Effect of TDZ on Various Plant Cultures

25

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Abstract

This chapter provides the effect of thidiazuron (TDZ) on various plant cultures. Plant cell cultures still remain to be of great benefit to many disciplines including studies, viz., physiology, mechanism, etc. Apart from plant potency, this supremacy can be attributed to the increase in number of plant growth regulators (PGRs). Growth regulators are the mile stones in plant tissue culture history. Plant growth regulators depict some interesting functions; they singly, in synergy or antagonistically, function in growth of plant. Also, their concentrations play pivotal role in plant response. These PGRs are categorized in one of the five classes of plant hormones: auxins, gibberellins (GAs), cytokinins (CKs), ethylene (C_2H_4), and abscisic acid (ABA). In recent years apart from natural PGRs available, different synthetic PGRs are made available. The use of thidiazuron (N-phenyl-N′-1,2,3-thiadiazol-5-ylurea) has been successfully demonstrated to promote axillary shoot proliferation and to encourage shoot formation in plants. Recalcitrant woody species have been great responders to TDZ, reason being its high cytokinin-like activity and better response. It facilitates initiation of multiple shoots in many recalcitrant woody tree species. It has been observed that lower concentrations (<1 μM) of TDZ show greater axillary proliferation compared to other cytokinins. Besides, it has many adverse effects on culture, viz., higher concentration of TDZ causes no shoot elongation. Thus, the present chapter reveals the effect of TDZ on various plant cultures.

Keywords

Thidiazuron · Cytokinin · Auxin · Plant growth regulator · Recalcitrant · Woody

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25.1 Introduction

Humans have a great deal of allure toward plants, due to obvious inclusions of many of them in food, clothing, medicine, and other purposes. Researchers have been studying on various aspects of the plants to understand their system. Optimization on availability of the plants throughout the season has been one among the many other challenges which has been answered efficiently. With the emergence of biotechnology, the things have been easy, especially plant tissue culture has played a pivotal role in optimizing the yield of plants. This was only possible by learning that plant growth can be regulated by adding some chemicals. Initial studies demonstrated that substances isolated from human urine can regulate the plant growth, which was later identified as indole acetic acid, a substance that had been known for decades (Mitchell and Rice 1942).

Cytokinins comprise of a separate class of growth promoters; they stimulate synthesis of proteins and actively take part in cell cycle control. Perhaps due to which they thought to promote chloroplast maturation and delay senescence in plants. Cytokinins when applied to plant tissue, biochemically, causes the treated part to act as a sink for amino acids, which then migrates to the nearby sites (George et al. 2008), thus causing the most noticeable effect of cytokinin in the tissue. They are always used in ratio with auxin to encourage cell division and manage morphogenesis. They are known to overcome apical dominance and proclaim lateral buds from dormant tissues.

25.2 Thidiazuron as Cytokinin

Naturally occurring cytokinin compounds and their derivatives are available in market. Kinetin was the first cytokinin to be discovered at professor Skoog's laboratory in the University of Wisconsin. This came out as a result out of the experiemnts to promote continuing growth of the callus which formed on tobacco stem sections in vitro. Further, as George et al. (2008) mentions that chromatography of alcohol-soluble yeast extract fractions proved to be purine, researches for supplementary sources of purines were evaluated to observe the potency toward callus growth. Until then, extracts of herring sperm DNA showed a molecule with similar spectrum and chemical behavior. Its isolation and crystallization from DNA samples under acidic conditions lead to a new growth factor "kinetin." This molecule stimulated cell division in cells which otherwise might have become multinuclear (Miller et al. 1955a, b; Miller 1961a, b) and was later identified as 6-furfuryl aminopurine. Skoog et al. (1965) proposed the general term "cytokinin" to envelop all molecules that show such similar activity.

Kinetin is not accepted as natural cytokinin and has arisen as structural rearrangements in original isolates (Hecht 1980). There are many cytokinins naturally available and identified which structurally resemble to kinetin. They are structurally either freebases, glucosides, ribosides, or nucleotides of kinetin (Entsch et al. 1980).

