ISSN 2226-3063 e-ISSN 2227-9555
Modern Phytomorphology **10**: 3–10, 2016

CYCLAMEN CAULOGENESIS, RHIZOGENESIS AND MICROTUBERIZATION

Jaime A. Teixeira da Silva

Abstract. The tissue culture of *Cyclamen persicum* Mill. of the Primulaceae family, and a popular ornamental pot plant, is well explored. This synopsis review provides an overview of the induction of shoots (caulogenesis), roots (rhizogenesis) and microtubers (microtuberization) *in vitro* as viable routes for the tissue culture of clonal material, primarily from the English literature.

Key words: *Cyclamen*, biotechnology, media, microtuber, plant growth regulator, tissue culture

P.O. Box 7, Miki-cho post office, Ikenobe 3011-2, Kagawa-ken, 761-0799, Japan; jaimetex@yahoo.com

Abbreviations

2,4-D – 2,4-diclorophenoxyacetic acid
2iP – N^6-(2-isopentenyl) adenine
AdS – adenine sulphate
BA – 6-benzyladenine
IAA – indole-3-acetic acid
Kin – kinetin
MS – Murashige and Skoog medium
NAA – α-naphthaleneacetic acid
N-69 – Nitsch & Nitsch basal medium
PGR – plant growth regulator
SIM – shoot induction media
TDZ – thidiazuron
TCLs – thin cell layers

Introduction

The tissue culture of members of the genus *Cyclamen* L. (Primulaceae), geophytes (perennial, herbaceous, with a over- or underground storage organs) that are widespread in countries surrounding the Mediterranean, is well explored (Jalali *et al.* 2012). This mini-review aims to look at three *in vitro* processes related to the tissue culture of *Cyclamen* species, focusing primarily on *C. persicum* Mill., or Persian cyclamen: shoot induction (caulogenesis), root induction (rhizogenesis), and microtuber formation (microtuberization). These processes are examined in detail, but explored from a historical perspective. Callogenesis and somatic embryogenesis, which are closely related, but which were recently covered elsewhere (Tagipur *et al.* 2016), will not be discussed in this manuscript.

The first two decades (1950s – 1970s)

The earliest known study on the *in vitro* culture of *C. persicum* was published by Mayer-Hörster (1956) in which cubes from tubers (but referred to incorrectly as corms) including cambial tissue were induced to form callus in the presence of 0.08 mg/L α-naphthaleneacetic acid (NAA) or 40 mg/L adenine sulphate (AdS) and 0.2 mg/L NAA on White's (1963) basal medium or only roots in the presence of 1 mg/L NAA. Stichel (1959) capitalized upon those initial findings, showing how only NAA up to 0.3 mg/L could induce shoots while an initial pulse of 0.3-0.5 mg/L NAA, followed by transfer onto medium with AdS induced both adventitious shoots and roots. Loewenberg (1969) also used tubers to prepare 4 mm^3 explants after surface disinfecting whole tubers in 0.03% mercuric chloride ($HgCl_2$) overnight, rinsing in 1% sodium hypochlorite (NaOCl) and then trimming away the outer layers. Using modified White's medium with 10 mg/L NAA, Loewenberg induced callus in the dark at 25 °C although indole-3-acetic acid (IAA) or 2,4-dichlorophenoxyacetic acid (2,4-D) could also induce callus. Tuber explants formed only

