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.
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).

References

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. 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.

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.

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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 neatly 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 excep- tionally 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. Freiherr, 1945) but which is still not resolved (cf. our own previous account, Manton and Clarke, 1957).

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-
F.A. LIONEL CLOWES

Localization of Nucleic Acid Synthesis in Root Meristems


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

Abstract Adenine-8-\(^{14}\)C 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 denoted and this suggests that there is as little cell interchange between the histogens as there is in roots with visibly discrete histogens.

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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, later \(^{14}\)C, and later \(^{1}\)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 the histogen giving rise to all the RAM tissues. Clowes finalizes his study with the QC clearly denoted and this suggests that there is as little cell interchange between the histogens as there is in roots with visibly discrete histogens.

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 (i) 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.

References


R.M. SPANSWICK, E.J. WILLIAMS

Effect of Na, K, and Cl Concentrations on the Membrane Potential of \textit{Nitella translucens}

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

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 \textit{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 $\alpha = P_{\text{Na}}/P_{\text{K}}$ 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 17 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 and 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+. Other dominant ions. Spanwick and Williams (1964) and Spanwick *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 followed 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.

References


By the end of the 1970s, it had been well established that some ROS were potentially mutagenic and could also produce 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 H2O2 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 Noriuki 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 apoplastic, with extracellular SOD favouring the conversion of superoxide to H2O2. 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 H2O2 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.

References


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Abstract

Ion fluxes have been measured in ‘isolated’ guard cells of Commelina communis L. using $^{86}$RbCl and $^{86}$KBr, 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 ($C_e$). The influx of $^{86}$Rb rose with concentration, to a $V_{max}$ of about 23 pmol mm$^{-2}$ h$^{-1}$. The efflux curve of $^{86}$Br could be well fitted by two exponential terms, with half-times of 38 min (independent of $C_e$), and 5–35 h (falling with increasing $C_e$). Bromide contents of cytoplasm and vacuole ($Q_o$ and $Q_v$), and fluxes at plasmalemma and tonoplast, were calculated from the efflux kinetics. Over $C_e$ 20–60 mM, as the aperture increased from 7 μm to 17 μm, the tonoplast flux (0.5–1.5 pmol mm$^{-2}$ h$^{-1}$) was always much less than the plasmalemma flux (7–77 pmol mm$^{-2}$ h$^{-1}$). $Q_o$ and $Q_v$ both increased with aperture. The increase in $Q_v$ 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.

ENID A.C. MACROBBIE

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


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

P. G. BLACKMAN, W. J. DAVIES

Journal of Experimental Botany, Volume 36, Issue 1, January 1985, Pages 39–48

Abstract Seedlings of Zea mays L. (John Innes hybrid) were grown with roots divided between two containers such that 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.

P. G. BLACKMAN and W. J. DAVIES

Department of Biological Sciences, University of Lancaster, Bailrigg, Lancaster, LA1 4YQ, U.K.

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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 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 excess of increased or reduced transport of some chemical compound will be felt in the shoots. Declining root activity may eventually result in a reduced supply of water and, if water loss is not restricted, in the development of water deficits. If chemicals moving from roots can exert some influence on plant water loss, this response may be obtained before any substantial change in the transport of water. It may be argued (Vonesch, 1983; Cowen, 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, 1984; Davies and Sharp, 1981; Bates and Hall, 1981), are flooded (Braddock and Hsiao, 1982; Jackson and Kowalski, 1983) or suffer changes in temperature (Simpson, 1982).

Different forms of deficit irrigation (‘partial rootzone irrigation’) are being developed to reduce water use by crops. 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 potential 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 irrigation’) are being developed to reduce water use by crops. 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).

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 re-circulated from roots. There is current discussion of roles that might be played by such ‘root signals’ (e.g. Maurer 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).
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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Biochemical Limitations to Carbon Assimilation in C3 Plants—A Retrospective Analysis of the A/C Curves from 109 Species

Abstract

Species-specific differences in the assimilation of atmospheric CO₂ depend 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 CO₂ 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 curves for 109 C₃ plant species. Based on this analysis, the maximum rate of carboxylation, Vcₘₐₓ, ranged from 6 μmol m⁻² s⁻¹ for the coniferous species Picea abies to 104 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, J₀ₙₐₓ, ranged from 17 μmol m⁻² s⁻¹ 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 Vcₘₐₓ and J₀ₙₐₓ indicated that the assimilation of CO₂ was regulated in a co-ordinated manner by these two component processes. Of the A/C curves analysed, 23 showed either an insensitivity or reversed-sensitivity to increasing CO₂ concentration, indicating that CO₂ 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 CO₂ assimilation, separating these species-specific results into those of broad 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.