Their utility in plant tissue culture work has been widely studied, viz., trans-zeatin (4-hydroxy-3-methyltrans-2-butenylaminopurine), iP (N6-Δ2isopentenyladenine), and dihydrozeatin (6-(4-hydroxy-3-methyl-trans-2-butenyl)aminopurine).

Higher costs of natural cytokinins (iP and zeatin) make them an unpopular choice for commercial routine laboratory practices; however, they are still a popular choice in research laboratories. Substituted purines and phenyl urea are two largely vouched groups of synthetic cytokinins, prevalent due to their potent cytokinin-like properties. Synthetic derivatives of natural occurring cytokinins are extremely potent; they chiefly include N6-substituted adenine derivatives. There are few other molecules to some extent structurally similar but possess such activity, such as 4-alkylaminopteridines (Iwamura et al. 1980a, b) and 6-benzyloxypurines. Some of these analogues are reported to be more active than kinetin or benzyladenine (BA) and are particularly effective in promoting morphogenesis (Wilcox et al. 1978, 1981). The 1-deaza analogue of zeatin riboside (Rogozinska et al. 1973; Rodaway and Lutz 1985; Kaminek et al. 1987) also has cytokinin activity. Current-day literature suggests that BA and its derivatives are widely accepted forms of cytokinins. Topolin is one such derived group which is aromatic and naturally occurring cytokinins.

Natural cytokinin, viz., yeast extract or coconut milk, is used in media as organic supplements. Shantz and Steward (1955) demonstrated that they contain physiologically active substances including natural cytokinin zeatin and 1,3-diphenylurea. The two molecules in the same series are 2Cl-4PU (N-(2-chloro-4-pyridyl)-N′-phenylurea) and 2,6Cl-4PU (N-(2,6-dichloro-4-pyridyl)-N′-phenylurea), which is supposed to be the most active.

A thiadiazole-substituted phenylurea, thidiazuron (TDZ) (N-phenyl-N′-1,2,3-thiadiazol-5-ylurea) which was earlier registered as a cotton defoliant (Arndt et al. 1976)with the product name “Dropp,” has demonstrated high cytokinin activity (Mok et al. 1982). Diphenylurea is a rather weakly active cytokinin (Bottomley et al. 1963; Miller 1960; Strong 1956), but particular derivatives of N-phenyl-N′-4-pyridylurea exhibit cytokinin activity equal to or exceeding that of zeatin in the tobacco callus bioassay (Takahashi et al. 1978).

Mok and coworkers (1982) studied cytokinin property of TDZ on *Phaseolus* species. The experiment was part of their study on cytokinin metabolism in species (Mok et al. 1978, 1979, 1980; Armstrong et al. 1981); they examined effects of TDZ and other substituted urea compounds on the growth of cytokinin-dependent callus tissues of *Phaseolus lunatus* cv. Kingston. It was understood that TDZ has more potent cytokinin activity compared to N-phenyl-N′-4-pyridylureaderivatives and most other active cytokinins of adenine type. It was also concluded that there are two derived classes of urea, (i) pyridylurea and (ii) thiadiazolyl urea, comprising of various compounds with cytokinin activity. It was also mentioned that these activities were equivalent or exceeding to that of the most active cytokinins of the adenine type (Mok et al. 1982).

25.3 Chemistry

In recent years, TDZ appeared as a very effectual PGR in plant tissue culture experiments for a wide range of species like herbs, shrubs, climbers, crop plants, and majorly trees (Murthy et al. 1998). Thidiazuron had a commercial impact for its strongest use in defoliating leaves to facilitate collection of bolls from cotton plant (Arndt et al. 1976). It has also proved to protect chlorophyll from degradation in detached leaves. TDZ exhibits a high level of activity at concentrations as low as 10 pM for a relatively short period (Bakulev et al.). Chemically it is 1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea. It has been demonstrated to have herbicidal properties. TDZ has a chemical formula $C_9H_8N_4OS$, molar mass of 220.251 g/mol, and density of 1.51 g/cm^3. Structurally it is different from both auxins and adenine-type cytokinins. It possesses two functional groups, phenyl and thiadiazole. It has been demonstrated that replacement or modifications in these groups will result in reduction of kinin activity of TDZ.

