AERATION STRESS IN PLANT TISSUE CULTURES

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Summary. The requirement for sterility in plant tissue cultures avoidance of dehydration can impose sealing requirements that severely limit the rate of gas exchange in and out of the culture vessel. Conditions within the culture vessel such as the depth of any water cover, the presence of gelling agents, the bulk and porosity of the tissue and the temperature also strongly influence *in vitro* rates of gas exchange, primarily driven by diffusion. This article uses elements of Fick's Law of Diffusion to identify key factors limiting gas exchange between culture and its immediate surroundings. In particular, it identifies static liquid media, gelling agents, large tissue mass and warm temperatures as imposing severe limits on diffusive flux for gases such as O_2, CO_2 and ethylene. The principle barrier to diffusive exchange of gases between the *in vitro* and *ex vitro* atmospheres is the wall of the enclosing vessel. This is invariably made of glass or plastic that is gas-impermeable and well-sealed against evaporative drying or entry of micro-organisms. Cultures enclosed in this way will, inevitably, asphyxiate unless a compensating pathway for diffusive gas exchange is contrived or replaced by some system of convective flow that carries gases to and from the tissue. Supplementing diffusive aeration with convective flow is the basis of most successful hydroponics systems for whole plants and may be a prerequisite for securing levels of aeration suitable for autotrophic cultures. The paramount consideration is the extent to which the total rate of consumption or production of a particular gas by the cultured tissues is matched by the maximum rates of gas transport imposed by the culture itself, its immediate surroundings and the ventilation and sealing system of the culture enclosure.

Introduction

Techniques of plant tissue culture are a stimulus for basic research and also a tool with potential and proven applications in agriculture, horticulture and forestry. Plant tissue

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culture has wide application, encompassing vector and vector-free genetic transformation of cells, embryo rescue, somatic embryogenesis and commercial clonal propagation. Much plant biotechnology requires at least one stage where plant tissue culture is obligatory (Thorpe, 2000). For success, the conditions under which cultures are housed and treated must be strictly adhered to and many papers record how minor changes to the conditions, especially chemical components of the culture medium, enable hitherto recalcitrant cultures to perform in the desired manner.

Ventilation is an issue because the technology of tissue culture is inextricably bound up with a need for sterility and preventing dehydration. Such methods almost always involve some form of sealing of culture vessels. Inevitably, this interferes with the free exchange of gases between the cultured material and the outside atmosphere. The problems can be exacerbated by use of liquid cultures where cells, callus or tissues can be immersed in liquid medium. Much of what is known about the consequences of the resulting poor aeration for healthy plant growth or survival can be inferred from studies of flooding or submergence experienced by plants in the outside world (Vartapetian and Jackson, 1987). Here too, gas exchange is severely compromised by virtue of a dramatic slowing of gas diffusion in and out of the inundated tissue imposed by water. Well-sealed plastic, glass culture vessels and the nutrient medium they contain impose much the same aeration stress since they also impede gas exchange severely. One major difference between the well-sealed tissue culture vessel and plant submergence (the most extreme form of flooding stress) is that in the later case, well-adapted species such as *Callitriche platyarpa*, rice (*Oryza sativa*) and *Rumex palustris* can escape by means of an invigorated underwater elongation that renews contact between shoot and aerial environment (Jackson 1990; Voesenek and Blom, 1999). This is obviously not an option for plant material growing in culture vessels.

Despite the self-evident importance of the gaseous environment for tissue cultures, it is an often neglected component of their technical specification. This shortcoming can have unwelcome consequences for culture performance because of the strong physiological impact of the gases involved, notably O_2, CO_2 and C_2H_4 (Jackson et al., 1994). This article summarises the processes of tissue culture aeration and highlights some recent developments intended to ensure its adequacy.

