Science with Impact
Article Collection

Journal of Experimental Botany

the plant journal

Plant Direct

Plant Biotech Journal

Conservation Physiology

Introduction to the Collection

To commemorate the Society for Experimental Biology's (SEB)'s Centenary year, this article collection showcases the impactful research published to date in the society's five academic journals: *Journal of Experimental Botany*, *The Plant Journal*, *Plant Biotechnology Journal*, *Plant Direct* and *Conservation Physiology*. Impact is considered based on scientific significance, public interest, and real-world applications of research. Content published throughout the journals' lifetimes is included, demonstrating how the journals have developed over the years, and illustrating wider scientific progress within diverse fields. Each article is accompanied by an introduction, written by the article's author or by another knowledgeable individual, which contextualises the work and explains its scientific significance.

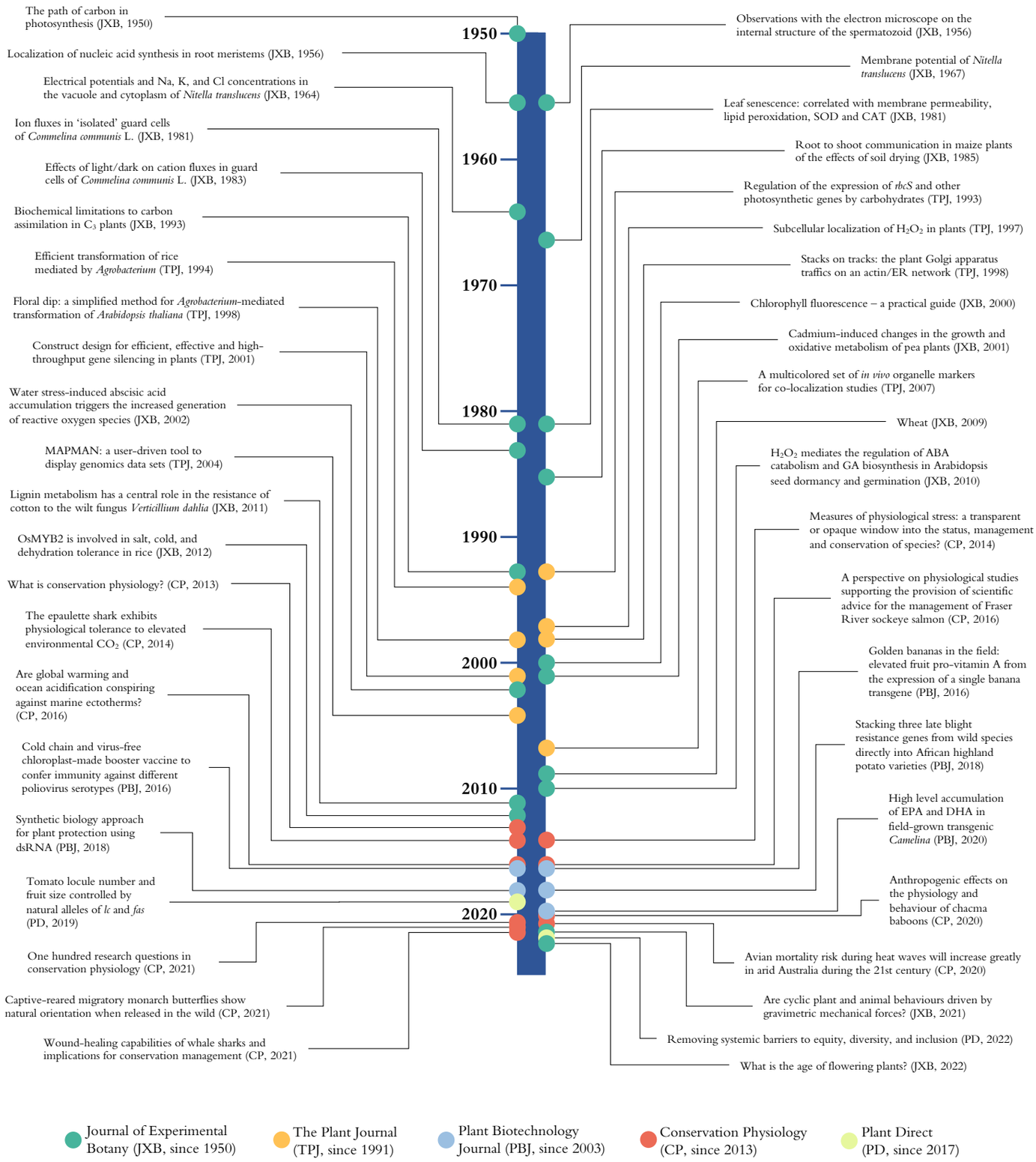
To produce the collection, article longlists were generated based on citation patterns and Altmetric data. Citations during the years after publication and in the present day were analysed in order to effectively represent both immediate and long-term impact. Altmetric data included media and social media attention, and mentions in policy documents and patents. SEB Editorial Office staff refined the longlists in association with journal Editors- in-Chief, SEB section chairs, and other respected names from various fields.

Of particular interest at this refinement stage were reports on major scientific or methodological breakthroughs, for example Benson and Calvin's seminal work describing the carbon fixation pathway in photosynthesis (Benson & Calvin, 1950, *Journal of Experimental Botany*); and development of the floral-dip method for *Agrobacterium*-mediated plant transformation (Clough & Bent, 2008, *The Plant Journal*), which greatly simplified the transformation process and which consequently remains a widely-employed technique. Also included in the collection are articles defining and shaping their research field, such as Cooke *et. al.'s* perspective which poses one hundred research questions for generating actionable evidence to inform conservation policy and practice (Cooke *et. al.* 2021, *Conservation Physiology*); and the Plant Science Research Network's report on recommendations for improving equity, diversity and inclusion in the plant sciences and beyond (Henkhaus *et. al.* 2022, *Plant Direct*). Another key criterion for inclusion was research relevant to combatting major global challenges such as climate change, biodiversity loss, food insecurity, and human health. For example, to combat vitamin-A deficiency, a major global health concern, Paul *et. al.* (2016, *Plant Biotechnology Journal*) developed a method for producing 'Golden bananas' with elevated fruit provitamin-A content using *Agrobacterium*-mediated genetic transformation. In the conservation area, Womersley *et. al.* (2021, *Conservation Physiology*) provides new insights on whale sharks' abilities to heal human-induced wounds and discusses the conservation implications of this work. By featuring articles such as these, this collection, as with the Centenary celebrations as a whole, looks to the future and to the technological advancements, emerging fields, and global issues which present fresh avenues for exploration and scientific impact.

Collection edited by Bridget O'Boyle, with thanks to the SEB editorial office team, especially to Mike Page and David Mansley. Thanks also to Tracy Lawson, journal Editors in Chief, and to all others who contributed to organising the collection and who provided article introductions.

Cover design by iKann editing.

A timeline of Science with Impact in the SEB's journals



Science with Impact
100 YEARS 1923 - 2023

Journal of Experimental Botany

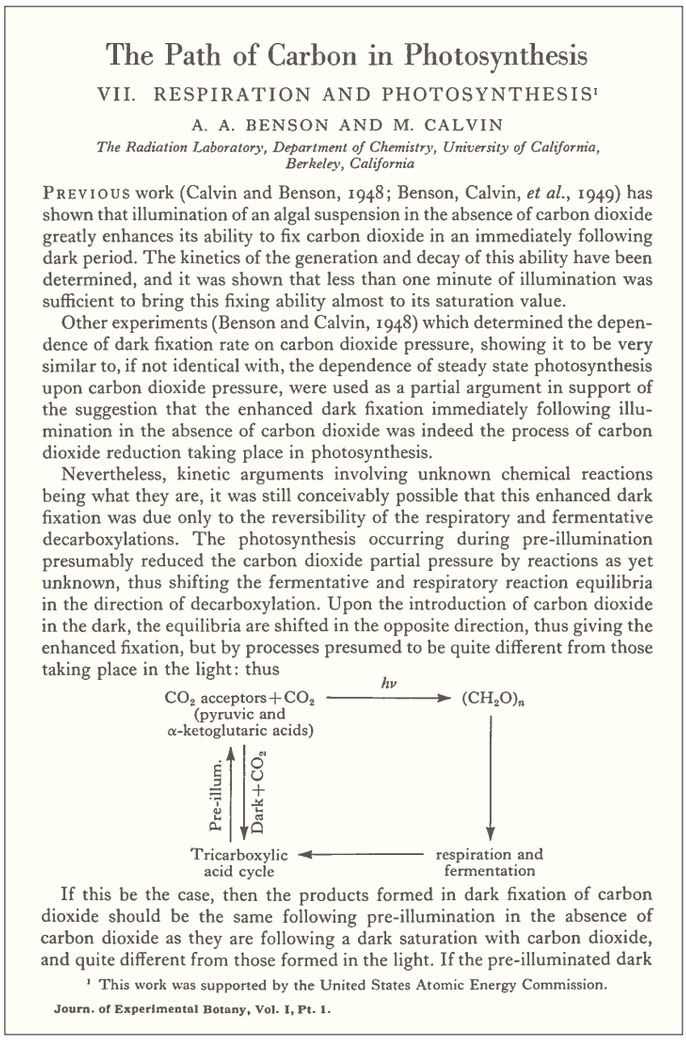
Journal of Experimental Botany

A. A. BENSON, M. CALVIN

The Path of Carbon in Photosynthesis: VII. RESPIRATION AND PHOTOSYNTHESIS

Journal of Experimental Botany, Volume 1, Issue 1, 1950, Pages 63–68

• Read the full article here: https://doi.org/10.1093/jxb/1.1.63



The Path of Carbon in Photosynthesis

VII. RESPIRATION AND PHOTOSYNTHESIS[1]

A. A. BENSON AND M. CALVIN

The Radiation Laboratory, Department of Chemistry, University of California, Berkeley, California

PREVIOUS work (Calvin and Benson, 1948; Benson, Calvin, *et al.*, 1949) has shown that illumination of an algal suspension in the absence of carbon dioxide greatly enhances its ability to fix carbon dioxide in an immediately following dark period. The kinetics of the generation and decay of this ability have been determined, and it was shown that less than one minute of illumination was sufficient to bring this fixing ability almost to its saturation value.

Other experiments (Benson and Calvin, 1948) which determined the dependence of dark fixation rate on carbon dioxide pressure, showing it to be very similar to, if not identical with, the dependence of steady state photosynthesis upon carbon dioxide pressure, were used as a partial argument in support of the suggestion that the enhanced dark fixation immediately following illumination in the absence of carbon dioxide was indeed the process of carbon dioxide reduction taking place in photosynthesis.

Nevertheless, kinetic arguments involving unknown chemical reactions being what they are, it was still conceivably possible that this enhanced dark fixation was due only to the reversibility of the respiratory and fermentative decarboxylations. The photosynthesis occurring during pre-illumination presumably reduced the carbon dioxide partial pressure by reactions as yet unknown, thus shifting the fermentative and respiratory reaction equilibria in the direction of decarboxylation. Upon the introduction of carbon dioxide in the dark, the equilibria are shifted in the opposite direction, thus giving the enhanced fixation, but by processes presumed to be quite different from those taking place in the light: thus

If this be the case, then the products formed in dark fixation of carbon dioxide should be the same following pre-illumination in the absence of carbon dioxide as they are following a dark saturation with carbon dioxide, and quite different from those formed in the light. If the pre-illuminated dark

[1] This work was supported by the United States Atomic Energy Commission.

Journ. of Experimental Botany, Vol. I, Pt. 1.

Introduced by
Douglas Orr • Lancaster University, UK

In the late 1940s, the pace of discovery of components of what would become known as the Calvin-Benson-Bassham cycle was prodigious. Yet there remained many unknowns, and indeed theories that would later be discarded as additional insights came about (for an excellent historical perspective see Sharkey 2019). Earlier work had investigated the impact of light-dark transitions and CO_2 presence on carbon fixation in the dark by algal suspensions (Calvin & Benson 1948, Calvin *et. al.* 1949). Following this, and with consideration of the evidence available, or as the authors put it "kinetic arguments involving unknown chemical reactions being what they are", in 1950 Benson and Calvin sought to gather further evidence on the potential reversibility of decarboxylations as an explanation for enhanced dark fixation under certain conditions (Benson & Calvin, 1950). In this paper Benson and Calvin present a series of radiograms of labelling experiments with algae and barley leaves to examine this question under varied light and gas conditions. From this and existing evidence of CO_2 fixation in the dark following strong illumination, the authors were able to confirm their earlier hypothesis that "all of the reactions lying between carbon dioxide and sucrose are dark reactions". In addition to validating and extending earlier work, of interest are the pointers to theories of the time and the then unrealised importance of some observations. Some, such as the involvement of reactions in the tricarboxylic acid cycle, would soon become disproven through additional labelling studies (Calvin & Massini 1952). Other observations, namely the labelling of phosphoglycolate, would take a further 20 years to fully understand, following the identification of the oxygenase activity of Rubisco as the first step in photorespiration (Bowes *et. al.* 1971).

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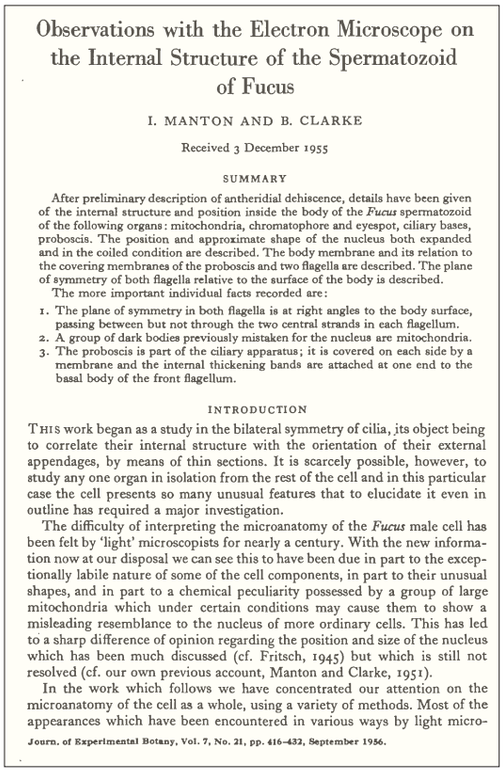
IRENE MANTON AND BRYAN CLARKE

Observations with the Electron Microscope on the Internal Structure of the Spermatoziod of *Fucus*

Journal of Experimental Botany, Volume 7, Issue 3, 1956, Pages 416–417

• Read the full article here: https://doi.org/10.1093/jxb/7.3.416



Observations with the Electron Microscope on the Internal Structure of the Spermatozoid of Fucus

I. MANTON AND B. CLARKE

Received 3 December 1955

SUMMARY

After preliminary description of antheridial dehiscence, details have been given of the internal structure and position inside the body of the *Fucus* spermatozoid of the following organs: mitochondria, chromatophore and eyespot, ciliary bases, proboscis. The position and approximate shape of the nucleus both expanded and in the coiled condition are described. The body membrane and its relation to the covering membranes of the proboscis and two flagella are described. The plane of symmetry of both flagella relative to the surface of the body is described.

The more important individual facts recorded are:

1. The plane of symmetry in both flagella is at right angles to the body surface, passing between but not through the two central strands in each flagellum.
2. A group of dark bodies previously mistaken for the nucleus are mitochondria.
3. The proboscis is part of the ciliary apparatus; it is covered on each side by a membrane and the internal thickening bands are attached at one end to the basal body of the front flagellum.

INTRODUCTION

THIS work began as a study in the bilateral symmetry of cilia, its object being to correlate their internal structure with the orientation of their external appendages, by means of thin sections. It is scarcely possible, however, to study any one organ in isolation from the rest of the cell and in this particular case the cell presents so many unusual features that to elucidate it even in outline has required a major investigation.

The difficulty of interpreting the microanatomy of the *Fucus* male cell has been felt by 'light' microscopists for nearly a century. With the new information now at our disposal we can see this to have been due in part to the exceptionally labile nature of some of the cell components, in part to their unusual shapes, and in part to a chemical peculiarity possessed by a group of large mitochondria which under certain conditions may cause them to show a misleading resemblance to the nucleus of more ordinary cells. This has led to a sharp difference of opinion regarding the position and size of the nucleus which has been much discussed (cf. Fritsch, 1945) but which is still not resolved (cf. our own previous account, Manton and Clarke, 1951).

In the work which follows we have concentrated our attention on the microanatomy of the cell as a whole, using a variety of methods. Most of the appearances which have been encountered in various ways by light micro-

Journ. of Experimental Botany, Vol. 7, No. 21, pp. 416–432, September 1956.

Abstract After preliminary description of antheridial dehiscene, details have been given of the internal structure and position inside the body of the *Fucus* spermatozoid of the following organs: mitochondria, chromatophore and eyespot, ciliary bases, proboscis. The position and approximate shape of the nucleus both expanded and in the coiled condition are described. The body membrane and its relation to the covering membranes of the proboscis and two flagella are described. The plane of symmetry of both flagella relative to the surface of the body is described.

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Introduced by
Colin Brownlee & Katherine Helliwell
• Marine Biological Association, UK & University of Exeter; Marine Biological Association, UK

Irene Manton began her studies of the structure of algal cells in the 1950s, seizing the opportunities afforded by the development of the electron microscope, which allowed unprecedented resolution not possible with light microscopy. Indeed, her early electron micrographs heralded the explosion of the use of the electron microscope in cell biology. This article by Irene Manton and Bryan Clarke is one of a series of 6 papers that provide some of the first descriptions of fundamental subcellular structures. Focussing on the easily obtainable *Fucus* sperm cell as a model, Manton described the core structure of cilia and flagella (the axoneme), providing the first reconstruction of the "9 + 2" organisation of the microtubules – arguably one of the most significant discoveries in cell biology of the 20th century. Subsequently, working with Mary Parke at the Marine Biological Association in Plymouth, Manton broadened her ultrastructural studies to the marine phytoplankton, leading to the discovery of the haptonema in the haptophyte algae, a structure superficially similar to flagella but with a different arrangement of microtubules. A key development at the time in electron microscopy was the preparation of ultrathin sections. Working with collaborators at the Rockerfeller Institute, the ubiquity of the "9 + 2" flagellar microtubule structure became apparent. Irene Manton's work also led to the confirmation that the Golgi apparatus was a major secretory organ in eukaryotic cells. This was enabled by careful studies of the production of the intricate calcium carbonate "coccoliths" that are found on the surface of the single-celled coccolithophorid phytoplankton, the globally important group of calcifying phytoplankton belonging to the haptophytes. Manton showed that coccoliths were produced inside Golgi cisternae (the coccolith vesicle) and secreted directly onto the cell surface by the process of exocytosis. Manton received many accolades in recognition of her important work, including being elected a Fellow of the Royal Society as well as being awarded the Linnean medal. Her career continued long after her retirement and involved many collaborations world-wide, continuing her focus on the marine phytoplankton. Her legacy lives on through the many cell biology studies that have been spawned through her discoveries. •

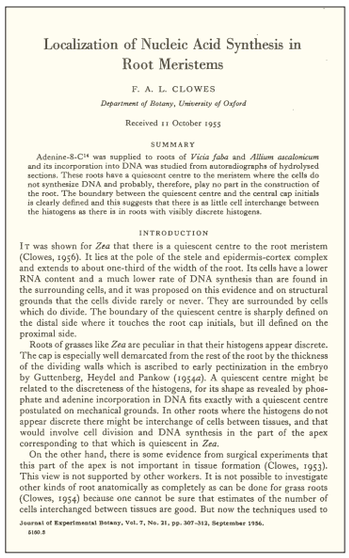
F.A. LIONEL CLOWES

Localization of Nucleic Acid Synthesis in Root Meristems

Journal of Experimental Botany, Volume 7, Issue 3, 1956, Pages 307–312

• Read the full article here: https://doi.org/10.1093/jxb/7.3.307

Abstract Adenine-8-C^{14} was supplied to roots of *Vicia faba* and *Allium ascalonicum* and its incorporation into DNA was studied from autoradiographs of hydrolysed sections. These roots have a quiescent centre to the meristem where the cells do not synthesize DNA and probably, therefore, play no part in the construction of the root. The boundary between the quiescent centre and the central cap initials is clearly denned and this suggests that there is as little cell interchange between the histogens as there is in roots with visibly discrete histogens.



Localization of Nucleic Acid Synthesis in Root Meristems

F. A. L. CLOWES

Department of Botany, University of Oxford

Received 11 October 1955

SUMMARY

Adenine-8-C^{14} was supplied to roots of *Vicia faba* and *Allium ascalonicum* and its incorporation into DNA was studied from autoradiographs of hydrolysed sections. These roots have a quiescent centre to the meristem where the cells do not synthesize DNA and probably, therefore, play no part in the construction of the root. The boundary between the quiescent centre and the central cap initials is clearly defined and this suggests that there is as little cell interchange between the histogens as there is in roots with visibly discrete histogens.

INTRODUCTION

It was shown for *Zea* that there is a quiescent centre to the root meristem (Clowes, 1956). It lies at the pole of the stele and epidermis-cortex complex and extends to about one-third of the width of the root. Its cells have a lower RNA content and a much lower rate of DNA synthesis than are found in the surrounding cells, and it was proposed on this evidence and on structural grounds that the cells divide rarely or never. They are surrounded by cells which do divide. The boundary of the quiescent centre is sharply defined on the distal side where it touches the root cap initials, but ill defined on the proximal side.

Roots of grasses like *Zea* are peculiar in that their histogens appear discrete. The cap is especially well demarcated from the rest of the root by the thickness of the dividing walls which is ascribed to early pectinization in the embryo by Guttenberg, Heydel and Pankow (1954a). A quiescent centre might be related to the discreteness of the histogens, for its shape as revealed by phosphate and adenine incorporation in DNA fits exactly with a quiescent centre postulated on mechanical grounds. In other roots where the histogens do not appear discrete there might be interchange of cells between tissues, and that would involve cell division and DNA synthesis in the part of the apex corresponding to that which is quiescent in *Zea*.

On the other hand, there is some evidence from surgical experiments that this part of the apex is not important in tissue formation (Clowes, 1953). This view is not supported by other workers. It is not possible to investigate other kinds of root anatomically as completely as can be done for grass roots (Clowes, 1954) because one cannot be sure that estimates of the number of cells interchanged between tissues are good. But now the techniques used to

Journal of Experimental Botany, Vol. 7, No. 21, pp. 307-312, September 1956.
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Introduced by
Joseph G. Dubrovsky • Universidad Nacional Autónoma de México (UNAM), Mexico

How the root apical meristem (RAM) is organized, how it functions and from where all the cells are coming are still not easy questions, but they are important to understand root growth and development. F.A.L. Clowes was one of the first who applied DNA radioactive precursors (first inorganic ^{32}P, then adenine-8-^{14}C, and later ^{3}H-thymidine) to study the RAM. In a milestone study published in 1956 he coined the term "the quiescent centre", describing the group of cells in the RAM that rarely divide and that have a lower RNA content than in the rest of the RAM (Clowes, 1956a). Now we know that the quiescent centre (QC) behaves as an organizer and consists of cells with stem cell functions (Dubrovsky and Ivanov, 2021).

This first study of Clowes on the QC was performed on maize (*Zea mays*) roots that have a closed-type RAM organization with recognizable initial cells. In this species, the QC coincided exactly with anatomically identified promeristem (Clowes, 1954) that includes initial cells giving rise to all the RAM tissues. Clowes finalizes his first work on the QC saying: "Such exact agreement of independent techniques makes it possible to try to use the autoradiograph method to investigate other types of root meristems where the cell organization is more difficult to interpret" (Clowes, 1956a, p33). So, his next study published in Journal of Experimental Botany (Clowes, 1956b) was dedicated precisely to demonstrating that the QC is formed also in such cases when the RAM organization is not clear based on anatomy.

In this study he applied radiolabeled adenine-8-^{14}C to roots of *Vicia faba* and *Allium ascalonicum*. In the first species with open-type RAM "none of the histogens is readily distinguished" (Clowes, 1956b, p309). Despite that, autoradiographs of the RAM longitudinal sections after feeding the root with labelled adenine permitted clear recognition of the QC in the RAM. One of the views of that time was that cells below the root columella (central root cap) initial cells form so called "transverse meristem" (Popham, 1955) which gives rise to all the RAM tissues. After identification of the QC in *V. faba* it became clear that these columella initial cells were always labelled and located below the QC, thus rejecting the possibility that transverse meristem was a source of cells for stele or cortex. In *A. ascalonicum* the origin of each tissue type was similarly unclear and "the files of columella cells sometimes appear continuous with those of the stele" (Clowes, 1956b, p310). Again, the autoradiograph technique showed the presence of the QC and evidenced that columella initials are heavily labelled. Therefore, a possibility for a common origin of columella and stele initials was again rejected, showing that anatomical or cell pattern analyses alone are not sufficient to understand the dynamics and origin of the root cell types. In this study, it was also reported for the first time, though without many details, that fern (*Azolla*) roots with a single apical cell do not show quiescence of the apical cells.

This study (Clowes, 1956b) thus showed some important aspects for understanding the RAM function. It revealed that (1) The QC formation is common for species with both open-type and closed-type RAM organization; (2) Columella initial cells are rapidly cycling cells and are not part of the QC; (3) The roots with a single apical cell do not show QC-like properties; (4) The QC "shape, size, and position in the meristem probably fluctuate" (Clowes, 1956b, p311); (5) Cells on the surface of the QC behave as initial cells giving rise to the RAM cells. With this work, a new direction was paved for root developmental studies that brought us to deeper understanding of the RAM organization, cell production, and root growth. The transition from static anatomical studies to a dynamic experimental approach was also evident.

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Science with Impact

Journal of Experimental Botany

R.M. SPANSWICK, E.J. WILLIAMS

Electrical Potentials and Na, K, and Cl Concentrations in the Vacuole and Cytoplasm of *Nitella translucens*

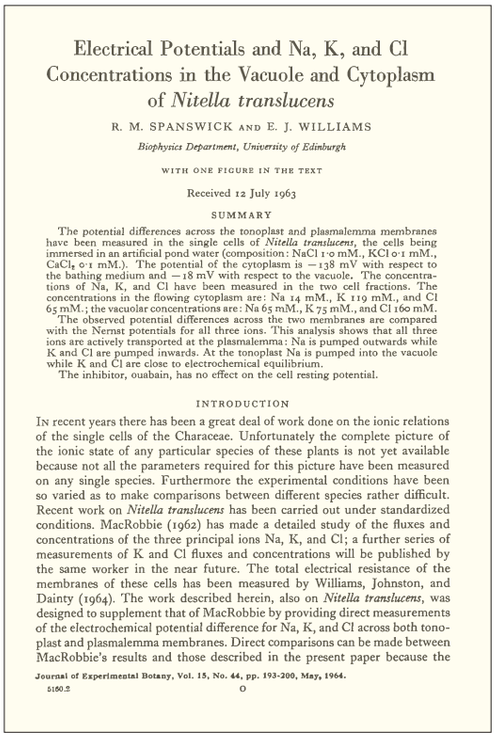
Journal of Experimental Botany, Volume 15, Issue 2, May 1964, Pages 193–200

• Read the full article here:
https://doi.org/10.1093/jxb/15.2.193

Abstract The potential differences across the tonoplast and plasmalemma membranes have been measured in the single cells of *Nitella translucens*, the cells being immersed in an artificial pond water (composition: NaCl 1.0 mM., KC1 0.1 mM., $CaCl_2$ 0.1 mM.). The potential of the cytoplasm is –138 m V with respect to the bathing medium and –18 mV with respect to the vacuole. The concentrations of Na, K, and Cl have been measured in the two cell fractions. The concentrations in the flowing cytoplasm are: Na 14 mM., K 119 mM., and Cl 65 mM.; the vacuolar concentrations are: Na 65 mM., K 75 mM.,and Cl 160 mM.

The observed potential differences across the two membranes are compared with the Nernst potentials for all three ions. This analysis shows that all three ions are actively transported at the plasmalemma: Na is pumped outwards while K and Cl are pumped inwards. At the tonoplast Na is pumped into the vacuole while K and Cl are close to electrochemical equilibrium.

The inhibitor, ouabain, has no effect on the cell resting potential.



Electrical Potentials and Na, K, and Cl Concentrations in the Vacuole and Cytoplasm of *Nitella translucens*

R. M. SPANSWICK AND E. J. WILLIAMS

Biophysics Department, University of Edinburgh

WITH ONE FIGURE IN THE TEXT

Received 12 July 1963

SUMMARY

The potential differences across the tonoplast and plasmalemma membranes have been measured in the single cells of *Nitella translucens*, the cells being immersed in an artificial pond water (composition: NaCl 1·0 mM., KCl 0·1 mM., $CaCl_2$ 0·1 mM.). The potential of the cytoplasm is −138 mV with respect to the bathing medium and −18 mV with respect to the vacuole. The concentrations of Na, K, and Cl have been measured in the two cell fractions. The concentrations in the flowing cytoplasm are: Na 14 mM., K 119 mM., and Cl 65 mM.; the vacuolar concentrations are: Na 65 mM., K 75 mM., and Cl 160 mM.

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The inhibitor, ouabain, has no effect on the cell resting potential.

INTRODUCTION

In recent years there has been a great deal of work done on the ionic relations of the single cells of the Characeae. Unfortunately the complete picture of the ionic state of any particular species of these plants is not yet available because not all the parameters required for this picture have been measured on any single species. Furthermore the experimental conditions have been so varied as to make comparisons between different species rather difficult. Recent work on *Nitella translucens* has been carried out under standardized conditions. MacRobbie (1962) has made a detailed study of the fluxes and concentrations of the three principal ions Na, K, and Cl; a further series of measurements of K and Cl fluxes and concentrations will be published by the same worker in the near future. The total electrical resistance of the membranes of these cells has been measured by Williams, Johnston, and Dainty (1964). The work described herein, also on *Nitella translucens*, was designed to supplement that of MacRobbie by providing direct measurements of the electrochemical potential difference for Na, K, and Cl across both tonoplast and plasmalemma membranes. Direct comparisons can be made between MacRobbie's results and those described in the present paper because the

Journal of Experimental Botany, Vol. 15, No. 44, pp. 193-200, May, 1964.

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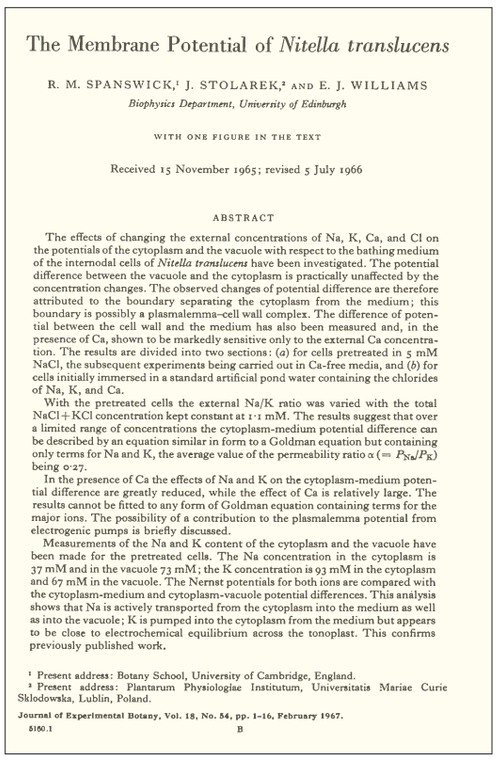
R.M. SPANSWICK, J. STOLARK, E.J. WILLIAMS

The Membrane Potential of *Nitella translucens*

Journal of Experimental Botany, Volume 18, Issue 1, February 1967, Pages 1–16

• Read the full article here:
https://doi.org/10.1093/jxb/18.1.1



The Membrane Potential of *Nitella translucens*

R. M. SPANSWICK,[1] J. STOLAREK,[2] AND E. J. WILLIAMS

Biophysics Department, University of Edinburgh

WITH ONE FIGURE IN THE TEXT

Received 15 November 1965; revised 5 July 1966

ABSTRACT

The effects of changing the external concentrations of Na, K, Ca, and Cl on the potentials of the cytoplasm and the vacuole with respect to the bathing medium of the internodal cells of *Nitella translucens* have been investigated. The potential difference between the vacuole and the cytoplasm is practically unaffected by the concentration changes. The observed changes of potential difference are therefore attributed to the boundary separating the cytoplasm from the medium; this boundary is possibly a plasmalemma–cell wall complex. The difference of potential between the cell wall and the medium has also been measured and, in the presence of Ca, shown to be markedly sensitive only to the external Ca concentration. The results are divided into two sections: (*a*) for cells pretreated in 5 mM NaCl, the subsequent experiments being carried out in Ca-free media, and (*b*) for cells initially immersed in a standard artificial pond water containing the chlorides of Na, K, and Ca.

With the pretreated cells the external Na/K ratio was varied with the total NaCl + KCl concentration kept constant at 1·1 mM. The results suggest that over a limited range of concentrations the cytoplasm-medium potential difference can be described by an equation similar in form to a Goldman equation but containing only terms for Na and K, the average value of the permeability ratio α (= P_{Na}/P_K) being 0·27.

In the presence of Ca the effects of Na and K on the cytoplasm-medium potential difference are greatly reduced, while the effect of Ca is relatively large. The results cannot be fitted to any form of Goldman equation containing terms for the major ions. The possibility of a contribution to the plasmalemma potential from electrogenic pumps is briefly discussed.

Measurements of the Na and K content of the cytoplasm and the vacuole have been made for the pretreated cells. The Na concentration in the cytoplasm is 37 mM and in the vacuole 73 mM; the K concentration is 93 mM in the cytoplasm and 67 mM in the vacuole. The Nernst potentials for both ions are compared with the cytoplasm-medium and cytoplasm-vacuole potential differences. This analysis shows that Na is actively transported from the cytoplasm into the medium as well as into the vacuole; K is pumped into the cytoplasm from the medium but appears to be close to electrochemical equilibrium across the tonoplast. This confirms previously published work.

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Journal of Experimental Botany, Vol. 18, No. 54, pp. 1–16, February 1967.