Thomas and Katterman (1986) demonstrated dose-dependent effect of thidiazuron in radish and soybean. They concluded that the general growth and cell division stimulation become saturated at low levels of TDZ. That is, TDZ was 10^2 times more effective in the radish and 10^4 times more effective in soybean compared to purine cytokinins (Thomas and Katterman 1986).

As per physical chemistry guidelines, TDZ is found as colorless, odorless crystals, white to yellow in color. It has a melting point of 210.5 °C and a vapor pressure of 4×10^{-6} mPa (25 °C). It is soluble in water at a rate of 31 mg/L in a neutral condition (pH 7) with temperature of 25 °C and also soluble in organic solvents (hexane, 0.002; methanol, 4.20; dichloromethane, 0.003; toluene, 0.400; acetone, 6.67; ethyl acetate, 1.1 g/L 20 °C). Interestingly it has been written that TDZ rapidly gets converted to photoisomer, 1-phenyl-3-(1,2.5-thiadiazol-3-yl)urea in the presence of light (λ >290 nm). TDZ is hydrolytically stable at room temperatures, at pH 5.9.

25.4 Mode of Action

TDZ has been used singly or in synergy with other PGRs, mainly auxins. Positive effects on culture of *Geranium* by replacing auxin and cytokinin with TDZ have been successfully been demonstrated (Visser et al. 1992). Further studies elucidated role of TDZ in induction and regeneration in many species (Murthy and Saxena 1998). Not only this but the recalcitrant cultures successfully responded and regenerated to TDZ (Malik and Saxena 1992b; Murthy and Saxena 1998).

The flow of actions on treatment of TDZ involves reprogramming and expression of morphological and genetic cell competent to undergo development which further leads to morphogenesis. Studies have showed association of TDZ in metabolism of PGRs. TDZ earlier was categorized as cytokinin; this was because of its natural cytokinin-like response. Metabolism of endogenous plant growth regulators has a direct relation to the presence of TDZ during morphogenesis and regulates endogenous growth. The role of TDZ in morphogenesis is intimately related to the

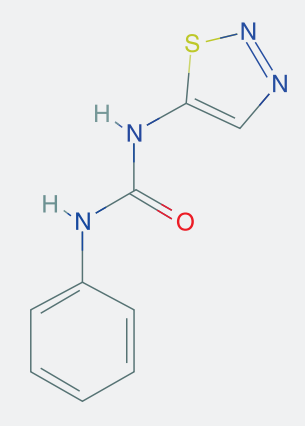

Fig. 25.1 Chemical structure of TDZ (Source: https://pubchem.ncbi.nlm.nih.gov/compound/thidiazuron#section=Top)

metabolism of endogenous growth regulators. In some independent experiments, increased levels of endogenous auxin, ethylene, and ABA were recorded in response to TDZ treatment (Murthy et al. 1995; Yip and Yang 1986; Capelle et al. 1983; Thomas and Katterman 1986; Mok et al. 1987; Ji and Wang 1988; Hutchinson and Saxena 1996b) (Fig. 25.1).

Actions of TDZ have been discussed by many workers in details; referring to most of the articles, it feels like TDZ can be compared in following ways to the classical PGRs to understand the exact mechanisms (Fig. 25.2).

