

# Plant Thin Cell Layers: Challenging the Concept

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## ABSTRACT

The concept of a thin cell layer, or TCL, was initially coined by Khiem Tranh Than Van in two key papers, one of which was in Nature, exactly 35 years ago. At that time *Nicotiana tabacum* had been used as a model plant to establish three main pathways for *de novo* organogenesis by establishing a flower, vegetative bud and root “programme” from pedicel tissue. Over the last 35 years, a wealth of research in plant tissue culture based on TCLs has emerged to fortify the importance of this very simple technique, highlighting its continued fundamental importance as a front-runner tool in plant cell and tissue differentiation and organ development. In this conceptual paper, I primarily wish to point out and explain the logic behind some of the inherent contradictions, paradoxes and incongruities of the concept, based on basic, fundamental definitions in cellular biology and botany, even though I have been one of its most avid supporters.

**Keywords:** definition, ITCL, tobacco, tTCL

**Abbreviations:** ITCL, longitudinal TCL; PLB, protocorm-like bodies; TCL, thin cell layer; tTCL, transverse TCL; TTL, thin tissue layer

## Thin Cell Layers: a concept is born

When nearly every plant scientist comes into contact for the first time with the almost “magical” world of plant tissue culture, it is one of the most central fundamentals that captivates each and every one of us: the notion that in some way we might be able to control the outcome of a developmental event by manipulating the plant cell or tissue or the environment into which it is placed to create something new and unique.

The mere notion that it is possible to take a plant part, any part, from an *in vivo* or *in planta milieu*, dissect it or use it whole to create a (clonal) product of the material from which it was first derived is the conceptual framework that underlines our capacity to try and culture, through *in vitro* manipulation, any target material, for whatever purpose we dream of creating. This same notion was without doubt what Gottlieb Haberlandt must have envisioned when he first laid claim to the possibility of culturing isolated tissues and what led pioneers like Toshio Murashige and Folke Skoog to seek a “universal” growth medium that would allow for the growth and culture of tissues from a broad range of species or that led Georges Morel to try and culture the first virus-free *Cymbidium* orchid when he noted that small bodies, or protocorms, formed from cultured shoot tip explants derived from plants that were originally infected with *Cymbidium mosaic virus* (Morel 1960). Furthermore, this notion may have been the underlying force of inspiration that led to what is still considered by many plant tissue culture scientists today to be the milestone publication of plant tissue culture by Edwin George and colleagues in 1987. Finally, it is without doubt that the same notion led Khiem Tranh Than Van to explore the notion that perhaps, by manipulating explant size, independent of position, would allow for a greater control of its organogenic potential. And hence the concept of a thin cell layer (TCL) was born and put into practice (Tran Thanh Van 1973a, 1973b).

## Thin Cell Layer → Thin Tissue Layer: is it a cell or is it a tissue?

TCLs represent, as the name suggests, a thin layer of cells. However, the most commonly accepted definition of a tissue in biology is a group of biological cells with similar structure and that performs a similar and specific function. Unlike plant science, a tissue in medical science need not form a layer but we will concern ourselves with plants here. This implies two things: those cells that form a simple tissue such as the epidermis, parenchyma, sclerenchyma or collenchyma are either similar in structure or their inherent nature (ultracellular, biochemical or genetic) is the same. Complex tissue consists of two or more different types of simple tissue. So, strictly-speaking, a thin layer of cells or a TCL would and should be, by definition, a tissue.

I wish to take vascular tissue as an example and turn to some Botany I concepts to make this point – perhaps – a little clearer. Vascular tissue is a complex tissue which is primarily composed of xylem and phloem, which have great functional and structural differences: even though they are both involved in the conduction of water, minerals and nutrients throughout the plant, the former is the principal water-conducting tissue while the latter is the principal food-conducting tissue. Xylem is itself composed of different kinds of cells, which are themselves structurally and functionally different: tracheids involved in conduction and support, vessel members used for conduction, fibers for support and parenchyma for food storage. In phloem, the conducting cells themselves are the sieve elements, but other cell types may exist such as companion, parenchyma, fiber, sclereid, and ray cells. Xylem and phloem are thus spatially related but physiologically differentiated.

