The Availability of Minerals in Plant Tissue Culture Media

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This paper examines some of the factors affecting the availability of minerals to plants in vitro. The growth of *Ptilotus exaltatus* explants decreased when minerals were supplied in the gel at some distance from the explants rather than in direct contact, suggesting that transport of minerals through the gel may be limiting mineral uptake. Explant growth in dry weight was proportional to the relative matric potential of the medium. Since water was lost from the culture vessels during the culture period, relative matric potential would also decrease. It is argued that mineral uptake in vitro, and hence plant growth, is limited by the declining water availability which in turn affects the rate of diffusion of minerals through the medium.

INTRODUCTION

The basic technique of plant tissue culture is now a well established commercial practice. Many plant species are routinely propagated through micropropagation. An essential part of the micropropagation process is formulation of the medium. Minerals are an important component of such media. The earlier pioneer tissue culturists (White, 1943; Heller, 1965; Murashige and Skoog, 1962; Gamborg et al., 1968) recognised the essential requirement for the supply of minerals for plant growth in vitro and developed the formulations on which most current media are based.

Increasing or decreasing the supply of minerals in the medium causes varying responses. Lee (1978) found that omission of individual elements such as N, K, P, Mg, or Fe from the culture medium significantly reduced plant growth. Barbas et al. (1993) reported growth of walnut shoot cultures dramatically decreased to almost nil after 28 days due to mineral deficiency. Increasing the supply of minerals in the medium increased the net uptake of minerals and the growth of *Ptilotus exaltatus* explants (Winney, 1988); and *Hemercallis* (George et al., 1987). However, other reports indicate that increasing mineral concentration in the medium does not always increase growth. Lumsden et al. (1990) reported that there was no significant effect of doubling the concentration of PO_4^{-3} on *Iris* growth in vitro. Inhibition of plant growth preceded the exhaustion of minerals in plant tissue. Likewise Williams et al. (1991) found that none of the essential minerals had been exhausted from the medium after growth of *Ptilotus* has ceased. Thus growth in vitro is not simply dependant on the supply of minerals provided in the medium, some other factor limits the utilisation of these minerals by the plant.

For many years little attention has been paid to the control of mineral availability and uptake in vitro until the topic was reviewed by Williams (1992, 1993). Mineral availability depends on a number of factors including the concentration of minerals in the medium, solubility of the ions, and transport of ions through the medium, as well as uptake by the plant. In this paper we are examining mineral transport through the gel.

There are two main mechanisms of mineral ion transport, diffusion and mass flow. Mass flow is where the ions are carried in solution as water moves through the system. Given the high humidity in tissue culture vessels, transpiration by the plant, and hence water flow through the plant, is low. Thus water flow through the medium to the plant would also be minimal. Therefore, it is assumed that transport through the gel must be predominantly by diffusion.

There is evidence to suggest that localised depletion of minerals occurs around plants in vitro (Lumsden et al., 1990). In fact, localised depletion has also been reported in stationary liquid cultures (Asher et al., 1965). This could occur if diffusion through the medium was slower than uptake by the plants. Romberger and Tabor (1971) argued that diffusion of macromolecules could be limited by the pore size of gelled medium. We have tested the importance of diffusion by supplying minerals either adjacent to or remote from the explants in vitro. Our hypothesis was *that mineral uptake (and hence growth) of plants in vitro will be less if the minerals are supplied at a distance from the plant rather than adjacent.*

Whether mineral transport is by diffusion or mass flow, the supply of water in the medium will be important. Diffusion requires "free" water in the pore spaces of the gel. The availability of water can be measured in terms of matric potential. The total water content and hence the matric potential of the medium would be expected to decrease over time as water is lost by evaporation from the culture vessel. It would also be expected that diffusion of minerals through gelled media would depend on mineral concentration, gel brand (Scherer et al., 1988), gel concentration (Romberger and Tabor, 1971), and water availability. The effects of gel concentration and water loss have also been investigated.

