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Effect of Medium pH on Shoot Regeneration from the Cotyledonary Explants of Tomato

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Abstract: To optimise medium pH for *in vitro* shoot regeneration and shoot growth from the cotyledon explants of tomato cv. Red Coat, the explants were inoculated onto MS medium which was maintained at a wide range of pH (4.5-7.5). Percentage shoot regeneration and number of shoots produced per explant were not significantly affected by the medium pH. The proportion of explants that produced callus and callus diameter remained unaffected by the medium pH. In contrast, the shoot height was significantly affected and taller shoots were only produced at a pH range of 5.5-6.0. Lower (4.5-5) and higher (6.5-7.5) medium pH significantly reduced shoot height. The results suggest that the explants of tomato Cv. Red Coat are moderately tolerant to a wide range of medium pH, as most of the traits studied remained unaffected by the change in media pH. However, the best regeneration and growth occurred only in the pH range 5.5–6.0.

Key words: *Lycopersicon esculentum*, micropropagation, morphogenesis, multiplication

INTRODUCTION

Improvements in shoot regeneration efficiency require a better understanding of the influence of culture conditions on shoot regeneration. Medium pH is extremely important, as it influences the uptake of nutrients and plant growth regulators by regulating their solubility in the culture media[1]. Medium pH also regulates a wide range of biochemical reactions occurring in plant tissues[2]. A pH higher than 6 gives a fairly hard medium and a pH below 5 does not allow satisfactory gelling. Thus, the effective range of pH for tissue culture medium is limited. The optimal pH of a culture medium must not disrupt the functioning of plant cell membranes, or the buffered pH of the cytoplasm. The cytoplasmic pH of the plant cells is tightly regulated and was earlier thought to be in the range of 6.5-8.0[3]; however, more recent studies suggest a pH range of 6.7-7.7[4,5]. Changes in external pH have a small transient effect on cytoplasmic pH but the cells are readily readjusted towards their original pH[6]. Thus the effect of external pH on cytoplasm is not long lasting. However this change may affect plant growth as follows. Exposure of cells to extreme low pH leads to conversion of inorganic phosphate into organic phosphate at the extracellular region. This is also accompanied by a reduction in ATPs which leads to reduced plant growth[7]. The detrimental effects of adverse medium pH are generally related to an imbalance in nutrient uptake rather than to direct cell damage (except at extreme pH changes).

The medium pH drops to 4.5 from 5.1–5.2 (which is the pH after autoclaving, if set to 5.8 before autoclaving) soon after the explants are inoculated and it rises slowly between 5 and 6 or above as the growth proceeds[1]. The change in pH, amongst other factors, is due to preferential uptake of NH_4^+ and/or NO_3^- from the medium, or an efflux of protons or hydroxy ions, respectively, caused by this uptake [8]. The uptake of anions is favoured at acidic pH, while that of cations is best achieved when the pH is increased[1].

For tomato regeneration, most researchers set the medium pH to 5.5-6[9]. In the present study efforts were made to optimise medium pH for tomato shoot regeneration and shoot growth.

MATERIALS AND METHODS

Explant: Seeds of the Red Coat cultivar of tomato were obtained from the Yates Vegetable Seeds Co. Ltd. (Milperra, New South Wales, Australia). The seeds were surface sterilised for 15 min using 1% sodium hypochlorite (v/v) and rinsed with sterile water before being transferred to autoclavable transparent culture tubes (25×80 mm) containing 5 mL MS basal medium solidified with 0.8% agar (Sigma Chemical Company, St Louis, MO, USA). Cotyledons were excised from

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one-week old seedlings and the whole cotyledons were inoculated onto medium. Explants were aseptically placed with the abaxial (lower) surface touching the medium. The culture tubes were incubated in a controlled environment room which was maintained at 25±2°C with a light intensity of 38 μmol m^{-2} s^{-1} and 16 h photoperiod provided by cool white fluorescent tubes (Sylvania Gro-Lux, Germany).

