

Effect of pH on Callus Induction and Shoot Regeneration from Cotyledon and Leaf and Hypocotyl Explants of Tomato

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ABSTRACT

To standardize medium pH for *in vitro* callus induction and shoot regeneration from cotyledon and leaf explants of genotype Punjab Upma and IPA-3 of tomato, the explants were cultured on the MS medium which was maintained at wide range of pH (4.5-7.0). Callus induction and shoot regeneration were significantly affected by medium pH. The callus induction and shoot regeneration were affected with lower pH (4.5-5.0) and higher (6.0-7.0) medium pH significantly. Among two explants used cotyledons shows the maximum callus induction as well as shoot regeneration. The best callus induction and shoot regeneration occurred in the medium pH 5.5-6.0.

Key words: Callus induction, Shoot regeneration explants.

INTRODUCTION

Tissue culture techniques have become an attractive field of biotechnological research. The benefits of these studies are particularly valuable in the areas of large-scale clonal propagation, crop improvement, the production of important plant compounds and the conservation of genetic resources. The development of shoot regeneration efficiency requires a better understanding of the influence of culture conditions on shoot generation. Plant cells and tissues require an optimum pH for growth and development in cultures. The pH affects nutrient uptake as well as enzymatic and hormonal activities in plants¹

The optimal pH level regulates the cytoplasmic activity that affects cell division and the growth of shoots and it does not interrupt the function of the cell membrane and the buffered pH of the cytoplasm². The changes in external pH have a small transient effect on cytoplasmic pH but the cells are readily readjusted towards their original pH⁵, 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.

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This is also accompanied by a reduction in ATPs which leads to reduced plant growth⁴. The detrimental effects of adverse pH are generally related to an imbalance in nutrient uptake rather than to direct cell damage. The change in pH, amongst other factors, due to preferential uptake of NH_4^+ or NO_3^- from the medium, or an efflux of protons or hydroxy ions, respectively, caused by this uptake⁶. The uptake of anions is favoured at acidic pH, while that of cations is the best achieved when pH is increased³. The pH also influences the status of the solidifying agent in a medium: a pH higher than 6 produces a very hard medium and a pH lower than 5 does not sufficiently solidify the medium¹. Therefore, it is necessary to optimize pH level for callus induction and shoot regeneration because pH level directly influence shoot regeneration. In the present study, cotyledon, leaf and hypocotyl explants of tomato were cultured in a MS medium with various pH levels. Based on the obtained results, pH level were determined that affected to the callus induction and efficient shoot regeneration

MATERIAL AND METHODS

The investigation was carried out at “Tissue Culture and Genetic Transformation” Laboratory in the School of Agricultural Biotechnology, Punjab Agricultural University, Ludhaina during 2006-2009. In this study, seeds of two tomato genotypes *viz*: Punjab Upma and IPA-3 were used. Seeds were surface sterilized in 2% sodium hypochlorite for 20 minutes in laminar air flow and rinsed 3 times with sterile distilled water. Seeds were then germinated on MS medium supplemented with 100 mg/l myo-inositol and 30 g/l sucrose. Hormones like NAA, IAA and BAP were added in varying combinations and concentrations. All cultures were maintained at $25 \pm 2^\circ \text{C}$ under 16 h light (2500 lux) and 8 h dark periods. Cotyledons were excised from 15-20 day, whereas leaf and hypocotyl explants 25-30 day old *in vitro* germinated seedlings used as explants for callus induction

were excised from the observations were recorded on per cent callus induction and per cent regeneration via callus cotyledon, leaf and hypocotyl. The data were analyzed using factorial CRD design. All the media including the control were supplemented for callus induction with MS + with 1.0 mg/l NAA and 3.0 mg/l BAP and plant regeneration were supplemented with MS + IAA 1.0 mg/l and BAP 2.0 mg/l. Leaf, cotyledon and hypocotyl were used to examine the effects of pH on callus induction and shoot regeneration. The pH of the medium was varied from 4.0 to 7.5 (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5) and the pH of the media was adjusted (prior to autoclaving) by using either 1M NaOH or 0.25 M HCl. To minimize condensation, the media were cooled to 40°C before the lids were tightened. No pH buffers were used. Observations were recorded after four weeks of inoculation and these included percent seed germination, callus induction percent, shoot regeneration percent and number of shoot produced by per cotyledon, leaf and hypocotyl.

