

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

5-AMINOURACIL
SYNCHRONIZATION OF THE CELL CYCLE OF VICIA FABA
ROOT TIP MERISTEMS

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

JENNIFER ANN BUTCHER

1977

ABSTRACT

This study was undertaken to try and find how the thymine analogue 5-aminouracil induces cell synchrony in the cell cycle of plant root meristems. It has previously been used as a synchronizing agent without knowing its mode of action.

The experiments confirmed the synchronization effect and that the removal of plants from 5AU stimulated the cells to divide. Results indicated that the late S and early G₂ phases of the cell cycle were the most affected, with DNA synthesis continuing in the presence of 5AU at a reduced rate. The inhibition of division caused by 5AU could be reversed by other bases and mixtures.

The G₁ phase was found not to be affected by 5AU but it was postulated that cells in early G₂ were slowed down or halted by the chemical. DNA density measurements were taken of nuclei treated continuously for varied times with 5AU, and these results confirmed a buildup of cells in the latter third of the S phase found by other workers. The presence of Feulgen-negative regions in chromatids of the 5AU treated tissue was noted and linked with possible interference in heterochromatin synthesis. The possibility of some enzyme function important in the final joining together of DNA units being interfered with by 5AU is also discussed. Suggestions are made for further possible avenues of work into DNA synthesis.

The significance of cell cycle studies and their experimental design has recently been reconsidered and is mentioned in view of this work and other cell population studies.

ACKNOWLEDGEMENTS

I would firstly like to thank my supervisor Dr E D Penny of the Botany Department, Massey University, for his continual guidance during the course of this study, and for his valued criticism of the manuscript.

My thanks also go to Dr K Giles of the Plant Physiology Division, DSIR, Palmerston North, for his unlimited assistance with the fluorescence photography and making the fluorescence microscope and several stains available for my use.

The generosity of Dr P Fitzgerald of the Cytogenetics Unit, Christchurch Hospital, in allowing my use of the Vickers M85 Integrating Microdensitometer is also greatly appreciated.

I would also like to thank Professor R Thomas for advise and discussion on different aspects of this study.

Finally to my husband Mike must go thanks for his continued encouragement, and especially for assistance with photography and diagrams.

TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS USED IN TEXT	x
INTRODUCTION	1
MATERIALS AND METHODS	7
2-01 Growth of Seedlings	7
2-02 5AU treatment	10
2-03 Cytological Procedures	11
2-04 Isotope Incorporation Studies	16
2-05 Reversal Experiments	18
2-06 Sectioning of Material	19
2-07 DNA content of cell nuclei	21
RESULTS	24
3-01 Synchronization Experiments	24
3-02 Longitudinal sections from synchrony experiments	31
3-03 Effect of 5AU on G ₁ and G ₂ phases	35
3-04 DNA synthesis after 5AU treatment	38
3-05 Effect of 5AU on DNA synthesis itself	42
3-06 Longitudinal sections from labeling experiments	47
3-07 Nuclear DNA density	49
3-08 Reversal Experiments	57
3-09 Quiescent Centre	63

3-10	Cell Cycle Duration	64
3-11	Growth Inhibition	66
DISCUSSION		69
CONCLUSION		88
REFERENCES		90
APPENDICES		101
1.	Staining Procedures	101
2.	Dehydration, Infiltration and Embedding							
	of Tissue	106

LIST OF TABLES

TABLE	PAGE
1. Hoagland's Mineral Nutrient Solution 2.	8
2. LI recorded in the presence or absence of 5AU solution over 7 hours ..	35
3. LI of mitotic figures observed over 11 hours of 5AU treatment	37
4. MI and percentage of labeled figures occurring over peak of division after a 12 hour 5AU treatment	40
5. LI and MI obtained after Pulse A using 'cold' thymidine, H ³ -TdR and H ³ -CdR	44
6. LI and MI during Continuous 5AU treatment	46
7. A selection of DNA densities of mitotic figures from several control slides	51
8. Mitotic figures seen in reversal experiments	59
9. Average cell lengths in microns at 2 positions in the root tip during different lengths of 5AU treatment	67
10. Variation in cell cycle times for <u>Vicia</u> <u>fab</u> a in the literature	84

LIST OF FIGURES

FIGURE	PAGE
1. <u>Vicia faba</u> plants growing in darkroom situation	9
2. Longitudinal median section through a root showing different cell types ..	20
3. Fluorescing nuclear DNA of cells in squash preparations stained with BAO ..	20
4. Graph showing recovery after a 24 hour 5AU treatment, using roots from one seedling per sample	25
5. Graph of results gained from first experiment using continuous 5AU	25
6A. Graph showing recovery after a 24 hour 5AU treatment	28
6B. Graph of MI during continuous 5AU treatments	28
6C. Graph showing recovery after a 12 hour 5AU treatment	28
7. Partially synchronized cell division 14 hours after a 24 hour 5AU treatment	30
8. No cells dividing at the end of 24 hours of 5AU treatment	30
9. Diagrams of longitudinal median sections of roots collected 10 to 15 hours after removal from a 24 hour 5AU treatment	32
10. Diagrams of longitudinal median sections of roots collected over peaks of divisions after a 12 hour 5AU treatment ..	34

FIGURE	PAGE
11. 21½ hours continuous 5AU preparation showing incorporation of label immediately after a half hour pulse ..	48
12. 42 hours continuous 5AU treatment showing large nuclei	48
13. Diagrams of longitudinal median sections of pulse labeled tissue showing distribution of label in the root tip	50
14. Histograms showing a change in nuclear DNA content during continuous 5AU treatment	53
15. Histograms showing range of DNA content in G ₁ and G ₂ cells treated with continuous 5AU	56
16. High mitotic index seen in reversal experiment using mixture of pyrimidine bases	60
17. 'Damaged' nuclei seen after prolonged treatment with 'cold' thymidine	60
18. Longitudinal section of pulse labeled tissue showing the quiescent centre	65
19. Close up of quiescent centre	65
20. Feulgen-negative regions visible in chromatids after 15 hours continuous 5AU treatment	78
21. Feulgen-negative regions visible in control tissue	78

ABBREVIATIONS USED IN TEXT

5AU	5-aminouracil
BAO	2,5-bis[4'-aminophenyl-(1')]7-1,3,4-oxadizole
CdR	deoxycytidine
H ³ -CdR	tritiated deoxycytidine
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
FUdR	5-fluorouracil-2-deoxyriboside
H ³	tritium
LI	Labeling Index
MI	Mitotic Index
ppm	parts per million
RNA	Ribonucleic acid
RNase	Ribonuclease
TdR	thymidine
H ³ -TdR	tritiated thymidine