Thidiazuron in Micropropagation of Aroid Plants

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Abstract

Thidiazuron (TDZ) or phenyl-N′-(1,2,3-thiadiazol-5-yl) urea is a synthetic phenyl urea derivative and possesses strong cytokinin-like activity exceeding that of most other commonly used adenine-type cytokinins in regulating plant morphogenesis. In this article, we devote our attention to the use of TDZ in micropropagation of plants in the family Araceae, commonly known as aroids. This family has 3750 recognized species across 114 genera. A large number of genera are important ornamental plants, particularly in the foliage plant industry. Some genera are produced for edible roots or used as medicinal plants, and a few others are aquatic plants. Aroids are traditionally propagated through cutting, division, rhizomes, or tubers. Vegetative propagation not only carries plant pathogens but also significantly slows the speed in the introduction of new cultivars. Our research over the years has focused on the development of methods for micropropagating aroid plants. TDZ has been shown to be an important plant growth regulator for efficient micropropagation of aroid plants via in vitro shoot culture and plant regeneration through the route of shoot organogenesis, somatic embryogenesis, and protocorm-like bodies (PLBs). Mechanisms underlying TDZ-mediated plant regeneration are still largely unknown, but the established regeneration systems derived from our work on aroids present valuable models for molecular analysis of TDZ-mediated plant morphogenesis.

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4.1 Introduction

Micropropagation is a form of tissue culture that rapidly propagates plants through in vitro shoot culture or regenerates plants through shoot organogenesis, somatic embryogenesis, and protocorm-like bodies (PLBs). In vitro shoot culture refers to in vitro propagation of plants through repeated enhanced formation of axillary shoots from shoot tips or lateral buds cultured on medium supplemented with plant growth regulators, primarily cytokinin (George et al. 2008). There are generally four distinct stages involved in shoot culture: culture initiation, shoot multiplication, in vitro rooting, and acclimatization (Murashige 1974; Rout et al. 2006).

Micropropagation through plant regeneration rests on the cell theory (Schleiden 1838; Schwann 1839) and started with Gottlieb Haberlandt's speculation of the totipotency of plant cells near the turn of the twentieth century (Haberlandt 1902). Totipotency is a single cell that has the genetic program to grow into an entire plant (Hartmann et al. 2002). Shoot organogenesis is the regeneration of plants directly from plant tissues or indirectly from callus derived from cells, tissues, and organs called explants cultured on artificial medium supplemented with plant growth regulators. In indirect shoot organogenesis, the cultured cells go through dedifferentiation, induction, and differentiation phases to produce plant shoots. Regeneration through somatic embryogenesis is a process through which undifferentiated cells are induced through the actions of cytokinins and auxins in the culture media to become embryogenically determined. When induced cells produce embryos without a callus phase, this is referred to as direct somatic embryogenesis. If cells produce callus preceding the formation of embryos, this is referred to as indirect embryogenesis. PLBs resemble protocorms structurally induced from explants or calluses which are composed of many meristematic centers that are able to differentiate into shoots and roots (Jones and Tisserat 1990; Cui et al. 2008).

Plant growth regulators, mainly cytokinins and auxins, play critical roles in both shoot culture and organogenesis. Skoog and Miller (1957) were the first to demonstrate the role of kinetin (6-furfuryladenine) in organogenesis. When the ratio of kinetin to auxin was higher, only shoots developed. Whereas when the ratio was lower, only roots were formed. Two groups of chemicals are known to have cytokinin activities: the N6-substituted adenine derivatives and certain substituted urea compounds. Thidiazuron (TDZ) or phenyl-N′-(1,2,3-thiadiazol-5-yl) urea, a substituted urea compound, was synthesized by German Schering Corporation for defoliation of cotton (*Gossypium hirsutum*) (Arndt et al. 1976). TDZ is known to have cytokinin activity (Mok et al. 1982) and to promote the growth of cytokinin-dependent callus cultures of *Phaseolus lunatus* (Capelle et al. 1983). The cytokinin activity of TDZ was reported to be similar to the most active cytokinins of the adenine

type (Huetteman and Preece 1993; Murthy et al. 1998). As a result, TDZ emerged as an effective growth regulator in cell and tissue cultures in a wide array of plant species (Li et al. 2000; Hosseini-Nasr and Rashid 2002; Yancheva et al. 2003; Matand and Prakash 2007; Guo et al. 2011). This article is intended to review TDZ applications in micropropagation of aroid plants and to document the importance of this plant growth regulator in improving propagation and production of aroid plants.