Culture media: All the media including the control were supplemented with 15 μM zeatin, 3% sucrose and solidified with 0.8% agar. The pH of the media was adjusted to 4, 4.5, 5, 5.5, 5.8, 6, 6.5, 7 and 7.5 (prior to autoclaving) using either 1M NaOH or 0.25 M HCl. The media were mechanically dispensed (5 mL per tube) into plastic culture tubes (25×80 mm) prior to autoclaving at 1.05 kg cm^{-2} (103.5 kPa) and 121°C for 15 min. To minimise condensation, the media were cooled to 40°C before the lids were tightened. No pH buffers were used.

Observations: Observations were recorded after four weeks of inoculation and these included frequency of explants showing organogenesis, number of shoots per explant, shoot height, number of explants showing callus formation and callus diameter.

Experimental design and statistical analysis: Thirty culture tubes per treatment were used and the tubes were incubated in a controlled environment room according to a completely randomised design. Each tube had a single explant and was considered as an experimental unit. The data were analysed using GenStat® statistical analysis software. For shoot regeneration and callus response, data were analysed using Generalised Linear Model (GLM)[10], assuming a binomial error and a logit link function. Pair-wise comparisons of the means were conducted on the logit scale as per the GenStat RPAIR procedure. For number of shoots per explant and shoot height and callus diameter, only those cultures in which shoot and/or callus was produced, were considered in the analysis. As such, these data were unbalanced and were analysed in GenStat using the procedure AUNBALANCED[11] to perform an analysis of variance. Pair-wise comparison of means was performed using the Least Significant Difference (LSD) test at the 95% confidence level. Graphs were prepared using Sigma Plot (SPSS Inc., USA).

RESULTS AND DISCUSSION

Medium pH had no statistically significant ($p < 0.364$) effect on percentage shoot regeneration response. However, there was a trend such that the better shoot regeneration occurred at the acidic pH (4.5-5.8) rather than at alkaline pH (6.5–7.5) (Fig. 1). The optimal pH was 5.5, at which 57% of the explants produced shoots. Statistically, no significant ($P = 0.489$) effect of pH was observed for number of shoots produced per explant (Fig. 2). However, there was a significant ($p < 0.001$) effect of pH on the shoot height (Fig. 3). Longer shoots were observed at the pH 5.5–6 while shorter shoots were regenerated at both higher (6.5-7) and lower (4.5–5) pH. There was no significant effect of pH on the callus response ($P = 0.746$) (Fig. 4) or the callus diameter ($P = 0.483$) (Fig. 5).

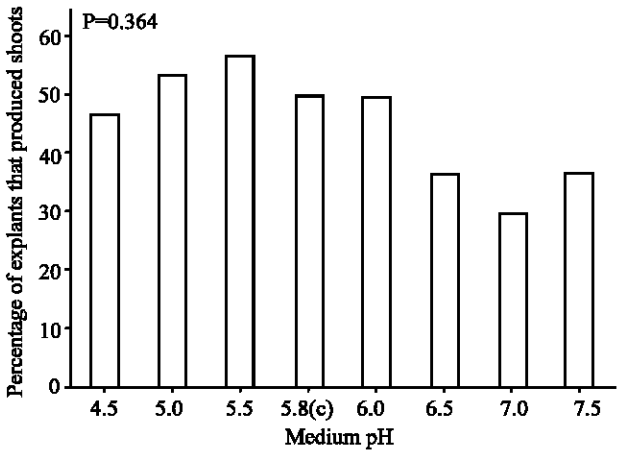

Fig. 1: Effect of medium pH on the percentage of cotyledonary explants of Cv. Red Coat that produced shoots in four weeks time (n = 30). LSD is not obtained as data was analysed using generalised linear model (see materials and methods) and means were compared using RPAIR procedure only if the P value was significant, C: control

