Guidelines for Measuring and Reporting Environmental Parameters in Controlled Environments used for Plant Tissue Culture Experiments

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ICCEG and UK CEUG

History of plant tissue culture

- Originally arose out of the quest to germinate orchid seeds – flasking
- Morel (1960) demonstrated virus-free cloning of *Cymbidium* orchids
- Street (1970s) demonstrated callus production from carrot and later regenerated plants from callus

History of PTC

- Empirical experimentation in defining PTC media
- Murashige and Skoog, 1962
- First stage in standardising PTC
- Ubiquitous medium – has led to standardisation of media preparation by big companies to ISO 9001 (and up) specifications

Four important elements of PTC

- medium
- aseptic handling and manipulation
- culture vessels
- incubation in a culture room

The stages of PTC

- Explant production from an *in-vivo* plant
- Media making – *macro- & micro-nutrients, sugar, hormones, agar/liquid, + additives (vitamins, anti-oxidising agents, slow release agents, antibiotics), pH adjustment*
- Autoclaving and pouring into culture vessels
- Sub-culturing – placing explants into vessels
- Incubation in CE
- Sub-culturing or rooting
- Weaning

Variation

- Variation in almost all of the previous factors can lead to variation in performance
- Thus need standardisation of processes and procedures
- Commercial manufacture of tissue ingredients is subject to Standards and Procedures (e.g. ISO 9001)

Applications of PTC

- Commercial micropropagation
  - 45 million plants per year produced for the home market in Holland alone
  - 75% of the Scottish seed potato crop is produced by micropropagation
- Research tool
  - PTC is the delivery technology for GM plants
  - PTC is a delivery technology for mutation
  - PTC is used as a germination technology for *Arabidopsis* propagation
  - PTC is used as an investigative tool for plant physiology

The basics of CEs for PTC

- Warm stable temperature (20 - 25°C) – most tend to overheat → air conditioning
- Fluorescent lights – more for photomorphogenesis than for photosynthesis thus often at low PPFD's less than 50 µmol m^{-2} s^{-1} PAR (lots of variation here!)
- Long photoperiods – 16 h
- Room RH not so important as culture vessels maintain 99%+ RH

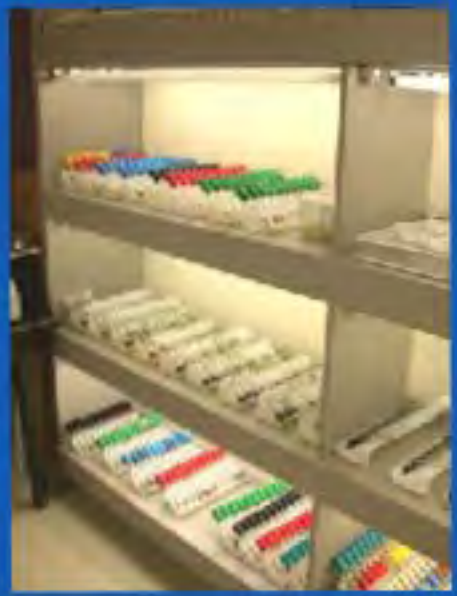
The need for accurate CE reporting

- Reproducible results
  - Commercially important – often will have more than 1 CE room for PTC
- Repeatable experiments
  - From researcher to researcher
  - From laboratory to laboratory
- For spotting artefacts or serendipity

Commercial micropropagation

- Large scale walk-in rooms
- Large numbers of lights
- Heat generation problems
- Big refrigeration plants and large volume air circulation

Research tool



Diversity in CEs abounds!

Innovations in CE design for PTC

- Shelf design – to remove excess heat on a shelf
  - Water cooled/heated shelves
- Efficiency of light usage – rotating shelves past vertical lights
- High volume air exchange
- Light quality and quantity

Innovations in CE design (contd)

- Media additives – e.g. antioxidants
- Vessel design
  - Glass - rigid plastic – gas permeable films
  - Vents
- Temporary immersion
- In-vessel measurements
- Manipulation of CO_2 levels

