E3. Hormones and the Regulation of Water Balance

Ian C. Dodd and William J. Davies
The Lancaster Environment Centre, Lancaster University, LA1 4YQ, UK
E-mail: W.Davies@lancaster.ac.uk

INTRODUCTION

Soil water status limits both species distribution and crop yield and a long-held view is that plant water status is the key variable that mediates the influence of soil drying. Plant water status can be a highly dynamic variable that fluctuates over varying time scales. For example, over the course of a day, plant water status of even a well-watered plant declines as solar noon approaches, as transpiration increases with increasing evaporative demand. The plant will hydrate again as temperatures (and transpiration) decrease towards the end of the day. In between rainfall (or irrigation) events, plant water status will generally decrease along with soil water status. In many circumstances, we are content with the suggestion that as soil dries, reduced uptake of water from drying soil results in shoot water deficit which closes stomata to restrict water loss and further shoot dehydration.

However, this is not always the case. Under some situations, some plants show so-called 'iso-hydric' behaviour, when water status is strongly regulated, usually by partial stomatal closure (Fig. 1). Here, it is necessary to suggest that the stomata are either very finely tuned to reductions in water supply to shoots or that they are responding to some other measure of soil water availability. In this chapter[1], we advance the hypothesis that chemical signalling between roots and shoots can tune stomatal (and other physiological and developmental) responses to soil drying such that plant water status can be effectively regulated.

Total plant water loss is the sum of water loss of each individual leaf and therefore limiting leaf expansion provides another means of limiting

[1] Abbreviations: A, CO_2 assimilation; ABP1, auxin-binding protein 1; ACC, aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinylglycine; g_s, stomatal conductance; ipt, isopentenyl transferase; Lp, root hydraulic conductivity; Ψ_{leaf}, leaf water potential; Ψ_{soil}, soil water potential; PRD, partial rootzone drying; θ, soil water content; WT , Wild-type; WUE, water use efficiency; [X-ABA], Xylem sap ABA concentration.

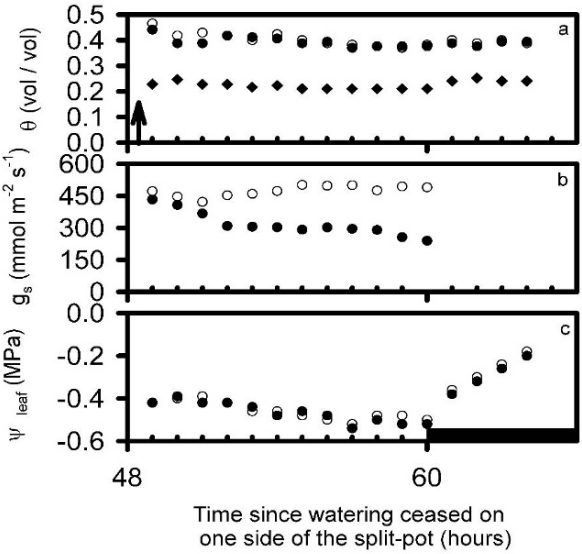

Figure 1. Soil moisture content of pots watered daily (indicated by arrow) on both sides of a split-pot (○), and of the watered (●) and drying (♦) sides of split-pots watered only on one side (a). Stomatal conductance (b) and leaf water potential (c) of plants watered daily on one (●) or both (○) sides of the split-pot (b-c). In (b), points are means of 5 leaflets per leaf. Dark shading on the time axis indicates the night period. (W.Y Sobeih, I.C. Dodd, M.A. Bacon & W.J. Davies, unpublished data).

plant water loss over periods of several days. Root growth and root activity can also exert considerable influence over plant water uptake. Integration of these processes (stomatal regulation, leaf expansion and root growth) will contribute to effective maintenance of plant water balance, and hormone-based signalling may be a means of achieving this. Such a central role for signalling in the plant's drought responses may provide targets for crop improvement.

STOMATAL REGULATION

Leaf conductance depends on the distribution of stomatal pore size across the leaf, and stomatal frequency. While leaf hormonal status can affect stomatal differentiation and frequency, we emphasise here the important role of chemical signalling in the dynamic regulation of stomatal aperture and water balance. Without regulation of this type, plants as we know them could not survive in the rapidly changing, challenging terrestrial environment into which they have evolved. Although exogenous hormone applications have demonstrated that stomata can respond to all 5 classical hormone classes (10) and several other chemical species, the role of ABA in eliciting stomatal closure is highlighted here. This is largely because of the now-recognised

potency of this hormone in the regulation of stomatal behaviour (across several orders of magnitude of concentration) and its stress-induced accumulation across a similar concentration range.

Abscisic Acid

Abscisic acid – receptors and signal transduction

Although the plant hormone concept suggests that a hormone molecule must interact with a receptor protein in order to effect a physiological response (Chapter A2), identification of an ABA receptor has proved elusive. Physiological experiments have sought to determine where in the stomatal guard cells such receptors might reside. ABA is not readily able to cross the cell membrane at pH 8, yet incubation of epidermal strips in solutions of ABA at this pH can elicit stomatal closure (14), which has been taken as evidence of extracellular ABA receptors. The presence of intracellular ABA receptors has also been suggested, as injection of ABA into individual guard cells causes stomatal closure, indicating that stomata can perceive symplastic hormone concentrations (1).

In terms of long-distance signalling between roots and shoots, the extracellular receptors must encounter hormones arriving in the apoplast of the leaf and therefore offer a sensitive link between hormone delivery and response. Irrespective of the location of ABA receptors, much effort has been expended in elucidating the intracellular signal transduction pathway resulting in stomatal closure. Observations that extracellular calcium and ABA acted synergistically in causing stomatal closure (Fig. 2) provided early clues that calcium acted as an important second messenger of ABA action. Perfusion of isolated guard cells with ABA increased the free calcium concentration in the cytosol (23). However, exposing plants to ABA at low temperature for 48 hours closed stomata in the absence of increases in concentration of free calcium in the cytosol (1), indicating that ABA-induced stomatal closure can use both calcium-dependent and calcium-independent pathways. Much research has concentrated on defining the intracellular source and dynamics of this elevated calcium, and its interaction with downstream parts of the ABA signal transduction pathway (Chapter D5).

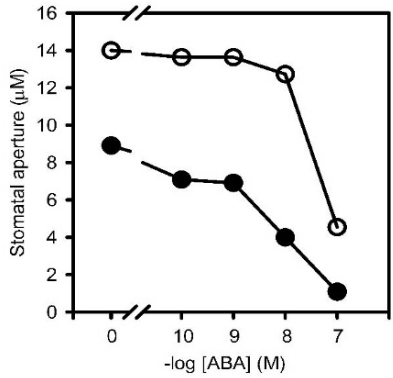

Figure 2. Stomatal aperture of *Commelina communis* as a function of ABA concentration when incubated with (●) and without (○) 10^{-4}M $CaCl_2$. Data re-drawn from (9).

Synthesis and distribution of abscisic acid

Much progress has been made in elucidating the pathway of ABA

biosynthesis (Chapter B5). Although it is important to note that even well-watered plants contain some ABA, bulk leaf ABA concentration increases in detached leaves in response to cellular dehydration (53). The capacity of leaf tissue to synthesise additional ABA varies between different genotypes (33), can be enhanced by nutrient deficiency and decreased by high (40°C) temperature (35). Although root tissue generally contains lower ABA concentrations than leaves, dehydration of detached roots from various species, and of various ages and branching orders also stimulated ABA synthesis (8).

Synthesis and transport of ABA conjugates provides another mechanism of increasing ABA concentrations in various compartments. Apoplastic glucosidases can liberate free ABA from the ABA glucose ester, which can be found in relatively high concentrations in the xylem sap (39). Xylem sap is also reported to contain an ABA precursor or adduct, which is hydrolysed at alkaline pH (28). Although such compounds may be important in long-distance transport, their contributions to the ABA budgets of specific organs have yet to be evaluated.

Since ABA is a weak acid (pKa = 4.75), its distribution in plant tissues will be governed by the Henderson-Hasselbach equation. At a cytosolic pH commonly found in many well watered plants (pH 7.3), ABA present in the protonated form moves into the alkaline chloroplast stroma (pH 7.9) where it dissociates to form an anion which is not as readily permeable (14). For this reason, most of the ABA in unstressed leaves is assumed to be in the chloroplasts (which act as an anion trap for ABA). Leaf dehydration alkalises the apoplast, increasing apoplastic ABA concentrations (14). Redistribution of ABA between different compartments of the leaf provides an attractive possibility for stomatal regulation in response to drought.

Prior to the quantification of the effects of drought on ABA accumulation and the demonstration of the effects of this hormone on stomata, it was widely assumed that stomatal response to soil drying was effectively a direct response to a change in leaf turgor. Stomatal closure was then attributed to the accumulation of ABA in leaves but careful work showed that the hormone accumulated only after leaf conductance declined (8, 54). Results such as these are not altogether surprising, given that most of the ABA in a leaf will be sequestered in the mesophyll chloroplasts and unavailable to active sites at or within the guard cell (14). It was then proposed that drought-induced changes in leaf water potential liberated ABA from the mesophyll chloroplasts, and that this ABA would move to the guard cells to initiate stomatal closure (22). However, this hypothesis does not explain stomatal closure without leaf dehydration (Fig. 1) and it is therefore necessary to argue for signalling from elsewhere in the plant to explain responses of this type.

ABA as a long distance signal of soil drying

For ABA to act as a root signal molecule that regulates stomatal behaviour, it was necessary to show increased delivery of the hormone into the leaf apoplast adjacent to putative extracellular binding sites on the guard cells. After overnight immersion of roots in an ABA solution (which was removed prior to the lights coming on), transpiring leaves showed stomatal closure during the following morning with the degree of closure being proportional to the accumulation of ABA in the leaf epidermis (8). More recently, using a combination of microdissection and a sensitive immunoassay, the accumulation of ABA has been measured in individual guard cells (57). These kinds of measurements cannot be made in any great numbers in crop level studies (as sample collection is labour intensive), but there is evidence that guard cell ABA accumulation can correlate with whole leaf and xylem ABA concentration, [X-ABA] (57).