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 (Vcₘₐₓ), and (2) regeneration of ribulose-1,5-bisphosphate by the Calvin Cycle which is limited by the maximum
electron transport rate ($J_{\text{max}}$). 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; $V_{\text{cmax}}$, $J_{\text{max}}$, 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 known as an $A/C_i$ curve. Wullschleger painstakingly extracted $A/C_i$ curves from published work and then derived $V_{\text{cmax}}$, $J_{\text{max}}$, 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 $V_{\text{cmax}}$ and $J_{\text{max}}$. 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.

References


• Science with Impact

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

Abstract The effect of growing pea (Pisum sativum L.) plants with CdCl₂ (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 CdCl₂ 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 CdCl₂ 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.
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.

References


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


• Read the full article here: https://doi.org/10.1093/jxb/erf090

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$_2$O$_2$, 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 defense system.

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$_2$O$_2$), 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$_2$O$_2$ production and H$_2$O$_2$-activated Ca$^{2+}$ channels are important mechanisms for ABA-induced stomatal closure (Pei et al. 2000). H$_2$O$_2$-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$_2$O$_2$ production, and both ABA and H$_2$O$_2$ induced the expression of cati, 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$_2$O$_2$, 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 (2000a), 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 Ca2+ 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 H2O2 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 H2O2 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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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.

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 2-3.7 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 polygenic 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 26% 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 caloric intake.
H$_2$O$_2$ mediates the regulation of ABA catabolism and GA biosynthesis in Arabidopsis seed dormancy and germination

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

Abstract

H$_2$O$_2$ is known as a signal molecule in plant cells, but its role in the regulation of abscisic acid (ABA) and gibberellic acid (GA) metabolism and hormonal balance is not yet clear. In this study, we found that H$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ 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.

Keywords: ABA, ABA catabolism, H$_2$O$_2$, GA biosynthesis, NO, seed dormancy.

Introduction

Many factors are involved in seed dormancy regulation, including some plant hormones, such as abscisic acid (ABA), gibberellic acid (GA), and ethylene (Bewley, 1997; Bewley and Black, 1994). Seeds of most angiosperms are incapable of a viable seed to germinate under favourable environmental factors, such as light intensity and low temperature (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, 2002). Seeds of most angiosperms are incapable of a viable seed to germinate under favourable environmental factors, such as light intensity and low temperature (Bewley, 1997).

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$_2$O$_2$ and NO affect the germination of dormant seeds, so we designed experiments to verify the regulatory relationship between them. Our results showed that H$_2$O$_2$ could accelerate the degradation of ABA by inducing the expression of ABA metabolic genes (CYP707A). Induction of H$_2$O$_2$ on ABA metabolic gene expression requires the involvement of another signaling molecule, NO. H$_2$O$_2$ 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$_2$O$_2$ does.

If GA synthesis is further suppressed, H$_2$O$_2$ is unable to release the strong dormancy phenotype of CYP707A2 mutants. These results suggest that H$_2$O$_2$-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 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.
Overall, a critical role for lignin was believed to contribute to the lignification and cross-linking of lignin in resistant cotton stems. Furthermore, quantitative analysis demonstrated increased lignification and cross-linking of lignin in resistant cotton. Histochemical analysis of lignin after inoculation with the wilt fungus *Verticillium dahliae* strain V991 showed an increased level of expression of lignin metabolism-related genes, phenylpropanoid metabolism was deduced to be involved in 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.

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; Vaamhestra 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 degradation 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 GhLac1 over-expressing cotton lines showed a broad resistance to cotton bollworm, cotton aphid and *V. dahliae*, due to lignin hyperaccumulation 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 (Ninkku 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. 2009; 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.

References


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

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-overexpressing 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. Overexpression of OsMYB2 enhanced up-regulation of genes encoding proline synthase and transporters. The OsMYB2-overexpressing plants accumulated less amounts of H2O2 and malondialdehyde. The enhanced activities of antioxidant enzymes, including peroxidase, superoxide dismutase, and catalase, may underlie the lower H2O2 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-overexpressing plants. These findings suggest that OsMYB2 encodes a stress-responsive MYB transcription factor that plays a regulatory role in tolerance of rice to salt, cold, and dehydration stress.

Plants are sessile organisms and are frequently exposed to variable environmental 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 biochemical and physiological changes during the abiotic stress response. These changes include accumulation of osmolytes and cryoprotectants 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 dehydration stress. Overexpression of OsMYB2 led to enhanced accumulation of compatible osmolytes, such as soluble sugars, free proline, and LEA proteins in rice, and suppressed the accumulation of malondialdehyde (MDA) and hydrogen peroxide (H2O2) under conditions of salt stress. The up-regulation of OsMYB2 promoted effective osmoregulation in rice plants via accumulation of compatible solutes and by minimizing oxidative damage during abiotic stress. More importantly, overexpression of OsMYB2 in rice seedlings did not affect their phenotypes under control conditions. Therefore, OsMYB2 provides a promising tool for improving the tolerance of rice to abiotic stress in general, and to salt stress in particular.

