



## THE UNTAPPED POTENTIAL OF PLANT THIN CELL LAYERS

### Research note

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### ABSTRACT

Thin cell layers (TCLs), which contain a small number of cells or tissues, are explants excised from different organs (stems, leaves, roots, inflorescences, flowers, cotyledons, hypocotyls/epicotyls, and embryos). After almost 45 years of research, this culture system has been used for several monocotyledonous and dicotyledonous plants of commercial importance, and for model plants. The limited amount of cells in a TCL is of paramount importance because marker molecules/genes of differentiation can be easily localized *in situ* in the target/responsive cells. Thus, the use of TCLs has allowed, and continues to allow, for the expansion of knowledge in plant research in a practical and applied manner into the fields of tissue culture and micropropagation, cell and organ genetics, molecular biology, biochemistry, and development. Starting from a brief historical background, the actual and potential uses of the TCL system are briefly reviewed.

**Key words:** model plants; organogenesis; plant biotechnology; TCLs

### TCLs: a brief background

The revolutionary finding by Tran Thanh Van (1973) that a tiny layer of pedicel epidermal cells of tobacco, the thin cell layers (TCLs), could lead to the formation of flowers, shoots, roots and callus, simply by manipulating the auxin to cytokinin ratio was truly an important event in plant tissue culture. TCLs serve as a simple, yet effective way to control the developmental pathway and organogenic outcome and provide, where tested, an effective way of mass producing clones of specific organs.

TCLs have been used for the effective tissue culture of several dozen plants with commercial importance, spanning model plants such as orchids (Teixeira da Silva 2013), tobacco, *Arabidopsis thaliana* (Della Rovere et al. 2013, 2015) or snapdragon, field crops (cereals, rape, rice) horticultural commodities (fruits, vegetables, ornamental plants), medicinal plants and herbs, and even forestry trees (*Pinus* sp.), and woody fruit plants, *Citrus* spp. and apple (Fig. 1) (Dobránszki & Teixeira da Silva 2011;

Teixeira da Silva & Dobránszki 2013). Yet, the lack of applications of such a simple model system for staple crops such as wheat or potato, or for a wider range of medicinally and pharmaceutically important species, or even to other woody tree species like sandalwood that may be facing extinction, suggests that the potential of this technique remains widely underexplored.

### TCLs: actual and potential uses

Even though research is increasingly turning toward more complex molecular techniques, there is still a constant need to mass produce plant tissues or organs in a sterile and controlled *in vitro* growth environment. TCLs hold particular value where specific organs might be required for highly specialized experiments, such as the production of *in vitro* flowers (Teixeira da Silva 2013; Teixeira da Silva et al. 2014) to make anthers or stigmas available at any time, but which, under natural conditions, would be severely restricted to specific seasons. Such applica-

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tions can easily be envisioned for saffron, which uses only the stigmas for the food, flavor, and fragrance industries.

Compared with conventional explants, the use of TCLs for morphogenesis (organogenesis, xylogenesis, or somatic embryogenesis) may result in higher productivity due to lower levels of endogenous plant growth regulators in the TCL, more effective transport of the medium components to the target cells, earlier morphogenesis, or a higher ratio of the morphogenic cells compared to the total number of cells of the explant (Tran Thanh Van 2003).

In an unpublished experiment, transverse TCLs (tTCLs) of stem segments from *in vitro* apple shoots of cv. Freedom induced adventitious shoots when cultured on regeneration medium (defined in Dobránszki & Teixeira da Silva 2011), containing 22.7  $\mu\text{M}$  thidiazuron (TDZ) and 1.07  $\mu\text{M}$   $\alpha$ -naphthaleneacetic acid (NAA). The number of regenerated shoots increased when stem tTCLs (0.2–0.3 mm thick) were used and the number of shoots per stem tTCL was as much as 39% higher than control stem explants (2–3 mm thick). Shoot initials were already well developed by the fourth week of regeneration on stem tTCLs cultured in light (L in Fig. 1). When stem tTCLs were cultured in light (L), the percentage of shoot-regenerating explants (i.e., regeneration percentage) increased 23% more than tTCLs or control explants cultured in the dark (D) during the first three weeks of regeneration (D+L). Moreover, inverse orientation of stem tTCLs was more favorable than the normal orientation, resulting in 30% higher regeneration. The number of shoots per regenerating explant (0 in L vs. 1.4–1.5 in D+L) was significantly lower on leaf tTCLs (Dobránszki & Teixeira da Silva 2011) than on stem TCLs (2.1–2.5).

