PROTECTIVE MECHANISMS IN NEW ZEALAND LIVERWORTS

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ABSTRACT

Liverworts were the first plants to successfully make the transition from water to land and thus had to develop morphological and physiological features to overcome novel environmental constraints. The relative simple structure of liverworts in comparison to vascular plants implies that they have to combat environmental stress at a cellular level. Liverworts are an important part of the New Zealand flora. Leafy liverworts such as *Jamesoniella colorata*, *Isotachis lyallii* and *Lepidolaena taylorii* display a spectacular range in colouration within a single population. The leaves can vary from pale green in shaded habitats to bright red in more exposed places. A thalloid species, *Monoclea forsteri*, lacks such pigmentation but it might possess some unique cellular features to deal with stressful conditions. This thesis aims to study the cellular characteristics that allow liverworts to cope with extreme environmental conditions, with emphasis on (red) auxiliary pigments.

Genetic analysis of liverwort populations revealed a highly clonal structure, which suggests that red colouration of liverwort leaves is a phenotypic response, and not a result of genotypic differences. Indeed, the red pigment could be induced in *I. lyallii* at relatively modest irradiance levels.

The red leaves absorbed about 10% more photosynthetically active radiation than green leaves from the same species. Although the chemical characteristics of the red pigment remained elusive, it has light attenuation characteristics similar to anthocyanins in vascular plants. Its cellular location inside the cell wall is ideal for intercepting potentially damaging light quanta and can thereby protect the underlying chloroplasts from high light fluxes. The light energy that is absorbed by the red pigment did not translate into an enhanced protection from chilling-stress through leaf warming.

The photosynthetic apparatus of liverworts has acclimated to the environmental conditions that these plants are most likely to experience in their habitat. Gametophytes from shady environments had lower chlorophyll *a* to *b* ratios than gametophytes found in sunnier places. This illustrates the need for a more efficient light-harvesting system in the former to collect all of the available light. In addition, plants from sunny environments had lower chlorophyll to

carotenoid ratios than plants found in shady environments, which suggests that the photo-protective properties of carotenoids are better developed in the former. This was confirmed by measurements of chlorophyll *a* fluorescence which clearly showed a greater potential for non-photochemical quenching in gametophytes from exposed habitats than gametophytes found in sheltered places, both during high light treatment and desiccation.

A better developed photo-protective system (*i.e.* non-photochemical processes and light-screening pigments) allowed the red-leaved gametophytes to better deal with high light stress as well as desiccation than their green-leaved counterparts. Measurements of photosynthetic responses revealed that red gametophytes were photoinhibited less and their recovery was more complete from a high light treatment of 1800 μmol/m^2/s than the greens. Similarly, measurements of photosynthetic responses and oxidative damage indicated that the red morph was better protected from high light fluxes that occur during drought-stress than the green morph. Determination of cell water relations showed that the red pigment in *J. colorata* did not influence the water balance during drought.

In conclusion, this study has presented evidence that liverworts from exposed habitats are better equipped to deal with abiotic stressors such as high light and dehydration than liverworts from more sheltered environments. Changes in pigment composition, concentration and location are likely to play an important role in liverwort protection from environmental stress – most noticeably a red pigment with photo-protective attributes.

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ABBREVIATIONS

SI (Système Internationale) abbreviations are used for chemical elements. Other abbreviations are listed below:

°C . . . . . degrees Celsius
$^3Chl^*$ . . . . . triplet (excited) chlorophyll
ε . . . . . extinction coefficient
µg . . . . . microgram(s)
µl . . . . . micro litre(s)
µM . . . . . micro molar
µmol . . . . . micromole(s)
Φ_{PSII} . . . . . efficiency of photochemistry within photosystem II
Ψp . . . . . turgor pressure
Ψw . . . . . water potential
Ψπ . . . . . osmotic potential
Ψπ,s . . . . . symplast osmotic potential
< . . . . . smaller-than
> . . . . . greater-than
≤ . . . . . smaller-than or equal to
≥ . . . . . greater-than or equal to
≈ . . . . . almost equal to

A . . . . . absorbance
ABA . . . . . abscisic acid
AMOVA . . . . . analysis of molecular variance
ANOVA . . . . . analysis of variance
APX . . . . . ascorbate peroxidase
ARI . . . . . anthocyanin reflectance index
AsA . . . . . ascorbate
ATP . . . . . adenosine triphosphate
BHT . . . . . butylated hydroxytoluene

bp . . . . . base pair(s)

c . . . . . circa

cm . . . . . centimetre(s)

