for the in vitro propagation of an endangered medicinal climber *Tylophora indica* through leaf explants. Different types of calli produced on BA and TDZ-augmented MS basal medium were selected for shoot induction and somatic embryogenesis studies. Calli when transferred from BA (5.0 μM) + TDZ (2.5 μM) to the MS medium containing BA (5.0 μM) resulted in high-frequency shoot induction (26.8 ± 0.97 shoots/culture) along with somatic embryogenesis (10.20 ± 0.37 embryoids/culture) up to three subculture passages. Embryoids transformed into complete plantlets when transferred to growth regulator-free half-strength MS medium.

Dhandapani et al. (2008) were able to achieve plant regeneration via somatic embryogenesis in *Catharanthus roseus*. The highest regeneration percentage through somatic embryogenesis was achieved from mature zygotic embryo on MS + TDZ (7.5 μM), and further the mature embryo also regenerated efficiently via organogenesis in MS medium fortified with TDZ (2.5 μM) + BA (2.2 μM). Joshi et al. (2008) found that failure of peanut somatic embryos to convert into plantlets is attributed to the abnormal development of the plumule. TDZ was effective in the conversion of peanut somatic embryos to plantlets by triggering morphogenetic activity in the abnormal plumules of the rooted somatic embryos. Bud-like projections appeared in the embryogenic masses when these were cultured in media containing combinations of 2,4-D and TDZ. These projections developed into buds, which subsequently formed shoots and plantlets. The response varied with the concentration and exposure of TDZ. At lower concentrations, the buds appeared in a defined row in the equatorial region of the explant, and with extended incubation, more and more buds appeared in rows alongside the initial row. Induction of multiple buds in a defined row in this specific site (equatorial region) suggested the presence of potent cells around this region. At higher concentrations, these projections appeared in large numbers spread over the whole upper part of the embryogenic mass starting from the equatorial region. The ability of embryogenic mass to convert into organogenic mass and to produce large number of organogenic buds provides an excellent system for basic studies and for the genetic transformation of peanut.

Mithila et al. (2003) observed TDZ-mediated regeneration using leaf and petiole explants from in vitro grown African violet plants. The response of cultures to other growth regulators over a range of 0.5–10 μM was 50% less than that observed with TDZ. A comparative study among several cultivars of African violet indicated that "Benjamin" and "William" had the highest regeneration potential. In "Benjamin," higher frequencies of shoot organogenesis (two fold) and somatic embryogenesis (a 50% increase) were observed from in vitro and greenhouse-grown plants, respectively. At concentrations lower than 2.5 μM, TDZ induced shoot organogenesis, whereas at higher doses (5–10 μM) somatic embryos were formed.

11.7 Conclusions

Regulation of cell division and cell differentiation is necessary for the morphogenesis either in vivo or in vitro. Auxin and cytokinin are believed to be responsible for this synergistic control. The present review deals the importance of TDZ, another class of plant growth regulators, significantly different from the cytokinin. It also attempts to integrate the vast amount of knowledge generated on TDZ-induced responses in a myriad of systems. Application of TDZ results in a wide variety of responses in in vitro cultured tissues, but the biochemical and physiological basis of the modulation of morphogenic response induced by TDZ are poorly understood. However, studies encompassing a wide array of species, techniques, and physiological responses have led to several tentative models to explain the regulatory role of TDZ. A complete picture concerning the mechanism of action of TDZ is not likely to occur, and many mysteries of auxin- and cytokinin-related morphogenesis are resolved. Nevertheless, the recent advancement in biochemical and molecular characterization of auxin and cytokinin mutants and general enthusiasm in plant growth regulator research promises very exciting results in the next decade. A complete understanding of the biochemical and physiological responses of plant tissues to TDZ will broaden our understanding of morphogenesis and further help in improvement of tissue culture technology.