Normal plant aeration

The gases of greatest concern are O_2, CO_2 and ethylene. Oxygen and CO_2 are principal substrates or products of aerobic respiration and photosynthesis and thus intrinsic to the most basic life-sustaining metabolic pathways of plant cells. By contrast, ethylene is a plant hormone capable of influencing developmental processes such as cell expansion, senescence and differentiation at relatively small concentrations [0.01–10 ppm (v/v)]. The gas is correctly considered as a growth regulator rather than a toxin. The lack of metabolic breakdown and the ease with which it can be measured at small

concentrations make it a suitable marker of aeration status (Jackson et al., 1987). Other gaseous growth regulators such as methyl jasmonate and nitric oxide may also influence tissue culture development but these have received relatively little research attention in this context.

All living tissues of higher plants, not just the photosynthetic parts, require unimpeded gas exchange to function normally. Figure 1 provides some approximate figures illustrating the scale of gas influx and efflux that underpins normal plant growth and development. Severe impediment to gas movement more than a few hours is usually fatal to growing cells, while partial interference can influence growth rate and the pattern of development. As the term gas exchange implies, both inward and outward efflux of gases are slowed or arrested. This leads not only to a shortage of gases normally provided by the environment but also to an internal accumulation of gases made by the plant and normally excreted at a similar rate by outward diffusion. Thus, when gas exchange is slowed by external conditions plant cultures will experience shortages of oxygen and (in the light) carbon dioxide while accumulating ethylene, a plant hormone that can have a range of responses depending on tissue sensitivities. Other plant-made volatiles such as ethanol and acetaldehyde (Righetti et al., 1990), the free radical nitric oxide and the hormone like molecule methyl jasmonate may also build up, effects of these have not been explored to any great extent but deserve investigation. Nitric oxide could be generated from nitrite produced by nitrate reductase activity. It can be harmful by causing oxidative damage via the production of oxygen superoxide (O_2^-) as well as being a possible signalling molecule for certain developmental processes (Sakihama et al., 2002). There is a Clarke type NO electrode and a fluorescence method for NO detection and it can also be measured by laser photoacoustics.

Aeration by diffusion

The principal mechanism that propels gas exchange in and out of a plant tissue is net diffusion down concentration gradients. The process is summed up by Fick's Law of Diffusion that can be expressed as $Q/t = D * A(C_{in} - C_{out})/Th$, where Q/t = amount of gas moved per second (flux density); D = Diffusion coefficient of the medium through which the gas is moving; A = cross sectional area of diffusion pathway; C_{in} = concentration at start of pathway; C_{out} = concentration at end of pathway; Th = length of diffusion pathway. Factors that influence the components of this equation and thus impact on the rate of gas transport (flux density) are now examined.

Flux density

Flux density (Q/t) is the first term in the Fick's Law equation ($\mathbf{Q/t} = D * A(C_{in} - C_{out})/Th$). Its value is affected by the physical environment of the tissue and the morphology of

the tissue itelf. Whatever the maximum possible flux of a gas to the surface of the tissue or cell turns out to be in a given set of circumstances, it must be judged against what the tissue or cell actually requires to sustain normal rates of metabolism or to keep internally generated gases below some activity threshold. Oxygen flux density in relation to root growth will be used as an example, in a situation where the maximum oxygen flux rate to a root is depressed by the high diffusion resistance imposed by submergence in water. Cereal root respiration rate in the elongating apex can be in the order of $1.56\,\mu mol.kg^{-1}.s^{-1}$ or 50 ng O_2 cm^{-2} of root surface min^{-1} at $10\,^{\circ}C$. The expression using surface area is especially useful because it highlights the importance of area available for gas capture or release. Using platinum electrodes, Blackwell and Wells (1983) showed that when the equilibrium concentration of dissolved oxygen in the flooded soil fell from 21 % to 15 %, in response to consumption by micro-organisms, the maximum possible inward flux density of O_2 toward the root decreased from > 80 ng O_2 cm^{-2} of root surface min^{-1} (the value for freshly flooded soil) to below 30 ng O_2 cm^{-2}.min^{-1}, a value close to the respiratory demand of the root. At this point, root elongation became retarded. They also showed that, for elongation to be stopped completely, the flux density must fall almost to zero. From this study we learn that (i) some loss of gas diffusion capability for O_2 can be sustained without damage (from > 80 ng O_2 cm^{-2}.min^{-1} down to < 30 ng O_2 cm^{-2}.min^{-1}, in this case), (ii) to depress growth, flux densities must fall to below those potentially generated by rate of respiration and (iii) an almost total arrest of O_2 influx is needed to stop growth and possibly kill the root.