Evidence that [X-ABA] was dynamically linked to changes in stomatal conductance (g_s) was first provided by Loveys (21). Subsequently, [X-ABA] was shown to increase much earlier and to a greater extent than bulk leaf ABA concentration during a soil drying cycle, and that this increase correlated with decreased g_s (54). Several comprehensive data sets from field and glasshouse studies indicate an excellent correlation between [X-ABA] and g_s (when xylem sap was collected from the same leaves in which g_s was measured) in a diverse range of species (46, 6, 4). Importantly, the

$$[\text{X-ABA}] / g_s$$

relationship for a given species is commonly unified across different growing conditions, from leaf to leaf on individual plants, and from day to day as the plant develops, providing good evidence of a causal relationship. There is considerable species to species variation in the [X-ABA] at which stomatal closure occurs (Fig. 3), suggesting it is not the absolute ABA concentration, but the relative increase in ABA concentration that is physio-logically important. A similar analysis can be applied to some ABA-deficient genotypes, which show a decreased capacity to synthesise ABA. Although such genotypes have a lower absolute ABA concentration (and thus higher g_s), they show a similar sensitivity of g_s to [X-ABA] (4).

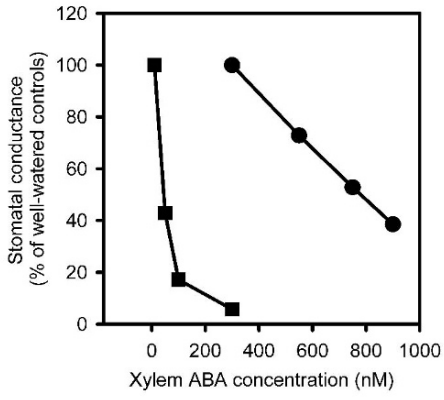

Figure 3. Relative stomatal conductance as a function of xylem ABA concentration for field-grown maize (■) and grape (●). Data re-drawn from (46) (■) and (6) (●).

Irrespective of the site of sap collection, as the soil dries, the concentration of all xylem sap

constituents should passively increase as transpirational fluxes decrease. Thus any solute, irrespective of any effect on stomata, could show a negative relationship with flux, similar to that shown between [X-ABA] and g_s (Fig. 3). Consequently, more rigorous tests of the physiological significance of ABA have been formulated. *Correlation* and *duplication* experiments have shown that soil application or stem injection of synthetic ABA to well-watered plants generates a similar relationship between [X-ABA] and g_s to that found in droughted plants. *Deletion* and *re-instatement* experiments test the specificity of hormone action by manipulating endogenous hormone levels. Removal of ABA from maize xylem sap using an immunoaffinity column eliminated its antitranspirant activity, as assessed using a detached leaf transpiration assay (8).

Although xylem sap is assumed to be in direct contact with the leaf apoplast (and thus available to extracellular guard cell receptors), the site and nature of xylem sap collection is important to accurately assess [X-ABA] (19). Xylem sap collected from leaves will more closely reflect apoplastic sap adjacent to the guard cells than sap collected from the root system, due to gains or losses in xylem solutes during long-distance transport through the stem. Leaf xylem sap can be obtained by growing plants in a whole plant pressure chamber or by pressurising entire detached leaves in a Scholander-type pressure chamber. If xylem sap must be collected from the root system, sap should flow at rates equivalent to whole plant transpiration rate, since the concentrations of many xylem constituents increase exponentially with decreasing sap flow rate (40). Sap collection from de-topped root systems under root pressure alone over-estimates true xylem sap concentration (40).

Even when xylem sap is collected from the same leaf in which g_s is measured, xylem sap can be modified en route to the leaf apoplast, by exchange with xylem parenchyma within the petiole, or by mesophyll catabolism and sequestration. The importance of the mesophyll tissue in modifying stomatal behaviour is revealed by experiments comparing dose-response relationships of stomata to apoplastic or xylem-supplied ABA (Fig. 4). In isolated epidermal strips, where hormone concentrations are controlled at the guard cell apoplast, stomatal closure occurred at ABA concentrations as low as 0.1 μM. In contrast, 1 μM ABA was needed to elicit substantial stomatal closure in detached leaves supplied with ABA via the xylem. Inhibition of ABA catabolism with tetcyclacis enhanced stomatal closure in leaf pieces (47). The implication is that xylem ABA concentrations are sufficient to keep stomata closed most of the time were it not for the mesophyll sequestering and breaking down ABA.

Although variation in [X-ABA] often accounts for variation in g_s, the origin of this ABA continues to attract debate. During some drying cycles, root ABA concen-tration and xylem sap concentration increase in parallel, prior to any increase in leaf ABA concentration (8), suggesting that xylem ABA is root-derived. However, considerable re-circulation of ABA between

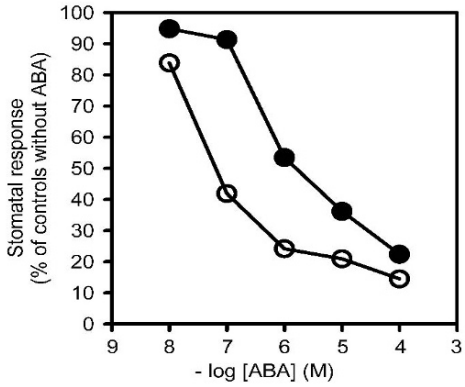

Figure 4. Relative stomatal response of *Commelina communis* as a function of incubation solution ABA concentration for epidermal strips (○) and ABA concentration supplied via the xylem to detached leaves (●). Data re-drawn from (47).

xylem and phloem can occur (52) thus not all ABA in a xylem sap sample is likely to be root-derived. Droughted plants can first lose turgor in older leaves (54), which might enhance any root-sourced ABA signal to younger leaves. In some species, ABA found in the rhizosphere can be efficiently transferred across the root tissues into the xylem (11). The importance of root-sourced ABA in mediating drought-induced stomatal closure has been addressed by reciprocal grafting of wild-type (WT) and ABA-deficient genotypes and comparing the stomatal responses and ABA concentrations of the graft combinations (15). Irrespective of whether WT shoots were grafted on WT or ABA-deficient roots, stomatal closure occurred in both graft combinations, despite a 4-fold difference in [X-ABA]. Such data suggests that other chemical species, in addition to ABA, can act as signals of the degree of soil drying and can be important in regulating stomatal behaviour.

Does apoplastic pH determine the ABA response ?

Several studies indicate that variation in [X-ABA] alone cannot always explain the extent of drought-induced stomatal closure. Studies with detached leaves have suggested the presence of other antitranspirant compounds in wheat and barley xylem sap (27). Also, drought-induced stomatal closure can precede increases in [X-ABA].

Alkalisation of xylem sap is a common response to various edaphic stresses (50) and supplying detached *Commelina* and tomato leaves with neutral or alkaline buffers (pH ≥ 7) via the transpiration stream can close stomata (Fig. 5). These alkaline buffers increased apoplastic pH, thus decreasing sequestration of ABA by mesophyll cells, causing increased apoplastic ABA concentrations, which closed stomata (51). Stomatal closure in response to xylem-supplied alkaline buffers was ABA-dependent, as leaves detached from an ABA-deficient mutant (*flacca* - *flc*) did not show stomatal closure when fed pH 7 buffers (Fig. 5), and in some cases transpiration actually increased (50). This pH response of detached *flc* leaves is consistent with g_s increasing when *flc* plants dried the soil within a certain range of soil water contents (15).

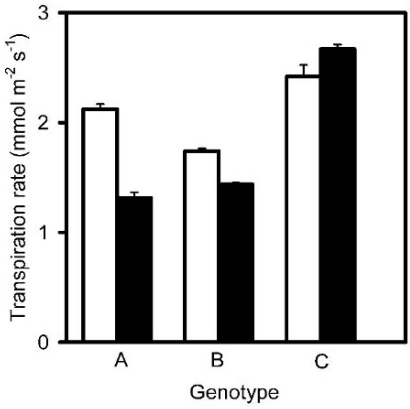

Figure 5. Transpiration rate of detached leaves fed phosphate buffers at pH 6-6.25 (hollow bars) and 7-7.75 (filled bars). Genotypes were *Commelina communis* (A), *Lycopersicon esculentum* cv. Ailsa Craig (B) and the ABA deficient tomato mutant *flacca* (C). Data re-drawn from (51) (*C. communis*) and (50) (*L. esculentum*).

Stomatal closure in response to sap alkalisation may help explain observations where stomatal closure could not be readily explained in terms of an increased [X-ABA]. During soil drying, changes in xylem sap pH may initiate stomatal closure as these changes may precede changes in [X-ABA]. Further, alkalisation of xylem sap may be responsible for the "unexplained" antitranspirant activity of some saps. However, in some species, xylem sap pH can acidify (*Ricinus communis;* 40) or show no change (*Nicotinia plumbaginifolia;* 4) in response to drought.

Variations in the aerial environment can also alkalise xylem sap. In well-watered *Forsythia x intermedia* plants grown in a greenhouse, more alkaline xylem sap pH was correlated with an increased light intensity and stomatal closure (7) although it not yet known which component of the aerial environment (e.g., light intensity, temperature, vapour pressure deficit) is responsible for the pH variation. Such phenomena allow integration of signals from both aerial and root environments, and may also explain observations showing that the effectiveness of ABA in closing stomata depends on prevailing environmental variables.

Environmental modification of ABA response

Stomatal responses to ABA vary considerably according to nutritional factors (14, 35) and environmental factors such as temperature and leaf water potential (Ψ_{leaf}). Such interactions may be important in the minute-by-minute control of stomatal aperture in a fluctuating environment, and allow drought-stressed plants to open their stomata to maximise photosynthesis under conditions (lower temperature and vapour pressure deficit) where transpirational losses can be minimised.