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Abstract The effects of changing the external concentrations of Na, K, Ca, and Cl on the potentials of the cytoplasm and the vacuole with respect to the bathing medium of the internodal cells of *Nitella translucens* have been investigated. The potential difference between the vacuole and the cytoplasm is practically unaffected by the concentration changes. The observed changes of potential difference are therefore attributed to the boundary separating the cytoplasm from the medium; this boundary is possibly a plasmalemma–cell wall complex. The difference of potential between the cell wall and the medium has also been measured and, in the presence of Ca, shown to be markedly sensitive only to the external Ca concentration. The results are divided into two sections: (a) for cells pretreated in 5 mM NaCl, the subsequent experiments being carried out in Ca-free media, and (b) for cells initially immersed in a standard artificial pond water containing the chlorides of Na, K, Ca. With the pretreated cells the external Na/K ratio was varied with the total NaCl + KCl concentration kept constant at 1.1 mM. The results suggest that over a limited range of concentrations the cytoplasm-medium potential difference can be described by an equation similar in form to a Goldman equation but containing only terms for Na and K, the average value of the permeability ratio α(= P_{Na}/P_K) being 0.27. In the presence of Ca the effects of Na and K on the cytoplasm-medium potential difference are greatly reduced, while the effect of Ca is relatively large. The results cannot be fitted to any form of Goldman equation containing terms for the major ions. The possibility of a contribution to the plasmalemma potential from electrogenic pumps is briefly discussed. Measurements of the Na and K content of the cytoplasm and the vacuole have been made for the pretreated cells. The Na concentration in the cytoplasm is 37 mM and in the vacuole 73 mM; the K concentration is 93 mM in the cytoplasm and 67 mM in the vacuole. The Nernst potentials for both ions are compared with the cytoplasm-medium and cytoplasm-vacuole potential differences. This analysis shows that Na is actively transported from the cytoplasm into the medium as well as into the vacuole; K is pumped into the cytoplasm from the medium but appears to be close to electrochemical equilibrium across the tonoplast. This confirms previously published work.

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Giant algal cells, because of their size and ease of handling, were attractive experimental models for electrophysiologists in the first decades of the 20th century. Studies by Umrath (1930, 1932) and Osterhout (1931) in the 1920s and 1930s showed that direct impalements on living cells were possible using fine glass microcapillaries. Cole and Curtis (1938) made use of *Nitella* in their analysis of action potentials. Through the 1940s technical advances in electrophysiology were dominated by work with plant cells such that the recordings of Alan Walker (Walker, 1955) on *Nitella* were carried out using an amplifier and microelectrodes that would be recognised today.

By the beginning of the 1950s, animal physiologists had adopted the microelectrode techniques pioneered by plant physiologists. Thereafter, efforts to explain the electrical properties of plant membranes were heavily influenced by the successes of work on animal cells. By the late 1950s, it was clear that the resting potentials of nerve and muscle cells were largely dictated by passive diffusion of K^+ and, to a lesser extent of Na^+, with the differential in the concentrations of these two ions maintained by a near electroneutral exchange of Na^+ with K^+ driven by ATP hydrolysis. Indeed, it would be another 30 years before the electrogenic properties of the Na^+/K^+-ATPase were widely accepted.

Hope and Walker (1961) initially suggested that the electrical properties of plant cell membranes might also arise from a passive diffusion of ions, at least in cells pretreated to remove external Ca^{2+}. However, with Ca^{2+} present, it was soon clear that diffusion alone was insufficient to account for the voltages recorded across the membranes of plants (Higinbotham *et al*. 1967) and fungi (Slayman and Slayman, 1962). Quantifying these voltages and ascribing them to one or more ionic conductances therefore posed a challenge.

As has often proven the case, arriving at the correct solution was a process of elimination. Ultimately, Kitasato (1968) linked the large effect of pH on membrane voltage with the concept of a high permeability to H^+ and an electrogenic H^+ pump in *Nitella*. To arrive at this conclusion, however, it was first necessary to establish the limits for diffusion of all other dominant ions. Spanswick and Williams (1964) and Spanswick *et al*. (1967) set the bar for such analyses, demonstrating how sets of measurements could be made across a range of ion concentrations, even from a single cell. They showed that the membrane voltage failed to follow the negative limit for diffusion set by the K^+ equilibrium voltage, EK, at low K^+ concentrations, suggesting that at submillimolar K^+ other conductances take precedence. These studies established the energetic limits for transport of K^+, but also for Na^+ that could be shown to require active pumping out of the cytosol. Finally, they confirmed that the vacuolar membrane maintains a smaller but significant voltage with the vacuole at a potential positive to the cytosol. The approaches introduced by these two papers, in particular establishing the voltage relationship to EK across a range of external ion concentrations for individual cells, provided a reference point for much research on plant membrane transport over the subsequent decades.

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RAJINDER S. DHINDSA, PAMELA PLUMB-DHINDSA, TREVOR A. THORPE

Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase

Journal of Experimental Botany, 1981, Volume 32, 93–101

- Read the full article here:

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Abstract The changes in membrane permeability (soluble leakage), lipid peroxidation, and activities of superoxide dismutase (SOD) and catalase have been studied during *in situ* senescence of leaves of *Nicotiana tabacum* L., cv. Wisconsin 38. After full leaf expansion was reached there was a rapid, almost linear increase in the rate of ^{86}Rb leakage from the preloaded leaf discs, with leaf age. Parallel with this increase in membrane permeability was a cumulative increase in the level of lipid peroxidation. At the same leaf age there were changes in the activities of SOD and catalase. SOD activity decreased on the basis of fresh weight but did not change when measured on the basis of protein content probably due to relative stability of SOD during the senescence-associated general decline in protein content. Catalase activity first increased parallel with the chlorophyll content of the leaf and then, after full leaf expansion, declined on the basis of both fresh weight and protein content. These changes in membrane permeability, lipid peroxidation, and the enzyme activities coincide in leaf age with the decline in protein and chlorophyll contents and in chlorophyll a:b ratio. When the senescence of the bottom-most leaves was reversed by removing the stem from immediately above them, the senescence-associated changes in protein and chlorophyll contents, lipid peroxidation, and the enzyme activities were also reversed. It is suggested that leaf senescence may be a consequence of cumulative membrane deterioration due to increasing level of lipid peroxidation probably controlled by, among other factors, the activities of SOD and catalase.

Journal of Experimental Botany, Vol. 32, No. 126, pp. 93–101, February 1981

Leaf Senescence: Correlated with Increased Levels of Membrane Permeability and Lipid Peroxidation, and Decreased Levels of Superoxide Dismutase and Catalase

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ABSTRACT

The changes in membrane permeability (soluble leakage), lipid peroxidation, and activities of superoxide dismutase (SOD) and catalase have been studied during *in situ* senescence of leaves of *Nicotiana tabacum* L., cv. Wisconsin 38. After full leaf expansion was reached there was a rapid, almost linear increase in the rate of ^{86}Rb leakage from the preloaded leaf discs, with leaf age. Parallel with this increase in membrane permeability was a cumulative increase in the level of lipid peroxidation. At the same leaf age there were changes in the activities of SOD and catalase. SOD activity decreased on the basis of fresh weight but did not change when measured on the basis of protein content probably due to relative stability of SOD during the senescence-associated general decline in protein content. Catalase activity first increased parallel with the chlorophyll content of the leaf and then, after full leaf expansion, declined on the basis of both fresh weight and protein content. These changes in membrane permeability, lipid peroxidation, and the enzyme activities coincide in leaf age with the decline in protein and chlorophyll contents and in chlorophyll a : b ratio. When the senescence of the bottom-most leaves was reversed by removing the stem from immediately above them, the senescence-associated changes in protein and chlorophyll contents, lipid peroxidation, and the enzyme activities were also reversed. It is suggested that leaf senescence may be a consequence of cumulative membrane deterioration due to increasing level of lipid peroxidation probably controlled by, among other factors, the activities of SOD and catalase.

INTRODUCTION

Senescence in plant tissues is known to be accompanied by changes in membrane permeability. Thus an increase in apparent free space and a loss in ability to retain solutes have been demonstrated during ripening of fruits (Sacher, 1973), accelerated ageing of seeds (Parrish and Leopold, 1978), and senescence of green plant tissues (Ferguson and Simon, 1973). These permeability changes during

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Rust never sleeps. The earth's atmosphere means that a fundamental property of most organisms living today is the ability to face up to the ever-present danger of uncontrolled oxidation. Even more potentially destructive than dioxygen itself, forms of oxygen that arise from energy transfer (singlet oxygen) or reduction (notably superoxide and H_2O_2) are produced at significant rates in cells. Now known as reactive oxygen species (ROS), these molecules or their derivatives can potentially lead to the oxidation of key components such as lipids, pigments, proteins, and nucleotides, altering their structure and affecting their function. Maintenance of cellular activities requires the removal of ROS by antioxidative systems, including superoxide dismutase (SOD) and catalase, two of the most rapid enzymes known.

By the end of the 1970s, it had been well established that some ROS were potentially mutagenic and could also induce lipid peroxidation, leading to impaired function and even death. Hence the "free radical theory of ageing" in the field of medicine. Within this context, attention began to be turned to the factors underlying leaf senescence, a programmed but environmentally sensitive process that in many plants allows resources to be reallocated to support the growth of newer tissues.

At that time, ROS were already acknowledged as part and parcel of plant cell physiology. They were known to be products of various metabolic processes, such as photosynthetic electron transport in the chloroplast (Allen and Hall, 1974; Asada *et al.* 1974). As in animals, work

in plants had analysed the potential of ROS to trigger peroxidation of membrane lipids (Heath and Packer, 1968) and fruit ripening had been linked to increases in ROS (Brennan and Frenkel, 1977). Likewise, leaf senescence was known to involve changes in membrane structure and permeability. But how were ROS and membrane integrity linked during leaf development? And what might explain the potential accumulation of ROS in senescing leaves?

Since the beginning of the present century, enormous attention has been paid to programmed production of ROS in response to various environmental or internal triggers. Over forty years ago, however, the first reports of such phenomena were yet to emerge. The study of Dhindsa *et al.* (1981) focused rather on the potential importance of decreased activities of antioxidative enzymes in allowing ROS accumulation. The authors reported that altered membrane permeability was correlated with increases in lipid peroxidation products in tobacco leaves and that both these changes were accompanied by diminished leaf activities of SOD and catalase. Further, experimentally reversing senescence overturned all these trends. The principal conclusions were two-fold: (1) lipid peroxidation triggered by ROS accumulation could be the cause of a loss of membrane integrity during senescence, and (2) ROS accumulation might at least partly result from decreases in antioxidative capacity.

The paper stimulated much further research and contributed significantly to emerging concepts in the field. Two years later, a barley mutant was reported in which decreased catalase activities led to leaf lesions and death in conditions favouring H_2O_2 production (Kendall *et al.* 1983). Both studies emphasised the roles of ROS as destructive compounds associated with ageing, disorder, and death. This concept remains valid, although it is now clear that ROS also function as signals in many developmental processes and responses to environmental shifts.

Shortly after the report by Dhindsa *et al.* (1981), the pioneering work of Noriyuki Doke provided evidence that ROS accumulated in an NADPH-linked manner in plants subjected to biotic challenge (Doke, 1983, 1985). Along with information from studies of mammalian systems, these findings eventually led to the genetic characterization in Arabidopsis of NADPH oxidases, systems that have evolved specifically to generate ROS in response to specific triggers. In addition to their roles in plants undergoing pathogen attack, NADPH oxidases are involved in phytohormone signalling and the regulation of plant growth (Torres *et al.* 2002; Foreman *et al.* 2003; Kwak *et al.* 2003). They are considered to produce superoxide mainly at the plasma membrane and apoplast, with extracellular SOD favouring the conversion of superoxide to H_2O_2. Inside the cell, many other compartments have a high capacity for ROS production, the actual rates of which are influenced by environmental conditions and metabolic status. Unlike the apoplast, much of the cell interior is policed by a powerful and intricate battery of antioxidative systems, with catalases and various types of peroxidases acting alongside SOD to keep both superoxide and H_2O_2 at concentrations that are compatible with cell function. Hence, intracellular processes that are sensitive to ROS concentrations may be influenced just as much by changes in the capacities of antioxidative systems as by altered rates of ROS production. In addition to implicating ROS in plant development, the paper by Dhindsa *et al.* (1981) drew attention to this fundamental point.

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ENID A.C. MACROBBIE

Ion Fluxes in 'Isolated' Guard Cells of *Commelina communis* L.

Journal of Experimental Botany, Volume 32, Issue 3, June 1981, Pages 545–562

• Read the full article here:
https://doi.org/10.1093/jxb/32.3.545

Abstract Ion fluxes have been measured in 'isolated' guard cells of *Commelina communis* L. using ^{86}RbCl and K^{82}Br, in epidermal strips in which all cells other than guard cells have been killed by treatment at low pH. To avoid problems of slow free space exchange most fluxes have been measured at pH 3.9, at which stomata open well in K(Rb) Cl(Br) and are stable for many hours. At pH 3.9 the intracellular ^{86}Rb exchanged as a single compartment with a half-time of 2–3 h, independent of external concentration (C_o). The influx of ^{86}Rb rose with concentration, to a V_{max} of about 23 pmol mm^{-2} h^{-1}. The efflux curve of ^{82}Br could be well fitted by two exponential terms, with half-times of 38 min (independent of C_o), and 5–35 h (falling with increasing C_o). Bromide contents of cytoplasm and vacuole (Q_c and Q_v), and fluxes at plasmalemma and tonoplast, were calculated from the efflux kinetics. Over C_o 20–60 mM, as the aperture increased from 7 µm to 17 µm, the tonoplast flux (0.5–11.5 pmol mm^{-2}h^{-1}) was always much less than the plasmalemma flux (7–77 pmol mm^{-2} h^{-1}). Q_c and Q_v both increased with aperture. The increase in Q_c of 10.3 pmol mm^{-2} µm^{-1} is adequate to account for the osmotic changes required to change the aperture, as previously estimated. However, the change in vacuolar content of only 5.9 pmol mm^{-2} µm^{-1} is much too small to account for the osmotic changes required, or to balance the cytoplasmic changes. It appears therefore that increasing KBr outside not only increases the cytoplasmic salt content, and the Br flux at the tonoplast, but also stimulates the vacuolar accumulation of some other solute.

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Effects of Light/Dark on Cation Fluxes in Guard Cells of *Commelina communis* L.

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Abstract The effects of light/dark on cation fluxes in isolated guard cells of *Commelina communis* L. have been studied, using ^{86}RbCl and ^{22}NaCl. Transfer to the dark has no effect on ^{86}Rb influx, but produces a marked transient stimulation of ^{86}Rb efflux, similar to that seen previously on adding ABA. The ^{86}Rb efflux falls on return to light only during the period of stimulated flux; after the transient, return to light has no effect on efflux. The ability to produce this transient stimulation on transfer to the dark is recovered in a subsequent light period. In general, in Na-loaded cells, the stimulated efflux is not seen and the cells do not close in the dark. The results are not consistent with a simple permeability or potential change, but suggest a specific ion excretion activated by the transfer to the dark.

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Guard cells regulate the aperture of stomata that control gas and water exchange in leaves. This regulation is effected through change in guard cell turgor that is achieved through intracellular ion exchange with the apoplast. In order to understand the mechanistic features of this ion exchange it is important to be able to measure the fluxes of relevant ions across both the plasma membrane and the tonoplast membrane that surrounds the vacuole – the major site of intracellular storage of ions. Using isotopes of Rb^+ and Br^- - which serve as proxies for the physiologically-relevant ions K^+ and Cl^-, respectively - Enid MacRobbie shows in the first of these papers that it is possible to discern the relevant two-way fluxes of cations and anions in strips of leaf epidermis that are devoid of all living cells except guard cells. This description is achieved through an elegant algebraic dissection of the influx and efflux kinetics for each ionic species. The combination of the practical approach to measuring ion fluxes specifically in guard cells, together with the development of a robust theoretical framework for interpretation of the results, laid the foundation for subsequent studies that addressed the mode of action of biotic and abiotic stimuli that open and close stomata.

In the second paper, MacRobbie builds on the combined experimental and theoretical framework that she established two years previously to address the impact of illumination on the cation component of salt uptake and release. Gas and water exchange in leaves is restricted by darkness through a decrease in stomatal aperture that is in turn achieved by net solute loss from guard cells and resultant loss of turgor. The cation fluxes underpinning this solute loss were investigated using an isotope of Rb^+ as a proxy for K^+. The results demonstrate that the impact of darkness is selectively and transiently to enhance unidirectional Rb^+ release and that there is no effect on unidirectional Rb^+ uptake. Interestingly, cells that have been loaded with Na^+ do not exhibit this dark-stimulated Rb^+ efflux, and accordingly stomata fail to close in such conditions. The findings reported in this paper challenged the notion that dark-stimulated loss of turgor is the result of general leakage of solutes from the cells. Rather, the loss of Rb^+ (and presumably K^+) leading to stomatal closure is ion-specific. Subsequent studies building on the results in this paper confirmed that individual classes of transport system are selectively responsive to illumination and darkness (Jezek and Blatt, 2017).

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P. G. BLACKMAN, W. J. DAVIES

Root to Shoot Communication in Maize Plants of the Effects of Soil Drying

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Abstract Seedlings of *Zea mays* L. (John Innes hybrid) were grown with roots divided between two containers such that part of the root system could reduce the water potential of the soil in its immediate vicinity while the rest of the root system was well supplied with water. When compared to plants rooted in two pots of moist soil, drying of part of the root system resulted in partial closure of stomata, even though leaf water potential, turgor and abscisic acid (ABA) content remained unaffected. When leaf pieces were removed from the two groups of plants and incubated under conditions favourable for stomatal opening, stomata of the 'half-watered' plants still showed restricted apertures. Incubation in kinetin (10 mmol m–3) or zeatin (100 mmol m–3) reversed the closure of stomata stimulated by soil drying. These results suggest that a continuous supply of cytokinin from roots may be necessary to sustain maximal stomatal opening and an interruption of this supply due to soil drying may act as an indicator of inhibited root activity, resulting in restricted stomatal opening and thereby restricted water use.

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Root to Shoot Communication in Maize Plants of the Effects of Soil Drying

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ABSTRACT

Seedlings of *Zea mays* L. (John Innes hybrid) were grown with roots divided between two containers such that part of the root system could reduce the water potential of the soil in its immediate vicinity while the rest of the root system was well supplied with water. When compared to plants rooted in two pots of moist soil, drying of part of the root system resulted in partial closure of stomata, even though leaf water potential, turgor and abscisic acid (ABA) content remained unaffected. When leaf pieces were removed from the two groups of plants and incubated under conditions favourable for stomatal opening, stomata of the 'half-watered' plants still showed restricted apertures. Incubation in kinetin (10 mmol m^{-3}) or zeatin (100 mmol m^{-3}) reversed the closure of stomata stimulated by soil drying. *These results suggest that a continuous supply of cytokinin from roots may be necessary to sustain* maximal stomatal opening and an interruption of this supply due to soil drying may act as an indicator of inhibited root activity, resulting in restricted stomatal opening and thereby restricted water use.

Key words: *Zea mays* L.; Soil drying; Stomata; Roots.

INTRODUCTION

In addition to water and minerals, roots provide shoots with a number of compounds which are essential for growth. In recent years, there has been interest in the possibility that the root can communicate to the shoot some indication of perturbation in the soil environment. It seems possible that if root activity declines for any reason the effects of increased or reduced transport of some chemical compound will be felt in the shoots. Declining root activity may eventually also result in a reduced supply of water and, if water loss is not restricted, in the development of water deficit. If chemicals moving from roots can exert some influence of plant water loss, this response may be detected *before* any substantial change in the transport of water. It may be argued (Jones, 1980; Cowan, 1982) that this type of indication to the shoot of a change in root activity may provide some measure of the future availability of soil water and enable the plant to develop a pattern of efficient, long-term utilization of water.

Several perturbations in the edaphic environment can result in restricted water supply and it has been suggested that plants may respond to 'signals' moving from roots when soils dry (Meyer and Gingrich, 1964; Davies and Sharp, 1981; Bates and Hall, 1981), are flooded (Bradford and Hsiao, 1982; Jackson and Kowalewska, 1983) or suffer changes in temperature (Steponkus, 1982).

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Peter Blackman's work on the possible effects of cytokinin signalling on stomatal behaviour of *Zea mays* seedlings came at a time when there was much discussion of a role for long-distance chemical signalling in the regulation of physiology, growth and development of plants experiencing reductions in soil water availability (e.g. Bates and Hall, 1981). Prior to this debate, emphasis had been on interpreting the impacts of soil drying on shoot growth and physiology solely as a function of variation in shoot water status. In the absence of any measurable shoot water deficit, Blackman suggests a role for the cytokinins in root to shoot signalling of the effects of soil drying. Many studies show that cytokinin levels are reduced in droughted plants (e.g. Zhang *et al.* 2009) and work by Blackman and others shows that cytokinins can reverse ABA-induced stomatal closure (Blackman and Davies, 1983). Subsequent work has quantified changes in xylem fluxes of a range of plant growth regulators (PGRs) in different plant species and linked these to variation in physiology and development (e.g. Jiang and Hartung, 2008; Wilkinson and Davies, 2010).

While there is little doubt about drought-induced changes in the titre of a range of regulators, there has been much debate over the nature of the signalling involved in physiological regulation. The source tissue of some of these regulators has been widely debated (e.g. Walton *et al.* 1976; Zeevaart and Boyer, 1984; Loveys, 1984; Slovik *et al.* 1995; Jiang and Hartung, 2008) but recent work has confirmed early suggestions that most ABA in the roots of drought-stressed plants is shoot-sourced (e.g. McAdam *et al.* 2016). Importantly, shoot growth and physiology of droughted plants still commonly appear to be related to xylem fluxes of (shoot-sourced) ABA, apparently recirculated from roots. There is current discussion of the roles that might be played by such 'root signals' (e.g. Maurel and Nacry, 2020). Whatever the source of these regulators, there are now good data showing drought-related changes in xylem fluxes of ABA, cytokinins, auxins and ethylene and other potential chemical regulators in the xylem of droughted plants and crops in dryland situations (e.g. Zhang and Davies, 1990; Kudoyarova *et al.* 2007; Perez-Perez *et al.* 2020) and there is much interest in linking chemical signalling with variation in crop physiology (Tardieu and Davies, 1993) and importantly, with crop yield (Yang *et al.* 2001).

A major impetus for work to elucidate the mechanisms regulating plant water loss when plants are water stressed is the increasing frequency of hot dry summers in many of the world's most-important crop production regions. Irrigated agriculture is now increasingly important as a means of sustaining crop yields but in many regions excess water abstraction for irrigation is leading to potentially catastrophic decreases in ground water levels (e.g. Kang *et al.* 2008). In these regions, farmers are often restricted in the amounts of water that they can use in a cropping season and thus there is now much interest in the use of deficit irrigation. Different forms of deficit irrigation ("partial rootzone

drying" (PRD) and "alternate wetting and drying" (AWD)), techniques which are directly derived from early PGR research, have been applied across multiple crops and growing systems. Commonly these techniques reduce water use (by 20 to 40%) but can increase yield compared to conventional deficit irrigation (by 30% to 50%) (Dodd, 2009; Zhang *et al.* 2009)). A number of groups have sought to understand the fundamental root-to-shoot plant hormone signalling mechanisms underpinning these yield responses.

In rice, an important (but thirsty) major crop, reductions of use of irrigation water (AWD) after flowering can promote plant senescence and importantly, increase grain filling (e.g. Zhang *et al.* 2009). Currently, around half of the rice fields in Jiangsu province in China have adopted AWD for more sustainable rice production. Physiological studies with rice have shown that ABA and ethylene are the key regulators in transmitting the soil drying signal (Yang *et al.* 2001). There is also recent work where plant growth-promoting bacteria associated with the roots (rhizobacteria) are used as a practical approach to attenuate the inhibitory effects of ethylene, or boost the promotive effects of cytokinins. Rhizobacteria that produce the enzyme ACC deaminase (that breaks down the precursor of ethylene, ACC) decrease root-to-shoot ethylene signalling (Belimov *et al.* 2009), while cytokinin-producing bacteria promote potentially beneficial root-to-shoot cytokinin signalling (Kudoyarova *et al.* 2014). There is now increasing use of rhizobacteria in world agriculture with the aim of altering root-to-shoot hormone signalling to enhance efficiency of water use and nutritional quality of a range of crops under rainfed cropping conditions.

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Science with Impact

Journal of Experimental Botany

STAN D. WULLSCHLEGER

Biochemical Limitations to Carbon Assimilation in C_3 Plants—A Retrospective Analysis of the A/C_i Curves from 109 Species

Journal of Experimental Botany, Volume 44, Issue 5, May 1993, Pages 907–920

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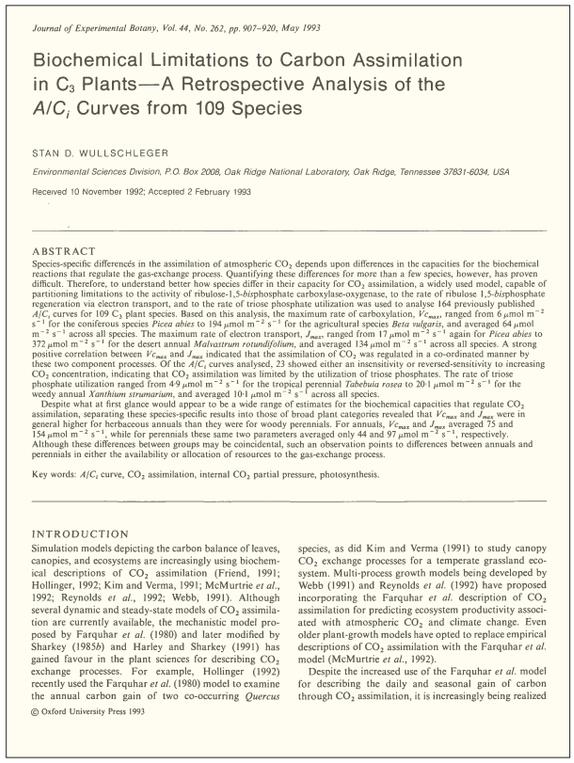
Abstract Species-specific differences in the assimilation of atmospheric CO_2 depends upon differences in the capacities for the biochemical reactions that regulate the gas-exchange process. Quantifying these differences for more than a few species, however, has proven difficult. Therefore, to understand better how species differ in their capacity for CO_2 assimilation, a widely used model, capable of partitioning limitations to the activity of ribulose-1,5-bisphosphate carboxylase-oxygenase, to the rate of ribulose 1,5-bisphosphate regeneration via electron transport, and to the rate of triose phosphate utilization was used to analyse 164 previously published A/C_i curves for 109 C_3 plant species. Based on this analysis, the maximum rate of carboxylation, Vc_{max}, ranged from 6 µmol m^{-2} s^{-1} for the coniferous species *Picea abies* to 194 µmol m^{-2} s^{-1} for the agricultural species *Beta vulgaris*, and averaged 64 µmol m^{-2} s^{-1} across all species. The maximum rate of electron transport, J_{max}, ranged from 17 µmol m^{-2} s^{-1} again for *Picea abies* to 372 µmol m^{-2} s^{-1} for the desert annual *Malvastrum rotundifolium*, and averaged 134 µmol m^{-2} s^{-1} across all species. A strong positive correlation between Vc_{max} and J_{max} indicated that the assimilation of CO_2 was regulated in a co-ordinated manner by these two component processes. Of the A/C_i curves analysed, 23 showed either an insensitivity or reversed-sensitivity to increasing CO_2 concentration, indicating that CO_2 assimilation was limited by the utilization of triose phosphates. The rate of triose phosphate utilization ranged from 4·9 µmol m^{-2} s^{-1} for the tropical perennial *Tabebuia rosea* to 20·1 µmol m^{-2} s^{-1} for the weedy annual *Xanthium strumarium*, and averaged 10·1 µmol m^{-2} s^{-1} across all species.

Despite what at first glance would appear to be a wide range of estimates for the biochemical capacities that regulate CO_2 assimilation, separating these species-specific results into those of broad plant categories revealed that Vc_{max} and J_{max} were in general higher for herbaceous annuals than they were for woody perennials. For annuals, Vc_{max} and J_{max} averaged 75 and 154 µmol m^{-2} s^{-1}, while for perennials these same two parameters averaged only 44 and 97 µmol m^{-2} s^{-1}, respectively. Although these differences between groups may be coincidental, such an observation points to differences between annuals and perennials in either the availability or allocation of resources to the gas-exchange process.



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Biochemical Limitations to Carbon Assimilation in C_3 Plants—A Retrospective Analysis of the A/C_i Curves from 109 Species

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ABSTRACT

Species-specific differencés in the assimilation of atmospheric CO_2 depends upon differences in the capacities for the biochemical reactions that regulate the gas-exchange process. Quantifying these differences for more than a few species, however, has proven difficult. Therefore, to understand better how species differ in their capacity for CO_2 assimilation, a widely used model, capable of partitioning limitations to the activity of ribulose-1,5-*bis*phosphate carboxylase-oxygenase, to the rate of ribulose 1,5-*bis*phosphate regeneration via electron transport, and to the rate of triose phosphate utilization was used to analyse 164 previously published A/C_i curves for 109 C_3 plant species. Based on this analysis, the maximum rate of carboxylation, Vc_{max}, ranged from 6 µmol m^{-2} s^{-1} for the coniferous species *Picea abies* to 194 µmol m^{-2} s^{-1} for the agricultural species *Beta vulgaris*, and averaged 64 µmol m^{-2} s^{-1} across all species. The maximum rate of electron transport, J_{max}, ranged from 17 µmol m^{-2} s^{-1} again for *Picea abies* to 372 µmol m^{-2} s^{-1} for the desert annual *Malvastrum rotundifolium*, and averaged 134 µmol m^{-2} s^{-1} across all species. A strong positive correlation between Vc_{max} and J_{max} indicated that the assimilation of CO_2 was regulated in a co-ordinated manner by these two component processes. Of the A/C_i curves analysed, 23 showed either an insensitivity or reversed-sensitivity to increasing CO_2 concentration, indicating that CO_2 assimilation was limited by the utilization of triose phosphates. The rate of triose phosphate utilization ranged from 4·9 µmol m^{-2} s^{-1} for the tropical perennial *Tabebuia rosea* to 20·1 µmol m^{-2} s^{-1} for the weedy annual *Xanthium strumarium*, and averaged 10·1 µmol m^{-2} s^{-1} across all species.

Despite what at first glance would appear to be a wide range of estimates for the biochemical capacities that regulate CO_2 assimilation, separating these species-specific results into those of broad plant categories revealed that Vc_{max} and J_{max} were in general higher for herbaceous annuals than they were for woody perennials. For annuals, Vc_{max} and J_{max} averaged 75 and 154 µmol m^{-2} s^{-1}, while for perennials these same two parameters averaged only 44 and 97 µmol m^{-2} s^{-1}, respectively. Although these differences between groups may be coincidental, such an observation points to differences between annuals and perennials in either the availability or allocation of resources to the gas-exchange process.

Key words: A/C_i curve, CO_2 assimilation, internal CO_2 partial pressure, photosynthesis.

INTRODUCTION

Simulation models depicting the carbon balance of leaves, canopies, and ecosystems are increasingly using biochemical descriptions of CO_2 assimilation (Friend, 1991; Hollinger, 1992; Kim and Verma, 1991; McMurtrie *et al.*, 1992; Reynolds *et al.*, 1992; Webb, 1991). Although several dynamic and steady-state models of CO_2 assimilation are currently available, the mechanistic model proposed by Farquhar *et al.* (1980) and later modified by Sharkey (1985*b*) and Harley and Sharkey (1991) has gained favour in the plant sciences for describing CO_2 exchange processes. For example, Hollinger (1992) recently used the Farquhar *et al.* (1980) model to examine the annual carbon gain of two co-occurring *Quercus* species, as did Kim and Verma (1991) to study canopy CO_2 exchange processes for a temperate grassland ecosystem. Multi-process growth models being developed by Webb (1991) and Reynolds *et al.* (1992) have proposed incorporating the Farquhar *et al.* description of CO_2 assimilation for predicting ecosystem productivity associated with atmospheric CO_2 and climate change. Even older plant-growth models have opted to replace empirical descriptions of CO_2 assimilation with the Farquhar *et al.* model (McMurtrie *et al.*, 1992).

Despite the increased use of the Farquhar *et al.* model for describing the daily and seasonal gain of carbon through CO_2 assimilation, it is increasingly being realized

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In the early 1990s physiologists and modelers were using the mechanistic model of photosynthesis proposed by Farquhar *et al.* (1980) to estimate photosynthesis at scales ranging from individual plants to global climate models. Wullschleger (1993) recognized an important limitation that hindered implementation of the Farquhar *et al.* (1980) model, "parameterizing this rather complex model for leaves of a single species, let alone for the many species that make up an ecosystem, is not straightforward". At the time Wullschleger started his literature search and subsequent analysis, climate models had begun to describe the vegetation using the concept of plant functional types that collapsed the structural and functional diversity of plants into a handful of functional types, e.g. a broad leaved deciduous tree. However, for photosynthesis, data were only available for a few species, limiting confidence in the parameterization of existing functional types, and preventing model representation of a more diverse range of plant functional types with different physiological characteristics.

In the original Farquhar *et al.* (1980) model, photosynthesis was determined by the minimum of two processes. (1) the maximum carboxylation rate of the enzyme Rubisco (Vc_{max}), and (2) regeneration of ribulose-1,5-bisphosphate by the Calvin Cycle which is limited by the maximum

electron transport rate (J_{max}). When photosynthetic rates exceed the capacity of the plant to use the triose phosphates produced by photosynthesis, a third limitation, known as triose phosphate utilization (TPU) limitation is observed (Sharkey, 1985). For a given species or plant functional type these three key parameters; Vc_{max}, J_{max}, and TPU, are necessary to accurately model carbon dioxide assimilation using the Farquhar *et al.* (1980) model. All three parameters can be estimated from the measured response of photosynthetic carbon dioxide assimilation (A) to the carbon dioxide concentration inside the leaf (C_i) — commonly know as an A/C_i curve. Wullschleger painstakingly extracted A/C_i curves from published work and then derived Vc_{max}, J_{max} and TPU. Fitting an A/C_i curve is taken for granted these days, but at the time Wullschleger's paper provided a foundational method for estimating these key parameters, a topic which continues to generate new approaches today.