DHA . . . . . dehydroascorbate

DHAR . . . . . dehydroascorbate reductase

DNA . . . . . deoxyribonucleic acid

dNTPs . . . . . 2'-deoxynucleotide 5'-triphosphate

D_{st} . . . . . average gene diversity over loci

DTT . . . . . dithiothreitol

DW . . . . . dry weight

EDTA . . . . . ethylene diamine tetra-acetic acid

etc . . . . . etcetera

Fd . . . . . ferrodoxin

fig(s) . . . . . figure(s)

F_m . . . . . maximal chlorophyll *a* fluorescence of dark acclimated tissue

F_m' . . . . . maximal chlorophyll *a* fluorescence of light acclimated tissue

F_o . . . . . minimal chlorophyll *a* fluorescence of dark acclimated tissue

F_o' . . . . . minimal chlorophyll *a* fluorescence of light acclimated tissue

F_s . . . . . stable chlorophyll *a* fluorescence of light acclimated tissue

F_v . . . . . variable chlorophyll *a* fluorescence of dark acclimated tissue

F_v/F_m . . . . . maximum quantum efficiency of photosytem II

FW . . . . . fresh weight

g . . . . . gram(s)

g . . . . . gravitational acceleration

GR . . . . . glutathione reductase

GSH . . . . . glutathione

GSSH . . . . . glutathione disulfide

H_2O_2 . . . . . hydrogen peroxide

hr(s) . . . . . hour(s)

Kb . . . . . kilo base(s)

km . . . . . kilometre(s)

lea . . . . . late-embryogenesis-abundant

m . . . . . meter(s)

M . . . . . molar (moles / litre)

MDA . . . . . malondialdehyde

$MDA^{\bullet}$ . . . . . monodehydroascorbate radical

MDAR . . . . . monodehydroascorbate reductase

mg . . . . . milligram(s)

min(s) . . . . . minute(s)

ml . . . . . millilitre(s)

mm . . . . . millimetre(s)

mM . . . . . milli molar

MPa . . . . . mega Pascal(s)

$NADP^{+}$ . . . . . nicotinamide adenine dinucleotide phosphate

NADPH . . . . . protonated form of nicotinamide adenine dinucleotide phosphate

n.d. . . . . not detectable

ng . . . . . nanogram(s)

nm . . . . . nanometre(s)

NPQ . . . . . non-photochemical quenching

O_2 . . . . . molecular oxygen

$O_2^{\bullet-}$ . . . . . superoxide

$^1O_2$ . . . . . singlet oxygen

$^{\bullet}OH$ . . . . . hydroxyl radical

PAR . . . . . photosynthetically active radiation (400-700 nm)

PCR . . . . . polymerase chain reaction

PRI . . . . . photochemical reflectance index

PSI . . . . . photosystem I

PSII . . . . . photosystem II

P-V . . . . . pressure-volume

Q_A . . . . . plastoquinone

qP . . . . . photochemical quenching

R . . . . . reflectance

R'a . . . . . apoplast volume

RAPDs . . . . . random amplified polymorphic DNA

REL . . . . . relative electrolyte leakage

RFLPs . . . . . restricted fragment length polymorphisms

RNA . . . . . ribonucleic acid

$ROO^{\bullet}$ . . . . . lipid peroxyl radical

ROS . . . . . reactive oxygen species

R_{red} / R_{green} . . . . . red / green reflectance ratio

Rubisco . . . . . ribulose biphosphate carboxylase / oxygenase

RWC . . . . . relative water content

s . . . . . second(s)

SEM . . . . . scanning electron microscopy

SOD . . . . . superoxide dismutase

sp . . . . . species (singular)

spp . . . . . species (plural)

SW . . . . . saturated weight

TBA . . . . . thiobarbituric acid

TBARS . . . . . thiobarbituric acid reactive substances

TBE . . . . . Tris-boric acid-EDTA

TCA . . . . . trichloroacetic acid

TEM . . . . . transmission electron microscopy

UV . . . . . ultraviolet

UV-B . . . . . ultraviolet-B (280-320 nm)

UV-VIS . . . . . ultraviolet-visible

$^{v}/_{v}$ . . . . . volume / volume

W . . . . . Watt

$^{w}/_{v}$ . . . . . weight / volume