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The Plant Growth Correction Factor. I. The Hypothetical and Philosophical Basis

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ABSTRACT

There are two possible reasons why regeneration ability in plant tissue culture (PTC) differs from study to study. In life, not all beings are born equal. In PTC, too, not all explants have the same regeneration capacity. A plethora of factors influence the organogenic outcome of an explant in PTC, but differences in production, yield and organogenic output are all measured by one factor, and one factor alone: the size of the explant. In this ground-breaking paper, we put forward a radical notion that would attempt to allow for the direct comparison of organogenic potential of PTCs of the same cultivar or species conducted in different studies or laboratories. The prototype concept, the growth correction factor or GCF, has been tested on a model species, apple (*Malus* sp.).

Keywords: abiotic factors; explant; plant tissue culture; *Malus* sp.
Abbreviations: GCF, growth correction factor; **PTC**, plant tissue culture; **TCL**, thin cell layer; **TDZ**, thidiazuron

THE GCF THEOREM: HYPOTHETICAL AND PHILOSPHICAL BASIS

Without a doubt, the explant is the most important factor in plant tissue culture (PTC). Without the explant, there is no experiment. Without the appropriate choice of explant, there is no successful regeneration. And although a million combinations of abiotic factors can be tested, it always reverts to the basic notion: that the explant is the most basic unit of PTC that makes the experiment work.

Now that the importance of the explant in PTC is out of the way, we now turn to what seems to be a gross violation in claims and distortions which could, in theory, be equivalent to fraud in human-human cases in a court of law. What we are referring to is the abuse of the terms "better than", "more than" or "higher than" which many – if not most – PTC scientists to describe their results when discussing them within the Discussion relative to the findings within the literature.

For example, if scientist A uses explant X and regenerates 50 shoots *in vitro* while scientist B uses explant Y and regenerates 100 shoots, indeed, at face value, scientist B could probably claim (and probably does claim) in their Abstract, Results and Discussion – and some scientists really make excessively emphatic claims – that their regeneration protocol is superior to that of scientist A. In theory, what scientist B is saying is correct, but in practice, closer examination of the experimental protocol might prove otherwise. Specifically, the size of the explant might not be identical, and thus not directly comparable, unless some sorts of correction factor were to be applied. To extend the logic of the above example, let us imagine that explant X used by scientist A was 1 mm in size (length) while explant Y of scientist B was 1 cm in size (length). Obviously, both volume and surface area would be radically different, and at least in terms of length, at face value, we would of course expect regeneration capacity to be higher in explant Y than in explant X, particularly if we consider that the surface area in explant Y would be superior to that in explant X and thus lead to higher yield. Thus, and although this might appear to be a perfectly redundant statement, we need to understand that 1 cm is 10 × 1 mm, i.e. explant Y is 10 times longer/larger than explant X. So, theoretically, and at a very crude level, we could expect that using the protocol of scientist A, that from 1 cm of plant tissue, that 10 explants could be created. Thus, in scientist A's experiment, 1 cm of tissue could theoretically yield 500 shoots from explant Y (assuming that both have the same regeneration potential). With this new value in hand, it would be extremely evident that the protocol as devised by scientist A is 5-fold superior to that of scientist B, even though scientist B claimed that his/her protocol was 2-fold superior to that of scientist A. Therefore, to make direct comparisons a growth correction factor (GCF) or 10 would be required to make the explants X and Y and experiments of scientists A and B directly comparable (**Fig. 1**).

At this point in the theoretical discussion, we ask the reader to briefly pause from the thought of the explant, and to reflect on the deeper consequences of what it is we are proposing and suggesting. If our concept of GCF were to somehow be true, then it would have far-reaching consequences not only on what was already reported and claimed by thousands if not tens of thousands of scientists in the literature to date, but also on the way in which PTC scientists would begin to report their data in the future *ad infinitum*. In essence, with a GCF, we would be able to prove that the claims of scientist B were false. How? Looking back, scientist B claimed to have a superior protocol because their explant Y produced 100 shoots from a 1-cm explant. However, if scientist A were to use the same length of tissue, i.e. 10 × explant X, then, *de facto*, scientist A can produce 5 times more shoots than scientist B. This would make scientist A's protocol superior to that of scientist B and scientist B's claims of a superior protocol false.