Wullschleger's comprehensive A/C_i curve analysis was one of the first to describe the marked natural variation in photosynthetic properties in a broad range of species and revealed that the differences in photosynthetic rate among species were attributable to their underlying biochemistry, i.e. their capacity for carboxylation, electron transport, and end product use. This work, and the summary table that runs for over three pages, provided an important new resource for the community and enabled parameterization of the Farquhar *et al.* (1980) model for a range of plant functional types.

Perhaps the most striking result was the observation of the linear relationship between Vc_{max} and J_{max}. Wullschleger noted that despite multi-fold variation in these two parameters across the data set, there was a tight coupling between investment in the processes of carboxylation and electron transport. Knowledge of the constant ratio between these two dominant controls on photosynthesis was integral to representation of photosynthesis in plant, ecosystem, and climate models. Several follow-on studies that included normalization to a common reference temperature, and the use of updated kinetic constants, built on Wullschleger's work and continue to inform model representation of photosynthesis.

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Science with Impact

Journal of Experimental Botany

KATE MAXWELL AND GILES N. JOHNSON

Chlorophyll fluorescence—a practical guide

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Journal of Experimental Botany

REVIEW ARTICLE

Chlorophyll fluorescence—a practical guide

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Abstract

Chlorophyll fluorescence analysis has become one of the most powerful and widely used techniques available to plant physiologists and ecophysiologists. This review aims to provide an introduction for the novice into the methodology and applications of chlorophyll fluorescence. After a brief introduction into the theoretical background of the technique, the methodology and some of the technical pitfalls that can be encountered are explained. A selection of examples is then used to illustrate the types of information that fluorescence can provide.

Key words: Chlorophyll fluorescence, electron transport, photoinhibition.

Introduction

In recent years, the technique of chlorophyll fluorescence has become ubiquitous in plant ecophysiology studies. No investigation into the photosynthetic performance of plants under field conditions seems complete without some fluorescence data. This trend has been fuelled to a large degree, by the introduction of a number of highly user-friendly (and portable) chlorophyll fluorometers. In spite of the simplicity of the measurements, however, the underlying theory and the interpretation of data remains complex and, in places, controversial. A number of excellent reviews exist that discuss the theoretical background of both measurement and analysis, however, these are typically written from a biophysicist's or a molecular plant physiologist's point of view (Horton and Bowyer, 1990; Krause and Weis, 1991; Govindjee, 1995). The aim of this review is to provide a simple, practical guide to chlorophyll fluorescence for those beginners who are interested in applying the technique in both field and laboratory situations. Whilst the principles behind the measurements will be discussed briefly, the emphasis will be on the applications and limitations of this technique in plant ecophysiology.

The basis of chlorophyll fluorescence measurements

The principle underlying chlorophyll fluorescence analysis is relatively straightforward. Light energy absorbed by chlorophyll molecules in a leaf can undergo one of three fates: it can be used to drive photosynthesis (photochemistry), excess energy can be dissipated as heat or it can be re-emitted as light—chlorophyll fluorescence. These three process occur in competition, such that any increase in the efficiency of one will result in a decrease in the yield of the other two. Hence, by measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be gained.

Although the total amount of chlorophyll fluorescence is very small (only 1 or 2% of total light absorbed), measurement is quite easy. The spectrum of fluorescence is different to that of absorbed light, with the peak of fluorescence emission being of longer wavelength than

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Abbreviations: F_v/F_m, ratio of variable to maximum fluorescence—the quantum efficiency of open photosystem II centres; F_m, maximum fluorescence yield; F_o, minimum fluorescence yield; F_s or F_t, steady-state fluorescence yield; J, photosynthetic electron transport rate (μequiv m^{-2} s^{-1}); NPQ, non-photochemical quenching; PFD, photon flux density (400–700 nm); PSII, photosystem II; q_P, photochemical quenching; q_E, energy-dependent quenching; q_I, photoinhibitory quenching; q_T, quenching related to state transitions; Q_A, primary quinone acceptor of photosystem II; ΔpH, transthylakoid pH gradient; Φ_{PSII}, quantum yield of photosystem II photochemistry.

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Chlorophyll fluorescence is an elegant non-contact set of methods for probing photosynthetic function, based around the fact that chlorophyll does not just absorb light like a sponge absorbs water. Rather, it processes light energy, rapidly passing it on to be used in photosynthetic electron transport or releasing it as heat in a photo-protective manner (preventing reaction with oxygen). A small proportion of the energy is re-released as light at longer wavelengths which can be readily measured. The efficiency of re-emission of such photons provides valuable information about both electron transport in photosystem II (PSII) and about photoprotection. This is chlorophyll fluorescence. Applications of the technique have become incredibly diverse and have led to development of fluorescence imaging at diverse scales, from microscopic to whole plant and field, all the way to remote sensing of fluorescence via satellites. Combined with direct gas exchange and other methods such as hyperspectral reflectance, fluorescence imaging enables a complete picture of photosynthetic functioning and efficiency of individual components, accurately predicting responses in diverse environments. PSII is one of the most sensitive components in plants to environmental changes and it has been used to measure environmental responses, stress, predictions of productivity, and the onset of disease. The influence of PSII measurements on photosynthetic research has been substantial; it is difficult to imagine where we would be without these.

The technique is highly accessible. As Maxwell and Johnson state, it enables photosynthesis to be measured by simply 'pointing a fluorometer at a leaf and flashing a light'. After the 1980s it quickly became a hugely popular method among plant scientists and ecologists. It also became apparent that there was a mismatch between the ease of taking measurements and interpreting the data which requires some appreciation of the complex underlying theory. While there were excellent papers available explaining the biophysics or biochemistry, it wasn't always easy for a scientist working in another field to quickly integrate the concepts in a way that would allow them to design experiments optimally or to interpret the data fully. Preventing such inappropriate use was no doubt one motivation for Maxwell and Johnson. Their paper was intended to be a user guide that would introduce the non-intuitive world of fluorescence quenching analysis in a way that did not 'dumb down' and that would, if used correctly, enable accurate interpretation for a particular experiment. Once understood, the reader was better equipped to explore further. As they said in the paper, 'what can chlorophyll fluorescence do for you?'.

After 23 years the article is still cited where credibility is required, despite the appearance of many updated and more in-depth reviews and multiple adaptations and improvements to the methodology. The principles remain the same and it is still often one of the first papers looked at by researchers approaching the topic. Whilst co-writing an updated fluorescence methods paper in 2013, I was told 'I wish I had this when I started my PhD'. Of course, this was the purpose of the original publication too. •

Science with Impact

Journal of Experimental Botany

LUISA M. SANDALIO, HUMBERTO C. DALURZO, MANUEL GÓMEZ, MARIA C. ROMERO-PUERTAS, LUIS A. DEL RÍO

Cadmium-induced changes in the growth and oxidative metabolism of pea plants

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Abstract The effect of growing pea (*Pisum sativum* L.) plants with $CdCl_2$ (0–50 μM) on different plant physiological parameters and antioxidative enzymes of leaves was studied in order to know the possible involvement of this metal in the generation of oxidative stress. In roots and leaves of pea plants Cd produced a significant inhibition of growth as well as a reduction in the transpiration and photosynthesis rate, chlorophyll content of leaves, and an alteration in the nutrient status in both roots and leaves. The ultrastructural analysis of leaves from plants grown with 50 μM $CdCl_2$, showed cell disturbances characterized by an increase of mesophyll cell size, and a reduction of intercellular spaces, as well as severe disturbances in chloroplast structure. Alterations in the activated oxygen metabolism of pea plants were also detected, as evidenced by an increase in lipid peroxidation and carbonyl-groups content, as well as a decrease in catalase, SOD and, to a lesser extent, guaiacol peroxidase activities. Glutathione reductase activity did not show significant changes as a result of Cd treatment. A strong reduction of chloroplastic and cytosolic Cu,Zn-SODs by Cd was found, and to a lesser extent of Fe-SOD, while Mn-SOD was only affected by the highest Cd concentrations. Catalase isoenzymes responded differentially, the most acidic isoforms being the most sensitive to Cd treatment. Results obtained suggest that growth of pea plants with $CdCl_2$ can induce a concentration-dependent oxidative stress situation in leaves, characterized by an accumulation of lipid peroxides and oxidized proteins as a result of the inhibition of the antioxidant systems. These results, together with the ultrastructural data, point to a possible induction of leaf senescence by cadmium.

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The heavy metal cadmium (Cd), which is toxic to humans, animals and plants, enters the environment mainly from industrial processes and phosphate fertilizers and is then transferred to the food chain. When the work of Sandalio *et al.* was published in 2001, little was known of the mechanisms involved in cadmium toxicity or of the plant cell responses to this heavy metal, although considerable advances have since been made in these areas. This pioneering study analysed the toxic effects of Cd, as well as plant responses to the metal, using a diverse range of approaches, including analyses of basic growth parameters, photosynthetic efficiency, oxidative stress parameters and antioxidative enzymes, together with ionomics and leaf ultrastructural studies.

The results obtained in this study suggest that $CdCl_2$ induces concentration-dependent oxidative stress in pea leaves, characterized by an accumulation of lipid peroxides, oxidized proteins and, possibly, H_2O_2 caused by the inhibition of the antioxidative enzymes catalase and peroxidase. Consequently, an induction of leaf senescence was observed, characterized by cessation of photosynthesis, disintegration of organelle structures, higher lipid peroxidation levels, a reduction in chloroplast size and an increase in the number and size of plastoglobuli in chloroplasts. The presence of Cd in the nutrient solution also disrupted micro- and macro-nutrient uptake in roots and leaves, thus affecting different metabolic pathways.

However, over the last 22 years, there has been a considerable increase in the volume of research devoted to Cd toxicity and plant responses to this heavy metal, mainly due to improvements in molecular marker analysis, as well as in genetic transformation and gene editing, thus increasing the availability of mutants of model plant species such as Arabidopsis. The development of omics-based approaches such as transcriptomics (Yang *et al.* 2020; Pacenza *et al.* 2021, Romero-Puertas *et al.* 2021; Yang *et al.* 2022), proteomics (Dupae *et al.* 2014), metabolomics and ionomics (Pacenza *et al.* 2021; Hafsi *et al.* 2022), as well as new cell biology techniques (Rodríguez-Serrano *et al.* 2016; Barón-Sola *et al.* 2021) has also contributed considerably to increase the scope of this field. In addition, the availability of integrated results (Zhou and Zheng, 2022) from different omics data sets through the use of bioinformatic tools will facilitate the establishment of models of Cd toxicity and cell response regulation with reactive oxygen species (ROS) and nitric oxide (NO) playing important roles as key regulators of cellular defence programs and metal

or macronutrient transport through transcriptional and post-translational modifications (Terrón-Camero *et al.* 2019; Nieves-Cordones, 2019; Hafsi *et al.* 2022). More recently, the involvement of autophagy in responses to Cd and the role of ROS in regulating this process have been reported (Calero-Muñoz *et al.* 2019). The information obtained in recent years should enable considerable improvements to be made in developing phytoremediation strategies for cleaning up contaminated soils and in selecting crop plants capable of preventing Cd accumulation in the edible parts of plants.

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MINGYI JIANG, JIANHUA ZHANG

Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves

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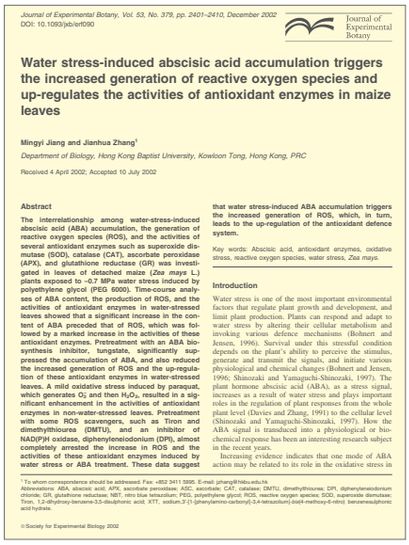
Abstract The interrelationship among water stress-induced abscisic acid (ABA) accumulation, the generation of reactive oxygen species (ROS), and the activities of several antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) was investigated in leaves of detached maize (*Zea mays* L.) plants exposed to –0.7 MPa water stress-induced by polyethylene glycol (PEG 6000). Time-course analyses of ABA content, the production of ROS, and the activities of antioxidant enzymes in water-stressed leaves showed that a significant increase in the content of ABA preceded that of ROS, which was followed by a marked increase in the activities of these antioxidant enzymes. Pretreatment with an ABA biosynthesis inhibitor, tungstate, significantly suppressed the accumulation of ABA, and also reduced the increased generation of ROS and the up-regulation of these antioxidant enzymes in water-stressed leaves. A mild oxidative stress induced by paraquat, which generates O_2^- and then H_2O_2, resulted in a significant enhancement in the activities of antioxidant enzymes in non-water-stressed leaves. Pretreatment with some ROS scavengers, such as Tiron and dimethylthiourea (DMTU), and an inhibitor of NAD(P)H oxidase, diphenyleneiodonium (DPI), almost completely arrested the increase in ROS and the activities of these antioxidant enzymes induced by water stress or ABA treatment. These data suggest that water stress-induced ABA accumulation triggers the increased generation of ROS, which, in turn, leads to the up-regulation of the antioxidant defence system.



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Journal of Experimental Botany

Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves

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Abstract

The interrelationship among water-stress-induced abscisic acid (ABA) accumulation, the generation of reactive oxygen species (ROS), and the activities of several antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) was investigated in leaves of detached maize (*Zea mays* L.) plants exposed to –0.7 MPa water stress induced by polyethylene glycol (PEG 6000). Time-course analyses of ABA content, the production of ROS, and the activities of antioxidant enzymes in water-stressed leaves showed that a significant increase in the content of ABA preceded that of ROS, which was followed by a marked increase in the activities of these antioxidant enzymes. Pretreatment with an ABA biosynthesis inhibitor, tungstate, significantly suppressed the accumulation of ABA, and also reduced the increased generation of ROS and the up-regulation of these antioxidant enzymes in water-stressed leaves. A mild oxidative stress induced by paraquat, which generates O_2^- and then H_2O_2, resulted in a significant enhancement in the activities of antioxidant enzymes in non-water-stressed leaves. Pretreatment with some ROS scavengers, such as Tiron and dimethylthiourea (DMTU), and an inhibitor of NAD(P)H oxidase, diphenyleneiodonium (DPI), almost completely arrested the increase in ROS and the activities of these antioxidant enzymes induced by water stress or ABA treatment. These data suggest that water stress-induced ABA accumulation triggers the increased generation of ROS, which, in turn, leads to the up-regulation of the antioxidant defence system.

Key words: Abscisic acid, antioxidant enzymes, oxidative stress, reactive oxygen species, water stress, *Zea mays*.

Introduction

Water stress is one of the most important environmental factors that regulate plant growth and development, and limit plant production. Plants can respond and adapt to water stress by altering their cellular metabolism and invoking various defence mechanisms (Bohnert and Jensen, 1996). Survival under this stressful condition depends on the plant's ability to perceive the stimulus, generate and transmit the signals, and initiate various physiological and chemical changes (Bohnert and Jensen, 1996; Shinozaki and Yamaguchi-Shinozaki, 1997). The plant hormone abscisic acid (ABA), as a stress signal, increases as a result of water stress and plays important roles in the regulation of plant responses from the whole plant level (Davies and Zhang, 1991) to the cellular level (Shinozaki and Yamaguchi-Shinozaki, 1997). How the ABA signal is transduced into a physiological or biochemical response has been an interesting research subject in the recent years.

Increasing evidence indicates that one mode of ABA action may be related to its role in the oxidative stress in

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Abbreviations: ABA, abscisic acid; APX, ascobate peroxidase; ASC, ascorbate; CAT, catalase; DMTU, dimethylthiourea; DPI, diphenyleneiodonium chloride; GR, glutathione reductase; NBT, nitro blue tetrazolium; PEG, polyethylene glycol; ROS, reactive oxygen species; SOD, superoxide dismutase; Tiron, 1,2-dihydroxy-benzene-3,5-disulphonic acid; XTT, sodium,3'-[1-[phenylamino-carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzenesulphonic acid hydrate.

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Mingyi Jiang • Nanjing Agricultural University, China

Drought and salinity are among the most important environmental factors affecting plant growth, development and productivity. To survive these stress conditions, plants can respond and adapt through various physiological and biochemical changes. The plant hormone abscisic acid (ABA), accumulated in plant cells exposed to drought and salinity, plays a crucial role in the tolerance of plants to these stresses.

In the early 2000s, several important papers reported that hydrogen peroxide (H_2O_2), a major reactive oxygen species (ROS), is involved in ABA signaling (Guan *et al.* 2000; Pei *et al.* 2000; Jiang and Zhang, 2001; Zhang *et al.* 2001). In guard cells of Arabidopsis, it was shown that ABA-induced H_2O_2 production and H_2O_2-activated Ca^{2+} channels are important mechanisms for ABA-induced stomatal closure (Pei *et al.* 2000). H_2O_2-dependent stomatal closure in ABA signaling was also observed in the guard cells of *Vicia faba* (Zhang *et al.* 2001). Meanwhile, in maize leaves, ABA induced H_2O_2 production, and both ABA and H_2O_2 induced the expression of *cat1*, which encodes catalase (CAT) isozyme 1 (Guan *et al.*, 2000). Moreover, ABA was also shown to induce production of superoxide radicals (O_2^-) and H_2O_2, and to enhance the capacity of whole antioxidant defense systems including enzymatic and non-enzymatic constituents in maize leaves (Jiang and Zhang, 2001). However, at that time, the interrelationships between ABA, ROS, and antioxidant defense in plants exposed to water stress were still not clear.

In this study by Jiang and Zhang (2002a), the authors systematically examined the interrelationships between ABA accumulation, ROS production, and the activities of several antioxidant enzymes such as superoxide dismutase (SOD), CAT, ascorbate peroxidase (APX), and glutathione reductase (GR) in leaves of maize plants exposed to water stress induced by polyethylene glycol (PEG 6000). Time-course analyses showed that a significant increase in ABA content preceded the production of ROS, which was followed by a marked increase in the activities of these antioxidant enzymes under water stress. Inhibition of ABA accumulation by the ABA biosynthesis inhibitor tungstate was accompanied by decreases in ROS production and in the activities of antioxidant enzymes in water-stressed leaves, and these decreases were fully prevented by the addition of ABA. Meanwhile, oxidative stress induced by paraquat also resulted in significant increases in the activities of antioxidant enzymes in non-water-stressed leaves. Pretreatment with ROS scavengers Tiron and dimethylthiourea (DMTU) and with NADPH oxidase inhibitor diphenyleneiodonium (DPI) almost completely blocked the increases in ROS production and in the activities of these antioxidant enzymes induced by water stress or ABA treatment. These results depict a mini-

mal chain of events initiated by water stress-induced ABA accumulation, which then triggers the production of ROS, resulting in the induction of antioxidant defense systems in plants.

This study together with that by Jiang and Zhang (2002b) also suggests that NADPH oxidase is an important source of ROS in ABA signaling. Subsequent genetic evidence has revealed that some members of the NADPH oxidase family play critical roles in ABA signaling and in water stress signaling. In Arabidopsis, it was demonstrated that AtRbohD and AtRbohF are major NADPH oxidase catalytic subunits that mediate ABA-induced ROS production, ABA activation of Ca^{2+} channels and ABA-induced stomatal closure (Kwak *et al*. 2003). In rice, it was shown that ABA induced increased expression of *OsRbohB* and *OsRbohE* (Zhang *et al*. 2014), and ABA-induced H_2O_2 production was impaired in *osrbohb/e* double mutants (Ni *et al.* 2019). A recent study demonstrated that both *OsRbohB* and *OsRbohE* are involved in ABA-induced H_2O_2 production and in ABA-regulated physiological processes, including seed germination, root growth, and tolerance to both water stress and oxidative stress, in which *OsRbohB* makes a major contribution to these ABA responses (Wang *et al.* 2023). Taken together, these results support the notion that water stress-induced ABA accumulation triggers the production of ROS by NADPH oxidase, which, in turn, leads to the induction of antioxidant defense systems against oxidative damage in plants.

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PETER R. SHEWRY

Wheat

Journal of Experimental Botany, Volume 60, Issue 6, April 2009, Pages 1537–1553

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Abstract Wheat is the dominant crop in temperate countries being used for human food and livestock feed. Its success depends partly on its adaptability and high yield potential but also on the gluten protein fraction which confers the viscoelastic properties that allow dough to be processed into bread, pasta, noodles, and other food products. Wheat also contributes essential amino acids, minerals, and vitamins, and beneficial phytochemicals and dietary fibre components to the human diet, and these are particularly enriched in whole-grain products. However, wheat products are also known or suggested to be responsible for a number of adverse reactions in humans, including intolerances (notably coeliac disease) and allergies (respiratory and food). Current and future concerns include sustaining wheat production and quality with reduced inputs of agrochemicals and developing lines with enhanced quality for specific end-uses, notably for biofuels and human nutrition.

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DARWIN REVIEW

Wheat

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Abstract

Wheat is the dominant crop in temperate countries being used for human food and livestock feed. Its success depends partly on its adaptability and high yield potential but also on the gluten protein fraction which confers the viscoelastic properties that allow dough to be processed into bread, pasta, noodles, and other food products. Wheat also contributes essential amino acids, minerals, and vitamins, and beneficial phytochemicals and dietary fibre components to the human diet, and these are particularly enriched in whole-grain products. However, wheat products are also known or suggested to be responsible for a number of adverse reactions in humans, including intolerances (notably coeliac disease) and allergies (respiratory and food). Current and future concerns include sustaining wheat production and quality with reduced inputs of agrochemicals and developing lines with enhanced quality for specific end-uses, notably for biofuels and human nutrition.

Key words: Allergy, bread, crop evolution, dietary fibre, flour, gluten proteins, grain, intolerance, nutrition, processing, wheat.

Introduction

Wheat is counted among the 'big three' cereal crops, with over 600 million tonnes being harvested annually. For example, in 2007, the total world harvest was about 607 m tonnes compared with 652 m tonnes of rice and 785 m tonnes of maize (http://faostat.fao.org/). However, wheat is unrivalled in its range of cultivation, from 67° N in Scandinavia and Russia to 45° S in Argentina, including elevated regions in the tropics and sub-tropics (Feldman, 1995). It is also unrivalled in its range of diversity and the extent to which it has become embedded in the culture and even the religion of diverse societies.

Most readers will be aware of the significance of bread in the Judaeo-Christian tradition including the use of matzo (hard flat bread) at the Jewish Passover and of bread to represent the 'host' at the Christian Eucharist (Holy Communion). The latter may be a thin unleavened wafer, similar to the Jewish matzo, in the Roman Catholic Church and some Protestant denominations, or leavened in other Protestant denominations and the Eastern Orthodox Church. But how many readers are aware that bread is treated as sacred in everyday life in the largely Muslim communities of Central Asia, such as Uzbekistan and Kyrgyzstan? In this culture, the leavened round breads (nan) are stamped before baking and must be treated with respect, including being kept upright and never left on the ground or thrown away in public. These customs almost certainly originate from earlier indigenous religions in the Middle East in which wheat played a similar role and was sometimes equated with the sun and its god.

Although such cultural and religious traditions are fascinating and will certainly reward further study, they are essentially outside the scope of this article which will examine why wheat has developed and continues to be so successful as a crop and food source.

Origin and evolution of wheat

The first cultivation of wheat occurred about 10 000 years ago, as part of the 'Neolithic Revolution', which saw a transition from hunting and gathering of food to settled agriculture. These earliest cultivated forms were diploid (genome AA) (einkorn) and tetraploid (genome AABB) (emmer) wheats and their genetic relationships indicate that they originated from the south-eastern part of Turkey (Heun *et al.*, 1997; Nesbitt, 1998; Dubcovsky and Dvorak, 2007). Cultivation spread to the Near East by about 9000

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Bread wheat has been the dominant arable crop in temperate countries for centuries. However, when this review was published, research on wheat was lagging behind that on the two other major cereal crops, rice and maize, due partly to the slow development of genomic resources. In particular, whereas assembled genome sequences for rice and maize were reported in 2002 and 2009, the genome assembly for the reference wheat genotype Chinese Spring was not reported until 2018. This slow progress resulted from several factors, including technical challenges associated with the large genome size (16 Gb compared to 400-430 Mb for rice and 2.3-2.7 Gb for maize), hexaploid constitution and high content (>80%) of repetitive DNA in wheat, but also due to limited investment by private sector biotechnology companies who focused on hybrid crops.

However, the last 15 years have seen a steady increase in the volume of research on wheat and it is currently the most accessed plant species in the Ensembl Plants portal (http://plants.ensembl.org), above rice and Arabidopsis. This increase has been fuelled by substantial public sector investments, including a series of 5 year coordinated research programmes funded by the UK Biological Sciences and Biotechnology Research Council (BBSRC), major programmes in other countries and the Wheat programme at CIMMYT (https://wheat.org). This investment has led to the wide availability of well-characterised germplasm resources (including land races, sequenced libraries of mutant lines and characterised populations and panels for mapping), high density molecular marker systems, increasing numbers of fully sequenced genomes and efficient systems for genetic transformation and gene editing. At the same time, it has become recognised that bread wheat exhibits a range of fascinating biological phenomena, particularly relating to genome organisation and evolution and the mechanisms controlling the operation of polyploid genomes.

This investment has already resulted in some practical benefits, notably the development of biofortified high zinc lines by scientists in CIMMYT. The next few decades should see even more benefits, not only for the global population (wheat accounting for about 20% of the global intakes of calories and protein) but particularly for countries where wheat is the major part of the diet, accounting for half or more of the total calorific intake. •

YINGGAO LIU, NENGHUI YE, RUI LIU, MOXIAN CHEN, JIANHUA ZHANG

H_2O_2 mediates the regulation of ABA catabolism and GA biosynthesis in Arabidopsis seed dormancy and germination

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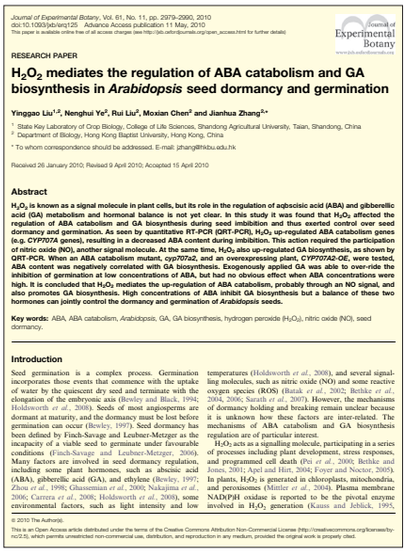

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Journal of Experimental Botany

RESEARCH PAPER

H_2O_2 mediates the regulation of ABA catabolism and GA biosynthesis in *Arabidopsis* seed dormancy and germination

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Abstract

H_2O_2 is known as a signal molecule in plant cells, but its role in the regulation of aqbscisic acid (ABA) and gibberellic acid (GA) metabolism and hormonal balance is not yet clear. In this study it was found that H_2O_2 affected the regulation of ABA catabolism and GA biosynthesis during seed imbibition and thus exerted control over seed dormancy and germination. As seen by quantitative RT-PCR (QRT-PCR), H_2O_2 up-regulated ABA catabolism genes (e.g. *CYP707A* genes), resulting in a decreased ABA content during imbibition. This action required the participation of nitric oxide (NO), another signal molecule. At the same time, H_2O_2 also up-regulated GA biosynthesis, as shown by QRT-PCR. When an ABA catabolism mutant, *cyp707a2*, and an overexpressing plant, *CYP707A2-OE*, were tested, ABA content was negatively correlated with GA biosynthesis. Exogenously applied GA was able to over-ride the inhibition of germination at low concentrations of ABA, but had no obvious effect when ABA concentrations were high. It is concluded that H_2O_2 mediates the up-regulation of ABA catabolism, probably through an NO signal, and also promotes GA biosynthesis. High concentrations of ABA inhibit GA biosynthesis but a balance of these two hormones can jointly control the dormancy and germination of *Arabidopsis* seeds.

Key words: ABA, ABA catabolism, *Arabidopsis*, GA, GA biosynthesis, hydrogen peroxide (H_2O_2), nitric oxide (NO), seed dormancy.

Introduction

Seed germination is a complex process. Germination incorporates those events that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis (Bewley and Black, 1994; Holdsworth *et al.*, 2008). Seeds of most angiosperms are dormant at maturity, and the dormancy must be lost before germination can occur (Bewley, 1997). Seed dormancy has been defined by Finch-Savage and Leubner-Metzger as the incapacity of a viable seed to germinate under favourable conditions (Finch-Savage and Leubner-Metzger, 2006). Many factors are involved in seed dormancy regulation, including some plant hormones, such as abscisic acid (ABA), gibberellic acid (GA), and ethylene (Bewley, 1997; Zhou *et al.*, 1998; Ghassemian *et al.*, 2000; Nakajima *et al.*, 2006; Carrera *et al.*, 2008; Holdsworth *et al.*, 2008), some environmental factors, such as light intensity and low temperatures (Holdsworth *et al.*, 2008), and several signalling molecules, such as nitric oxide (NO) and some reactive oxygen species (ROS) (Batak *et al.*, 2002; Bethke *et al.*, 2004, 2006; Sarath *et al.*, 2007). However, the mechanisms of dormancy holding and breaking remain unclear because it is unknown how these factors are inter-related. The mechanisms of ABA catabolism and GA biosynthesis regulation are of particular interest.

H_2O_2 acts as a signalling molecule, participating in a series of processes including plant development, stress responses, and programmed cell death (Pei *et al.*, 2000; Bethke and Jones, 2001; Apel and Hirt, 2004; Foyer and Noctor, 2005). In plants, H_2O_2 is generated in chloroplasts, mitochondria, and peroxisomes (Mittler *et al.*, 2004). Plasma membrane NADPH oxidase is reported to be the pivotal enzyme involved in H_2O_2 generation (Kauss and Jeblick, 1995,

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Abstract H_2O_2 is known as a signal molecule in plant cells, but its role in the regulation of aqbscisic acid (ABA) and gibberellic acid (GA) metabolism and hormonal balance is not yet clear. In this study it was found that H_2O_2 affected the regulation of ABA catabolism and GA biosynthesis during seed imbibition and thus exerted control over seed dormancy and germination. As seen by quantitative RT-PCR (qRT-PCR), H_2O_2 up-regulated ABA catabolism genes (e.g. *CYP707A* genes), resulting in a decreased ABA content during imbibition. This action required the participation of nitric oxide (NO), another signal molecule. At the same time, H_2O_2 also up-regulated GA biosynthesis, as shown by qRT-PCR. When an ABA catabolism mutant, *cyp707a2*, and an overexpressing plant, *CYP707A2-OE*, were tested, ABA content was negatively correlated with GA biosynthesis. Exogenously applied GA was able to over-ride the inhibition of germination at low concentrations of ABA, but had no obvious effect when ABA concentrations were high. It is concluded that H_2O_2 mediates the up-regulation of ABA catabolism, probably through an NO signal, and also promotes GA biosynthesis. High concentrations of ABA inhibit GA biosynthesis but a balance of these two hormones can jointly control the dormancy and germination of Arabidopsis seeds.

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Seed dormancy requires ABA degradation and GA accumulation, but it is not clear how ABA degradation and GA synthesis are regulated during this process. We found that both signal molecules H_2O_2 and NO affect the germination of dormant seeds, so we designed experiments to verify the regulatory relationship between them. Our results showed that H_2O_2 could accelerate the degradation of ABA by inducing the expression of ABA metabolic genes (*CYP707As*). Induction of H_2O_2 on ABA metabolic gene expression requires the involvement of another signaling molecule, NO. H_2O_2 can also break seed dormancy by inducing GA synthesis, a process that does not require the involvement of NO. We also found that NO does not release the strong dormancy phenotype of the *CYP707A2* mutant, while H_2O_2 does. If GA synthesis is further suppressed, H_2O_2 is unable to release the strong dormancy phenotype of *CYP707A2* mutants. These results suggest that H_2O_2-breaking seed dormancy is associated with both alleviating ABA inhibition and promoting GA synthesis. In addition, we found that ABA treatment could inhibit GA synthesis and the expression of response genes, which explained why the inhibitory effect of low ABA concentration on seed germination could be relieved by high GA concentration, but the inhibitory effect of high ABA concentration on seed germination could not be relieved by high GA concentration. Overall, our findings provide insights into the complex regulation of seed dormancy by multiple signaling molecules, provide some experimental evidence for understanding the complexity of seed dormancy and germination, and shed light on potential targets for improving seed germination in agriculture. •

Science with Impact

Journal of Experimental Botany

LI XU, LONGFU ZHU, LILI TU, LINLIN LIU, DAOJUN YUAN, LI JIN, LU LONG, XIANLONG ZHANG

Lignin metabolism has a central role in the resistance of cotton to the wilt fungus *Verticillium dahliae* as revealed by RNA-Seq-dependent transcriptional analysis and histochemistry

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RESEARCH PAPER

Lignin metabolism has a central role in the resistance of cotton to the wilt fungus *Verticillium dahliae* as revealed by RNA-Seq-dependent transcriptional analysis and histochemistry

Li Xu, Longfu Zhu, Lili Tu, Linlin Liu, Daojun Yuan, Li Jin, Lu Long and Xianlong Zhang*

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Abstract

The incompatible pathosystem between resistant cotton (*Gossypium barbadense* cv. 7124) and *Verticillium dahliae* strain V991 was used to study the cotton transcriptome changes after pathogen inoculation by RNA-Seq. Of 32 774 genes detected by mapping the tags to assembly cotton contigs, 3442 defence-responsive genes were identified. Gene cluster analyses and functional assignments of differentially expressed genes indicated a significant transcriptional complexity. Quantitative real-time PCR (qPCR) was performed on selected genes with different expression levels and functional assignments to demonstrate the utility of RNA-Seq for gene expression profiles during the cotton defence response. Detailed elucidation of responses of leucine-rich repeat receptor-like kinases (LRR-RLKs), phytohormone signalling-related genes, and transcription factors described the interplay of signals that allowed the plant to fine-tune defence responses. On the basis of global gene regulation of phenylpropanoid metabolism-related genes, phenylpropanoid metabolism was deduced to be involved in the cotton defence response. A closer look at the expression of these genes, enzyme activity, and lignin levels revealed differences between resistant and susceptible cotton plants. Both types of plants showed an increased level of expression of lignin synthesis-related genes and increased phenylalanine-ammonia lyase (PAL) and peroxidase (POD) enzyme activity after inoculation with *V. dahliae*, but the increase was greater and faster in the resistant line. Histochemical analysis of lignin revealed that the resistant cotton not only retains its vascular structure, but also accumulates high levels of lignin. Furthermore, quantitative analysis demonstrated increased lignification and cross-linking of lignin in resistant cotton stems. Overall, a critical role for lignin was believed to contribute to the resistance of cotton to disease.

