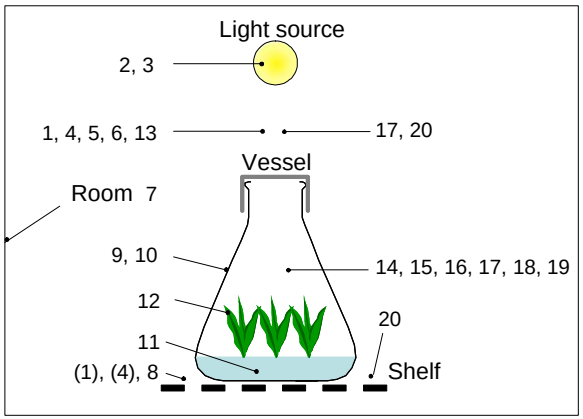
Introduction to guidelines

Tissue culture is a very significant tool for plant propagation and biotechnology 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 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 experimental 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.

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	**Notes:**	
9.	Vessel - properties	Shelf level = as close as possible to	
10.	Vessel - alignment	top of shelf. Vessel level = above but	
11.	Culture medium	as close as possible to top of vessel.	
12.	Number of explants	Specialist parameters 14 to 19 are	
13.	CO_2 concentration	measured inside a vessel. () = optional	

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 m² floor area and 2.1 m ceiling height), with horizontal air circulation through perforated sidewalls and four stacked steel-mesh shelves (24 m² 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. 319-369. 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

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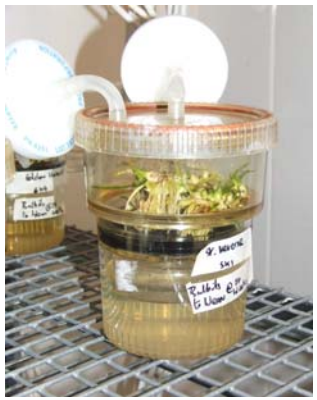

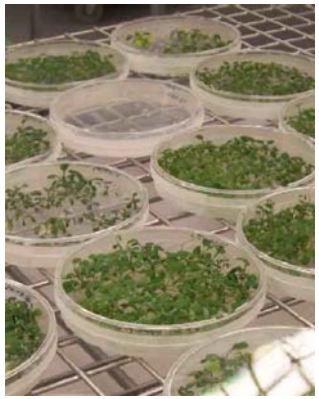

Measuring and Reporting Environmental Parameters for Experiments in Plant Tissue Culture Facilities: Table of Primary Parameters

International Committee for Controlled Environment Guidelines

What to measure	Units	Where to measure	When to measure	What to report
Radiation (PAR[1])	µmol m^{-2} s^{-1}	a) At vessel level, at uniform height throughout. (See Figure)	At start of experiment, and every 4 weeks [2]	Mean and standard deviation. Radiation sources (type, model and manufacturer, and distance from shelf)
		b) Optional, at shelf level, at centre of empty shelf [3]	As above	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	Daily during each light and dark period, at least 1 hour after light/dark changeovers	Mean and standard deviation for light and dark periods
		b) Optional, at shelf level, at centre of shelf, outside container	As above	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	Types (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 combinations). Type and make of gelling agent. pH Volume per vessel (mL) Mineral composition (macro- and micro-nutrients) 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 but only if CO_2 enrichment is installed within facility	Mean and standard deviation

[1] 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

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

[3] 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