MATERIALS AND METHODS

Experiment 1. To test the hypothesis on mineral proximity, *P. exaltatus* shoot explants were cultured on a double-layered gel system. For the treatments, minerals were supplied in either the top or bottom layer only (Fig. 1). The control had minerals in both layers. Each layer was composed of 30 ml of modified MS-basal medium with 3% sucrose and 0.8% Difco Bitek™ agar with or without minerals as required. Medium pH was adjusted to 5.5 before agar addition. Then medium was autoclaved at 101 KPs, 120C for 15 min.

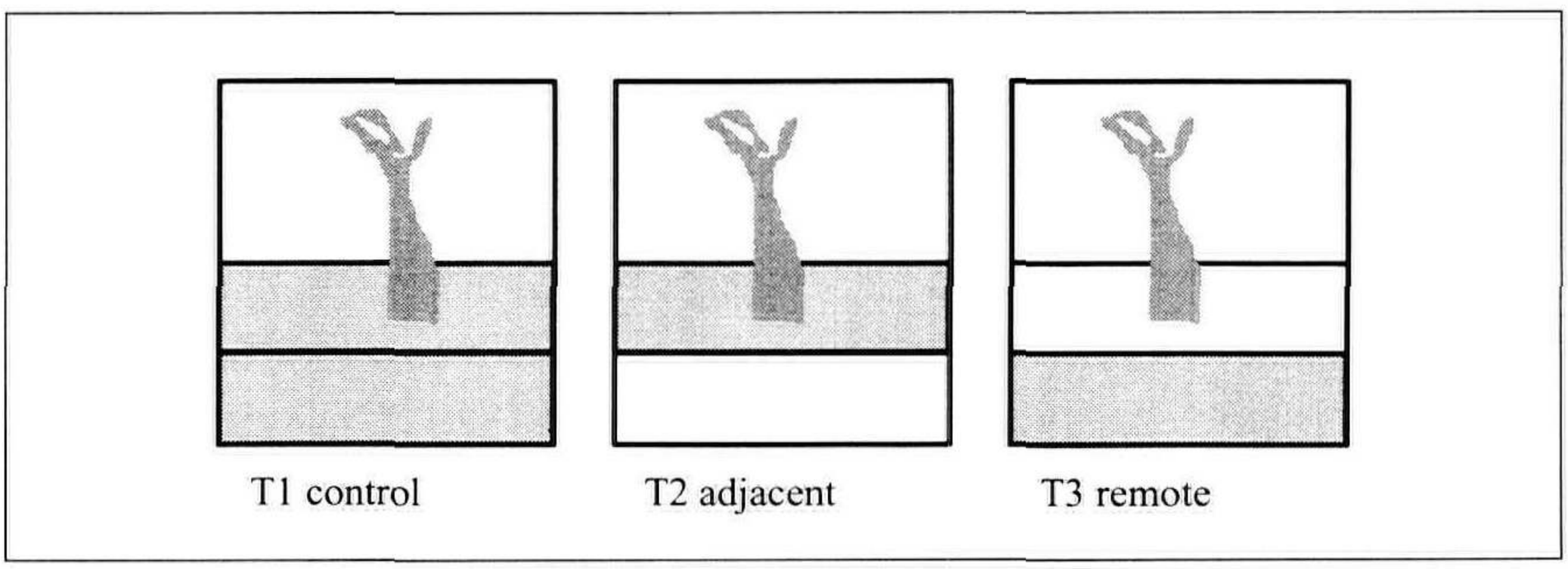

Figure 1. Layout of the two-layer media with minerals either adjacent to or remote from the plants.

Experiment 2. In a separate experiment different concentrations of gelling agents were used to modify the water potential of the medium. The relative matric potential (RMP) was determined using the technique of Owens et al. (1991) in which the rate of water absorption by air-dry filter paper discs is measured. Discs of Whatman No. 3 filter paper 5.5 mm diameter were weighted and moistened to about 90% of saturation by adding liquid medium equal to 2.60 times the initial weight of dry filter paper. The moistened paper disc was placed on the surface of the medium. The weight of liquid absorbed or lost was compared to the amount of liquid initially added to the paper. The amount of relative gain or loss of water from the paper filter was considered as RMP.