Key words: Cotton, defence response, phenylpropanoid, lignin, RNA-Seq, signal transduction, Verticillium dahliae.

Introduction

Deep-sequencing technologies have become a revolutionary tool to better understand the complicated eukaryote transcriptomes, and they include three widely used deep-sequencing platforms throughout the world, the Roche 454 Life Sciences, the Illumina Genome Analyzer, and Applied Biosystems' SOLiD (Ansorge, 2009). Highly specific, sensitive, and quantitative measurements enable the deep-sequencing technologies to overcome the shortcomings of traditional hybridization-based approaches. Different from the traditional hybridization-based approaches, high-throughput sequencing technologies, referring to as RNA-Seq, have much greater power to distinguish between paralogous genes, detect low or high abundance transcripts, and allow replicate quantification based on the number of sequences obtained (Wang *et al.* 2009). Furthermore, RNA-Seq can identify transcript sequence polymorphisms and novel trans-splicing and splice isoforms; in addition, there is no strict requirement for a reference genome

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Undoubtedly, the cell wall is the first physical barrier to defend against pathogen invasions, and it is also involved in sensing external stresses and transferring the corresponding signal to stimulate defense responses (Rui and Dinneny 2020; Vaahtera *et al.* 2019; Wan *et al.* 2021; Zhao and Dixon 2014). Thus, the cell wall is more than a passive physical barrier, but also an initial monitoring system for perceiving invaders and inducing defense responses (Vaahtera *et al.* 2019; Wan *et al.* 2021; Bacete *et al.* 2018). Lignin, a hydrophobic aromatic polymer derived from the phenylpropanoid pathway and polymerized by oxidative combinatorial coupling of 4-hydroxyphenylpropanoids, is usually present on the secondary cell wall of vascular plants, where it serves to increase the strength of the wall and to protect it from the degradative enzymes secreted by pathogens (Liu *et al.* 2018; Vanholme *et al.*. 2010). As shown in our previous work, lignin metabolism also has a central role in resistance of cotton to *Verticillium dahliae*. Increased cell wall lignification has been observed in both resistant and susceptible cotton plants exposed to *V. dahliae*, but the increase was greater and faster in the resistant line, suggesting that lignin deposition is a unified response of plants in resistance to pathogens, with resistant varieties potentially having stronger lignin synthesis capabilities (Xu *et al.* 2011). Moreover, we also found that the G (guaiacyl lignin)/S (syringyl lignin) ratio was increased in the resistant cotton line but decreased in the susceptible cotton line, which suggested that the composition of lignin is also critical in defense responses, and that G lignin plays a dominant role in cotton resistance to *V. dahliae* (Xu *et al.* 2011). A similar study was conducted in GhLac1transgenic cotton plants (Hu *et al.* 2018). *GhLac1* is a laccase protein (EC. 1.10.3.2) involved in the oxidative polymerization of monolignols. *GhLac1* over-expressing cotton lines showed a broad resistance to cotton bollworm, cotton aphid and *V. dahliae*, due to lignin hyper-accumulation in the cell wall (Hu *et al.* 2018). This provides effective evidence of lignin as a passive defense system. It is noteworthy that the *GhLac1* RNAi plants also exhibited enhanced resistance to cotton bollworm and *V. dahliae*, with decreased lignin content and the relaxation of the cell wall structure leading to jasmonic acid accumulation (Hu *et al.* 2018). In a sense, this belongs to the initial responses resulting from the modified cell wall structure. Of course, this phenomenon is no accident. The mutants of lignin synthesis genes such as *PAL, C4H, 4CL,* the soluble *C3H, HCT, CCoAOMTs, COMTs, F5H, CAD, CCR*, peroxidases, laccases and other regulators showed great differences in lignin content, composition and resistance to different pathogens. This was inconsistent between different plant species, indicating that lignin is complex and capricious in plants' defense responses (Ninkuu *et al.* 2022; Xie *et al.* 2018). How plant

cells perceive the structural change of the cell wall, and what kind of signal molecules are generated and eventually transferred into disease resistance responses, remain unclear and worth studying, especially in mutants with reduced lignin content but increased resistance to pathogens (Gallego-Giraldo *et al*. 2018). On the other hand, phydroxyphenyl (H), G, and S lignin were the only known lignin monomers, but increasing evidence has shown that the three classical hydroxycinnamyl alcohols are not the only compounds that can be incorporated into natural lignin, and additional monomers such as catechyl (C) and 5-Hydroxy-guaiacyl (5H) monomers also contribute to lignin polymerization in some plant species (Dixon and Barros 2019; Ralph *et al.* 2001; Weng *et al.* 2010; Vanholme *et al.* 2010; Zhong *et al.* 2019; Quan *et al.* 2019). Studies to date suggest that the spatial control of lignin chemistry depends on different combinations of laccases with nonredundant activities immobilized in specific cell types and cell wall layers, but the impact on lignin composition of their modified expression *in planta* cannot necessarily be predicted (Wang *et al.* 2020; Zhao *et al.* 2013; Zhuo *et al.* 2022). Therefore, when studying lignin and plant resistance, we need to consider not only changes in lignin content, but also composition and spatial specificity.

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Science with Impact

Journal of Experimental Botany

AN YANG, XIAOYAN DAI, WEN-HAO ZHANG

A R2R3-type MYB gene, *OsMYB2*, is involved in salt, cold, and dehydration tolerance in rice

Journal of Experimental Botany, Volume 63, Issue 7, April 2012, Pages 2541–2556

• Read the full article here: https://doi.org/10.1093/jxb/err431

Abstract MYB-type transcription factors play a diverse role in plant development and response to abiotic stress. This study isolated a rice R2R3-type MYB gene, *OsMYB2*, and functionally characterized its role in tolerance to abiotic stress by generating transgenic rice plants with overexpressing and RNA interference *OsMYB2*. Expression of *OsMYB2* was up-regulated by salt, cold, and dehydration stress. *OsMYB2* was localized in the nucleus with transactivation activity. No difference in growth and development between the *OsMYB2*-overexpressing and wild-type plants was observed under normal growth conditions, but the *OsMYB2*-over-expressing plants were more tolerant to salt, cold, and dehydration stresses and more sensitive to abscisic acid than wild-type plants. The *OsMYB2*-overexpressing plants accumulated greater amounts of soluble sugars and proline than wild-type plants under salt stress. Overexpres-sion of *OsMYB2* enhanced up-regulation of genes encoding proline syn-thase and transporters. The *OsMYB2*-overexpressing plants accumulated less amounts of H_2O_2 and malondialdehyde. The enhanced activities of antioxidant enzymes, including peroxidase, superoxide dismutase, and catalase, may underlie the lower H_2O_2 contents in *OsMYB2*-overexpressing plants. There was greater up-regulation of stress-related genes, including *OsLEA3, OsRab16A,* and *OsDREB2A*, in the *OsMYB2*-overexpressing plants. Microarray analysis showed that expression of numerous genes involving diverse functions in stress response was altered in the *OsMYB2*-overex-pressing plants. These findings suggest that *OsMYB2* encodes a stress-re-sponsive MYB transcription factor that plays a regulatory role in toler-ance of rice to salt, cold, and dehydration stress.

Introduced by **Wen-Hao Zhang** • Institute of Botany, Chinese Academy of Sciences

Plants are sessile organ-isms and are frequently exposed to variable envi-ronmental stressors that adversely affect their growth and agricultural productivity. To cope with these stressors, plants have evolved efficient mechanisms to sense and rapidly adapt to challenging environmental conditions. Plants undergo numerous bi-ochemical and physiological changes during the abiotic stress response. These chang-es include accumulation of osmolytes and cryopro-tectants such as sugars and proline (Xin & Browse, 1998) to facilitate osmoregulation and to prevent oxidative damage caused by disruption of reactive oxygen species (ROS) homeostasis (Suzuki & Mittler, 2006). In addition, many genes are activated, leading to accumulation of numerous proteins involved in resistance to abiotic stress, such as Late Embryogenesis Abundant proteins (LEAs; Ma *et al.* 2010). The expression of stress-induced genes is largely regulated by specific transcription factors, such as MYB transcription factors (Hu *et al.* 2008).

We identified one such transcription factor, *OsMYB2*, that functions as a positive regulator of stress response genes in order to mediate tolerance of rice seedlings to salt, cold, and dehydra-tion stress. Overexpression of *OsMYB2* led to enhanced accumulation of compatible osmolytes, such as soluble sugars, free proline, and LEA proteins in rice, and sup-pressed the accumulation of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) under conditions of salt stress. The up-regulation of *OsMYB2* promoted effective osmoregulation in rice plants via accumulation of compati-ble solutes and by minimizing oxidative damage during abi-otic stress. More importantly, overexpression of *OsMYB2* in rice seedlings did not affect their phenotypes under con-trol conditions. Therefore, *OsMYB2* provides a promising tool for improving the toler-ance of rice to abiotic stress in general, and to salt stress in particular.

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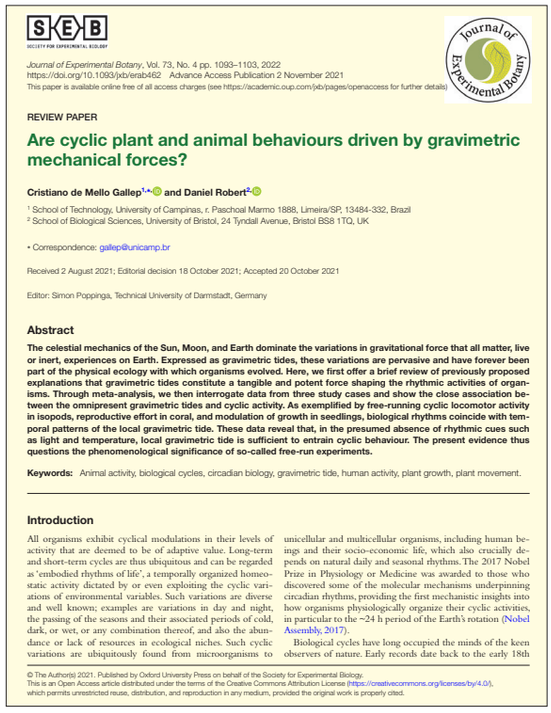
Are cyclic plant and animal behaviours driven by gravimetric mechanical forces?

Journal of Experimental Botany, Volume 73, Issue 4, 2021, Pages 1093–1103

• Read the full article here: https://doi.org/10.1093/jxb/erab462

Abstract The celestial mechanics of the Sun, Moon, and Earth dominate the variations in gravitational force that all matter, live or inert, experiences on Earth. Expressed as gravimetric tides, these variations are pervasive and have forever been part of the physical ecology with which organisms evolved. Here, we first offer a brief review of previously proposed explanations that gravimetric tides constitute a tangible and potent force shaping the rhythmic activities of organisms. Through meta-analysis, we then interrogate data from three study cases and show the close association between the omnipresent gravimetric tides and cyclic activity. As exemplified by free-running cyclic locomotor activity in isopods, reproductive effort in coral, and modulation of growth in seedlings, biological rhythms coincide with temporal patterns of the local gravimetric tide. These data reveal that, in the presumed absence of rhythmic cues such as light and temperature, local gravimetric tide is sufficient to entrain cyclic behaviour. The present evidence thus questions the phenomenological significance of so-called free-run experiments.



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REVIEW PAPER

Are cyclic plant and animal behaviours driven by gravimetric mechanical forces?

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Abstract

The celestial mechanics of the Sun, Moon, and Earth dominate the variations in gravitational force that all matter, live or inert, experiences on Earth. Expressed as gravimetric tides, these variations are pervasive and have forever been part of the physical ecology with which organisms evolved. Here, we first offer a brief review of previously proposed explanations that gravimetric tides constitute a tangible and potent force shaping the rhythmic activities of organisms. Through meta-analysis, we then interrogate data from three study cases and show the close association between the omnipresent gravimetric tides and cyclic activity. As exemplified by free-running cyclic locomotor activity in isopods, reproductive effort in coral, and modulation of growth in seedlings, biological rhythms coincide with temporal patterns of the local gravimetric tide. These data reveal that, in the presumed absence of rhythmic cues such as light and temperature, local gravimetric tide is sufficient to entrain cyclic behaviour. The present evidence thus questions the phenomenological significance of so-called free-run experiments.

Keywords: Animal activity, biological cycles, circadian biology, gravimetric tide, human activity, plant growth, plant movement.

Introduction

All organisms exhibit cyclical modulations in their levels of activity that are deemed to be of adaptive value. Long-term and short-term cycles are thus ubiquitous and can be regarded as 'embodied rhythms of life', a temporally organized homeostatic activity dictated by or even exploiting the cyclic variations of environmental variables. Such variations are diverse and well known; examples are variations in day and night, the passing of the seasons and their associated periods of cold, dark, or wet, or any combination thereof, and also the abundance or lack of resources in ecological niches. Such cyclic variations are ubiquitously found from microorganisms to unicellular and multicellular organisms, including human beings and their socio-economic life, which also crucially depends on natural daily and seasonal rhythms. The 2017 Nobel Prize in Physiology or Medicine was awarded to those who discovered some of the molecular mechanisms underpinning circadian rhythms, providing the first mechanistic insights into how organisms physiologically organize their cyclic activities, in particular to the ~24 h period of the Earth's rotation (Nobel Assembly, 2017).

Biological cycles have long occupied the minds of the keen observers of nature. Early records date back to the early 18th

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Introduced by

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• University of Campinas, Brazil & University of Bristol, UK

The Sun, Earth and Moon have been under each other's cyclical gravitational influence for billions of years. On Earth, such gravitational interplay between the Sun and Moon is most evident through its powerful action on oceanic water masses. Yet, all other terrestrial objects are affected by gravity, from continents and conifers to wombats and water. Gravity acts on all organisms, yet the effects on organisms of the smaller variations in gravitational acceleration due to the lunisolar tide (known as g-tide) are less obvious and rarely considered. Thus, whilst microscale variations in gravitational acceleration are pervasive, their actions are not always readily observable in the natural world. In effect, this sweeping lunisolar g-tide can easily be quantified and predicted, and indeed has always been part of the natural environment, since before life began. The existing literature on the subject is significant and informative, and in view of novel data, renewed attention is very worthwhile. This article by Gallep and Robert offers a brief and focused review of evidence of the rhythmic effects of lunisolar tides on various organisms. Through a meta analysis, the article presents in more detail three cases of putative rhythmic cycles driven by g-tide. These examples pertain to the timing of 1. isopod locomotor activity, 2. coral reproductive effort and 3. seedling growth. A common feature of these studies is that they were performed in controlled lab conditions, crucially providing accurate dates and times of experiments. These three examples are used to bring to the fore the influence of the local g-tide on the temporal organisation of organismal activity. This evidence shows that without external temporal cues provided by light, temperature or resource availability, gravimetric tide is a sufficient cue to contribute to the generation of rhythmic behaviour in all organisms.

This evidence challenges the operational framework of so called "free-run" experiments in circadian rhythm research. The challenge pertains to the interpretation and significance of free-run experiments, which assume that no rhythmic cues are present when the organism is tested in darkness, constant temperature, or other possible experimental perturbations. One overt conclusion from both previous research and the present article is that the observed rhythmic behaviour in free run experiments is commensurate with that of lunisolar microgravitational variations. This phenomenology therefore suggests that the organisms tested thus far, mostly plants and animals, have the competence to detect small rhythmic variations in gravitational acceleration. As an open invitation, this evidence highlights the need for more research to further our understanding of the complete set of drivers, or zeitgebers in the parlance of circadian research, that contribute to the rhythms of life. Plant research is particularly well placed to warrant progress as photosynthetic organisms exhibit gene regulation, physiological processes and behaviours that are intimately linked to two salient and periodic features of the Sun and Moon: light and gravitational pull. •

HERVÉ SAUQUET, SANTIAGO RAMÍREZ-BARAHONA, SUSANA MAGALLÓN

What is the age of flowering plants?

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FLOWERING NEWSLETTER REVIEW

What is the age of flowering plants?

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Abstract

The origin of flowering plants (angiosperms) was one of the most transformative events in the history of our planet. Despite considerable interest from multiple research fields, numerous questions remain, including the age of the group as a whole. Recent studies have reported a perplexing range of estimates for the crown-group age of angiosperms, from ~140 million years (Ma; Early Cretaceous) to 270 Ma (Permian). Both ends of the spectrum are now supported by both macroevolutionary analyses of the fossil record and fossil-calibrated molecular dating analyses. Here, we first clarify and distinguish among the three ages of angiosperms: the age of their divergence with acrogymnosperms (stem age); the age(s) of emergence of their unique, distinctive features including flowers (morphological age); and the age of the most recent common ancestor of all their living species (crown age). We then demonstrate, based on recent studies, that fossil-calibrated molecular dating estimates of the crown-group age of angiosperms have little to do with either the amount of molecular data or the number of internal fossil calibrations included. Instead, we argue that this age is almost entirely conditioned by its own prior distribution (typically a calibration density set by the user in Bayesian analyses). Lastly, we discuss which future discoveries or novel types of analyses are most likely to bring more definitive answers. In the meantime, we propose that the age of angiosperms is best described as largely unknown (140–270 Ma) and that contrasting age estimates in the literature mostly reflect conflicting prior distributions. We also suggest that future work that depends on the time scale of flowering plant diversification be designed to integrate over this vexing uncertainty.

Keywords: Angiosperms, crown age, divergence times, fossil record, morphological age, priors, stem age, uncertainty.

Introduction

Flowering plants (angiosperms) today dominate most terrestrial ecosystems and provide food and habitat to an extraordinary diversity of other life forms. Although the exact number of described species is not yet known (and new species continue to be described every year), estimates of ~300 000 living species indicate that they represent ~90% of all land plants (embryophytes). It has long been known, based on the plant fossil record, that flowering plants are a relatively recent phenomenon on the

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Abstract The origin of flowering plants (angiosperms) was one of the most transformative events in the history of our planet. Despite considerable interest from multiple research fields, numerous questions remain, including the age of the group as a whole. Recent studies have reported a perplexing range of estimates for the crown-group age of angiosperms, from ~140 million years (Ma; Early Cretaceous) to 270 Ma (Permian). Both ends of the spectrum are now supported by both macroevolutionary analyses of the fossil record and fossil-calibrated molecular dating analyses. Here, we first clarify and distinguish among the three ages of angiosperms: the age of their divergence with acrogymnosperms (stem age); the age(s) of emergence of their unique, distinctive features including flowers (morphological age); and the age of the most recent common ancestor of all their living species (crown age). We then demonstrate, based on recent studies, that fossil-calibrated molecular dating estimates of the crown-group age of angiosperms have little to do with either the amount of molecular data or the number of internal fossil calibrations included. Instead, we argue that this age is almost entirely conditioned by its own prior distribution (typically a calibration density set by the user in Bayesian analyses). Lastly, we discuss which future discoveries or novel types of analyses are most likely to bring more definitive answers. In the meantime, we propose that the age of angiosperms is best described as largely unknown (140–270 Ma) and that contrasting age estimates in the literature mostly reflect conflicting prior distributions. We also suggest that future work that depends on the time scale of flowering plant diversification be designed to integrate over this vexing uncertainty.

Introduced by
Rainer Melzer • University College Dublin, Ireland

Without flowering plants, the world would look very different. Food, clothing, ecosystems, even the *Journal of Experimental Botany*, all heavily rely on flowering plants. Yet the origin and evolution of this most species-rich of all land plant groups is surprisingly unclear. This particularly relates to the age of the flowering plants. In their review, Sauquet *et al.* discuss this topic in great detail and outline different approaches for estimating the age of flowering plants. They settle on a relatively large bracket of 140-270 million years ago, and also explain why some uncertainty in age estimates may persist for the foreseeable future.

Though mainly discussing the age of flowering plants, the article is relevant far beyond that topic as it also provides a comprehensive overview of different approaches for estimating the age of taxonomic groups. It especially highlights the importance of fossils and the significant impact of prior assumptions in age estimates. Sauquet *et al.* is therefore an excellent primer for anyone interested in age estimates of taxonomic groups.

Age estimates themselves shape how we think about the world and the evolution of living beings and are therefore inherently interesting. However, as outlined by Sauquet *et al.* the age of flowering plants also has important implications for other research questions like the co-evolution of plants with animals. Indeed, in a new publication by the research group they demonstrate that the first flowering plants were probably insect pollinated, and that time estimates for shifts towards other pollination syndromes are difficult to obtain due to the uncertain age of flowering plants (Stephens *et al.* 2023).

Discussions on the age of different plants and animal groups will continue for the foreseeable future, but with Sauquet *et al.* we have an important guide at hand that navigates the plethora of different approaches and will remain essential reading, perhaps not for 140 million years but for quite some time to come.

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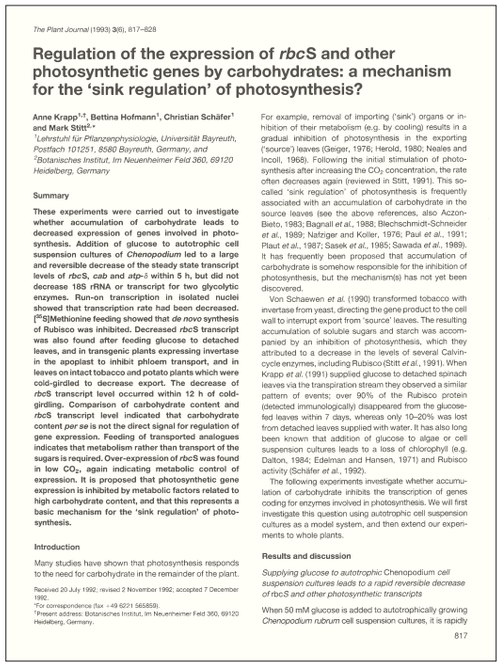
ANNE KRAPP, BETTINA HOFMANN, CHRISTIAN SCHÄFER, MARK STITT

Regulation of the expression of *rbcS* and other photosynthetic genes by carbohydrates: a mechanism for the 'sink regulation' of photosynthesis?

The Plant Journal, 1993, 3: 817-828

• Read the full article here: https://doi.org/10.1111/j.1365-313X.1993.00817.x

Abstract These experiments were carried out to investigate whether accumulation of carbohydrate leads to decreased expression of genes involved in photosynthesis. Addition of glucose to autotrophic cell suspension cultures of *Chenopodium* led to a large and reversible decrease of the steady state transcript levels of *rbcS, cab* and *atp*-& within 5 h, but did not decrease 18S rRNA or transcript for two glycolytic enzymes. Run-on transcription in isolated nuclei showed that transcription rate had been decreased. [^{35}S]Methionine feeding showed that de novo synthesis of Rubisco was inhibited. Decreased *rbcS* transcript was also found after feeding glucose to detached leaves, and in transgenic plants expressing invertase in the apoplast to inhibit phloem transport, and in leaves on intact tobacco and potato plants which were cold-girdled to decrease export. The decrease of *rbcS* transcript level occurred within 12 h of coldgirdling. Comparison of carbohydrate content and *rbcS* transcript level indicated that carbohydrate content per se is not the direct signal for regulation of gene expression. Feeding of transported analogues indicates that metabolism rather than transport of the sugars is required. Over-expression of *rbcS* was found in low CO_2, again indicating metabolic control of expression. It is proposed that photosynthetic gene expression is inhibited by metabolic factors related to high carbohydrate content, and that this represents a basic mechanism for the 'sink regulation' of photo-synthesis.



The Plant Journal (1993) **3**(6), 817–828

Regulation of the expression of *rbc*S and other photosynthetic genes by carbohydrates: a mechanism for the 'sink regulation' of photosynthesis?

Anne Krapp[1,†], Bettina Hofmann[1], Christian Schäfer[1] and Mark Stitt[2,*]

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Summary

These experiments were carried out to investigate whether accumulation of carbohydrate leads to decreased expression of genes involved in photosynthesis. Addition of glucose to autotrophic cell suspension cultures of *Chenopodium* led to a large and reversible decrease of the steady state transcript levels of *rbcS*, *cab* and *atp*-5 within 5 h, but did not decrease 18S rRNA or transcript for two glycolytic enzymes. Run-on transcription in isolated nuclei showed that transcription rate had been decreased. [^{35}S]Methionine feeding showed that *de novo* synthesis of Rubisco was inhibited. Decreased *rbc*S transcript was also found after feeding glucose to detached leaves, and in transgenic plants expressing invertase in the apoplast to inhibit phloem transport, and in leaves on intact tobacco and potato plants which were cold-girdled to decrease export. The decrease of *rbc*S transcript level occurred within 12 h of cold-girdling. Comparison of carbohydrate content and *rbc*S transcript level indicated that carbohydrate content *per se* is not the direct signal for regulation of gene expression. Feeding of transported analogues indicates that metabolism rather than transport of the sugars is required. Over-expression of *rbc*S was found in low CO_2, again indicating metabolic control of expression. It is proposed that photosynthetic gene expression is inhibited by metabolic factors related to high carbohydrate content, and that this represents a basic mechanism for the 'sink regulation' of photo-synthesis.

Introduction

Many studies have shown that photosynthesis responds to the need for carbohydrate in the remainder of the plant.

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For example, removal of importing ('sink') organs or inhibition of their metabolism (e.g. by cooling) results in a gradual inhibition of photosynthesis in the exporting ('source') leaves (Geiger, 1976; Herold, 1980; Neales and Incoll, 1968). Following the initial stimulation of photosynthesis after increasing the CO_2 concentration, the rate often decreases again (reviewed in Stitt, 1991). This so-called 'sink regulation' of photosynthesis is frequently associated with an accumulation of carbohydrate in the source leaves (see the above references, also Azcon-Bieto, 1983; Bagnall *et al.*, 1988; Blechschmidt-Schneider *et al.*, 1989; Nafziger and Koller, 1976; Paul *et al.*, 1991; Plaut *et al.*, 1987; Sasek *et al.*, 1985; Sawada *et al.*, 1989). It has frequently been proposed that accumulation of carbohydrate is somehow responsible for the inhibition of photosynthesis, but the mechanism(s) has not yet been discovered.

Von Schaewen *et al.* (1990) transformed tobacco with invertase from yeast, directing the gene product to the cell wall to interrupt export from 'source' leaves. The resulting accumulation of soluble sugars and starch was accompanied by an inhibition of photosynthesis, which they attributed to a decrease in the levels of several Calvin-cycle enzymes, including Rubisco (Stitt *et al.*, 1991). When Krapp *et al.* (1991) supplied glucose to detached spinach leaves via the transpiration stream they observed a similar pattern of events; over 90% of the Rubisco protein (detected immunologically) disappeared from the glucose-fed leaves within 7 days, whereas only 10–20% was lost from detached leaves supplied with water. It has also long been known that addition of glucose to algae or cell suspension cultures leads to a loss of chlorophyll (e.g. Dalton, 1984; Edelman and Hansen, 1971) and Rubisco activity (Schäfer *et al.*, 1992).

The following experiments investigate whether accumulation of carbohydrate inhibits the transcription of genes coding for enzymes involved in photosynthesis. We will first investigate this question using autotrophic cell suspension cultures as a model system, and then extend our experiments to whole plants.

Results and discussion

Supplying glucose to autotrophic Chenopodium *cell suspension cultures leads to a rapid reversible decrease of rbcS and other photosynthetic transcripts*

When 50 mM glucose is added to autotrophically growing *Chenopodium rubrum* cell suspension cultures, it is rapidly

Introduced by

Elena Baena-Gonzalez • University of Oxford, UK

Photosynthesis powers almost all life on earth and is responsible for the production of most of our food. The mechanisms that regulate photosynthesis have therefore been an area of intense research, as their understanding can provide the means to increase photosynthetic efficiency and thereby plant growth and productivity. In this seminal study, Krapp and colleagues found that the end-products of photosynthesis, sugars, inhibit photosynthesis by reducing the expression of genes involved in this process. This provided a mechanism for a long-observed correlation between the demand for sugars in non-photosynthetic organs (aka. sinks) and the rates of photosynthesis in leaves. This study further paved the way for the search for components involved in sugar perception and signalling. At present, the relationship between sugar consumption by sink organs and photosynthetic efficiency constitutes one of the most promising areas of research in the quest to improve crop productivity.•

YUKOH HIEI, SHOZO OHTA, TOSHIHIKO KOMARI, TAKASHI KUMASHIRO

Efficient transformation of rice (*Oryza sativa* L.) mediated by Agrobacterium and sequence analysis of the boundaries of the T-DNA

The Plant Journal, 1994, 6: 271-282

• Read the full article here:
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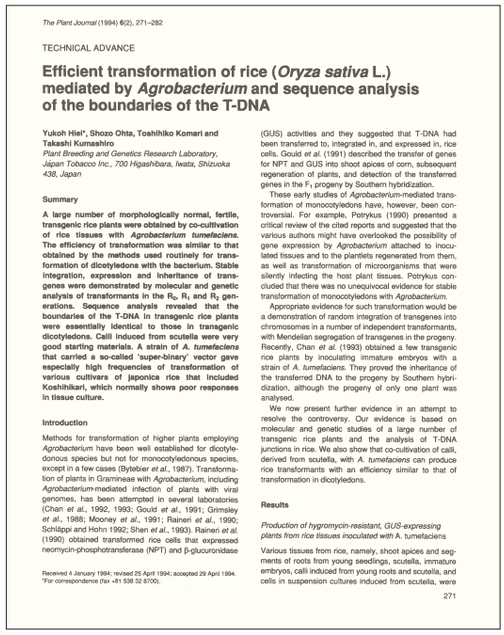

The Plant Journal (1994) 6(2), 271–282

TECHNICAL ADVANCE

Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA

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Summary

A large number of morphologically normal, fertile, transgenic rice plants were obtained by co-cultivation of rice tissues with *Agrobacterium tumefaciens*. The efficiency of transformation was similar to that obtained by the methods used routinely for transformation of dicotyledons with the bacterium. Stable integration, expression and inheritance of transgenes were demonstrated by molecular and genetic analysis of transformants in the R_0, R_1 and R_2 generations. Sequence analysis revealed that the boundaries of the T-DNA in transgenic rice plants were essentially identical to those in transgenic dicotyledons. Calli induced from scutella were very good starting materials. A strain of *A. tumefaciens* that carried a so-called 'super-binary' vector gave especially high frequencies of transformation of various cultivars of japonica rice that included Koshihikari, which normally shows poor responses in tissue culture.

Introduction

Methods for transformation of higher plants employing *Agrobacterium* have been well established for dicotyledonous species but not for monocotyledonous species, except in a few cases (Bytebier *et al.*, 1987). Transformation of plants in Gramineae with *Agrobacterium*, including *Agrobacterium*-mediated infection of plants with viral genomes, has been attempted in several laboratories (Chan *et al.*, 1992, 1993; Gould *et al.*, 1991; Grimsley *et al.*, 1988; Mooney *et al.*, 1991; Raineri *et al.*, 1990; Schläppi and Hohn 1992; Shen *et al.*, 1993). Raineri *et al.* (1990) obtained transformed rice cells that expressed neomycin-phosphotransferase (NPT) and β-glucuronidase (GUS) activities and they suggested that T-DNA had been transferred to, integrated in, and expressed in, rice cells. Gould *et al.* (1991) described the transfer of genes for NPT and GUS into shoot apices of corn, subsequent regeneration of plants, and detection of the transferred genes in the F_1 progeny by Southern hybridization.

These early studies of *Agrobacterium*-mediated transformation of monocotyledons have, however, been controversial. For example, Potrykus (1990) presented a critical review of the cited reports and suggested that the various authors might have overlooked the possibility of gene expression by *Agrobacterium* attached to inoculated tissues and to the plantlets regenerated from them, as well as transformation of microorganisms that were silently infecting the host plant tissues. Potrykus concluded that there was no unequivocal evidence for stable transformation of monocotyledons with *Agrobacterium*.

Appropriate evidence for such transformation would be a demonstration of random integration of transgenes into chromosomes in a number of independent transformants, with Mendelian segregation of transgenes in the progeny. Recently, Chan *et al.* (1993) obtained a few transgenic rice plants by inoculating immature embryos with a strain of *A. tumefaciens*. They proved the inheritance of the transferred DNA to the progeny by Southern hybridization, although the progeny of only one plant was analysed.

We now present further evidence in an attempt to resolve the controversy. Our evidence is based on molecular and genetic studies of a large number of transgenic rice plants and the analysis of T-DNA junctions in rice. We also show that co-cultivation of calli, derived from scutella, with *A. tumefaciens* can produce rice transformants with an efficiency similar to that of transformation in dicotyledons.