Since temperature and vapour pressure deficit and thus whole plant transpiration rate co-vary, an increase in temperature is likely to increase ABA delivery to the shoot. For this reason, demonstrations that the effects of ABA are truly temperature-dependent have often used epidermal strips to control apoplastic ABA concentrations. In maize epidermal strips, application of ABA stimulated stomatal opening below a threshold

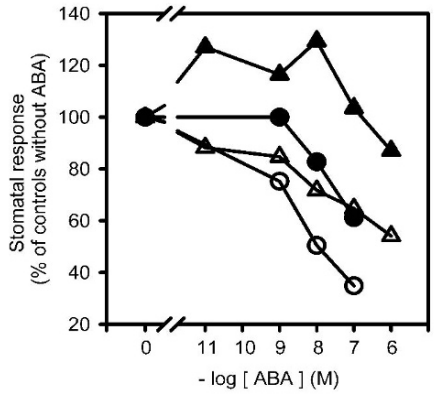

Figure 6. Relative stomatal response as a function of ABA concentration for epidermal strips of *Bellis perennis* incubated at 10°C (▲) and 30°C (Δ) and *Commelina communis* incubated at 7°C (●) and 25°C (○). Data re-drawn from (16) (*B. perennis*) and (49) (*C. communis*).

temperature, yet caused stomatal closure as temperature increased (36). Similarly, stomata of several species (*Bellis perennis, Cardamine pratensis, Commelina communis*) were relatively insensitive to ABA when incubated at 10°C (Fig. 6) and in some cases showed stomatal opening, but showed normal ABA-induced stomatal closure at 20°C or 30°C (16). The pre-treatment history of the plants can also be important, as a 48 hour exposure to cold temperatures (< 15°C) decreased the rate of stomatal closure of plants subsequently challenged with ABA (1). The decreased stomatal response to ABA at chilling temperatures was not related to thermotolerance in a comparison of two species (49): cold-tolerant *Commelina communis* showed decreased stomatal sensitivity to ABA at low temperature while cold-intolerant *Nicotinia rustica* showed a similar stomatal sensitivity at both 7°C and 27°C. The physiological bases for temperature-dependent ABA-induced stomatal closure, and for such genotypic differences, remain poorly defined.

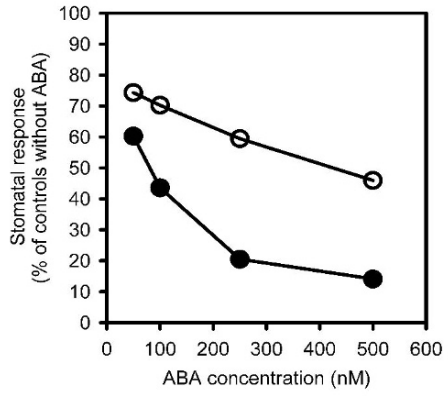

Figure 7. Relative stomatal response as a function of ABA concentration for *Commelina communis* epidermal strips incubated on solutions with an osmotic potential of –0.3 MPa (○) and –0.5 MPa (●). Modified from (46).

In field-grown maize, the relationship between [X-ABA] and g_s varied diurnally, with the most sensitive stomatal closure occurring at lower Ψ_{leaf} (46). Since an increased Ψ_{leaf} increases the rate of catabolism of xylem-supplied ABA (20), differences in the amounts of ABA reaching the guard cells might occur at different Ψ_{leaf}. However, increased stomatal sensitivity to ABA was seen when *Commelina* epidermes were incubated on ABA solutions of decreasing medium osmotic potential (Fig. 7). This interaction may be

thought of as a sensitive dynamic feedback control mechanism to ensure homeostasis of Ψ_{leaf}. Any decrease in Ψ_{leaf} (e.g., caused by the sun appearing from behind a cloud) will enhance stomatal response to ABA thus decreasing transpiration and returning Ψ_{leaf} to its original value. Interactions between ABA concentration and the environmental and plant variables discussed above enable the plant to integrate stomatal responses to a wide range of factors, all of which have the potential to modify plant water balance.

Auxins

Although attention has been given to stomatal responses to auxin, there is still far too little information on the effects of water stress on auxin delivery to the shoot, and leaf auxin concentrations. Abaxial stomata of *Commelina communis* can be relatively insensitive to IAA concentration in CO_2-free air, yet increasing IAA concentrations abolishes CO_2-induced stomatal closure (Fig. 8). IAA-induced stomatal opening can be an indirect effect of auxin-induced ethylene production. An inhibitor of ACC synthase, aminoethoxyvinylglycine (AVG), prevented auxin-induced stomatal opening in *Vicia faba* epidermes (24). Addition of ACC (the immediate precursor of ethylene) restored auxin-induced opening in a concentration-dependent manner when AVG was also present in the incubation solution (24).

Evidence that IAA can affect stomatal behaviour via ethylene-independent pathways is provided by the observation that antibodies to auxin-binding protein 1 (ABP1), a putative auxin receptor, induced stomatal opening (12). Overexpression of ABP1 in transgenic plants enhanced the sensitivity of guard cells to auxin, as measured by the changes in the auxin dose-response of inward and outwardly rectifying K+ currents (3).

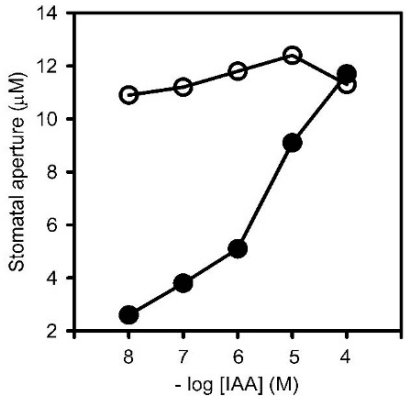

Figure 8. Stomatal aperture of *Commelina communis* as a function of IAA concentration at 700 μL L^{-1} CO_2 (●) or without CO_2 (○). Modified from (44).

While stomatal responses to auxin are often studied in isolation, it seems likely that the interaction of auxin with other hormones will be important *in vivo*. Incubation of *Commelina* epidermes in solutions containing 10 or 100 μM IAA antagonised ABA-induced stomatal closure (10). IAA overproducing and underproducing transgenics may allow IAA antagonism of ABA-induced stomatal closure to be evaluated *in planta*, and it is

important to give attention to potential hormonal interactions.

Cytokinins (CKs)

Stomatal responses to CK application are rather variable (Fig. 9) according to the species and the particular CK applied (10, 18). In isolated epidermes, micromolar CK concentrations are usually required to stimulate stomatal opening, but stomata can respond to CK concentrations as low as 10 nM (10). Xylem CK concentrations typically range between 1-50 nM according to the species, growth conditions and perhaps most importantly, sap flow rate during collection. When supplied via the xylem to detached leaves, nanomolar CK concentrations stimulate transpiration of some monocotyledonous species (18), yet much higher CK concentrations apparently have no effect on dicotyledonous species (Fig. 9). The apparent insensitivity of stomata to CKs in some studies may occur because endogenous CK concentrations are already optimal for stomatal opening. Another explanation is that CKs most effectively promote stomatal opening in ageing leaves and most studies use the youngest fully expanded leaf (18), suggesting that stomata might become sensitive to CKs only once endogenous concentrations decrease.

Perhaps the most convincing demonstration that endogenous CKs increase g_s and plant transpiration is provided by plant transformation with bacterial *ipt* (isopentenyl transferase, which catalyses *de novo* CK biosynthesis) or *zmp* (which encodes a protein capable of cleaving CK glucosides into active forms) genes (32). However, such transformations can also alter the concentrations of other hormones, which might affect g_s. Tobacco *ipt* and *zmp* transformants showed decreased and increased leaf IAA levels respectively, yet both showed increased g_s and increased leaf CK concentrations (32), suggesting that CKs and not auxin caused the observed stomatal phenotype.

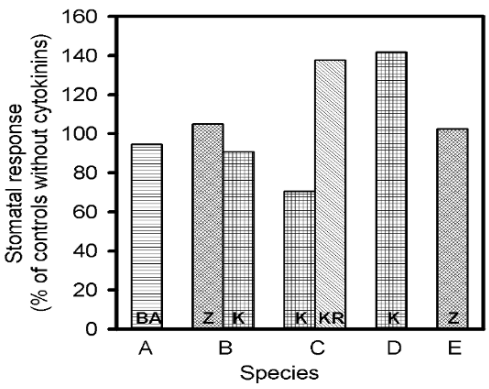

Figure 9. Relative stomatal responses to 1 μM solutions of various CKs in different species. Epidermal strips of *Commelina benghalensis* (A), *C. communis* (B) and *Vicia faba* (C) were incubated in the light in CK solutions; while detached barley (D) and lupin (E) leaves were supplied with CKs via the xylem. CKs applied were BA, benzyladenine; K, kinetin; KR, kinetin riboside and Z, zeatin. Data re-drawn from references in (10).

Although CKs can promote stomatal opening when applied in isolation, they can also antagonise ABA-induced stomatal closure.

Incubation of *Commelina* epidermes in solutions containing 50 μM benzyladenine and incubation of maize leaf pieces in solutions containing 10 or 100 μM zeatin or kinetin antagonised ABA-induced stomatal closure (10). However, similar experiments supplying CKs via the xylem to detached leaves have not always shown similar results, suggesting that xylem CK concentrations can be less important to stomatal responses than leaf (or apoplastic) CK concentrations. Supply of 0.1-10 μM kinetin to detached barley leaves and supply of 0.1-10 μM zeatin to detached lupin (*Lupinus cosentinii)* leaves did not reverse ABA-induced stomatal closure (10), perhaps due to rapid CK metabolism by the mesophyll. More recently, ABA-induced stomatal closure in detached sunflower leaves was partially reversed by nanomolar concentrations of zeatin riboside, which are typically found in sunflower xylem sap (13). The importance of CK antagonism of ABA-induced stomatal closure to *in vivo* responses might be tested by submitting CK overproducing transgenics along with WT plants to gradual soil drying and analysing stomatal sensitivity to [X-ABA].

ROOT GROWTH AND WATER UPTAKE

`

While stomata can regulate water loss from the shoot to slow the development of harmful water deficits, such regulation relies on the root system to sustain an adequate water supply. Mild soil drying commonly first depletes the water content of the soil immediately adjacent to the root and plant water uptake can therefore be restricted, even at quite high bulk soil water content. Under these circumstances, variation in root hydraulic conductivity (Lp) will only have a small impact on plant water uptake but it may be important in certain circumstances that root Lp does increase as soil dries (perhaps as a result of changed aquaporin activity) and that this response can be mimicked by ABA application to roots (56). However, prolonged and more severe soil drying decreases root Lp due to cell death and structural modifications such as suberisation to prevent water loss from dehydrated roots.

Although drought usually decreases overall plant (and root system) size, the proportional allocation of dry matter to the roots is usually increased (31) and root elongation can be increased (41). While drying of surface soil layers may cause local root mortality, root exploration of wetter and sometimes deeper parts of the soil profile may act to minimise the development of high soil/root interface resistances for water uptake and as such can help to maintain plant water status.

