STUDIES ON THE MECHANISM OF PLANT CELL EXPANSION

A thesis presented in partial fulfilment
of the requirements for the degree
of Doctor of Philosophy
in Botany at
Massey University

David William Pearce
1983

ABSTRACT

The mechanism of elongation of segments of hypocotyl of light-grown seedlings of lupin (Lupinus angustifolius cv. N.Z. Bitter Blue) has been investigated. The approach was three-fold: biophysical analysis of growth responses; an investigation of the role of individual tissues in elongation; and tests of predictions of the acid-growth hypothesis.

In biophysical studies, a method was developed to measure the half-times of transients in elongation rate in response to application of a compressive load. For loads of 4-18g (equivalent to applied pressures of about 0.1-0.5 bars) half-times for the return of elongation rate to a steady value after loading were 3-15 minutes for segments incubated without IAA, and 6-13 minutes for IAA-treated segments. Half-times after removing the load were 2-7 minutes for non-IAA-treated segments. Results were analysed according to the diagnostic scheme of Cosgrove (1981, Plant Physiol. 68:1439-1446), and suggested that IAA promoted elongation through an effect on either the tissue free energy diffusivity of water (D), or on extensibility. It was not possible to distinguish between these alternatives on the evidence available.

In studies on the role of different tissues in elongation, the effect of removing specific tissues from non-IAA-treated segments was first determined. The epidermis apparently limited elongation of intact segments, since a burst of extension occurred when it was removed by peeling. In peeled segments, the stele (vascular tissue and pith) apparently limited the rate of extension since its removal resulted in very rapid extension of the remaining cylinder of cortex. On IAA treatment, the response of segments with the stele removed was initially similar to that obtained with intact segments, suggesting that the epidermis and cortex only were involved in the initial response. In segments where the epidermis had previously been removed this initial response to IAA was absent, but there was a longer term response. These results suggest that the response of intact segments to IAA consisted of two superimposed phases. The first was the result

of epidermal "relaxation", and the second was an independent elongation controlled by the cortex.

The acid-growth hypothesis predicts that treatment with acid solutions will promote elongation to IAA-induced levels. Tests of this prediction with hollow cylinders and peeled segments of lupin hypocotyl showed that the most IAA-responsive preparation (hollow cylinders with the epidermis intact) was the least acid-responsive, with little elongation response at pH 5. Treatment at pH 4 was needed to promote elongation to IAA-induced rate. The cortex alone responded strongly to acid treatment (pH 5), suggesting that the epidermis was limiting response when it was present. Peeled segments elongated in response to IAA treatment, but did not elongate in response to acid treatment (pH 5) (if pretreated in water), perhaps because response was limited by restricted diffusion of hydrogen ions through the starch sheath and into the stele. However, peeled segments elongated rapidly initially after treatment with acid if first pretreated in buffer (1mM K_2HPO_4-citric acid, pH 6.6). These results show that acid-induced elongation of segments may be influenced by differential response of tissues, by barriers to diffusion of hydrogen ions, and by treatment with buffered solutions. The results suggest that unless IAA action in intact segments causes pH in the walls of the outermost cell layers to fall to to about pH 4, then it is unlikely that IAA-induced elongation is mediated (initially) by hydrogen ions.

ACKNOWLEDGEMENTS

Dr David Penny, Dr Paul Gandar, and Professor R.G. Thomas supervised this study. I am sincerely grateful for their expert and patient guidance, and especially thank my chief supervisor, Dr Penny, for his help.

I thank Mrs Pauline Patchell and Kirsty Stalker for assistance with typing; Mr Richard Pearce for drawing some of the figures; Mr Jim Napier for helping with proof-reading; and Mr Chong-Lon Kan for some technical assistance.

Special thanks to Mr and Mrs Les Ford for their encouragement and support.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES