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影响培养基 pH 值变化的因素

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摘要 植物组织培养基的 pH 值一旦外植体植入就开始发生改变,直到达到某一平衡点。不同种的植物有其各自的 pH 平衡点。用 6 种植物(*Ptilotus exaltatus* Dees ex Lehm, *Lechenaultia formosus* R. Br., *Rosa canina* L., *Melaleuca alternifolia* (Maiden & E. Betche) Cheel, *Anigozanthos flavidus* DC., *Zieria cytisoides* Sm.)培养在 1/2 MS 琼脂培养基中,pH 平衡点的范围通常在 2.8~4.3 之间,pH 的变化率也有差异。外植体植入到培养基中的前两周,植物体的重量会减少。在外植体植入前将培养基 pH 值调节至平衡点,这样避免了外植体植入后的 pH 变化。但是,这并没有克服外植体植入后前两周植物体重量的减少。矿物质的获取不受培养基 pH 值的影响。

关键词 pH 变化,矿物质获取,植物组织培养

FACTORS AFFECTING pH CHANGES OF *IN VITRO* MEDIA

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Abstract It has been shown that the pH of agar medium changed steadily from the time when the explants were introduced until an equilibrium was reached. The equilibrium pH differed among plant species. Six species, *Ptilotus exaltatus* Dees ex Lehm, *Lechenaultia formosus* R. Br., *Rosa canina* L., *Melaleuca alternifolia* (Maiden & E. Betche) Cheel, *Anigozanthos flavidus* DC. and *Zieria cytisoides* Sm., cultured on 1/2 MS agar had pH equilibria in the range of 2.8 to 4.3. There were also differences in the rate of pH change. Explants lost weight over the first two weeks of culture. Equilibration of the pH of the medium before inserting the explants prevented the pH change but did not prevent the loss of plant weight. Mineral availability was not affected by the medium pH.

Key words pH change, Mineral availability, Plant tissue culture

Most plant tissue culture media protocols suggest that the pH of the nutrient solution be adjusted to a specific value, usually in the range of 5.5 to 6.0, prior to the addition of a gelling agent. The rationale for this is based on our knowledge of the effect of pH on the solubility of mineral salts in water and the dissociation of mineral ions in soil or potting media. The recommended pH may be based on empirical experimentation to find the optimum for a particular culture system. In most cases no further mention is made of medium pH.

A number of papers have reported on the changes in pH which occur in plant tissue culture me-

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dia both during media preparation and during the culture cycle[1~4] but little has been done to validate the basic assumption that pH has the same effect on mineral availability *in vitro*, particularly with gelled media compared to solution culture. Similarly, we have limited knowledge of the interaction among the tissue culture plant, the medium, the pH and plant growth responses. The available information is mainly from studies of cell suspension culture.

It has previously been shown that the pH of the medium changes steadily from the time the explant is introduced until an equilibrium pH is reached[2,3]. This equilibrium varies among species. The equilibrium pH for *Ptilotus exaltatus* shoot cultures is approximately 4.2. Different plant species have distinct pH optima for growth and/or rooting rates[5]. There is a net loss of minerals from explants following transferring to new medium[4]. One explanation is that the efflux of minerals is a result of the pH imbalance. Therefore, this effect should be overcome if the medium pH is adjusted to the equilibrium prior to the culture period. We have investigated several of the factors which affect the pattern of pH change during the culture cycle and how this affects mineral availability.

1 MATERIALS AND METHODS

The standard culture system consisted of 30 mL of agar-gelled medium containing minerals and organic components based on DeFossard 1976[6], in a 250 mL screw-capped, polycarbonate culture vessel. The pH of the nutrient solution was adjusted to 5.8 prior to the addition of gelling agent and autoclaving. Our reference cultures were shoot explants of *Ptilotus exaltatus*, 4 per container, subcultured on a 6~8 week cycle. Growth room conditions included a room air temperature of approximately (23±1) ℃ with 16 h per day of 30 to 50 mE white fluorescent light.

Individual experiments involved comparisons among levels of minerals supplied, different plant species, types of gelling agent, numbers of explants per culture, different initial medium pH, pH at different distances from the explant, and the effects of pH on mineral availability (solubility).

The pH of media was normally measured by stirring the gel with a glass combination electrode until a steady reading was obtained. For conductivity measurements, 20 mL of deionised water were blended with the medium then filtered off and the conductivity meter probe was inserted into the filtrate. Fresh weight (FW) and dry weight (DW) of plant were determined as required.