An experimentally convenient system to analyse root growth responses to soil water deficit, that circumvents the influence of the shoot controlling carbon supply to the roots, is the growth of primary roots in the dark at fixed soil water potentials (Ψ_{soil}). ABA seems to have an important role in root

growth maintenance at low Ψ_{soil} since genetic or chemical reductions in ABA accumulation inhibited primary root elongation of dark-grown maize seedlings (Fig. 10). In contrast, at high Ψ_{soil}, ABA application to WT plants inhibited primary root elongation (42). At low Ψ_{soil}, ABA-deficient roots were not only shorter but thicker, suggesting a drought-induced increase in ethylene synthesis, which has been confirmed (43). Further work suggests that ABA accumulation in roots at low Ψ_{soil} limits their ethylene synthesis, and that this allows some root extension even at very low soil/tissue water potentials.

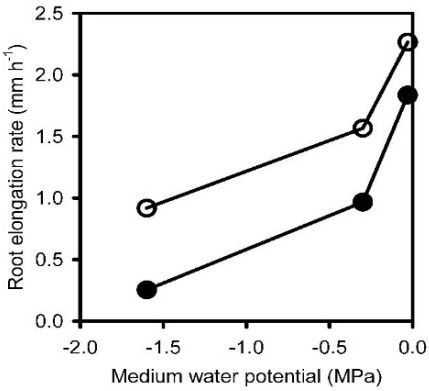

Figure 10. Primary root elongation rate as a function of medium water potential for the ABA deficient mutant *vp5* (●) and WT maize (○). Data re-drawn from (37, 42).

In light-grown plants, root growth is influenced by shoot control of assimilate availability. Growth analysis of ABA-deficient mutants provides some support for another role for ABA in maintaining root growth under various soil stresses, as the mutants often partition a decreased proportion of assimilates to the roots compared to WT plants (26), perhaps because the mutants are experiencing water deficit as indicated by a decreased Ψ_{leaf}. Reciprocal grafting experiments may avoid confounding effects of shoot water and ABA status. When WT scions were double-grafted onto both ABA-deficient (*sitiens*) and WT rootstocks and exposed to several cycles of soil drying, the *sitiens* root system accumulated 1.45-fold more biomass than the WT root system (15), suggesting that ABA deficient roots had a greater sink strength independent of shoot water status. Thus the role of root ABA status in whole plant resource partitioning requires further evaluation.

LEAF GROWTH

While stomatal closure limits water loss from existing leaf area, another water-saving measure is to decrease the extent of leaf area expansion. Several research groups have now demonstrated that leaf expansion of plants grown in drying soil can be decreased even when plant water status is maintained (8). In such situations, chemical signals generated as a result of the interaction between root systems and drying soil can inhibit leaf growth. Although many studies suggest a dominant role for the plant hormone ABA

in the control of stomatal behaviour under drought (4), the role of ABA in the control of leaf growth is still debated.

Early experiments showed that incubation of leaf discs on ABA solutions decreased leaf expansion by decreasing cell wall extensibility (48). The relationship between leaf growth inhibition and [X-ABA] was similar for maize plants fed ABA hydroponically in a nutrient solution and those from which water was withheld (55), indicating a possible regulatory role for ABA in controlling leaf growth of droughted plants. Detached maize shoots supplied with ABA via the xylem showed a similar relationship to that generated in intact plants between [X-ABA] and leaf growth (Fig. 11). However, in some cases leaf growth is apparently restricted prior to an increased [X-ABA] (26).

Recently, it was demonstrated that increased xylem sap pH correlated with drought-induced leaf growth inhibition, and that feeding leaves more alkaline buffers via the xylem inhibited leaf growth (2). This response is directly analogous to the effect of alkaline buffers on detached leaf transpiration (discussed earlier). Feeding more alkaline buffers to an ABA-deficient barley mutant (*Az34*) did not inhibit leaf growth unless an ABA concentration typical of well-watered plants was also present in the buffer. It was suggested that alkaline pH allowed ABA access to sites of action within the leaf elongation zone, inhibiting growth. At a well-watered, more acidic apoplastic pH, ABA is presumably partitioned into alkaline compartments in the symplast and away from sites of action regulating leaf growth. Apoplastic pH variation proves to be extremely sensitive to reduction in soil water availability and allows redistribution of ABA into different compartments to modify plant behaviour without the necessity for substantial *de novo* ABA synthesis.

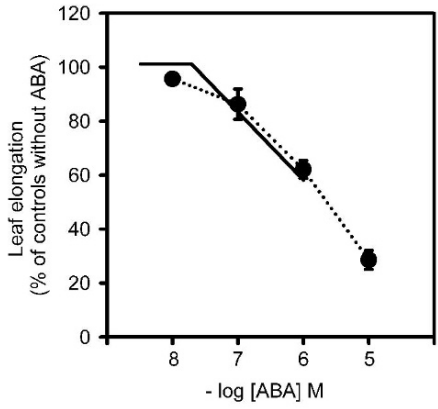

Figure 11. Response of leaf elongation to [X-ABA] in intact maize plants (solid line) and detached shoots (●). Solid line, redrawn from (55), was for whole plants grown in drying soil or fed a nutrient solution containing ABA. Symbols are means ± S.E. of n experiments for 10^{-8}M (n=3), 10^{-7}M (n=9), 10^{-6}M (n=11) and 10^{-5}M (n=8) ABA (I.C. Dodd, unpublished data).

Initial work growing ABA-deficient mutants at low Ψ_{soil} supported the contention that drought-induced ABA accumulation inhibits shoot growth. Shoots of the *vp5* mutant grew more than WT shoots when transplanted to vermiculite at –0.3 MPa (37). However, extending the experiment beyond 60 hours after transplanting resulted in the WT shoots growing faster than *vp5*, indicating that the effects of

ABA varied with the stage of plant development (43).

Further work with ABA-deficient mutants has reinforced the view that an important function of ABA is to restrict ethylene synthesis (43). Several changes in the soil environment such as soil compaction (17) can increase ethylene evolution, inhibiting shoot growth in most circumstances. In one report, intact droughted plants did not show increased ethylene evolution (25). However, soil drying can also increase soil strength, and it therefore seems possible that many soil drying episodes can perturb ethylene evolution, thus modifying leaf growth.

HORMONAL TARGETS TO INFLUENCE CROP WATER BALANCE AND YIELD ?

A framework (Fig. 12) to analyse the effects of manipulating hormone status or response on crop yield has been formulated (29), stating that:

Yield = Water transpired x Efficiency of water use x Harvest Index.

Increasing the amount of water transpired (moving along the slope – arrow A in Fig. 12) can increase yield, as can increasing the carbon gain per unit of water transpired (increasing the water use efficiency - WUE) (arrow B in Fig. 12). An increasingly important agricultural objective in many water-limited areas is to decrease the amount of water used (by decreasing water supplied to the crop) with minimal yield penalty (arrow C in Fig. 12). Can manipulating plant hormone status or response deliver any of these outcomes?

Manipulating leaf ABA accumulation has been the target of several crop breeding programs (34). ABA-induced stomatal closure in flag leaves could result in stomatal limitation of photosynthesis and decrease assimilate supply to the developing grains, since yield of cereal crops is often proportional to flag leaf photosynthesis. Although some field studies indicated that decreased ABA accumulation was associated with higher yields, others showed that enhancing ABA accumulation was beneficial to crop yield (34). The discussion above shows that there are many possible explanations for both types of response and that temperature, evaporative demand, nutrient supply and many other variables might determine the impact of a genetic modification in hormone accumulation. It seems likely that to generate a predictable impact on

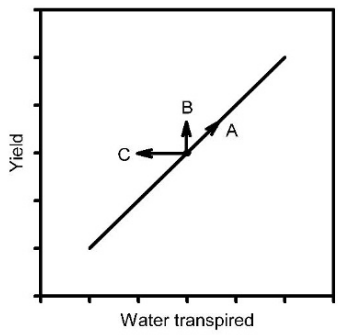

Figure 12. Relationship between crop yield and transpiration (from 29). For description of arrows A, B, C see text.

plant water balance and yield, any change in hormone production and/or distribution will have to be specifically targeted to a specific plant part, stage of development or even a specific climatic regime.

One problem with much breeding for variation in ABA accumulation is that variation is often defined by a standard laboratory test for ABA accumulation following a given degree of leaf dehydration (33). Lines may behave differently in the field where leaf ABA accumulation will depend on leaf water status and the contribution of xylem-supplied ABA from the roots. For this reason, some breeding programs have used field ABA accumulation as a selection criterion. In some cases, lines with low leaf ABA accumulation have a higher leaf water status (presumably due in part to an increased ability to extract soil water) which is correlated with an increased force required to uproot the plant (38). In this way, leaf ABA accumulation indirectly (and fortuitously) acts as a selection criterion for a potentially desirable root system property that might increase yield in dry soil. Although ethylene synthesis can limit elongation of roots in drying soil (43), roots of ethylene insensitive or deficient lines are less able to penetrate compacted soil (5, 17) and therefore manipulation of ethylene synthesis may not always confer agronomic advantages in the field.

Desirable root system properties are likely to depend on the environment, specifically soil water availability throughout the cropping period. Increased partitioning of resources to roots may restrict shoot growth and yield and may be of limited benefit to an individual growing in a monoculture in environments where water supply is assured. In water-limited environments, root systems that are able to explore deeper horizons of the soil profile (as a means of sustaining crop water use throughout the entire season) might be useful. It is argued (30) that in such climates where the soil is wet at the beginning of the season and dries thereafter (Mediterranean environments), a desirable plant profile (crop ideotype) would:

a) cover the ground rapidly to avoid soil water loss when water is available. This saves water for later flowering and fruiting, which is vitally important to yield.
b) have roots that will continue to slowly explore the soil and have a high resistance to water flow so that soil water is not used too fast.
c) show some turgor maintenance but also limitation of leaf growth later so the plant has resources for reproductive development.
d) show high WUE. However, since high resource use efficiency is usually linked with slow growth, this link must be broken early in the season.

It should be clear that while one of these characters can be changed via manipulation of plant hormone status or response, achieving all desirable traits would require manipulation of many specific genes at specific times during the crop life cycle. Given physiological uncertainties in the value of a crop ideotype approach, and the biotechnological complexity required to

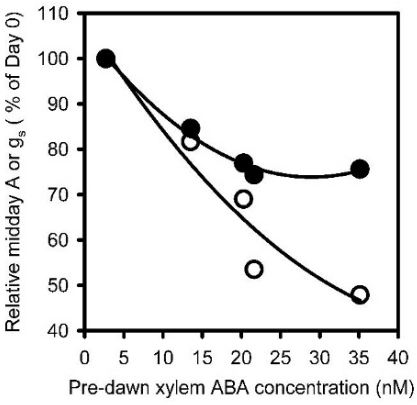

Figure 13. Relative responses of stomatal conductance (○) and CO_2 assimilation (●) to changes in pre-dawn [X-ABA] in *Trifolium subterraneum.* Data re-drawn from (45).

achieve it, perhaps agronomic manipulation provides better opportunities to apply our knowledge of hormone responses to crop improvement.