References

Ahmad N, Anis M (2007) Rapid clonal multiplication of a woody tree, *Vitex negundo* L. through axillary shoots proliferation. Agrofor Syst 71:195–200

Ahmed MR, Anis M (2012) Role of TDZ in the quick regeneration of multiple shoots from nodal explants of *Vitex trifolia* L. – an important medicinal plant. Appl Biochem Biotechnol 168:957–966

Ahmed MR, Anis M (2014) Changes in activity of antioxidant enzymes and photosynthetic machinery during acclimatization of micropropagated *Cassia alata* L. plantlets. In Vitro Cell Dev Biol-Plant 50:601–609

Arndt FR, Rusch R, Stillfried HV, Hanisch B, Martin WC (1976) A new cotton defoliant. Plant Physiol 57:S-99

Augustine AC, D'Souza L (1997) Micropropagation of an endangered forest tree *Zanthoxylum rhetsa* Roxb. Phytomorphology 47:319–323

Babaei N, Abdullah NAP, Saleh G, Abdullah TL (2014) An efficient in vitro plantlet regeneration from shoot tip cultures of *Curculigo latifolia*, a medicinal plant. Hindawi Publishing Corporation http://dx.doi.org/10.1155/2014/275028

Banerjee S, Tripathi J, Verma PC, Dwivedi PD, Khanuja SPS, Bagchi GD (2004) Thidiazuron-induced high-frequency shoot proliferation in *Cineraria maritima*. Linn Curr Sci 87:1287–1290

Baskaran P, Staden JV (2014) Plant regeneration via somatic embryogenesis in *Drimia robusta*. Plant Cell Tissue Organ Cult 119:281–288

Baskaran P, Staden JV (2017) Ultra structure of somatic embryo development and plant propagation for *Lachenalia montana*. South Afr J Bot 109:269–274

Baskaran P, Kumari A, Naido D, Staden JV (2016) In vitro propagation and ultrastructural studies of somatic embryogenesis of *Ledebouria ovatifolia*. In Vitro Cell Dev Biol Plant. https://doi.org/10.1007/s11627-016-9762-9

Bates S, Preece JE, Navarrete NE, Sambeek JWV, Gaffney GR (1992) Thidiazuron stimulates shoot organogenesis and somatic embryogenesis in white ash (*Fraxinus americana* L.) Plant Cell Tissue Organ Cult 31:21–29

Benelli C, Germana MA, Camino T, Beghe D, Fabbri A (2010) Morphological and anatomical observations of abnormal somatic embryos from anther cultures of *Citrus reticulate*. Biol Plant 54:224–230

Capelle SC, Mok DWS, Kirchner SC, Mok MC (1983) Effects of thidiazuron on cytokinin autonomy and the metabolism of N6-(DELTA2-isopentenyl) [8- 14C] adenosine in callus tissues of Phareolus *Zunahs* L. Plant Physiol 73:796–802

Casanova E, Valdes E, Fernandez B, Moysset L, Trillas M (2004) Levels and in situ localization of endogenous cytokinins in thiadizuron induced shoot organogenesis in carnation. J Plant Physiol 16:95–104

Casanova E, Moysset L, Trillas M (2008) Effect of agar concentration and vessel closure on the organogenesis and hyperhydricity of adventitious carnation shoots. Biol Plant 52:1–8

Casas J, Olmos E, Piqueras A (2010) In vitro propagation of carnation (*Dianthus caryophyllus* L.) In: Protocols for in vitro propagation of ornamental plants. Methods Mol Biol 589:109–116

Chen Y, Zhang Y, Cheng O, Niu M, Liang H, Yan H, Zhang X, Silva JAT, Ma G (2016) Plant regeneration via direct and callus-mediated organogenesis from leaf explants of *Chirita swinglei* (Merr.) W. T. Wang. In Vitro Cell Dev Biol-Plant 52:521–529

Chhabra G, Chaudhary D, Varma M, Sainger M, Jaiwal PK (2008) TDZ induced direct shoot organogenesis and somatic embryogenesis on cotyledoray node explants of lentil (*Lens culinaris Medik*.) Physiol Mol Biol Plants 14:347–353