4.2 Aroid Plants

The family Araceae, commonly known as aroids, encompasses 114 genera and more than 3750 species that are mostly herbaceous either as terrestrial, aquatic, or epiphytic (Mayo et al. 1997; Bown 2000). Most are indigenous to the tropics of America, Southeast Asia, the Malay Archipelago (Malaysia, Indonesia, the Philippines, Papua New Guinea, Singapore, and Brunei), and tropical Africa (Mayo et al. 1997). Some species, such as *Amorphophallus paeoniifolius*, *Colocasia esculenta*, *Cyrtosperma merkusii*, and *Xanthosoma sagittifolium*, are cultivated as sources of carbohydrate foods (Chen et al. 2003; Bown 2000) (Table 4.1). Some including *Arisaema heterophyllum*, *Pinellia ternata*, and *Typhoniumi trilobatum* are important medicinal plants (Mayo et al. 1997; Bown 2000; Chen et al. 2007). A large number of them are ornamental foliage plants, such as *Aglaonema*, *Anthurium*, *Dieffenbachia*, *Epipremnum*, *Philodendron*, *Spathiphyllum*, and *Syngonium* (Mayo et al. 1997; Bown 2000; Henny and Chen 2003; Chen et al. 2005). This group of plants is prized for their beautiful leaf forms, textures, colors, and variegation patterns as well as colorful spathes present in some genera (Chen et al. 2003; Bown 2000). Ornamental aroids are a major component of the foliage plant industry and account for about one-third of total ornamental foliage plant sales in the United States (Henny et al. 2004). Ornamental aroids have been widely used as living specimens for interior decoration or interiorscaping because of their ability to maintain an aesthetically pleasing appearance under interior low light conditions. Interior decoration with ornamental aroids brings beauty and comfort to our surroundings and also reminds us of nature (Chen et al. 2005). In addition, ornamental aroids grown in interior environments can act as natural humidifiers and have been shown to reduce indoor air pollutants (Wolverton et al. 1984; Oyabu et al. 2003).

Aroids were traditionally propagated through vegetative means, such as cuttings or divisions (Chen and Stamps 2006). Vegetatively propagated plants are often associated with the spread of diseases such as dasheen mosaic virus that can be difficult to eradicate by chemical or physical treatment (Hartman 1974) and bacterial wilt (*Ralstonia solanacearum*) carried over through the cuttings of pothos (*Epipremnum aureum*) (Norman and Yuen 1998). Hartman (1974) was the first to report the use of micropropagation for producing *Caladium bicolor*, *Xanthosoma sagittifolium*, and *Colocasia esculenta* that were free of dasheen mosaic virus. Micropropagation decreases greenhouse space needed for stock plant production and provides growers with liners (tissue-cultured plantlets grown in cell plug trays) on a year-round basis (Chen and Henny 2008). Adoption of in vitro propagation has reduced the wait for

Table 4.1 Important genera of aroids, methods of propagation, and their economic use

Genera	Common name	Conventional propagation	Economic value
Acorus	Sweet flag	Seed or rhizome division	Aquatic plants with medicinal and aromatic value
Aglaonema	Chinese evergreen	Cutting or division	Ornamental plants
Alocasia	Elephant's ear	Rhizome division, cutting, seed	Ornamental or edible plants
Amorphophallus	Voodoo lily, titan arum	Division or seed	Ornamental or edible plants
Anthurium	Flamingo flower, laceleaf	Seed or division	Ornamental plants
Caladium	Angel wings	Root tubers	Ornamental plants
Colocasia	Taro, cocoyam	Corms or root tubers	Ornamental or edible plants
Dieffenbachia	Dumb cane	Stem cutting or division	Ornamental plants
Epipremnum	Pothos	Stem cutting	Ornamental plants
Homalomena	Homalomena	Division	Ornamental plants
Monstera	Swiss cheese plant	Cutting	Ornamental plants
Lemna	Duckweed	Division	Aquatic plants
Philodendron	Philodendron	Cutting or division	Ornamental plants
Pinellia	Green dragon	Bulbils, seeds, tubers	Medicinal plants
Spathiphyllum	Peace lily	Division or seed	Ornamental plants
Spirodela	Giant duckweed, duckweed	Asexual budding, seeds	Aquatic plants
Syngonium	Arrowhead vine	Cutting	Ornamental plants
Xanthosoma	Arrowleaf elephant's ear	Corms or root tubers	Ornamental and edible plants
Zantedeschia	Calla lily, arum lily	Root tubers	Ornamental plants

new plant introduction and new cultivar release. Using tissue culture methods, a new aroid hybrid cultivar can be increased rapidly enough to reach commercial production levels within 2–3 years instead of the 5–7 years previously as required using traditional cutting or division techniques (Henny and Chen 2003). Furthermore, ornamental aroids produced from in vitro propagated plantlets show desirable growth habits when compared to plants produced from traditional propagation methods such as cuttings and division. *Anthurium*, *Dieffenbachia*, *Spathiphyllum*, and *Syngonium* often develop multiple basal shoots when grown from in vitro propagated liners and produce finished plants that are fuller and more compact than plants produced by other methods (Chen and Henny 2008). As a result of the increase of basal shoots, *Anthurium* and *Spathiphyllum* produced from in vitro-propagated liners usually have more flowers (Henny and Chen 2003). More than 132 million aroid plantlets were produced annually through micropropagation with wholesale values up to 107 million US dollars (Chen and Henny 2008).