The seeming tolerance of quite substantial decreases in O_2 flux density is partly attributable to the large amount of O_2 present in the air (21 % v/v)**.** This ensures a maximum flux density in water that can often exceed the needs of respiring tissue (see below for qualifications). This can be true, in well-stirred water, despite the limited solubility of O_2 in water that reduces the concentration from 8.31 mol.m^{-2} in air to 0.25 mol.m^{-3} in water (at $25\,^{\circ}C$). An exception may be roots in tissue culture growing at optimal temperatures and supplied *in vitro* with external sugar (Asplund and Curtis, 2001). Here, local root-tip respiration is so intense that fluxes through well-stirred water in equilibrium with air are too slow to sustain maximum repiration rates. A second explanation for the insensitivity of tissue to modest depressions in O_2 availability is that the affinity for oxygen shown by cytochrome oxidase, the terminal oxidase in mitochondrial electron transport, is extraordinarily strong (K_m approx. 0.14 mmol.m^{-3}). It is instructive to compare these figures with those for CO_2, the photosynthetic progenitor of environmental O_2. CO_2 flux densities required to support photosynthesis are similar or higher than those required to support respiration with external O_2 (Fig. 1). Although CO_2 is also much more soluble in water than O_2; at $25\,^{\circ}C$ (the ratio of dissolved CO_2/gas-phase being approximately 0.76 at neutral pH, while the ratio for oxygen is only 0.03), the small amount of CO_2 in air (approx. 360 ppm, v/v) is insufficient to generate dissolved concentrations that match those of dissolved O_2. At
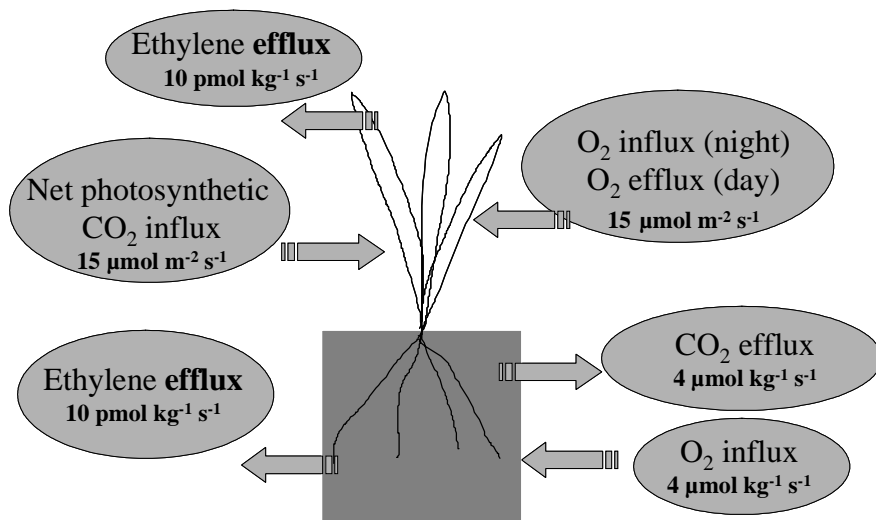
Fig. 1. Schematic illustration of the pattern and approximate scale of gas efflux and influx that underlies normal plant growth and development. Sources for gas flux data include: Haupt-Herting and Fock (2002); Jackson (1980), Armstrong *et al* (1991)

$25\,^{\circ}C$, CO_2 concentrations may attain only $11.4\,mmol.m^{-3}$ compared with the concentration of O_2 in aerated water of $0.266\,mmol.m^{-3}$, a difference of almost 2000. Since the inward flux densities of CO_2 needed for photosynthesis are similar or greater than those of O_2 needed for respiration it is clear that autotrophic plants are much more at risk from CO_2 starvation than from O_2 starvation. A further contributing factor to this greater sensitivity to decreased availability of CO_2 is the low affinity for CO_2 of Rubisco, the enzyme largely responsible for capturing photosynthetic CO_2 (K_m for CO_2 – $10\,mmol.m^{-3}$ compared to $0.14\,mmol.m^{-3}$ for O_2). This high K_m for CO_2 by Rubisco is almost the same as that for the concentration of CO_2 dissolved water in equilibrium with air, indicating the impossiblility of submerged leaves from photosynthesising normally. Data for O_2 and CO_2 used in these comparisons can be found in Armstrong (1979).