The formulation of the Tissue Culture Guidelines

- Followed on from the ICCEG's 'Minimum Guidelines' published in 2004
- International sub-committee established
  - Jacques Boccon-Gibod, Institut Nationale d'Horticulture, France
  - Geoff Holroyd (first chairman), University of Lancaster, UK
  - Julian Franklin, Rothamsted Research, UK
  - Yoshi Kitaya, Osaka University, Japan
  - Chieri Kubota, University of Arizona, UK
  - Philip Larkin, CSIRO Canberra, Australia
  - Mick Fuller, University of Plymouth, UK
  - Steve Millam (second chairman), Chichester College, UK
  - Lynton Incoll (third chairman), University of Leeds, UK

Issues of debate

- What to measure?
  - Temperature, PFD, photoperiod, RH, CO_2, CE construction, shelf construction, air circulation
- Where to measure?
  - Room space, shelf space, in-vessel, air intake/outlet
- How often is it necessary to measure?
  - Beginning and end of experiment, constantly
- What else to report?
  - Details of vessels, media, lighting sources

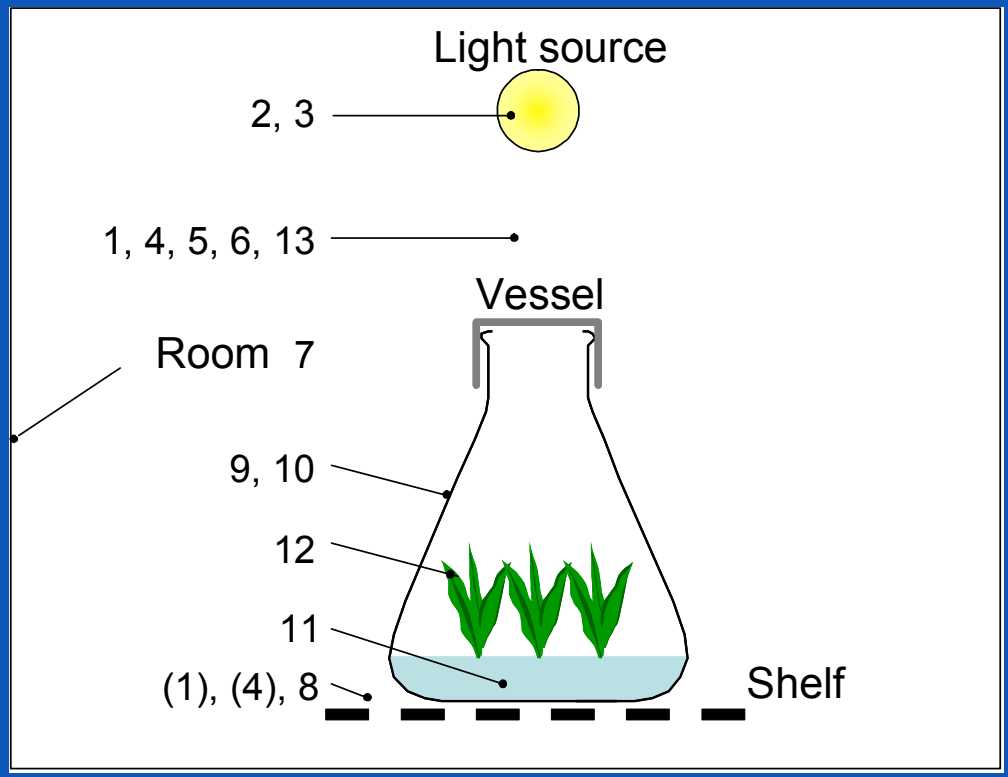
Resolution of the debates

- Acknowledge that these were not always unanimous and a lot of email traffic has passed over the cyberspace and resolutions sometimes gave way to what is possible rather than what is desirable! e.g. in-vessel measurements
- Decided to split parameters into 2 groups
  - Primary Parameters
  - Specialist Parameters

Basic layout of the Guidelines

- Endorsement by the 3 CE groups (Europe, USA and Australasia)
- Explanation as to why the guidelines are necessary
- What to measure, Where to measure, When to measure and What to report (including units)
  - (Table format for easy reference)
- An illustration of where to measure
- An example of a good practice write-up in a published paper

What and where to measure



1. Radiation
2. Light source - properties
3. Photoperiod
4. Air temperature
5. Atmospheric moisture
6. Air circulation
7. Room – properties
8. Shelf - properties
9. Vessel - properties
10. Vessel - alignment
11. Culture medium
12. Number of explants
13. CO_2 concentration