25.4.1 Cytokinin-Like Action

According to Mok et al. (1982), TDZ holds cytokinin-like activity, and also in several other bioassays, the application of TDZ elicited effects in association with cytokinins (Thomas and Katterman 1986; Visser et al. 1995). There are reports suggesting TDZ promoted synthesis and/or accumulation of endogenous cytokinins (Thomas and Katterman 1986; Murthy et al. 1995; Hutchinson and Saxena 1996b). Kefford et al. (1968) reported that this may potentially occur due to (a) increase in synthesis, (b) decrease in catabolism, or (c) release of biologically active cytokinin molecules from non-active storage forms. Interconversion of labeled cytokinin ribonucleosides and ribonucleotides in the presence of TDZ and zeatin was studied by Mok et al. (1987)). Here, TDZ inhibited formation of nucleotides, whereas zeatin sustained in production of nucleotides from ribonucleosides. This indicated involvement of TDZ in regulating metabolism for production of endogenous cytokinins (Mok et al. 1987). Its cytokinin-like action was also evident when tissue of *Phaseolus lunatus* callus proliferated on medium with TDZ and on a cytokinin-free medium. This indicated positive alteration and involvement of TDZ in pathways for production of cytokinin-active adenine derivatives bearing N6-isoprenoid side chains (Capelle et al. 1983).

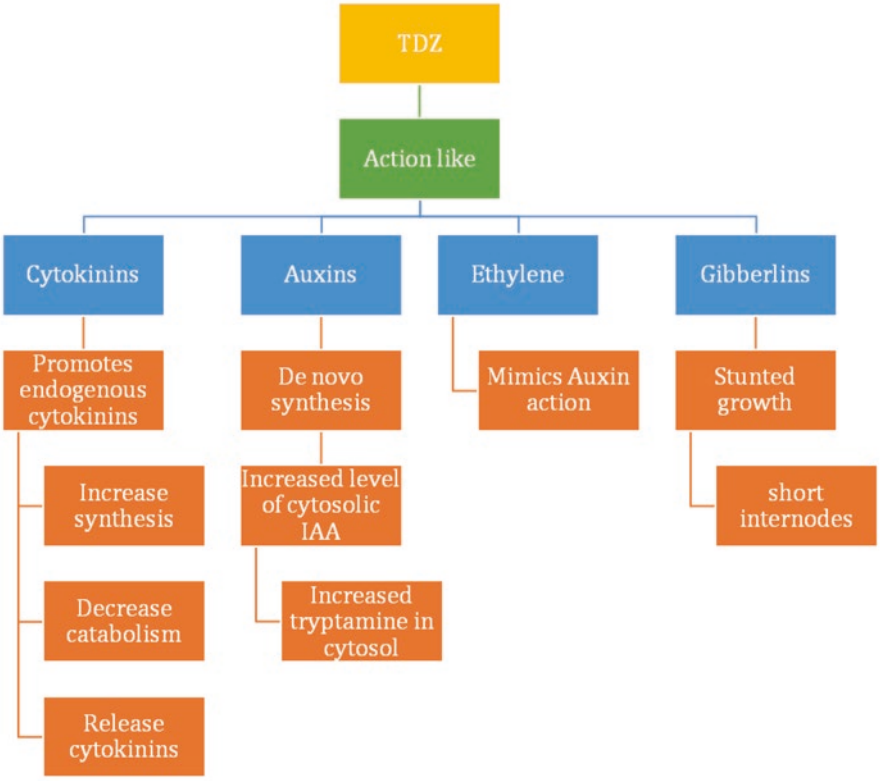

Fig. 25.2 Various PGR-like actions of TDZ

Thidiazuron is structurally dissimilar to any other naturally occurring cytokinins especially purine based. Further, its action to induce somatic embryogenesis (SE) makes it different from any other purine-based cytokinins, wherein the later alone has never been reported to induce SE. Therefore, properties of TDZ might be apparent via different mechanisms. The biological responses induced with purine-based cytokinins and phenylureas in the presence of competitive inhibitors and the measurement of a relationship between activity and structure suggested a common site of action for these two groups of growth regulators (Kefford et al. 1968; Iwamura et al. 1980b). Recently, Nagata et al. (1993) isolated a cytokinin-specific binding protein (CSBP) from etiolated mung bean seedlings and showed that the association constant of CSBP for CPPU, a phenylurea derivative, was higher than that for BA. This finding clearly demonstrated the existence of a common cytokinin-specific binding protein for both types of compounds.