Experiment 3. In the third experiment the water loss from *Ptilotus* shoot cultures was measured. Each 250 ml screw-capped, polyethylene culture vessel contained 30 ml of gelled medium, with or without 4 explants. Culture vessels were weighed periodically over the 8-week culture period.

RESULTS

The *Ptilotus* explants in all cultures increased little in dry weight over the first two weeks following subculture but the control explants grew more rapidly between weeks 2 and 8 (Fig. 2). Final growth in the two treatments was about half that of the control, i.e., it was approximately proportional to the total mineral supply. The divergence of the growth curves indicates differences in growth rate up to week 8 after which growth stopped in all treatments. The growth rate was greater when the minerals were adjacent to the plant and hence final plant weight was nearly twice that of the remote treatment.

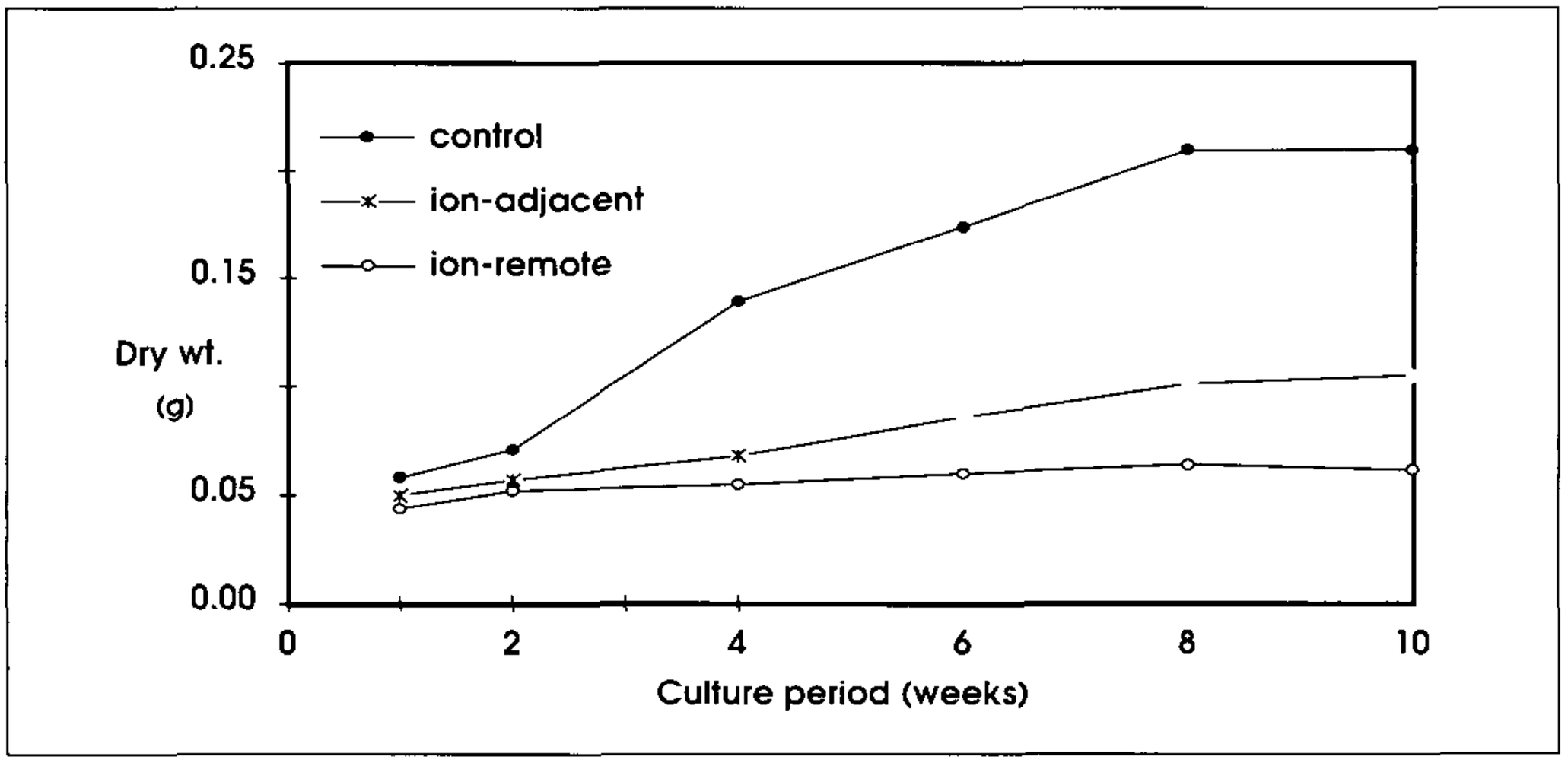

Figure 2. The effect of mineral proximity to explant on growth (dry weight) of *Ptilotus exaltatus* shoots in vitro.

Explant growth in dry weight was proportional to the RMP (Fig. 3). Growth was greatest when RMP was highest, i.e., when water availability was greatest.

The total water content of cultures declined over the culture period (Fig. 4).