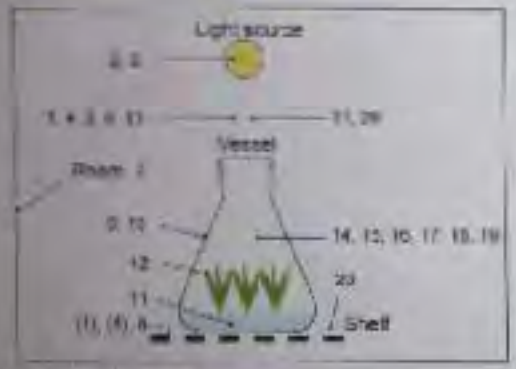
( ) *Optional measurement*

Introduction to guidelines

Tissue culture is a very significant tool for plant propagation and horticulture and is a research technique for plant physiology and molecular biology. Facilities vary from low-tech equipment through off-the-shelf incubators to state-of-the-art suites of cabinets and rooms. In all cases, accurate environmental records are essential to standardise and maximise growth of cultures and to facilitate valid replication of experiments between different facilities.

The primary critical parameters common to most plant tissue culture facilities can be monitored and recorded relatively easily. The Table (over page) gives guidance on how to monitor and record these primary parameters, most of which ideally should be monitored at the location of the cultures (see Figure below). Facilities with automatic control systems usually measure temperature at the air re-circulation intake, which can differ significantly from that

Where to measure parameters



Key to Figure:

Primary parameters		Specialist parameters	
1	Radiation	14	Air temperature
2	Light source properties	15	Atmospheric moisture
3	Photoperiod	16	Radiation
4	Air temperature	17	Spectral distribution of radiation
5	Atmospheric moisture	18	CO_2 concentration
6	Air circulation	19	Air exchange rate of vessels
7	Room - properties	20	Air velocity
8	Shelf - properties		
9	Vessel - properties		
10	Vessel - arrangement		
11	Culture medium		
12	Position of cultures		
13	CO_2 concentration		

Notes:
Shelf level = as close as possible to top of shelf. Vessel level = about mid-way between shelf and top of vessel.
Specialist parameters 14 to 19 are important inside a vessel. (): optional

above a shelf. If several shelves are used in an experiment, then each shelf should be monitored.

Many research facilities have more elaborate recording equipment, or may be able to record a wider range of environmental parameters. The real environment of a tissue culture is inside the vessel. The most advanced facilities may have equipment available to carry out extremely detailed and technically difficult measurements inside vessels, including spectral distribution of radiation. The thirteen primary parameters and the seven most important specialist environmental parameters are identified and the location of their measurement is illustrated (see Figure). For details on the specialist parameters and their interactions with primary parameters see Fujiwara and Kozai (1995) [1]. If specialist parameters are measured they should be reported.

How to report your experimental conditions

Here is an example of a report suitable for publication:

"The experiment was conducted in a walk-in growth room (model, manufacturer) (11.2 m^2 floor area and 2.1 m ceiling height), with horizontal air circulation through perforated sidewalls and four stacked steel-mesh shelves (24 m^2 total shelf space). Sufficient outdoor make-up air was provided to maintain ambient CO_2 concentrations in the room. Cool white fluorescent lamps (model, manufacturer) mounted 40 cm above each shelf provided an average photosynthetically active radiation (PAR) of 50 (s.d. ±7) μmol m^{-2} s^{-1} above the culture vessels during the 16-h photoperiod. Air temperature above the culture vessels was 25/20 (s.d. ±1)°C during the light/dark period. Relative humidity above the culture vessels was 67 (s.d. ±10)%.

Ten plantlets were cultured in 200 mL glass Erlenmeyer flasks sealed with translucent plastic film. Each flask contained 40 mL of medium with Murashige and Skoog (1962) basal components, 30 g L^{-1} sucrose, 5 g L^{-1} of activated charcoal and 8 g L^{-1} agar. The pH of the medium was adjusted to 5.8. The flasks were in a single layer on each shelf with sufficient spacing to allow adequate air movement around each flask. No environmental parameters were recorded inside the flasks."

