THE INFLUENCE OF AUXINS AND MINERALS ON ROOT MORPHOGENESIS OF *EUCALYPTUS FICIFOLIA* F. MUELL *IN VITRO*

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Abstract. When several auxins were tested individually in the rooting medium of seedling cultures of *Eucalyptus ficifolia* distinct differences were found in root morphology and were linked to auxin structure Auxins having a phenolic oxygen between the aromatic ring and the side chain induced callus formation, whereas auxins without this oxygen promoted the development of a good root system Within this last category, however, different auxins gave rise to distinct root systems

Several nutrient groups were also tested in a rooting medium containing indole-3-butyric acid (IBA) as the only auxin and there was an interaction between the nutrients and IBA

INTRODUCTION

Morphogenesis, the origin of form, is a little understood phenomenon, which even recent advances in biochemical techniques and increased knowledge at the molecular and genetic levels, have failed to explain. Ultimately, an understanding of morphogenesis must involve a synthesis of all the individual details of plant function but at this level too, there are still large gaps in our knowledge, and much basic research is required.

The tissue culture system provides a unique opportunity for studying many aspects of plant growth and development under well-defined conditions In particular it should be exploited much further in studying the effect of various growth substances on morphology In recent years, there has been considerable research into plant cell cultures and concomitant genetic manipulation. The progress made, in terms of being able to achieve an end product, i.e. a whole plant from a single cell, has been disappointingly slow, and there is an increasing realization that a better understanding of plant developmental biology, through basic tissue culture study, is vital to the success of applied levels of research (4)

In this paper, a small aspect of plant development — the differentiation of roots at a macroscopic level — is discussed. The study is not definitive, it serves to emphasise the importance of cooperation between workers in the areas of plant physiology and biochemistry

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The work described here, follows on from a project commenced in 1976 designed to devise a suitable tissue culture method for the propagation of *E. ficifolia*. The basic principle applied to the initial research was that founded upon the Broad Spectrum experiment (5,10). The objective was to find a particular combination of the interacting categories and concentrations for use as a basal medium, suitable to achieve a particular response, e.g. callus, multiplication, rooting. Experimentation was then done to define specific constituents in the basal medium which were important to the achievement of the desired response.

The research on seedling cultured material was in its final stages, when, in large experiments with individual growth factors (6,8), the significance of riboflavin, especially in relation to the rooting response of cultures, was first discovered under light incubation. The presence of riboflavin in the culture medium, led to the development of a root system in which there were one to three, occasionally more, long, subsurface roots with short or no laterals, callus development at the base of the cultures was minimal. In comparison, absence of riboflavin led to the development of a root system in which there were many short roots and teratomas, associated with fairly heavy callusing, there was a tendency for the roots to grow close to, or on, the surface of the medium. Under dark incubation, the presence of riboflavin had no effect. Subsequent experimentation (15) to determine which constituents of the medium were affecting the rooting response, showed that riboflavin and indole-3-butyric acid (IBA), were linked in a distinct riboflavin-induced change in root morphology, in the light.

When activated by visible light, riboflavin gains a high oxidising potential, and reduced riboflavin, resulting from the oxidation of organic substances, can be readily re-oxidised by oxygen (12), thus having a role analogous to the one it plays in respiration. It has long been known that riboflavin can sensitize the degradation of indole-3-acetic acid (IAA) (1,11,12,13,14). It has also been reported to sensitize the degradation of 2-4-dichlorophenoxyacetic acid (2,4-D) (3), and α-naphthaleneacetic acid (α-NAA) (16), and it would thus seem reasonable to assume that a similar degradation was occurring with IBA

The results of the experiment to determine which constituents of the culture medium were affecting the rooting response, suggested two interesting avenues for further experimentation. a) to examine the effect of riboflavin together with other auxins, on root formation, b) to examine the interaction between riboflavin, IBA, and minerals on root formation.