Results

Production of hygromycin-resistant, GUS-expressing plants from rice tissues inoculated with A. tumefaciens

Various tissues from rice, namely, shoot apices and segments of roots from young seedlings, scutella, immature embryos, calli induced from young roots and scutella, and cells in suspension cultures induced from scutella, were

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Introduced by

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When the groundbreaking study by Hiei *et al.* was published in 1994, the *Agrobacterium*-mediated plant transformation method was well-established and routinely used for dicots but not monocots. In this study, the authors reported an efficient method for transformation of the model monocot species rice, mediated by *Agrobacterium* co-cultivation. The study complemented and added to the small number of existing studies (Rueb and Hensgens 1989; Raineri *et al.* 1990; Chan *et al.* 1992, 1993) in essential aspects such as tissue culture media and growth condition optimization, use of more efficient *Agrobacterium* strains, and choice of explant. At this time, there were very few scattered reports on the topic, with no equivocal evidence of using *Agrobacterium tumefaciens* for stable transformation and integration of genes of interest in monocot species. This study, however, achieved transformation efficiencies in rice equivalent to that in dicots. Another significant feature of the research is that it highlighted the 'starting material', i.e. the rice explant, which could be used for raising stable and fertile transformants. Scutella-derived calli co-cultivated with LBA4404 cells carrying the 'super-binary' vector pTOK233 gave the highest frequency of GUS-expressing and hygromycin-resistant cells even in varieties such as Koshihikari which otherwise responded poorly to tissue culture. Yet another remarkable feature is that the tissue culture conditions optimized and the transformation method established in this study were the first to generate a substantially large number of fertile, morphologically normal, and stable transformants with minimal rearrangements throughout the R2 generation in three varieties of japonica, as confirmed through Southern blotting and other molecular analyses. The stable integration of the desired gene was additionally confirmed using sequence analysis of the adjacent boundaries of T-DNA. Notably, the T-DNA sequences in transgenic rice were shown to be similar to those routinely found in dicots.

This study was impactful because it made an exceedingly advantageous method a routine laboratory protocol. *Agrobacterium*-mediated transformation in monocots, especially rice, soon became the preferred method for raising transgenics carrying useful traits such as abiotic stress tolerance, improved nutritional content, and higher yield. Since then, many different explants have been experimented with, in a host of genotypes with strains carrying different plasmids, and substantial transformation efficiencies have been achieved in each of the studies (reviewed by Sah *et al.* 2014).

This study, published in one of the early volumes of *The Plant Journal* in 1994, indeed represents a significant contribution to plant research. For example, the work led to yet another important study wherein rapid and efficient transformation and regeneration was achieved in different indica cultivars using a technically-advanced protocol with optimized media and growth conditions (Sahoo *et al.* 2011).

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Science with Impact

the plant journal

HANS THORDAL-CHRISTENSEN, ZIGUO ZHANG, YANGDOU WEI, DAVID B. COLLINGE

Subcellular localization of H_2O_2 in plants. H_2O_2 accumulation in papillae and hypersensitive response during the barley—powdery mildew interaction

The Plant Journal, 1997, Volume 11, Issue 6, 1187–1194

• Read the full article here:
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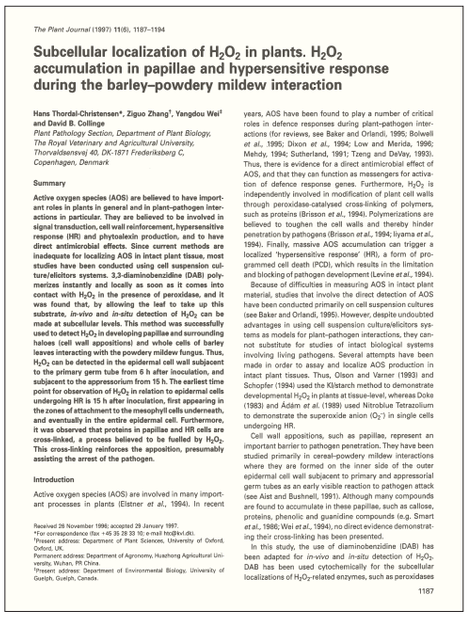
Abstract Active oxygen species (AOS) are believed to have important roles in plants in general and in plant—pathogen interactions in particular. They are believed to be involved in signal transduction, cell wall reinforcement, hypersensitive response (HR) and phytoalexin production, and to have direct antimicrobial effects. Since current methods are inadequate for localizing AOS in intact plant tissue, most studies have been conducted using cell suspension culture/elicitors systems. 3,3-diaminobenzidine (DAB) polymerizes instantly and locally as soon as it comes into contact with H_2O_2 in the presence of peroxidase, and it was found that, by allowing the leaf to take up this substrate, *in-vivo* and *in-situ* detection of H_2O_2 can be made at subcellular levels. This method was successfully used to detect H_2O_2 in developing papillae and surrounding haloes (cell wall appositions) and whole cells of barley leaves interacting with the powdery mildew fungus. Thus, H_2O_2 can be detected in the epidermal cell wall subjacent to the primary germ tube from 6 h after inoculation, and subjacent to the appressorium from 15 h. The earliest time point for observation of H_2O_2 in relation to epidermal cells undergoing HR is 15 h after inoculation, first appearing in the zones of attachment to the mesophyll cells underneath, and eventually in the entire epidermal cell. Furthermore, it was observed that proteins in papillae and HR cells are cross-linked, a process believed to be fuelled by H_2O_2. This cross-linking reinforces the apposition, presumably assisting the arrest of the pathogen.



The Plant Journal (1997) 11(6), 1187–1194

Subcellular localization of H_2O_2 in plants. H_2O_2 accumulation in papillae and hypersensitive response during the barley–powdery mildew interaction

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Summary

Active oxygen species (AOS) are believed to have important roles in plants in general and in plant–pathogen interactions in particular. They are believed to be involved in signal transduction, cell wall reinforcement, hypersensitive response (HR) and phytoalexin production, and to have direct antimicrobial effects. Since current methods are inadequate for localizing AOS in intact plant tissue, most studies have been conducted using cell suspension culture/elicitors systems. 3,3-diaminobenzidine (DAB) polymerizes instantly and locally as soon as it comes into contact with H_2O_2 in the presence of peroxidase, and it was found that, by allowing the leaf to take up this substrate, *in-vivo* and *in-situ* detection of H_2O_2 can be made at subcellular levels. This method was successfully used to detect H_2O_2 in developing papillae and surrounding haloes (cell wall appositions) and whole cells of barley leaves interacting with the powdery mildew fungus. Thus, H_2O_2 can be detected in the epidermal cell wall subjacent to the primary germ tube from 6 h after inoculation, and subjacent to the appressorium from 15 h. The earliest time point for observation of H_2O_2 in relation to epidermal cells undergoing HR is 15 h after inoculation, first appearing in the zones of attachment to the mesophyll cells underneath, and eventually in the entire epidermal cell. Furthermore, it was observed that proteins in papillae and HR cells are cross-linked, a process believed to be fuelled by H_2O_2. This cross-linking reinforces the apposition, presumably assisting the arrest of the pathogen.

Introduction

Active oxygen species (AOS) are involved in many important processes in plants (Elstner *et al.*, 1994). In recent years, AOS have been found to play a number of critical roles in defence responses during plant–pathogen interactions (for reviews, see Baker and Orlandi, 1995; Bolwell *et al.*, 1995; Dixon *et al.*, 1994; Low and Merida, 1996; Mehdy, 1994; Sutherland, 1991; Tzeng and DeVay, 1993). Thus, there is evidence for a direct antimicrobial effect of AOS, and that they can function as messengers for activation of defence response genes. Furthermore, H_2O_2 is independently involved in modification of plant cell walls through peroxidase-catalysed cross-linking of polymers, such as proteins (Brisson *et al.*, 1994). Polymerizations are believed to toughen the cell walls and thereby hinder penetration by pathogens (Brisson *et al.*, 1994; Iyama *et al.*, 1994). Finally, massive AOS accumulation can trigger a localized 'hypersensitive response' (HR), a form of programmed cell death (PCD), which results in the limitation and blocking of pathogen development (Levine *et al.*, 1994).

Because of difficulties in measuring AOS in intact plant material, studies that involve the direct detection of AOS have been conducted primarily on cell suspension cultures (see Baker and Orlandi, 1995). However, despite undoubted advantages in using cell suspension culture/elicitors systems as models for plant–pathogen interactions, they cannot substitute for studies of intact biological systems involving living pathogens. Several attempts have been made in order to assay and localize AOS production in intact plant tissues. Thus, Olson and Varner (1993) and Schopfer (1994) used the KI/starch method to demonstrate developmental H_2O_2 in plants at tissue-level, whereas Doke (1983) and Adám *et al.* (1989) used Nitroblue Tetrazolium to demonstrate the superoxide anion (O_2^-) in single cells undergoing HR.

Cell wall appositions, such as papillae, represent an important barrier to pathogen penetration. They have been studied primarily in cereal–powdery mildew interactions where they are formed on the inner side of the outer epidermal cell wall subjacent to primary and appressorial germ tubes as an early visible reaction to pathogen attack (see Aist and Bushnell, 1991). Although many compounds are found to accumulate in these papillae, such as callose, proteins, phenolic and guanidine compounds (e.g. Smart *et al.*, 1986; Wei *et al.*, 1994), no direct evidence demonstrating their cross-linking has been presented.

In this study, the use of diaminobenzidine (DAB) has been adapted for *in-vivo* and *in-situ* detection of H_2O_2. DAB has been used cytochemically for the subcellular localizations of H_2O_2-related enzymes, such as peroxidases

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Plants are sessile organisms that lack a nervous system, but they still respond rapidly to environmental threats such as fungal invasion by sending signals from the site of perception systemically throughout distal tissues in order to trigger appropriate defence responses. The generation of reactive oxygen species (ROS), or active oxygen species (AOS) as denoted in the article by Thordal-Christensen *et al.*, (1997), plays a central role in plant defences. This paper was based on a solid foundation of literature evidence showing that ROS, particularly hydrogen peroxide, are generated in response to pathogen attack. The hypothesis that ROS were important signal transducing molecules still remained to be validated at the time of publication. The accepted concept was that ROS were largely harmful metabolic by-products, although they were known to be involved in plant defence responses. A key issue at this time concerned accurate methods of ROS detection, a problem that in fact still persists today. Thordal-Christensen *et al.*, (1997) used *in situ* staining methods to show that hydrogen peroxide was produced and accumulated only at specific sites within barley leaves infected with the powdery mildew fungus, *Erysiphe graminis*. Using a 3,3-diaminobenzideine (DAB) stain together with high precision microscopy, the authors showed that hydrogen peroxide accumulated only in places where defensive cell wall reinforcement was occurring through the deposition of material at appositions (papillae) and at the points of contact with adjacent cells, as well as around some cells at specific locations. This paper demonstrated for the first time that the sites of pathogen-induced hydrogen peroxide generation were highly specific, occurring only at specific locations at the cell periphery and not throughout the attacked cells. The authors speculated that this specificity was related to oxidative cross-linking of the cell wall that is essential to prevent spread of the pathogen. This seminal work provided a step change in our understanding of how plants use targeted ROS production in pathogen defences. These authors not only demonstrated the power of diaminobenzideine (DAB) staining to localise hydrogen peroxide accumulation in different cellular locations, but the findings also paved the way for current concepts of how the apoplastic oxidative burst and associated ROS waves contribute to auto-propagating cell-to-cell signalling pathways. •

PETRA BOEVINK, KARL OPARKA, SIMON SANTA CRUZ, BARRY MARTIN, ALAN BETTERIDGE, CHRIS HAWES

Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network

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The Plant Journal (1998) **15**(3), 441–447

SHORT COMMUNICATION

Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network†

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Summary

We have visualized the relationship between the endoplasmic reticulum (ER) and Golgi in leaf cells of *Nicotiana clevelandii* by expression of two Golgi proteins fused to green fluorescent protein (GFP). A fusion of the *trans*-membrane domain (signal anchor sequence) of a rat sialyl transferase to GFP was targeted to the Golgi stacks. A second construct that expressed the *Arabidopsis* H/KDEL receptor homologue aERD2, fused to GFP, was targeted to both the Golgi apparatus and ER, allowing the relationship between these two organelles to be studied in living cells for the first time. The Golgi stacks were shown to move rapidly and extensively along the polygonal cortical ER network of leaf epidermal cells, without departing from the ER tubules. Co-localization of F-actin in the GFP-expressing cells revealed an underlying actin cytoskeleton that matched precisely the architecture of the ER network, while treatment of cells with the inhibitors cytochalasin D and *N*-ethylmaleimide revealed the dependency of Golgi movement on actin cables. These observations suggest that the leaf Golgi complex functions as a motile system of actin-directed stacks whose function is to pick up products from a relatively stationary ER system. Also, we demonstrate for the first time *in vivo* brefeldin A-induced retrograde transport of Golgi membrane protein to the ER.

Introduction

It is generally accepted that bidirectional transport of proteins between the endoplasmic reticulum (ER) and the Golgi in plant cells is by means of transport vesicles (Denecke, 1996; Staehelin and Moore, 1995), although direct membrane connections have been described in some cell types (Satiat-Jeunemaitre *et al.*, 1996a). The driving forces behind such transport and the molecular mechanisms underlying it are far from clear. However, in animal and yeast cells, the pathways of membrane and protein flow between the ER and Golgi have been dissected morphologically and biochemically (Farquhar and Palade, 1998). It is generally accepted that secretory product flow from the ER is by means of vesicle vectors transporting newly synthesized proteins and glycoproteins to an 'intermediate' compartment between the exit site on the ER and the *cis*-Golgi, variously termed as vesicular tubular clusters (VTC), sorting exosomes or *cis*-Golgi compartments (Aridor and Balch, 1996). Here, the first sorting occurs for the return of proteins in the retrograde direction back to the ER. Subsequently, and depending on the model currently in vogue, this compartment either delivers its cargo to the *cis*-Golgi via COP-coated vesicles or matures into a *cis*-Golgi cisternum (Bannykh *et al.*, 1998).

Recent studies, utilizing a fusion of the green fluorescent protein (GFP) to a viral glycoprotein, have revealed the animal Golgi complex to be a relatively stationary structure, with rapidly forming pre-Golgi structures (VTC) being unidirectionally translocated by microtubules, inwards from the ER to the Golgi (Presley *et al.*, 1997). This new approach permits the *in vivo* observation of endomembrane dynamics and has been used previously to demonstrate the diffusional mobility of Golgi membrane proteins including GFP-tagged glycosyl transferases (Cole *et al.*, 1996).

We have demonstrated previously that GFP can be used for the *in vivo* study of the plant endomembrane system by targeting it to the ER lumen in tobacco leaf cells using a virus-based expression system (Boevink *et al.*, 1996). Here, in order to investigate the relationship between the leaf Golgi apparatus and ER we have expressed, in leaf cells, GFP spliced to the transmembrane domain (signal anchor sequence) of a rat sialyl transferase (Munro, 1995) and to the *Arabidopsis* homologue of the H/KDEL receptor (Lee *et al.*, 1993). We show that both these proteins target the tobacco leaf Golgi, which are highly motile and track in an actin-dependent manner over the polygonal network of cortical ER. This close relationship between the ER and Golgi is highlighted by a brefeldin A-induced retrograde transport of the sialyl transferase construct into the ER.

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†Golgi movies can be found at the following web site: http://www.brookes.ac.uk/schools/bms/research/molcell/hawesgfp/gfp.html.

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Abstract We have visualized the relationship between the endoplasmic reticulum (ER) and Golgi in leaf cells of *Nicotiana clevelandii* by expression of two Golgi proteins fused to green fluorescent protein (GFP). A fusion of the trans-membrane domain (signal anchor sequence) of a rat sialyl transferase to GFP was targeted to the Golgi stacks. A second construct that expressed the *Arabidopsis* H/KDEL receptor homologue aERD2, fused to GFP, was targeted to both the Golgi apparatus and ER, allowing the relationship between these two organelles to be studied in living cells for the first time. The Golgi stacks were shown to move rapidly and extensively along the polygonal cortical ER network of leaf epidermal cells, without departing from the ER tubules. Co-localization of F-actin in the GFP-expressing cells revealed an underlying actin cytoskeleton that matched precisely the architecture of the ER network, while treatment of cells with the inhibitors cytochalasin D and N-ethylmaleimide revealed the dependency of Golgi movement on actin cables. These observations suggest that the leaf Golgi complex functions as a motile system of actin-directed stacks whose function is to pick up products from a relatively stationary ER system. Also, we demonstrate for the first time *in vivo* brefeldin A-induced retrograde transport of Golgi membrane protein to the ER.

Introduced by

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Almost 250 years ago, it was reported that plant cells exhibit a fascinating cytoskeleton-dependent movement of organelles, commonly referred to as cytoplasmic streaming. Since then, in the absence of specific organelle labels, teasing apart the relationships between organelles and cytoskeleton in live plant cells has been particularly difficult. In this pioneering work, Boevink and colleagues investigated the relationship between the leaf Golgi apparatus and endoplasmic reticulum (ER) with the cytoskeleton. In very early days since the adoption of the green fluorescent protein (GFP) in cell biology analyses at large, the authors developed two probes for the ER/Golgi and the Golgi apparatus, using a plant protein (H/KDEL receptor) and a chimaera with the transmembrane domain and cytosolic tail of a mammalian protein (rat sialyl transferase), respectively. The study was the first to demonstrate that the tobacco leaf Golgi stacks are highly mobile and track in an actin-dependent manner over the polygonal network of cortical ER. These findings have stood the test of time and paved the way for exciting research to understand the molecular underpinnings of the endomembrane traffic in plant cells. •

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Construct design for efficient, effective and high-throughput gene silencing in plants

The Plant Journal, 2001, 27: 581–590

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Abstract Post-transcriptional silencing of plant genes using anti-sense or co-suppression constructs usually results in only a modest proportion of silenced individuals. Recent work has demonstrated the potential for constructs encoding self-complementary 'hairpin' RNA (hpRNA) to efficiently silence genes. In this study we examine design rules for efficient gene silencing, in terms of both the proportion of independent transgenic plants showing silencing, and the degree of silencing. Using hpRNA constructs containing sense/anti-sense arms ranging from 98 to 853 nt gave efficient silencing in a wide range of plant species, and inclusion of an intron in these constructs had a consistently enhancing effect. Intron-containing constructs (ihpRNA) generally gave 90–100% of independent transgenic plants showing silencing. The degree of silencing with these constructs was much greater than that obtained using either co-suppression or anti-sense constructs. We have made a generic vector, pHANNIBAL, that allows a simple, single PCR product from a gene of interest to be easily converted into a highly effective ihpRNA silencing construct. We have also created a high-throughput vector, pHELLSGATE, that should facilitate the cloning of gene libraries or large numbers of defined genes, such as those in EST collections, using an in vitro recombinase system. This system may facilitate the large-scale determination and discovery of plant gene functions in the same way as RNAi is being used to examine gene function in *Caenorhabditis elegans*.

Introduced by
Bin Yu • University of Nebraska–Lincoln, USA

In the late 1990s, plant scientists exploited the post transcriptional gene silencing phenomenon (PTGS) to silence gene expression (Hamilton *et al.* 1998, Waterhouse *et al.* 1998). They discovered that constructs generating self-complementary hairpin RNAs (hpRNAs) effectively mute the expression of target genes containing the sequence of the hpRNA duplex stem (Hamilton *et al.* 1998, Waterhouse *et al.* 1998). A similar phenomenon known as RNA Interference was discovered in C. elegans, in which injection of double-stranded RNAs causes sequence-specific gene silencing (Fire *et al.* 1998). These findings point to a promising strategy for controlling gene expression. In this article, Wesley et al. investigated the principles for designing hpRNAs that can trigger efficient silencing in terms of the proportion of silenced individual plants and the degree of silencing. The findings revealed that hpRNAs with stem arm lengths ranging from 98nt to 853nt produce effective silencing in a variety of plant species. Furthermore, inclusion of an intron in the designs (ihpRNA) consistently boosts silencing efficacy, resulting in silencing in 90-100% of independent transgenic plants. More importantly, this study generated a generic vector that can be easily used to construct ihpRNA vectors targeting genes of interest, as well as a high-throughput hpRNA vector that can be used to build libraries targeting a large number of genes. In summary, this research lays the ground for dissecting gene function and improving crop traits by gene expression manipulation.

The Plant Journal (2001) 27(6), 581–590

TECHNICAL ADVANCE

Construct design for efficient, effective and high-throughput gene silencing in plants

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Summary

Post-transcriptional silencing of plant genes using anti-sense or co-suppression constructs usually results in only a modest proportion of silenced individuals. Recent work has demonstrated the potential for constructs encoding self-complementary 'hairpin' RNA (hpRNA) to efficiently silence genes. In this study we examine design rules for efficient gene silencing, in terms of both the proportion of independent transgenic plants showing silencing, and the degree of silencing. Using hpRNA constructs containing sense/anti-sense arms ranging from 98 to 853 nt gave efficient silencing in a wide range of plant species, and inclusion of an intron in these constructs had a consistently enhancing effect. Intron-containing constructs (ihpRNA) generally gave 90–100% of independent transgenic plants showing silencing. The degree of silencing with these constructs was much greater than that obtained using either co-suppression or anti-sense constructs. We have made a generic vector, pHANNIBAL, that allows a simple, single PCR product from a gene of interest to be easily converted into a highly effective ihpRNA silencing construct. We have also created a high-throughput vector, pHELLSGATE, that should facilitate the cloning of gene libraries or large numbers of defined genes, such as those in EST collections, using an in vitro recombinase system. This system may facilitate the large-scale determination and discovery of plant gene functions in the same way as RNAi is being used to examine gene function in *Caenorhabditis elegans*.

Keywords: PTGS, RNAi, genomics, vector, ihpRNA, Gateway.

Introduction

The ultimate goal of current genome projects is to identify the biological function of every gene in the genome. Whole genomes of several organisms (including *Arabidopsis*, http://www.arabidopsis.org), have been completely sequenced, providing a wealth of information. The functions of some of the genes have been identified directly by the appropriate assay, or have been inferred by homology to genes of known function in other organisms. Loss-of-function mutants, from insertional mutagenesis or transposable elements, have also been very informative about the role of some of these genes (Aspinol,eshan and Feldmann, 1997; Martienssen, 1998), particularly in the large-scale analysis of the yeast genome (Ross-Macdonald *et al.*, 1999). However, the functions of a large proportion of genes remain unknown.

Injection or ingestion of dsRNA into nematodes can trigger specific RNA degradation, in a process known as RNA-interference (RNAi; Fire *et al.*, 1998). This process facilitates targeted post-transcriptional gene silencing (PTGS) and has recently been harnessed to study the function of over 4000 genes on chromosomes I and III

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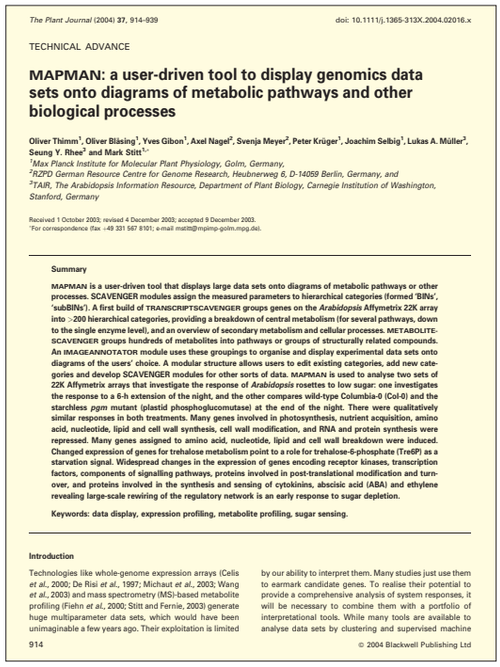
OLIVER THIMM, OLIVER BLÄSING, YVES GIBON, AXEL NAGEL, SVENJA MEYER, PETER KRÜGER, JOACHIM SELBIG, LUKAS A. MÜLLER, SEUNG Y. RHEE AND MARK STITT

MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes

The Plant Journal, 2004, 37: 914–939

• Read the full article here:
https://doi.org/10.1111/j.1365-313X.2004.02016.x

Abstract MAPMAN is a user-driven tool that displays large data sets onto diagrams of metabolic pathways or other processes. SCAVENGER modules assign the measured parameters to hierarchical categories (formed 'BINs', 'subBINs'). A first build of TRANSCRIPTSCAVENGER groups genes on the *Arabidopsis* Affymetrix 22K array into >200 hierarchical categories, providing a breakdown of central metabolism (for several pathways, down to the single enzyme level), and an overview of secondary metabolism and cellular processes. METABOLITESCAVENGER groups hundreds of metabolites into pathways or groups of structurally related compounds. An IMAGEANNOTATOR module uses these groupings to organise and display experimental data sets onto diagrams of the users' choice. A modular structure allows users to edit existing categories, add new categories and develop SCAVENGER modules for other sorts of data. MAPMAN is used to analyse two sets of 22K Affymetrix arrays that investigate the response of *Arabidopsis* rosettes to low sugar: one investigates the response to a 6-h extension of the night, and the other compares wild-type Columbia-0 (Col-0) and the starchless *pgm* mutant (plastid phosphoglucomutase) at the end of the night. There were qualitatively similar responses in both treatments. Many genes involved in photosynthesis, nutrient acquisition, amino acid, nucleotide, lipid and cell wall synthesis, cell wall modification, and RNA and protein synthesis were repressed. Many genes assigned to amino acid, nucleotide, lipid and cell wall breakdown were induced. Changed expression of genes for trehalose metabolism point to a role for trehalose-6-phosphate (Tre6P) as a starvation signal. Widespread changes in the expression of genes encoding receptor kinases, transcription factors, components of signalling pathways, proteins involved in post-translational modification and turnover, and proteins involved in the synthesis and sensing of cytokinins, abscisic acid (ABA) and ethylene revealing large-scale rewiring of the regulatory network is an early response to sugar depletion.



The Plant Journal (2004) **37**, 914–939 doi: 10.1111/j.1365-313X.2004.02016.x

TECHNICAL ADVANCE

MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes

Oliver Thimm[1], Oliver Bläsing[1], Yves Gibon[1], Axel Nagel[2], Svenja Meyer[2], Peter Krüger[1], Joachim Selbig[1], Lukas A. Müller[2], Seung Y. Rhee[3] and Mark Stitt[1,*]

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Summary

MAPMAN is a user-driven tool that displays large data sets onto diagrams of metabolic pathways or other processes. SCAVENGER modules assign the measured parameters to hierarchical categories (formed 'BINs', 'subBINs'). A first build of TRANSCRIPTSCAVENGER groups genes on the *Arabidopsis* Affymetrix 22K array into >200 hierarchical categories, providing a breakdown of central metabolism (for several pathways, down to the single enzyme level), and an overview of secondary metabolism and cellular processes. METABOLITESCAVENGER groups hundreds of metabolites into pathways or groups of structurally related compounds. An IMAGEANNOTATOR module uses these groupings to organise and display experimental data sets onto diagrams of the users' choice. A modular structure allows users to edit existing categories, add new categories and develop SCAVENGER modules for other sorts of data. MAPMAN is used to analyse two sets of 22K Affymetrix arrays that investigate the response of *Arabidopsis* rosettes to low sugar: one investigates the response to a 6-h extension of the night, and the other compares wild-type Columbia-0 (Col-0) and the starchless *pgm* mutant (plastid phosphoglucomutase) at the end of the night. There were qualitatively similar responses in both treatments. Many genes involved in photosynthesis, nutrient acquisition, amino acid, nucleotide, lipid and cell wall synthesis, cell wall modification, and RNA and protein synthesis were repressed. Many genes assigned to amino acid, nucleotide, lipid and cell wall breakdown were induced. Changed expression of genes for trehalose metabolism point to a role for trehalose-6-phosphate (Tre6P) as a starvation signal. Widespread changes in the expression of genes encoding receptor kinases, transcription factors, components of signalling pathways, proteins involved in post-translational modification and turnover, and proteins involved in the synthesis and sensing of cytokinins, abscisic acid (ABA) and ethylene revealing large-scale rewiring of the regulatory network is an early response to sugar depletion.

Keywords: data display, expression profiling, metabolite profiling, sugar sensing.

Introduction

Technologies like whole-genome expression arrays (Celis *et al.*, 2000; De Risi *et al.*, 1997; Michaut *et al.*, 2003; Wang *et al.*, 2003) and mass spectrometry (MS)-based metabolite profiling (Fiehn *et al.*, 2000; Stitt and Fernie, 2003) generate huge multiparameter data sets, which would have been unimaginable a few years ago. Their exploitation is limited by our ability to interpret them. Many studies just use them to earmark candidate genes. To realise their potential to provide a comprehensive analysis of system responses, it will be necessary to combine them with a portfolio of interpretational tools. While many tools are available to analyse data sets by clustering and supervised machine

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Introduced by **Alisdair Fernie** • Max Planck Institute of Molecular Plant Physiology, Germany

Thim *et al.*'s MAPMAN paper published in 2004 is highly deserving of its mammoth number of citations simply because it was so far ahead of its time. At the turn of the century, microarrays had revolutionized biology and the amount of data that they provided was well in excess of what biologists were used to. Whilst researchers worked out the best controls for these multi-parallel analyses relatively quickly, they were much less rapid at achieving automated methods to analyse the data that they produced. Mark Stitt´s group thus initiated what became a suite of programs for transcriptomics. MAPMAN provided both non-redundant gene ontologies and ready drawn maps placing genes within defined metabolic or regulatory pathways, with the former being expertly assigned based on the knowledge of the lab. The MAPMAN software described in the paper was made freely available and it was not long before MAPMAN figures were appearing across the full spectrum of plant science journals and beyond. Indeed, these figures became nearly as omnipresent as Mark himself was at conferences, wearing a T-Shirt gifted to him by his lab and sporting a cartoon of MAPMAN paying homage to a similarly titled video console game. Despite the replacement of microarrays by RNAseq and the widespread adoption of GO ontogologies, MAPMAN is still widely used today with the current iteration supporting far more species than Arabidopsis. •

the plant journal

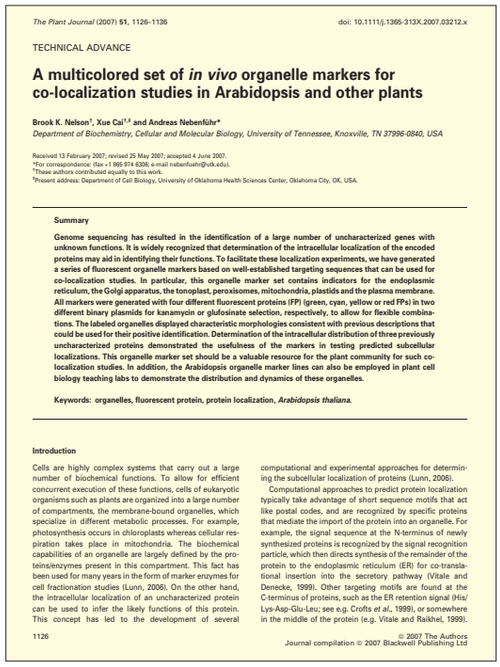
BROOK K. NELSON, XUE CAI, ANDREAS NEBENFÜHR

A multicolored set of *in vivo* organelle markers for co-localization studies in Arabidopsis and other plants.

The Plant Journal, 2007, 51: 1126–1136

• Read the full article here:
https://doi.org/10.1111/j.1365-313X.2007.03212.x

Abstract Genome sequencing has resulted in the identification of a large number of uncharacterized genes with unknown functions. It is widely recognized that determination of the intracellular localization of the encoded proteins may aid in identifying their functions. To facilitate these localization experiments, we have generated a series of fluorescent organelle markers based on well-established targeting sequences that can be used for co-localization studies. In particular, this organelle marker set contains indicators for the endoplasmic reticulum, the Golgi apparatus, the tonoplast, peroxisomes, mitochondria, plastids and the plasma membrane. All markers were generated with four different fluorescent proteins (FP) (green, cyan, yellow or red FPs) in two different binary plasmids for kanamycin or glufosinate selection, respectively, to allow for flexible combinations. The labeled organelles displayed characteristic morphologies consistent with previous descriptions that could be used for their positive identification. Determination of the intracellular distribution of three previously uncharacterized proteins demonstrated the usefulness of the markers in testing predicted subcellular localizations. This organelle marker set should be a valuable resource for the plant community for such co-localization studies. In addition, the Arabidopsis organelle marker lines can also be employed in plant cell biology teaching labs to demonstrate the distribution and dynamics of these organelles.



The Plant Journal (2007) **51**, 1126–1136 doi: 10.1111/j.1365-313X.2007.03212.x

TECHNICAL ADVANCE

A multicolored set of *in vivo* organelle markers for co-localization studies in Arabidopsis and other plants

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Summary

Genome sequencing has resulted in the identification of a large number of uncharacterized genes with unknown functions. It is widely recognized that determination of the intracellular localization of the encoded proteins may aid in identifying their functions. To facilitate these localization experiments, we have generated a series of fluorescent organelle markers based on well-established targeting sequences that can be used for co-localization studies. In particular, this organelle marker set contains indicators for the endoplasmic reticulum, the Golgi apparatus, the tonoplast, peroxisomes, mitochondria, plastids and the plasma membrane. All markers were generated with four different fluorescent proteins (FP) (green, cyan, yellow or red FPs) in two different binary plasmids for kanamycin or glufosinate selection, respectively, to allow for flexible combinations. The labeled organelles displayed characteristic morphologies consistent with previous descriptions that could be used for their positive identification. Determination of the intracellular distribution of three previously uncharacterized proteins demonstrated the usefulness of the markers in testing predicted subcellular localizations. This organelle marker set should be a valuable resource for the plant community for such co-localization studies. In addition, the Arabidopsis organelle marker lines can also be employed in plant cell biology teaching labs to demonstrate the distribution and dynamics of these organelles.

Keywords: organelles, fluorescent protein, protein localization, *Arabidopsis thaliana*.

