

Baseline data generated on soil properties, microbial biodiversity, activity and respiration in the IHR will assist in understanding the mechanisms and controlling factors of SOC decomposition in the region. Through modeling of these soil microbial community data along with other climate factors, the results will help us in developing long-term strategies to monitor climate change impacts and propose policy briefs/management strategies to manage microbial systems for mitigation of climate change.

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Pamela Bhattacharya, Gautam Talukdar, Gopal Singh Rawat and Samrat Mondol* are in the Wildlife Institute of India, Chandrabani, Dehradun 248 001, India.
*e-mail: samrat@wii.gov.in

Declaring the commercial source and grade of chemicals, and equipment, in a scientific paper

Jaime A. Teixeira da Silva and Judit Dobránszki

Scientific biomedical papers widely use chemicals, reagents and/or equipment. These are described in the materials and methods section. The source of these methodological props needs to be precisely defined for scientific and proprietary reasons. The commercial source or grade of a chemical can affect the quality and outcome of an analysis, e.g. in plant tissue culture. Failure to recognize the commercial source deprives a company of its due proprietary investment in a product, reduces reproducibility and thus constitutes an incomplete or erroneous methodology. Such errors should be corrected, which should be the responsibility of authors, editors and publishers.

A methodological prop or tool (MPT) is defined here as any chemical, utensil, or equipment (CUE) that serves to support a methodology within a scientific manuscript. Not only do MPTs serve as important and fundamental tools for completing a method, their commercial source can, in select cases, also influence the outcome of a scientific manuscript. This note aims, using plant tissue culture, to (a) highlight the importance of specifying the commercial source and grade of CUEs; (b) show through select and concrete examples, how specific CUEs from different sources, or of different quality, can lead to qualitative and quantitative differences in the outcome of an experiment; (c) encourage authors, editors and publishers to correct the literature, to correct the weaknesses of traditional peer review¹, through post-publication peer

review (PPPR), in a bid to make the methodological sections as accurate and precise as possible. In doing so, reproducibility of weak, unclear or unstated methodological flaws might increase. However, efforts to increase reproducibility will be in vain, unless all parties are involved².

Using select examples of plant tissue culture, a branch of plant biotechnology, we demonstrate how differences in the choice of MPTs and CUEs can influence the outcome of an experiment. Thus, defining these elements is a central aspect of reproducibility of a protocol. This concept is fortified by a respectable leading Society in the plant science community – The American Society for Horticultural Science³, which states that ‘In general, refer to trade or brand names only parenthetically with the active in-

redient, chemical formula, purity, and diluent or solvent stated clearly in the text and emphasized in preference to the commercial product; also, include the name, city, and state/country of the company that produces the product.’

The effect of chemicals, vessels, or medium components on analytical and developmental outcome in plant tissue culture

Plant cells and tissues can grow and develop *in vitro* on different media containing inorganic and organic nutrients and plant growth regulators that are added, creating an artificial growth environment, and either benefiting or negatively affecting growth. However, such nutrients may also contain impurities in the

chemicals. Therefore, the commercial source and grade of chemicals should be described precisely in any scientific paper. Macro- and micronutrients, which are fundamental constituents of a plant tissue culture medium, serve not only as nutrients but also regulate morphogenesis by signalling and modifying hormone synthesis in plants^{4,5}. Moreover, there is crosstalk between macro- and microelements⁶. Different nutrients, mainly micronutrients, however, may be added to the tissue culture medium also as impurities of the chemicals used for preparation of the medium solutions. One of the most common elements which is essential for plants, i.e. nickel (Ni), is added to media as an impurity⁷. Earlier studies have documented that the source, brand and quality of agar-agar can affect the growth and developmental outcome in plant tissue culture⁸⁻¹⁰ due to differences in their impurities and mineral composition as well as variation in their physico-chemical characteristics, such as gel strength and diffusion properties^{9,11,12}. Except for liquid culture systems and bioreactors, most plant tissue culture systems require a gelling agent that serves as a solid base for the explant. Studying seven different commercially available agars, Scholten and Pierik¹³ detected major differences in their mineral elements content, mainly Na, Cl, Fe, Ca, K, Mn and several trace elements (Cu, Cr, Cd, Ni). These mineral elements affected the growth and developmental outcome of a plant tissue or caused different growth disorders due to their mobilization into plant tissues. Scholten and Pierik¹⁴ further studied the effect of seven commercially available agars on shoot and root development of 20 plant species. Eighteen out of 20 species were sensitive to agar quality, but axillary shoot development of *Gerbera jamesonii* 'Joyce' and *Syringa vulgaris* 'rootstock A2' was insensitive to the agar source. The degree of sensitivity was found to depend not only on the plant species, but also on the developmental process. Adventitious shoot or root regeneration was the most sensitive developmental process compared to axillary shoot development or bulblet formation. The authors further concluded that in general more purified agars resulted in better growth. Those early findings were later confirmed by plant tissue culture studies of other plant species. There are several examples including *Ranunculus asiaticus* L. 'Elet-

tra'¹⁵, Marubakaido apple rootstock (*Malus prunifolia* (Willd.) Borkh.)¹⁶, rootstock Quince A (*Cydonia oblonga* Mill.)¹², and banana (*Musa × paradisiaca* L. 'Grand Naine')¹⁷.