MATERIALS AND METHODS

Plant material. The methods used to initiate and develop seedling cultures, have been described previously (2,6,7,9). The seedlings were repeatedly subcultured at approximately two month intervals over a four year period on the multiplication medium (6). Cultures were maintained in a Sherer growth cabinet (25 ± 2°C, 12 h photoperiod supplied by Powertube cool white fluorescent lights of approximately 20 $\mu Em^{-2}s^{-1}$). They were grown in UC30P polycarbonate tubes fitted with polypropylene screw-on lids, and containing 10 ml of medium, sterilized at 121°C and 104 kPa, for 20 min

Experimental media: The constituents of the rooting medium are given in Table 1. Deletions and additions to this medium were made according to the nature of individual experiments.

Experimental media were prepared in subdued light, and stored no longer than 24 h prior to use, in darkness. Explants, which consisted of 1 cm long shoot tips with several nodes, were transferred from the multiplication medium to the experimental media in a darkened room, with a small point light source, to illuminate the working area. Such precautions prevented the breakdown of riboflavin and any light-stimulated reactions between riboflavin and other constituents of the media.

(i) Auxin experiment.

The basal medium was that described in Table 1, except that various auxins were substituted for IBA. The auxin treatments were as follows*:

No auxins, six auxins (IAA, IBA, α-NAA, β-NOA, pCPA, 2,4-D), each at 5 μM. These are the components of the auxin category of the Broad Spectrum (BS) experiment; 5 μM of the following auxins tested individually, IBA, IAA, pCPA, 2,4-D, β-NOA, α-NAA, β-NAA, IPropA, IPyrA, 2,4,5-T.

Each of these auxins was tested in the presence and absence of 10 μM riboflavin, thus giving a total of 12 × 2 = 24 treatments Ten replicates of each treatment were incubated in the dark, and ten were given a 24 h exposure to light of approximately 110 $\mu Em^{-2}s^{-1}$, supplied by 40 W Sieray white fluorescent lights These replicates were then transferred to dark incubation. The cultures were examined after a total incubation period of 34 days.

* The following abbreviations, not already defined were used β-NOA — β-naphthoxyacetic acid, pCPA — p-chlorophenoxy-acetic acid, IPropA — indole-3-propionic acid, IPyrA — indole-3-pyruvic acid, 2,4,5-T — 2,4,5-trichlorophenoxyacetic acid

(ii) IBA/Mineral experiment

The basal medium was that described in Table 1; except that macro- and micronutrients were tested in the groups indicated in Table 1 as follows:

No macro- and micronutrients; Groups 1-5; Group 1; Group 2; Group 3; Group 4; Group 5.

Each of the mineral groupings was tested in the presence and absence of 5 μM IBA and 10 μM riboflavin, thus giving a total of $7 \times 2 \times 2 = 28$ treatments. There were ten replicates and all cultures were given a 24 h exposure to light before dark incubation. The cultures were examined after a total incubation period of 34 days.

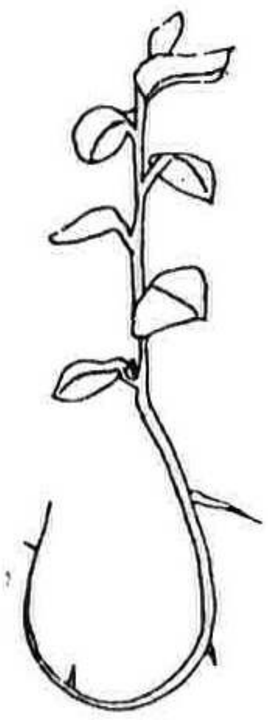
RESULTS

(i) Auxin experiment

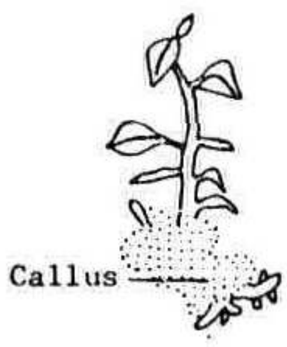
The effects of the individual auxin treatments, in the absence of riboflavin, in the light and the dark, are represented in Figure 1. In the absence of auxins, the percentage of explants forming roots was low (approximately 50%), and the root system was characterized by the appearance of one or two long roots with some lateral development; there was little or no callus; β-NAA, α-NAA, IPropA and IPyrA generally, encouraged 100% rooting of explants and the root system consisted of two or three long roots with fairly extensive lateral development.