[1] Fujiwara, K. and Kozai, T. (1995) Physical microenvironment and its effects. p. 305-369. In: J. Aitken-Christie, T. Kozai and M.A.L. Smith (eds) Automation and Environmental Control in Plant Tissue Culture. Kluwer Academic Publishers, Dordrecht, Netherlands

International Committee for Controlled Environment Guidelines

Guidelines for Measuring and Reporting Environmental Parameters for Experiments in Plant Tissue Culture Facilities

Sponsored by and published for the UK Controlled Environment Users' Group, the North American Committee on Controlled Environment Technology and Use (NCERA-101), and the Australasian Controlled Environment Working Group

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Measuring and Reporting Environmental Parameters for Experiments in Plant Tissue Culture Facilities: *Table of Primary Parameters*

International Committee for Controlled Environment Guidelines

What to measure	Units	Where to measure	When to measure	What to report
Radiation (PAR*)	µmol m^{-2} s^{-1}	a) At vessel level, at uniform height throughout. (See Figure) b) Optional, at shelf level, at centre of empty shelf**	At start of experiment, and every 4 weeks (***) As above	Mean and standard deviation. Radiation sources (type, model and manufacturer), and distance from shelf) As above
Photoperiod	h		At start of experiment	Duration of light and dark periods
Air temperature	°C	a) At vessel level Location of sensor is crucial, and should be independent of the facility's temperature control sensor b) Optional, at shelf level, at centre of shelf, outside container	Daily during each light and dark period, at least 1 hour after light/dark changeovers As above	Mean and standard deviation for light and dark periods As above
Atmospheric moisture (relative humidity or vapour pressure deficit)	% or kPa	At vessel level and independently of the facility's humidity control sensor	Daily during each light and dark period, at least 1 hour after light/dark changeovers	Mean and standard deviation for light and dark periods
Air circulation		At vessel level	At start of experiment	Record whether perforated shelves, walls, ceiling, floor or ducts, and horizontal or vertical flow. Record source of fresh air
Room or cabinet properties			At start of experiment	Size (floor area m^2, ceiling height (m) and type (walk in/reach in) Manufacturer and model if available. Indicate if it has special features e.g. rotating shelves, light reflectors, bottom cooling of shelves
Shelf properties			At start of experiment	Area (m^2), type (solid or mesh, steel, wood or transparent), number (stacked, not stacked) and construction. Note if shelves are bottom cooled by air or water
Vessel specifications (It is appreciated that a range of vessels may be in use)			At start of experiment	Type (flasks, dishes, bottles, jars) and materials (glass, plastic) Size/volume (mL) Closure type and additional seal or vent
Vessel alignment		On each shelf	At start of experiment	Number of vessels and number of layers (if vessels are stacked) per shelf
Culture medium			At start of experiment	Solid, gel, or liquid (or combination). Type and make of gelling agent, pH Volume per vessel (mL) Mineral composition (macro- and micro-elements) Carbon source, growth regulators, vitamins and their concentrations, also whether activated carbon and other additional substrates are in use
Number of explants		In each vessel	At start of experiment	Initial number of explants
Atmospheric CO_2 concentration	µmol mol^{-1}	At vessel level, at centre of shelf	Daily (or only if CO_2 enrichment is installed within facility	Mean and standard deviation

* Referred to as photosynthetically active radiation (PAR; 400-700 nm) for general usage and described as photosynthetic photon flux density (PPFD) by many journals, professional societies and manufacturers of quantum sensors.

** If lamps are arranged at the back of the shelf rather than above the shelf this should be stated and PAR measured at the back and the front of an empty shelf.

*** Fluorescent lamp efficiency declines significantly within weeks of installation and gradually thereafter and such lamps therefore require a regular monitoring and replacement programme.

Other forms of output

- Large Poster
  - For display at other meetings, seminars and conferences – together with copies of the guidelines leaflet
- Small Poster
  - For display around CE facilities, in laboratories and for dissemination by post to all members and others
- Web addresses where the Guidelines and the Posters can be found

Importance of dissemination

The guidelines will only make an impact if:

- Everyone puts a poster up in their facility
- If future users (students) are made aware of them
- If authors of papers refer to them in their manuscripts
- If non-members users are made aware of them
  - At other meetings
  - By mailing
  - By articles and advertising in plant journals

Don’t forget to

- Pick up your copies of the leaflet or order some through your user group
- Visit the poster display
- Disseminate
  - To your colleagues
  - To your students
  - To your users

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- Lynton Incoll
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