CHAPTER 4

REGULATING PLANT WATER STATUS BY STOMATAL CONTROL

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Abstract: The regulation of gas exchange at the leaf level is a key factor for plant survival under a fluctuating environment (Buckley, 2005). In this context, control of stomatal opening and closure is the evolutionary solution to balance water loss with CO_2 uptake and yield. A decrease in leaf/root water potential resulting from soil drought is typically accompanied by an elevated level of abscisic acid (ABA), which is well established as a stress hormone (Davies et al., 2005). ABA is a central component in drought-stress sensing leading to efficient stomatal control, thereby avoiding deleterious yield losses during stress conditions. Depending on the crop species, or its growing environment, different strategies for yield-optimization need to be chosen (Araus et al., 2002; Chaves and Oliveira, 2004). ABA effects are modulated by the levels of and sensitivity to other hormones, in an interdependent network. Unraveling the complex regulatory mechanisms of stomatal control between hormones, second messengers, ion channels and other classes of implicated proteins will lead to new insights in how to tailor plants to take maximum advantage of the available natural resources (Li et al., 2006). Possible strategies are either to trigger an earlier stress response without a negative impact on yield, or to attenuate the plant stress response so that assimilation will increase. These desired traits can be brought about by overexpressing or downregulating the expression of specific genes involved in the complex and possibly redundant signaling network of stomatal responses.

This chapter provides an overview of the mechanisms behind the changes in stomatal movements under water-limiting conditions, including hormonal regulation and developmental influences

Keywords: drought stress; screening; stomata; transpiration

1. INTRODUCTION

Aperture control of the microscopic pores at the leaf surface helps a plant to achieve growth, while avoiding dehydration (Buckley, 2005). Environmental parameters including air humidity, light intensity, temperature, air movement and concentration of atmospheric CO_2, but also endogenous hormonal and hydraulic signals regulate

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stomatal movements, and influence stomatal development and density (Hetherington and Woodward, 2003; Woodward et al., 2002). This multi-parameter control maximizes net photosynthesis, and allows the plant to effectively use the available water. Water use efficiency (WUE) is a parameter defined as the CO_2 assimilation per unit water transpired, which serves as a measure of plant yield (Condon et al., 2004). Control of stomatal aperture is a rapid adaptive response, while the effects on stomatal development (including density) are a longer-term response affecting newly emerging leaves. The stomatal control mechanism can however not be seen separately from the control of water transport at the root/soil interface or in the vascular system (Buckley, 2005; Davies et al., 2005; Jones, 1998). Therefore, despite the fact that WUE is most often determined at the leaf level, only whole crop WUE provides a correct picture of the whole plant system (Chaerle et al., 2005; Condon et al., 2004). An overview of the mechanisms exploited by plants to control stomatal aperture will be given, and the methods available to reveal these responses will be discussed.

2. STOMATAL CONTROL MECHANISMS

Drought stress is the major cause of stomatal closure. As outlined in the introduction, maintaining adequate photosynthesis and thus avoiding yield loss under adverse conditions is the primary route towards crop improvement. Multiple players in the complex regulatory system of leaf gas exchange have been identified and will be discussed in this section. An overview is given in Figure 1. As mentioned above, ABA has a central role in drought responses (Li et al., 2006); however, at least 4 independent drought signaling pathways exist, two of which are ABA-independent (Riera et al., 2005; Valliyodan and Nguyen, 2006). Stomatal perception of ABA induces a sequence of events initiated by a cytosolic pH increase, and followed by the accumulation of reactive oxygen species (ROS), nitric oxide (NO) synthesis, increase in the concentration of cytosolic calcium ions, synthesis of lipid-derived second messengers, activation of protein kinases and phosphatases, and finally, modulation of ion channel activity (both at the vacuolar and plasma membrane) (Garcia-Mata and Lamattina, 2003; Himmelbach et al., 2003). In addition, ABA induces a variety of transcription factors (TFs) that regulate the expression of stress-related genes; however, ABA-independent induction of TFs is an equally crucial component of stress tolerance (Riera et al., 2005). Stability and processing of mRNAs of ABA-responsive genes (Lee et al., 2006; Zhang et al., 2006) represents another level of regulation of stomatal opening (Riera et al., 2006; Verslues et al., 2006), but this is covered in another chapter of this book.

It is important to note that not all plant species follow the same strategy of stomatal control. In general, a division is made into two categories: isohydric and non-isohydric plants (Jones and Tardieu, 1998). Isohydric plants stabilize their leaf water contents by adjusting stomatal aperture; non-isohydric plants have a much slower stomatal reaction to drought stress.