4.3 TDZ in Shoot Culture

Micropropagation starts with shoot culture. *Amorphophallus rivieri*, an aroid, was actually the first monocotyledon to be successfully cultured in vitro (Morel and Wetmore 1951). Dasheen mosaic virus was eradicated from *C. bicolor*, *X. sagittifolium*, and *C. esculenta* through in vitro shoot culture (Hartman 1974). Subsequently, methods for shoot culture of *Alocasia*, *Anthurium*, *Dieffenbachia*, *Philodendron*, *Spathiphyllum*, and *Syngonium* were developed (Henny et al. 1981). The cultures were based primarily on MS medium (Murashige and Skoog 1962) supplemented with 6-benzylaminopurine (BA), 6-(γ, γ-dimethylallylamino) purine (2iP), or kinetin with or without auxins (Hartman 1974; Henny et al. 1981).

Since the discovery of TDZ as a plant growth regulator in the 1980s, TDZ has also been used for in vitro shoot culture of aroid plants including *Acorus*, *Aglaonema*, *Alocasia*, *Amorphophallus*, *Colocasia*, *Syngonium, Xanthosoma*, and *Zantedeschia* (Table 4.2). *Aglaonema* is one of the most popular ornamental foliage plant genera.

Table 4.2 Aroid plants micropropagated through in vitro shoot culture using TDZ as a cytokinin in the culture medium

Scientific name	Explant	Protocol	References
Acorus calamus, Acorus gramineus	Seedling	MS + 4.54 μM TDZ	Lee and Han (2011)
Aglaonema var. Cochin	Shoot	MS + 6.81 μM TDZ or MS + 6.81 μM TDZ + 13.32 μM BA	Mariani et al. (2011)
Aglaonema 'Lady Valentine'	Node	MS + 9.08 μM TDZ + 2.69 μM NAA	Fang et al. (2013)
Aglaonema 'White Tip' and 'Emerald Beauty'	Inflorescence	MS + 10 μM TDZ + 5–10 μM Dicamba	Yeh et al. (2007)
Alocasia amazonica	Corm	MS + 2.27 μM TDZ	Jo et al. (2008)
Alocasia cadieri	Shoot tip	MS + 2.27 μM TDZ+ 2.69 μM NAA	Han et al. (2004)
Amorphophallus muelleri	Shoot	MS + 0.91 μM TDZ + 2.22 μM BA	Imelda et al. (2007)
Colocasia esculenta	Meristem	Modified MS + 4.54 μM TDZ or MS + 0.45 μM TDZ + 13.32 μM BA	Chand et al. (1999)
Colocasia esculenta	Shoot or node	MS + 4.09 μM TDZ + 57.08 μM IAA	Seetohul et al. (2009)
Colocasia esculenta	Shoot tip	MS + 4.09 μM TDZ + 8.88 μM BA	Seetohul et al. (2007)
Syngonium podophyllum	Node	MS + 0.90 μM TDZ + 4.44 μM BA	Kalimuthu and Prabakaran (2014)
Xanthosoma sagittifolium	Shoot tip	Modified B5 + 2 μM TDZ + 0.05 μM NAA + 20 μM BA	Sama et al. (2012)
Xanthosoma sagittifolium	Shoot tip, corm	Modified B5 + 2 μM TDZ + 0.05 μM NAA	Sama et al. (2015)
Zantedeschia albomaculata	Shoot tip, tuber eye	MS + 4.54 μM TDZ	Chang et al. (2003)

TDZ at low concentrations (4 μM or lower) induced more axillary shoots of *Aglaonema* 'White Tip' than BA at concentrations lower than 10 μM (Chen and Yeh 2007). The genus *Acorus* is a perennial hydrophyte used as a medicinal and aromatic plant. In vitro shoot culture of two species showed that 17.8 μM BA induced 5.4 axillary shoots per explants, whereas 4.5 μM TDZ induced 11.0 shoots for *A. calamus*. The same concentrations of BA and TDZ produced 2.7 and 3.9 shoots, respectively, for *A. gramineus* (Lee and Han 2011). *Colocasia esculenta*, commonly known as taro, is an important edible crop throughout the Pacific Islands (Chand et al. 1999). Meristems of six cultivars were cultured on a modified MS medium supplemented with TDZ. In experiments with the cultivar Niue, explants cultured on the modified MS medium with 2.6 μM TDZ grew more vigorously than on the medium including BA. Subculture of explants on medium containing 4.3 μM TDZ gave a 15–25-fold increase in production of plantlets per 4-week culture period compared to a fourfold increase with BA (Chand et al. 1999). TDZ also significantly increased the shoot proliferation rate in *Alocasia amazonica* (Jo et al. 2008).