All explants on a medium containing IBA, produced a root system consisting of many short roots, with extensive lateral development and a tendency for abundant root hairs, where roots had not penetrated the medium; IAA also caused a rather "stunted" root system to develop (in 100% of explants), but lateral development was minimal. It is interesting to note the difference in the root systems formed by IBA, IAA and IPropA. These three homologous auxins differ only in the number of CH_2 groups in the side chain and yet produced three distinct responses.

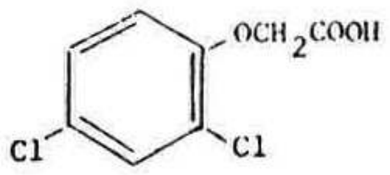
In the case of 2,4-D, 2,4,5-T, pCPA, β-NOA and the six BS auxins, 100% of explants produced a mass of basal, nodular callus, which occasionally developed teratomas (root-like extrusions) The addition of riboflavin to the medium had no effect on cultures incubated in the dark. However, with the exposure to light, cultures containing pCPA, β-NOA, β-NAA, α-NAA, IBA, IAA, IPropA, or IPyrA, produced a root system identical with that formed on cultures in a medium without any auxins; i.e. these auxins appeared to be inactivated in the presence of riboflavin in the light The riboflavin/light interaction caused a reduction in callus in cultures on a medium



