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Thermogenesis in plants: the mode of heating and regulation in hot flowers

Nicole Michele Grant
University of Wollongong

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Thermogenesis in plants: the mode of heating and regulation in hot flowers

**A thesis presented in fulfilment of the requirements for the
award of the degree of**

Doctor of Philosophy

from

University of Wollongong

by

Nicole Michele Grant

BSc. (Hons.)

School of Biological Sciences

2010

DECLARATION

I, Nicole Michele Grant, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced and acknowledged below. This document has not been submitted for qualifications at any other academic institution.

- Mitochondrial protein isolation and stable isotope measurements of *N. nucifera* and *P. bipinnatifidum* tissues needed to be done simultaneously; therefore, stable isotope measurements in Chapters 2, 4 & 5 were carried out by S. Robinson, J. Watling and R. Miller.
- In Chapter 2, R. Miller assisted with carbohydrate measurements.
- AOX protein sequencing in Chapter 3 was performed in Japan by K. Ito, Y. Kakizaki and J. Watling. Y. Onda assisted with experimental design.
- Chapter 5 was co-authored with R. Miller, who also aided with lipid and carbohydrate measurements and analysis.
- Due to the short Australian flowering season of *P. bipinnatifidum* in 2008, elevated O₂ stable isotope measurements were undertaken in California by S. Robinson, J. Watling, R. Miller, L. Giles and J. Berry.
- Respiration measurements of *D. vulgaris* in Chapter 6 were performed by R. Seymour.

I carried out all other field measurements, sample collection, experimental design, protein measurements, mitochondrial biochemistry, carbohydrate and fatty acid measurements, data analysis and writing.

Please see print copy for image



The lotus flower is regarded as a spiritual flower that inspires a person taking root in muddy, murky water, the darkest of places, to where he or she rises above to bloom beautiful, powerful on the surface. Anon

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ABSTRACT

Thermogenesis or self heating in plants has been known to scientists for more than 100 years. It occurs across a number of different plant families, most commonly in the Araceae family, however questions remain as to which tissues heat, the heating mechanisms and the respiratory substrates used for heating in the majority of species. Heat production is thought to occur via the alternative oxidase (AOX), the plant uncoupling protein (pUCP), or perhaps a combination of the two. In this study four thermogenic plant species were investigated: three species from the Araceae family, *Amorphophallus titanum*, *Dracunculus vulgaris* and *Philodendron bipinnatifidum*; and one from Nelumbonaceae, *Nelumbo nucifera*.

Nelumbo nucifera receptacles, petals and stamens were all found to heat independently of one another. The receptacle heated most above non-thermogenic tissue temperature ($8.1 \pm 1.9^\circ\text{C}$), stamens intermediate and petals the least ($2.8 \pm 4.2^\circ\text{C}$). In *P. bipinnatifidum* both the sterile male florets and the fertile male florets heated independently, whilst the female florets did not heat. Heating in fertile male florets was characterised by a pattern similar to sterile male florets but with a lower mean peak temperature, regulated at 35.7°C ; compared with the sterile male peak temperature which was regulated at $> 40^\circ\text{C}$. The sterile and fertile male florets continued to heat more than 10°C above laboratory temperature (22°C) for up to 30 hrs following removal from the plant. The pattern of heating (a peak, followed by a dip and then a regulatory plateau) continued despite removal from the plant indicating that all resources for the thermogenic phase are within the florets themselves. The male florets of *D. vulgaris* heated $5.6 \pm 0.9^\circ\text{C}$ above ambient while the appendix did not heat despite high respiratory flux values. Unlike the male florets, the large appendix is not insulated by the spathe, thus it is possible that heat loss exceeds heat production in the appendix of this species.

AOX protein was found in the thermogenic tissues of all study species during the thermogenic period. In *N. nucifera*, *D. vulgaris* and *P. bipinnatifidum* there was a significant increase in AOX protein with the onset of thermogenesis in thermogenic tissues and a significant decrease at the end of the thermogenic period. In contrast, the non-thermogenic female florets of *P. bipinnatifidum* showed little change in AOX protein levels during floral development. *In vivo* flux through the AOX pathway in

N. nucifera and *P. bipinnatifidum* was quantified using stable oxygen isotope methodology and a significant positive relationship between AOX flux and the amount of heating above ambient was confirmed. During the thermogenic period AOX flux accounted for up to 99% of the total flux in *P. bipinnatifidum* and 93% in *N. nucifera*. The localisation of AOX protein in the various floral parts and possible evolutionary rationale is discussed.