An important characteristic in shoot culture of aroids is that TDZ concentration should be lower than other commonly used cytokinins such as BA and 2iP. High TDZ concentration could either reduce multiplication rates or cause phytotoxicity. For example, TDZ at high concentrations were shown to inhibit shoot proliferation in *Spathiphyllum cannifolium* (Dewir et al. 2006). Higher concentrations of TDZ (4 or 20 μM) inhibited shoot elongation of *Aglaonema* (Chen and Yeh 2007). This phenomenon could be attributed to the following reasons: (1) TDZ is a more effective growth regulator than the commonly used other cytokinins (Preece et al. 1991). It has been shown that exposure of plant tissue to TDZ for a relatively short period is sufficient to stimulate regeneration (Visser et al. 1992; Hutchinson and Saxena 1996). Thus, a low concentration should be used for axillary shoot induction. (2) TDZ is rather stable in culture medium (Murthy et al. 1998). Radiolabeled TDZ in tissue culture remains structurally intact for up to 48 h, suggesting that the intact structure is important for its activity (Mok et al. 1982; Mok and Mok 1985). Furthermore, its activity could be the induction of cascade reactions in plant cells (Kou et al. 2016; Guo et al. 2017) as TDZ action could be retained even after transfer to fresh basal medium (Capelle et al. 1983).

4.4 TDZ in Shoot Organogenesis

TDZ has been used as a cytokinin for regeneration of plants from 11 aroid genera (Table 4.3). Explants used for the regeneration include anther, corm, leaves, petioles, spathe, and stems. Adventitious shoots are predominantly produced through callus phase, i.e., indirect shoot organogenesis. There is only one report of direct shoot organogenesis, in which adventitious shoots of a *Dieffenbachia* cultivar were induced directly from petiole explants cultured on MS medium. About 15.4% of petioles cultured with TDZ at 4.5 μM with 5.4 μM NAA produced buds compared to 10.2% of petioles cultured with 4.4 μM BA with 4.5 μM 2, 4-D (2, 4-dichlorophenoxyacetic acid) (Orlikowska et al. 1995). This study, however, did not provide any details about the claimed direct shoot organogenesis.

Table 4.3 Aroid plants regenerated through indirect organogenesis with TDZ as cytokinin in the culture medium

Scientific name	Explant	Protocol	References
Aglaonema 'Lady Valentine'	Node	MS + 9.08 μM TDZ + 2.69 μM NAA	Fang et al. (2013)
Aglaonema	Inflorescence	MS + 10 μM TDZ + 5–10 μM Dicamba	Yeh et al. (2007)
Anthurium andraeanum	Leaf	Modified MS + 1.82 μM TDZ	Gu et al. (2012)
Anthurium andraeanum	Callus	Modified MS + 0.05 μM TDZ	Kumari et al. (2011)
Anthurium spp.	Leaf	Modified MS + 2.27 μM TDZ + 2.69 μM NAA	Orlikowska and Zawadzka (2010)
Anthurium andraeanum	Node	Modified MS + 0.92 μM TDZ	Bhattacharya et al. (2015)
Anthurium andraeanum	Anther	WRM + 4.54 μM TDZ + 3.33 μM BA	Winarto et al. (2010)
Anthurium andraeanum	Half-anthers	WT-1 + 2.27 μM TDZ + 0.05 μM NAA + 4.44 μM BA	Winarto et al. (2011b)
Anthurium andraeanum	Half-anthers	NWT-3 + 6.81 μM TDZ + 0.11 μM NAA + 3.33 μM BA	Winarto and da Silva (2012)
Anthurium andraeanum	Callus	NWT + 6.81 μM TDZ + 1.13 μM 2,4-D + 0.11 μM NAA + 3.33 μM BA	Winarto et al. (2011a)
Anthurium andraeanum	Shoot	MS + 4.54 μM TDZ or 44.39 μM BA	Han and Goo (2003)
Colocasia esculenta	Corm	½ MS + 4.54 μM TDZ	Deo et al. (2009)
Colocasia esculenta	Shoot	First step: MS + 2 μM TDZ + 10 μM 2, 4-D	Verma and Cho (2007)
	Callus	Second step: MS + 5 μM TDZ	
Colocasia esculenta	Shoot tip	½ MS + 4.54 μM TDZ	Du et al. (2006)
Colocasia esculenta	Corm	First step: ½ MS + 12.67 μM 2, 4-D	Deo et al. (2010)
		Second step: ½ MS + 4.54 μM TDZ	
	Callus	MS + 4.54 μM TDZ + 2.26 μM 2,4-D	
	Callus	MS + 4.54 μM TDZ + 2.26 μM 2,4-D	
	Suspension cells	MS + 0.45 μM TDZ + 0.23 μM 2, 4-D	
Colocasia esculenta	Embryogenic callus	MS + 0.45 μM TDZ + 0.23 μM 2.4 D	Fitriani et al. (2016)
Dieffenbachia spp.	Petiole	MS + 4.54 μM TDZ + 5.37 μM NAA + 4.44 μM BAP + 4.52 μM 2, 4 D	Orlikowska et al. (1995)

(continued)

Table 4.3 (continued)