The purity and quality of carbohydrates added to tissue culture medium may affect the growth and developmental outcome. Kodym and Zapata-Arias¹⁸ compared the effects of a Sigma sucrose (S5391; Sigma Chem., St. Louis, USA) and 13 commercial sugars originated from different sources on the micropropagation rate of banana (*Musa* 'Grande Naine'). They found that dark sugars resulted in a lower rate of micropropagation, very likely due to the presence of impurities (minerals, organic compounds, inhibitors) in them. Only 2 of the 13 commercial sucroses tested were comparable with the Sigma sucrose in terms of the micropropagation rate. A similar result was reported by Placide¹⁹, who detected that laboratory-grade sucrose (85% purity) was superior to table sugar in terms of the growth of banana (cv. 'Injogo') plantlets; both the growth rate and fresh weight of *in vitro* banana shoots were higher when laboratory-grade sucrose was used.

The iron chelate formula is also an important factor for plant development *in vitro*, as was proved for different plant species such as *Rosa hybrida* L. 'Moneyway'²⁰, pear rootstock 'OHF 333' (ref. 21), peach rootstock GF-677 (ref. 22), *Carlina onopordifolia*²³, and hybrid hazelnut (*C. avellana* L. × *C. americana* M. 'Geneva')²⁴. The purity and grade of different chemicals used for studying plant morphology or function are also important for scientific reproducibility. Carmine is a half-synthetic dye partly originated from *Dactylopius coccus* Costa, and used to stain nuclei or chromosomes. Its quality may differ according to the geography, agricultural practices, years and producers. The different staining quality of carmine from different sources is a well-known problem that can be minimized, but not totally eliminated²⁵.

Equipment and utensils used in a laboratory also have an effect on the growth and developmental outcome of plant material. The type and closure of culture vessels influence the quantity and quality of plant material by affecting the physical environment, such as light quantity and quality, temperature, ventilation, air/gas exchange and relative humidity,

thus directly affecting the growth and development of plant cells, tissues and organs²⁶. McClelland and Smith²⁷ studied the growth and developmental responses of five woody plant species (*Amelanchier spicata* (Lam.) C. Koch, *Acer rubrum* L. 'Red Sunset', *Forsythia × intermedia* Zab. 'Sunrise', *Malus × domestica* Borkh. 'McIntosh' and *Betula nigra* L.) in three vessel types, namely GA7 polypropylene vessels (350 ml), baby-food glass jars (200 ml) and glass tubes (60 ml). They concluded that the factor that significantly affected plant growth was the size/volume of vessels. Larger vessels showed a higher fresh weight and leaf area of shoot cultures in all species; however, the number and length of shoots also depended on the plant species. Rooting of shoots was better when the latter were previously cultured in either GA7 vessels or glass jars. When vessels were closed by parafilm, the growth response of plants was species-dependent; shoot length and density of *Betula nigra* increased when vessels were sealed with parafilm. Ironically, parafilm is a registered trademark of Pecheny Plastics Packaging, but the authors fail to describe the product as Parafilm[®], further emphasizing the details required about commercial products in scientific papers associated with plant tissue culture, in particular the aspect related to proprietary property, discussed briefly below.

Light transmittance and quality of light inside growth vessels, and more importantly, the gas exchange between vessels and the environment were different when four types of culture vessels (jam jars with metal caps, baby-food jars with metal caps, baby-food jars with Magenta B-caps, Magenta GA7 vessels) were used for the *in vitro* growth of four *Dianthus caryophyllus* cultivars ('Scania', 'White Sim', 'Angeline', 'Pink Calypso')²⁸. These differences significantly affected the multiplication coefficient (number of normal nodal segments per explant), and the number of normal shoots in three cultivars and shoot length in one cultivar.

Vessel closure (normal or perforated caps) and thus ventilation, both affected the growth (biomass, shoot length, number of leaves) of *in vitro* plantlets of *Artemisia annua* L. and the growth of microstructures, such as non-glandular and glandular trichomes on leaves²⁹. It is

thus imperative to define the commercial source of all utensils and equipment.