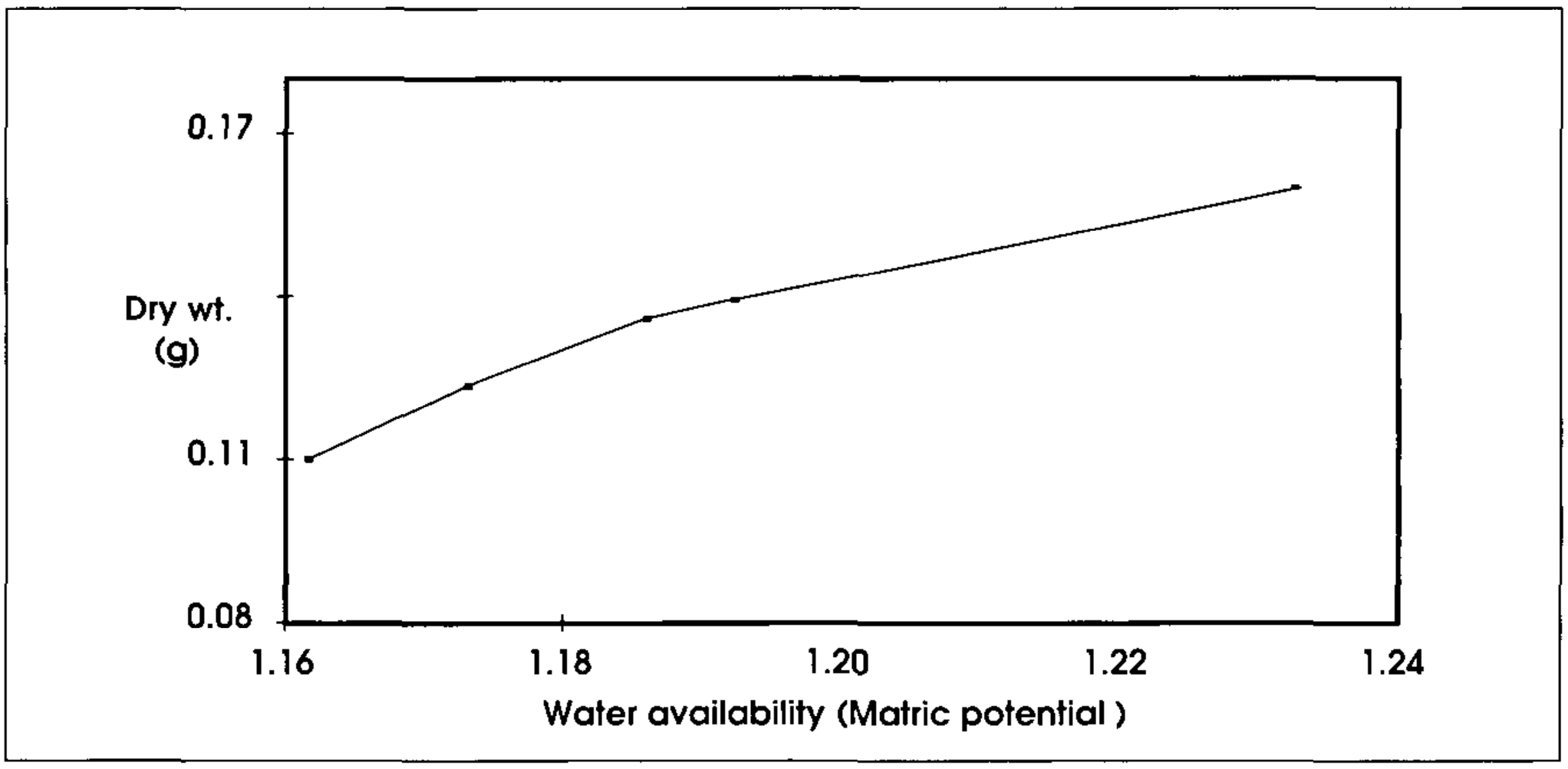

Figure 3. The relationship between plant growth and water availability in vitro.