1.1 Plant species and gel type

Species of six plant genera were cultured on basal medium for eight weeks and the medium pH measured after each 2 weeks. The species were *Ptilotus exaltatus* Dees ex Lehm, *Lechenaultia formosus* R.Br., *Rosa canina* L., *Melaleuca alternifolia* (Maiden & E.Betche) Cheel, *Anigozanthos flavidus* DC., and *Zieria cytisoides* Sm. In a second experiment 7 species, including *Boronia filifolia* F. Muell, *Stirlingia latifolia* and *Begonia rex* Putze were each grown on either agar or gelcarin based medium (see Table 2).

1.2 Adjustment of initial pH to equilibrium

Standard medium (pH adjusted to 5.8 prior to autoclaving) was compared with media where the pH was adjusted to 4.2 after autoclaving. Four *P. exaltatus* shoot explants were placed in each container. The pH of the medium was measured in independent containers at the beginning of the culture period and after 2,4 and 6 weeks.

1.3 Explant number and proximity

The number of *P. exaltatus* shoot explants per culture was varied from 0 to 8 and medium pH measured after 2, 4 and 6 weeks to determine whether explant number affected the rate of pH change or the equilibrium pH. A micro-combination electrode with a tip diameter of 1 mm was used to measure the localized pH. This was either inserted into the hole from which the explant was removed or

directly into the medium at distances of 5, 10 and 20 mm from the explant position. Readings were taken 2 minutes after insertion of the electrode to allow the pH to stabilize. The electrode was washed in distilled water between readings.

1.4 pH and mineral availability

During media preparation, before addition of agar and autoclaving, the pH of the nutrient solution was adjusted to specific values from 3.5 to 7.5 by the addition of appropriate amounts of either 1 mol/L NaOH or 1 mol/L HCl. The gel was cut into 1 cm^3 pieces and washed with 20 mL of distilled water at pH 4.2. The pH of the medium was measured after mixing with the water and before the filtrate was collected for conductivity measurements.

2 RESULTS

2.1 Plant species and gel type

The medium pH generally decreased over the 8-week-period but the actual pattern and final pH differed among species (Fig.1 and Table 1). With *R. canina* and *M. alternifolia* the medium pH dropped steadily (from 4.1 and 5.0 respectively at the first week) to approximately a value of pH = 2.9 after 8 weeks. With *P. exaltatus* and *Lechenautia formosus* the medium pH remained approximately the same (with *P. exaltatus* more variable than *L. formosus*), still at 4.2 after 8

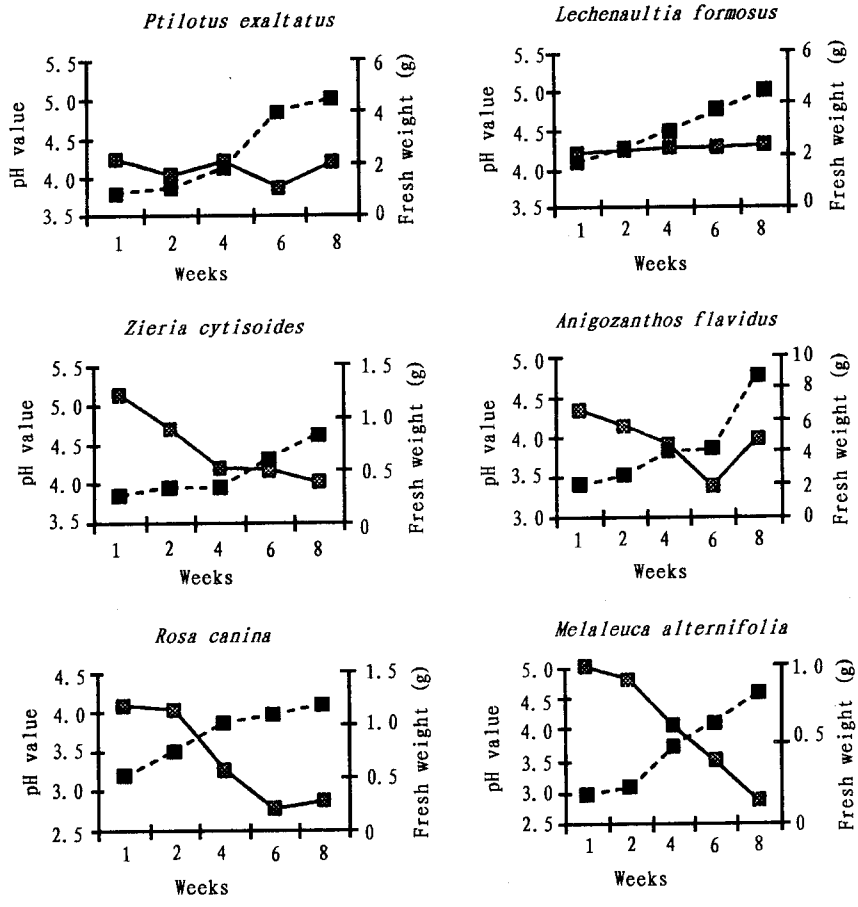

Fig.1 pH equilibria and growth of different species
—pH; - - FW.

weeks. With *A. flavidus* and *Z. cytisoides* the medium pH decreased steadily (from 4.3 and 5.1 to 4.1 and 3.5 respectively) after the 6th week, then with *A. flavidus* it rose during the 8th week, also ending at about 4.1.