This analysis makes it apparent that while plants can sustain considerable interference in ventilation before mainstream respiration is strongly inhibited, almost no interference in aeration is possible in autotrophic plants before photosynthesis is depressed. This means that diffusion-driven CO_2 supply is rarely adequate for photosynthesis in tissue cultures, imposing the necessity of the sugar supplements almost universally used in tissue cultures. Even for oxygen, the leeway in diffusion losses can be sustained before damage sets-in, is strongly eroded by other factors such as warmer temperatures and decreasing surface to volume ratios. Thus, although the physical processes of diffusion is largely unresponsive to temperature *per se*, the flux required by the tissue increases dramatically with warming, typically doubling every $10\,^{\circ}C$
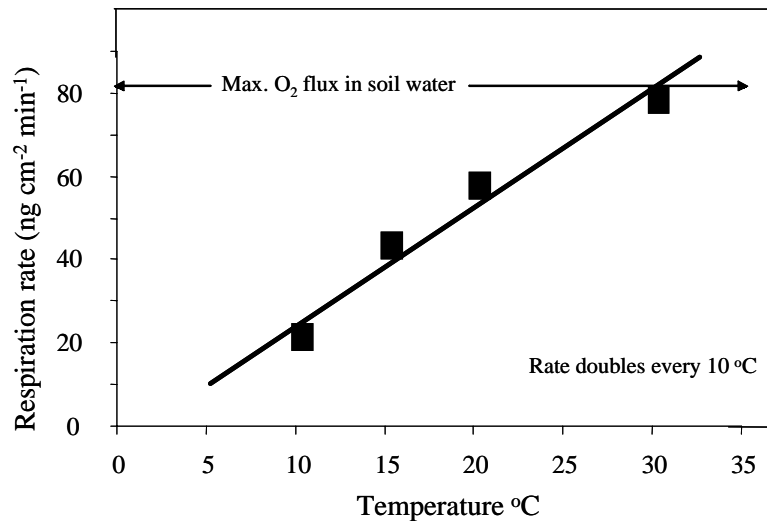
Fig. 2. Relationship between temperature and the rate of respiration expressed in terms of an inward flux density of O_2 required to service respiratory demand of plant tissue such as a cereal root axis. While at cool temperatures (e.g., 15 °C) the requirement is smaller than the maximum flux density possible in water equilibrated with air (approx. 80 ng.cm^{-2}.min^{-1}), it is barely inadequate to service the complete needs of roots at 30 °C.

(Fig. 2). At about 30 °C, demand by a cereal root matches the maximum flux that it possible from water in equilibrium with air. Clearly, increasing temperature markedly increases the required flux, placing a much larger demand on the gas exchange capacity of the surroundings. The flux required is also raised very considerably by radial growth of the tissue. This comes about because, as tissue bulk increases, the surface area available to service the gas exchange needs of each unit of tissue volume decreases. In a tissue culture, the leeway for how much aeration can be impaired before a tissue culture will suffer from O_2 shortage is abolished as size of tissue mass increases (Fig. 3). The effect can only be ameliorated if the tissue mass is highly porous and permeated by gas-filled intercellular spaces, which is uncommon. It may be concluded that any margin that exists before restricted aeration damages tissue cultures through O_2 shortage can be lost to warmer temperatures and to increasing tissue volume.