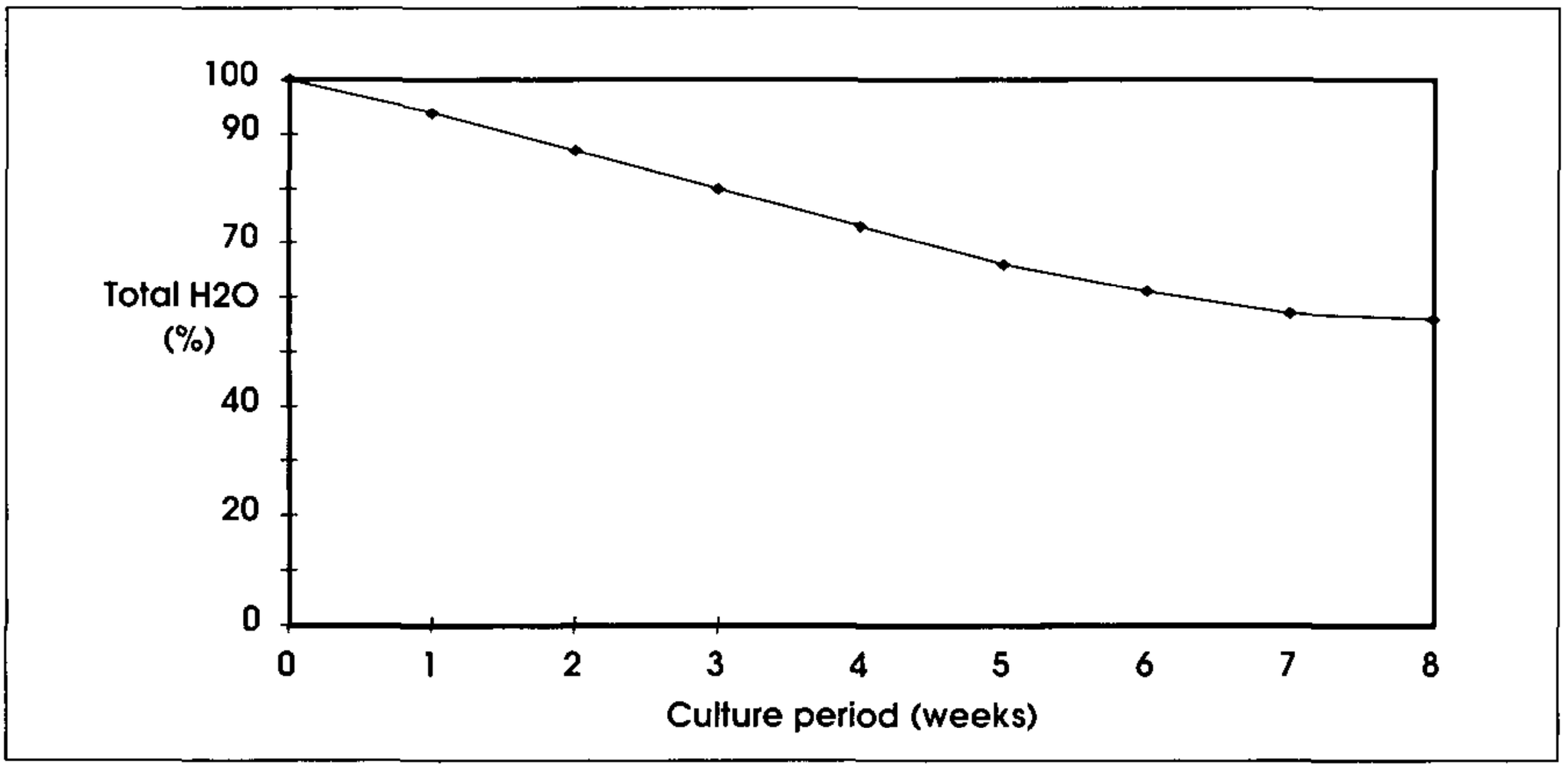

Figure 4. Water loss during the culture period.

DISCUSSION

The critical comparison in the first experiment is the adjacent versus the remote mineral supply. Growth was less when the supply was remote. This is consistent with the hypothesis that mineral uptake, and hence growth, is limited by the ability of minerals to diffuse through the gel. However, when the adjacent treatment is compared with the control it is clear that the total mineral supply has an even greater effect on growth.

It would be expected that total mineral supply would affect the final weight of the explants. A greater supply of minerals would take longer to be depleted thus supporting a longer period of growth and hence a greater final weight. But this does not explain the pattern of response observed. The greater final growth was due to a more rapid growth rate over the culture period with growth stopping after 8 weeks in all cases (Fig. 2). It has been previously shown that growth in vitro is dependant on the concentration of minerals in the medium (Pryce et al., 1993; Winney, 1988).

The concentration of minerals was initially the same in each of the mineral layers of the first experiment. However, if minerals do diffuse through the gel, the concentration would have been reduced to half in the two treatments as the minerals were distributed throughout both layers of gel. This might also explain the overall difference in growth in the control compared to the two treatments. Thus the cessation of growth in the control after 8 weeks could be due to a similar level of depletion of minerals in the gel; twice the growth rate would deplete twice the total amount of minerals over the same period.

The correlation between increased dry weight and RMP or water availability (Fig. 3), may be explained in different ways. One possibility is that explant growth is directly dependant on the availability of water and that mineral uptake follows growth. However, it has been reported that mineral uptake by *Ptilotus* shoot cultures (Williams, 1993) and *Juglans* (Barbas et al., 1993) declines before plant growth rate. Alternatively, the availability of water may directly affect the availability of minerals. This effect could be via a change in mineral solubility or a change in rate of ion mobility through the medium.

Water is lost from the gel over the culture period (Fig. 4). As this water is lost the RMP, and hence water availability, must decrease. A progressive reduction in water availability could in turn reduce the rate of mineral diffusion and thus account for the decline in growth rate seen after 8 weeks in these experiments (Fig. 2). This again suggests that water availability may be the prime limiting factor for growth in vitro. As a matter of fact, Murashige and Skoog in their classic 1962 paper, also suggest that the growth of tobacco callus was limited by the loss of water from the cultures.

This paper highlights the complexity of mineral nutrition in vitro. We have only covered some of the factors affecting mineral availability. It has been demonstrated that the availability of minerals in vitro is dependant on their rate of movement through the medium. Mineral availability is also dependant on the availability of water. We still need to determine the mechanisms involved.

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