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Environmental Factors Affecting Plant Tissue Cultures ©

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INTRODUCTION

Environmental factors have many effects on plant growth and development. Indeed, plant propagators often take great care in managing the nursery environment to optimise plant propagation and growth. The environment includes physical or chemical (abiotic) and biotic components. In the nursery the biotic factors include not only insects and microorganisms but also other plants, including weeds. What may be less obvious in the nursery is that plants in turn may affect their environment. This interaction between the plant and its environment is the scientific discipline of "ecology." The main message of this paper is that plants growing in plant tissue culture (or in vitro) are also subject to these same interactions, hence the research field of "in vitro ecology." In this short paper I am going to focus on two aspects of the culture environment, light and gas exchange (ventilation).

Light can be described and measured by several characteristics, each having various effects on plants: quantity (intensity x duration), photoperiod (light-dark cycles), quality (colour or wavelength), and direction. Each of these parameters of light are associated with particular aspects of plant growth and development.

PHOTOSYNTHESIS AND PHOTOAUTOTROPHY

The best known effect of light is as an energy source for plant growth via photosynthesis. Light of particular wavelengths is absorbed by the pigment chlorophyll to convert carbon dioxide and water to carbohydrate. The importance of photosynthesis for plants in vitro has gone full circle from the early assumption that it was insignificant and unnecessary, because sugar is provided in the media, to current recognition that it is not only possible but can provide substantial benefits, under the right conditions. Photoautotrophy, the ability of cultures to obtain their sugar (carbohydrate and energy) through photosynthesis, has been demonstrated for many species and is routinely practiced in micropropagation laboratories. It requires a reduction or removal of sugar in the medium, higher light intensity, and aeration (venting) of the containers to enable gas exchange, particularly the supply of carbon dioxide. When used during the final culture cycles, the reduced humidity inside the container has the additional advantage of hardening the plants ready for deflasking. This aspect of light in vitro has been well covered at previous I.P.P.S. meetings, e.g., the article by Chieri Kubota (Kubota, 2002).

Photosynthesis is dependent on the total input of light within the photosynthetically active radiation (PAR) range. This is a function of the light intensity and duration of exposure of the cultures and is best described by the term PPFD (photosynthetic photon flux density) — a measure of the total number of photons (units of light energy) supplied to the plants. In practice it depends on the output of the light source, the distance from the plant surface, and the material of the culture vessel. The PPFD required for efficient photosynthesis is higher than that usually supplied in growth rooms (Table 1).

Table 1. Typical light intensities.

Light source	$\mu mol \cdot m^{-2}s^{-1}$
White fluorescent tube	10–50
Compensation point	20
Autotrophic cultures	65–250
Glasshouse	100–1500
Sunlight	>2000

PHOTOPERIOD

Photoperiod, the relative duration of the daily periods of light and dark, is a well established phenomenon in the control of plant growth. It is particularly known for its effects on control of flowering but may also influence vegetative growth cycles and the occurrence of dormancy. A related but distinct effect is that the total duration of light also affects the cumulative light input for photosynthesis.

LIGHT QUALITY

The term "light quality" refers to its colour or the range of wavelengths. Light, or more correctly radiant energy, occurs across a wide spectrum of wavelength only part of which is visible to humans (Fig. 1). Plants respond to light because particular pigments in the cells absorb light at certain wavelengths. White light includes the radiation across the visible spectrum but may be accompanied by invisible wavelengths, including infra red and ultra violet (UV). Radiation in the invisible bands contributes to the heat load and particular wavelengths may also be toxic to plants.

There is considerable interest in the use of various shade cloths and screening materials to modify the light input to plants in the nursery and even in the field. The type of light source and the light transmission characteristics of culture containers affect the quality of the light reaching plants in vitro (Fig. 1). Modifying the spectral composition can alter the growth rate or morphology of the plant. It can promote or inhibit flowering. The actual response varies widely between plant species. Some general response are listed in Table 2, but there are many exceptions with individual plant species.

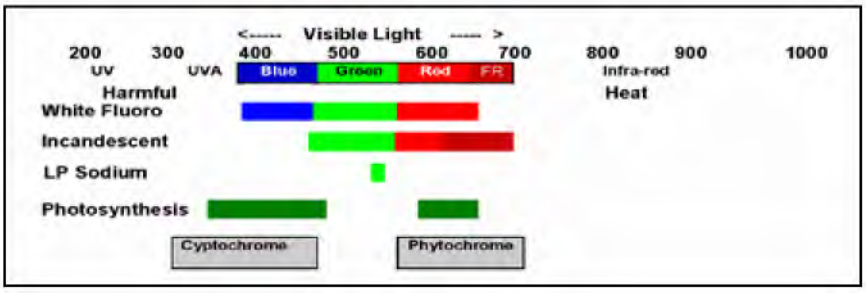

Figure 1. The radiant energy spectrum with bands of light that affect plant growth.

Table 2. General responses of plants to light quality.

Red + blue	Increased net photosynthesis
Blue or blue + far red	Reduced photosynthesis
Red or red + far red	Increased stem length by internode elongation not leaf number
Blue or blue + far red	Reduced internode elongation
Red + Far red	Reduced leaf area

Growth responses to light (photomorphogenesis) are mostly regulated by the relative supply of light in the blue, red, and far-red bands of the spectrum. Note that fluorescent lights lack light in the red-far red range and therefore an additional light source (incandescent globe) is required for photomorphogenic responses. Photomorphogenesis is regulated via specilised chemicals that are activated or de-activated by specific wavelengths of light. Only small quantities of light are required.