Diffusion coefficient of the diffusion medium

The diffusion coefficient (D) of the medium or barrier surrounding the tissue is the second component of the Fick's Law equation ($Q/t = \mathbf{D} * A\ (C_{in} - C_{out})/Th$). This value quantifies the relative ease with which the gas diffuses through a given medium (e.g., air, water, tissue) along its path to the tissue or cell. The very small coefficient of gas diffusion in water compared to that in air makes water a principle enemy of tissue
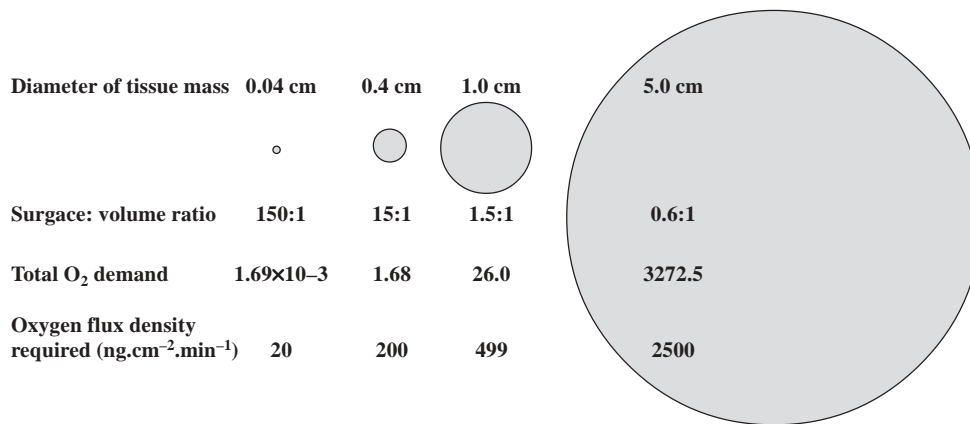
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Diameter of tissue mass	0.04 cm	0.4 cm	1.0 cm	5.0 cm
Surgace: volume ratio	150:1	15:1	1.5:1	0.6:1
Total O_2 demand	$1.69 \times 10-3$	1.68	26.0	3272.5
Oxygen flux density required (ng.cm^{-2}.min^{-1})	20	200	499	2500

Fig. 3. Illustration of the impact of increasing tissue bulk on surface area: volume ratio, total respiration and required oxygen flux densities per unit surface area (based on spheres).

aeration. For O_2, ethylene and CO_2, the diffusion coefficient in water is almost 10,000 times smaller than in air (in air: $0.201 \, cm^2.s^{-1}$; in water: $2.1 \times 10^{-5} \, cm^2.s^{-1}$). Adding a solid matrix such as agar or similar setting agent increases the barrier to gas diffusion even more. It also imposes a large unstirred layer that, in effect, strongly decreases the concentration of externally derived gases at the tissue surface thus slowing the rate of gaseous diffusion into the cells. This effect also enhances the trapping effect of water on metabolically generated gases such as ethylene. The work of Barrett-Lennard and Dracup (1988) and Verslues, Ober and Sharp (1998) illustrates the highly damaging impact of gelling agents on aeration and growth.

In most tissue culture systems, the strongest diffusion barrier is the container itself, which is usually fabricated from material that is totally impermeable to gases. For cultures to do well in such containers they must either be very large in relation to the size of the culture or be made to leak gas in some way. Obvious ways to achieve leakage include loosening the closure or inserting a gas-permeable membrane (e.g., polypropylene). Cultures of *Ficus elastica* in 305 ml plastic Magenta boxes were found to benefit from leakage rates that gave a half time of gas replacement of about 8 h. The benefit was attributed to a decrease in accumulated ethylene, resulting in enhanced leaf expansion (Jackson et al., 1991).

Cross sectional area of the diffusion pathway

The cross sectional area of the diffusion pathway (A) of the medium or barrier is the third component of the Fick's Law equation ($Q/t = D*A \, (C_{in} - C_{out})/Th$). Obviously, the larger the area across which gases can diffuse the more gas can be moved in total in a given time. One important aspect of this effect is the influence of the size and format of the tissue mass; a larger mass creating a smaller area for diffusion per unit

volume of tissue. The impact of cross sectionl area also means that the amount of *internal* surface area of tissue that is in contact with a gas phase (e.g., that created by aerenchyma) will also affect the gaseous flux in proportion to the diffusive surface area this creates. These effects have already been considered above.