Scientific name	Explant	Protocol	References
Dieffenbachia cv. Camouflage	Leaf	Ms + 5 μM TDZ + 1 μM 2, 4-D	Shen et al. (2007a)
Dieffenbachia	Leaf	Ms + 5 μM TDZ + 1 μM 2, 4-D	Shen et al. (2007b)
Dieffenbachia spp.	Leaf	Modified MS + 5 μM TDZ + 1 μM 2, 4-D	Shen et al. (2008)
Epipremnum aureum	Leaf	MS + 5 μM TDZ + 0.5 μM NAA	Qu et al. (2002)
	Petiole	MS + 10 μM TDZ + 0.5 μM NAA	
Lemna gibba	Callus	B5 + 4.54 μM TDZ + 0.1% sucrose	Li et al. (2004)
Philodendron spp.	Stem	MS + 2.27 μM TDZ	Chen et al. (2012)
Spathiphyllum wallisii	Petiole	MS + 4.54 μM TDZ + 0.90 μM 2,4 D	Lakshmanan et al. (2011)
		MS + 9.08 μM TDZ + 4.52 μM 2,4 D	
Spathiphyllum	Petiole	MS + 4.54 μM TDZ	Orlikowska et al. (1995)
	Pith	MS + 2.27 μM TDZ	
Spirodela oligorrhiza	Callus	WP + 4.54 μM TDZ	Li et al. (2004)
Spirodela punctata	Callus	WP + 2.27 μM TDZ + 4.52 μM 2,4-D + 26.85 μM NAA	Li et al. (2004)
Syngonium podophyllum	Petiole	MS + 9.08 μM TDZ + 2.26 μM 2,4-D	Cui et al. (2008)
Xanthosoma sagittifolium	Shoot tip, petiole	B5 + MS + 0.045 μM TDZ + 13.5 μM Dicamba	Nyochembeng and Garton (1998)

Indirect shoot organogenesis has been shown to be an effective way of producing plantlets. Nyochembeng and Garton (1998) reported that addition of TDZ in a culture medium supplemented with dicamba (3,6-dichloro-2-methoxybenzoic acid) enhanced callus production from petioles of *X. sagittifolium*, but subsequent adventitious shoot regeneration occurred when the callus was cultured with dicamba alone, 2,4-D with kinetin, or dicamba with kinetin. The first detailed report on TDZ-mediated indirect shoot organogenesis came from a study from Qu et al. (2002) where adventitious shoots were regenerated from leaf and petiole explants of pothos (*E. aureum* 'Jade'). Among TDZ, 2iP, and zeatin [6-(4-Hydroxy-3-methylbut-2-enylamino) purine] tested, TDZ was the best cytokinin for pothos regeneration. Regeneration frequencies were 18% and 50% for leaf and petiole explants, respectively, on medium containing TDZ and NAA (1-naphthalene acetic acid) after only 30 days of culture, and responding explants regardless of leaf or petiole explants produced approximately 30 adventitious shoots. Regeneration on medium containing either 2iP or zeatin with NAA produced a maximum of four shoots during a 50-day culture period. The methods of TDZ-mediated shoot

organogenesis and plant regeneration were then modified and used for regeneration of *Aglaonema*, *Alocasia*, *Anthurium*, *Colocasia*, *Dieffenbachia*, *Lemna*, *Philodendron*, *Spathiphyllum*, *Spirodela*, and *Syngonium* (Table 4.3).

TDZ-mediated indirect shoot organogenesis in aroid plants exhibits the following characteristics: (1) Calluses induced are usually nodular-like (Shen et al. 2007a, 2008), which are different from friable calluses. (2) TDZ is more effective in induction of callus formation than other cytokinins. In a study of *Dieffenbachia*, BA, N-(2-chloro-4- pyridyl)-N-phenylurea (CPPU), kinetin, dicamba, 4-amino-3,5,6-trichloro-2pyridinecarboxylic acid (picloram), and TDZ in combination with either 2,4-D or NAA were used for callus induction. TDZ with 2,4-D induced up to 96% of leaf explants to produce callus, while other combinations failed to produce calluses (Shen et al. 2007a). (3) TDZ alone can induce callus formation. Gu et al. (2012) reported TDZ alone induced callus formation in *Anthurium*. This could be attributed to the fact that TDZ could elicit both auxin and cytokinin responses as documented by Murthy et al. (1995), and also TDZ is a highly stable cytokinin and is resistant to degradation by cytokinin oxidase (Mok et al. 1987). (4) Medium with TDZ usually shortens the time for callus induction and adventitious regeneration such as the aforementioned pothos (Qu et al. 2002). (5) Genotypes vary in response to TDZ induction. TDZ in combination with 2,4-D was found to be an effective combination for *Dieffenbachia* shoot organogenesis; callus formation frequency and shoot numbers per callus were 96% and 6.7 for cultivar Camouflage, but these frequencies were 62% and 4.4 for 'Camille,' 66% and 0 for 'Octopus,' and 52% and 3.5 for 'Star Bright' (Shen et al. 2008). (6) Somaclonal variation could occur in regenerated populations. In general, more somaclonal variants are observed in plants regenerated via callus phase (Larkin and Scowcroft 1981; Chen et al. 2003). Three somaclonal variants were identified from regenerated populations of *Dieffenbachia* 'Camouflage' and one from *Dieffenbachia* 'Star Bright' (Shen et al. 2007b).