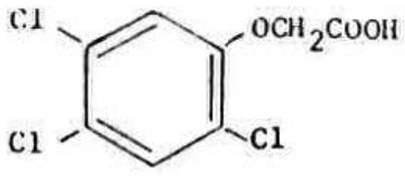
NO AUXINS



SIX BS AUXINS



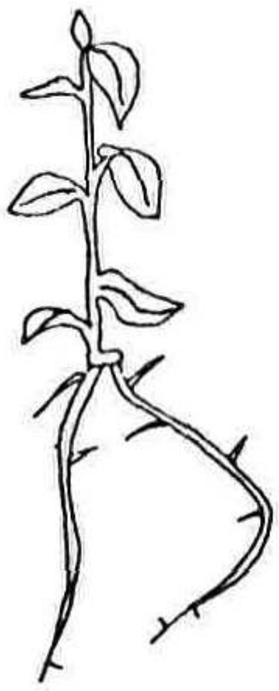
2,4-D



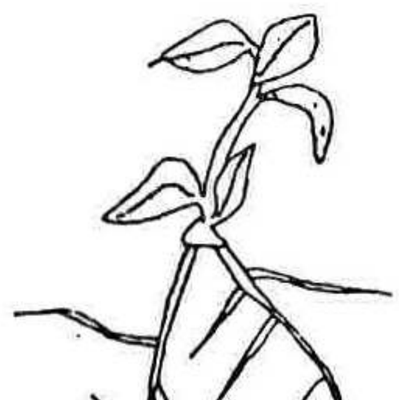
2,4,5-T

pCPA

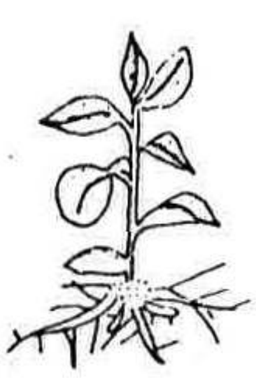
β-NOA



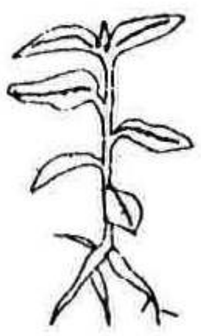
β-NAA



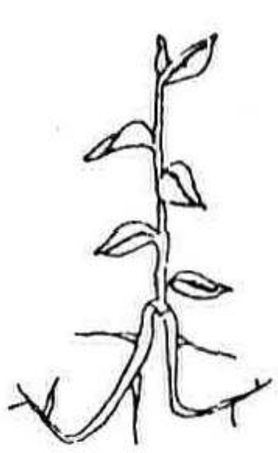
α-NAA



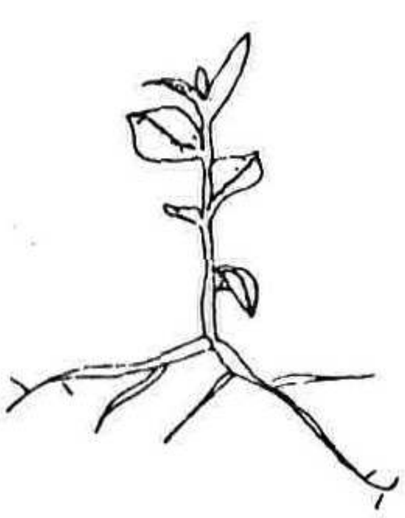
IBA



IAA



IPropA



IPyrA

Figure 1. The effect of individual auxins in the rooting medium on root morphology of seedling shoot tip cultures. These results were obtained in the light and in the dark when riboflavin was excluded from the medium. The six BS auxins are IBA, IAA, α-NOA, pCPA and 2,4-D.

containing 2,4-D, and short, thick roots were formed. Explants on media containing 2,4,5-T or the six BS auxins were unaffected by the presence of riboflavin in the light.

The most obvious point emerging from the experiment in terms of the effects of individual auxins, is that auxins containing a phenolic oxygen cause the formation of heavy callus and few or no roots This point is brought home dramatically in the comparison of the effects of β-NONA and β-NAA; there are two entirely different responses in the explants and yet the only difference between the auxins is in the oxygen link.

It would be interesting to test α-NOA which differs from α-NAA only in having a phenolic oxygen If the conclusion about structure and plant response holds true, one would expect the α-NOA to give a similar response to β-NOA.

(ii) IBA/Mineral experiment

The effects of individual treatments in the absence of riboflavin, are represented in Figure 2. It can be seen that in the absence of minerals and auxins, explants produced a root system identical to that described in the Auxin experiment for explants on the treatment without auxins, i.e. one or two long roots with some lateral development and little or no callus. Interestingly, this root system type appeared in all the mineral treatments where IBA was excluded from the medium (either in the presence or absence of riboflavin), or where IBA and riboflavin were present together, i.e. by themselves, individual nutrients did not affect morphogenesis.

In the presence of IBA, there were distinct morphogenetic responses to individual nutrients Explants on media containing no minerals, NaH_2PO_4, or KCl + $CaCl_2$, produced similar root systems, i.e. short, thick roots with fairly well developed laterals, with NH_4NO_3 + $MgSO_4$ present in the medium, explants produced a mass of very short roots — almost teratomas, $FeSO_4$ + Na_2EDTA, led to the formation of two or three long roots covered in a mass of very short laterals, and the remaining micronutrients (Group 4), gave a similar response although lateral development was not as pronounced.

DISCUSSION

The aims of the two experiments were to look at the effects of the riboflavin/light/auxin interaction on root morphogenesis and to this end, it has been shown that riboflavin caused inactivation of all the auxins tested, with the exception of 2,4,5-T and, to some extent, 2,4-D It is interesting that the two other callus promoting auxins, pCPA and β-NOA, were inactivated by riboflavin; the reasons for this difference need to be sought at the biochemical level. The incidental but more