Length of the diffusion pathway

The length of the diffusion pathway (Th) is the final term in the Fick's Law equation $(Q/t = D * A \, (C_{in} - C_{out})/\textbf{Th})$**.** The longer the path the greater the total resistance to gaseous diffusion. Tissue itself impedes its own aeration. After all, cells are mostly water. If tissues enlarge radially, the total path length to the centre of the mass also grows. For this reason, a large tissue mass may result in a poorly aerated interiors leading possible to anaerobic or hypoxic cores while more peripheral cells remain better aerated. There is an added problem connected with the thickness of the tissue barrier. For oxygen, it is that more and more of it is consumed as the pathway lengthens. This adds to the probability of deficiency deeper into the tissue since the concentration gradient driving further inward flow drops away as more and more is used in respiration. These affects are additional to the small surface to volume ratios that typify larger masses of tissue and raise detrimentally the requirements for gas exchange at the tissue external surface (as discussed above). Diffusion, within tissue can also be impeded more strongly by certain kinds of differentiation (e.g., lignified parts). These can impose sharply internally delineated radial-diffusion barriers (Armstrong et al., 2000).

Outside the tissue, the thickness (depth) of any water covering also impacts on gas diffusion. The effect is illustrated in calculations for ethylene accumulating at the surface of a root producing ethylene at a known rate (Jackson, 1979). When the radius of a water covering over the root of radius 0.025 cm is increased from 0.25 cm to 2.0 cm, ethylene build up at the root surface is enhanced 10-fold. A similar effect in reverse is seen for O_2. Thus, there is a benefit to aeration from maintaining water cover that is as shallow as possible or is intermittent to allow periods of relief from gas entrapment or exclusion.

Driving force for diffusion

The driving force for directional net diffusion is the fourth bracketed term in the Fick's Law equation $(Q/t = D * A \, (\textbf{C}_{\textbf{in}} - \textbf{C}_{\textbf{out}})/Th)$. It comprises the difference in concentration between source and sink. For O_2 and CO_2 it is appropriate to consider C_{out} as the atmospheric concentrations and C_{in} usually lying somewhere between atmospheric and zero. For ethylene we take C_{out} to be zero and C_{in} to be up to tens of parts per million and thus possibly above physiologically active levels. Clearly, C_i is the physiologically active component and is affected by all the factors considered above that

influence gaseous flux densisty. Thus, managing tissue culture aeration is about managing C_i to avoid physiologically damaging concentrations developing.

Approaches towards improving tissue culture aeration

The key to adequately aerating the cultured tissue itself is to maximise the concentration gradient for the gas between the interior and the immediate exterior of the tissue and to minimise the flux density per unit surface area neeed to sustain normal growth, respiration and where appropriate, photosynthesis. Our analysis of components of the Fick's Law equation has indicated this can be achieved by increasing diffusive ventilation of culture volume, minimising resistances to the movement of gas down that gradient, keeping cultures cool, minimising thickness of any water cover and avoiding solid media such as agar. A further important consideration is minimizing tissue volumes. This both decreases the flux density per unit surface area of tissue required to aerated the tissue and shortens internal diffusion pathways. Actual success is determined by achieving the flux density of gas needed to support the desired growth rate or pattern of development. This is especially demanding if the cultures are to be autotrophic since CO_2 is almost always in short supply The aim is to optimise C_i for key gases such as CO_2, O_2 and ethylene. This aim can be helped simply by having the vessels large in relation to the amount of tissue since this provides greater reserves of O_2 or CO_2 and a more effective dilution of metabolically generated gases such as ethylene.