25.4.2 Auxin-Like Action

Auxins have a vital role in differentiation of cell aggregates which are preliminary requirement for regeneration. TDZ has proved to induce SE in *Arachis hypogea* and *Azadirachta indica* (Murthy and Saxena 1998). In an independent study, Murthy et al. (1995) observed de novo synthesis of auxins in peanut seedlings grown on TDZ-containing medium. It was observed that there was an increased level of IAA and other monoamine alkaloid compound tryptamine in cytoplasm. It was further observed that the use of PCIB (2-(p-chlorophenoxy)-2-methylpropionic acid) popularly known as clofibric acid which is a herbicide functioning against auxin biosynthesis, for reduction of TDZ-induced somatic embryogenesis in both *Geranium* (Hutchinson et al. 1996) and *Arachis hypogea*. Similarly, TIBA (2, 3, 5-triiodobenzoic acid), an inhibitor of polar auxin transport (Thomson et al. 1973), reduced SE but failed to decrease auxin levels in TDZ-treated plant tissues (Hutchinson et al. 1996).

25.4.3 Ethylene-Like Action

Promotive and inhibitory effects of ethylene in somatic embryogenesis have been reported (Biddington 1992). Supplementation of media with TDZ for induction of *Geranium* somatic embryogenesis results in elevated levels of ethylene in the space of culture vessel (Hutchinson et al. 1997a). On the other hand, decrease in ethylene level by its inhibitor AVG (aminoethoxyvinylglycine) improved the embryogenic response in geranium hypocotyls. Thus, indicating ethylene can be produced as a negative by-product of TDZ-mediated metabolic cascade (Hutchinson et al. 1997a). Utilization of exogenous ethylene or 1-aminocyclopropane-l-carboxylic acid, an ethylene forerunner, diminished the embryogenic result to an undistinguished level as observed in the TDZ-actuated culture (Hutchinson et al. 1997a). Auxin-like metabolic response of TDZ was based on the observation of Suttle (1985, 1986) on 2-(p-chlorophenoxy)-2-methylpropionic acid (PCIB) that inhibited TDZ-mediated ethylene production in cotton. Since auxin treatment additionally brought about expanded ethylene generation (Suttle 1984; Yip and Yang 1986), it is conceivable to infer that the leaf abscission may not be an immediate aftereffect of TDZ treatment yet rather a consequence of the auxin reaction instigated by TDZ.

25.4.4 Gibberellin-Like Action

There are not really any reports observed on coordinated balance of endogenous gibberellins by TDZ. In any case, a few reports propose change of TDZ-prompted somatic embryogenesis in geraniums (Hutchinson et al. 1997b) by GA synthesis inhibitors (triazoles and ancymidol), supporting that gibberellins are affected by TDZ. Legume seedlings growing on TDZ medium exhibited stunted growth habit (Murthy et al. 1995, 1998); also foliar spray or soil drenching of TDZ significantly

affected stem elongation making them stunted in ginseng and geranium (Sanago et al. 1995; Proctor et al. 1996). Woody plants regenerating into adventitious shoots on TDZ medium will be dwarf with short internodes (Lu 1993; Pai et al. 2017), which indicates changed metabolism in gibberellins. Although to what extent it explains connection of TDZ with gibberellins remains to be the area to be examined.

25.5 Thidiazuron and Its Applications

European Union banned the use of thidiazuron in agriculture. It was one of the agricultural chemicals in the framework of the European Pesticides Directive 91/414/EEC that must have an environmental and health assessment to obtain a new authorization (Wikipedia). In spite of the fact being known that TDZ is modestly poisonous to fish and aquatic organisms and also that it is not easily biodegradable, yet it is utilized in different parts of the world, including United States, Australia, and Mexico. Since it is not extremely toxic to birds or bees, the World Health Organization has made substance classification as: "It was rated unlikely to present acute hazard in normal use."

25.5.1 Plant-Based Applications

Each living cell of a plant body has a capacity to grow into a whole plant (totipotency) by means of de novo formation of organs or somatic embryos. Plant growth substances (viz., auxins and cytokinins) mainly regulate the whole process of acquisition of competency, dedifferentiation, and redifferentiation. Further, the above processes in tissue cell cultures are effectively governed by TDZ singly or in combination with other PGRs.