Decreasing the amount of irrigation water applied (deficit irrigation) is a legitimate irrigation strategy to decrease plant water use, but can restrict yield or at least result in some lesion in plant growth. A recent development of deficit irrigation is partial root drying (PRD). The technique works in drip or furrow irrigated crops where each side of the row can be watered independently. When the crop is irrigated, only one half of the plant root system receives water and the other is allowed to dry the soil. Water use is commonly halved under PRD irrigation. The wet side of the root system is able to supply enough water to the shoot to prevent harmful shoot water deficits. In response to drying soil, the dry part of the plant root system produces chemical signals, which are transmitted to the shoots to close the stomata (decreasing water loss). Given the relationship between yield and water use (Fig. 12), the projected decrease in water use (caused by stomatal closure) by PRD crops should decrease crop yield. However, stomatal conductance is more responsive to increased xylem ABA concentration than are changes in photosynthesis (Fig. 13), allowing considerable decreases in stomatal conductance to occur with minimal impacts on CO_2 assimilation. Also, plants grown with PRD may allocate proportionally more dry matter to the harvested portion of the crop (increasing the harvest index). Much remains to be learnt about whether (and how) drought-induced changes in plant hormone status alter biomass allocation within the plant. However, early indications from PRD trials in different parts of the world (7) suggest that agronomic exploitation of plant long-distance signalling may allow more efficient water use in agriculture.

Ackowledgements

We thank BBSRC for a ROPA award for continued support of research on soil drying.

References

1. Allan AC, Fricker MD, Ward JL, Beale MH, Trewavas AJ (1994) Two transduction pathways mediate rapid effects of abscisic acid in *Commelina* guard cells. Plant Cell 6: 1319-1328

2. Bacon MA, Wilkinson S, Davies WJ (1998) pH-regulated leaf cell expansion in droughted plants is abscisic acid dependent. Plant Physiol 118: 1507-1515
3. Bauly JM, Sealy IM, Macdonald H, Brearley J, Droge S, Hillmer S, Robinson DG, Venis MA, Blatt MR, Lazarus CM, Napier RM (2000) Overexpression of auxin-binding protein enhances the sensitivity of guard cells to auxin. Plant Physiol 124: 1229-1238
4. Borel C, Frey A, Marion-Poll A, Simmoneau T, Tardieu F (2001) Does engineering abscisic acid biosynthesis in *Nicotiana plumbaginifolia* modify stomatal response to drought ? Plant, Cell Env 24: 477-489
5. Clark DG, Gubrium EK, Barrett JE, Nell TA, Klee HJ (1999) Root formation in ethylene-insensitive plants. Plant Physiol 121: 53-59
6. Correia MJ, Pereira JS, Chaves MM, Rodrigues ML, Pacheco CA (1995) ABA xylem concentrations determine maximum daily leaf conductance of field-grown *Vitis vinifera* L. plants. Plant, Cell Env 18: 511-521
7. Davies WJ, Wilkinson S, Loveys B (2002) Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. New Phytol 153: 449-460
8. Davies WJ, Zhang J (1991) Root signals and the regulation of growth and development of plants in drying soil. Ann Rev Plant Physiol Plant Mol Biol 42: 55-76
9. De Silva DLR, Hetherington AM, Mansfield TA (1985) Synergism between calcium ions and abscisic acid in preventing stomatal opening. New Phytol 100: 473-482
10. Dodd IC (2003) Hormonal interactions and stomatal responses. J Plant Growth Reg 22, 32-46
11. Freundl E, Steudle E, Hartung W (1998) Water uptake by roots of maize and sunflower affects the radial transport of abscisic acid and its concentration in the xylem. Planta 207: 8-19
12. Gehring CA, McConchie RM, Venis MA, Parish RW (1998) Auxin-binding-protein antibodies and peptides influence stomatal opening and alter cytoplasmic pH. Planta 205: 581-586
13. Hansen H, Dörffling K (2003) Root-derived trans-zeatin riboside and abscisic acid in drought-stressed and rewatered sunflower plants: interaction in the control of leaf diffusive resistance? Funct Plant Biol 30: 365-375
14. Hartung W, Slovik S (1991) Physicochemical properties of plant growth regulators and plant tissues determine their distribution and redistribution - stomatal regulation by abscisic acid in leaves. New Phytol 119: 361-382
15. Holbrook NM, Shashidhar VR, James RA, Munns R (2002) Stomatal control in tomato with ABA-deficient roots: response of grafted plants to soil drying. J Exp Bot 53: 1503-1514
16. Honour SJ, Webb AAR, Mansfield TA (1995) The responses of stomata to abscisic acid and temperature are interrelated. Proc Royal Soc London B 259: 301-306
17. Hussain A, Black CR, Taylor IB, Roberts JR (1999) Soil compaction. A role for ethylene in regulating leaf expansion and shoot growth in tomato? Plant Physiol 121:1227-1237
18. Incoll LD, Jewer PC (1987) Cytokins and stomata *In* E Zeiger, GD Farquhar , IR Cowan, *eds*, Stomatal Function, Stanford University Press, Stanford, pp 281-292
19. Jackson MB (1993) Are plant hormones involved in root to shoot communication ? Adv Bot Res 19: 103-187.
20. Jia WS, Zhang JH (1997) Comparison of exportation and metabolism of xylem-delivered ABA in maize leaves at different water status and xylem sap pH. Plant Growth Reg 21, 43-49
21. Loveys BR (1984) Diurnal changes in water relations and abscisic acid in field-grown *Vitis vinifera* cultivars. III. The influence of xylem-derived abscisic acid on leaf gas exchange. New Phytol 98: 563-573
22. Mansfield TA, Davies WJ (1981) Stomata and stomatal mechanisms. *In* LG Paleg, D Aspinall, *eds*, The Physiology and Biochemistry of Drought Resistance, Academic Press,

Sydney, pp 315-346
23. McAinsh MR, Brownlee C, Hetherington AM (1990) Abscisic acid-induced elevation of guard cell cytosolic Ca^{2+} precedes stomatal closure. Nature 343: 186-188.
24. Merritt F, Kemper A, Tallman G (2001) Inhibitors of ethylene synthesis inhibit auxin-induced stomatal opening in epidermis detached from leaves of *Vicia faba* L. Plant Cell Physiol 42: 223-230.
25. Morgan PW, He C-J, De Greef JA, De Proft MP (1990) Does water deficit stress promote ethylene synthesis by intact plants ? Plant Physiol 94: 1616-1624.
26. Munns R, Cramer GR (1996) Is coordination of leaf and root growth mediated by abscisic acid? Opinion. Plant Soil 185: 33-49
27. Munns R, King RW (1988) Abscisic acid is not the only stomatal inhibitor in the transpiration stream of wheat plants. Plant Physiol 88: 703-708
28. Netting AG (2000) pH, abscisic acid and the integration of metabolism in plants under stressed and non-stressed conditions: cellular responses to stress and their implication for plant water relations. J Exp Bot 51: 147-158
29. Passioura JB (1977) Grain yield, harvest index and water use of wheat. J Aust Inst Agric Sci 43: 117-121
30. Passioura JB, Condon AG, Richards RA (1993) Water deficits, the development of leaf area and crop productivity *In* JAC Smith & HG Griffiths, *eds*, Water Deficits : Plant responses from cell to community, Bios Scientific Publishers, Oxford, pp 253-264
31. Poorter H, Nagel O (2000) The role of biomass allocation in the growth response to different levels of light, CO_2, nutrients and water : a quantitative review. Aust J Plant Physiol 27: 595-607
32. Pospisilova J, Synkova H, Machackova I, Catsky J (1998) Photosynthesis in different types of transgenic tobacco plants with elevated cytokinin content. Biol Plant 40: 81-89
33. Quarrie SA (1981) Genetic variability and heritability of drought-induced abscisic acid accumulation in spring wheat. Plant, Cell Env 4: 147-151
34. Quarrie SA (1991) Implications of genetic differences in ABA accumulation for crop production. *In* WJ Davies, HG Jones, *eds*, Abscisic Acid : Physiology and Biochemistry, Bios Scientific Publishers, Oxford, pp 137-152
35. Radin JW, Parker LL, Guinn G (1982) Water relations of cotton plants under nitrogen deficiency. V. Environmental control of abscisic acid accumulation and stomatal sensitivity to abscisic acid. Plant Physiol 70: 1066-1070
36. Rodriguez JL, Davies WJ (1982) The effects of temperature and ABA on stomata of *Zea mays* L. J Exp Bot 33: 977-987
37. Saab IN, Sharp RE, Pritchard J, Voetberg GS (1990) Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. Plant Physiol 93: 1329-1336
38. Sanguineti MC, Tuberosa R, Landi P, Salvi DS, Maccaferri M, Casarini E, Conti S (1999) QTL analysis of drought related traits and grain yield in relation to genetic variation for leaf abscisic acid concentration in field-grown maize. J Exp Bot 50: 1289-1297
39. Sauter A, Dietz KJ, Hartung W (2002) A possible stress physiological role of abscisic acid conjugates in root-to-shoot signalling. Plant, Cell Env 25: 223-228
40. Schurr U, Schulze E-D (1995) The concentration of xylem sap constituents in root exudate, and in sap from intact, transpiring castor bean plants (*Ricinus communis* L.). Plant, Cell Env 18, 409-420
41. Sharp RE, Davies WJ (1979) Solute regulation and growth by roots and shoots of water-stressed maize plants. Planta 147: 43-49
42. Sharp RE, Wu Y, Voetberg GS, Saab IN, LeNoble ME (1994) Confirmation that abscisic acid accumulation is required for maize primary root elongation at low water potentials. J Exp Bot 45: 1743-1751
43. Sharp RE, LeNoble ME (2002) ABA, ethylene and the control of shoot and root growth