a very small amount of callus on medium free of plant growth regulators (PGRs). Loewenberg (1969) also found that White's medium was more conducive to callus induction than Heller (Gautheret 1954), Miller (1961), Nitsch (1969), and Murashige & Skoog (1962) basal media. The 1969 Loewenberg *in vitro* trials also revealed that 6% sucrose produced more callus than 0.7% or 2% sucrose, and that sucrose induced more callus than fructose, galactose, glucose, maltose, glycerol or mannitol as a carbon source. Finally, Loewenberg showed that while AdS stimulated callus, asparagine and glutamic acid inhibited its formation. Curiously, in the same year, but on the other side of the world, Okumoto & Takabayashi (1969) surface disinfected two-year old tubers that had just completed flowering by rinsing them in lukewarm water, dipping them in a solution of 14% NaOCl for 30 min, repeating the entire process, peeling away outer layers, and using explants 10 mm in diameter and 10 mm long from the core of surface disinfected tubers to induce root or shoots in the presence of 1 or 0.1 mg/L NAA, respectively when cultured in MS basal medium at 20 °C/10 °C (day/night). At this temperature, contamination and explant necrosis were reduced by about 10% relative to untreated (i.e., not surface disinfected) controls. Shoot buds could also be induced to form from tuber tissue when 1 mg/L NAA was combined with 20 or 100 mg/L AdS. As many as 30 shoot buds/explant could be induced. The surface disinfection process negatively affected organ formation (Okumoto & Takabayashi 1969; Pierik 1975). Even the use of achromycin, an antibiotic, by Stichel (1959) was able to control bacterial infection. Geier (1977), however, controlled 75% or 100% contamination when 50 and 100 mg/L achromycin were used in medium. Prior to that, Geier surface disinfected tubers, leaves, petioles and closed flower buds from 12-18 month old plants by immersing them in 3% NaOCl for 5-10 min, rinsing three times with sterile water, peeling outer tissues then leaving explants to soak in 0.03% $HgCl_2$ overnight, as suggested by Loewenberg (1969). After three rinses with sterile water, tubers were cut into 8 mm^3 explants, anthers were excised from closed buds and plated intact, 40 mm^2 (8 × 5 mm) leaf explants with midrib tissue were placed abaxial side down on medium while 8 mm long petioles were also plated on Nitsch basal medium. This protocol resulted in 100% contamination-free cultures. Although all cultures were initially placed in the dark at 24 °C, when roots or shoots formed, cultures were placed in a 15-h photoperiod at 10000-15000 lux. When tubers served as the explant, NAA at 0.5 or 2.5 mg/L with 0.5 or 1 mg/L kinetin (Kin) formed primarily callus, IAA at 0.1 or 0.5 mg/L with 0.5 mg/L Kin formed shoot buds, or shoot buds and roots when 2.5 mg/L IAA and 0.5 or 1 mg/L Kin were applied, while 0.1 mg/L 2,4-D with 0.5 mg/L Kin induced shoot buds. In several of these cases, organogenesis was possible in 100% of explants. Leaves, petioles and anthers were not as responsive as tuber tissue, although shoots could be induced from anthers with 1-5 mg/L IAA and 1-2.5 mg/L 6-benzyladenine (BA; see notes in Teixeira da Silva 2012) in 25% of explants, but also formed petaloid organs and anther-like structures. In the same study, callus could be induced from somatic anther tissue by 1 mg/L each of NAA and Kin, while organogenesis from leaves was always indirect, i.e., via intermediate callus formation. Although Geier (1977) claimed to test three varieties, he did not describe the response of each variety *in vitro*.

The first two decades thus showed that *in vitro* cultures could be established, albeit with difficulties in the control of infection, and that primarily corm tissue was responsive *in vitro*, when placed in suitable basal medium with carefully selected PGRs.

The 1980s

Many important basic findings of the response of different explants to *in vitro* culture were examined in the first three decades of *Cyclamen* tissue culture, focusing primarily on disinfection and explant responses to PGRs, but the 1980s and 1990s saw a few additional new ideas and progress in this field. Most likely the most obvious one was the discovery

by Wicart *et al.* (1984), following a detailed histological analysis, that microtubers do not share any vascular continuity with the explant tissue, similar to somatic embryos (i.e., bipolar structures), and unlike shoot buds or adventitious roots (unipolar structures). Given the variable terminology that has emerged to describe different structures that have formed from *Cyclamen* explants *in vitro*, histological verification in *in vitro* studies is essential. Ando & Murasaki (1983) induced shoots of cv. 'Vuurbaak' from ~75% of etiolated petioles (distal half of 10-cm long petioles) in the dark on third-strength MS medium (⅓ MS) containing 500 μM adenine hydrochloride and 10 μM each of NAA and BA although the number of shoots formed per explant was not quantified. Despite the use of a simple explant disinfection procedure that involved rinsing in running tap water and dipping in 1% NaOCl for 15 min, the authors reported 0% contamination unlike 12.5% and 83% contamination in normal petioles and tubers, respectively. Expanding on that earlier protocol, Murasaki & Tsurushima (1988) transferred shoots that formed from etiolated petioles on ⅓ MS with 0.037 mg/L NAA and 0.23 mg/L BA to a 16-h photoperiod at 2500 lux. Cv. 'Vuurbaak' was more receptive than cv. 'Victoria', with 74% vs 63% of explants forming shoots, respectively. Roots formed on 70% of explants and 95% of resulting plantlets that were transferred to the greenhouse survived. Fukui *et al.* (1988), without providing any surface disinfection protocol, and also using cv. 'Vuurbaak', induced most and largest microtubers (which the authors referred to as tuber-like-organs) on ½ MS medium supplemented with 10 μM NAA or BA from shoot tips, pedicels and apical tuber sections, the latter referred to incorrectly as bulbs, although the range of 0.1 to 1 μM of either NAA or BA also resulted in the formation of microtubers; even the control treatment, i.e., the lack of any PGRs, resulted in the formation of microtubers. However, the authors also claimed the formation of callus and somatic embryos at the same concentrations and from the same explants, but did not provide any histological proof. Schwenkel & Grunewaldt (1988) surface disinfected leaves, petioles and peduncles by dipping them for 1 min in ethanol, 20 min in 2.6% NaOCl, and then washed them with autoclaved tap water. Cutting leaves into 8 mm^2 explants and petioles and peduncles into 6 mm long explants, the authors claimed the formation of shoots and callus from peduncles, and even the formation of plantlets, but many aspects of the study were unclear, and high levels of contamination (as much as 50% of explants) occurred. Using the Wainwright & Harwood (1985) protocol for establishing tissue culture from aseptic seedlings, Hawkes & Wainwright (1987) induced more adventitious leaves from cotyledons and tubers than from petioles and root explants derived from aseptic cv. 'Rosamunde' seedlings (0.62, 0.53, 0.22, 0.16 leaves/explant for cotyledons, tubers, petioles and roots, respectively) when cultured in light on ½ MS medium supplemented with 1 mg/L BA and 1 mg/L NAA. Subculture of leaves on ½ MS medium with 1 or 2.5 mg/L BA could not induce adventitious shoots.