25.5.1.1 Callus Formation

The presence of auxins in cell culture media helps in the induction of cell proliferation and callus growth. Weedicides such as 2,4-dichlorophenoxyacetic acid along with other synthetic auxins [naphthaleneacetic acid (NAA) and others] have successfully and extensively been utilized in tissue culture. The importance of another synthetic compound "TDZ" has been observed in the induction of callus formation in assorted plant cultures, which has also exhibited higher rate of cell proliferation compared to other PGRs. For example, TDZ induced a 30-fold increase in the growth of callus cultures over other plant growth regulators (Capelle et al. 1983). In addition, the callus absorbed less TDZ than other plant growth regulators, thereby indicating a relatively high intrinsic activity of TDZ (Capelle et al. 1983). Apart from its cytokinin-like activity, TDZ is also used in the formation of callus. Table 25.1 enlists few such recent examples of plants in which TDZ has been used in combination with other PGRs from production or maintenance of callus.

Table 25.1 Use of TDZ in formation of callus

Plant name	TDZ (mg/l)	Other PGR (mg/l)	References
Phalaenopsis spp.	0.5	2,4 D (0.5)	Chen et al. (2000)
Crocus spp.	4.0	NAA (4.0)	Verma et al. (2016)
Aconitum balfourii	0.5	NAA (1.0)	Gondval et al. (2016)
Fragaria × *ananassa*	0.2 and 0.5	2, 4 D (0.02)	Cappelletti et al. (2016)
Hypericum triquetrifolium	0.4	IAA (0.5)	Azeez et al. (2017)
Curcuma soloensis	2.5 uM	BA (1.2 uM), 2, 4 D (1.2 uM)	Zhang et al. (2011)
Cymbidium spp.	0.01	NAA (0.1)	Huan et al. (2004)
Artemisia absinthium	2.0	NAA (1.0)	Tariq et al. (2014)
Digitalis spp.	0.5	IAA (0.25)	Cingoz et al. (2014)
Mangosteen	2.25 uM	BA (2.22)	Te-chato and Lim (2000)

25.5.1.2 Shoot Formation

There is generous confirmation that TDZ not only helps in the induction of bud (axillary) break and production of adventitious buds but also plays a major role in shoot production of diverse crops ranging from tropical fruit trees to roots and tuber crops. Medium fortified with high levels of cytokinins used for culturing explants stimulates multiple shoots or bud formation. A comprehensive review of plants that have been micropropagated using TDZ as growth regulator has been published (Huetteman and Preece 1993; Lu 1993). TDZ has been effectively utilized in regeneration of wood plant species (Briggs et al. 1988; Preece and Imel 1991; Baker and Bhatia 1993), but without the help of high concentration of adenine type of cytokinins, organogenesis is not possible. Also, TDZ has been utilized adequately in species in which purine-type cytokinins were incapable. In any case, there have been reports of issues with transformation of TDZ-induced shoots into complete plantlets, poor shoot elongation, and inadequate rooting (Huetteman and Preece 1993; Lu 1993). This debilitated development of TDZ-induced regenerants may come about because of utilization of supraoptimal levels of TDZ in the media or the presence of the compound in cultured tissues. Table 25.2 provides an insight in the published recent literature, wherein TDZ has been used in shoot formation.

25.5.1.3 Somatic Embryogenesis

Exogenous auxin to cytokinin ratio plays an important role in production of embryogenetic tissues especially somatic embryos. TDZ singly has been found to substitute for both the auxin and cytokinin prerequisite of substantial embryogenesis in numerous species (Saxena et al. 1992; Visser et al. 1992; Gill et al. 1993). Addition of TDZ in culture media invigorated in vitro somatic embryogenesis in *Nicotiana* (Gill et al. 1993), *Arachis hypogea* (Saxena et al. 1992; Murthy et al. 1995), geranium (Visser et al. 1992), chickpea (Murthy et al. 1996), neem (Murthy and Saxena 1998), and St. John's wort (Murch et al. unpublished information), at a significantly higher rate compared to the known phytohormones. In different cases, concurrent