under water stress. J Exp Bot 53: 33-37
44. Snaith PJ, Mansfield TA (1982a) Control of the CO_2 responses of stomata by indol-3ylacetic acid and abscisic acid. J Exp Bot 33: 360-365
45. Socias X, Correia MJ, Chaves M, Medrano H (1997) The role of abscisic acid and water relations in drought responses of subterranean clover. J Exp Bot 48: 1281-1288.
46. Tardieu F, Davies WJ (1992) Stomatal response to abscisic acid is a function of current plant water status. Plant Physiol 98: 540-545
47. Trejo CL, Davies WJ, Ruiz LMP (1993) Sensitivity of stomata to abscisic acid. An effect of the mesophyll. Plant Physiol 102: 497-502
48. Van Volkenburgh E, Davies WJ (1983) Inhibition of light-stimulated leaf expansion by abscisic acid. J Exp Bot 34: 835-845.
49. Wilkinson S, Clephan AL, Davies WJ (2001) Rapid low temperature-induced stomatal closure occurs in cold-tolerant *Commelina communis* leaves but not in cold-sensitive tobacco leaves, via a mechanism that involves apoplastic calcium but not abscisic acid. Plant Physiol 126: 1566-1578
50. Wilkinson S, Corlett JE, Oger L, Davies WJ (1998) Effects of xylem pH on transpiration from wild-type and *flacca* tomato leaves : a vital role for abscisic acid in preventing excessive water loss even from well-watered plants. Plant Physiol 117: 703-709
51. Wilkinson S, Davies WJ (1997) Xylem sap pH increase: A drought signal received at the apoplastic face of the guard cell which involves the suppression of saturable ABA uptake by the epidermal symplast. Plant Physiol 113: 559-573
52. Wolf O, Jeschke WD, Hartung W (1990) Long distance transport of abscisic acid in NaCl-treated intact plants of *Lupinus albus*. J Exp Bot 41: 593-600
53. Wright STC, Hiron RWP (1969) (+) abscisic acid, the growth inhibitor induced in detached wheat leaves by a period of wilting. Nature 224: 719-720
54. Zhang J, Davies WJ (1989) Sequential response of whole plant water relations to prolonged soil drying and the involvement of xylem sap ABA in the regulation of stomatal behaviour of sunflower plants. New Phytol 113: 167-174
55. Zhang J, Davies WJ (1990) Does ABA in the xylem control the rate of leaf growth in soil-dried maize and sunflower plants ? J Exp Bot 41: 1125-1132
56. Zhang JH, Zhang XP, Liang JS (1995) Exudation rate and hydraulic conductivity of maize roots are enhanced by soil drying and abscisic acid treatment. New Phytol 131: 329-336
57. Zhang SQ, Outlaw WH, Aghoram K (2001) Relationship between changes in the guard cell abscisic acid content and other stress related physiological parameters in intact plants. J Exp Bot 52: 301-308

E3A. The Flowering Hormone - Florigen: A Protein Hormone

Brian G. Ayre
Department of Biological Sciences, University of North Texas, Denton, Texas, 76203 USA. E-mail: brian.ayre@unt.edu

INTRODUCTION

Phytohormones are classically defined as small, mobile compounds that, in trace quantities, influence growth and development in tissues distant from the sites of synthesis (11). The first five hormones discovered, auxin, cytokinin, gibberellins, abscisic acid and ethylene, have been joined more recently by other compounds that more-or-less fit the classical definition, such as polyamines, brassinosteroids, jasmonic acid, salicylic acid, and peptide hormones. For many of these, the physiological impacts of the hormone were known for years or even decades prior to identifying the active compound (11). Florigen, the flowering hormone, is inarguably the most celebrated example of a hormone-like signal for which the physiological effects are represented by vast numbers of publications spanning many decades, but whose chemical nature was only revealed in 2007 (10, 41). The fundamental concept of a flowering hormone goes back to experiments by Julius Sachs (published in 1865) showing that illuminated leaves of Nasturtium (*Tropaeoloum majus*) and Morning Glory (*Ipomoea purpurea)* promoted flowering in darkened shoots (45). The term "florigen" was coined by Mikhail Chailakhyan in 1937 for a graft-transmissible, hormone-like substance that is produced in leaves in response to photoperiod, and that migrates to apical meristems to induce reproductive growth (*i.e.,* flowering) (8). However, despite extensive effort, identifying the active compound was hindered largely due to limitations in technology and to a bias that it would be similar to canonical phytohormones (5, 8, 13, 44). Recent progress in dissecting the genetics and molecular biology of flowering has culminated in identifying a 175 amino-acid protein encoded by *FLOWERING LOCUS T* (*FT* [1]) as florigen, or to be at least a predominant component of the florigen signal (22, 42). As a 19.8 kDa protein, florigen differs from canonical

[1] Abbreviations: *CO*, *CONSTANS*; *FT*, *FLOWERING LOCUS T*; *FD*, *FD*; *AP1, APETALA 1*; *SOC1*, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*; *LFY, LEAFY; PEBP, PHOSPHATIDYLETHANOLAMINE-BINDING PROTEIN*; *SP*, *SELF PRUNING*; *SFT, SINGLE FLOWER TRUSS; TFL1, TERMINAL FLOWER 1*

phytohormones by virtue of its size and transport, which appears to be entirely within the symplast without extracellular steps. In hindsight, it is clear why classical approaches of applying extracts from flowering plants to leaves or buds of non-flowering plants were not fruitful in identifying the active compound.

PHOTOPERIODISM AND THE TRANSITION TO FLOWERING

The transition to flowering occurs when vegetative meristems cease indeterminate production of leaves, axillary buds, nodes, and internodes, and instead produce determinate flowers. This transition is fundamental to producing offspring and must be coordinated with seasonal change to optimize lineage survival. Many plants monitor seasonal progression through changes in photoperiod - the light/dark cycles that occur over a twenty-four hour period. The first comprehensive model on flowering in response to photoperiod was published in 1920 by Henry Allard and Wightman Garner of the United States Department of Agriculture (14). They observed that Maryland Mammoth, a new tobacco (*Nicotiana tabacum*) variety, grew to five meters without flowering during the summer, but in the greenhouse during winter, flowered profusely when less than one meter tall. Related experiments with soybean brought them to the conclusion that Maryland Mammoth tobacco and soybean transitioned to reproductive growth when days were shorter than a critical length, and referred to these as short-day plants. They further established that other plants flowered when days were longer than a critical period, and referred to these as long-day plants. We now understand that it is the dark period that is measured, such that long nights induce short-day plants to flower and short nights induce long-day plants to flower. Plants that do not exhibit photoperiodism in their flowering response are day-neutral plants (14).

However, apical meristems are commonly covered by protective juvenile leaves and are thus poor tissues for detecting changes in light conditions. Work published in 1934 established that leaves perceive photoperiod and that a signal is transmitted to the bud to initiate reproductive growth. Chailakhyan tested this directly by grafting plants that were not flowering to those that were flowering, and maintaining the chimera under non-inductive conditions. Flowering was observed in the non-induced stems, indicating that a signal crossed the graft junction. Subsequent experiments showed that transport was in the phloem. Grafts between different species showed that the signal is conserved across a broad range of angiosperms, irrespective of whether they are short-day, long-day, or day-neutral plants. For example, reciprocal grafts between short-day tobacco and long-day black henbane (*Hyoscamus niger*) caused the black henbane to flower if the tobacco was stimulated with short days, and conversely, the tobacco flowered if the black henbane was induced with long days (5, 8, 44).

Attempts to define the chemical nature of florigen were unsuccessful in part because it was assumed that florigen was a small, hormone-like molecule that could mediate a response from the apoplast. Prior to the advent of molecular biology, phytohormone activity was typically assessed by applying extracts exogenously and recording the response in a bioassay. These techniques are still very useful, but knowing now that florigen is a phloem-mobile protein, it is apparent why they were not successful in the specific case of the flowering hormone: it is unlikely that a protein, if it retained activity during extract preparation, would cross the plasma membrane to enter the phloem for long-distance transport.

THE GENETICS AND MOLECULAR BIOLOGY OF FLORIGEN

Advances in genetics and molecular biology, and adoption of Arabodopsis *thaliana* as a model plant, provided new tools for identifying florigen. Arabidopsis is particularly useful because of the vast genetic resources available and because it is a facultative long-day plant (synonymous with quantitative long-day plant), meaning that the transition to flowering is accelerated in long days but will occur eventually in short days after an extended vegetative phase. In Arabidopsis, a series of mutants that flower late under long days but are identical to wild type under short days define a genetic pathway that promotes flowering in long-day photoperiods. One of these genes, *CONSTANS* (*CO*), encodes a transcriptional regulator that coordinates external light stimuli with the plant's internal circadian rhythms (35, 38). *CO* mRNA levels oscillate with a circadian rhythm. They begin to accumulate approximately ten hours after "dawn" and then drop back down shortly after "dawn" the following day (Figure 1). Importantly, *CO* mRNA accumulation initiates during the illuminated period in long days but begins accumulating during the dark period in short days. Light regulates CO activity by stabilizing the protein: CO protein is stable while plants are illuminated, but in darkness, CO interacts with COP1 (encoded by *CONSTITUTIVE PHOTOMORPHOGENIC 1*), which acts as an E3 ubiquitin-ligase and targets CO for ubiquitin-mediated degradation (20, 28). This combination of transcriptional regulation by circadian rhythms and post-translational regulation by light provides an elegant mechanism that ensures that active CO protein is available only during long days. This is an example of an external coincidence model for response regulation (42), since the light stimulus must coincide with a particular phase of the circadian oscillations to manifest an effect. In rice (*Oryza sativa*), *HEADING DATE 1* (*Hd1*) is the *CO* ortholog and, along with other factors, participates in controlling the transition to reproductive growth (40). Rice however is a short-day plant and a well-supported model for how *Hd1* promotes flowering in short days but prevents flowering in long days has yet to emerge (42).