The 1990s

Ishizaka & Uematsu (1992) ushered in the 1990s with a study in which ovules derived from interspecific hybrids of *C. persicum* and *C. hederifolium* Aiton 30 days after pollination were cultured on either MS, White's or Nitsch (1951) basal media at 25°C in the light (16-h photoperiod, 3000 lux). In their study, ovaries were first disinfected with 70% ethanol for 60 s, 2% NaOCl for 30 min, then rinsed twice in sterilized water. After peeling off the ovary wall, the ovules with placenta attached served as the explants. Plantlets were obtained in 17.8% of excised ovules when 6% sucrose was used in MS medium although 3-10% sucrose also supported lower levels of plant regeneration (0.4-16.5%) while plantlets could not form on White's or Nitsch basal media containing any sucrose concentration, nor could the addition of coconut milk improve regeneration percentage. The technique of ovule culture by Ishizaka & Uematsu (1992) is very important because the authors showed that all embryos in the ovules had degenerated by 43 days after

pollination when diploid *C. persicum* was used in the cross (and by 35 days when tetraploid *C. persicum* was used), with 0% seed set, so ovule culture serves as a valuable method to rescue valuable hybrid *Cyclamen* material when hybridizations are unsuccessful. Takamura *et al.* (1993) established callus and shoot cultures from seedling tissues of an F_2 cross between cv. 'Pure White' and a yellow variety. Seeds were washed in 2% NaOCl for 10 min, rinsed three times with sterile distilled water (SDW), then plated on PGR-free ⅓ MS with 3% sucrose for 21 days in the dark and then a further 21 days in light (12-h photoperiod; 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$; Takamura & Miyajima 1997). Cotyledons, leaf petioles, tubers and roots were isolated from seedlings and plated on ⅓ MS with 1 μM BA and 0.1 μM NAA in the dark for 35 days and then in a 16-h photoperiod. At most, 70% of cotyledons formed shoots (3.8/explant) while 93.8% of petioles or tubers formed shoots (6.0 or 6.8/explant, respectively) but no photographic evidence of *in vitro* organogenesis was provided. Dillen *et al.* (1996) induced callus from young leaf explants after surface disinfecting 45 mm × 60 mm leaves in 70% ethanol (brief dip), 1.5% NaOCl for 15 min and four rinses in sterile water. Leaf explants (1 cm^2), when plated on MS basal medium with 3-4 mg/L BA, 3-4 mg/L Kin and 2 mg/L NAA, callus formed after 17 weeks in the dark. Callus was cut into 1 cm^3 pieces and subcultured three times on the same medium for 8, 9 and 10 weeks, then subcultured five times (6-7 weeks each subculture) on MS basal medium with 4 mg/L BA, 4 mg/L Kin and 3 mg/L NAA, forming etiolated shoots (12/explant). In Nitsch-based medium, 88% of shoots rooted after 4 weeks while only 53% of shoots rooted on MS medium in the light (5000-6000 lux), but the latter needed 6% sucrose. Dillen *et al.* (1996) then conducted a scaled-up trial, acclimatizing 1200 rooted shoots (i.e., plantlets) from 18 genotypes, showing 90% rooting and 78% acclimatization, although the substrate used was not defined. Also, in a bid to assess the cytological stability of regenerants after two-year callus culture, as much as 10-20% of plants in three genotypes showed variations in ploidy. Bach *et al.* (1998) were able to induce shoots directly from 35-38% of leaves and hypocotyls derived from aseptic seedlings of *C. persicum* F_1 cv. 'Medium' in the presence of 50 μM 2,4-D and 4 μM N^6-(2-isopentenyl) adenine (2iP) when placed on MS basal medium and maintained at 25 °C in the dark. When basal medium contained 2.5 μM NAA and 25 μM 2iP, 75% of leaf explants, 30% of hypocotyls and 45% of root explants formed shoots.

