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Gene expression in the precocious germination of late maturation *Phaseolus vulgaris* L. seeds.

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Plant Biology at Massey University

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TABLE OF CONTENTS

			Page
TITLE PAGE			i
ACKNOWLEDGEMENTS			ii
TABLE OF CONTENTS			iii
LIST OF FIGURES			iv
LIST OF TABLES			vi
LIST OF ABBREVIATIONS			vii
ABSTRACT		**************************************	1
INTRODUCTION	*		2
METHODS		and the state of t	9
RESULTS		180	21
DISCUSSION			43
REFERENCES			68
APPENDICES			76

LIST OF FIGURES

		Page
FIGURE 1	Stages of pod and seed development in <i>Phaseolus vulgaris</i> .	10
FIGURE 2	Gas-tight containers used for the incubation of <i>Phaseolus vulgaris</i> embryos.	11
FIGURE 3	Typical responses of <i>Phaseolus vulgaris</i> seeds and embryos to the experimental treatments.	22
FIGURE 4	Fresh weight changes and radicle elongation of <i>Phaseolus vulgaris</i> embryos and seeds during incubation.	23
FIGURE 5	Agarose gel electrophoresis of <i>Phaseolus</i> vulgaris RNA isolated using guanidinium and phenol/chloroform extraction procedures(Gel A).	26
FIGURE 6	Agarose gel electrophoresis of <i>Phaseolus</i> vulgaris RNA isolated using guanidinium and phenol/chloroform extraction procedures(Gel B).	28
FIGURE 7	Gradient SDS-polyacrylamide gel electrophoresis of <u>in vitro</u> translation products.	33
FIGURE 8	Protein content of putative protein-body enriched and protein-body depleted fractions over development and germination.	37
FIGURE 9	SDS-polyacrylamide gel electrophoresis of protein-body depleted fractions prepared from development and germination stages of <i>Phaseolus vulgaris</i> .	39
FIGURE 10	SDS-polyacrylamide gel electrophoresis of protein-body enriched fractions prepared from development and germination stages of <i>Phaseolus vulgaris</i> .	40
FIGURE 11	The response to energy capture by individual silver halide grains in film.	46

FIGURE 12	Agarose gel electrophoresis of RNA extracted from axes and cotyledons of <i>Phaseolus vulgaris</i> .	77
FIGURE 13	Agarose gel electrophoresis of RNA extracted from axes and cotyledons of <i>Phaseolus vulgaris</i> .	79
FIGURE 14	Isolation from <i>Phaseolus vulgaris</i> total RNA of a poly(A) ⁺ fraction by the technique of oligo-dT-cellulose chromatography.	81

LIST OF TABLES

		Page
TABLE 1	Yields and purity of <i>Phaseolus vulgaris</i> RNA isolated using guanidinium and phenol/chloroform extraction procedures.	25
TABLE 2	Incorporation of ³⁵ S-methionine into TCA-precipitable protein by <u>in vitro</u> translation of <i>Phaseolus vulgaris</i> RNA.	30
TABLE 3	Incorporation of ³⁵ S-methionine into TCA-precipitable protein by <u>in vitro</u> translation of <i>Phaseolus vulgaris</i> RNA.	31

LIST OF ABBREVIATIONS

1-D one dimensional

2-D two dimensional

A₂₆₀ absorbance at 260nm

ABA abscisic acid

AU absorbance unit

AU*mm absorbance unit times millimetres(a measure of peak area)

cpm counts per minute

DAA days after anthesis

DEPC diethylpyrocarbonate

EDTA ethylenediaminetetraacetic acid

FW fresh weight

kb kilobase

kD kilodalton

LEA late embryogenesis abundant(protein)

MOPS 3-[N-Morpholino]propanesulfonic acid

MW molecular weight

PCR polymerase chain reaction

pI isoelectric point

ppm parts per million
PPO 2,5-diphenyloxazole

CDC

SDS sodium dodecylsulphate

SDS-PAGE sodium dodecylsulphate polyacrylamide gel electrophoresis

TBE Tris-borate EDTA

TCA trichloroacetic acid

TEMED N,N,N',N',-tetramethylethylenediamine

TMV tobacco mosaic virus

ABSTRACT

Ethylene induces precocious germination in late maturation embryos (32-40 days after anthesis) of Phaseolus vulgaris L. cv. Seminole, thus overriding the endogenous controls which normally maintain quiescence. The possibility that ethylene exerts its effects at the gene expression level was investigated by in vitro translation of RNA extracted from embryo axis tissue of seeds induced to germinate precociously by incubation with ethylene. 35S-labelled products so produced were analyzed by electrophoresis, fluorography, and scanning densitometry. Results were compared with normally germinating seeds and with embryos incubated in the absence of ethylene. Ethylene was found to induce a qualitative and quantitative change in gene expression in late maturation embryos detectable within 6 hours of ethylene exposure. Two products (37-38kD and 27kD) were up-regulated within 24 hours in both ethylene-induced precocious germination and normal germination. products (71kD, 67-68kD, 65-66kD, and 41-42kD) which appeared in normal germination were evidently not required for ethylene-induced precocious germination. In contrast with the findings of Misra & Bewley (1985;1986) for maize(Zea mays L.) no products could be identified as being unique to the developmental phase, however two products (38-39kD and 28kD) were strongly present in development but disappeared shortly after germination. A product of 22-23kD was apparently unique to the ethylene-induced precocious germination treatment and may represent a gene regulated by ethylene. This product was not seen until 24 hours after ethylene introduction. An attempt was made using SDS-PAGE to identify the major storage proteins of P.vulgaris to use as markers of the developmental phase, however this was only partially successful. Suggestions are made as to approaches and methods for future research.