Thus, when we consider a more functional approach, different cells with different functional end-points will naturally result in functionally different tissues. Whether this translates into a visibly detectable difference in the final structure, the organ such as a root, shoot or flower, depends on too many variables, which is far beyond the scope of what I am trying to achieve here. The focus, therefore of this conceptual challenge, is at the level of the cell and tis-

sue.

Now that the concepts of a cell, simple and complex tissues are a little more clearly defined, I turn my attention to TCLs. Two kinds of TCLs were originally defined by Tran Thanh Van (1973a, 1973b): longitudinal TCLs or ITCLs, or transversal TCLs or tTCLs. In her words “The TCL system consisted of explants of small sizes which are excised from different plant organs... They are excised either longitudinally – the explants are designed as longitudinal TCLs (ITCL) – or transversally – designed as transverse TCLs (tTCL). The ITCLs (1 mm × 0.5 or 10 mm) include only one tissue-type for example a monolayer of epidermal cells (which could be peeled off the organs) or several (3-6) layers of cortical cells whereas the tTCLs (0.2/0.5 or a few mm of thickness) include a small number of cells of different tissue-types (epidermal, cortical, cambium, perivascular and medullar tissue as well as parenchyma cells.” (Tran Thanh Van 2003). However, by that definition alone, it is clear that a tTCL would automatically include several cell types with distinct morphological, structural and functional differences (i.e., a simple or complex tissue) within a single section or explant, independent of the size, and independent of the organ from which the tTCL derived. This then directly contradicts the basic term itself: thin cell layer. I here propose that the term TCL be re-defined as a thin tissue layer or TTL on a botanical basis rather than an ideological basis as the original concept was established, and as such tTCL should be correctly (botanically) termed a tTTL. Similarly, an ITCL, as would be typified by an epidermal explant, would still be a tissue, albeit a simple one, but a tissue nonetheless, and hence be called or redefined as an ITTL. To avoid confusion, I will refer to the term TCL uniformly throughout this text from this point onwards, although the intended meaning is TTL.

### Does size matter? The principle of exclusion

As mentioned above, Tran Thanh Van (2003) defines the size of an ITCL as 1 mm × 0.5 or 10 mm. Although she does not use the term “size” in this particular quotation, it is implied, if we consider that the word “size” is a physical magnitude of something that is based on graduated or defined measurements. If this were the case, then to be more precise, she was referring to the area, rather than to the volume. This basal definition has a few major fundamental flaws but which I will cluster as referring to “size”: the assumption that she makes is that independent of the thickness, an explant of this area (0.5 or 1 cm<sup>2</sup>) would be the basal definition of an ITCL. Tran Thanh Van does not specifically address the actual size that a tTCL should be nor does she specifically state whether, if an explant has twice that area would or could be considered a TCL. Rather, by interpreting her comment further “The common trait of ITCL and tTCL is to be “thin” i.e. an inoculum with as small a number of cells as possible.” (Tran Thanh Van 2003), she would therefore imply such a wide variability which would not allow for standardization of a definition, so vital to today’s plant science driven by precision and accuracy.

To take a hypothetical example, if we were to prepare an ITCL (ITTL) from a stem, for example, would an explant of this dimension (area = 0.5 or 1 cm<sup>2</sup>) but of differing depths (i.e. of differing volumes) result in different organogenic responses? If the basic concept of a TCL were sound, then the organogenic outcome would be identical, or at worst, similar, even with as much as a 100% coefficient of variation. However, and much to my surprise, despite 35 years of the use of “TCL technology”, no such elaborate experiments exist in the main-stream literature or even on the Internet. Initial experiments which I established that looked at the effect of ITCLs created from the surface tissue of 2-month-old protocorm-like bodies (PLBs) of several *Cymbidium* hybrids, and which led to the successful establishment of different organogenic programmes (Teixeira da Silva and Tanaka 2006) for these often difficult-to-propagate orchids, indicated that whereas area of an ITCL played no significant