The 2000s

Karam & Al-Majathoub (2000a) regenerated shoots directly from the leaves of wild Jordanian *C. persicum in vitro* seedlings on ½ MS medium with 0.22 mg/L thidiazuron (TDZ) and 0.1 mg/L NAA in the dark. The authors also tested different explant sizes and locations of *in vitro*-derived leaves (wounded blades, blades with midrib, blades without midrib, central lamina, and petiolated lamina), noting that blades with a midrib showed the highest shoot induction ability (88% vs 81%, 44%, 34% and 0% from wounded blades, central lamina, petiolated lamina, and blades without midrib, respectively), forming a maximum of 2.9 shoots/explant. Also within the same study, the authors studied the influence of explant type (*in vitro* seedling-derived tuber, petiole, cotyledon, and root) on the outcome of microtuberization, noting that root explants showed highest tuberization (42% of explants) in response to 1 mg/L BA in ½ MS medium (relative to 4% in cotyledons and 0% tuberization from tubers and petioles). Optimal sucrose concentration was 3%, decreasing when 6, 9 or 12% were tested, forming microtubers in 100% of explants and 6 mictotubers/explant with roots in response to 3% sucrose, and 37.5% of explants and 1.63 mictotubers/explant when 6% sucrose was used). In a separate paper by the same authors (Karam & Al-Majathoub 2000b), the shoot inductive ability of other explants (leaf discs, petioles, petals, and peduncles) derived from wild *ex vitro* plants at the flowering stage was tested. Unlike their other study (Karam & Al-Majathou 2000a), here they used ⅓ MS with 3% sucrose and 0.1 mg/L NAA as the basal medium, noting that

peduncles formed most shoots (2.13 shoots/explant in 54% of explants) in this basal medium supplemented with 0.022 or 0.22 mg/L TDZ while petals formed most shoots (1.07 shoots/explant in 38% of explants) when 0.022 mg/L TDZ was applied; the values for petioles were 0.16 shoots/explant in 7% of explants while leaf discs did not form any shoots, in sharp contrast to their previose findings (Karam & Al-Majathoub 2000a) where *in vitro*-derived shoots from seedlings were found to be most productive. Callus formed in peduncles, petals and petioles at all concentrations of TDZ tested. A separate trial in that study showed that etiolated leaf petioles formed more shoots (0.86 shoots/explant in 32% of explants) than non-etiolated leaf petioles (0.16 shoots/explant in 7% of explants).

The explant disinfection protocols used in the Karam & Al-Majathoub (2000a, 2000b) studies deserve special mention due to their precision and detail. In Karam & Al-Majathoub (2000a), seeds were extracted from mature fruits, washed with water for 15 min, dipped in 70% ethanol for 1 min, added to 20% NaOCl for 20 min, then rinsed in SDW. Surface-disinfected seeds were plated in the dark at 15 °C until germination, and then transferred to light conditions (16-h photoperiod and 50-60 μmol · m^{-2} · s^{-1}) at 22 °C. In their other study (Karam & Al-Majathoub 2000b), they first washed explants in water for 10 min, dipped them in 70% ethanol for 30 s, added them to 4% NaOCl for 15 min, then rinsed them in SDW. All explants were placed in the dark at 22 °C. Unlike most *Cyclamen in vitro* studies from the 1950s – 1990s, these two studies did not (regrettably) report on the level of explant or culture contamination. Using the same surface disinfection procedure as Karam & Al-Majathoub (2000a), Mohannad & Karam (2000) tested the shoot induction ability of *in vitro* seedling-derived tubers, petioles, cotyledons, and roots in response to BA when placed on MS basal medium. Most shoots formed from tuber segments in response to 2 mg/L BA and 0.1 mg/L NAA (3.4 shoots/explant in 54% of explants) although both shoots and roots also formed from the other three explant types, but with lower shoot induction ability. Shoots were proliferated in the presence of 2 mg/L BA, forming 7.4 shoots/shoot, and individual shoots were rooted in 0.5 or 1 mg/L NAA in 80-100% of explants, although IBA was just as effective as NAA. Plantlets were acclimatized in a peat + perlite (1:1, v/v) substrate and no visible abnormalities were observed.

Abu-Qaoud (2004) tested the ability of tuber sections and petioles from *in vitro* cv. 'Concert' seedlings to form shoots. Seeds were initially surface disinfected in 5% NaOCl for 10 min, rinsed three times in SDW, then germinated on MS basal medium at 22-24 °C and cultured in a 16-h photoperiod at 50 μmol · m^{-2} · s^{-1}. While no shoots formed on petioles, 58.3% of tuber explants formed shoots (10.8/explant) in response to 5.4 μM NAA and 4 μM TDZ. Even though the author claimed successful acclimatization with 30% normal flowering, no visual or quantitative proof was provided.