In the second experiment we compared the pH changes of a range of species on agar medium versus gelcarin. There was no overall difference between the gels but in 2 species, *Stirlingia latifolia* and *Lechanaultia formosus*, pH was lower on the agar medium (Table 2).

Table 1 Equilibrium pH for different species after 8 weeks on agar

Plant species	pH value
Zieria cytisoides	4.1
Lechenaultia formosus	4.2
Anigozanthos flavidus	4.3
Rosa canina	2.8
Ptilotus exaltatus	4.1
Melaleuca alternifolia	2.8

Table 2 Effect of gel type on pH*

Plant species	Agar	Gelcarin
Zieria cytisoides	5.23	5.06
Lechenaultia formosus	4.03*	4.76
Anigozanthos flavidus	3.83	3.75
Rosa canina	3.02	3.01
Stirlingia latifolia	4.24*	4.62
Boronia filifolia	5.47	5.35
Begonia rex	4.11	4.22

* Significant difference at $P = 0.01$.

2.2 Adjustment of initial pH to equilibrium

The pH of the control (initially 5.8) decreased rapidly during the first 2 weeks to equilibrate at about 4.4 and eventually equalled that of the other treatment (initially 4.2), which remained about 4.1 to 4.2. Growth (FW and DW) was similar in both treatments, declining over the first 2 weeks then increasing steadily up to week 6 (Fig.2).

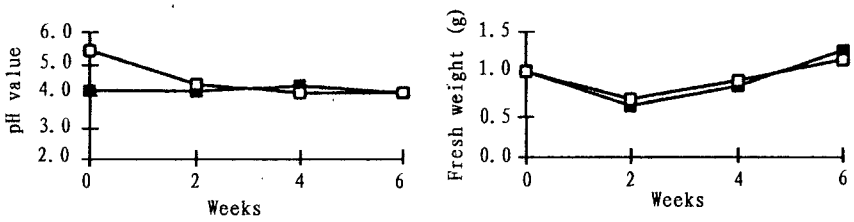

Fig.2 Effect of equilibration of pH on pH change and shoot growth of *Ptilotus exaltatus in vitro*
□Control; ■Equilibrated.

2.3 Explant number and proximity

In the absence of plant material the pH decreased from 5.8 to 5.3 within two weeks but changed little between weeks 2 and 6 (Table 3). With explants present the medium pH decreased

Table 3 Effect of plant number on pH of *Ptilotus exaltatus* cultures

Plant No.	2 weeks	4 weeks	6 weeks
0	5.33	5.22	5.15
1	4.91	4.58	4.54
2	4.65	4.43	4.52
4	4.34	4.18	4.36
6	4.27	4.12	4.38
8	4.28	4.12	4.31

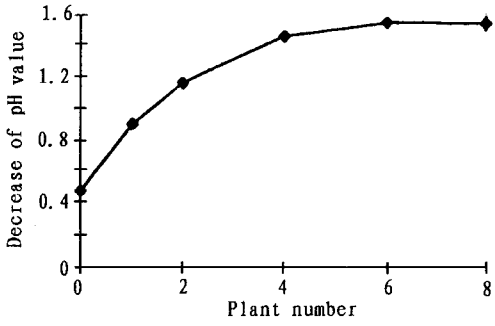

Fig.3 Effect of explant number on the rate of pH change over the first 2 weeks of *Ptilotus exaltatus* culture

steadily over the first 4 weeks with the rate of decrease increasing with the number of explants, up to 6 (Fig. 3). The pH then increased slightly between weeks 4 and 6 where 2 or more explants were present. There was little difference in the pH measured at up to 20 mm from the position of the explant (data not shown).

2.4 pH and mineral availability

The availability of minerals, as indicated by their solubility and hence the conductivity of the leachate, increased with the medium pH after 24 hours but there was little difference between the response to pH by day 7 (Fig.4).