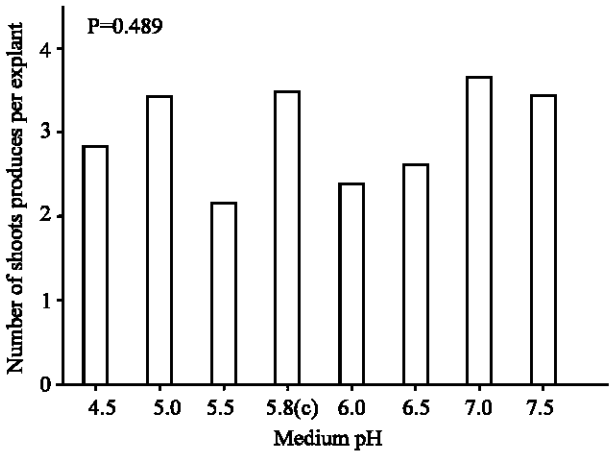

Fig. 2: Effect of medium pH on the number of shoots produced per cotyledonary explant of Cv. Red Coat in four weeks time (n = 9-21), C: control

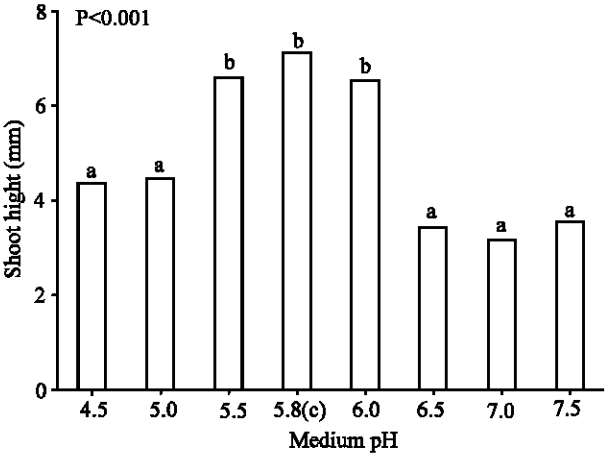

Fig. 3: Effect of medium pH on height of regenerated shoots developed from cotyledonary explant of Cv. Red Coat in four weeks time (n = 28-57), C: control

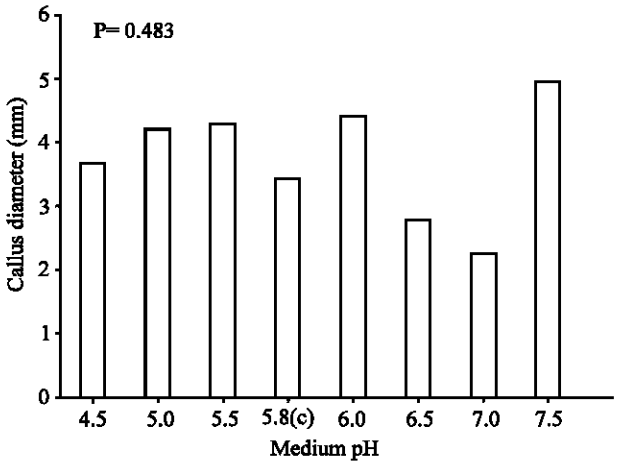

Fig. 5: Effect of medium pH on callus diameter developed from cotyledonary explants of Cv. Red Coat in four weeks time (n = 13-18), C: control