Prange *et al.* (2008) were determined to induce shoots from *C. mirabile* Hildebrand, *C. coum* Miller, *C. graecum* Link and *C. hederifolium* from three explant types (cotyledons, tubers and roots) derived from aseptic seedlings using two shoot induction media (SIM): SIM I, with 0.5 mg/L NAA and 1 mg/L BA; SIM II, with 0.5 mg/L IAA, 1 mg/L BA, 1 mg/L 2iP and 1 mg/L Kin. Seed germination and shoot induction were conducted in the dark at 20 °C. After 8 weeks, *C. mirabile* tubers formed shoots in 22% of explants on SIM I, 20% of *C. coum* cotyledons in SIM II, 58% of *C. graecum* cotyledons on SIM I, and 43% of *C. hederifolium* cotyledons on SIM I. Values pertaining to the success of shoot induction differed considerably depending on whether explants were rated after 4, 8 or 16 weeks, an important issue in plant tissue culture (Teixeira da Silva & Dobránszki 2013a).

Yamaner & Erdag (2008) were able to induce shoots from *in vitro* tubers of *C. mirabile* Hildebr., an endemic Turkish species, on a wide range of basal media (Bourgin & Nitsch 1967, MS, ½ MS, Linsmaier & Skoog 1965, and Loewenberg 1969) containing

0.1 mg/L IAA or 0.5 mg/L NAA and 0.5 mg/L Kin. Initially, seeds from mature fruits were washed in running tap water for 1 h, dipped in 70% ethanol for 10 min, added to 4.5% NaOCl for 25 min, then rinsed in SDW 3-4 times for 10 min. Disinfected seeds were germinated in the dark at 15 °C on PGR-free ½ MS or MS medium with 3% sucrose, and once seedlings had germinated, these were transferred to light conditions (16-h photoperiod) at 24 °C. A maximum of 53.3% of seeds germinated on ½ MS medium after 10 weeks, while only 41.6% at most germinated on MS medium in the same period. Leaf lamina, petioles, roots, intact tubers and tuber segments were prepared from 8-week-old seedlings. Callus was induced from petioles in ½ MS medium with 2 mg/L NAA, but callus could not be induced to differentiate and no regeneration was possible from leaf or root explants. The most responsive explant were intact tubers, forming 12.2 shoots/explant and 6 microtubers/explant in the presence of 1 mg/L BA with 0.1 mg/L NAA and 2 mg/L BA with 0.1 mg/L NAA, respectively. No additional plantlet formation beyond microtuberization was reported.

Bürün & Şahin (2009) germinated *C. alpinum* Sprenger seeds *in vitro* that had been stored for 8 months by surface disinfection in 4.5% NaOCl for 10 min, 5-6 rinses with SDW, and then plating on PGR-free MS medium. Seeds stored in capsules at 15 °C or 20 °C showed a lower germination percentage than seeds that were first excised from capsules prior to storage (60% vs 70%, respectively), but *in vivo* germination in Petri dishes was superior to *in vitro* germination trials (92% from seeds stored in capsules, 54% of seeds excised from capsules prior to storage).

Seyring *et al.* (2009) attempted to induce shoots in several *Cyclamen* species (*C. africanum* Boiss. et Reut., *C. cilicium* Boiss. et Heldr., *C. coum* Mill., *C. hederifolium* Ait., *C. persicum* Mill., and *C. purpurascens* Mill.). In all cases, explants were derived from young leaves, petioles, flower buds and peduncles of adult greenhouse-derived plants. Leaves with petioles were disinfected for 3 min in 0.8% $AgNO_3$ (silver nitrate) and rinsed three times (5 min each time) in SDW while peduncles and flower buds were dipped for 30 s in 70% alcohol, for 20 min in 3% NaOCl, and three rinses (5 min each time) in autoclaved tap water. However, shoots formed only in 12.5% of *C. africanum* petioles and in 20-25% of peduncles, leaves and petioles of *C. purpurascens* when explants were cultured on MS with 9.05 μM 2,4-D and 3.94 μM 2iP in the dark at 25°C. The authors also devised a second, more complex, protocol that involved Nitsch & Nitsch (N-69; 1969) basal medium that included 6.66 μM BA, 5.71 μM indolyl-3-acedic acid (IES) and 888.24 μM adenine for 6 weeks at 20 °C in the dark. This was followed by 2 weeks under a 16-h photoperiod (46 $\mu mol \cdot m^{-2} \cdot s^{-1}$) then transfer to N-69 with 4.44 μM BA, 5.71 μM IES and 10.74 μM 1-naphthylacetic acid. It is unclear if IES and 1-naphthylacetic acid refer to IAA and NAA, respectively. Using this second protocol, the following percentage of explants formed shoots: *C. africanum* (placenta: 8.3%; peduncle: 6.3%; leaf: 75%; petiole: 18.8%), *C. hederifolium* ssp. *confusum* (leaf: 40%; petiole: 36.8%), *C. hederifolium* cv. 'Perlenteppich' (leaf: 25%; petiole: 5%), *C. hederifolium* cv. 'Rosenteppich' (peduncle: 12.5%; leaf: 8.3%; petiole: 6.3%), *C. persicum* (peduncle: 18.8%; leaf: 25%; petiole: 7.1%), pink *C. persicum* (placenta: 50%; peduncle: 45%; leaf: 25%), and *C. purpurascens* (peduncle: 20%; leaf: 50%; petiole: 5.3%). Shoots did not form in *C. cilicium* or in *C. coum*, or in any explant of the other genotypes listed above.