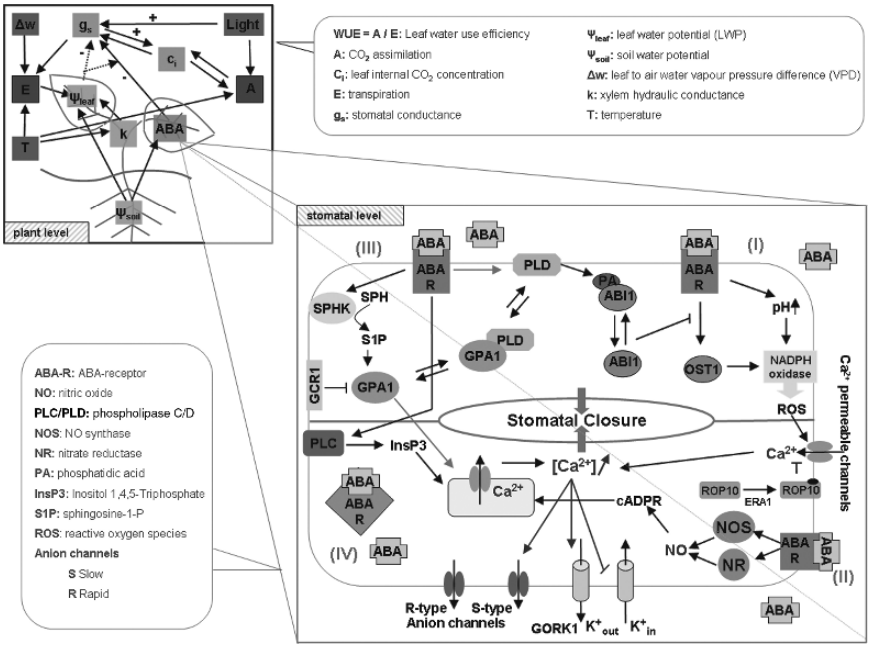

Figure 1. Schematic overview of stomatal aperture regulation

The top inset shows the response at the plant level to the varying environmental factors. The ABA response is worked out in detail in the main picture. ABA is either transported to the guard cells from the roots or vascular tissue, and binds to cell surface receptors (I-II-III). ABA synthesized in the guard cells is presumably recognized by an internal receptor (IV). Stomatal perception of ABA (I) leads to on accumulation of reactive oxygen species (ROS), through a cytosolic pH increase or mediated by the OST1 kinase. The ROS subsequently activate Ca2+ influx, leading to stomatal closure. The small G protein ROP10 is activated by the ERA farnesyltransferase and blocks Ca2+ influx. Stomatal perception of ABA (II) also results in the synthesis of NO, and the accumulation of cADPR (by Ca2+ activation of an ADP ribosyl cyclase). cADPR activates Ca2+ release from the vacuole, which amplifies the increases in cytoplasmic Ca2+, further promoting stomatal closure by modulation of the ion channels. Stomatal perception of ABA (III) liberates lipid-derived secondary messengers. Inositol-1,4,5- triphosphate (InsP3; derived from lipids through PLC activity), phosphatidic acid (PA; derived from ABA-activated PLD) and sphingosine-1-P (S1P). S1P induces stomatal closure in a process dependent on GPA1 (a Gα-subunit protein), whose function is inhibited by GCR1, a G protein coupled receptor-like protein. GPA1 interacts with PLD; free GPA1 inhibits stomatal opening. ABI1 is sequestered to the plasma membrane by PA, and thus cannot inhibit stomatal closure.

2.1. Signaling Factors (Secondary Messengers)

ABA-induced reactive oxygen species (ROS) production is catalyzed by two NADPH oxidases, encoded by AtrbohD and AtrbohF (Kwak et al., 2003). ROS production is induced through the action of the OST1 kinase (Mustilli et al., 2002) (also see below). A number of regulatory steps converge on the mobilization of $Ca^{2+}$from internal stores (Hetherington and Brownlee, 2004), followed by activation of ion

channels. Lipid-derived signaling components such as phytosphingosine-1-phosphate (Coursol et al., 2005) and phosphatidic acid (PA) produced by phospholipase D (Bargmann and Munnik, 2006; Mishra et al., 2006) function in a signaling cascade that negatively regulates the ABI1 kinase activity. ABI1 (abscisic acid insensitive) promotes stomatal opening (Merlot et al., 2001). A second pathway leads to nitric oxide (NO) accumulation. NO is the first signaling intermediate in the reactions leading to changes in Ca^{2+} levels (Garcia-Mata and Lamattina, 2003; Li et al., 2006).