Table 25.2 Use of TDZ in shoot formation in different plant species

Plant name	TDZ (mg/l)	Other PGRs (mg/l)	References
Phalaenopsis spp.	0.5	2,4 D (0.5)	Chen et al. (2000)
Aconitum balfourii	0.5	–	Gondval et al. (2016)
Solanum tuberosum	2.0	–	Sherkar and Chavan
Oryza sativa	0.5	BAP (0.5), Kn (1.5), NAA (0.5)	Dina et al. (2016)
Brassica oleracea	0.33 and 0.088	Adenine (79.70) and IAA (0.22)	Gambhir et al. (2017)
Curcuma soloensis	2.5 uM	BA (9.0 µM), NAA (1.2 µM)	Zhang et al. (2011)
Swertia lawii	3.0	IBA (0.3)	Kshirsagar et al. (2015)
Ancistrocladus heyneanus	6.81 uM	BAP (13.31 uM)	Pai et al. (2008)
Achyranthes aspera	0.1	BAP (3.0)	Pai et al. (2017)
Strawberry	0.5	2, 4 D (0.02)	Cappelletti et al. (2016)
Blueberry	0.5	2iP (0.2)	Cappelletti et al. (2016)
Agapanthus praecox	4.5 uM	BA (22.2 uM), IAA (2.9 uM)	Baskaran and Van Staden (2013)

production of shoots and somatic embryos has additionally been recorded (Bates et al. 1992). Despite the fact that the activity of TDZ as a cytokinin-like compound is all around archived, the previously mentioned reports of somatic embryogenesis give proof to a part of TDZ in regulation of auxin metabolism, as the enlistment of somatic embryogenesis is a response usually connected with auxins.

A novel arrangement of TDZ-induced regeneration is the improvement of somatic embryos on intact seedlings (Malik and Saxena 1992b). Somatic embryos developed at various sites on intact pea, peanut, and chickpea seedlings germinated on TDZ-fortified media. In purine cytokinin BA (N 6-benzyladenine) utilization as a part of a similar procedure, de novo shoots appeared at the regenerative area, demonstrating that the TDZ-initiated somatic embryogenesis is not exclusively a cytokinin-dependent response. Despite the fact that the seedling culture system was initially produced for recovering substantial seeded legumes, viz., pea, bean, and peanut, this system has been therefore utilized as a part of a wide assortment of different plants including geranium (Quresbi and Saxena 1992) and neem (Murthy and Saxena 2015). The following table depicts the use of different concentrations of TDZ along with other PGRs to achieve somatic embryos (Table 25.3).

25.5.1.4 Protoplast Culture

Plant regeneration from protoplasts more often than not continues through a callus stage; in any case, somatic embryogenesis might be started from the protoplast-derived cells (Song et al. 1990). The significance of the nearness of both auxin and cytokinin in the cultures to stimulate protoplast division and development is well studied (Cook and Meyer 1981), and henceforth, most protoplast culture media contain mixture of auxins and cytokinins. TDZ (in combination with auxins like NAA,

Table 25.3 Use of TDZ in induction of somatic embryos in various plant species

Plant name	TDZ (mg/l)	Other PGRs (mg/l)	References
Crocus spp.	2.0	IAA (2.0) + BAP (2.0)	Verma et al. (2016)
Mirabilis jalapa	0.5	BAP (2.0)	Rohela et al. (2016)
Lachenalia montana	1 uM	2, 4 D (0.5 uM)	Baskaran and Van Staden (2017)
Crocus olivieri	2.0	IAA (2.0)	Verma et al. (2016)
Malaxis densiflora	6.80	2, 4 D (3.39)	Mahendran and Bai (2016)
Crocus sativus	2.5 uM	Picloram (2.00 uM)	Devi et al. (2014)
Lachenalia viridiflora	1.0 uM	Picloram (2.50 uM)	Kumar et al. (2016)
Digitalis trojana	1.0	IAA (0.5)	Verma et al. (2012)
Cajanus cajan	10.0 uM	–	Singh et al. (2003)
Azadirachta sp.	1.0 uM	–	Gairi and Rashid (2004)