The spectrum of light can be manipulated using assorted filters or screening materials; however a more precise method is now becoming economic for small-scale purposes, such as in tissue culture growth rooms, using light emitting diodes (LEDs). These solid-state electronic devices produce light in very specific wavelengths. They have the added advantage of a low energy requirement and conversely produce very little heat but because the light output is small, large numbers may be needed to get adequate coverage.

Research recently published on chrysanthemum (Kim et al., 2003) illustrates the type of responses to light quality using LEDs (Fig. 2). The shoots are elongated and leaf size is reduced under red or blue light but growth is more normal under blue + red. Note also the differences in root growth. With strawberry 70% red + 30% blue gave the best dry weight and increased leaf number (i.e., internode number) whereas 100% red gave the longest internodes (Nhut et al., 2003). The principle here is that the shape of the plant can be manipulated by varying the mixture of red and blue in the light source.

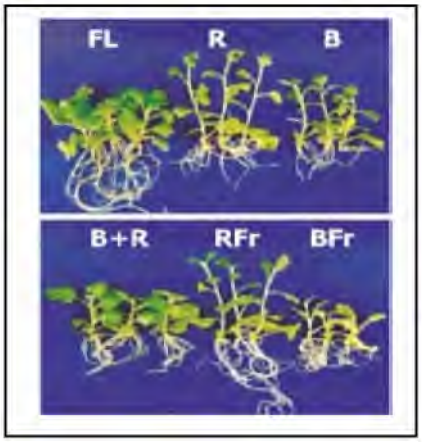

Figure 2. Light quality affects *Chrysanthemum* plantlet form.

FL=fluorescent; B=blue; R=Red; FR=FarRed light. From Kim et al. (2003).

Overall plant growth is dependent on the supply of carbohydrate and energy. Perhaps more importantly, the survival of plants during that critical period following deflasking is dependent on the supply of carbohydrate stored in the plant tissues. Traditionally this has been supplied to in vitro plants as sugar in the medium but, as discussed above, autotrophy is possible under the right conditions. However, the quality of light can also affect net photosyn-

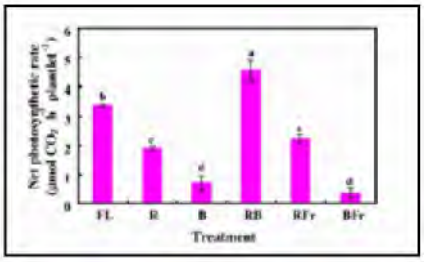

Figure 3. Effect of light quality on photosynthesis in chrysanthemum cultures. From Kim et al. (2003).

thesis. In particular, exposure to red + blue increased photosynthesis of chrysanthemum plantlets in culture (Fig. 3) and more importantly, these plants still had 30% more dry weight 45 days after planting out.

VENTILATION

Tissue culture containers are traditionally sealed to exclude microorganisms and to conserve moisture, but we need to reconsider. Firstly, as mentioned above, one factor limiting photosynthesis in vitro is gas exchange to maintain the carbon dioxide levels in the headspace of the container, e.g., with grapevine cultures (Shim et al., 2003) (Fig. 4). The conundrum is that increasing ventilation of culture vessels increases water loss. Secondly, it has been amply demonstrated that reducing the humidity in the culture vessel in the culture cycle before deflasking helps to harden the transplants against water stress. How do we manage this conundrum?

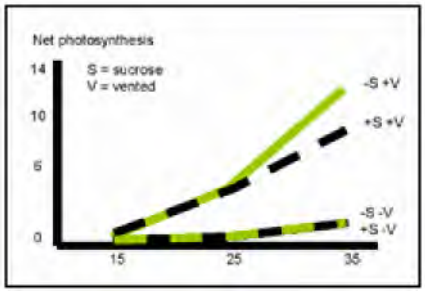

Figure 4. Ventilation effects on grapevine shoot cultures. From Shim et al. (2003).

Fortuitously, for autotrophic culture we can (need to) delete sugar from the medium. This alone greatly reduces the risk of microbial contamination. The balance between gas exchange and conservation of water can be maintained by the use of semi-permeable enclosures that allow gas exchange but limit water loss. There are various films and membrane vents that allow good gas transfer but little water. There are custom-made culture vessels incorporating this technology. In practice, small changes in the sealing of culture containers can have a significant effect. Often just leaving the lids slightly loose is sufficient.

Note also that the extent of ventilation can also have marked effects on the growth pattern of the plantlets, e.g., with *Annona* (Zobayed et al., 2002) (Table 3).

Table 3. Ventilation effects on *Annona* cultures.

	Sealed	Natural	Forced	N + F*
Days to initiation	6.0	8.5	13.5	
No. shoot + buds	0	48.7	25.5	39.2
Shoot length (mm)	-	5.0	17.0	12.0
No. nodes	-	1.1	3.0	1.6
Leaf area/shoot	-	0.5	2.0	1.2

*N-2 weeks then F-5 weeks

From Zobayed et al., 2003.

THE MESSAGE

I have only briefly covered selected aspects of "in vitro ecology" and the implications for plant tissue culture practice. An important practical message here is that often subtle differences in technique of handling the cultures can make a difference to the performance of the plants. Often these differences go unnoticed, but they may well explain some of the variability laboratories and nurseries experience between batches of plants.

The quality (and intensity) of light can change as the light source ages. Stray light from a window or the glow from a nearby warning light (that new exit sign above the door!) may be sufficient. What about the paint on the culture room walls? This could affect the quality of reflected light. Changes in the composition or colour of containers or lids affect the light transmitted into the plants as well as the pattern of gas exchange.

The tissue culture environment is complex and dynamic and has marked effects on plant growth both during culture and after planting out. Different plant species also respond differently. We still have much to learn but we can start by being aware of the possibilities.

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