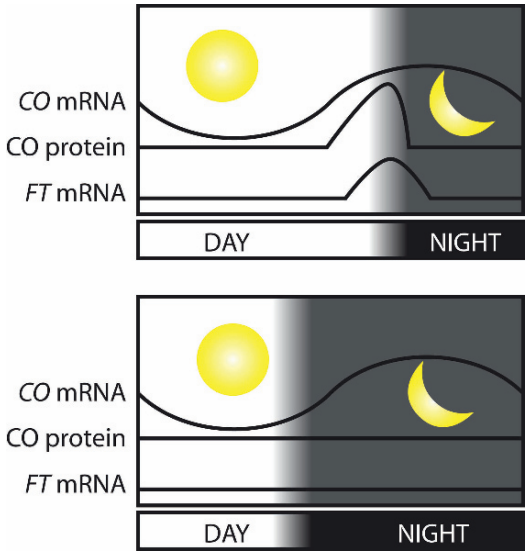

Figure 1. External coincidence model for integrating the circadian clock with photoperiod via *CO* in the flowering response of the facultative long-day plant, *Arabidopsis thaliana*. Top: In long-day conditions, *CO* mRNA levels oscillate with a circadian rhythm, beginning to accumulate eight to ten hours after dawn and dropping down again shortly after dawn the following day. CO protein is translated, and is stable while the plant is illuminated, but rapidly degrades in darkness. *FT* is activated by CO, and transcribed only while CO is present. Bottom: In short-day conditions, *CO* mRNA begins accumulating after dusk and translated protein is rapidly degraded. Consequently, expression of *FT* is not induced.

CO encodes a transcriptional regulator and is expressed predominantly in the vascular tissues of leaves and stems (39). In transgenic Arabidopsis, *CO* expression in the phloem from the *AtSUC2* promoter, or specifically in the companion cells of minor veins from the *CmGAS1* promoter, is sufficient to overcome *co* mutations, demonstrating that expression in the phloem is sufficient to generate the florigen signal (3, 4). In addition, using these transgenic plants in grafts with *co* mutant scions demonstrated that the *CO*-derived signal is graft transmissible. In long-day conditions, *FLOWERING LOCUS T* (*FT*) appears to be the sole target for activation by CO (43), which is consistent with earlier genetic evidence that *FT* is in the photoperiodic-flowering pathway (*i.e., ft* mutants flower late in long days and transgenic plants overexpressing *FT* flower early in short days), and that it functions downstream of *CO* (21). *FT* is naturally expressed in leaf-vascular tissues in a pattern more restrictive than observed for *CO* (39), and *FT* expressed from either the *AtSUC2* or *CmGAS1* promoter overcomes a *ft* mutation, demonstrating that *FT* expression in the phloem is sufficient to generate the florigenic signal (10).

The movement of RNA and protein in the phloem is well documented (29), and it was suggested that *FT* RNA moved through the phloem to induce flowering at the meristem. This was subsequently established as erroneous (6), and there is instead substantial evidence that it is the protein moves long-

distance to promote flowering [readers are referred to other articles for research addressing the movement of *FT* RNA (42)]. FT protein has been detected in phloem exudates from several species (2, 15, 26). The green fluorescent protein, which has a greater molecular weight than FT, also readily enters the phloem (18) showing that the size of FT is not a barrier to movement. Evidence that FT movement is required for photoperiodic flowering was obtained in experiments where entry into the translocation stream was blocked by either incorporating a strong nuclear-localization signal or fusing it to larger proteins to prevent it from passing into sieve elements through plasmodesmata. In these experiments, early flowering was prevented, and it was shown that it was the inability of FT to move long-distance that prevented flowering (10, 19, 30).

FT is one of seven genes in Arabidopsis that contain a core motif of phosphatidylethanolamine-binding proteins (PEBP). In animals, PEBPs participate in signaling by mediating protein-protein interactions, but in plants their biochemical activity is unknown. Using genetic suppressor screens and yeast two-hybrid interaction screens, it was established that FT protein interacts with a bZIP transcription factor encoded by *FD*[2] (1, 43). Plants with *fd* mutations flower late in long days, showing genetically that *FD* is a component of the photoperiodic flowering pathway. It was further established that as a complex, the two proteins localize to the nuclei of plant cells and activate the floral meristem identity gene *APETALA1* (*AP1*) by binding to its promoter (1, 43). The FT/FD complex is also implicated in activating *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), *SEPALLATA* 3 (*SEP3*), and *FRUITFUL* (*FUL*), all of which participate in controlling floral meristem identity (31, 42). The product of *SOC1*, for example, directly interacts with the product of *AGAMOUS-LIKE 24* (*AGL24*) to promote expression of another floral meristem identity gene, *LEAFY* (*LFY*) (24, 27). Consistent with the FT/FD complex promoting gene expression in the meristem, *FD* expression is strongest in the shoot apex whereas *FT*, as described above, is expressed in leaf veins and the protein is transported to the meristem.

The prevailing model for FT protein functioning as the flowering hormone in Arabidopsis (Figure 2) is therefore that FT protein is produced in source-leaf veins in response to transcriptional activation by CO protein. CO protein is only present in long-day conditions when the increase in transcript, regulated by circadian oscillations, coincides with light-mediated protein stabilization (or perhaps more accurately, the lack of dark-mediated degradation). This regulation also explains the "night-break" phenomenon, in which a short exposure to red light in the middle of an otherwise long night is interpreted as a short night, since mRNA levels are high throughout the night and brief illumination would stabilize CO protein sufficiently to activate *FT* transcription. FT protein produced in phloem companion cells

[2] *FD* is the full name of the gene, and should not be confused with *FLOWERING LOCUS D* (*FLD*)

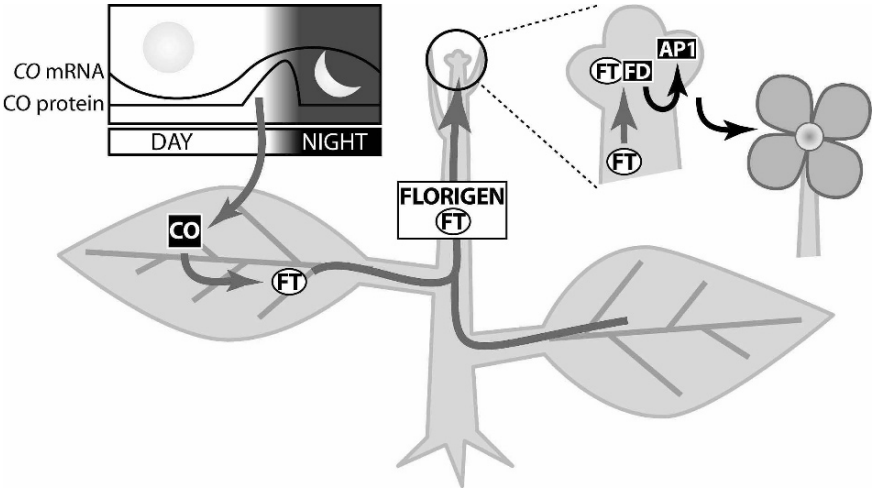

Figure 2. (Color plate page CP17) The prevailing model for FT protein function as florigen, the flowering hormone. CO protein accumulates in the companion cells of leaf phloem under long-day conditions, and triggers the transcription and subsequent translation of FT. FT protein probably diffuses through plasmodesmata into the sieve elements for long-distance transport, and then most likely moves cell to cell, also by diffusion through plasmodesmata, to the meristem, where it interacts with FD protein. The FT/FD complex translocates to the nucleus, and triggers expression of several genes, including the floral meristem identity gene, *AP1*.

needs to enter the sieve elements for long-distance transport, and this is probably, but not certainly, by passive diffusion through plasmodesmata. Cell-to-cell movement from the termini of the sieve tubes to the meristem is similarly most likely by diffusion through plasmodesmata. Once in the meristem, FT associates with FD, translocates to the nuclei and initiates a cascade of gene expression that ultimately results in the development of flowers.

IS FT THE UNIVERSAL FLORIGEN SIGNAL?

Classical research carried out by Chailakhyan and others showed that if plants were sufficiently compatible to be grafted, they were sufficiently compatible for graft transmission of flowering signals (5, 8, 44). Through molecular biology, the ability to express an *FT* ortholog from one species in a very distant species has greatly expanded the test for the universality of the florigen signal. For example, overexpressing *HEADING DATE 3a* (*Hd3a)*, the *FT* ortholog of rice, in Arabidopsis in short days was as effective at promoting early flowering as overexpressing *FT* (23). Additionally, *FT* orthologs from a spectrum of eudicots have been expressed in Arabidopsis and induced early flowering, and *FT* orthologs expressed in species other than Arabidopsis have similarly promoted early flowering. The *FT* ortholog of tomato (*Solanum lycopersicum*) is *SINGLE FLOWER TRUSS* (*SFT*) and

tomato plants overexpressing *SFT* flower early and *sft* mutants flower late (25). *SFT* from tomato (an Astrid) promoted early flowering in Arabidopsis (a Rosid) in short-day conditions when expressed from a leaf-specific promoter. In addition, tomato plants overexpressing *SFT* grafted to short-day Maryland Mammoth tobacco caused the tobacco to flower under non-inductive, long-day conditions (25). These experiments are especially satisfying, since they bring the florigen story full-circle back to the photoperiod work of Garner and Allard, and the interspecific grafts of Chailakhyan. It is particularly noteworthy that *FT* orthologs, when expressed in heterologous species and uncoupled from photoperiodic control, promote early flowering whether the host is a long-day, short-day, or day-neutral species. FT protein therefore does appear to be the universal flowering hormone, the abundance of which is regulated by upstream factors (*e.g., CO* orthologs). It is the regulation of these upstream factors that have evolved to suit the reproductive needs of the individual species.

IS FT PROTEIN MORE THAN FLORIGEN?

Two additional findings warrant discussion on FT protein as the flowering hormone. First, photoperiod is one of two principal external stimuli contributing to the transition to flowering, with the second being vernalization (the prolonged exposure to cold temperatures). Vernalization is required among winter annuals, including many accessions of Arabidopsis, to signal the passing of winter and the onset of spring (31). The main target for vernalization is *FLOWERING LOCUS C* (*FLC*), which encodes a transcription factor that represses expression of the main photoperiodic genes *FT*, *FD*, and *SOC1* (36), such that prior to winter, photoperiodic flowering is blocked. *FLC* expression decreases during prolonged cold treatment, and remains low when temperatures increase (32, 33). *FLC* is most strongly expressed in meristems to block expression of *FD* and *SOC1*, but is also expressed in leaves to block *FT* expression (36). Since the repression of *FT* needs to be removed by vernalization for photoperiodic flowering, FT acts as an integrator of the photoperiod and vernalization pathways, in addition to acting as the flowering hormone (45).