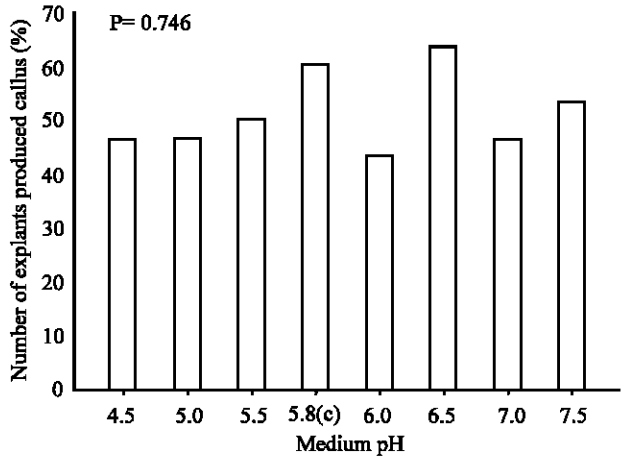

Fig. 4: Effect of medium pH on the percentage of cotyledonary explants of Cv. Red Coat that produced callus in four weeks time (n = 30). LSD is not obtained as data was analysed using generalised linear model (see materials and methods) and means were compared using RPAIR procedure only if the P value was significant, C: control

All the traits, except shoot height remained unaffected by the changes in the media pH. These results suggested that tomato Cv. Red Coat is tolerant to a wide range of pH. This response in tissue culture can be compared to that in the field where tomatoes are successfully grown in soils having a wide range of pH[12]. Tomato tissues are able to tolerate a broad range of pH in two ways. Acidic pH is tolerated by exporting out the protons (H^+) from the cytoplasm to the extracellular space in exchange for anions, or the cells growing in an acidic environment degrade cytoplasmic organic acid to raise the pH[1]. Conversely, alkaline pH is tolerated by the synthesis of organic acids, such as malate from a neutral precursor[13]. These mechanisms are likely to ensure that tomato plants tolerate a range of pH in *in vitro* culture.

Another likely reason for the lack of any response to pH for most of the traits studied here is the self-stabilisation of the medium pH. Skirvin *et al.*[14] for *Cucumis melo* culture reported that MS medium tended to become more acidic with time and the pH of the medium was self stabilised within a pH range of 4.6–4.9 irrespective of the initial pH (3.3–8.0). The changes in medium pH during culture can be explained by the differential uptake of nitrogen sources; the uptake of NO_3^- leads to a drift towards an alkaline pH, while the uptake of NH_4^+ results in a shift towards acidity[1]. The pH drop may lead to preferential uptake of cations[15] and to the exudation of organic acids from plant materials[15-17]. However, Williams *et al.*[18] reported that changes occur in the pH of *in vitro* nutrient media during preparation and over the culture period. The direction and extent of the changes depend upon the initial pH and the presence or type of gelling agent. Agar-based medium was progressively acidified in the presence of a living *Ptilotus exaltatus* explant which was not a response to wounding.

In the present study, the only trait that responded to pH was shoot height. Reduced shoot height was observed at both high and low extremes of pH. Modification in growth and development of cells by the medium pH occurs mainly by affecting ammonium and nitrate utilisation and sucrose uptake, either of which could affect cell differentiation[19,20]. Martin and Rose[19] reported that the uptake of ammonium and sugar by *Ipomea* cell suspension was lower at pH 4.8 than at pH

5.6. At low pH, cells release H^+ to the extracellular environment affecting the absorption of nutrients, especially the NH_4^+[21]. At high pH, cells release OH^- ions thus the absorption of NO_3^- is adversely affected[22]. Based on the current report, it is hypothesised that the effect of pH on shoot height is likely to be due to reduced uptake of NH_4^+ and NO_3^- at low and high pH, respectively.

In conclusion, the results of this experiment demonstrated that tomato Cv. Red Coat tissues can tolerate a wide range of pH as its shoot response, number of shoots produced per explant and callus response were not affected by the medium pH. However, better shoot height (growth) could only be achieved in the pH range 5.5-6 and the shoot height reduced at lower and higher pH indicating that the pH was influencing tomato growth indirectly via possibly affecting nutrient uptake at both extremes of pH. The reduction in nutrient uptake seems to be just enough to affect shoot height and it may not be drastic enough to affect the other traits studied. Further research is needed to elucidate the mechanism by which either the low pH (2.5–3) or high pH (8) affect growth in tomato and to determine whether other tomato cultivars respond similar to the Red Coat cultivar.

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