The use of *Arabidopsis* longitudinal TCLs (lTCLs) has assisted in the study of the genetic and hormonal control underlying adventitious root formation. *Arabidopsis* lTCLs are formed by the stem inflorescence tissues external to the vascular system, i.e. uniseriate epidermis, three or four cortical layers, a monolayered endodermis, and one or two fiber layers. The formation of adventitious roots was

induced by indole-3-butyric acid (10  $\mu\text{M}$ ) plus kinetin (0.1  $\mu\text{M}$ ) under continuous darkness for at least two weeks of culture. The explants were cultured horizontally, epidermal side up, and the adventitious roots appeared all along their surface and originated from a unique tissue, i.e., the stem endodermis (Falasca et al. 2004), by superimposed periclinal divisions and occurred at the same time of expansion and cell-to-cell separation events in the cortex (Fig. 2A). The endodermis periclinal derivatives either organize root meristemoids, further developing into roots (i.e., a rhizogenic response), or proliferate into superimposed callus-like layers with xylary clumps or strands (xylogenesis) (Fig. 2B) (Della Rovere et al. 2013, 2015). By using numerous mutants and transgenic lines it has been demonstrated that *Arabidopsis* plants form adventitious roots *in planta* starting from the pericycle of the hypocotyl, with no involvement of the hypocotyl's endodermis. Their formation involves auxin accumulation, and related gene expression, in the initiating cells, derived by periclinal and anticlinal divisions of the pericycle, and in the meristem cells of the primordium and mature root apex (Della Rovere et al. 2013, 2015). However, under the same hormonal treatment of lTCLs, the pericycle derivatives may be alternatively involved in ectopic xylary development (Della Rovere et al. 2015). The response *in vitro* of the lTCLs excised from the same mutant and transgenic lines allowed to understand that when the adventitious roots are formed by different founder cells *in planta*, the stem endodermal cells express the same genes, and hormonal control, necessary for their development *in planta*. This demonstrates that the founder tissue may not be a determinant for the correct development of an adventitious root. Interestingly, the use of lTCLs has also been useful for demonstrating that adventitious root formation and xylogenesis (Fig. 2B) originated from the stem endodermis and that these two developmental programs are inversely related, since the same programs take place in the pericycle *in planta*, with the same transcription factors involved in the initiation programs, and the same auxin influx carrier in their switching (Della Rovere et al. 2015).

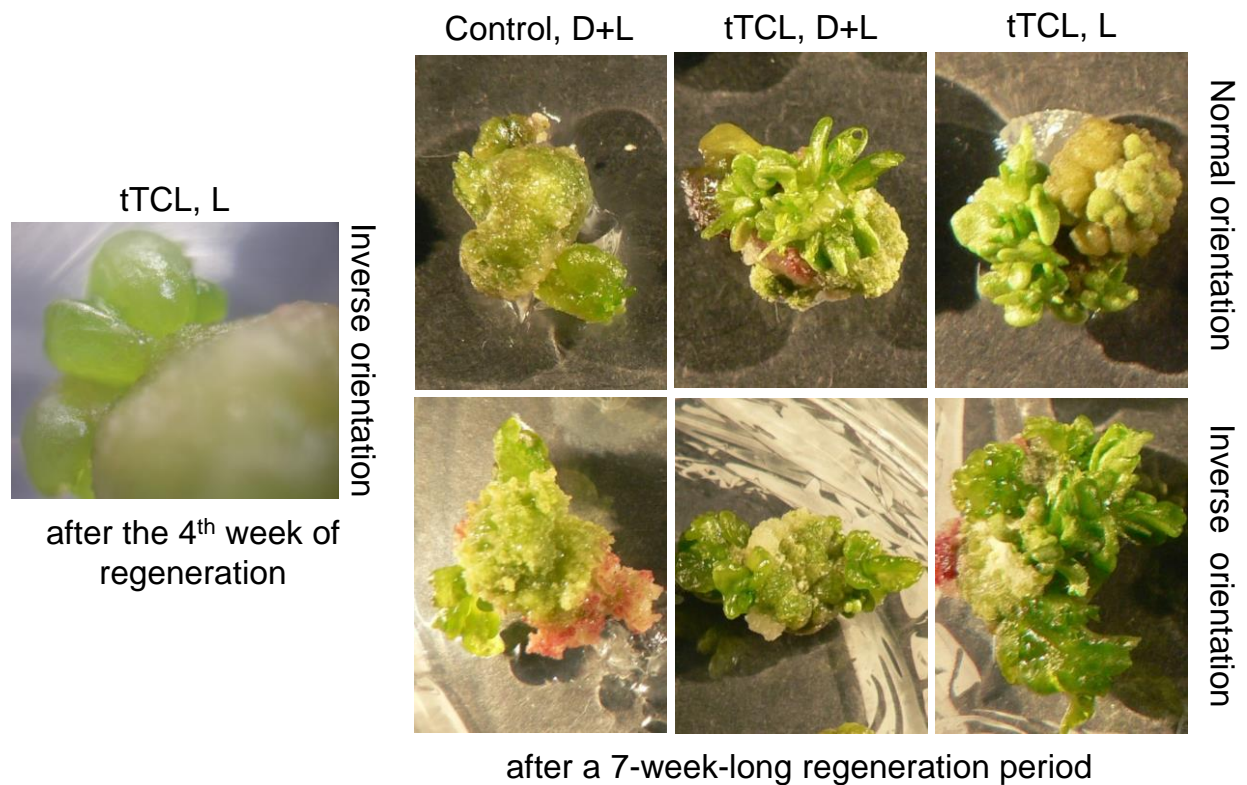


Fig. 1 Adventitious shoot initials from apple (*Malus × domestica*) 'Freedom' forming from stem transverse thin cell layers of *in vitro* shoots after 4 weeks in culture (left photo) and adventitious shoots after 7 weeks of regeneration (right photo plate)