naphthoxyacetic acid (NOA), or 2,4-D) at various levels (0.001–20 btM) has been utilized amid introductory periods of cell wall formation around protoplasts, initiation of cell division (Chupeau et al. 1993; Reustle et al. 1995), and in later stages to complete the recovery from protoplast-derived callus (Lenzner et al. 1995). Wallin and Johansson (1989) showed that TDZ supported division of leaf protoplasts of apple at a superior rate than either BA or zeatin. Likewise, TDZ was more viable than kinetin and zeatin for development of willow (*Salix viminalis* L.) protoplast cultures (Vahala and Eriksson 1991). Chupeau et al. (1993) reported that TDZ was more successful at lower levels compared to adenine-type cytokinins. The role of TDZ in induction of regeneration from protoplast cultures is by all accounts to a great extent a cytokinin-like response, yet the correct mechanism for this impact stays undetermined.

25.5.1.5 In Vivo Regeneration

The effectiveness of TDZ as an inductive molecule for morphogenesis is not restricted to tissue culture systems. Sanago et al. (1995) found that TDZ actuated the arrangement of regenerative outgrowths on root tissues and adventitious shoots at the crown region of greenhouse-grown geraniums. Also, pot-developed *Spathiphyllum* plants splashed with TDZ created countless shoots, both at and beneath soil levels (Henny 1995). Proctor et al. (1996) observed the arrangement of adventitious buds on the shoulders of ginseng tap roots when TDZ was applied either as foliar shower or soil soaked. This solid relationship among TDZ and morphogenic forms instigated in vitro and in vivo is exclusive to the molecule and gives a few experimental systems for surveying the biochemical responses to TDZ and additionally researching the variables that manage plant morphogenesis.

Thidiazuron does not degrade by cytokinin oxidase and is stable (Mok et al. 1987). It is considered more active compared to BAP or zeatin, and a lower concentration of it is effective in tissue culture experiments. TDZ is more efficient in most of the species yet had made a noteworthy accomplishment in woody species. A portion of the impediments revealed in a few species include hyperhydricity of shoots

(Debergh et al. 1992; Briggs et al. 1988; Cousineau and Donnelly 1991), anomalous leaf morphology (van Nieuwkerk et al. 1986; Cambecedes et al. 1991), shorter internodes and smaller shoots (Fasolo et al. 1989; Meyer and van Staden 1988; Desai et al. 2016), and trouble in prolongation and establishing of recovered shoots (Meyer and Kerns 1986; Meyer and van Staden 1988). In such cases, hyperhydricity can be overwhelmed by utilizing unlocked profound petri dishes in the shoot initiation stage, vented caps in the shoot lengthening stage, or higher levels of gelling agent. Shoot quality can be enhanced by utilizing a mixture of TDZ and purine cytokinin (Briggs et al. 1988). Preece and Imel (1991) accounted that the majority of shoots regenerated on TDZ medium were short, yet elongated after shifting them to medium containing IBA and 2iP. To overcome other issues mentioned above, explants ought to be prompted with the most minimal successful TDZ concentration and continued TDZ medium for the slightest time that is required for each species (Lu 1993). In another investigation, Pawar et al. (2015) discovered unfavorable impact of TDZ on nature of callus and content of proline and glutamine in rice.

Conclusively, in years of its usage since its revelation, TDZ has demonstrated to advance in its application from basic cytokinin to every single other feature of plant tissue culture. Impact of any PGR rely upon many factors as fixation, levels of different endogenous PGR, ecological conditions, signaling factors, and for the most part affectability of plant species. In this manner, it is hard to anticipate the activity of exogenous utilization of any PGR including TDZ for new plant system. A scrutiny of writing uncovers that it has effectively been utilized to induce axillary or adventitious shoot multiplication in various plant species including herbaceous and perennials. Less has been comprehended about the activity of TDZ on morphogenesis and furthermore its biochemical and physiological basis. In any case, studies done on different species and tissue culture response of TDZ have laid more extensive application in plant tissue culture.

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