RESULTS AND DISCUSSION

pH had statistically significant effect on percent shoots regeneration response. However there was a trend such the better shoot regeneration occurs at the acidic pH (4.5-6.0) rather than the alkaline (6.5-7.5) (Table 1). The optimal pH was 5.5 at which 65.30% of the explants produced shoots. Statistically significant (0.48) effect was observed for the number of shoots produced per explants. The highest number of shoots/cotyledon (10.00) was recorded in pH level 6.0 the lowest number of shoot/cotyledon (5.4) was recorded when the medium was adjusted to pH 4.0 and 7.5 respectively. The effect of varieties and pH level was also showed statistically significant difference in respect of percent shoot regeneration and number of shoots produced per cotyledons (Table 2). Both the genotypes Punjab Upma and IPA-3 produced highest percent shoot

regeneration 68.20% and 62.40% respectively when the medium contained pH 5.5. The pH of the culture medium is an important factor for proliferating shoots *in vitro*. In the absence of pH regulation, the ionization of acidic and basic groups causes considerable changes in structure that affect their function at the cellular level⁴. The present study suggested that shoot regeneration is affected by the changes in the media pH. Every species requires an optimum pH which can promote maximum shoot formation. In the present study, a better performance in all parameters on shoot development was found at pH 5.5 in a MS medium containing 0.5 mg/l IAA and 3mg/l BAP. 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 (George1993). Conversely, alkaline pH is tolerated by the synthesis of organic acids, such as malate from a neutral precursor. The changes in pH during culture can be explained by the differential uptake of nitrogen sources; the uptake of NO₃⁻ leads to a drift towards an alkaline pH, while the uptake of NH₄⁺ results in a shift towards acidity³. The pH drop may lead to

preferential uptake of cations and to the exudation of organic acids from plant materials 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* explants which was not a response to wounding. Meanwhile, the best shoot multiplication at pH 5.8 level in a medicinal plant, *Calaphyllum apetalum*⁵. Lower and higher pH levels showed low performance for the induction and elongation of shoots from leaf derived calli of *A. elata*. The main reason for these results seems to be the solid status of the medium: a higher pH level resulted in a hard medium, while a lower pH resulted in unsatisfactory solidification of the agar. In this study, the pH level played an effective role in enzyme and growth regulator activities that affect the function of cells as well as whole plants. The role that optimum pH plays in enhancing the activities of growth regulators and enzymes have been suggested⁵. In conclusion, the results of this experiment demonstrated that this system also ensures base line for production of transgenic tomato.

Table 1: Callus induction, shoot regeneration and number of shoots from cotyledon explant of tomato in MS media containing different pH levels

pH	Callus induction (%)	Shoot regeneration (%)	No. of shoots/cotyledon
4.0	41.25	9.12	0.81
4.5	45.23	10.23	0.91
5.0	51.26	12.36	1.15
5.3	66.32	13.69	1.25
5.5	70.25	18.56	1.89
5.8	71.23	19.23	2.14
6.0	72.14	21.56	2.56
6.3	65.21	17.26	2.11
6.5	62.14	15.24	1.89
6.8	54.23	13.56	1.75
7.0	46.32	13.26	1.11

Table 2: Combined effects of variety and different pH level on percent shoot regeneration and number of shoot/cotyledon

Variety	pH	Callus induction (%)	Shoot regeneration (%)	No. of shoots/cotyledon
Punjab Upma	4.0	41.25	9.12	0.81
	4.5	45.23	10.23	0.91
	5.0	51.26	12.36	1.15
	5.3	66.32	13.69	1.25
	5.5	70.25	18.56	1.89
IPA-3	5.8	71.23	19.23	2.14
	6.0	72.14	21.56	2.56
	6.3	65.21	17.26	2.11
	6.5	62.14	15.24	1.89
	6.8	54.23	13.56	1.75
	7.0	46.32	13.26	1.11

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