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CYTOKININS AND THE GROWTH OF SPIRODELA OLIGORRHIZA

A thesis submitted

for the degree of

Doctor of Philosophy

by

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SUMMARY

1 Spirodela oligorrhiza on sterile glucose-mineral medium ceased to grow three days after transfer into darkness.

2 Cytokinins, supplied in the medium, allowed continuous growth of Spirodela after transfer to darkness. Other plant growth substances, or adenine analogues, were ineffective.

3 Kinetin stimulated production of new fronds after a 24 hour lag period when added to dormant cultures although it was rapidly taken up. Kinetin reached a constant concentration in the plantlets within 30 to 60 minutes of addition to the medium.

4 In the absence of cytokinin, dormancy continued for three or four weeks after which growth spontaneously resumed in darkness. The growth rate then reached almost half that achieved with optimum kinetin concentrations in darkness. Growth continued in darkness for at least eight weeks without cytokinin.

5 Pretreatment in the light with either metabolic inhibitors, kinetin, abscisic acid, or high or low temperature, essentially eliminated the period of dormancy of Spirodela transferred to darkness in the absence of cytokinin. Growth was reduced 50% to 90% during the pretreatments.

6 The pretreatments designed to affect plastid RNA, protein or ATP production were the most effective. The fastest growth rate achieved in darkness without cytokinin after pretreatment was 10% less than that promoted by optimum kinetin.

7 Dormant Spirodela in darkness continued to incorporate precursors into RNA, DNA and protein at a rate 50% that in growing (plus kinetin) cultures. Net rates of macromolecule accumulation were extremely slow, indicating extensive degradation.

8 Addition of kinetin to non-growing Spirodela in darkness stimulated the synthesis of RNA, DNA and protein simultaneously after a lag of approximately one hour. The rates of precursor incorporation increased to equal those in continuously growing cultures.

9 Non-growing Spirodela in darkness rapidly accumulated starch. Kinetin had little or no effect on accumulation and mobilization of starch.

10 ^{14}C-glucose was taken up by growing (plus kinetin) and non-growing (minus kinetin) Spirodela in darkness, and was metabolized equally in each. Three times as much ^{14}C-glucose entered starch in the non-growing cultures.

11 A model scheme for the control of dormancy in Spirodela is proposed based on an inhibitory mediator. The mediator may be similar to the hypothetical mediator of the pleiotypic response shown by animal cells.

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ABBREVIATIONS AND TRIVIAL NAMES

ABA	abscisic acid
Ach	acetylcholine
3BA	3-benzyladenine
6BA	6-benzylaminopurine
BSA	bovine serium albumen
CAP	chloramphenicol
C	Curie
DEAE	diethylaminoethyl
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
f.w.	fresh weight
FUDR	5-fluorodeoxyuridine
g	acceleration due to gravity
GA	gibberellic acid
gm	gram
IAA	indoleacetic acid
kinetin	6-furfurylaminopurine
m, u, n	milli (10^{-3}), micro (10^{-6}), nano (10^{-9}) respectively
M	molar
MAK	methylated albumen bound to celite
ml, ul	millilitre, microlitre respectively
NDS	naphthalene disulphonate
nm	nanometres
OD	optical density
PVP	polyvinylpyrollidone
rRNA, tRNA, mRNA	ribosomal, transfer and messenger RNA respectively
RNA	ribonucleic acid
SDS	sodium dodecyl sulphate
Spec. act.	specific activity
TCA	trichloroacetic acid
Tris	tris(hydroxymethyl)aminomethane
uv	ultraviolet