However, in practice satisfying the aeration needs of tissue cultures by facilitating diffusive aeration and maximising vessel volumes alone is almost impossible, especially if autotrophic cultures are needed. Thus, additional measures are required. One approach is to side-step some of the problems. The most widespread such approach is to overcome the need for adequate flux of external CO_2 by supplying respirable sugars such as sucrose. This is widely practiced and does not warrant further discussion here accept to point out the heightened need for total sterility the use of sugar demands and the problems of adaptation this practice poses for any later move to autotrophic life outside the culture vessel. A lack of photosynthetic CO_2 fixation also deprives the cultures of the O_2 this generates. A second example of side-stepping to minimise C_{in} of ethylene by reducing C_{out} with an absorbant such as alkaline potassium permanganate or mercuric perchlorate. Enhanced leaf expansion rates in *F. lyrata* have reported using this approach (Jackson et al., 1987; 1991). An closely related measure is to incorporate an inhibitor of ethylene action such as silver nitrate (e.g., Armstrong et al., 1997) or 1-methylcyclopropene, a recently developed and very effective gaseous inhibitor of ethylene action (Sisler et al., 1996)

More satisfactory than side-stepping is to introduce an element of convection flow (mass flow) of the air (or water where liquid cultures are uses) to augment the contribution from diffusion alone. With this mechanism of aeration, it is useful to consider

the surrounding water or air as a physical carrier of gases to and from the tissue rather than a diffusion medium or barrier. For convention to be effective, the air or water must move across the cultured material. Various methods have been adopted in attempts to achieve this simply and effectively.

Harnessing convective flow

An elegant non-mechanized solution to the problem of how to ventilate tissue culture vessels with convection is provided by Armstrong and colleagues (Armstrong et al., 1997; Zobayed et al., 2001). It is based on a mechanism operating in whole plants of wetland species such as *Phragmites australis*. Here, mass flow of gases is driven initially by diffusion of N_2 and O_2 through stomata into the humid interior of the leaf down a concentration gradient. This gradient arises as a result of extra dilution of N_2 and O_2 in the plant's interior by abundant water vapour. The pore size of stomata is such that the resistance they offer to inward diffusion is less than outward mass flow of gas. Thus, inward diffusion of N_2 and O_2 (and other more minor components of air) results in an internal pressurization of the plant's gas spaces. This force is then capable of driving mass flow along interconnected gas-filled spaces. This mechanism of humidity-induced convective throughflow can be harnessed to ventilate tissue culture vessels with a sterile flow of air. Pressurization is achieved by connecting the culture vessel to a port with an overlying microporous membrane with a small pore size (0.03 µm) overlying but no quite touching water held in a small reservoir. This creates a humidity-driven inflow and pressurizes the culture vessel. A second port protected by a membrane of larger pore size (0.2 µm) creates an exhaust of low resistance to mass flow (Fig. 4). The outcome is a convective throughflow of air that is fast enough to clear half any accumulated ethylene within 30 min and to sustain greater rates of dry matter accumulation through enhanced CO_2 supply (Zobayed et al., 2001).

For tissue cultures where liquid medium is used and especially where mass production of somatic embryos in bioreactors is required, highly mechanised systems of introducing convective aeration have been devised. Here, three new elements are intro duced to supplement diffusive aeration with mass flows. Firstly, air is pumped over the cultures to carry O_2 and CO_2 to the plants and substrate and aid removal of metabolically produced gases from the tissues. Secondly, the air flow is fertilized with CO_2 to encourage further photosynthetic assimilation if convention flow of air does not provide a CO_2 flux density for the desired rate of photosynthesis Thirdly, the roots are greown in a porous medium to promote their aeration by diffusion. This is also periodically flushed with aerated nutrient solution to bring these resources to the roots by mass flow. A set-up such as this is described by Afreen et al. (2002). The plants produced by such a system were notable for their rapid adaptation to *ex vitro* conditions