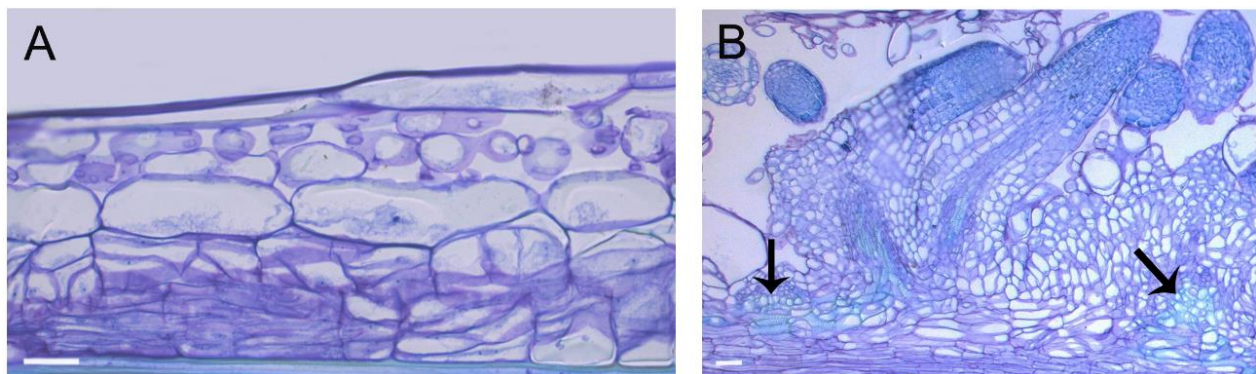


Fig. 2 Histological stages of adventitious root formation from *Arabidopsis thaliana* ITCLs cultured with indole-3-butyric acid (10  $\mu$ M) plus kinetin (0.1  $\mu$ M) under continuous darkness. **A** – superimposed layers of derivatives from the stem endodermis (lower part of the explant), and expanded and separated cells of the cortical layers (upper part of the explant) (day 5 of culture). **B** – Adventitious roots in longitudinal and cross-section at the end of culture (day 14). Some of them protrude from the explant. Superimposed cell layers of endodermal derivatives are shown in the lower part of the image with the roots arising from the outermost of these derivatives. Xylary cells in these derivatives (xylogenesis) are also visible (arrows). Radial longitudinal sections (bright-field images, toluidine blue staining). Scale bars = 30  $\mu$ m

In addition, the secret to the developmental success of a TCL *in vitro* lies, not only in the tissue types it might incorporate, either by cutting transversally or longitudinally, but also in the area and volume of the explant itself. Using three horticultural crops (*Cymbidium*, apple, and chrysanthemum), the plant growth correction factor was used to extrapolate the productivity of a TCL relative to a conventional explant, indicating that even though the face-value organogenic outcome was less in TCLs than in conventional explants, once the size, shape, and volume of the explant were factored in, the productivity of the TCL was, in fact, several fold higher than a conventional explant (Teixeira da Silva & Dobránszki 2014). This little known fact by many plant scientists, together with the smallest of developmental treasures, the TCLs, would serve the plant science community well, if applied more widely to a greater range of plant species. Finally, the use of TCLs in model species would serve to improve knowledge about the genetic control of plant development, and its plasticity, as the recent papers on *Arabidopsis* TCLs demonstrate (Della Rovere et al. 2013, 2015).

### TCLs: new developments

TCLs continue to serve as a model cellular developmental system. Since our last reviews (Teixeira da Silva 2013; Teixeira da Silva & Dobránszki 2013), eight studies that employed TCLs have emerged in the literature. The majority of these new studies targeted new plant species that had not yet tested the use of TCLs for *in vitro* regeneration, or mass propagation by organogenesis or somatic embryogenesis. These plants include three regional medicinal plants, i.e. ipecacuanha (*Psychotria ipecacuanha* Brot.) (Botero Giraldo et al. 2015), bulbous ceropogia (*Ceropegia bulbosa* Roxb.) (Dhir & Shekhawat 2014), and *Paris polyphylla* Sm. (Rao-mai et al. 2015), an ornamental, *Brasilidium forbesii* (Hook.) Campacci (Gomes et al. 2015), an orchid (Teixeira da Silva 2014), *Agave fourcroydes* Lem., a plant used in the fiber industry (Monja-Mio & Robert 2014), and *Stevia rebaudiana* Bertoni (Ramírez-Mosqueda & Iglesias-Andreu 2015), used in the food industry. Moreover, TCLs were used as the regeneration system of choice for pyrethrum

(*Tanacetum cinerariifolium*, (Trev.) Sch. Bip) in *Agrobacterium*-mediated transformation (Mao et al. 2014). These studies attest to the continued importance of TCLs as a simple, yet effective model regeneration system for a wide range of plant species, including horticultural crops.

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