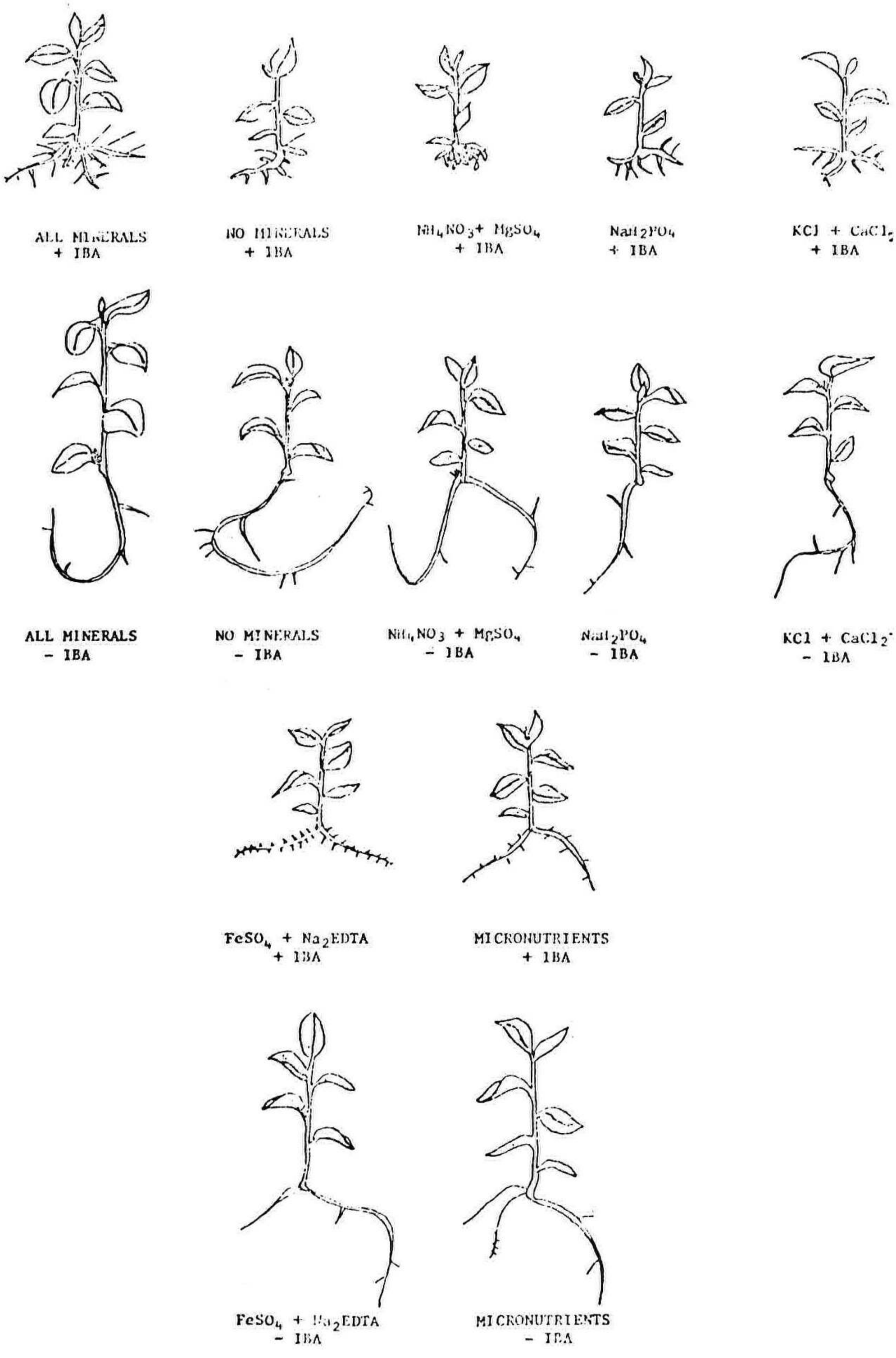


Figure 2. The effect of various nutrients in the rooting medium on root morphology of seedling shoot tip cultures, in the presence and absence of IBA. All minerals refers to the macro- and micronutrients described in Table 1.

interesting results of the experiments showed firstly that there is strong evidence for an auxin structure/plant response relationship, and secondly that specific nutrients and auxin interact to influence morphogenesis.