Chitra DSV, Padmaja G (2005) Shoot regeneration via direct organogenesis from in vitro derived leaves of mulberry using thidiazuron and 6-benzylaminopurine. Sci Hortic 106:593–602

Choffe K, Victor JMR, Murch SJ, Saxena PK (2000) In vitro regeneration of *Echinacea Purpurea* L.: direct somatic embryogenesis and indirect shoot organogenesis in petiole culture. In Vitro Cell Dev Biol 36:30–36

Cocu S, Urabey S, Iek A, Khawar KM, Sarihan EO, Kaya MD, Parmaksiz ÖS (2004) Adventitious shoot regeneration and micropropagation in *Calendula officinalis* L. Biol Plant 48:449–451

Deore AC, Johnson TS (2008) High-frequency plant regeneration from leaf-disc cultures of *Jatropha curcas* L.: an important biodiesel plant. Plant Biotechnology Reports 2(1):7–11

Dhandapani M, Kim DH, Hong SB (2008) Efficient plant regeneration via somatic embryogenesis and organogenesis from the explants of *Catharanthus roseus*. In Vitro Cell Dev Biol Plant 44:18–25

Faisal M, Anis M (2005) An efficient in vitro method for mass propagation of *Tylophora indica*. Biol Plant 49:257–260

Faisal M, Anis M (2006) Thidiazuron induced high frequency axillary shoot multiplication in *Psoralea corylifolia*. Biol Plant 50:437–440

Faisal M, Ahmad N, Anis M (2005) Shoot multiplication in *Rauvolfia tetraphylla* L. using thidiazuron. Plant Cell Tissue Organ Cult 80:187–190

Faisal M, Shahzad A, Anis M (2008) Somatic embryogenesis and plant regeneration from nodal explants in *Psoralea corylifolia* L. IJPDB 2(2):111–113

Fengyen L, Han L (2002) Effect of exogenous hormones on micropropagation of in vitro virus free potato plantlets. Chines Potato J 16:214–216

Ganeshan S, Monica B, Bryan LH, Brian GR, Graham JS, Ravindra NC (2003) Production of multiple shoots from thidiazuron-treated mature embryos and leafbase/apical meristem of barley (*Hordeum vulgare*). Plant Cell Tissue Organ Cult 73:57–64

Graner ME, Oberschelp JPG, Brondani EG, Batagin-Piotto DK, de Almeida VC, de Almeida M (2013) TDZ pulsing evaluation on the in vitro morphogenesis of peach palm. Physiol Mol Biol Plants 19:283–288

Guo B, He W, Zhao Y, Wu Y, Fu Y, Guo J, Wei Y (2017) Changes in endogenous hormones and H_2O_2 burst during shoot organogenesis in TDZ-treated *Saussurea involucrata* explants. Plant Cell Tissue Organ Cult 128:1–8