Introduction

Cells are highly complex systems that carry out a large number of biochemical functions. To allow for efficient concurrent execution of these functions, cells of eukaryotic organisms such as plants are organized into a large number of compartments, the membrane-bound organelles, which specialize in different metabolic processes. For example, photosynthesis occurs in chloroplasts whereas cellular respiration takes place in mitochondria. The biochemical capabilities of an organelle are largely defined by the proteins/enzymes present in this compartment. This fact has been used for many years in the form of marker enzymes for cell fractionation studies (Lunn, 2006). On the other hand, the intracellular localization of an uncharacterized protein can be used to infer the likely functions of this protein. This concept has led to the development of several computational and experimental approaches for determining the subcellular localization of proteins (Lunn, 2006).

Computational approaches to predict protein localization typically take advantage of short sequence motifs that act like postal codes, and are recognized by specific proteins that mediate the import of the protein into an organelle. For example, the signal sequence at the N-terminus of newly synthesized proteins is recognized by the signal recognition particle, which then directs synthesis of the remainder of the protein to the endoplasmic reticulum (ER) for co-translational insertion into the secretory pathway (Vitale and Denecke, 1999). Other targeting motifs are found at the C-terminus of proteins, such as the ER retention signal (His/Lys-Asp-Glu-Leu; see e.g. Crofts *et al.*, 1999), or somewhere in the middle of the protein (e.g. Vitale and Raikhel, 1999).

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One of the most exciting and important aspects of biological research is finding new roles for proteins, and with that often new functions and layers of life. Starting with approaches such as determining protein localisation within the cell gives new insights into their potential functions or pathways.

The work by Brook Nelson, Xue Cai and Andreas Nebenführ prepared and tested a library of organellar markers to allow for direct visualisation of different organelles. These markers are proteins with known localisations destined for organelles such as plastids, mitochondria, the endoplasmic reticulum, and Golgi bodies. The marker proteins can be fused to fluorescent proteins and expressed in plant cells in a transient or stable manner. Previously uncharacterised proteins can be expressed together with these markers to enable identification of their cellular localisation.

This work is not only of great value for plant cell biology research, but has also been used in cell biology teaching. For example, growing Arabidopsis lines expressing markers in a stable manner allows students to see and appreciate the incredible dynamics of plant organelles.

This work has been cited by over 1900 articles, and has informed various novel projects and research fields including hormonal pathways and plant growth, microRNAs, combinatorial analysis of membrane compartments, cell wall biosynthesis, and many others. •

STEVEN J. CLOUGH, ANDREW F. BENT

Floral dip: a simplified method for Agrobacterium -mediated transformation of Arabidopsis thaliana

The Plant Journal, 2008, 16: 735-743

• Read the full article here: https://doi.org/10.1046/j.1365-313x.1998.00343.x

The Plant Journal (1998) **16**(6), 735–743

TECHNICAL ADVANCE

Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*

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Summary

The *Agrobacterium* vacuum infiltration method has made it possible to transform *Arabidopsis thaliana* without plant tissue culture or regeneration. In the present study, this method was evaluated and a substantially modified transformation method was developed. The labor-intensive vacuum infiltration process was eliminated in favor of simple dipping of developing floral tissues into a solution containing *Agrobacterium tumefaciens*, 5% sucrose and 500 microliters per litre of surfactant Silwet L-77. Sucrose and surfactant were critical to the success of the floral dip method. Plants inoculated when numerous immature floral buds and few siliques were present produced transformed progeny at the highest rate. Plant tissue culture media, the hormone benzylamino purine and pH adjustment were unnecessary, and *Agrobacterium* could be applied to plants at a range of cell densities. Repeated application of *Agrobacterium* improved transformation rates and overall yield of transformants approximately twofold. Covering plants for 1 day to retain humidity after inoculation also raised transformation rates twofold. Multiple ecotypes were transformable by this method. The modified method should facilitate high-throughput transformation of *Arabidopsis* for efforts such as T-DNA gene tagging, positional cloning, or attempts at targeted gene replacement.

Introduction

Plant transformation technology offers an array of opportunities for basic scientific research and for modification of food and fiber crops. Transgenic plants are typically produced by complex methods that require careful preparation of plant cells or tissues, introduction of DNA using *Agrobacterium tumefaciens* or particle bombardment, selection of transformed cell lines, and regeneration of plants (Christou, 1996; Hooykaas and Schilperoort, 1992; Siemens and Schieder, 1996; Weising *et al.*, 1988). These transformation methods require time, skilled labor and relatively expensive laboratory facilities. In contrast, the 'Agrobacterium vacuum infiltration' method is a relatively new and simple procedure for transformation of *Arabidopsis thaliana* (Bechtold *et al.*, 1993). In its original form, the method involved the growth of *Arabidopsis* to flowering stage, uprooting of plants, application of *Agrobacterium* to whole plants via vacuum infiltration in a sucrose/hormone growth medium, re-planting, collection of seed a few weeks later, and identification of transformed progeny by selection on media containing antibiotic or herbicide (Bechtold *et al.*, 1993). The technique, which can be viewed as an extension of earlier *in planta* transformation methods (Chang *et al.*, 1994; Feldmann and Marks, 1987; Feldmann, 1992; Katavic *et al.*, 1994), offered a substitute for widely utilized *Arabidopsis* transformation methods that involved root tissue culture and plant regeneration (e.g. Valvekens *et al.*, 1988). With vacuum infiltration and other *in planta* transformation methods, most transformed progeny are genetically uniform (non-chimeric) and the somaclonal variation associated with tissue culture and regeneration is minimized. Transformed progeny are typically hemizygous for the transgene at a given locus, suggesting that transformation occurs after the divergence of anther and ovary cell lineages (Bechtold *et al.*, 1993; Feldmann, 1992). Likely targets of heritable transformation are therefore the gametophyte-progenitor tissues, mature gametophytes, or recently fertilized embryos.

The primary reasons for the popularity of the *Agrobacterium* vacuum infiltration method have been its simplicity and reliability. The elimination of tissue culture and regeneration greatly reduces hands-on time, and success can be achieved by non-experts (Bechtold *et al.*, 1993; Bent and Clough, 1998). Transformed plants can be obtained at sufficiently high rates so that the procedure can be used not only to introduce specific gene constructs into plants, but also as a random mutagenesis method for gene-tagging (e.g. Azpiroz-Leehan and Feldmann, 1997; Hirsch *et al.*, 1998; Koncz *et al.*, 1989; Mollier *et al.*, 1995; Richardson *et al.*, 1998). The primary drawback of the method is that successful application has only been reported for one plant species, *Arabidopsis thaliana*.

To improve this very widely used *Arabidopsis* transformation method and to gain insights that may facilitate transformation of other plant species, we sought to test a number of parameters involved in the transformation

Received 26 June 1998; revised 15 October 1998; accepted 26 October 1998.
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Abstract The *Agrobacterium* vacuum infiltration method has made it possible to transform *Arabidopsis thaliana* without plant tissue culture or regeneration. In the present study, this method was evaluated and a substantially modified transformation method was developed. The labor-intensive vacuum infiltration process was eliminated in favor of simple dipping of developing floral tissues into a solution containing *Agrobacterium tumefaciens*, 5% sucrose and 500 microliters per litre of surfactant Silwet L-77. Sucrose and surfactant were critical to the success of the floral dip method. Plants inoculated when numerous immature floral buds and few siliques were present produced transformed progeny at the highest rate. Plant tissue culture media, the hormone benzylamino purine and pH adjustment were unnecessary, and *Agrobacterium* could be applied to plants at a range of cell densities. Repeated application of *Agrobacterium* improved transformation rates and overall yield of transformants approximately twofold. Covering plants for 1 day to retain humidity after inoculation also raised transformation rates twofold. Multiple ecotypes were transformable by this method. The modified method should facilitate high-throughput transformation of *Arabidopsis* for efforts such as T-DNA gene tagging, positional cloning, or attempts at targeted gene replacement.

Introduced by

Federica Brandizzi • Michigan State University, USA

Manipulation of plant genomes is foundational to understand the biology of plants and develop new traits for a variety of applications. Therefore, the development of straightforward transformation protocols is needed for analyses of individual genes and high-throughput studies. In this work, Clough and Bent addressed this challenge by generating a simplified method to transform the model plant species, *Arabidopsis thaliana*, which is widely used in plant laboratories worldwide. The protocol improved an earlier, solid, but laborious transformation method based on *Agrobacterium*-mediated vacuum transformation. The method put forward in Clough and Bent's article applies simple dipping of flowering plants in *Agrobacterium* cells that are suspended in a solution containing sucrose (or glucose) and surfactant, followed by plant incubation under a dome up to a day after bacterial inoculation. The simplicity and efficacy of this method, dubbed "floral dip method" by the authors, has led to its widely broad adoption for *Arabidopsis* studies. Its simplicity is remains unmatched to date. •

Science with Impact

Plant Biotechnology Journal

Plant Biotech Journal

JEAN-YVES PAUL, HARJEET KHANNA, JENNIFER KLEIDON, PHUONG HOANG, JASON GEIJSKES, JEFF DANIELLS, ELLA ZAPLIN, YVONNE ROSENBERG, ANTHONY JAMES, BULUKANI MLALAZI, PRADEEP DEO, GEOFREY ARINAITWE, PRIVER NAMANYA, DOUGLAS BECKER, JAMES TINDAMANYIRE, WILBERFORCE TUSHEMEREIRWE, ROBERT HARDING, JAMES DALE

Golden bananas in the field: elevated fruit pro-vitamin A from the expression of a single banana transgene

Plant Biotechnology Journal, 2016, 15: 520–532

• Read the full article here: https://doi.org/10.1111/pbi.12650

Abstract Vitamin A deficiency remains one of the world's major public health problems despite food fortification and supplements strategies. Biofortification of staple crops with enhanced levels of pro-vitamin A (PVA) offers a sustainable alternative strategy to both food fortification and supplementation. As a proof of concept, PVA-biofortified transgenic Cavendish bananas were generated and field trialed in Australia with the aim of achieving a target level of 20 μg/g of dry weight (dw) β-carotene equivalent (β-CE) in the fruit. Expression of a Fe'i banana-derived phytoene synthase 2a (*MtPsy2a*) gene resulted in the generation of lines with PVA levels exceeding the target level with one line reaching 55 μg/g dw β-CE. Expression of the maize phytoene synthase 1 (*ZmPsy1*) gene, used to develop 'Golden Rice 2', also resulted in increased fruit PVA levels although many lines displayed undesirable phenotypes. Constitutive expression of either transgene with the maize polyubiquitin promoter increased PVA accumulation from the earliest stage of fruit development. In contrast, PVA accumulation was restricted to the late stages of fruit development when either the banana 1-aminocyclopropane-1-carboxylate oxidase or the expansin 1 promoters were used to drive the same transgenes. Wild-type plants with the longest fruit development time had also the highest fruit PVA concentrations. The results from this study suggest that early activation of the rate-limiting enzyme in the carotenoid biosynthetic pathway and extended fruit maturation time are essential factors to achieve optimal PVA concentrations in banana fruit.


Plant Biotechnology Journal

Golden bananas in the field: elevated fruit pro-vitamin A from the expression of a single banana transgene

Abstract

Introduction

Introduced by

Jean-Yves Paul • Centre for Agriculture and the Bioeconomy, Queensland University of Technology, Brisbane, Australia

This article describes the results of the initial research and development phase of Banana21, a project aimed at alleviating vitamin A deficiency (VAD) in Uganda through the biofortification of their staple crop, banana. Micronutrient deficiencies, and VAD in particular, are unacceptably high in sub-Saharan Africa despite decades of unsustainable interventions. The availability of nutrient-dense staple foods is an effective addition to current interventions targeting micronutrient-deficient rural populations, which often rely on household-produced, micronutrient-poor staples for the majority of their diet. Therefore, the development of pro-vitamin A (pVA)-rich bananas was proposed as a complementary approach to help address the high incidence of VAD in Uganda and other countries where bananas are a staple food.

Banana21 commenced in 2005 and, with ongoing support from the Bill & Melinda Gates Foundation, has progressed from the initial proof-of-concept described in the manuscript into a technology transfer phase and, at the present time, a product development phase. Since September 2019, the National Agricultural Research Organisation (NARO) of Uganda has carried out multi-location field trials of genetically modified pVA-biofortified East African highland banana (EAHB) lines in four distinct agro-ecological zones of the country (Zawedde *et al.* 2018). From these trials, a lead pVA-biofortified event of the EAHB cultivar "Nakitembe" with enhanced pVA levels has been identified. Extensive biosafety data is currently being collected from this event and compiled into a dossier for food and environmental safety assessments in Uganda.

Uganda currently does not have a biosafety bill in place that would allow the progression of the lead event through to deregulation and ultimately farmer release, unlike neighbouring countries such as Kenya and Rwanda (Ongu *et al.* 2023). Kenya enacted the Biosafety Act in 2009, overseen by the National Biosafety Authority, while Rwanda passed the Law on Biosafety in 2019, administered by the Rwanda Environment Management Authority. The absence of such an Act in Uganda has hindered significant research opportunities in this developing nation, which is in great need of agricultural advancements and innovation. The Banana21 project has certainly been impacted in that regard. Importantly, with the status quo and considering the porous nature of national borders in the region, Uganda is exposed to the risk of unregulated GMOs being grown within its borders. The long-awaited implementation of biosafety regulations would enable Uganda to explore and harness the tremendous benefits of genetically modified crops, providing valuable opportunities for progress and development.

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Zawedde BM, Kwehangana M, Oloka HK. 2018. Readiness for Environmental Release of Genetically Engineered (GE) Plants in Uganda [Policy and Practice Reviews]. Frontiers in Bioengineering and Biotechnology, 6, DOI 10.3389/fbioe.2018.00152. •

ANNETTE NIEHL, MARJUKKA SOININEN, MINNA M. PORANEN, MANFRED HEINLEIN

Synthetic biology approach for plant protection using dsRNA

Plant Biotechnology Journal, 2018, 16: 1679–1687

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https://doi.org/10.1111/pbi.12904

Plant Biotechnology Journal

Plant Biotechnology Journal (2018) **16**, pp. 1679–1687 doi: 10.1111/pbi.12904

Synthetic biology approach for plant protection using dsRNA

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Summary
Pathogens induce severe damages on cultivated plants and represent a serious threat to global food security. Emerging strategies for crop protection involve the external treatment of plants with double-stranded (ds)RNA to trigger RNA interference. However, applying this technology in greenhouses and fields depends on dsRNA quality, stability and efficient large-scale production. Using components of the bacteriophage phi6, we engineered a stable and accurate *in vivo* dsRNA production system in *Pseudomonas syringae* bacteria. Unlike other *in vitro* or *in vivo* dsRNA production systems that rely on DNA transcription and postsynthetic alignment of single-stranded RNA molecules, the phi6 system is based on the replication of dsRNA by an RNA-dependent RNA polymerase, thus allowing production of high-quality, long dsRNA molecules. The phi6 replication complex was reprogrammed to multiply dsRNA sequences homologous to tobacco mosaic virus (TMV) by replacing the coding regions within two of the three phi6 genome segments with TMV sequences and introduction of these constructs into *P. syringae* together with the third phi6 segment, which encodes the components of the phi6 replication complex. The stable production of TMV dsRNA was achieved by combining all the three phi6 genome segments and by maintaining the natural dsRNA sizes and sequence elements required for efficient replication and packaging of the segments. The produced TMV-derived dsRNAs inhibited TMV propagation when applied to infected *Nicotiana benthamiana* plants. The established dsRNA production system enables the broad application of dsRNA molecules as an efficient, highly flexible, nontransgenic and environmentally friendly approach for protecting crops against viruses and other pathogens.

Keywords: RNAi, dsRNA production technology, sustainable crop protection.

Introduction

Agricultural yields are greatly affected by various pathogens and pests which decrease production of crops worldwide (Oerke, 2005) with losses adding up to around $US100 billion per year (Fletcher *et al.*, 2006) and potentially reaching U$540 billion per year if the spreading of invasive pests and pathogens is not controlled (Kew Royal Botanical Gardens, 2017; Pairs *et al.*, 2016). To protect plants against pathogens and pests, agriculture relies on the widespread use of chemical pesticides that are applied to the environment in large amounts. These intense applications of chemical pesticides pose potential risks of human health, beneficial organisms and the environment (Nicolopoulou-Stamati *et al.*, 2016). Thus, there is a need for novel tools and alternative methods to control disease epidemics. Indeed, legislation in Europe is aiming at reducing the use of chemical plant protection products and calling for an in-depth reconsideration of crop protection solutions (Union, 2009). A promising new approach with strong potential for protecting plants against viruses and other pathogens involves the application of double-stranded (ds)RNA.

dsRNA is a natural product that occurs in most, if not all, organisms in nature. It triggers RNA silencing, also known as RNA interference (RNAi), which is a conserved eukaryotic mechanism playing a crucial role in growth, development, host defence against viruses and transposon inactivation, across plant, fungi and animal kingdoms. RNAi plays a natural role in antiviral defence by targeting the invading viral RNA genomes and viral transcripts for cleavage and translational repression in a sequence-specific manner (Ding, 2010; Pumplin and Voinnet, 2013). Consistent with the central role of dsRNA in triggering RNAi, numerous studies have shown that plants treated with virus-specific dsRNA or single-stranded RNA (ssRNA) molecules that fold into hairpin conformation showed antiviral resistance (Carbonell *et al.*, 2008; Gan *et al.*, 2010; Konakalla *et al.*, 2016; Tenllado and Díaz-Ruiz, 2001; Tenllado *et al.*, 2003a,b; Yin *et al.*, 2009). dsRNAs were also shown to be effective against fungi (Koch *et al.*, 2016; Wang *et al.*, 2016, 2017) and insects (Baum *et al.*, 2007; Ghosh *et al.*, 2017; Li *et al.*, 2013; Luo *et al.*, 2017), thus providing important implications for dsRNA-triggered RNAi as an emerging novel approach for crop protection. RNAi has been deployed for plant protection since it was discovered. A plethora of studies has demonstrated the feasibility of RNAi-based approaches, and numerous virus-resistant crops have been approved for commercial release (Khalid *et al.*, 2017). However, unlike these previous approaches, which depend on transgenes, the external application of dsRNA will realize crop protection without any modification of the plant genome and, therefore, may be regulated differently compared to crop protection involving genetically modified organisms. Moreover, in being independent of any time-consuming genetic modification, the application of dsRNA is much more flexible and allows faster actions against new emerging diseases.

Applying dsRNA may have numerous advantages over the use of chemical compounds. Unlike chemical compounds, which act via a

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Abstract Pathogens induce severe damages on cultivated plants and represent a serious threat to global food security. Emerging strategies for crop protection involve the external treatment of plants with double-stranded (ds)RNA to trigger RNA interference. However, applying this technology in greenhouses and fields depends on dsRNA quality, stability and efficient large-scale production. Using components of the bacteriophage phi6, we engineered a stable and accurate *in vivo* dsRNA production system in *Pseudomonas syringae* bacteria. Unlike other *in vitro* or *in vivo* dsRNA production systems that rely on DNA transcription and postsynthetic alignment of single-stranded RNA molecules, the phi6 system is based on the replication of dsRNA by an RNA-dependent RNA polymerase, thus allowing production of high-quality, long dsRNA molecules. The phi6 replication complex was reprogrammed to multiply dsRNA sequences homologous to tobacco mosaic virus (TMV) by replacing the coding regions within two of the three phi6 genome segments with TMV sequences and introduction of these constructs into *P. syringae* together with the third phi6 segment, which encodes the components of the phi6 replication complex. The stable production of TMV dsRNA was achieved by combining all the three phi6 genome segments and by maintaining the natural dsRNA sizes and sequence elements required for efficient replication and packaging of the segments. The produced TMV-derived dsRNAs inhibited TMV propagation when applied to infected *Nicotiana benthamiana* plants. The established dsRNA production system enables the broad application of dsRNA molecules as an efficient, highly flexible, nontransgenic and environmentally friendly approach for protecting crops against viruses and other pathogens.

Introduced by
Manfred Heinlein • University of Strasbourg, France

The growing world population and climate instability challenge the ability of mankind to manage pests and diseases of cultivated plants. Moreover, concerns over negative effects of pesticides on the environment and on human health cause an increasing demand for environmentally sustainable production systems. In their paper, Niehl *et al.* (2018) studied the possibility to activate the plant's own defense systems to protect plants against viral pathogens. For this, they treated plants with double-stranded (ds)RNA which shares nucleotide sequence identity with the RNA genome of a pathogenic virus. Within the plant cell, dsRNA is digested into small interfering (si)RNA molecules which guide the cellular RNA interference machinery to target single-stranded (ss)RNA that is homologous in sequence to the produced siRNAs. This results in the degradation of the targeted viral RNA, thus preventing viral infection. By monitoring the GFP signal in plants infected with a GFP-tagged virus, the authors could demonstrate antiviral activity of the virus-specific dsRNA topically applied by either mechanical inoculation or spraying. The antiviral effect was sequence- specific as non-specific dsRNA, used as a control, did not reduce viral infection.

As a part of their study, Niehl *et al.* (2018) constructed a unique, synthetic dsRNA-producing platform which enables dsRNA amplification in bacteria using components derived from dsRNA bacteriophage phi6. Unlike many other bacterial systems that produce dsRNAs by post-synthetic annealing of ssRNA, the synthetic platform developed by Niehl *et al.* (2018) avoids the potentially erroneous post-synthetic annealing by using the phage RNA-dependent RNA polymerase to generate fully double-stranded, high-quality(hq)-dsRNAs. The bacteria continuously amplifying dsRNA can be grown in fermenters thus providing a sustained source of target-specific hq-dsRNA. The platform is highly flexible and can be adapted to any pathogen or pest target through just a few DNA cloning steps. While there is a need to further develop the hq-dsRNA purification from bacterial cells to reduce the overall production costs, the scalability and dsRNA quality achieved by the phi6-based production system are important arguments for further development, marketing and agricultural application. •

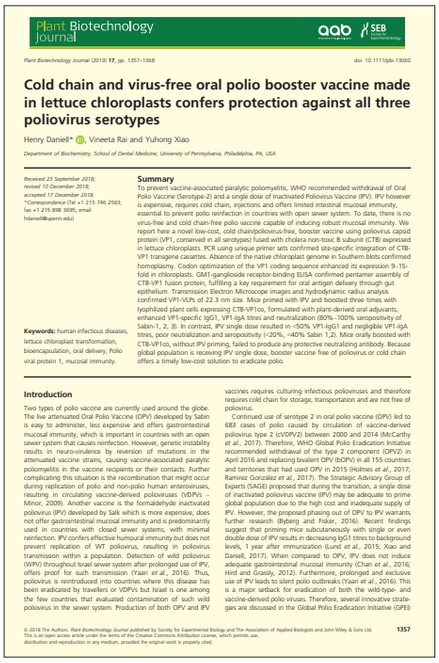
HENRY DANIELL, VINEETA RAI, YUHONG XIAO

Cold chain and virus-free oral polio booster vaccine made in lettuce chloroplasts confers protection against all three poliovirus serotypes

Plant Biotechnology Journal, 2018, 17: 1357-1368

• Read the full article here:
https://doi.org/10.1111/pbi.13060

Abstract To prevent vaccine-associated paralytic poliomyelitis, WHO recommended withdrawal of Oral Polio Vaccine (Serotype-2) and a single dose of Inactivated Poliovirus Vaccine (IPV). IPV however is expensive, requires cold chain, injections and offers limited intestinal mucosal immunity, essential to prevent polio reinfection in countries with open sewer system. To date, there is no virus-free and cold chain-free polio vaccine capable of inducing robust mucosal immunity. We report here a novel low-cost, cold chain/poliovirus-free, booster vaccine using poliovirus capsid protein (VP1, conserved in all serotypes) fused with cholera non-toxic B subunit (CTB) expressed in lettuce chloroplasts. PCR using unique primer sets confirmed site-specific integration of CTB-VP1 transgene cassettes. Absence of the native chloroplast genome in Southern blots confirmed homoplasmy. Codon optimization of the VP1 coding sequence enhanced its expression 9–15-fold in chloroplasts. GM1-ganglioside receptor-binding ELISA confirmed pentamer assembly of CTB-VP1 fusion protein, fulfilling a key requirement for oral antigen delivery through gut epithelium. Transmission Electron Microscope images and hydrodynamic radius analysis confirmed VP1-VLPs of 22.3 nm size. Mice primed with IPV and boosted three times with lyophilized plant cells expressing CTB-VP1co, formulated with plant-derived oral adjuvants, enhanced VP1-specific IgG1, VP1-IgA titres and neutralization (80%–100% seropositivity of Sabin-1, 2, 3). In contrast, IPV single dose resulted in <50% VP1-IgG1 and negligible VP1-IgA titres, poor neutralization and seropositivity (<20%, <40% Sabin 1,2). Mice orally boosted with CTB-VP1co, without IPV priming, failed to produce any protective neutralizing antibody. Because global population is receiving IPV single dose, booster vaccine free of poliovirus or cold chain offers a timely low-cost solution to eradicate polio.


Plant Biotechnology Journal

Plant Biotechnology Journal (2019) 17, pp. 1357–1368 doi: 10.1111/pbi.13060

Cold chain and virus-free oral polio booster vaccine made in lettuce chloroplasts confers protection against all three poliovirus serotypes

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Summary
To prevent vaccine-associated paralytic poliomyelitis, WHO recommended withdrawal of Oral Polio Vaccine (Serotype-2) and a single dose of Inactivated Poliovirus Vaccine (IPV). IPV however is expensive, requires cold chain, injections and offers limited intestinal mucosal immunity, essential to prevent polio reinfection in countries with open sewer system. To date, there is no virus-free and cold chain-free polio vaccine capable of inducing robust mucosal immunity. We report here a novel low-cost, cold chain/poliovirus-free, booster vaccine using poliovirus capsid protein (VP1, conserved in all serotypes) fused with cholera non-toxic B subunit (CTB) expressed in lettuce chloroplasts. PCR using unique primer sets confirmed site-specific integration of CTB-VP1 transgene cassettes. Absence of the native chloroplast genome in Southern blots confirmed homoplasmy. Codon optimization of the VP1 coding sequence enhanced its expression 9–15-fold in chloroplasts. GM1-ganglioside receptor-binding ELISA confirmed pentamer assembly of CTB-VP1 fusion protein, fulfilling a key requirement for oral antigen delivery through gut epithelium. Transmission Electron Microscope images and hydrodynamic radius analysis confirmed VP1-VLPs of 22.3 nm size. Mice primed with IPV and boosted three times with lyophilized plant cells expressing CTB-VP1co, formulated with plant-derived oral adjuvants, enhanced VP1-specific IgG1, VP1-IgA titres and neutralization (80%–100% seropositivity of Sabin-1, 2, 3). In contrast, IPV single dose resulted in <50% VP1-IgG1 and negligible VP1-IgA titres, poor neutralization and seropositivity (<20%, <40% Sabin 1,2). Mice orally boosted with CTB-VP1co, without IPV priming, failed to produce any protective neutralizing antibody. Because global population is receiving IPV single dose, booster vaccine free of poliovirus or cold chain offers a timely low-cost solution to eradicate polio.

Keywords: human infectious diseases, lettuce chloroplast transformation, bioencapsulation, oral delivery, Polio viral protein 1, mucosal immunity.

Introduction
Two types of polio vaccine are currently used around the globe. The live attenuated Oral Polio Vaccine (OPV) developed by Sabin is easy to administer, less expensive and offers gastrointestinal mucosal immunity, which is important in countries with an open sewer system that causes reinfection. However, genetic instability results in neuro-virulence by reversion of mutations in the attenuated vaccine strains, causing vaccine-associated paralytic poliomyelitis in the vaccine recipients or their contacts. Further complicating this situation is the recombination that might occur during replication of polio and non-polio human enteroviruses, resulting in circulating vaccine-derived polioviruses (VDPVs – Minor, 2009). Another vaccine is the formaldehyde inactivated poliovirus (IPV) developed by Salk which is more expensive, does not offer gastrointestinal mucosal immunity and is predominantly used in countries with closed sewer systems, with minimal reinfection. IPV confers effective humoural immunity but does not prevent replication of WT poliovirus, resulting in poliovirus transmission within a population. Detection of wild poliovirus (WPV) throughout Israel sewer system after prolonged use of IPV, offers proof for such transmission (Yaari et al., 2016). Thus, poliovirus is reintroduced into countries where this disease has been eradicated by travellers or VDPVs but Israel is one among the few countries that evaluated contamination of such wild poliovirus in the sewer system. Production of both OPV and IPV vaccines requires culturing infectious polioviruses and therefore requires cold chain for storage, transportation and are not free of poliovirus.

Continued use of serotype 2 in oral polio vaccine (OPV) led to 683 cases of polio caused by circulation of vaccine-derived poliovirus type 2 (cVDPV2) between 2000 and 2014 (McCarthy et al., 2017). Therefore, WHO Global Polio Eradication Initiative recommended withdrawal of the type 2 component (OPV2) in April 2016 and replacing bivalent OPV (bOPV) in all 155 countries and territories that had used OPV in 2015 (Holmes et al., 2017; Ramirez Gonzalez et al., 2017). The Strategic Advisory Group of Experts (SAGE) proposed that during the transition, a single dose of inactivated poliovirus vaccine (IPV) may be adequate to prime global population due to the high cost and inadequate supply of IPV. However, the proposed phasing out of OPV to IPV warrants further research (Ryberg and Fiskar, 2016). Recent findings suggest that priming mice subcutaneously with single or even double dose of IPV results in decreasing IgG1 titres to background levels, 1 year after immunization (Lund et al., 2015; Xiao and Daniell, 2017). When compared to OPV, IPV does not induce adequate gastrointestinal mucosal immunity (Chan et al., 2016; Hird and Grassly, 2012). Furthermore, prolonged and exclusive use of IPV leads to silent polio outbreaks (Yaari et al., 2016). This is a major setback for eradication of both the wild-type- and vaccine-derived polio viruses. Therefore, several innovative strategies are discussed in the Global Polio Eradication Initiative (GPEI)

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Henry Daniell • University of Pennsylvania, USA; Plant Biotechnology Journal Founding Editor (2002–), Editor in Chief (2012–2022), Editor Emeritus (2023–)

The Daniell lab at the University of Pennsylvania, in collaboration with FDA and CDC laboratories, reported the first cold chain and virus free booster vaccine, expressed in lettuce chloroplasts, conferring immunity against all three polio virus serotypes (Chan *et. al.* 2016). This publication attracted global news coverage, and received one of the highest Altmetric scores of articles published in *Plant Biotechnology Journal (PBJ)*. The significance of the booster vaccine was further realized during the recent pandemic, when SARS-CoV-2 required several booster shots. Most importantly, the need for cold chain free vaccines became even more apparent when 20 million doses of COVID-19 vaccines were discarded in Africa due to inadequate cold chain systems. More recently, a SARS-CoV-2 antigen (virus spike protein) booster vaccine expressed in lettuce chloroplasts was reported in *PBJ* (Singh *et. al.* 2022). Most importantly, Angiotensin Converting Enzyme expressed in lettuce chloroplasts and embedded in a chewing gum was used successfully to trap SARS-Cov-2 in human saliva in order to prevent self-infection and transmission. This product is the first engineered protein in edible plant cells to receive FDA approval (IND 154807), free of cold chain or purification, and human clinical trials are now in progress (NCT 05433181). This publication (Daniell *et. al.* 2022) received an impressive Altmetric score (1833); >2 million Twitter exchanges in numerous global languages; and was featured in the public press including the National Geographic, Scientific American, WebMD and a TED talk.

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Plant Biotech Journal

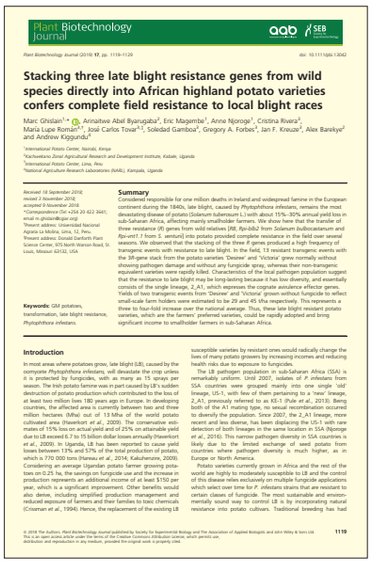
MARC GHISLAIN, ARINAITWE ABEL BYARUGABA, ERIC MAGEMBE, ANNE NJOROGE, CRISTINA RIVERA, MARÍA LUPE ROMÁN, JOSÉ CARLOS TOVAR, SOLEDAD GAMBOA, GREGORY A. FORBES, JAN F. KREUZE, ALEX BAREKYE, ANDREW KIGGUNDU

Stacking three late blight resistance genes from wild species directly into African highland potato varieties confers complete field resistance to local blight races

Plant Biotechnology Journal, 2019, 17(6), 1119–1129

• Read the full article here:
https://doi.org/10.1111/pbi.13042

Abstract Considered responsible for one million deaths in Ireland and widespread famine in the European continent during the 1840s, late blight, caused by *Phytophthora infestans*, remains the most devastating disease of potato (*Solanum tuberosum* L.) with about 15%–30% annual yield loss in sub-Saharan Africa, affecting mainly smallholder farmers. We show here that the transfer of three resistance (*R*) genes from wild relatives [*RB, Rpi-blb2* from *Solanum bulbocastanum* and *Rpi-vnt1.1* from *S. venturii*] into potato provided complete resistance in the field over several seasons. We observed that the stacking of the three *R* genes produced a high frequency of transgenic events with resistance to late blight. In the field, 13 resistant transgenic events with the 3*R*-gene stack from the potato varieties 'Desiree' and 'Victoria' grew normally without showing pathogen damage and without any fungicide spray, whereas their non-transgenic equivalent varieties were rapidly killed. Characteristics of the local pathogen population suggest that the resistance to late blight may be long-lasting because it has low diversity, and essentially consists of the single lineage, 2_A1, which expresses the cognate avirulence effector genes. Yields of two transgenic events from 'Desiree' and 'Victoria' grown without fungicide to reflect small-scale farm holders were estimated to be 29 and 45 t/ha respectively. This represents a three to four-fold increase over the national average. Thus, these late blight resistant potato varieties, which are the farmers' preferred varieties, could be rapidly adopted and bring significant income to smallholder farmers in sub-Saharan Africa



Plant Biotechnology Journal

Stacking three late blight resistance genes from wild species directly into African highland potato varieties confers complete field resistance to local blight races

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Summary
Considered responsible for one million deaths in Ireland and widespread famine in the European continent during the 1840s, late blight, caused by *Phytophthora infestans*, remains the most devastating disease of potato (*Solanum tuberosum* L.) with about 15%–30% annual yield loss in sub-Saharan Africa, affecting mainly smallholder farmers. We show here that the transfer of three resistance (*R*) genes from wild relatives [*RB, Rpi-blb2* from *Solanum bulbocastanum* and *Rpi-vnt1.1* from *S. venturii*] into potato provided complete resistance in the field over several seasons. We observed that the stacking of the three *R* genes produced a high frequency of transgenic events with resistance to late blight. In the field, 13 resistant transgenic events with the 3*R*-gene stack from the potato varieties 'Desiree' and 'Victoria' grew normally without showing pathogen damage and without any fungicide spray, whereas their non-transgenic equivalent varieties were rapidly killed. Characteristics of the local pathogen population suggest that the resistance to late blight may be long-lasting because it has low diversity, and essentially consists of the single lineage, 2_A1, which expresses the cognate avirulence effector genes. Yields of two transgenic events from 'Desiree' and 'Victoria' grown without fungicide to reflect small-scale farm holders were estimated to be 29 and 45 t/ha respectively. This represents a three to four-fold increase over the national average. Thus, these late blight resistant potato varieties, which are the farmers' preferred varieties, could be rapidly adopted and bring significant income to smallholder farmers in sub-Saharan Africa.