Nhut *et al.* (2012) used thin cell layers (TCLs) 1 mm thick derived from peduncles to induce callus in 100% of explants when cultured on MS medium supplemented with 0.2 mg/L TDZ and 1.0 mg/L 2,4-D. The transfer of 8-week old callus to MS medium containing 0.5 mg/L BA and 0.7 mg/L IBA resulted in the formation of adventitious shoots (39.4/explant). Shoots could also be induced from callus in the presence of 0.5 mg/L Kin and 0.1 to 0.7 mg/L IAA or NAA, but the numbers were significantly lower than the BA+IBA combination. These shoots (2-3 cm long), when cut off at the base of the callus and transferred to MS medium with 1.0 mg/L IBA, formed a robust root system. TCLs are showing great promise for new plant crops and for species

for which no effective tissue culture or *in vitro* regeneration system exists (Teixeira da Silva & Dobránszki 2015a; Teixeira da Silva *et al.* 2015).

Conclusions

Although the production of callus or shoots directly *in vitro* may be an attractive developmental model, for practical purposes, the production of pathogen-free microtubers for pot, greenhouse of field production is desirable. Such material can then be used to derive new microtubers that are free of infection which is a desirable form of clonal propagation since the initial tissue disinfection process of *in vivo*-derived tubers or other material can still result in heavy contamination of tissue cultures. Even though *Cyclamen* micropropagation (Jalali *et al.* 2012) and somatic embryogenesis (Tagipur *et al.* 2016) are well studied and have a rich history, several aspects still need to be explored, such as *in vitro* flowering (Teixeira da Silva *et al.* 2014), the use of ultrasound or sonication to induce organogenesis (Teixeira da Silva & Dobránszki 2014), the use of TCL to maximize organogenic output (Teixeira da Silva & Dobránszki 2013b), or the use of magnetic fields to study growth and development (Teixeira da Silva & Dobránszki 2015b).

Acknowledgements

The author thanks Dr. Neda Jalali, Dr. Roohangiz Naderi and Dr. Ali Shahi-Gharahlar (Department of Horticultural Sciences, Faculty of Agriculture, University of Tehran, Iran) and Dr. Tolga İzgü and Prof. Yesim Yalcin Mendi (Department of Horticulture, Faculty of Agriculture, University of Cukurova Balcalı, Turkey) for providing difficult-to-access literature related to *Cyclamen*. The author also thanks Prof. Andrzej Joachimiak, Editor-in-Chief of *Acta Biologica Cracoviensia Series Botanica*, for providing a copy of Bach *et al.* (1998), and Prof. Margrethe Serek and Prof. Traud Winkelmann (Institute of Horticultural Production Systems, Leibniz University, Hannover, FR Germany) for providing a copy of Prange *et al.* (2008).

References

Abu-Qaoud H. 2004. Direct regeneration in *Cyclamen persicum* Mill. using seedling tissues. *An-Najah Univ. J. Res. (Nat. Sci.)* **18**: 147–156.

Ando T., Murasaki K. 1983. *In vitro* propagation of *Cyclamen* by the use of etiolated petioles. *Tech. Bull. Fac. Hort. Chiba Univ.* **32**: 1–5.

Bach A., Malik M., Zolneczko B. 1998. Organogenesis and embryogenesis in cultures of *Cyclamen persicum* Mill, F-1 'Medium'. *Acta Biol. Cracov. Ser. Bot.* **40**: 47–51

Bourgin J.P., Nitsch J.P. 1967. Production of haploid *Nicotiana* from excised stamens. *Ann. Physiol. Veg.* **9**: 377–382.

Bürün B., Şahin O. 2009. *In vitro* and *in vivo* germination of *Cyclamen alpinum* seeds. *Turk. J. Bot.* **33**: 277–283. doi: 10.3906/bot-0709-8.

Dillen W., Dijkstra I., Oud J. 1996. Shoot regeneration in long-term callus cultures derived from mature flowering plants of *Cyclamen persicum* Mill. *Plant Cell Reports* **15**: 545–548. doi: 10.1007/BF00232991.