Hare PD, Cress WA (1997) Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul 21:79–102
Huetteman CA, Preece JE (1993) Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell Tissue Organ Cult 33:105–119
Husain MK, Anis M, Shahzad A (2007) In vitro propagation of Indian Kino (Pterocarpus marsupium Roxb.) using Thidiazuron. In Vitro Cel Dev Biol 43:59–64
Jahan AA, Anis M (2009) In vitro rapid multiplication and propagation of *Cardiospermum halicacabum* L. through axillary bud culture. Acta Physiol Plant 31:133–138
Jahan AA, Anis M, Aref MI (2011) Assessment of factors affecting micropropagation and ex vitro acclimatization of *Nyctanthes arbor-tristis* L. Acta Biol Hung 62:45–56
Ji A, Geng X, Zhang Y, Yang H, Wu G (2011) Advances in somatic embryogenesis research of horticultural plants. American J Plant Sci 2:727–732
Jones MPA, Yi Z, Murch SJ, Saxena PK (2007) Thidiazuron-induced regeneration of *Echinacea purpurea* L- micropropagation in solid and liquid culture systems. Plant Cell Rep 26:13–19
Joshi M, Sujatha K, Hazra S (2008) Effect of TDZ and 2,4-D on peanut somatic embryogenesis and in vitro bud development. Plant Cell Tissue Organ Cult 94:85–90
Kumari KG, Ganesan M, Jayabalan N (2008) Somatic organogenesis and plant regeneration in *Ricinus communis*. Biol Plant 52:17–25
Lata H, Chandra S, Khan I, ElSohly MA (2009) Thidiazuron-induced high-frequency direct shoot organogenesis of *Cannabis sativa* L. In Vitro Cell Dev Biol-Plant 45:12–19
Lata H, Chandra S, Wang YH, Raman V, Khan IA (2013) TDZ-induced high frequency plant regeneration through direct shoot organogenesis in *Stevia rebaudiana* Bertoni: an important medicinal plant and a natural sweetener. Amer J Plant Sci 4:117–128
Ledbetter DI, Preece JE (2004) Thidiazuron stimulates adventitious shoot production from *Hydrangea quercifolia* Bart. Leaf explants. Sci Hortic 101:121–126
Lee JH, Pijut PM (2017) Adventitious shoot regeneration from in vitro leaf explants of *Fraxinus nigra*. Plant Cell Tissue Organ Cult 130:335–343
Liu CZ, Murch SJ, Demerdash ME, Saxena PK (2003) Regeneration of Egyptian medicinal plant *Artemisia judaica* L. Plant Cell Rep 21:525–530
Lu CY (1993) The use of thidiazuron in tissue culture. In Vitro Cell Dev Biol 29:92–96
Ma G, da Silva JAT, Lü J, Zhang X, Zhao J (2011) Shoot organogenesis and plant regeneration in *Metabriggsia ovalifolia*. Plant Cell Tissue Organ Cult 105:355–361
Makara AM, Rubaihayo PR, Magambo MJS (2010) Carry-over effect of Thidiazuron on banana in vitro proliferation at different culture cycles and light incubation conditions. Afr J Biotechnol 9:3079–3085
Meratan AA, Ghaffari SM, Niknam V (2009) In vitro organogenesis and antioxidant enzymes activity in *Acanthophyllum sordidum*. Biol Plant 53:5–10
Mithila J, Hall JC, Victor JMR, Saxena PK (2003) Thidiazuron induces shoot organogenesis at low concentrations and somatic embryogenesis at high concentrations on leaf and petiole explants of African violet (*Saintpaulia ionantha* Wendl.) Plant Cell 21:408–414
Mok MC, Mok DWS, Turner JE, Mujer CV (1987) Biological and biochemical effects of cytokinin-active phenylurea derivatives in tissue culture system. Hortic Sci 22:1194–1197
Mroginski E, Rey HY, Gonzalez AM, Luis A, Mroginski (2004) Thidiazuron promotes in vitro plant regeneration of *Arachis correntina* (Leguminosae) via organogenesis. J Plant Growth Regul 23:129–134
Murch SJ, Choffe KL, Victor JMR, Slimmon TY, Raj SK, Saxena PK (2000) Thidiazuron-induced plant regeneration from hypocotyl cultures of St. John's wort (*Hypericum perforatum*'). Plant Cell Rep 19:576–581
Murthy BNS, Victor J, Singh R, Fletcher RA, Saxena PK (1996) In vitro regeneration of chickpea (*Cicer arietimrn* L.) stimulation of direct organogenesis and somatic embryogenesis by thidiaairon. Plant Growth Regul 19:233–240
Murthy BNS, Murch SJ, Saxena PK (1998) Thidiazuron: a potent regulator of in vitro plant morphogenesis. In Vitro Cell Dev Biol Plant 34:267–275

Naaty B, Mbithe CM, Nyende AB, Njenga P, Muli JK (2017) In vitro regeneration via somatic embryogenesis of *Schizozygia Coffaeoides* Baill (Mpelepele) Amer. J Plant Biol 2:66–72

Naaz R, Anis M, Aref IM (2012) Assessment of the potentiality of TDZ on multiple shoot induction in *Bauhinia tomentosa* L., a woody legume. Acta Biol Hung 63:474–482