A quite different approach to overcoming aeration problems is taken by Barry-Etienne et al. (2002). These authors recognise that the *in vitro* nature of culture systems
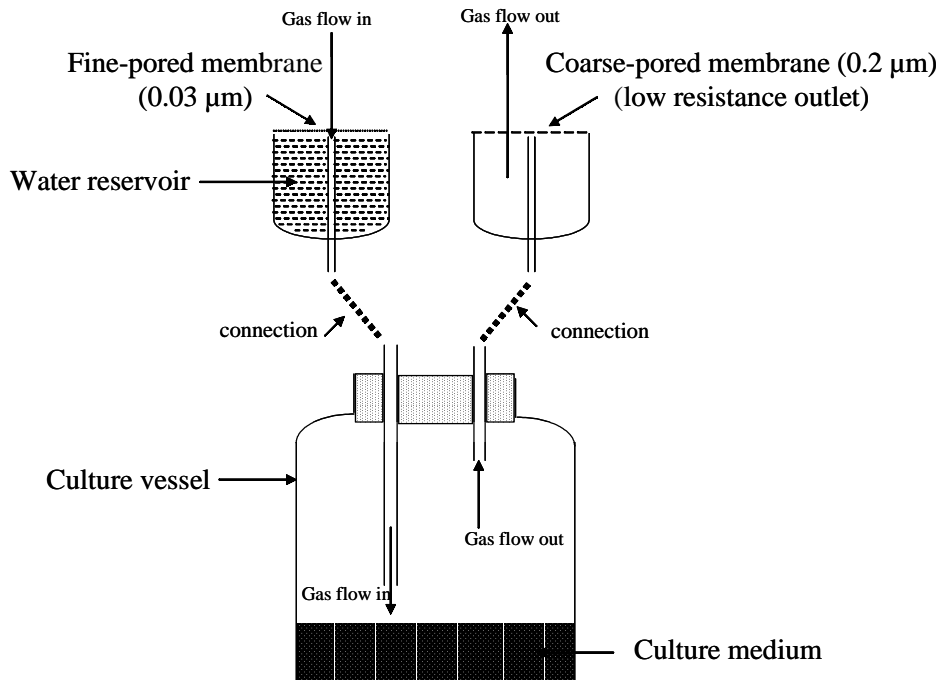
Gas flow in   Gas flow out

Fine-pored membrane   Coarse-pored membrane (0.2 µm)
(0.03 µm)   (low resistance outlet)

Water reservoir

connection   connection

Culture vessel

Gas flow out

Gas flow in

Culture medium

Fig. 4. Diagrammatic illustration of how humidity-driven inward diffusion of air across an membrane of fine pores can create a pressure driven flow of sterile air through the culture vessel and out through a second port protected by a membrane of larger pore size. Taken from Armstrong *et al* 1997.

is the principle problem for aeration. Thus, they propose keeping any *in vitro* phase in micropropagation as short as possible and returning the tissues to open septic environment at the earliest opportunity. This philosophy was anticipated several years earlier by Firn et al. (1994). In the system of Barry-Etienne, autotrophic somatic embryos, generated in a sophisticated illuminated bioreactor are transferred at an early stage (the cotyledon stage) to high density cells of sterilised soil-containing compost in a glasshouse under shade and carefully watered. The 60 % that survived grew on quite quickly to form well-rooted normal plants with no need for troublesome rehabilitation.

Conclusion

Gas exchange in tissue culture can involve two different although not entirely unconnected processes. These are simple diffusion down concentration gradients and/or convective (mass) flow. In the latter, gases are delivered to plant tissue by a flow of solvent (water) or carrier gas (air) that is propelled over the surface of cells or tissue by an external force, usually a pressure gradient. However, within the tissue mass itself, gas

movement is effected almost exclusively by diffusion. Only in whole plants growing in the natural environment where extensive interconnected porous tissue (aerenchyma) is present, can internal convective flow play a significant role in aeration (e.g., in *Phragmites* – Armstrong, et al., 1992). However, convective flow within culture vessels can readily be harnessed to increase rates of tissue culture gas exchange by contriving slow pressure-driven gas flows (e.g., Zobayed et al., 2001) or by flowing or stirring aerated medium over the cultured tissue. Opportunities for enhancing the diffusive aeration of tissue cultures are many and include temporary immersion set-ups, fitting gas permeable ports to culture vessels, adopting loose-fitting lids. The demands on the ventilation system can be minimised by restricting the depth of culture medium to thin films, keeping tissue bulk to a minimum and adopting cool temperatures. The biological effectiveness of any particular system will be affected by many factors. Paramount is the total rate of consumption or production of a particular gas by the culture in relation to the influx and efflux rates achievable by the enclosing environment and the ventilation system adopted.

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