The most extensively studied ABA response mutants are the above mentioned *abi1* and the enhanced response to ABA (era) mutant, which is affected in a farnesyltransferase subunit (Pei et al., 1998). Farnesyltransferases posttranslationally modify proteins by farnesylation, resulting in membrane anchoring. Loss of the ERA1 gene not only leads to a lower transpiration level, but also to a reduction of growth (Pei et al., 1998). However, downregulating the expression of the farnesyltransferase gene proved to be a successful approach in engineering drought tolerance (Wang et al., 2005)

2.2. Kinase and Protease Regulatory Systems

Protein phosphatases of the 2C class, such as the ABI1 and 2 proteins, were identified as negative regulators of stomatal opening. The phylogeny of PP2C's revealed that ABI1, ABI2 and HAB are closely related (Saez et al., 2004). The loss of function mutant *hab1* (hypersensitive to ABA1) had a transpiration level similar to the wild type, likely due to the complementation of phosphatase activity by ABI1 and/or 2. However, overexpression led to a higher sensitivity to drought stress. Conversely, plants with knock-out mutations in both *HAB1* and *ABI1* had a high tolerance to drought stress (Saez et al., 2006).

The Snf1 (yeast sucrose non-fermenting)-related kinase 2 (SnRK2) OST1/SNRK2E positively regulates ABA signal transduction (Belin et al., 2006). Loss of function mutants of *OST1* (open stomata) have an increased transpiration (Mustilli et al., 2002). In addition, over-expression of several other protein kinases (such as SRK2C (Umezawa et al., 2004) and NPK1 (Shou et al., 2004)) resulted in enhanced dehydration tolerance confirming their implication in the regulation of water usage.

2.3. Channels and Transporters

Stomatal guard cells are a model system to study the regulation of ion channels, central to the osmotic regulation of stomatal movements (Hetherington, 2001). It is therefore expected that modulation of ion channel activity will influence plant transpiration. The patch clamp technique in combination with a pharmacological approach is the method of choice to investigate activities of compounds that modulate ion channel activity. Such measurements at the single-cell level or at the microscopic scale (stomatal aperture determination) are complemented by measurements on the leaf or whole plant scale (transpiration).

Ion channels play a crucial role in changing the osmolyte content of guard cells and thus generate the driving force for turgor changes. Inward and outward

movements of the key electrolytes K^+, Ca^{2+}, and Cl^- are regulated by separate ion-specific channels. Potassium selective channels cause the increase or decrease in guard cell osmotic potential that drives the change in cell volume and the subsequent pore aperture change. Five classes of K^+-channels have been identified in plants, all of which share homology with the "Shaker" type of K+-channels in animal systems. The inward rectifying channels cause an inward transport of K+ (KAT, AKT), while outward rectifying channels cause the transport of K+ out of cells. The guard cell outward rectifying K^+ channel GORK1 is the major voltage-gated potassium channel in the guard cell membrane. Not only the amplitude of the response (determining the degree of opening), but also the speed of stomatal movement in response to fluctuating water availability can be an important parameter in the optimization of plant yield (Hosy et al., 2003). By analogy with their importance as a target in animal and human physiology, signaling through plant ion channels could be modulated to fine-tune plant stress tolerance (Okuma and Murata, 2004).

Modulation of guard cell ion channel activities involves ABC-transporter proteins (multidrug resistance associated protein (MRP) type ATP binding cassette transporters). *MRP4* and *MRP5* knockout mutants (both T-DNA insertion mutants) were isolated to assess the effect on stomatal regulation of a defective ABC transporter (Klein et al., 2004; Klein et al., 2003). Absence of MRP4 expression causes stomata to be more opened both in light and in darkness as compared to wild type plants. A loss-of-function mutation of the *MRP5* gene resulted in reduced transpiration, but importantly also had a higher WUE compared to the wild type (as determined by continuous gas exchange measurements). Furthermore, *mrp5* plants are characterized by increased auxin levels (Gaedeke et al., 2001), pointing at the complex interplay of different regulatory mechanisms.

Efficient water use at the cellular level also involves activation of specific water channels. Water transport through the lipophilic cell membranes is facilitated by water-channels termed aquaporins, belonging to the class of plasma-membrane intrinsic proteins (PIPs) (Luu and Maurel, 2005). These channels are present throughout the plant, and their opening is induced by ABA (Ye and Steudle, 2006). Overexpression of a *Brassica napus* BnPIP in tobacco conferred increased drought tolerance, while expressing the antisense BnPIP had the opposite effect (Yu et al., 2005).