The way in which auxins act in the plant is still not fully understood. IAA is probably the major natural plant auxin and Thimann and Koepfli (19) were the first to suggest that IAA was the root-forming hormone. Synthetic auxins have been used for the induction of roots on cuttings, since they appear to be more stable in the plant than IAA, IBA and α-NAA, particularly, are commonly used in horticultural practice. The morphology of roots formed by the different auxins has not been widely noted. Pearse (18) found, in a variety of fruit tree cuttings, that IBA gave a finely-branched fibrous root system, while α-NAA gave thick, fleshy roots with few branches. Van Overbeek (20) noted that auxins generally promoted a compact root system composed of many short roots, whilst there were a few long roots when rooting was "left to nature". Certainly this latter point has been illustrated in the experiments described here

Connections between auxin structure and root morphology have not been widely reported. Kaethner (17) postulated that the hormonal activity of auxins was due to the ability of the bound ligand to undergo a simultaneous conformational change or re-orientation with its receptor. This theory may account for the observed differences between auxins, and other groups such as cytokinins, but it does not account for any differences in response elicited by different auxins.

Table 1. Composition of the culture medium for the induction of roots in seedling cultures of *Eucalyptus ficifolia*. (The nutrients are combined from 5 pre-stock solutions (5) and these groupings are shown)

Macronutrients (mM)	NH_4NO_3 (5), $MgSO_4$ (0.5)	— Group 1
	NaH_2PO_4 (1)	— Group 2
	KCl (1 9), $CaCl_2$ (1)	— Group 3
Micronutrients (μM)	H_3BO_3 (150), $MnSO_4$ (100), $ZnSO_4$ (40), $CuSO_4$ (1 5), Na_2MoO_4	— Group 4
	$FeSO_4$ (100), Na_2EDTA (100)	— Group 5
Auxins (μM)	IBA (5)	
Growth Factors (μM)	± Riboflavin (10)	
Main Carbon Source (mM)	Sucrose (120)	
Agar (g/l)	'Fluka' (9)	

The pH of all culture media was adjusted to 5 5 with 1M NaOH prior to autoclaving

Similar problems are encountered when considering the nutrient story and explanations for the interaction of IBA with individual groups of nutrients, are disappointingly elusive. One could speculate that concentration gradients created within the root cells, play some part

The work reported here has raised many questions for further research. The problem is to select an approach which will give interpretable and useful results. Structure-activity or hormone-nutrient studies are not likely to give answers on the mechanism of auxins in the initial biochemical event leading to root formation because the assay of root formation is too far removed biochemically from this event However, studies of this type are likely to produce results which are highly specific and useful for the plant propagator.

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THE INFLUENCE OF PLANT HORMONES AND GROWTH FACTORS ON GROWTH OF ERIOSTEMON AUSTRALASIUS PERS. IN TISSUE CULTURE

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Abstract. Following standard disinfestation treatments, cultures of apical and axillary buds of *Eriostemon australasius* Pers can be initiated on a simple minerals-sucrose-agar medium, i e , MZZ [ZM] and rapid multiplication can be induced in cultures transfered to medium — $[MH_{Fe}]Z$ $BAP_{31\ 6\mu M}$ $[H_{4+R}M]$ Apically-dominant growth can be induced on transfer of cultures to medium — $[MH_{Fe}]M$ KINETIN $_{10\mu M}$ $[H_{4+R}M]$, and roots can be induced to form on some cultures on medium — $[MH_{Fe}]$ $NAA_{31\ 6\mu M}$ $BAP_{0\ 0316\mu M}[M_{ALL\text{-}R}M]$

Only BAP and PBA were able to induce adventitious bud formation and these cytokinins had, in common, a benzyl ring as a substituent in the N^6 position

Interactions of auxins, cytokinins, riboflavin and other growth factors in producing various growth forms in culture are discussed

INTRODUCTION

The research done by Lilien-Kipnis and de Fossard (unpublished results) was primarily aimed at finding methods for the clonal propagation of *Eriostemon australasius* Pers. It succeeded in developing a high multiplication rate, but only one experiment was done on root induction (Lilien-Kipnis and de Fossard, unpublished results). Of great interest was the induc-

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