The second finding that warrants discussion is the role of FT protein as a general growth regulator. *FT* acts antagonistically to another member of the PEBP-gene family, *TERMINAL FLOWER 1* (*TFL1*). *TFL1* expression maintains the indeterminate vegetative nature of inflorescence meristems (34) while *FT* promotes differentiation to determinate floral meristems. In Arabidopsis, *TFL1* is expressed in the inflorescence apical meristem and restricts expression of *LFY* to the lateral meristems, such that the inflorescence apical meristem of the raceme remains indeterminate and lateral meristems form flowers (9, 34). FT and TFL1 proteins are 60% identical, and a single amino acid change is sufficient to convert TFL1 to a protein with FT-like properties (promotes flowering rather than maintaining

vegetative growth), and *vice versa* (16). Because of the high sequence similarity in the PEBP-gene family, it is not surprising that FT and TFL1 can both interact with the FD protein (1, 43), and suggests a model in which the two compete for FD binding to control whether the meristem is indeterminate or determinate. If this is accurate, FT would be a direct inhibitor of vegetative growth in addition to a flowering hormone.

In tomato, which has a perennial growth habit, *SFT* expression levels are balanced with *SELF PRUNING* (*SP*), the tomato ortholog of *TFL1*, to influence diverse aspects of plant architecture (37). High ratios of *SFT* to *SP* activity reduce vegetative growth in both the main stem and subsequent sympodial branches by promoting the transition of the indeterminate, vegetative meristems to determinate inflorescence meristems; reduce compound leaf complexity to give rise to leaves with fewer leaflets and less lobing; and decrease radial expansion of stems (37). Furthermore, in perennial trees, specifically poplar (*Populus sp.)*and citrus (*Citrus sp.*), constitutive *FT* expression accelerates the transition to flowering by shortening the juvenile phase and influencing the seasonal vegetative and reproductive growth cycles characteristic of perennials (7, 12, 17). Available evidence therefore indicates that florigen regulates both vegetative and reproductive growth cycles, and also functions as a more general growth hormone with broader influence than strictly as a flowering regulator.

Acknowledgements

The author thanks Róisín C M^{c}Garry (University of North Texas, USA) for reviewing the manuscript.

References

1. Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science 309: 1052–1056
2. Aki T, Shigyo M, Nakano R, Yoneyama T, Yanagisawa S (2008) Nano scale proteomics revealed the presence of regulatory proteins including three FT-Like proteins in phloem and xylem saps from rice. Plant Cell Physiol 49: 767–790
3. An HL, Roussot C, Suarez-Lopez P, Corbesler L, Vincent C, Pineiro M, Hepworth S, Mouradov A, Justin S, Turnbull C, Coupland G (2004) CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. Development 131: 3615–3626
4. Ayre BG, Turgeon R (2004) Graft transmission of a floral stimulant derived from *CONSTANS*. Plant Physiol 135: 2271–2278
5. Bernier G (1988) The control of floral evocation and morphogenesis. Annu Rev Plant Physiol Plant Mol Biol 39: 175–219
6. Bohlenius H, Eriksson S, Parcy F, Nilsson O (2007) Retraction of Huang, T, et al., The mRNA of the Arabidopsis gene FT moves from leaf to shoot apex and induces flowering. Science 316: 367b
7. Bohlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH, Nilsson O (2006) CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312: 1040–1043
8. Chailakhyan MK (1968) Internal factors of plant flowering. Annu Rev Plant Physiol 19: 1–37

9. Conti L, Bradley D (2007) TERMINAL FLOWER1 is a mobile signal controlling Arabidopsis architecture. Plant Cell 19: 767–778
10. Corbesier L, Vincent C, Jang SH, Fornara F, Fan QZ, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C, Coupland G (2007) FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science 316: 1030–1033
11. Davies PJ (2004) The plant hormones: their nature, occurence and function. In PJ Davies, ed, Plant Hormones: Biosynthesis, Signal Transduction, Action! 3 edn. Kluwer Academic Publishers, Dordrecht, pp 1–15,
12. Endo T, Shimada T, Fujii H, Kobayashi Y, Araki T, Omura M (2005) Ectopic expression of an *FT* homolog from citrus confers an early flowering phenotype on trifoliate orange (*Poncirus trifoliata* L. Raf.). Transgenic Res 14: 703–712
13. Evans LT (1971) Flower induction and the florigen concept. Annu Rev Plant Physiol 22: 365–394
14. Garner WW, Allard HA (1920) Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. J Agric Res 18: 553–606
15. Giavalisco P, Kapitza K, Kolasa A, Buhtz A, Kehr J (2006) Towards the proteome of Brassica napus phloem sap. Proteomics 6: 896–909
16. Hanzawa Y, Money T, Bradley D (2005) A single amino acid converts a repressor to an activator of flowering. Proc Natl Acad Sci U S A 102: 7748–7753
17. Hsu CY, Liu YX, Luthe DS, Yuceer C (2006) Poplar FT2 shortens the juvenile phase and promotes seasonal flowering. Plant Cell 18: 1846–1861
18. Imlau A, Truernit E, Sauer N (1999) Cell-to-cell and long-distance trafficking of the green fluorescent protein in the phloem and symplastic unloading of the protein into sink tissues. Plant Cell 11: 309–322
19. Jaeger KE, Wigge PA (2007) FT protein acts as a long-range signal in Arabidopsis. Curr Biol 17: 1050–1054
20. Jang S, Marchal V, Panigrahi KCS, Wenkel S, Soppe W, Deng XW, Valverde F, Coupland G (2008) Arabidopsis COP1 shapes the temporal pattern of CO accumulation conferring a photoperiodic flowering response. EMBO J 27: 1277–1288
21. Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D (1999) Activation tagging of the floral inducer *FT*. Science 286: 1962–1965
22. Kobayashi Y, Weigel D (2007) Move on up, it's time for change – mobile signals controlling photoperiod-dependent flowering. Genes Dev 21: 2371–2384
23. Kojima S, Takahashi Y, Kobayashi Y, Monna L, Sasaki T, Araki T, Yano M (2002) *Hd3a*, a rice ortholog of the Arabidopsis *FT* gene, promotes transition to flowering downstream of *Hd1* under short-day conditions. Plant Cell Physiol 43: 1096–1105
24. Lee J, Oh M, Park H, Lee I (2008) SOC1 translocated to the nucleus by interaction with AGL24 directly regulates *LEAFY*. Plant J 55: 832–843
25. Lifschitz E, Eviatar T, Rozman A, Shalit A, Goldshmidt A, Amsellem Z, Alvarez JP, Eshed Y (2006) The tomato *FT* ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. Proc Natl Acad Sci U S A 103: 6398–6403
26. Lin M-K, Belanger H, Lee Y-J, Varkonyi-Gasic E, Taoka K-I, Miura E, Xoconostle-Cazares B, Gendler K, Jorgensen RA, Phinney B, Lough TJ, Lucas WJ (2007) FLOWERING LOCUS T protein may act as the long-distance florigenic signal in the cucurbits. Plant Cell 19: 1488–1506
27. Liu C, Chen H, Er HL, Soo HM, Kumar PP, Han JH, Liou YC, Yu H (2008) Direct interaction of AGL24 and SOC1 integrates flowering signals in Arabidopsis. Development 135: 1481–1491

28. Liu LJ, Zhang YC, Li QH, Sang Y, Mao J, Lian HL, Wang L, Yang HQ (2008) COP1-mediated ubiquitination of CONSTANS is implicated in cryptochrome regulation of flowering in Arabidopsis. Plant Cell 20: 292–306
29. Lough TJ, Lucas WJ (2006) Integrative plant biology: Role of phloem long-distance macromolecular trafficking. Annu Rev Plant Biol 57: 203–232
30. Mathieu J, Warthmann N, Kuttner F, Schmid M (2007) Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. Curr Biol 17: 1055–1060
31. Michaels SD (2009) Flowering time regulation produces much fruit. Curr Opin Plant Biol 12: 75–80
32. Michaels SD, Amasino RM (2000) Memories of winter: vernalization and the competence to flower. Plant Cell Environ 23: 1145–1153
33. Michaels SD, Himelblau E, Kim SY, Schomburg FM, Amasino RM (2005) Integration of flowering signals in winter-annual Arabidopsis. Plant Physiol 137: 149–156
34. Prusinkiewicz P, Erasmus Y, Lane B, Harder LD, Coen E (2007) Evolution and development of inflorescence architectures. Science 316: 1452–1456
35. Putterill J, Robson F, Lee K, Simon R, Coupland G (1995) The *CONSTANS* gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell 80: 847–857
36. Searle I, He YH, Turck F, Vincent C, Fornara F, Krober S, Amasino RA, Coupland G (2006) The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. Genes Dev 20: 898–912
37. Shalit A, Rozman A, Goldshmidt A, Alvarez JP, Bowman JL, Eshed Y, Lifschitz E (2009) The flowering hormone florigen functions as a general systemic regulator of growth and termination. Proc Natl Acad Sci U S A 106: 8392–8397
38. Suarez-Lopez P, Wheatley K, Robson F, Onouchi H, Valverde F, Coupland G (2001) *CONSTANS* mediates between the circadian clock and the control of flowering in Arabidopsis. Nature 410: 1116–1120
39. Takada S, Goto K (2003) *TERMINAL FLOWER2*, an Arabidopsis homolog of *HETEROCHROMATIN PROTEIN1*, counteracts the activation of *FLOWERING LOCUS T* by *CONSTANS* in the vascular tissues of leaves to regulate flowering time. Plant Cell 15: 2856–2865
40. Takahashi Y, Teshima KM, Yokoi S, Innan H, Shimamoto K (2009) Variations in Hd1 proteins, *Hd3a* promoters, and *Ehd1* expression levels contribute to diversity of flowering time in cultivated rice. Proc Natl Acad Sci U S A 106: 4555–4560
41. Tamaki S, Matsuo S, Wong HL, Yokoi S, Shimamoto K (2007) Hd3a protein is a mobile flowering signal in rice. Science 316: 1033–1036
42. Turck F, Fornara F, Coupland G (2008) Regulation and identity of florigen: *FLOWERING LOCUS T* moves center stage. Annu Rev Plant Biol 59: 573–594
43. Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D (2005) Integration of spatial and temporal information during floral induction in Arabidopsis. Science 309: 1056–1059
44. Zeevaart JAD (1976) Physiology of flower formation. Annu Rev Plant Physiol 27: 321–348
45. Zeevaart JAD (2008) Leaf-produced floral signals. Curr Opin Plant Biol 11: 541–547