Introduction

Introduced by
Marc Ghislain • International Potato Center, Nairobi, Kenya

Defeating one of the oldest and most widespread crop diseases in a sustainable manner is a key milestone in plant disease history. The article by Ghislain *et al.* (2019), published in Plant Biotechnology Journal, reports the development of complete resistance to late blight disease in potato, building on about 45 years of research on plant-pathogen molecular interactions. This achievement is not entirely new; previous research advances were reported a decade ago (Jones *et al.* 2014; Haverkort *et al.* 2016). However, these innovations came at the wrong time and the wrong place. Europe's agricultural priorities had excluded genetic engineering for crop improvement long before a policy was adopted in 2022 to halve the use and risks of chemical pesticides in agriculture by 2030. In this article, the authors focused on those without access to modern agrochemicals for controlling the disease. Late blight disease, the cause of the 1840s Irish potato famine, remains widespread, affecting potato production worldwide. The sole effective control for this disease is to apply environmentally-damaging chemicals, such as copper-based fungicides used in organic farming, or modern chemicals used in conventional agriculture. However, a genetic engineering solution now exists, providing immunity-like protection which can be durable: something that has yet to be achieved by conventional breeding. Since Flor's ground-breaking hypothesis of the gene-for-gene interaction between rust fungus and flax (Flor, 1971), pathogen effector and host resistance genes have been discovered and characterized for numerous crop diseases. A judicious choice of three late blight disease resistance (*R*) genes and their stacking by genetic engineering into African farmers' preferred potato varieties is an inexpensive, sustainable innovation. Indeed, small-farm holders in underdeveloped regions suffer regular losses in potato production, threatening their already low income and food security. A rotation of stacks with different *R* genes will ensure the durability of resistance to late blight disease. This innovation will reach the African market through small-farm holders thanks to leadership by wise local political leaders and agriculture development sponsors. The future will tell us whether the innovation will hold its promises or whether the European anti-genetic engineering policies will have spread in Africa, preventing this innovation from realizing its promises.

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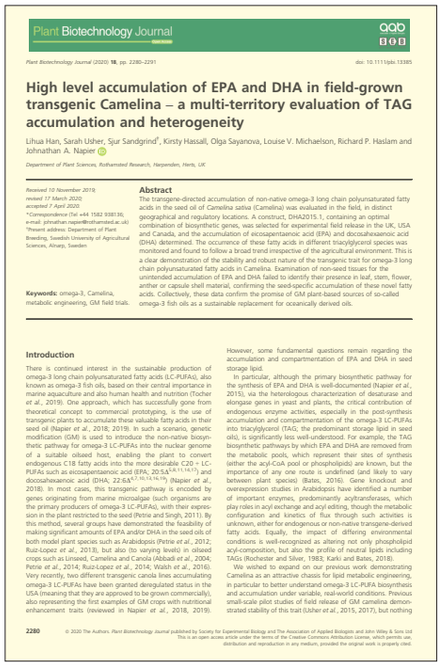
LIHUA HAN, SARAH USHER, SJUR SANDGRIND, KIRSTY HASSALL, OLGA SAYANOVA, LOUISE V. MICHAELSON, RICHARD P. HASLAM, JOHNATHAN A. NAPIER

High level accumulation of EPA and DHA in field-grown transgenic Camelina – a multi-territory evaluation of TAG accumulation and heterogeneity

Plant Biotechnology Journal, 2020, 18: 2280–2291

- Read the full article here: https://doi.org/10.1111/pbi.13385

Abstract The transgene-directed accumulation of non-native omega-3 long chain polyunsaturated fatty acids in the seed oil of *Camelina sativa* (Camelina) was evaluated in the field, in distinct geographical and regulatory locations. A construct, DHA2015.1, containing an optimal combination of biosynthetic genes, was selected for experimental field release in the UK, USA and Canada, and the accumulation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) determined. The occurrence of these fatty acids in different triacylglycerol species was monitored and found to follow a broad trend irrespective of the agricultural environment. This is a clear demonstration of the stability and robust nature of the transgenic trait for omega-3 long chain polyunsaturated fatty acids in Camelina. Examination of non-seed tissues for the unintended accumulation of EPA and DHA failed to identify their presence in leaf, stem, flower, anther or capsule shell material, confirming the seed-specific accumulation of these novel fatty acids. Collectively, these data confirm the promise of GM plant-based sources of so-called omega-3 fish oils as a sustainable replacement for oceanically derived oils.



Plant Biotechnology Journal

Plant Biotechnology Journal (2020) **18**, pp. 2280–2291 doi: 10.1111/pbi.13385

High level accumulation of EPA and DHA in field-grown transgenic Camelina – a multi-territory evaluation of TAG accumulation and heterogeneity

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Abstract
The transgene-directed accumulation of non-native omega-3 long chain polyunsaturated fatty acids in the seed oil of *Camelina sativa* (Camelina) was evaluated in the field, in distinct geographical and regulatory locations. A construct, DHA2015.1, containing an optimal combination of biosynthetic genes, was selected for experimental field release in the UK, USA and Canada, and the accumulation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) determined. The occurrence of these fatty acids in different triacylglycerol species was monitored and found to follow a broad trend irrespective of the agricultural environment. This is a clear demonstration of the stability and robust nature of the transgenic trait for omega-3 long chain polyunsaturated fatty acids in Camelina. Examination of non-seed tissues for the unintended accumulation of EPA and DHA failed to identify their presence in leaf, stem, flower, anther or capsule shell material, confirming the seed-specific accumulation of these novel fatty acids. Collectively, these data confirm the promise of GM plant-based sources of so-called omega-3 fish oils as a sustainable replacement for oceanically derived oils.

Keywords: omega-3, Camelina, metabolic engineering, GM field trials.

Introduction

There is continued interest in the sustainable production of omega-3 long chain polyunsaturated fatty acids (LC-PUFAs), also known as omega-3 fish oils, based on their central importance in marine aquaculture and also human health and nutrition (Tocher *et al.*, 2019). One approach, which has successfully gone from theoretical concept to commercial prototyping, is the use of transgenic plants to accumulate these valuable fatty acids in their seed oil (Napier *et al.*, 2018; 2019). In such a scenario, genetic modification (GM) is used to introduce the non-native biosynthetic pathway for omega-3 LC-PUFAs into the nuclear genome of a suitable oilseed host, enabling the plant to convert endogenous C18 fatty acids into the more desirable C20 + LC-PUFAs such as eicosapentaenoic acid (EPA; $20:5\Delta^{5,8,11,14,17}$) and docosahexaenoic acid (DHA; $22:6\Delta^{4,7,10,14,19}$) (Napier *et al.*, 2018). In most cases, this transgenic pathway is encoded by genes originating from marine microalgae (such organisms are the primary producers of omega-3 LC-PUFAs), with their expression in the plant restricted to the seed (Petrie and Singh, 2011). By this method, several groups have demonstrated the feasibility of making significant amounts of EPA and/or DHA in the seed oils of both model plant species such as Arabidopsis (Petrie *et al.*, 2012; Ruiz-Lopez *et al.*, 2013), but also (to varying levels) in oilseed crops such as Linseed, Camelina and Canola (Abbadi *et al.*, 2004; Petrie *et al.*, 2014; Ruiz-Lopez *et al.*, 2014; Walsh *et al.*, 2016). Very recently, two different transgenic canola lines accumulating omega-3 LC-PUFAs have been granted deregulated status in the USA (meaning that they are approved to be grown commercially), also representing the first examples of GM crops with nutritional enhancement traits (reviewed in Napier *et al.*, 2018, 2019). However, some fundamental questions remain regarding the accumulation and compartmentation of EPA and DHA in seed storage lipid.

In particular, although the primary biosynthetic pathway for the synthesis of EPA and DHA is well-documented (Napier *et al.*, 2015), via the heterologous characterization of desaturase and elongase genes in yeast and plants, the critical contribution of endogenous enzyme activities, especially in the post-synthesis accumulation and compartmentation of the omega-3 LC-PUFAs into triacylglycerol (TAG, the predominant storage lipid in seed oils), is significantly less well-understood. For example, the TAG biosynthetic pathways by which EPA and DHA are removed from the metabolic pools, which represent their sites of synthesis (either the acyl-CoA pool or phospholipids) are known, but the importance of any one route is undefined (and likely to vary between plant species) (Bates, 2016). Gene knockout and overexpression studies in Arabidopsis have identified a number of important enzymes, predominantly acyltransferases, which play roles in acyl exchange and acyl editing, though the metabolic configuration and kinetics of flux through such activities is unknown, either for endogenous or non-native transgene-derived fatty acids. Equally, the impact of differing environmental conditions is well-recognized as altering not only phospholipid acyl-composition, but also the profile of neutral lipids including TAGs (Rochester and Silver, 1983; Karki and Bates, 2018).

We wished to expand on our previous work demonstrating Camelina as an attractive chassis for lipid metabolic engineering, in particular to better understand omega-3 LC-PUFA biosynthesis and accumulation under variable, real-world conditions. Previous small-scale pilot studies of field release of GM camelina demonstrated stability of this trait (Usher *et al.*, 2015, 2017), but nothing

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Johnathan Napier • Rothamsted Research, Harpenden, UK; Editor in Chief, Plant Biotechnology Journal

Fish oils are a rich source of omega-3 long chain polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), whereas higher plants completely lack the capacity to synthesise these lipids. EPA and DHA are important components of a healthy diet, but vertebrates have a very limited capacity to make these fatty acids and need to obtain them from dietary sources. It is for this (counterintuitive) reason that most of the fish oils extracted from our oceans are used for aquaculture, since marine fish also lack the capacity to make EPA and DHA. However, growth in fish farming, driven in part by the ever-increasing global population, means that there is a pressing need for new sources of EPA and DHA that don't rely on further depleting our oceans (since most fish oil is harvested from pre-existing fish stocks). Over two decades ago, we and others proposed that transgenic oilseeds, engineered with the biosynthetic pathway for EPA and DHA, could represent a new sustainable source of omega-3 fish oils, and subsequently we demonstrated that Camelina was a superior host for the accumulation of omega-3 fish oils. But to validate this as being a *bona fide* solution to the lack of these lipids required real-world testing and field trialling in different locations. Here, we report data from GM field trials of Camelina in the UK, USA and Canada, showing that the EPA and DHA trait is stable irrespective of where the plants are grown and that the oil profile from our GM line is unaffected by the different environments. Collectively these data show the utility of our new plant-based source of omega-3 fish oils and represent a greener, sustainable path to better nutrition. •

Plant Direct

Science with Impact

Plant Direct

YI-HSUAN CHU, JYAN-CHYUN JANG, ZEJUN HUANG, ESTHER VAN DER KNAAP

Tomato locule number and fruit size controlled by natural alleles of lc and fas

Plant Direct, Volume 3, Issue 7, 2019, e00142

• Read the full article here: https://doi.org/10.1002/pld3.142

Abstract Improving yield by increasing the size of produce is an important selection criterion during the domestication of fruit and vegetable crops. Genes controlling meristem organization and organ formation work in concert to regulate the size of reproductive organs. In tomato, *lc* and *fas* control locule number, which often leads to enlarged fruits compared to the wild progenitors. *LC* is encoded by the tomato ortholog of WUSCHEL (WUS), whereas *FAS* is encoded by the tomato ortholog of CLAVATA3 (CLV3). The critical role of the WUS-CLV3 feedback loop in meristem organization has been demonstrated in several plant species. We show that mutant alleles for both loci in tomato led to an expansion of the *SlWUS* expression domain in young floral buds 2–3 days after initiation. Single and double mutant alleles of *lc* and *fas* maintain higher *SlWUS* expression during the development of the carpel primordia in the floral bud. This augmentation and altered spatial expression of *SlWUS* provided a mechanistic basis for the formation of multilocular and large fruits. Our results indicated that *lc* and *fas* are gain-of-function and partially loss-of-function alleles, respectively, while both mutations positively affect the size of tomato floral meristems. In addition, expression profiling showed that *lc* and *fas* affected the expression of several genes in biological processes including those involved in meristem/flower development, patterning, microtubule binding activity, and sterol biosynthesis. Several differentially expressed genes co-expressed with *SlWUS* have been identified, and they are enriched for functions in meristem regulation. Our results provide new insights into the transcriptional regulation of genes that modulate meristem maintenance and floral organ determinacy in tomato.

Introduced by **Björn Usadel** • IBG-4 Bioinformatics, Forschungszentrum Jülich, Germany; Institute for Biological Data Science, Heinrich Heine University, Germany.

Domestication has played a pivotal role in shaping the characteristics of modern tomato varieties, including fruit size. Wild tomatoes have small, marble-sized fruits. However, through selective breeding over millennia, humans have substantially increased the size of tomato fruits. The dimensions and shape of tomatoes, in part, hinge on the locule number, referring to the individual compartments within a tomato fruit that house the seeds. Notably, many wild and small tomato varieties exhibit fewer locules compared to the majority of tomatoes we consume today (Li *et. al.* 2017).

Two quantitative trait loci (QTLs), namely *fasciated (fas)* and *locule number (lc)*, explain a significant portion of the variation in locule number. The genetic basis of *fas* centers around a chromosomal inversion spanning 294 kilobases encompassing the *CLAVATA3* gene (Huang & van der Knaap, 2011). The *lc* QTL on the other hand was shown to be located at the 3' end of *WUSCHEL* (Muños *et. al.* 2011). Both WUSCHEL and CLAVATA3 are integral components of a conserved feedback loop (Schoof *et. al.* 2000), in which WUSCHEL triggers the expression of CLAVATA3, which subsequently represses WUSCHEL. This evolutionarily conserved loop has played a pivotal role in enhancing yields across several crop species.

Given that both loci are part of this loop, it is not surprising that there is interaction between the two underlying QTLs. Chu *et. al.* (2019) set out to clarify the role of these QTLs and to disentangle their regulation. By introgressing the *fas* and *lc* alleles into the small-fruited *Solanum pimpinellifolium*, they demonstrated that only the double mutation had a significant impact on tomato fruit weight, while *fas* alone increased tomato fruit area. Furthermore, Chu *et. al.* could show that both mutations led to an expansion of the domain where *WUSCHEL* is expressed in young floral buds and concluded that consequently *lc* is a gain of function mutation, whereas *fas* represents a partial loss-of-function mutation.

To delve deeper into the regulatory mechanisms, Chu *et. al.* conducted an extensive analysis of gene expression in the mutants across various developmental stages. They identified many hundred genes showing a significant genotypic effect, but only 13 genes with a significant genotype by development interaction effect. Notably, this set of 13 genes included *WUSCHEL* and *CLAVATA* as well as an ERF/AP2 transcription factor (Solyc03g117230) which was subsequently shown to be the underlying gene responsible for the *excessive number of floral organs (eno)* mutation, which also shows larger, multilocular fruits (Yuste-Lisbona *et. al.* 2020).

In conclusion, the interaction between the mutants *fas* and *lc* within the regulatory loop involving WUSCHEL and CLAVATA3 plays a pivotal role in shaping tomato fruit size, and Chu *et. al.* have shed light on the underlying genetic regulation.

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Science with Impact

Plant Direct

NATALIE A. HENKHAUS, WOLFGANG BUSCH, ANGELA CHEN, ADÁN COLÓN-CARMONA, MAYA COTHRAN, NICOLAS DIAZ, JOSE PABLO DUNDORE-ARIAS, MICHAEL GONZALES, DENITA HADZIABDIC, REBECCA A. HAYES, GUSTAVO C. MACINTOSH, ALI NA, BLESSING NYAMASOKA-MAGONZIWA, DIANNE PATER, F. CHRISTOPHER PERITORE-GALVE, TARA PHELPS-DURR, KERRY ROUHIER, DELANIE B. SICKLER, JOHN H. STARNES, QUENTIN R. TYLER, EVELYN VALDEZ-WARD, MIGUEL E. VEGA-SÁNCHEZ, RON R. WALCOTT, JOY K. WARD, SARAH E. WYATT, FELIPE ZAPATA, ASH T. ZEMENICK, DAVID B. STERN

Removing systemic barriers to equity, diversity, and inclusion: Report of the 2019 Plant Science Research Network workshop "Inclusivity in the Plant Sciences"

Plant Direct, Volume 6, Issue 8, 2022, e432.

• Read the full article here:
https://doi.org/10.1002/pld3.432

Abstract A future in which scientific discoveries are valued and trusted by the general public cannot be achieved without greater inclusion and participation of diverse communities. To envision a path towards this future, in January 2019 a diverse group of researchers, educators, students, and administrators gathered to hear and share personal perspectives on equity, diversity, and inclusion (EDI) in the plant sciences. From these broad perspectives, the group developed strategies and identified tactics to facilitate and support EDI within and beyond the plant science community. The workshop leveraged scenario planning and the richness of its participants to develop recommendations aimed at promoting systemic change at the institutional level through the actions of scientific societies, universities, and individuals and through new funding models to support research and training. While these initiatives were formulated specifically for the plant science community, they can also serve as a model to advance EDI in other disciplines. The proposed actions are thematically broad, integrating into discovery, applied and translational science, requiring and embracing multidisciplinarity, and giving voice to previously unheard perspectives. We offer a vision of barrier-free access to participation in science, and a plant science community that reflects the diversity of our rapidly changing nation, and supports and invests in the training and well-being of all its members. The relevance and robustness of our recommendations has been tested by dramatic and global events since the workshop. The time to act upon them is now.

Introduced by
Natalie Henkhaus • Bio-Rad Laboratories Inc

This report published last year shares recommendations for promoting systemic change to allow barrier-free access to participation in science. This outcome would transpire through the actions of scientific societies, universities, individuals, and new funding models to support research and training. The recommendations were developed by the Plant Science Research Network (PSRN) (plantae.org/education/PSRN), a research coordination network funded by the National Science Foundation from 2015-2021. While these initiatives were formulated specifically for the plant science community, they can also serve as a model to advance equity, diversity, and inclusion in other disciplines.

The specific recommendations aim to increase visibility of diverse participants by promoting early STEM exposure and improving retention of diverse scientists. Among the recommendations are actions to improve inclusive and equitable mentoring, improved transparency of diverse career opportunities, and fostering representation in positions of leadership and influence. Recommendations include early STEM exposure, relating to others through storytelling of personal narratives, broadening career opportunities through shifting mentorship norms, and calling out barriers to participation ingrained in our institutional structures and practices.

Addressing systemic issues is not simple. What is most important is to recognize these barriers for what they are: facets of a system long designed and operated by a narrow demographic group, and therefore a system that must reinvent itself if it wishes to embrace diversity. People in positions of influence have an additional responsibility of learning to recognize those behaviors and biases and to seek positive paths for change through reward rather than punishment. Read the full article to learn more about how you can help to drive meaningful change in your organization.

Science with Impact
100 YEARS 1923 - 2023

Conservation Physiology

Conservation Physiology

STEVEN J. COOKE, LAWREN SACK, CRAIG E. FRANKLIN, ANTHONY P. FARRELL, JOHN BEARDALL, MARTIN WIKELSKI, STEVEN L. CHOWN

What is conservation physiology? Perspectives on an increasingly integrated and essential science

Conservation Physiology, Volume 1, Issue 1, 2013, cot001

- Read the full article here: https://doi.org/10.1093/conphys/cot001

Abstract Globally, ecosystems and their constituent flora and fauna face the localized and broad-scale influence of human activities. Conservation practitioners and environmental managers struggle to identify and mitigate threats, reverse species declines, restore degraded ecosystems, and manage natural resources sustainably. Scientific research and evidence are increasingly regarded as the foundation for new regulations, conservation actions, and management interventions. Conservation biologists and managers have traditionally focused on the characteristics (e.g. abundance, structure, trends) of populations, species, communities, and ecosystems, and simple indicators of the responses to environmental perturbations and other human activities. However, an understanding of the specific mechanisms underlying conservation problems is becoming increasingly important for decision-making, in part because physiological tools and knowledge are especially useful for developing cause-and-effect relationships, and for identifying the optimal range of habitats and stressor thresholds for different organisms. When physiological knowledge is incorporated into ecological models, it can improve predictions of organism responses to environmental change and provide tools to support management decisions. Without such knowledge, we may be left with simple associations. 'Conservation physiology' has been defined previously with a focus on vertebrates, but here we redefine the concept universally, for application to the diversity of taxa from microbes to plants, to animals, and to natural resources. We also consider 'physiology' in the broadest possible terms; i.e. how an organism functions, and any associated mechanisms, from development to bioenergetics, to environmental interactions, through to fitness. Moreover, we consider conservation physiology to include a wide range of applications beyond assisting imperiled populations, and include, for example, the eradication of invasive species, refinement of resource management strategies to minimize impacts, and evaluation of restoration plans. This concept of conservation physiology emphasizes the basis, importance, and ecological relevance of physiological diversity at a variety of scales. Real advances in conservation and resource management require integration and inter-disciplinarity. Conservation physiology and its suite of tools and concepts is a key part of the evidence base needed to address pressing environmental challenges.

Conservation Physiology Volume 1 • 2013 10.1093/conphys/cot001 SEB Society for Experimental Biology

Perspective

What is conservation physiology? Perspectives on an increasingly integrated and essential science†

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† Inaugural paper for *Conservation Physiology*.

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Globally, ecosystems and their constituent flora and fauna face the localized and broad-scale influence of human activities. Conservation practitioners and environmental managers struggle to identify and mitigate threats, reverse species declines, restore degraded ecosystems, and manage natural resources sustainably. Scientific research and evidence are increasingly regarded as the foundation for new regulations, conservation actions, and management interventions. Conservation biologists and managers have traditionally focused on the characteristics (e.g. abundance, structure, trends) of populations, species, communities, and ecosystems, and simple indicators of the responses to environmental perturbations and other human activities. However, an understanding of the specific mechanisms underlying conservation problems is becoming increasingly important for decision-making, in part because physiological tools and knowledge are especially useful for developing cause-and-effect relationships, and for identifying the optimal range of habitats and stressor thresholds for different organisms. When physiological knowledge is incorporated into ecological models, it can improve predictions of organism responses to environmental change and provide tools to support management decisions. Without such knowledge, we may be left with simple associations. 'Conservation physiology' has been defined previously with a focus on vertebrates, but here we redefine the concept universally, for application to the diversity of taxa from microbes to plants, to animals, and to natural resources. We also consider 'physiology' in the broadest possible terms; i.e. how an organism functions, and any associated mechanisms, from development to bioenergetics, to environmental interactions, through to fitness. Moreover, we consider conservation physiology to include a wide range of applications beyond assisting imperiled populations, and include, for example, the eradication of invasive species, refinement of resource management strategies to minimize impacts, and evaluation of restoration plans. This concept of conservation physiology emphasizes the basis, importance, and ecological relevance of physiological diversity at a variety of scales. Real advances in conservation and resource management require integration and inter-disciplinarity. Conservation physiology and its suite of tools and concepts is a key part of the evidence base needed to address pressing environmental challenges.

Key words: Conservation physiology, conservation science, environment, mechanisms, resource management

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Introduced by
Steven J. Cooke • Carleton University, Canada; Editor in Chief, Conservation Physiology

When the journal *Conservation Physiology* was launched in 2013, the first task was to provide a clear signal to potential contributors what was meant by the term "conservation physiology", and therefore, what defined the remit of the journal. Building from several earlier definitional papers, we revisited the concept and provided what has become "the" definition. The paper remains one of the most highly read and cited in the history of the journal, with over 400 citations. Notably, it has been assigned as study material for PhD doctoral exams and as course material for undergraduate courses in conservation physiology. •

STEVEN J COOKE, JORDANNA N BERGMAN, CHRISTINE L MADLIGER, REBECCA L CRAMP, JOHN BEARDALL, GARY BURNESS, TIMOTHY D CLARK, BEN DANTZER, ERICK DE LA BARRERA, NANN A FANGUE, CRAIG E FRANKLIN, ANDREA FULLER, LUCY A HAWKES, KEVIN R HULTINE, KATHLEEN E HUNT, OLIVER P LOVE, HEATH A MACMILLAN, JOHN W MANDELMAN, FELIX C MARK, LYNN B MARTIN, AMY E M NEWMAN, ADRIENNE B NICOTRA, GRAHAM D RABY, SHARON A ROBINSON, YAN ROPERT-COUDERT, JODIE L RUMMER, FRANK SEEBACHER, ANNE E TODGHAM, SEAN TOMLINSON, STEVEN L CHOWN

One hundred research questions in conservation physiology for generating actionable evidence to inform conservation policy and practice

Conservation Physiology, Volume 9, Issue 1, 2021, coab009

- Read the full article here: https://doi.org/10.1093/conphys/coab009


Conservation Physiology
Volume 9 • 2021
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Perspective
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Abstract Environmental change and biodiversity loss are but two of the complex challenges facing conservation practitioners and policy makers. Relevant and robust scientific knowledge is critical for providing decision-makers with the actionable evidence needed to inform conservation decisions. In the Anthropocene, science that leads to meaningful improvements in biodiversity conservation, restoration and management is desperately needed. Conservation Physiology has emerged as a discipline that is well-positioned to identify the mechanisms underpinning population declines, predict responses to environmental change and test different *in situ* and *ex situ* conservation interventions for diverse taxa and ecosystems. Here we present a consensus list of 10 priority research themes. Within each theme we identify specific research questions (100 in total), answers to which will address conservation problems and should improve the management of biological resources. The themes frame a set of research questions related to the following: (i) adaptation and phenotypic plasticity; (ii) human–induced environmental change; (iii) human–wildlife interactions; (iv) invasive species; (v) methods, biomarkers and monitoring; (vi) policy, engagement and communication; (vii) pollution; (viii) restoration actions; (ix) threatened species; and (x) urban systems. The themes and questions will hopefully guide and inspire researchers while also helping to demonstrate to practitioners and policy makers the many ways in which physiology can help to support their decisions.

Introduced by

Steven J. Cooke • Carleton University, Canada; Editor in Chief, Conservation Physiology

Taking pause to conduct a horizon scan where one reflects on current knowledge and considers future needs can help to guide researchers. For that reason, the Editorial Board of *Conservation Physiology* did just that with a focus on identifying research questions that, if answered, would meaningfully benefit biodiversity conservation and the sustainable management of biological resources. Each of the one hundred questions identified could easily be the basis for a PhD thesis or even an entire career. The paper was intended to inspire individuals, especially early career scholars, to consider how their skills could be leveraged and applied to diverse conservation challenges. While it is difficult to quantify the impact of such a paper, anecdotal comments from early career researchers has revealed that the paper is having its desired goal of providing ideas on how physiological tools, concepts and knowledge can be applied to complex (or seemingly simple) conservation problems.

Science with Impact

Conservation Physiology

DENNIS D.U. HEINRICH, JODIE L. RUMMER, ANDREA J. MORASH, SUE-ANN WATSON, COLIN A. SIMPFENDORFER, MICHELLE R. HEUPEL, PHILIP L. MUNDAY

A product of its environment: the epaulette shark *(Hemiscyllium ocellatum)* exhibits physiological tolerance to elevated environmental CO_2

Conservation Physiology, Volume 2, Issue 1, 2014, cou047

• Read the full article here: https://doi.org/10.1093/conphys/cou047

Abstract Ocean acidification, resulting from increasing anthropogenic CO_2 emissions, is predicted to affect the physiological performance of many marine species. Recent studies have shown substantial reductions in aerobic performance in some teleost fish species, but no change or even enhanced performance in others. Notably lacking, however, are studies on the effects of near-future CO_2 conditions on larger meso and apex predators, such as elasmobranchs. The epaulette shark (*Hemiscyllium ocellatum*) lives on shallow coral reef flats and in lagoons, where it may frequently encounter short-term periods of environmental hypoxia and elevated CO_2, especially during nocturnal low tides. Indeed, *H. ocellatum* is remarkably tolerant to short periods (hours) of hypoxia, and possibly hypercapnia, but nothing is known about its response to prolonged exposure. We exposed *H. ocellatum* individuals to control (390 μatm) or one of two near-future CO_2 treatments (600 or 880 μatm) for a minimum of 60 days and then measured key aspects of their respiratory physiology, namely the resting oxygen consumption rate, which is used to estimate resting metabolic rate, and critical oxygen tension, a proxy for hypoxia sensitivity. Neither of these respiratory attributes was affected by the long-term exposure to elevated CO_2. Furthermore, there was no change in citrate synthase activity, a cellular indicator of aerobic energy production. Plasma bicarbonate concentrations were significantly elevated in sharks exposed to 600 and 880 μatm CO_2 treatments, indicating that acidosis was probably prevented by regulatory changes in acid–base relevant ions. Epaulette sharks may therefore possess adaptations that confer tolerance to CO_2 levels projected to occur in the ocean by the end of this century. It remains uncertain whether other elasmobranchs, especially pelagic species that do not experience such diurnal fluctuations in their environment, will be equally tolerant.

Introduced by
Jodie L. Rummer • James Cook University, Australia

Our team started researching epaulette sharks (*Hemiscyllium ocellatum*) in 2012, given that this small, shallow-water, tropical species is amenable to both captivity and controlled laboratory experiments and can be easily accessed in the field, where they are endemic to the waters of the Great Barrier Reef. Since then, our research program has expanded to include all life stages of this oviparous species, a captive breeding colony at our James Cook University research facility, field studies, an array of anthropogenic stressors, and multidisciplinary approaches involving collaborators worldwide, all of which has contributed to myriad graduate students' degrees and accolades.

Our first paper from our research program, which was published in *Conservation Physiology*, represents the early stages of our research to understand how epaulette sharks respond to climate change stressors, specifically elevated CO_2 simulating future ocean acidification conditions. We found that these small, benthic, walking sharks are a product of their environment; that is, they are likely robust to elevated CO_2 conditions because they experience it every night when the corals, algae, and other benthic organisms switch from photosynthesis to respiration, which naturally creates low oxygen, high CO_2 conditions (Heinrich *et al.* 2014). This study helped us to identify some key physiological traits that, perhaps, other small shark species may need to tolerate such conditions, and led us to also examine embryonic and juvenile life stages (Johnson *et al.* 2016) and to test fitness related behaviours, such as foraging and sheltering (Heinrich *et al.* 2016), which are also unaffected by elevated CO_2.

Our later work focusses on the effects of elevated temperatures on epaulette sharks because of the extremes that they already face in their natural habitats. We showed that, when epaulette shark embryos are reared *in ovo* at elevated temperatures (e.g., 31°C), they hatch 24 days earlier, are smaller, exhibit a reduced aerobic metabolic scope, utilise stored energy (i.e. yolk) much faster, require longer to recover from exercise (Wheeler *et al.* 2021), and display altered colouration and patterns (Gervais *et al.* 2016) As charismatic organisms with an underlying societal and cultural value and concrete evidence of impacts related to climate change factors, this species, and sharks in general, represent a great flagship for public engagement in the climate change crisis and an umbrella for the conservation of marine biodiversity in a rapidly changing future.

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BEN DANTZER, QUINN E. FLETCHER, RUDY BOONSTRA, MICHAEL J. SHERIFF

Measures of physiological stress: a transparent or opaque window into the status, management, and conservation of a species

Conservation Physiology, 2(1), 2014, cou023

• Read the full article here:
https://doi.org/10.1093/conphys/cou023

Abstract Conservation physiology proposes that measures of physiological stress (glucocorticoid levels) can be used to assess the status and future fate of natural populations. Increases in glucocorticoids may reflect a more challenging environment, suggesting that the influence of human activities on free-living animals could be quantified by measuring glucocorticoids. Biomedical studies suggest that chronic increases in glucocorticoids can have detrimental effects on survival and reproduction, which could influence the viability of populations. Here, we discuss the use of measurements of glucocorticoids in conservation physiology. We first provide an overview of the different methods to quantify glucocorticoids and their utility in conservation physiology. We then discuss five questions we think are essential for conservation physiologists to address. We highlight how intrinsic (e.g. sex, reproductive status, age, recent experiences) and ecological factors (e.g. predation, food availability, snowfall) can, by themselves or through their interactions with anthropogenic disturbances, affect the physiological stress response and mask any general patterns about the effects of anthropogenic disturbances on glucocorticoids. Using a meta-analysis, we show that anthropogenic disturbances are consistently associated with increased glucocorticoids regardless of the type of human disturbance. We also show that males may be more sensitive to anthropogenic disturbances than females and that faecal glucocorticoids, but not baseline plasma glucocorticoids, consistently increase in response to anthropogenic disturbances. Finally, we discuss how increases in glucocorticoids in free-living animals can sometimes enhance survival and reproduction. Unfortunately, our literature analysis indicates that this observation has not yet gained traction, and very few studies have shown that increases in glucocorticoid levels resulting from anthropogenic disturbances decrease survival or reproduction. We think that the use of measures of glucocorticoids in conservation physiology has tremendous potential, but there are still a number of methodological concerns, in addition to several crucial questions that should be addressed.

