A Modified Medium for the Enchancement of *in vitro* Xylogenesis in the Presence of Plant Hormone.

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INTRODUCTION

The minimal requirements for the induction of xylogenesis in explants of nondividing parenchymatous tissue are assumed to be an auxin, a cytokinin, and a carbohydrate (1). There has been little or no work on the nutritional requirements for the induction of xylogenesis *in vitro* aside from the hormonal and carbohydrate supplements. A recent study has shown that the exogenous carbohydrate requirement can be replaced by alternative carbon sources (2). The present investigation represents the first attempt to devise a basal nutrient medium for the induction of optimal numbers of tracheary elements *in vitro*. Our study is based on a modification of the Murashige and Skoog (MS) (3) basal medium.

PROCEDURE

Cylindrical explants (2 x 5 mm) were aseptically prepared from the pith parenchyma core removed from head of lettuce (*Lactuca sativa* L. Romána) as previously described (2). The explants were dark cultured (27 C. ± 1) on a Whatman No. 1 filter paper platform in glass vials (22 ml capacity) containing aproximately 5 ml of a liquid medium devised of induce tracheary element differentiation. The medium consisted of various modifications of the MS medium, IAA (10 mg/1), kinetin (0.1 mg / 1). and sucrose (2 % w/v). The final pH was

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adjusted to 5.5 and periodically tested during the experiment. After 7 days culture cell counts were made from each explants of newly-formed tracheary elements and total cell numbers (2).

RESLTS

Before testing the individual components of each of the stocks (macronutrients, iron supplement, micronutrients, and organic supplement), an experiment was performed using only IAA, kinetin, sucrose, and sterile double-distilled water. This treatment resulted in a greater percentage of tracheary elements than produced by a complete MS mixture plus the hormones and sugar. Our preliminary reslts therefore suggested that the standard MS medium does not provide optimal nutritional conditions for *in vitro* xylogenesis in lettuce pith explants.

Macronutrients

The macronutrients of the MS medium cnsist of NH_4NO_3, KNO_3, $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, and KH_2PO_4. Experiments were made using various media deficient in a single salt, and in addition, meda cnsisting of only a single macronutrient salt. The only salt showing a stimulatory effect on xylogenesis was $NH_4\ NO_3$, which gave optinum reslts at a concentration of 165 mg /1 (0.1 cncentration of normal MS medium).

Iron

All concentrations of the MS iron ($FeSO_4 \cdot 7H_2O$, Na_2 EDTA) stock tested (1.0, 0.5, 0.2, 0.1, 0.01) were inhibitory to xylogenesis. The application of Na_2 EDTA alone had a stimulatory effect at the normal MS concentration of 37.3 mg / 1.

Micronutrients

The micronutrients of the MS medium cnsist of $MnSO_4 \cdot 4H_2O$, $ZnSO_4 \cdot 4H_2O$, H_3BO_3, KI, $Na_2MoO_4 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, and $CoCl_2 \cdot 6H_2O$. KI and $Na_2MoO_4 \cdot 2H_2O$, tested in the same manner as the mactonutrients, were inhibitory at all concentrations (1.0 - 0.01). The remaining salts H_3BO_3, $MnSO_4 \cdot$

$4H_2O$, and $ZnSO_4 \cdot 7H_2O$ were most effective in the same concentration as the original MS formulation. Copper was most effective at 10x (0.25 mg / 1) and cobalt at 100x (2.5 mg / 1).

Organic Supplement

The MS vitamin spplement consists of glycine, nicotinic acid, pyridoxine HCl, and thiamine HCl. The cyclitol *myo*-inositol is added separately. Of the organic compounds tested, only nicotinic acid and myo-inositol were stimulatory to xylogenesis.

A Note on pH

The pH of the original MS medium is adjusted to 5.7 - 5.8. Unfortunately thiamine HCl is rapidly decomposed on autoclaving at pH values above 5.5 (4). For this reason, all of the experiments outlined in this study were performed at pH 5.5, and all of the media were autoclaved.

Conclusions

On the basis of the above experiments, the most effective nutrient medium for the induction of tracheary element formation in explants of lettuce pith consisted of the following:

Component	***Concentration*** (mg / 1)
NH_4NO_3	165
H_3BO_3	6.2
$MnSO_4 \cdot 4H_2O$	22.3
$ZnSO_4 \cdot 7H_2O$	8.6
$CuSO_4 \cdot 5H_2O$	0.25
$CoCl_2 \cdot 6H_2O$	2.5
Na_2EDTA	37.3
myo-inositol	100
nicotinic acid	0.5

The effectiveness of this xylogenesis-enhancement medium on tissue systems other than lettuce pith has not been examined to date.

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