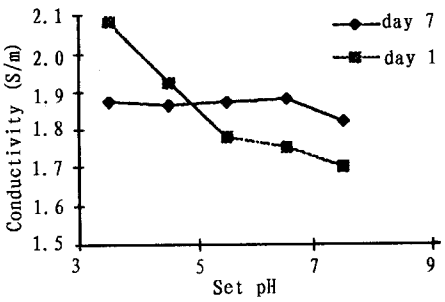

Fig.4 Effect of medium pH on mineral solubility (conductivity of leachate)

3 DISCUSSION

While the medium pH generally decreased after explants were placed in the gel, the actual pattern of pH change differed among species. A pH change in the medium must involve either the release or uptake of compounds by the explants[7]. There may be direct movement of anions and cations or release of organic compounds which causes reactions in the medium. Differential uptake of ammonium or nitrate ions[1,8], accumulation of CO_2[9], and the secretion of organic acids[10], have been reported to affect medium pH. If the pH change is due to influx or efflux from the explants, it would be expected that the rate of change would be greater with more explants. This was true over the first two weeks for up to 4 explants of *P. exaltatus* (Fig.3).

Skirvin *et al*[2] suggested that plant material could establish an optimum pH in its immediate environment and the direction and extent of pH change might be influenced by the parent plant's *in vivo* pH optimum. In our experiments, the medium pH of two species *R. canina* and *M. alternifolia* reduced as low as 2.9 after 8 weeks. It has not yet been known of why their equilibrium was so low and whether the acid pH affected the growth of the plants. The fresh weight of *R. canina* increased steadily and that of *M. alternifolia* increased slowly. The *M. alternifolia* cultures were subsequently found to be contaminated which might explain their low pH. It is hard to predict what the optimum pH is for a particular species.

It has been shown that the pH of the medium may change steadily from the time the explant is introduced until an equilibrium is reached[3]. There is also a net loss of minerals from the explants following transferring to fresh medium[4]. One explanlation is that the efflux of minerals is a result of the pH imbalance. Therefore, the loss of minerals could be prevented if the medium pH is adjusted to the equilibrium prior to the culture period. Adjustment of the medium pH to the equilibrium point for the species before introduction of the explant overcame the change in pH but did not prevent the loss of plant weight during the first two weeks. Thus the theory that the loss of minerals from the explants following subculture is due to pH induced ion efflux is disproved.

The initial pH of the medium may cause a change in the ionic balance of the nutrients supplied in the medium, leading to less soluble forms or perhaps binding of specific ions to the gelling agent[4]. The similarity of the conductivity of the leachates indicates no change in overall ion solubility at different pH and therefore does not support the idea of a change in mineral availability due to the pH value.

REFERENCES

1 Singha S, Oberly G H, Townsend E C. Changes in nutrient composition and pH of the culture medium during *in vitro* shoot pro-

liferation of crab-apple and pear. *Plant Cell Tiss Org Cult*, 1987. **11**:209 ~ 220
2 Skirvin R M, Chu M C, Mann M L *et al*. Stability of tissue culture medium pH as a function of autoclaving, time, and cultured plant material. *Plant Cell Rep*, 1986. **5**:292 ~ 294
3 Williams R R, Taji A M, Winnery K A. The effect of *Ptilotus* plant tissue on pH of *in vitro* media. *Plant Cell Tiss Org Cult*, 1990. **22**:153 ~ 158
4 Williams R R. Mineral nutrition *in vitro*—A mechanistic approach. *Aust J Bot*, 1993. **41**:237 ~ 251
5 Leifert C, Pryce S, Lumsden P J *et al*. Effect of medium acidity on growth and rooting of different plant species growing *in vitro*. *Tiss Org Cult*, 1992. **30**:171 ~ 179
6 DeFossard R A. Tissue Culture for Plant Propagators. Armidale: University of New England Press, 1976.
7 Butenko R G, Lipsky A K, Chernyak N D *et al*. Changes in culture medium pH by cell suspension cultures of *Dioscorea deltoidea*. *Plant Sci Lett*, 1984. **35**:207 ~ 212
8 McDonald K A, Jackman A P. Bioreactor studies of growth and nutrient utilization in alfalfa suspension cultures. *Plant Cell Rep*, 1989. **8**:455 ~ 458
9 Leva A R, Barrosom M, Murillo J M. La moltiplicazione del melo con la teinica della micropropagazione. Variazione del pH in substrati diversi durante la fase dimoltiplicazione. *Riv della Ortoflorofrutticoltura Ital*, 1984. **68**:483 ~ 492
10 Yoshihara T, Hanyu H. pH changes in culture medium with progress of growing stages, callus, multiple shoot and intact plant of strawberry. *Acta Horticulturae*, 1992. 319