Proprietary considerations

Sometimes companies invest several million US dollars in product research and development. Apart from returns on business transactions, in the form of sales of products to scientists, one of the most important returns for a company is to have its product displayed publicly in successful research, within a scientific manuscript. Thus, a successful result, or interesting research, that uses an equally specific set of MPTs and CUEs, will inherently attract new clients, since other scientists who wish to repeat those experiments for other hypotheses, will require the same to do so. Thus, when the exact commercial source, grade and quality or purity are not defined, not only does the reproducibility factor slump, there is also failure in the due recognition of the proprietary owner of those MPTs and CUEs. This could, in real terms, translate into financial losses (or the lack of financial gains had the products been correctly indicated). This note does not aim to examine the legal aspects of the issue, but most certainly one can easily imagine that improper or incorrect product descriptions, not only negatively impact the reproducibility of a protocol, but may equally negatively impact the proprietary benefits of a scientific company, not unlike copyright.

Post-publication peer review as one possible solution to the problem

Given the importance of defining the commercial source of MPTs and CUEs, a tool is required to identify, and correct, the errors and gaps that exist in the literature. PPPR is a simple but effective way to identify problems with the literature, including gaps in knowledge related to these gaps in information³⁰⁻³⁴. Yet, like any other tool, its effectiveness is only measurable if there is a suitable channel to correct the literature after such errors or gaps are reported. Sadly, however, some editors or publishers are unresponsive to correcting the literature or are academically irresponsible, even if there is a mechanism available to correct errors. Thus, in such cases where policies are not in place to hold editors, journals

or publishers accountable for such gaps in the literature, correction of the literature can only come about when there is a mass change in consciousness among the peer community for that field of study. It is thus essential, as part of a painful and laborious process of PPPR, to begin to indicate, through the meta-analysis of small pockets of the literature, how widespread the problem of the lack of details regarding the use of CUEs as MPTs may be. Wherever possible, the authors should be contacted and the details of missing information, which may be increasing the irreproducibility factor, need to be published as corrigenda.

The situation for Committee of Publication Ethics (COPE) member journals or publishers might be quite a different matter, however. The COPE mandatory code of conduct for journal editors³⁵ states, among other clauses, the following, which could be interpreted as being relevant to the issue at hand, i.e. the need to clearly define the commercial source of chemicals, reagents, equipment and any other MPT/CUE:

‘1.1. Editors should be accountable for everything published in their journals.

1.2. Strive to meet the needs of readers and authors;

1.4. Have processes in place to assure the quality of the material they publish;

1.6. Maintain the integrity of the academic record;

1.8. Always be willing to publish corrections, clarifications, retractions and apologies when needed;

8.1. Editors should take all reasonable steps to ensure the quality of the material they publish;

12.1. Errors, inaccurate or misleading statements must be corrected promptly and with due prominence;

14.1. Editors should encourage and be willing to consider cogent criticisms of work published in their journal.’

In the same COPE code of conduct for journal editors, the following best practice is also listed: ‘ensuring that appropriate reviewers are selected for submissions’ (p. 3 of the code). This implies that any reviewer (and also by association, handling editor and even editor-in-chief (EIC) who provided final approval) who has not indicated to the authors that the commercial source of any MPT/CUE needs to be defined is either: (a) incompetent; (b) poorly vetted; or (c) not completing the required

reviewer/editorial responsibilities efficiently³⁶. Thus, if a member of the public peer pool requests a journal to indicate the commercial source of any MPT/CUE that is missing in its journal, then it is the responsibility of the editor, or EIC, to contact the authors to obtain that information. In the case where such information cannot be obtained, or where the authors have not responded (e.g. retired or deceased authors), then it is the responsibility of the editor/EIC to issue an expression of concern if the lack of information prohibits confident replication of the protocol, either subjectively expressed, or implicitly shown through negative results. If this situation exists in a COPE-member journal, irrespective of the complainant (named or anonymous), and the editor/EIC fails to correct the academic record, then that editor/EIC is in direct contravention of the mandatory COPE code of conduct for journal editors, and should at first face a warning. If the record continues uncorrected, such editors should face disciplinary action. The publisher then has the responsibility of stepping in to ensure that the code of conduct for journal editors is respected, and fully implemented, removing such an editor/EIC, if necessary. In such a case, the onus of correcting the literature then falls directly on the publisher.

Conflict of interest. The authors declare that this study was conducted in the absence of any commercial, financial or other relationships that could be construed as a potential conflict of interest.

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Jaime A. Teixeira da Silva*, P. O. Box 7, Miki-cho Post Office, Ikenobe 3011-2, Kagawa-ken, 761-0799, Japan; Judit Dobránszki is at the Research Institute of Nyíregyháza, University of Debrecen, Nyíregyháza, P.O. Box 12, H-4400, Hungary.
*e-mail: jaimetex@yahoo.com