2.4. Transcription Factors

Transcription factors (TF) are grouped into classes depending on their conserved DNA-binding domain. The Apetala 2/ethylene-responsive element binding factor (AP2/ERF) class is one of the major TF families in plants (Shukla et al., 2006). A subfamily thereof, the dehydration response element binding protein /C-repeat binding factor (DREB/CBF) are implicated in ABA-independent regulation. DREB transcription factors activate DRE or C-repeat containing genes (Liu et al., 1998). Overexpression of the DREB/CBF transcription factor CBF4 resulted in drought stress tolerance (Haake et al., 2002). Likewise, a constitutively active form of DREB2A also leads to significant drought tolerance (Sakuma et al., 2006).

The bZIP family of TF is a second class of TF implicated in drought response. Within the bZIP family of TFs, several subfamilies were described (Bensmihen et al., 2005). One subgroup consists of TFs that bind to the conserved cis-acting sequences known as ABA-responsive elements (ABRE); these TFs are hence termed ABRE binding factors (ABFs). In rice, constitutively expressing CBF3/DREB1A or ABF3 did result in enhanced drought stress without growth penalty (Oh et al., 2005), in contrast to stunting seen in Arabidopsis (Liu et al., 1998).

A third important class is composed of the MYB-TF. One member of this transcription factor family, AtMYB60, an R2R3-MYB guard-cell specific TF, is down-regulated during drought stress, since knockout of AtMYB60 resulted in a constitutive reduction of stomatal opening, and consequently decreased wilting under water stress conditions (Cominelli et al., 2005). Another example of TF downregulation is provided by the disruption of the AP2-like ABA repressor 1 (ABR1) gene, leading to a higher level of ABA and thus drought resistance (Pandey et al., 2005). Importantly, the mutant was indistinguishable from the wild type under control conditions.

2.5. Metabolism

Stomata are characterized by a specialized physiology and an accordingly regulated metabolism (Outlaw, 2003). Malate is one of the solutes responsible for the turgor increase needed to open stomata and keep them open. Upregulating malate catabolism effectively reduced stomatal water loss and led to an increased WUE (Laporte et al., 2002). This was achieved in tobacco by expressing the maize NADP-malic enzyme (ME), which converts malate and NADP to pyruvate, NADPH, and CO_2.

Regulation of catabolic and anabolic enzymes also modulates ABA concentrations. Upon water deficit ABA is synthesized in roots and shoots and subsequently redistributed to the guard cells, where it triggers stomatal closure. After drought stress, ABA pools were detected in Arabidopsis shoot vasculature and in stomata, by using ABA-specific promotors coupled to the luciferase (LUC) reporter (Christmann et al., 2005). In Arabidopsis, expression of aldehyde oxidase 3 (AAO3), an enzyme involved in ABA synthesis, was revealed in root tips, root and shoot vasculature and in stomata (Koiwai et al., 2004). There is evidence for the existence of two pools of ABA, differing in their synthesis pathway and in their dynamics upon stress (Nambara and Marion-Poll, 2005; Seo and Koshiba, 2002). Foliar ABA was shown to be produced via the methyl-erythritol phosphate (MEP) pathway, but also the direct MEP-independent synthesis likely occurs in leaves (Nambara and Marion-Poll, 2005). This MEP-derived ABA pool was shown to regulate stomatal opening in response to rapid changes in water status. Inhibition of the MEP pathway resulted in an increase in leaf transpiration linked to a decrease in ABA-content (Barta and Loreto, 2006). However, the MEP-pathway derived ABA was shown not be involved in responses to high CO_2 or darkness. The regulation of ABA

levels linked to the diurnal light/dark cycle was related to the cytochrome P450 mono-oxygenase enzyme (CytP450), that catabolises the endogenous guard cell ABA to 8'-hydroxy-ABA (Tallman, 2004). Manipulation of the expression of ABA 8'-hydroxylases might be the preferred strategy to modulate ABA levels and thus water usage, since overexpression of ABA-synthesizing genes induces an increased ABA catabolic activity that annihilates the desired ABA-effect (Yang and Zeevaart, 2006). ABA is also subject to inactivation by conjugation (glucosylation), which limits the timeframe in which an ABA signal exerts its effects (Priest et al., 2005). Agronomic use of glucosylation and hydroxylation resistant ABA-analogues with long lasting effect was proposed (Priest et al., 2005). A recent study provides indications for a role of *XERICO*, an Arabidopsis RING-H2 gene (really interesting new gene zinc finger protein), in ABA homeostasis. Constitutive overexpression of *XERICO* resulted in an accelerated response of ABA biosynthesis upon drought stress (Ko et al., 2006).