Ouyang Y, Chen Y, Lü J, Teixeira da Silva JA, Zhang X, Ma G (2016) Somatic embryogenesis and enhanced shoot organogenesis in *Metabriggsia ovalifolia* W. T. Wang. Sci Rep 19:6–24

Parveen S, Shahzad A (2010) TDZ-induced high frequency shoot regeneration in *Cassia sophera* Linn. via cotyledonary node explants. Physiol Mol Biol Plants 16:201–206

Parveen S, Shahzad A (2011) A micropropagation protocol for *Cassia angustifolia* Vahl. from root explants. Acta Physiol Plant 33:789–796

Parveen S, Shahzad A, Saema S (2010) In vitro plant regeneration system for *Cassia siamea* Lam., a leguminous tree of economic importance. Agrofor Syst 80:109–116

Pelah D, Kaushik RA, Mizrahi Y, Sitrit Y (2002) Organogenesis in the vine cactus *Selenicereus megalanthus* using thidiazuron. Plant Cell Tissue Organ Cult 71:81–84

Phippen W, Simon JE (2000) Shoot regeneration of young leaf explants from basil (*Ocimum basilicum* L.) In Vitro Cell Dev Biol Plant 36:250–254

Prathanturarug S, Pheakkoet R, Jenjittikul T, Chuakul W, Saralamp P (2012) In vitro propagation of *Stemona hutanguriana* W. Chuakul, an endangered medicinal plant. Physiol Mol Biol Plants 18:281–286

Radhakrishnan R, Ramachandran A, Kumari BDR (2009) Rooting and shooting: dual function of Thidiazuron in vitro regeneration of soybean (*Glycine max*. L.) Acta Physiol Plant 31:1213–1217

Radhika K, Sujatha M, Nageshwar Rao T (2006) Thidiazuron stimulates adventitious shoot regeneration in different safflower explants. Biol Plant 50:174–179

Raghu AV, Geetha SP, Martin G, Ravindran PN (2006) Direct shoot organogenesis from leaf explants of Embelia ribes Burm. f.: a vulnerable medicinal plant. J Forest Res 11:57–60

Reddy CM, Bramhachari PV, Murthy KSR (2015) Optimized plant tissue culture protocol for in vitro morphogenesis of an endangered medicinal herb *Ceropegia ensifolia* Bedd. Trop Subtrop Agroecosyst 18:95–101

Rida A, Shibli AM, Suwwan A, Ajlouni M (2001) In vitro multiplication of virus free Spunta potato. Pak J Bot 33:35–41

Saeed T, Shahzad A (2015) High frequency plant regeneration in Indian Siris via cyclic somatic embryogenesis with biochemical, histological and SEM investigations. Ind Crop Prod 76:623–637

Sahai A, Shahzad A, Sharma S (2010) Histology of organogenesis and somatic embryogenesis in excised root cultures of an endangered species *Tylophora indica*. Aus J Bot 58:198–205

Shahzad A, Ahmad N, Anis M (2006) An improved method of organogenesis from cotyledon callus of *Acacia sinuata* (Lour.) Merr. using thidiazuron. J Plant Biotechnol 8:1–5

Sharma R, Shahzad A (2008) Thiadiazuron (TDZ) induced regeneration from cotyledonary node explant of Abelmoschus moschatus Medik. L. (a valuable medicinal plant). World J Agr Sci 4:449–452

Shirani S, Mahdavi F, Maziah M (2009) Morphological abnormality among regenerated shoots of banana and plantain (*Musa* spp.) after in vitro multiplication with TDZ and BAP from excised shoot-tips. African J Biotechnol 8:5755–5761

Siddique I, Anis M (2007a) Rapid micropropagation of *Ocimum basilicum* L. using shoot tip explants pre-cultured in thidiazuron supplemented liquid medium. Biol Plant 51:757–790