References


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An Yang, Xiaoyan Dai, Wen-Hao Zhang

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

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.

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.

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 organisinal 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.

Cristiano de Mello Gallep & Daniel Robert
University of Campinas, Brazil & University of Bristol, UK

Keywords: Animal activity, biological cycles, circadian biology, gravimetric tide, human activity, plant growth, plant movement.
HERVÉ SAUQUET, SANTIAGO RAMÍREZ-BARAHONA, SUSANA MAGALLÓN
What is the age of flowering plants?
Journal of Experimental Botany, Volume 73, Issue 12, 2022, 3840–3853

• Read the full article here: https://doi.org/10.1093/jxb/erac130

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 ago (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 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.

Introduction

Flowering plants (angiosperms) are the dominant group of terrestrial higher plants. They include ~270 000 described species (of which ~120 000 are flowering plants), ~40 000 of which are in eudicotyledons (such as the grassy monocots, or grasses) and ~34 000 in eudicotyledons (such as the grassy monocots, or grasses). The flowering plants are the dominant group of terrestrial higher plants, and they are the dominant group of terrestrial higher plants, and they are the dominant group of terrestrial higher plants, and they are

Without flowering plants, the world would look very different. Food, clothing, ecosystems, even the Journal of Experimental Botany, all 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.

References

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, TOSHIKO 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: https://doi.org/10.1046/j.1365-313X.1994.6020271.x

Abstract

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 R₀, R₁ and R₂ 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.

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 transgensics 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).

References


Sahoo KK, Tripathi AK, Pareek A, Sopory SK, Pareek SL. 2011. An improved protocol for efficient transformation and regeneration of diverse indica rice cultivars. Plant Methods 7, 49. *
An abstract of a scientific paper discussing the localization of H2O2 in plants, particularly in papillae and hypersensitive response during the barley—powdery mildew interaction.

**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 H2O2 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 H2O2 can be made at subcellular levels. This method was successfully used to detect H2O2 in developing papillae and surrounding haloes (cell wall appositions) and whole cells of barley leaves interacting with the powdery mildew fungus. Thus, H2O2 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 H2O2 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 H2O2. This cross-linking reinforces the apposition, presumably assisting the arrest of the pathogen.

**Introduction**

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-diaminobenzidine (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 diaminobenzidine (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.

**Read the full article here:**

https://doi.org/10.1046/j.1365-313X.1997.11061187.x
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-di rected 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.

Introductions

It is generally accepted that functional aspects of organelles are intimately linked to their cytoskeletal microenvironment. The role of the ER in maintaining the structural and functional integrity of the Golgi apparatus has been well documented (Feldherr, 1978; לפי Feldherr, 1978). More recently, it was shown that the Golgi apparatus is connected to the ER via proteinaceous bridges (Feldherr, 1978). The role of the Golgi apparatus in the transport of newly synthesized proteins from the ER to the Golgi has been well documented (Rothman, 1987). The ER and Golgi are also known to be connected by direct membrane connections (Denecke, 1996; Staehelin and Moore, 1995), although these connections have not been well characterized. The Golgi complex functions as a highly mobile system of vesicles that permit the in vivo observation of endomembrane traffic between the various compartments that make up the endomembrane system (Bannykh et al., 1998). Recent studies, utilizing a fusion of the green fluorescent protein (GFP) to a viral glycoprotein, have revealed the existence of a highly mobile and dynamic Golgi apparatus in plant cells (Boevink et al., 1998). The close relationship between the ER and Golgi is highlighted by a brefeldin A-induced retrograde transport of ER and Golgi membrane proteins (Bannykh et al., 1998). The relationship between the ER and Golgi has been extensively studied in animal systems, particularly in the fruitfly *Drosophila melanogaster* (Brown, 1998; Bannykh et al., 1998). In mammalian systems, the relationship between the ER and Golgi is mediated by direct membrane connections (Denecke, 1996; Staehelin and Moore, 1995), although these connections have not been well characterized. The ER and Golgi are also known to be connected by direct membrane connections (Denecke, 1996; Staehelin and Moore, 1995), although these connections have not been well characterized.

Aim of the study

The aim of this study was to better understand the relationship between the ER and Golgi in plant cells. In particular, we wanted to determine whether the relationship between the ER and Golgi in plant cells is mediated by direct membrane connections or by vesicular transport. We also wanted to determine whether the relationship between the ER and Golgi in plant cells is mediated by direct membrane connections or by vesicular transport. We also wanted to determine whether the relationship between the ER and Golgi in plant cells is mediated by direct membrane connections or by vesicular transport.