**Table 1** Effect of the Z-plane on organogenic outcome of ‘Twilight Moon’ protocorm-like body (PLB) tTCLs.

Explant plane (size in mm)	№ PLBs/PLB tTCL
X = 0.5, Y = 0.5, Z = 0.5	7.86 a
X = 0.5, Y = 0.5, Z = 1.0	8.26 a
X = 0.5, Y = 0.5, Z = 2.0	5.71 b
X = 0.5, Y = 0.5, Z = 2.0	3.66 c

X = length, Y = width, Z = thickness. n = 30 × 3 repetitions. Mean separation following ANOVA using Duncan’s New Multiple Range Test at P = 0.05.

effect on the number of PLBs generated and quantity of embryogenic callus generated, volume did (Teixeira da Silva, unpublished data, to be published elsewhere). As regards the tTCL, the trend of the effect is similar, although the quantification of the result is not significant, and the organogenic outcome depends more strongly on the source of the explant rather than its volume. This fundamental difference in the size of the explant (and also the origin or position of the explant) was shown more clearly with *Lilium longiflorum* bulb scale TCLs (Nhut *et al.* 2003a).

This implies that the Z-plane of the TCL is not really considered by Tran Thanh Van, or at least not openly discussed, and very rarely, if at all considered by all users of the TCL technology to date. However, in the above paper on *Cymbidium* orchids, I clearly define all three dimensions of the explant, and thus allow for reproducibility of the experiment. Almost every single TCL-related study ever published fails to address the Z-plane, and hence the limitations in the original definition of the TCL. I therefore propose to define clearly here what should be considered as the new guidelines and terminology for TCLs based on previous studies I conducted on *Cymbidium*, tobacco and chrysanthemum, and on unpublished data for *Cymbidium* (Table 1):

TCL → TTL (thin tissue layer)

tTTL = transverse thin tissue layer (i.e. symmetrical cross-section through a donor explant tissue) = 5-10 mm in length and diameter, maximum 1 mm in thickness;

ITTL = longitudinal thin tissue layer (i.e. longitudinal section through a donor explant tissue) = 5-10 mm in length and diameter, maximum 1 mm in thickness;

μtTTL/μITTL = TTLs prepared with a microtome under aseptic conditions = 5-10 mm in length and diameter, 10-100 μm in thickness.

If TCLs/TTLs are re-defined more clearly than they were originally proposed, then it makes the choice of definition much clearer, it reduces the chances of fraudulent claims, and allows for repetition of the methodology in a much stricter manner. Thus, based on the above dimensions – independent of the treatment applied, of the source of the explant, or of the skill of the tissue culture scientist – an explant with larger dimensions (area or volume) should NOT be considered as thin, and thus should not be considered as a TCL or a TTL.

In conclusion, size does matter, but more importantly, so do area and volume.

### Quo vadis?

TCL technology has been widely applied across dozens of plant families to induce clear and successful regeneration protocols, superior to when larger sized, more conventional explants are used (reviewed extensively in Nhut *et al.* 2003b, 2006; Teixeira da Silva 2007). Within these reviews, we also show the application of TCLs in genetic engineering, *in vitro* flowering, establishment of cultures for standardized secondary metabolite production, and the use of these small explants in studying genetic, differentiation and biochemical events.

To maintain the central dogma of TCL technology as defined by the founder herself, Tran Thanh Van, in 2003 “...the TCL system (is one) that one can reprogram differentiated cells into multi-programmable patterns with a specific spatial/temporal sequence” true to its original and

intended meaning, it is vital that an update of the terminology and limits to the use of the term be defined now so as to avoid murky limits between what is a TCL and what is a conventional explant. This, especially with an increasing number of papers related to the use of TCLs as a simple, but effective technology.

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