Fukui H., Yamamoto T., Asano T., Nakamura M. 1988. Effect of plant growth regulators on *in vitro* organogenesis of cyclamen (*Cyclamen persicum* Mill.). *Res. Bull. Faculty Agricult. Gifu Univ.* **53**: 139–145.

Gautherert J. 1954. Catalogue des cultures de tissus vegetaux. *Rev. Gen. Bot.* **61**: 672–702.

Geier T. 1977. Morphogenesis and plant regeneration from cultured organ fragments of *Cyclamen persicum. Acta Hortic.* **78**: 167–174. doi: 10.17660/ActaHortic.1977.78.20.

Hawkes H.Y., Wainwright H. 1987. *In vitro* organogenesis of *Cyclamen persicum* Mill. seedling tissue. *Acta Hortic.* **212**: 711–714. doi: 10.17660/ActaHortic.1987.212.122.

Ishizaka H., Uematsu J. 1992. Production of interspecific hybrids of *Cyclamen persicum* Mill and *C. hederifolium* Aiton by ovule culture. *Jpn. J. Breed.* **42 (2)**: 354–366. doi: 10.1270/jsbbs1951.42.353.

Jalali N., Naderi R., Ali S.-G., Teixeira da Silva J.A. 2012. Tissue culture of *Cyclamen* spp. *Sci. Hortic.* **137**: 11–19. doi: 10.1016/j.scienta.2012.01.015.

Karam N.S., Al-Majathoub M. 2000a. Direct shoot regeneration and microtuberization in wild *Cyclamen persicum* Mill. using seedling tissue. *Sci. Hortic.* **86**: 235-246. doi: 10.1016/S0304-4238(00)00146-1.

Karam N.S., Al-Majathoub M. 200b. *In vitro* shoot regeneration from mature tissue of wild *Cyclamen persicum* Mill. *Sci. Hortic.* **86**: 323–333. doi: 10.1016/S0304-4238(00)00160-6.

Linsmaier E.M., Skoog F. 1965. Organic growth factor requirements of tobacco tissue cultures. *Physiol. Plant.* **18**: 100–127. doi: 10.1111/j.1399-3054.1965.tb06874.x.

LOEWENBERG J.R. 1969. Cyclamen callus culture. *Can. J. Bot.* **47 (12)**: 2065–2067. doi: 10.1139/b69-298

MAYER-HÖRSTER L. 1956. Wachstum und Organbildung an *in vitro* kultivierten Segmenten von *Pelargonium zonale* und *Cyclamen persicum*. *Planta* **47**: 401–446. doi: 10.1007/BF01911864.

MILLER C.O. 1961. A kinetin like compound in maize. *Proc. Nat. Acad. Sci. U.S.A.* **47 (2)**: 170–174.

MOHANNAD A.M., KARAM N.S. 2000. *In vitro* propagation of wild *Cyclamen persicum* Mill. from seedling tissue. *Acta Hortic.* **530**: 243–252. doi: 10.17660/ActaHortic.2000.530.29.

MURASAKI K., TSURUSHIMA H. 1988. Improvement on clonal propagation of cyclamen *in vitro* by the use of etiolated petioles. *Acta Hortic.* **226**: 721–724. doi: 10.17660/ActaHortic.1988.226.105.

MURASHIGE T., SKOOG F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* **15**: 473–497.

NHUT D.T., THU H.T.M., VINH B.V.T., BINH N.V., LUAN V.Q. 2012. Thin cell layer technology in regeneration and micropropagation of *Cyclamen persicum* Mill. *Propag. Ornam. Plants* **12 (2)**: 89–95.

NITSCH J.P. 1951. Growth and development *in vitro* of excised ovaries. *Amer. J. Bot.* **38**: 566–577.

NITSCH J.P. 1969. Experimental androgenesis in *Nicotiana*. *Phytomorphol.* **19**: 389–404.

NITSCH J.P., NITSCH C. 1969. Haploid plants from pollen grains. *Science* **163 (3862)**: 85–89.

OKUMOTO H., TAKABAYASHI S. 1969. Aseptic culture of cyclamen tuber tissue. *J. Jpn. Soc. Hortic. Sci.* **38 (2)**: 178–187. doi: 10.2503/jjshs.38.178.

PIERIK R.L.M. 1975. Vegetatieve vermeerdering van *Cyclamen*. *Vakblad voor Bloemisterij* **30 (28)**: 13.

PRANGE A.N.S, SEREK M., WINKELMANN T. 2008. Vegetative propagation of different cyclamen species via adventitious shoot formation from seedling tissue. *Propag. Ornam. Plants* **8 (4)**: 204–209.