Methods

Plant material

Leaf epidermal cells of *Nicotiana clevelandii* were used in this study. *Nicotiana clevelandii* is a member of the family Solanaceae and is a common model organism for the study of plant cell biology. The leaves of *Nicotiana clevelandii* were used in this study because they are rich in ER and Golgi membranes and provide a convenient system for the visualization of membrane traffic between these organelles. The leaves were excised from young leaves of *Nicotiana clevelandii* and were used within 24 hours of excision. The leaves were placed on a glass coverslip in a Petri dish and were photographed using an inverted microscope (Nikon TE300). The leaves were then incubated at 4°C for 24 hours to allow the Golgi apparatus to move away from the ER. The leaves were then photographed again using an inverted microscope (Nikon TE300).

GFP expression

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.

Results

GFP expression

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Discussion

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


Read the full article here: https://doi.org/10.1046/j.1365-313X.2001.0105.x

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, pHANIBAL, 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.

References


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 IMAGENNOTATOR 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 pointed 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 depletation.

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


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

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 ontologies, MAPMAN is still widely used today with the current iteration supporting far more species than Arabidopsis.
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

The work by Brook Nelson, Xue Cai and Andreas Nebenführ prepared and tested a library of organelar 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.
Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana

STEVEN J. CLOUGH, ANDREW F. BENT

The Plant Journal, 2008, 16: 735–743

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 benzylaminopurine 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.

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

Jean-Yves Paul, Harjeet Khanha, Jennifer Kledion, Phuong Hoang, Jason Geijskes, Jeff Daniells, Ella Zaplin, Yvonne Rosenberg, Anthony James, Bulukani Mlalazi, Pradeep Deo, Geoffrey Arinaitwe, Priver Namanya, Douglas Becker, James Tindamanyire, Wilberforce Tushemereirwe, Robert Harding, James Dale

This article describes the results of the initial research and development phase of Bananaz, 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.

Bananaz 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 Bananaz 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.

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 Fei1 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 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 promoter 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.

References


Synthetic biology approach for plant protection using dsRNA

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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 post synthetic 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
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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/polio-virus-free, booster vaccine using poliovirus capsid protein (VP1, conserved in all serotypes) fused with cholela 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 L2). 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.

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 poliovirus 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 05433811). This publication (Daniell et al. 2022) received an impressive Altmetric score (i333): >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.

References
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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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-vent1 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 3R-gene stack from the potato variety ‘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 43 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.

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.

References


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


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.

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.

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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
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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-CLV5 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 domain in young floral buds 2–3 days after initiation. Single and double mutant alleles of lc and fas maintain higher SIWUS expression during the development of the carpel primordia in the floral bud. This augmentation and altered spatial expression of SIWUS 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 SIWUS 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.

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 k 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 k 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 (Slyco03g117230) 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 CLAVATA5 plays a pivotal role in shaping tomato fruit size, and Chu et al. have shed light on the underlying genetic regulation.

References


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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.
Conservation Physiology
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. 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 avoiding jeopardized 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.
One hundred research questions in conservation physiology for generating actionable evidence to inform conservation policy and practice

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.
Abstract Ocean acidification, resulting from increasing anthropogenic CO₂ 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 CO₂ 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 CO₂, 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 CO₂ 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 CO₂. 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 CO₂ treatment, indicating that acidosis was probably prevented by regulatory changes in acid–base relevant ions. Epaulette sharks may therefore possess adaptations that confer tolerance to CO₂ 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.

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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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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂.
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, and 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.

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 475 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.

References


SJANNIE LEFEVRE

Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO2 and their interaction

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- 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 CO2 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 CO2 affects resting oxygen uptake MO2rest and AAS; and (iii) whether there is an interaction between elevated temperature and CO2. The behavioural effects of CO2 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, CO2 regime or duration of CO2 treatment. When CO2 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 CO2 could complicate experimental determination of respiratory performance. Overall, this meta-analysis reveals heterogeneity in the responses to elevated temperature and CO2 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.

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 CO2 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 CO2 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 CO2 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.
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)

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**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 an interdisciplinary team an unusual opportunity to provide relevant scientific advice that can 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.

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 interaction 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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Introducing 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.

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

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

Abstract

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. Intense heat waves during current climate will still generally increase greatly, particularly in smaller species (~10–42 g) and those inhabiting the far western parts of the continent. By 2080, zebra finches will become intensely hot enough to cause lethal dehydration risk with more than 99% probability of daily mortality. By 2100 CE, zebra finches and other small passerines will experience the risks of lethal hyperthermia and dehydration for 10 Australian species in common with South Africa, indicating the impact of warming over recent decades and the novelty of current conditions. Conradie et al.’s (2020) study 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.

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

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• Read the full article here:
https://doi.org/10.1093/consphys/coaa120

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 80% 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.

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

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

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Read the full article here: https://doi.org/10.1093/conphys/coab032

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 (47% RH, 8L: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.

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.

References


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