However, it is highly likely that within the Discussion of the manuscript submitted by scientist B, that the claim "our experiment was superior to the protocol used by scientist A because we produced 100 shoots while scientist A only produced 50 shoots." Naturally, such a strongly-worded and confident claim would sway the opinion of the

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Hypothesis Paper

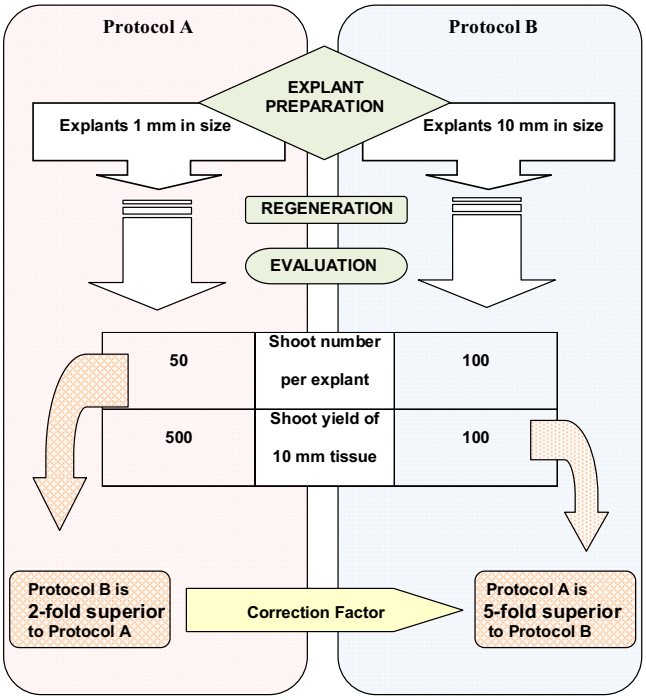

Fig. 1 Schematic diagram showing the differences between a conventional protocol and one with the GCF applied.

peer reviewer, and in recognition of such originality and superior output, that manuscript would most likely be accepted. In retrospect, had a GCF existed at the time, and had the peer reviewer been able to understand that the true comparison was not 50 vs 100 (A vs B) but rather 100 vs 500 (B vs A), most likely the decision to publish scientist B's paper might have been completely different, i.e. to reject on the ground of sufficient improvement of a former protocol.

APPLE: A PERFECT MODEL SPECIES

Apple is an excellent model species because its regeneration *in vitro* is extremely well explored and studied (Dobránszki and Teixeira da Silva 2010; Magyar-Tábori *et al*. 2010), despite being a hardwood species. We recently showed (unpublished data) how, in response to the cytokinin-like compound, thidiazuron, or TDZ, conventional apple leaf explants could produce a maximum of 12.1 shoots per explant in 'Royal Gala' after 9 weeks of culture on medium containing 0.5 µM TDZ. In this case, the explant was a strip of half-leaf 5-mm wide derived from the second leaf from the apex. However, when a transverse thin cell layer, or tTCL (0.1-0.3 mm thick), was used the from the exact same leaf source, and from the same scion (cultivar), and placed on medium with the same concentration of TDZ i.e., 0.5 µM, only 4.1. shoots formed. The TCL is an excellent model for studying fine-scale organogenesis in apple and other species (Teixeira da Silva *et al*. 2007; Dobránszki and Teixeira da Silva 2011). However, at 5 µM of TDZ, 6.5 shoots could be produced per tTCL. This represents one spot of data from the experiment, but will serve for the basis for the explanation of how a GCF is necessary.

In our experiment on apple (unpublished data), using a conventional rationale, we could, if we wanted to, state that conventional leaf explants produce more shoots (i.e. 12.1) than tTCLs (i.e. 6.5). Statistically, the data we report shows this superiority of the explant. And to reader, making the claim would be – at least to untrained eyes – correct and validated. However, and this is the nasty twist of the tale, we need to extrapolate one step further, and this is the fundamental basis of the GCF we are proposing. One leaf can yield two explants using a conventional protocol (Dobránszki *et al*. 2005). However, one leaf can yield 50 tTCLs. Therefore, although not the actual value measured, one leaf made up of two conventional explants could yield (theoretically) 24.2 shoots per leaf whereas one leaf cut into 50 tTCLs could yield (theoretically) 325 shoots, i.e. 13 times more shoots.

Reverting back to our scientist A vs B and explant X vs Y analogy, in reality we can report that explant A (here the conventional leaf) is superior in regeneration capacity and yield than explant B (tTCLs). In theory, however, it is more than evident that the opposite is true. The GCF is thus 13 while the **true** shoot yield per leaf was calculated according to the following two formulae:

$[2 \times (R\%_{control} \times SN_{control})/100]$

for conventional (control) explants and

$[50 \times (R\%_{tTCL} \times SN_{tTCL})/100]$ for tTCL explants.

where R% = percentage of explants that regenerated shoots; SN = shoot number per explant.

This paper is the first of several, the blue-print so to speak. In ensuing papers, we will be proposing more concrete GCFs for explants of different sizes or shapes, providing more data for more model plants (such as *Cymbidium* and chrysanthemum), and showing, from the literature, how claims by some scientists may have been incorrect simply because, at that time, no CGF existed.

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