Siddique I, Anis M (2007b) High frequency multiple shoot regeneration and plantlet formation in *Cassia angustifolia* (Vahl.) using thidiazuron. Med Arom Plant Sci Biotechnol 2:282–284

Siddique I, Anis M, Aref IM (2010) In vitro adventitious shoot regeneration via indirect organogenesis from petiole explants of *Cassia angustifolia* Vahl. – a potential medicinal plant. Appl Biochem Biotechnol 162:2067–2074

Singh CK, Raj SR, Jaiswal PS, Patil VR, Punwar PS, Chavda JC, Subhash N (2016) Effect of plant growth regulators on in vitro plant regeneration of sandalwood (*Santalum album* L.) via organogenesis. Agrofor Syst 90:281–288

Sivanesan L, Song JY, Hwang SJ, Jeong BR (2011) Micropropagation of *Cotoneaster wilsonii* Nakai – a rare endemic ornamental plant. Plant Cell Tissue Organ Cult 105:55–63

Sujatha M, Dinesh Kumar V (2007) In vitro bud regeneration of *Carthamus tinctorius* and wild Carthamus species from leaf explants and axillary buds. Biol Plant 51:782–786

Tejavathi DH, Raveesha HR, Shobha K (2011) Organogenesis is from the cultures of *Nothapodytes foetida* (Wight) Sleumer raised on TDZ supplemented media. Indian J Biotechnol 11:205–209

Thomas TD, Philip B (2005) Thidiazuron-induced high frequency shoot organogenesis from leaf derived callus of a medicinal climber, *Tylophora indica* (Burm. f.) Merrill. In Vitro Cell Dev Biol Plant 41:124–128

Thomas TD, Puthur JT (2004a) Thidiazuron induced high frequency shoot organogenesis in callus from Kigelia pinnata L. Botl Bull Acad Sin 45:307–313

Thomas TD, Puthur JT (2004b) Thidiazuron induced high frequency shoot organogenesis in callus from *Kigelia pinnata L.* Bot Bull Acad Sin 45:307–313

Tiwari V, Tiwari KN, Singh BD (2001) Comparative studies of cytokinins on in vitro propagation of *Bacopa monniera*. Plant Cell Tissue Organ Cult 66:9–16

Tulac S, Lejak-Levanic D, Krsnik-Rasol M, Jelaska S (2002) Effect of BAP, TDZ and CPPU on multiple shoot formation in pea (*Pisum sativum* L.) in culture in vitro. Acta Biol Craco Series Bot 44:161–168

Uranbey S (2005) Thidiazuron induced adventitious shoot regeneration in *Hyoscyamus niger*. Biol Plant 49:427–430

Varutharaju K, Soundar CR, Thilip C, Aslam A, Shajahan A (2014) High efficiency direct shoot organogenesis from leaf segments of Aerva lanata (L.) Juss. Ex Schult by using Thidiazuron. Sci World J 2014:1. Article ID 652919, 6 pages

Wang SY, Steffens GL, Faust M (1986) Breaking bud dormancy in apple with a plant bioregulator, thidiazuron. Phytochemistry 25:311–317

Wang SY, Iio HJ, Faust M (1991a) Changes in metabolic enzyme activities during thidiazuron-induced lateral bud break in apple. Hortic Sci 26:171–173

Wang SY, Jiao HJ, Faust M (1991b) Changes in activities of catalase, peroxidase and polyphenol oxîdase in apple buds during bud break induced by thidiaziron. J Plant Growth Regul 10:33–39

Xie D, Hong Y (2001) In vitro regeneration of *Acacia mangium* via organogenesis. Plant Cell Tissue Organ Cult 66:167–173

Zeng SJ, Duan J, LI LN (2008) Plant regeneration from leaf explants of *Tigridiopalma magnifica* (Melastomataceae). Pak J Bot 40:1179–1184

Zhang CL, Chen DF, Elliott MC, Slater A (2001) Thidiazuron- induce organogenesis and somatic embryogenesis in sugar beet (*Beta vulgaris* L.) In Vitro Cell Dev Biol Plant 37:305–310