Amino acid sequencing of the *N. nucifera* receptacle AOX protein revealed two isoforms, NnAOX1a and NnAOX1b. In both isoforms, the N-terminal regulatory cysteine residue found in most plant AOXs was replaced with a serine residue. Accordingly, the protein was activated by succinate, not the α -keto acid pyruvate, and the majority of protein could not be oxidised with diamide. Similarly, the majority of AOX protein isolated from *N. nucifera* petals and stamens could not be oxidised by diamide either, suggesting that AOX in these tissues also lacks the first regulatory cysteine. By contrast, AOX from *P. bipinnatifidum* fertile male and sterile male florets could be partly oxidised while AOX from the non-thermogenic female florets was almost fully oxidised by diamide. Functional differences between AOX from thermogenic and non-thermogenic tissues are discussed.

Plant uncoupling proteins were not detected in *N. nucifera* or *A. titanum* tissues. In *P. bipinnatifidum*, pUCPs were detected in both thermogenic and non-thermogenic tissues, however there was no significant change in pUCP expression during floral development in any tissue. Flux through the energy conserving COX (cytochrome oxidase) pathway was not correlated with heating or COX protein levels. The lack of change in either flux through the COX pathway or pUCP expression during thermogenesis, as well as the extremely high fluxes through the AOX pathway during peak heating events suggests that pUCPs are unlikely to contribute to heating in these species.

Lipids and carbohydrates were investigated as possible substrates for thermogenesis. In *N. nucifera* floral tissues carbohydrates decreased by 90% during the thermogenic period while lipids remained stable, suggesting that carbohydrates, not lipids, are the respiratory substrate in this species. In *P. bipinnatifidum*, storage lipids (triacylglycerides) in sterile male florets significantly decreased, while lipids in the

fertile male florets were low and did not significantly change during thermogenesis. Conversely, fertile male florets had significantly higher concentrations of starch than sterile male florets. Starch concentration in fertile male florets decreased by 82% during the thermogenic period while there was no change in the sterile male florets. In this species it appears that both lipids and carbohydrates are used as respiratory substrates for thermogenesis.

Advancing our understanding of the physiology and biochemistry of several thermogenic and thermoregulatory plant species, this study provides compelling evidence that AOX, rather than pUCP, plays a role in thermogenesis in the species studied. The methods utilised could be used to identify the involvement of the AOX pathway in other thermogenic species and thus further our knowledge of plant respiration in general.

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ABBREVIATIONS

AEST	Australian Eastern Standard Time
Ala	alanine
ANOVA	analysis of variance
AOX	alternative oxidase
BAT	brown adipose tissue
BSA	bovine serum albumin
CN	cyanide
COX	cytochrome c oxidase
Cys	cysteine
D	isotopic discrimination factors
DMSO	dimethyl sulfoxide
DTT	dithiothreitol
EGS	ethylene glycol-bis(succinimidylsuccinate)
FA	fatty acids
fru	fructose
fw	fresh weight
g	acceleration due to gravity
glc	glucose
Glyox	glyoxylate
KCN	potassium cyanide
kDa	kilo daltons
LA	linoleic acid
LDH	lactate dehydrogenase
Myr	million years
NADH	reduced nicotinamide adenine dinucleotide
<i>n</i>	sample number
<i>n</i> -PG	<i>n</i> -propyl gallate
PL	phospholipid
pUCP	plant uncoupling protein
redox	reduction/oxidation
ROS	reactive oxygen species
SA	surface area
sd	standard deviation
SDS	sodium dodecyl sulfate
se	standard error
Ser	serine
SHAM	salicylhydroxamic acid
suc	sucrose
Succ	succinate
<i>syn</i>	synonym
TAG	triacylglyceride
TBST	TBS-Tween
TCA	tricarboxylic acid cycle
UCP	uncoupling protein
V	volume
v/v	volume to volume ratio
w/v	weight to volume ratio