SCHWENKEL H.G., GRUNEWALDT J. 1988. *In vitro* propagation of *Cyclamen persicum* Mill. *Acta Hortic.* **226**: 659–662. doi: 10.17660/ActaHortic.1988.226.92.

SEYRING M., EWALD A., MUELLER A., HAENSCH K.-T. 2009. Screening for propagation suitability *in vitro* of different *Cyclamen* species. *Electron. J. Biotechnol.* **12 (4)**. doi: 10.2225/vol12-issue4-fulltext-7.

STICHEL E. 1959. Gleichzeitige Induktion von Sporossen und Wurzeln und *in vitro* Kultivierten Gewebestücken von *Cyclamen persicum*. *Planta* **53**: 293–317. doi: 10.1007/BF01881794.

TAGIPUR E.M., SEKER G., TEIXEIRA DA SILVA J.A., YELCIN MENDI Y. 2016. Somatic embryogenesis, cryopreservation and *in vitro* mutagenesis in *Cyclamen*. In: MUJIB A. (ed.), Somatic embryogenesis in ornamentals and its applications: 155–167. Springer, India. doi: 10.1007/978-81-322-2683-3_10.

TAKAMURA T., MIYAJIMA I., MAEHARA T. 1993. Seedling selection and micropropagation for the breeding of yellow-flowered *Cyclamen* cultivars. *J. Fac. Agric. Kyushu Univ.* **37 (3/4)**: 265–271.

TAKAMURA T., MIYAJIMA I. 1997. Micropropagation of *Cyclamen persicum* Mill. In: Bajaj Y.P.S (ed.), High-tech and micropropagation VI. *Biotechnology in Agriculture and Forestry* **40**: 96–112. Springer, Berlin. doi: 10.1007/978-3-662-03354-8_8.

TEIXEIRA DA SILVA J.A. 2012. Is BA (6-benzyladenine) BAP (6-benzylaminopurine)? *Asian Australas. J. Plant Sci. Biotechnol.* **6 (Special Issue 1)**: 121–124.

TEIXEIRA DA SILVA J.A., ALTAMURA M.M., DOBRÁNSZKI J. 2015. The untapped potential of plant thin cell layers. *J. Hortic. Res.* **23 (2)**: 127–131. doi: 10.2478/johr-2015-0024.

TEIXEIRA DA SILVA J.A., DOBRÁNSZKI J. 2013a. How timing of sampling can affect the outcome of the quantitative assessment of plant organogenesis. *Sci. Hortic.* **159**: 59–66. doi: 10.1016/j.scienta.2013.05.001.

TEIXEIRA DA SILVA J.A., DOBRÁNSZKI J. 2013b. Plant thin cell layers: a 40-year celebration. *J. Plant Growth Regul.* **32 (4)**: 922–943. doi: 10.1007/s00344-013-9336-6.

TEIXEIRA DA SILVA J.A., DOBRÁNSZKI J. 2014. Sonication and ultrasound: impact on plant growth and development. *Plant Cell Tissue Organ Cult.* **117 (2)**: 131–143. doi: 10.1007/s11240-014-0429-0.

TEIXEIRA DA SILVA J.A., DOBRÁNSZKI J. 2015a. Plant thin cell layers: update and perspectives. *Folia Hort.* **27 (2)**: 183–190. doi: 10.1515/fhort-2015-0029.

TEIXEIRA DA SILVA J.A., DOBRÁNSZKI J. 2015b. How do magnetic fields affect plants *in vitro*? *In Vitro Cell Dev. Biol. Plant.* **51 (3)**: 233–240. doi: 10.1007/s11627-015-9675-z.

TEIXEIRA DA SILVA J.A., KERBAUY G.B., ZENG S-J., CHEN Z-L., DUAN J. 2014. *In vitro* flowering of orchids. *Crit. Rev. Biotechnol.* **34 (1)**: 56–76. doi: 10.3109/07388551.2013.807219.

WAINWRIGHT H., HARWOOD A.C. 1985. *In vitro* organogenesis and plant regeneration of *Cyclamen persicum* Mill. using seedling tissue. *J. Hortic. Sci.* **60**: 397–403. doi: 10.1080/14620316.1985.11515644.

WHITE P.R. 1963. The cultivation of animal and plant cells. Ronald Press, New York.

WICART G., MOURAS A., LUTZ A. 1984. Histological study of organogenesis and embryogenesis in *Cyclamen persicum* Mill. tissue culture: evidence for a single organogenesis pattern. *Protoplasma* **119**: 159–167. doi: 10.1007/BF01288870.

YAMANER Ö., ERDAG B. 2008. Direct shoot formation and microtuberization from aseptic seedlings of *Cyclamen mirabile* Hildebr. *Biotechnol.* **7**: 328–332. doi: 10.3923/biotech.2008.328.332.