4.5 TDZ in Somatic Embryogenesis

Micropropagation through somatic embryogenesis has advantages over both shoot culture and organogenesis because a large number of plantlets can be produced and it can potentially scale up propagation for bioreactors and produce synthetic seeds (Rani and Raina 2000). Somatic embryos are also desirable materials for genetic transformation and cryopreservation.

Anthurium andraeanum is the first aroid species that was regenerated through somatic embryogenesis (Kuehnle et al. 1992). Leaf explants were cultured on half-strength MS medium supplemented with kinetin and 2,4-D, and embryo conversion occurred and plantlets were produced. Subsequently, plants were also regenerated from other *Anthurium* via somatic embryogenesis when culture medium was supplemented with kinetin and 2,4-D (Matsumoto et al. 1996). The use of 2,4-D alone or in combination with other growth regulators was a standard practice for inducing somatic embryos from the 1960s to the 1990s (Raghavan 2004). This is attributed to

the work of Halperin and Wetherell (1964) who demonstrated that a callus induced from any vegetative part of carrot (*Daucus carota*) cultured on a medium containing a high concentration of 2,4-D could form somatic embryos upon transfer to the medium with a reduced level of the auxin (Raghavan 2004).

With the recognition that TDZ in combination with 2, 4-D was able to induce somatic embryogenesis in the 1990s, such as white ash (*Fraxinus americana* L.) (Bates et al. 1992) and watermelon (*Citrullus lanatus*) (Compton and Gray 1993), TDZ was introduced for inducing somatic embryogenesis in aroids. Somatic embryos were induced from *S. wallisii* Regel 'Speedy' when anther filaments were cultured on a modified basal medium supplemented with TDZ with 2,4-D (Eeckhaut et al. 2004). This protocol (culture media supplemented with TDZ and 2,4-D) has been successfully used for inducing somatic embryogenesis of *C. esculenta* (Deo et al. 2009; Verma and Cho 2007), *Dieffenbachia* (Shen and Lee 2009), and *Spathiphyllum* 'Supreme' (Zhao et al. 2012a) (Table 4.4).

The protocol making 2,4-D a necessary requirement for somatic embryo induction was changed in aroid plants when Werbrouck et al. (2000) reported that somatic embryos could be induced from anther filaments of *Spathiphyllum* cultured on a modified basal medium supplemented with TDZ and NAA. This TDZ and NAA combination was further refined by Zhang et al. (2005) in regeneration of *E. aureum* 'Golden Pothos.' Somatic embryos were directly induced from leaf, petiole, and stem explants cultured on MS or MK [MS ingredients in combination with Kao medium (Kao 1977) vitamins] medium supplemented with TDZ and NAA (Fig. 4.1). The frequencies of explants with embryos and explants with embryo conversion were as high as 61%, 89%, and 86% for leaf, petiole, and stem explants. The success of the protocols (culture media supplemented with TDZ with NAA) developed by Zhang et al. (2005) in 'Golden Pothos' has been modified and used for somatic embryogenesis and plant regeneration of *Syngonium podophyllum* (Zhang et al. 2006), *Zantedeschia* (Duquenne et al. 2006), and *Epipremnum aureum* 'Marble Queen' (Zhao et al. 2012b) (Table 4.4).

TDZ alone can induce somatic embryogenesis in some aroid plants such as *Colocasia* (Verma and Cho 2007). TDZ often induces direct somatic embryogenesis in aroids including *Epipremnum* (Zhang et al. 2005; Zhao et al. 2012b), *Spathiphyllum* (Eeckhaut et al. 2004; Zhao et al. 2012a), and *Syngonium* (Zhang et al. 2006). Plant species and explant types affect TDZ-mediated somatic embryogenesis. The genotype had no obvious effect on somatic embryogenesis of *C. esculenta* (Deo et al. 2009). However, the most effective combination for inducing somatic embryogenesis from leaf explants of *E. aureum* 'Marble Queen' was 4.54 μM TDZ with 1.07 μM NAA, whereas for petiole explants, it was 9.08 μM TDZ with 1.07 μM NAA (Zhao et al. 2012b). Furthermore, six times more plantlets were regenerated from petiole explants than those of leaf explants in 'Marble Queen' (Zhao et al. 2012b). Somatic embryogenesis often requires TDZ at relatively higher concentrations than those used for organogenesis. For example, somatic embryogenesis of *Epipremnum* required TDZ concentrations at 4.5 μM or higher (Zhang et al. 2005; Zhao et al. 2012b) and above 9.0 μM for *S. podophyllum* 'Variegatum' (Zhang et al. 2006).