Introduced by
Rudy Boonstra & Ben Dantzer • University of Toronto Scarborough, Canada & University of Michigan, USA

Many animal populations are under enormous pressure and are declining because of unremitting human-induced changes to their habitat; direct human environmental impacts such as hunting, noise, plastics and artificial chemicals; and climate change. Our paper asks relatively simple questions: can we quantify how animals are responding to these pressures by measurements of a key integrator of environmental change: the stress axis; do these changes affect animal survival and reproduction; and can we use this evidence to aid in their conservation?

The stress axis plays a central role in allowing animals to respond to environmental stressors, whether biotic or abiotic. It is one of the key physiological systems that mediate the relationship of the organism to its environment, in terms of both short-term responses and long-term evolutionary responses to particular ecological and environmental challenges. In this review, we summarize the enormous amount of previous research which aimed to quantify anthropogenic impacts on the stress axis of animals using corticosteroid levels in plasma and feces. Our conclusions: 1. human impacts increase corticosteroid levels regardless of the nature of the disturbance; 2. males are more sensitive to stress than females; and 3. fecal corticosteroid levels are a better indicator of stress than baseline plasma corticosteroid levels. However, the link between these increased corticosteroid levels and decreased survival and reproduction is not clear. This is key and may partly be related to a lack of simultaneous quantification of detailed demography. In addition, it is critical to perform comparative research on wild, natural populations versus those impacted by humans.

Our paper has had a major impact, having been cited 415 times (Google Scholar) across the gamut of vertebrates, from fish to mammals. It thus serves as a benchmark for where the field was ten years ago, and as an incentive to stimulate further rigorous research on the impacts of anthropogenic stressors. Since the review was published, further work has shown that noise from natural gas fields increases the corticosterone response and reduces fitness in three bird species (Kleist *et al*. 2018). The work has additionally inspired several quantitative meta-analyses examining the impacts of human activities on the stress axis (for example Iglesias-Carrasco *et al*. 2020; Injaian *et al*. 2020), updating our original meta-analysis with new research. Importantly, we posed the question of how measures of stress axis activity might be applied to wildlife conservation. Studies such as Madliger *et al*. (2018) and Jerem & Matthews (2021) have since considered this aspect, showing that our conclusions have informed conservation management.

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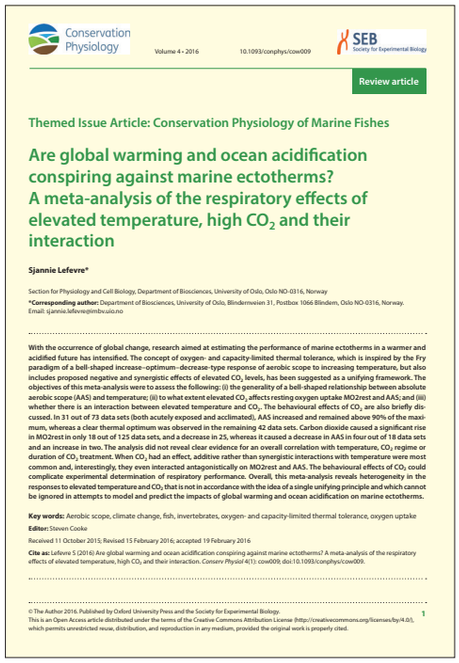
SJANNIE LEFEVRE

Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO_2 and their interaction

Conservation Physiology, Volume 4, Issue 1, 2016, cow009

- Read the full article here: https://doi.org/10.1093/conphys/cow009

Abstract With the occurrence of global change, research aimed at estimating the performance of marine ectotherms in a warmer and acidified future has intensified. The concept of oxygen- and capacity-limited thermal tolerance, which is inspired by the Fry paradigm of a bell-shaped increase–optimum–decrease-type response of aerobic scope to increasing temperature, but also includes proposed negative and synergistic effects of elevated CO_2 levels, has been suggested as a unifying framework. The objectives of this meta-analysis were to assess the following: (i) the generality of a bell-shaped relationship between absolute aerobic scope (AAS) and temperature; (ii) to what extent elevated CO_2 affects resting oxygen uptake MO2rest and AAS; and (iii) whether there is an interaction between elevated temperature and CO_2. The behavioural effects of CO_2 are also briefly discussed. In 31 out of 73 data sets (both acutely exposed and acclimated), AAS increased and remained above 90% of the maximum, whereas a clear thermal optimum was observed in the remaining 42 data sets. Carbon dioxide caused a significant rise in MO2rest in only 18 out of 125 data sets, and a decrease in 25, whereas it caused a decrease in AAS in four out of 18 data sets and an increase in two. The analysis did not reveal clear evidence for an overall correlation with temperature, CO_2 regime or duration of CO_2 treatment. When CO_2 had an effect, additive rather than synergistic interactions with temperature were most common and, interestingly, they even interacted antagonistically on MO2rest and AAS. The behavioural effects of CO_2 could complicate experimental determination of respiratory performance. Overall, this meta-analysis reveals heterogeneity in the responses to elevated temperature and CO_2 that is not in accordance with the idea of a single unifying principle and which cannot be ignored in attempts to model and predict the impacts of global warming and ocean acidification on marine ectotherms.


Conservation Physiology Volume 4 • 2016 10.1093/conphys/cow009 SEB Society for Experimental Biology

Review article

Themed Issue Article: Conservation Physiology of Marine Fishes

Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO_2 and their interaction

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With the occurrence of global change, research aimed at estimating the performance of marine ectotherms in a warmer and acidified future has intensified. The concept of oxygen- and capacity-limited thermal tolerance, which is inspired by the Fry paradigm of a bell-shaped increase–optimum–decrease-type response of aerobic scope to increasing temperature, but also includes proposed negative and synergistic effects of elevated CO_2 levels, has been suggested as a unifying framework. The objectives of this meta-analysis were to assess the following: (i) the generality of a bell-shaped relationship between absolute aerobic scope (AAS) and temperature; (ii) to what extent elevated CO_2 affects resting oxygen uptake MO2rest and AAS; and (iii) whether there is an interaction between elevated temperature and CO_2. The behavioural effects of CO_2 are also briefly discussed. In 31 out of 73 data sets (both acutely exposed and acclimated), AAS increased and remained above 90% of the maximum, whereas a clear thermal optimum was observed in the remaining 42 data sets. Carbon dioxide caused a significant rise in MO2rest in only 18 out of 125 data sets, and a decrease in 25, whereas it caused a decrease in AAS in four out of 18 data sets and an increase in two. The analysis did not reveal clear evidence for an overall correlation with temperature, CO_2 regime or duration of CO_2 treatment. When CO_2 had an effect, additive rather than synergistic interactions with temperature were most common and, interestingly, they even interacted antagonistically on MO2rest and AAS. The behavioural effects of CO_2 could complicate experimental determination of respiratory performance. Overall, this meta-analysis reveals heterogeneity in the responses to elevated temperature and CO_2 that is not in accordance with the idea of a single unifying principle and which cannot be ignored in attempts to model and predict the impacts of global warming and ocean acidification on marine ectotherms.

Key words: Aerobic scope, climate change, fish, invertebrates, oxygen- and capacity-limited thermal tolerance, oxygen uptake

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Introduced by **Sjannie Lefevre** • University of Oslo, Norway

The concept of oxygen- and capacity-limited thermal tolerance (OCLTT) is rooted in the Fry paradigm of a bell-shaped thermal performance curve for aerobic scope, but also includes proposed negative and synergistic effects of elevated CO_2 levels. Two basic predictions of the concept warranted investigation. One was whether data generally supported the notion that most ectotherms show a clear increase and then decrease for aerobic scope within the ecologically relevant temperature range. The other was whether data confirmed the proposed synergistic effects of elevated CO_2 and warming - are these two climate-change villains indeed conspiring against marine ectotherms? Lefevre (2016) set out to investigate these questions using an explorative, meta-analytical approach. From the comprehensive literature review and data exploration it was clear that for many species, aerobic scope would continue to increase until very close to critical temperatures and well beyond any ecologically relevant temperatures. This indicates that simply looking at aerobic scope for many species will not be useful in terms of predicting what will happen in a climate change warming scenario. The second main finding was that overall, there were few cases where elevated CO_2 seemed to exacerbate effects of warming, and responses appeared to be largely species-specific. Taken together, the study served as an important reminder that generalisation across species should be made with caution, and that aerobic scope alone may not be sufficient to predict wider effects on fitness and hence changes in population distributions in response to climate change. The paper continues to be actively used by the community, serving as a reference for studies investigating aerobic scope responses, as these continue to accumulate, or as a reference for the debate around OCLTT and how to predict the effects of future warming. •

Science with Impact

Conservation Physiology

DAVID A. PATTERSON, STEVEN J. COOKE, SCOTT G. HINCH, KENDRA A. ROBINSON, NATHAN YOUNG, ANTHONY P. FARRELL, KRISTINA M. MILLER

A perspective on physiological studies supporting the provision of scientific advice for the management of Fraser River sockeye salmon (*Oncorhynchus nerka*)

Conservation Physiology, Volume 4, Issue 1, 2016, cow026

• Read the full article here: https://doi.org/10.1093/conphys/cow026

Abstract The inability of physiologists to effect change in fisheries management has been the source of frustration for many decades. Close collaboration between fisheries managers and researchers has afforded our interdisciplinary team an unusual opportunity to evaluate the emerging impact that physiology can have in providing relevant and credible scientific advice to assist in management decisions. We categorize the quality of scientific advice given to management into five levels based on the type of scientific activity and resulting advice (notions, observations, descriptions, predictions and prescriptions). We argue that, ideally, both managers and researchers have concomitant but separate responsibilities for increasing the level of scientific advice provided. The responsibility of managers involves clear communication of management objectives to researchers, including exact descriptions of knowledge needs and researchable problems. The role of the researcher is to provide scientific advice based on the current state of scientific information and the level of integration with management. The examples of scientific advice discussed herein relate to physiological research on the impact of high discharge and water temperature, pathogens, sex and fisheries interactions on in-river migration success of adult Fraser River sockeye salmon (*Oncorhynchus nerka*) and the increased understanding and quality of scientific advice that emerges. We submit that success in increasing the quality of scientific advice is a function of political motivation linked to funding, legal clarity in management objectives, collaborative structures in government and academia, personal relationships, access to interdisciplinary experts and scientific peer acceptance. The major challenges with advancing scientific advice include uncertainty in results, lack of integration with management needs and institutional caution in adopting new research. We hope that conservation physiologists can learn from our experiences of providing scientific advice to management to increase the potential for this growing field of research to have a positive influence on resource management.

Introduced by
David Patterson • Fisheries and Oceans Canada, Simon Fraser University, Canada

Conservation physiologists have often struggled to effect change within the management systems they work in. Resource managers continue to ask researchers how they can incorporate environmental and biological research into their best management practices and decisions. Patterson *et al.* (2016) deconstructed this problem from both a science and management perspective, identifying major challenges and positive conditions to advancing scientific advice based on physiological research. The basis of this perspective was an evaluation of the 100 years of scientific advice given to, and used by, management regarding key factors influencing upstream adult migration survival of the iconic Fraser River sockeye salmon (*Oncorhynchus nerka*). This work highlighted that success was a joint responsibility driven by organic and formal collaborative structures between management and researchers, and that failure was often linked to unclear management objectives and scientific uncertainty. The authors of this work have continued to make incremental advancements in their scientific understanding in all four main research areas covered in the paper: discharge and temperature impacts (Cooke *et al.* 2021), fishing interactions (Patterson *et al.* 2017), sex differences (Hinch *et al.* 2021), and the role of pathogens (Teffer *et al.* 2022). However, progress on integration with management has been slow, and the authors are still facing a list of expected challenges that include institutional inertia to change, lack of direct connection to current management objectives, and limited explanatory power of the predictions. In addition, there are novel challenges, such as changes in the management objectives, and the interjection of user groups into management decisions and research as the implications of the scientific advice become more apparent. These novel challenges have led to changes in research direction, such as a renewed focus on social science research (e.g. Nguyen *et al.* 2019) and a switch from strictly supporting regulatory changes to more emphasis on educational or best practices approaches (e.g. Cook *et al.* 2019). Overall, the lessons learned from this original paper are still relevant today; there are several paths, but no quick fixes, to ensuring your best scientific advice is being used effectively by management.

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SHAHRINA CHOWDHURY, JANINE BROWN, LARISSA SWEDELL

Anthropogenic effects on the physiology and behaviour of chacma baboons in the Cape Peninsula of South Africa

Conservation Physiology, Volume 8, Issue 1, 2020, coaa066

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Conservation Physiology Volume 8 • 2020 10.1093/conphys/coaa066

Research article

Anthropogenic effects on the physiology and behaviour of chacma baboons in the Cape Peninsula of South Africa

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As animals increasingly occupy habitats in proximity to humans, with home ranges a mosaic of natural and anthropogenic landscapes, it becomes imperative from a conservation perspective to understand the impacts of human activities on wildlife. Many non-human primates share habitats with humans, an ability stemming largely from shared ecological needs due to our close evolutionary relationship. Such proximity, however, is often accompanied by direct conflict between humans and wildlife, leading to higher stress levels, injuries, mortality and behavioural changes, with detrimental effects on long-term health and fitness. Glucocorticoid (GC) hormones, which are released in response to ecological and social challenges, are increasingly employed to understand responses to anthropogenic disturbance. Here we investigate physiological and behavioural responses of female chacma baboons (*Papio ursinus*) to variation in spatial overlap and conflict with humans in their natural home range. The baboons resided in the Tokai Section of Table Mountain National Park in the Cape Peninsula of South Africa, where their home range included four types of habitats: protected, suburban, agricultural and mosaic areas. We studied the largest group of baboons in Tokai (70 individuals) and examined the effects of ranging in these different habitats on the faecal GCs and behaviour of all adult females in the troop (N = 16). We found time spent ranging in more anthropogenic habitats to be associated with higher levels of GCs, more aggression, less time socializing and shorter grooming bouts. Self-directed behaviour, however, varied and did not necessarily reflect physiological measures of stress. Taken together, the results of this study highlight the risks associated with ranging in anthropogenic environments and point to the need for a multifaceted approach to studying the negative impacts of human activities on animals so as to better inform conservation practices.

Key words: anthropogenic disturbance, glucocorticoids, self-directed behavior, stress, baboon

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Abstract As animals increasingly occupy habitats in proximity to humans, with home ranges a mosaic of natural and anthropogenic landscapes, it becomes imperative from a conservation perspective to understand the impacts of human activities on wildlife. Many non-human primates share habitats with humans, an ability stemming largely from shared ecological needs due to our close evolutionary relationship. Such proximity, however, is often accompanied by direct conflict between humans and wildlife, leading to higher stress levels, injuries, mortality and behavioural changes, with detrimental effects on long-term health and fitness. Glucocorticoid (GC) hormones, which are released in response to ecological and social challenges, are increasingly employed to understand responses to anthropogenic disturbance. Here we investigate physiological and behavioural responses of female chacma baboons (*Papio ursinus*) to variation in spatial overlap and conflict with humans in their natural home range. The baboons resided in the Tokai Section of Table Mountain National Park in the Cape Peninsula of South Africa, where their home range included four types of habitats: protected, suburban, agricultural and mosaic areas. We studied the largest group of baboons in Tokai (70 individuals) and examined the effects of ranging in these different habitats on the faecal GCs and behaviour of all adult females in the troop (N = 16). We found time spent ranging in more anthropogenic habitats to be associated with higher levels of GCs, more aggression, less time socializing and shorter grooming bouts. Self-directed behaviour, however, varied and did not necessarily reflect physiological measures of stress. Taken together, the results of this study highlight the risks associated with ranging in anthropogenic environments and point to the need for a multifaceted approach to studying the negative impacts of human activities on animals so as to better inform conservation practices.

Introduced by

Shahrina Chowdhury

- Department of Anthropology, Brooklyn College, City University of New York; Anthropology Program, Graduate Center, City University of New York; New York Consortium in Evolutionary Primatology (NYCEP)

This study arose out of the well-documented problem of baboon-human conflict in the Cape Peninsula of South Africa. We wanted to understand the potential negative impacts of wildlife management strategies on the behavior and physiology of nonhuman primates that live in close proximity to people, feed on anthropogenic foods, and have negative interactions with humans. In the Cape Peninsula, most of the >16 troops of baboons live in anthropogenic habitats. The study group of baboons occupies a mosaic habitat in the southern suburbs of Cape Town, where they range in protected, urban, and agricultural areas and are exposed to varying levels of anthropogenic stress. The population is managed by the City of Cape Town, which employs rangers to manage the baboons' ranging patterns in order to minimize baboon-human conflict, and which also euthanizes problematic animals that raid. This study was unique in that, unlike other studies which compared groups of primates living in natural areas to entirely different groups living in anthropogenic habitats, we compared variation in stress responses (cortisol levels, self-directed behaviors and activity budgets) within the same individuals over time according to their time spent ranging in anthropogenic versus protected habitats. We found that a greater amount of time in more anthropogenic habitats was associated with higher cortisol, reduced sociality, and higher levels of aggression, all of which are negative outcomes for the baboons.

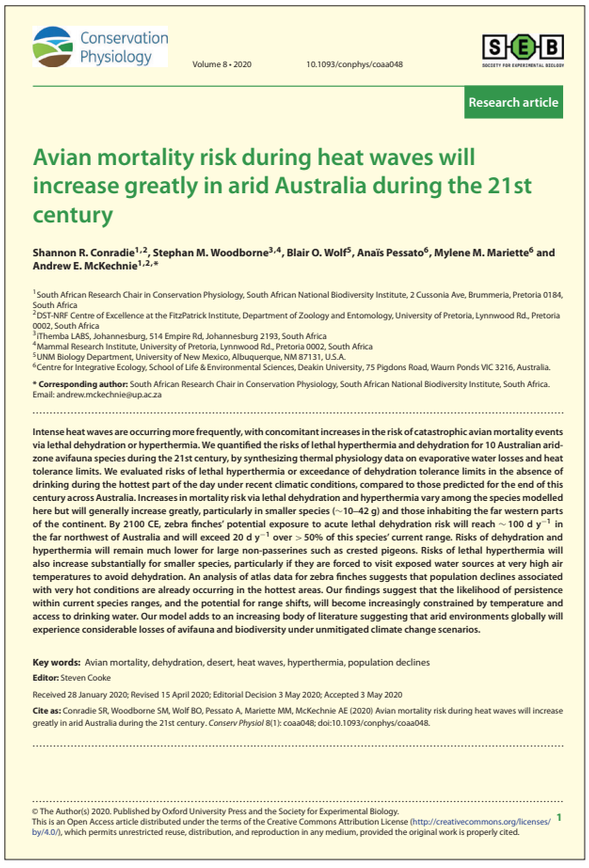
Soon after this study was completed, South African National Parks restricted research on the population in order to limit additional interactions of baboons with humans beyond what the population was experiencing naturally due to their daily ranging. Given that most primates now live in human-impacted habitats, studies like this are crucial for understanding the long-term impacts of anthropogenic disturbance. The results of this study are further alarming given that baboons are considered to be among the more adaptable primates, and the negative effects on their biology suggest that other less generalist and less adaptable species may be even more vulnerable. The findings of this study point to the need for more such studies, and the lead author of this paper plans to conduct similar research on specialist leaf-eating primates – langurs – in Bangladesh where habitat disturbance and loss have occurred at an unprecedented rate leaving highly fragmented and degraded natural habitats for primates. We plan to apply the results of these studies to inform conservation policies in Bangladesh working with local conservation authorities. •

SHANNON R CONRADIE, STEPHAN M WOODBORNE, BLAIR O WOLF, ANAÏS PESSATO, MYLENE M MARIETTE, ANDREW E MCKECHNIE

Avian mortality risk during heat waves will increase greatly in arid Australia during the 21st century

Conservation Physiology, Volume 8, Issue 1, 2020, coaa048

- Read the full article here: https://doi.org/10.1093/conphys/coaa048



Conservation Physiology Volume 8 • 2020 10.1093/conphys/coaa048

Research article

Avian mortality risk during heat waves will increase greatly in arid Australia during the 21st century

Shannon R. Conradie[1,2], Stephan M. Woodborne[3,4], Blair O. Wolf[5], Anaïs Pessato[6], Mylene M. Mariette[6] and Andrew E. McKechnie[1,2,*]

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Intense heat waves are occurring more frequently, with concomitant increases in the risk of catastrophic avian mortality events via lethal dehydration or hyperthermia. We quantified the risks of lethal hyperthermia and dehydration for 10 Australian arid-zone avifauna species during the 21st century, by synthesizing thermal physiology data on evaporative water losses and heat tolerance limits. We evaluated risks of lethal hyperthermia or exceedance of dehydration tolerance limits in the absence of drinking during the hottest part of the day under recent climatic conditions, compared to those predicted for the end of this century across Australia. Increases in mortality risk via lethal dehydration and hyperthermia vary among the species modelled here but will generally increase greatly, particularly in smaller species (~10–42 g) and those inhabiting the far western parts of the continent. By 2100 CE, zebra finches' potential exposure to acute lethal dehydration risk will reach ~100 d y^{-1} in the far northwest of Australia and will exceed 20 d y^{-1} over >50% of this species' current range. Risks of dehydration and hyperthermia will remain much lower for large non-passerines such as crested pigeons. Risks of lethal hyperthermia will also increase substantially for smaller species, particularly if they are forced to visit exposed water sources at very high air temperatures to avoid dehydration. An analysis of atlas data for zebra finches suggests that population declines associated with very hot conditions are already occurring in the hottest areas. Our findings suggest that the likelihood of persistence within current species ranges, and the potential for range shifts, will become increasingly constrained by temperature and access to drinking water. Our model adds to an increasing body of literature suggesting that arid environments globally will experience considerable losses of avifauna and biodiversity under unmitigated climate change scenarios.

Key words: Avian mortality, dehydration, desert, heat waves, hyperthermia, population declines

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Introduced by **Andrew McKechnie** • University of Pretoria, South Africa

Extreme heat waves can cause catastrophic mortality among birds, a phenomenon starkly illustrated by historical accounts from Australia and, increasingly, heat-related mortality events on other continents. To quantify how the risks of lethal dehydration and hyperthermia will change for Australian species in coming decades, Conradie *et al.* (2020) combined species-specific physiological data and projected climate change scenarios in a spatial model for ten Australian species. Their findings paint a sobering picture; for small-bodied taxa like finches and honeyeaters exposure to potentially lethal conditions will increase greatly in terms of both frequency and spatial extent. For instance, by the 2080s Zebra finches (*Taeniopygia guttata*) will experience more than 20 days per year hot enough to cause lethal dehydration over 50 percent of their current range, and over 100 days per year in the intensely hot northwestern parts of the continent. The study also highlighted that Australian birds are currently experiencing hotter conditions than during the 20th Century, emphasizing the impact of warming over recent decades and the novelty of current conditions. Conradie *et al.*'s work proved timely, with South Africa experiencing its first major heat-related mortality event involving wild birds five months after the study was published. In addition, two of the authors recently contributed to a global analysis of overlap between current protected area networks and future arid-zone avian biodiversity refugia, revealing the importance of this work for conservation planning on a warming planet. •

FREYA WOMERSLEY, JAMES HANCOCK, CAMERON T PERRY, DAVID ROWAT

Wound-healing capabilities of whale sharks (*Rhincodon typus*) and implications for conservation management

Conservation Physiology, Volume 9, Issue 1, 2021, coaa120

- Read the full article here:

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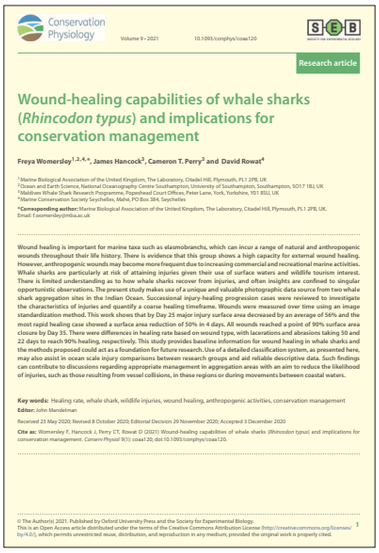

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Research article

Wound-healing capabilities of whale sharks (*Rhincodon typus*) and implications for conservation management

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Wound healing is important for marine taxa such as elasmobranchs, which can incur a range of natural and anthropogenic wounds throughout their life history. There is evidence that this group shows a high capacity for external wound healing. However, anthropogenic wounds may become more frequent due to increasing commercial and recreational marine activities. Whale sharks are particularly at risk of attaining injuries given their use of surface waters and wildlife tourism interest. There is limited understanding as to how whale sharks recover from injuries, and often insights are confined to singular opportunistic observations. The present study makes use of a unique and valuable photographic data source from two whale shark aggregation sites in the Indian Ocean. Successional injury-healing progression cases were reviewed to investigate the characteristics of injuries and quantify a coarse healing timeframe. Wounds were measured over time using an image standardization method. This work shows that by Day 25 major injury surface area decreased by an average of 56% and the most rapid healing case showed a surface area reduction of 50% in 4 days. All wounds reached a point of 90% surface area closure by Day 35. There were differences in healing rate based on wound type, with lacerations and abrasions taking 50 and 22 days to reach 90% healing, respectively. This study provides baseline information for wound healing in whale sharks and the methods proposed could act as a foundation for future research. Use of a detailed classification system, as presented here, may also assist in ocean scale injury comparisons between research groups and aid reliable descriptive data. Such findings can contribute to discussions regarding appropriate management in aggregation areas with an aim to reduce the likelihood of injuries, such as those resulting from vessel collisions, in these regions or during movements between coastal waters.

Key words: Healing rate, whale shark, wildlife injuries, wound healing, anthropogenic activities, conservation management
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Abstract Wound healing is important for marine taxa such as elasmobranchs, which can incur a range of natural and anthropogenic wounds throughout their life history. There is evidence that this group shows a high capacity for external wound healing. However, anthropogenic wounds may become more frequent due to increasing commercial and recreational marine activities. Whale sharks are particularly at risk of attaining injuries given their use of surface waters and wildlife tourism interest. There is limited understanding as to how whale sharks recover from injuries, and often insights are confined to singular opportunistic observations. The present study makes use of a unique and valuable photographic data source from two whale shark aggregation sites in the Indian Ocean. Successional injury-healing progression cases were reviewed to investigate the characteristics of injuries and quantify a coarse healing timeframe. Wounds were measured over time using an image standardization method. This work shows that by Day 25 major injury surface area decreased by an average of 56% and the most rapid healing case showed a surface area reduction of 50% in 4 days. All wounds reached a point of 90% surface area closure by Day 35. There were differences in healing rate based on wound type, with lacerations and abrasions taking 50 and 22 days to reach 90% healing, respectively. This study provides baseline information for wound healing in whale sharks and the methods proposed could act as a foundation for future research. Use of a detailed classification system, as presented here, may also assist in ocean scale injury comparisons between research groups and aid reliable descriptive data. Such findings can contribute to discussions regarding appropriate management in aggregation areas with an aim to reduce the likelihood of injuries, such as those resulting from vessel collisions, in these regions or during movements between coastal waters.

Introduced by
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Whale sharks have lost >60% of their global population in just three generations, and until recently it has been somewhat unclear as to why. The species is listed on several global treaties and is largely prohibited from being fished, sold and traded, yet numbers are still falling. This study published in Conservation Physiology used an image database of shark injuries to shed light on potentially 'hidden' interactions with humans that are not directly witnessed or monitored. It was conceived during a field season in Djibouti, Africa where a large number of sharks were observed with human-related injuries such as propeller lacerations or signs of fishing gear entanglement. Some sharks were seen with fresh injuries while others had wounds that had completely healed from previous years. This led to questions related to how long these animals take to recover and whether injuries can act as a threat quantification tool. Research found that even severe, seemingly debilitating wounds were survivable and open wounds fully closed in a matter of months. Although this suggests some innate resilience in whale sharks, the article strongly advised that regulations should be strengthened in places where the species comes into contact with humans due to the sub-lethal impacts that injuries may have on long-term survivorship.

Examples of wounded sharks from the article were widely shared on social media to raise awareness and to help promote new 'go-slow' initiatives in heavily used areas. In addition, the research uncovered a new set of questions, most notably, what about the sharks that do not survive? While injury quantification offers a tool to monitor interactions with small vessels and other potentially damaging human activities, this technique can mask events from long in the past which the shark has since healed from or, more importantly, events from which the shark did not recover. This article provided a key stepping stone for developing novel methods to address the latter; and in 2022 a global study involving over 60 researchers set out to explore the impacts of large vessels, such as those in the global transport fleet, on populations. Interestingly, the collision risk calculated therein did not relate to how many vessel-related injuries were recorded at aggregation sites. This further indicates that, while an important tool to classify some threats, measuring sub-lethal injuries alone cannot capture the true impacts that some human activities have on the species. This prompted new and important discussions in the wider science and policy communities, suggesting that the threat of shipping may be worse than is currently recognised. Collectively, these research outputs provide a strong incentive for improving upon current spatial protection for the species in order to protect against interactions with both small and large vessels and to help slow population declines in the future. •

Science with Impact

Conservation Physiology

ALANA A.E. WILCOX, AMY E.M. NEWMAN, NIGEL E. RAINE, GREG W. MITCHELL, D. RYAN NORRIS

Captive-reared migratory monarch butterflies show natural orientation when released in the wild

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Abstract Eastern North American migratory monarch butterflies (*Danaus plexippus*) have faced sharp declines over the past two decades. Captive rearing of monarch butterflies is a popular and widely used approach for both public education and conservation. However, recent evidence suggests that captive-reared monarchs may lose their capacity to orient southward during fall migration to their Mexican overwintering sites, raising questions about the value and ethics of this activity undertaken by tens of thousands of North American citizens, educators, volunteers and conservationists each year. We raised offspring of wild-caught monarchs on swamp milkweed (*Asclepias incarnata*) indoors at 29°C during the day and 23°C at night (~77% RH, 18L:6D), and after eclosion, individuals were either tested in a flight simulator or radio tracked in the wild using an array of automated telemetry towers. While 26% (10/39) of monarchs tested in the flight simulator showed a weakly concentrated southward orientation, 97% (28/29) of the radio-tracked individuals that could be reliably detected by automated towers flew in a south to southeast direction from the release site and were detected at distances of up to 200 km away. Our results suggest that, although captive rearing of monarch butterflies may cause temporary disorientation, proper orientation is likely established after exposure to natural skylight cues.

Introduced by
Ania A. Majewska • University of Georgia, USA

The impact of captive rearing on the migratory behavior of the North American Monarch butterfly (*Danaus plexippus*) is of considerable interest. Over the past decade, drastic population declines have been documented at overwintering sites in Mexico and coastal California. These declines have prompted nature enthusiasts to rear eggs and caterpillars to adulthood indoors to increase the chances of survival and to contribute to the overall population recovery. However, indoor environments, particularly in the fall, provide the developing monarchs with different environmental conditions (such as temperature and light) to those experienced in the wild. These indoor conditions can profoundly affect the probability of monarchs emerging in a suitable physiological state for a successful migration. At the same time, we have a limited understanding of the environmental cues experienced at the egg and caterpillar stages that induce migration in adult monarchs.

Whether monarchs reared indoors during the fall emerge ready for migration and behave as wild counterparts upon release has been called into question. Previous work has indicated that monarch butterflies reared in captivity in fall conditions do not show flights directed towards the expected migratory route (Tenger-Trolander *et al.* 2019; Tenger-Trolander *et al.* 2020). However, the flight simulator method used to determine the directionality of flight tends to yield relatively small sample sizes. This method involves invasive surgery to tether the monarch to a thin rod inside a flight simulator, a large plastic cylinder. The surgical procedure is complex; a substantial proportion of monarchs perish following the surgery, and others fail to fly in the simulator, therefore providing data for a limited number of monarchs. In the study by Wilcox *et al.* (2021), the authors used the flight simulator to assess flight directionality in captive-reared monarchs. They found that most monarchs raised in captivity under conditions simulating fall-like temperatures and day lengths did not fly in the expected south to southeast direction. The authors also released captive-reared monarchs with radio trackers into the wild to quantify orientation behavior over time and distance. The radio tracking data indicated that nearly all the captive-reared butterflies showed flight paths toward their migratory route. However, two questions remain: does exposure to natural fall conditions at release allow captive-reared monarchs to regain directional flight towards overwintering sites, and does the flight simulator method provide unreliable orientation measurements? Arguably, radio tracking of released adults captures a more realistic scenario that monarchs experience in the wild during the fall: adults gauge environmental cues such as temperature, barometric pressure changes, and the sun's position.

While the Wilcox *et al.* (2021) findings reveal that monarch butterflies reared in lab conditions mimicking the fall weather can orient correctly following release, the success rate of these monarchs reaching the overwintering destination remains unclear. Indeed, a monarch's journey to overwintering sites is met with multiple challenges, including loss and diminished quality of stopover and overnight roosting sites, and chances of vehicle strikes. More recent work on wild monarchs captured during fall migration showed that nighttime lights, which monarchs experience throughout the migratory flight due to light pollution, can disrupt flight directionality (Parlin *et al.* 2022). Successful fall migration is a complex and multifaceted phenomenon, and further work is needed to determine how captive rearing can be used for monarch conservation. As radio tracking technology advances and very lightweight tags are developed, our understanding of monarch migration biology and behavior will improve. Nonetheless, work by Wilcox *et al.* (2021) provides valuable insights into the ability of captive-reared monarchs to engage in fall migration and, at the same time, opens additional questions about the conservation of this iconic butterfly species.

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