Table 4.4 Aroid plants regenerated through somatic embryogenesis with TDZ as cytokinin in the culture medium

Scientific name	Explant	Protocol	References
Colocasia esculenta	Callus	MS + 5 μM TDZ	Verma and Cho (2007)
Colocasia esculenta	Suspension cells	MS + 0.45 μM TDZ + 0.23 μM 2,4-D	Deo et al. (2010)
Dieffenbachia 'Tiki'	Male inflorescence	½ MS + 2.27 or 4.54 μM TDZ + 18.09 μM 2,4-D	Shen and Lee (2009)
Epipremnum aureum	Leaf	MS + 4.54 μM TDZ + 1.07 μM NAA	Zhao et al. (2012b)
	Petiole	MS + 9.08 μM TDZ + 1.07 μM NAA	
Epipremnum aureum	Leaf, petiole	MS + 9.10 μM TDZ + 1.10 μM NAA	Zhao et al. (2013)
Epipremnum aureum	Petiole	MS + 11.35 μM TDZ + 2.69 μM NAA	Zhang et al. (2005)
	Stem	MS + 11.35 μM TDZ + 2.69 μM NAA	
Epipremnum aureum	Petiole	MS + 11.35 μM TDZ + 2.69 μM NAA	Wang et al. (2007)
Spathiphyllum wallisii	Anther filament	BMS + 2.5 μM TDZ + 10 μM 2,4-D	Werbrouck et al. (2000)
Syngonium podophyllum	Petiole	MS + 11.35 μM TDZ + 2.69 μM NAA	Zhang et al. (2006)
Spathiphyllum 'Supreme'	Leaf	MS + 9.08 μM TDZ + 2.26 μM 2,4-D	Zhao et al. (2012a)
	Petiole	MS + 4.54 μM TDZ + 2.26 μM 2,4-D	
Spathiphyllum wallisii	Ovules	BM + 4 μM TDZ + 15 μM IMA	Eeckhaut et al. (2001)
Syngonium podophyllum	Petiole	MS + 11.35 μM TDZ + 2.69 μM NAA	Wang et al. (2007)
Zantedeschia hybrids	Anthers	MS + 0.22 μM TDZ + 10.74 μM NAA	Duquenne et al. (2006)

Somatic embryogenesis, particularly direct somatic embryogenesis, has a low frequency of chimeras and a low probability of somaclonal variation. DNA flow cytometry analysis of randomly selected plantlets of 'Marble Queen' regenerated via direct somatic embryogenesis showed a single peak, indicating there were no mixoploids among the regenerated plantlets (Zhao et al. 2012a, b). Histological analysis of somatic embryos derived from ornamental aroids was also reported (Matsumoto et al. 1996; Hamidah et al. 1997; Zhao et al. 2012a). Longitudinal sections of a fully mature *Anthurium* somatic embryo showed clear bipolarity, with both shoot and root poles, as well as a continuous procambium and an epidermis (Matsumoto et al. 1996). Observation by Matsumoto et al. (1996) showed that somatic embryos of *A. andraeanum* originate within the mesophyll via direct embryogenesis.

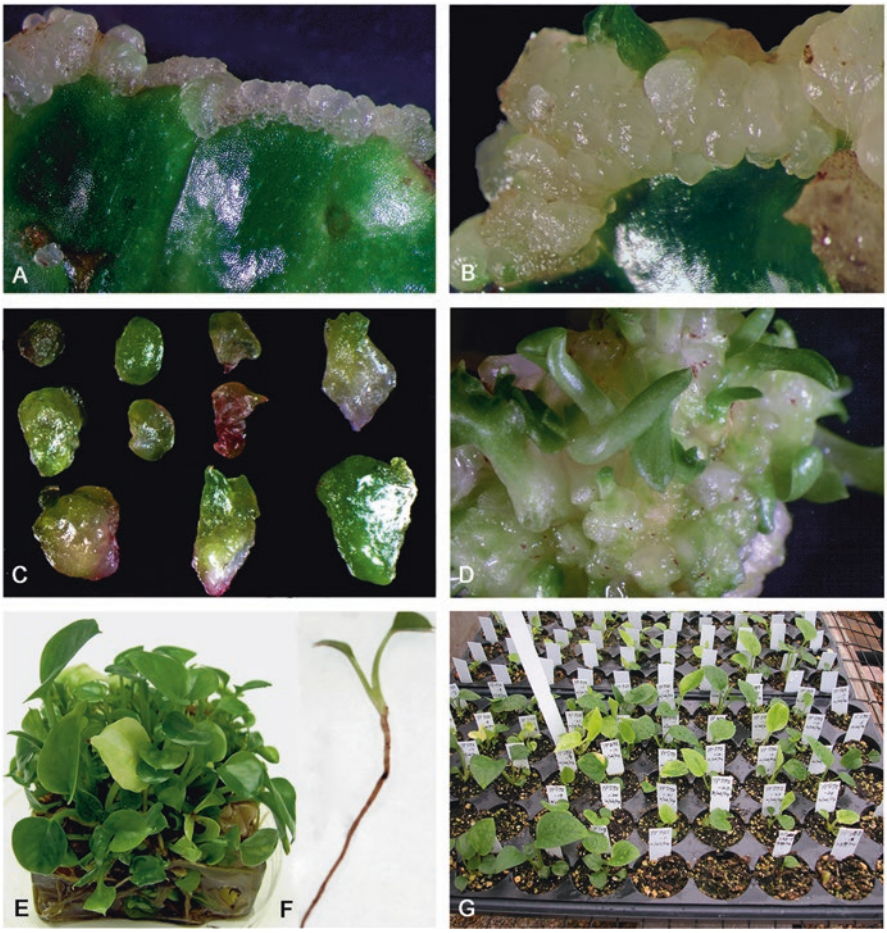

Fig. 4.1 Regeneration of *Epipremnum aureum* 'Golden Pothos' from leaf explants through direct somatic embryogenesis. Somatic embryos directly appeared from cut end of a leaf explants cultured on Murashige Skoog (MS) medium supplemented with 11.35 μM TDZ with 2.69 μM NAA (**a**). Embryos developed or produced secondary embryos and appeared in clusters (**b**). Embryos were well developed structures and easy to separate; an assortment of embryos including globular, scutellum, and torpedo stages are presented (**c**). Somatic embryos were able to convert to shoots (**d**) and produced roots (**e**), which looked like seedlings (**f**). Plantlets or seedlings were transplanted singly into cell-plug trays and grown healthily in a shaded greenhouse (**g**)

4.6 TDZ-Induced Regeneration Through PLBs

TDZ has been shown to induce PLBs in some aroid plants (Table 4.5). PLBs are composed of many meristematic centers that are able to differentiate into shoots and roots (Da Silva et al. 2000). Cui et al. (2008) documented that PLBs were formed from nodal explants of *S. podophyllum* 'White Butterfly' cultured on MS medium

Table 4.5 Aroid plants regenerated through protocorm-like bodies with TDZ as cytokinin in the culture medium

Scientific name	Explant	Protocol	References
Anthurium andraeanum cv.	Shoot tip-ends	MS + 5.0 μM TDZ	Gantait et al. (2012)
Syngonium podophyllum	Node	MS + 9.08 μM TDZ + 1.07 μM NAA	Cui et al. (2008)

supplemented with TDZ and 2,4-D. Adventitious shoots were formed from PLBs and roots formed thereafter. A popular opinion about PLBs in orchid propagation is that they are somatic embryos. However, PLBs are distinguished from somatic embryos by the lack of a single embryonic axis (Norstog 1979). A recent molecular analysis of PLBs in *Phalaenopsis aphrodite* indicated that PLBs do not follow the embryogenesis pattern (Fang et al. 2016). Instead, the authors proposed that *SHOOT MERISTEMLESS*, a class I KNOTTED-LIKE HOMEOBOX gene, is likely to play a role in PLB regeneration. Thus, PLBs differ from somatic embryos. An advantage for propagation through PLBs is that a large number of plantlets (shoots with roots) can be regenerated thus enhancing propagation efficiency.

4.7 TDZ Action in Micropropagation

The effectiveness of TDZ in plant micropropagation has been attributed to its high level of activity and stability in culture media. However, the mode of action of TDZ-mediated micropropagation is still unclear. TDZ was considered as a cytokinin for its induction of natural cytokinin-like responses. Increases in endogenous auxin, ethylene, and abscisic acid (ABA) in peanut were found to be related to TDZ treatment (Murthy et al. 1995; Murch and Saxena 1997). Some evidence suggests that the action mechanism of TDZ could be closely associated with the biosynthesis and transportation of indole-3-acetic acid (IAA) (Chhabra et al. 2008). Guo et al. (2017) proposed that a combination of increased GA_3, zeatin, and H_2O_2 concentration is the basis for enhanced shoot morphogenesis in response to TDZ treatment. In a study of TDZ-mediated regeneration of rose (*Rosa canina* L.), TDZ administration affected the level of endogenous auxins and cytokinins, converted the cell fate of rhizoid tips, and triggered PLB formation and plantlet regeneration (Kou et al. 2016). Nevertheless, molecular mechanisms concerning TDZ-mediated morphogenesis are largely unknown. However, the established shoot culture and regeneration methods through shoot organogenesis, somatic embryogenesis, and PLBs in aroid plants could be valuable systems for further dissecting the molecular basis underlying shoot culture and each of the regeneration pathways.

4.8 Conclusion

Aroids are economically and environmentally high value crops. Commercial production of this group of crops was traditionally limited due to the lack of healthy starting materials. It was the application of micropropagation techniques that lead to increased commercial availability and production of healthy and disease-free propagules year-round. Aroid plants such as *Amorphophallus rivieri* were among the first reports of plants successfully micropropagated (Morel and Wetmore 1951). Since the discovery of TDZ as an effective plant growth regulator, TDZ has been used for in vitro shoot culture and for regeneration of aroid plants through shoot organogenesis, somatic embryogenesis, and PLBs. Millions of plantlets from *Alocasia*, *Aglaonema*, *Anthurium*, *Dieffenbachia*, *Homalomena*, *Philodendron*, *Spathiphyllum*, and *Syngonium* have been produced. Through our continued research on TDZ-based aroid micropropagation, more aroid plants will be in vitro cultured, and more protocols will be developed. With the advance of omics technologies, in combination with the developed protocols, the molecular basis for TDZ-mediated regeneration will be uncovered in the near future.

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