3. CROSSTALK OF STRESS PATHWAYS

Crosstalk and thus overlap between biotic and abiotic stress pathways is highly common; however plants have also evolved mechanisms that prioritize drought (or more general abiotic) stress responses to biotic responses (Fujita et al., 2006). As a consequence of this cross-talk, selection for drought resistance can have effects on the pathogen resistance traits of a crop (Timmusk and Wagner, 1999). Drought stress is the most prevalent cause of stomatal closure and subsequent leaf surface temperature increase. However, other stresses influencing the water status of plants can 'mimic' the drought response. Infections such as fungal and bacterial wilting diseases directly impinge on the water-use efficiency of plants, resulting in a decrease thereof (Guimaraes and Stotz, 2004). The toxin fusicoccin, commonly used to study stomatal responses of plant mutants, is released by these pathogens to divert plant resources to pathogen growth.

Wilting diseases typically block water transport in the plant leading to a higher leaf temperature (Pinter et al., 1979). In sunflower, the effect of a wilting disease (Verticillium) was found to resemble drought response (Sadras et al., 2000). In this non-isohydric plant, which by definition has a slow stomatal response to hydric stress (see above), drought (and wilting disease) can be quantified by a decrease in leaf area. Overexpression of the activated disease resistance 1 (*ADR1*) gene, which encodes a coiled-coil (CC)-nucleotide-binding site (NBS)-leucine-rich repeat (LRR) protein, confers in addition to broad-spectrum pathogen resistance also drought resistance, but also results in enhanced susceptibility to heat and salt stress (Chini et al., 2004). The *rcd1* (radical-induced cell death) mutant displays rapid programmed cell death upon ozon exposure, which is reminiscent of pathogen resistance by the hypersensitive response. In addition, *rcd1* has a higher transpiration rate than wild type, is less sensitive to ABA, ethylene and jasmonate, and is thus implicated in multiple hormone-and stress signaling pathways (Ahlfors et al., 2004).

RCD1 belongs to the (ADP-ribosyl)transferase domain-containing subfamily of the WWE protein-protein interaction domain protein family. An unexpected implication of disease resistance response to powdery mildew in barley was the loss of stomatal control due to epidermal cell death around the stomata through which pathogen ingress occurred (Prats et al., 2006). The lack of turgor pressure from the epidermal cells left the stomata continuously open, leaving the plants exposed to severe levels of drought stress.

As ABA, the plant hormone ethylene is often involved in stress responses (De Paepe and Van Der Straeten, 2005). Ethylene inhibits ABA-induced stomatal closure, and ethylene overproducing mutants have a higher transpiration rate (Tanaka et al., 2005). A decrease in ethylene sensitivity is one of the mechanisms by which overexpression of Hahb-4, an HD-Zip protein from *Helianthus annuus*, increases drought tolerance of Arabidopsis (Manavella et al., 2006). A similar effect was observed in maize ACC synthase (ZmACS6) loss-of-function mutants, which are affected in the first regulatory step of ethylene biosynthesis (Young et al., 2004).

Hormonal cross-talk with the ABA pathway in relation to stomatal regulation is not limited to ethylene. Levels of auxins and cytokinins, hormones known to promote stomatal opening (Tanaka et al., 2006), display pronounced diurnal patterns which follow reverse trends compared to the corresponding ABA levels (Novakova et al., 2005).

Understanding the integration of chemical, electrical and hydraulic signals as a response to (coinciding) stresses at the whole plant level is a challenge for the future (Brenner et al., 2006).

4. CUTICULAR AND STOMATAL TRANSPIRATION

Transpiration is determined by both regulation of stomatal aperture and stomatal density. The latter parameters and stomatal size, are largely determined by the developmental program, but are also influenced by hormonal signals (Bergmann, 2006; Chaerle et al., 2005). When grown at low humidity, plants adaptively increase cuticle wax load (Holroyd et al., 2002). In contrast, high humidity conditions result in a lower stomatal density (Bergmann, 2004).

Modification of the epidermal surface (wax load) affects the survival of plants under severe drought stress, when stomata are completely closed (Zhang et al., 2005). The *shn* (shine) gain-of-function mutant has an altered wax composition of the leaf cuticula, responsible for its shiny appearance (Aharoni et al., 2004). The *shn* leaf epidermis is more permeable, resulting in a higher cuticular transpiration, and is characterized by a lower stomatal density. The combined effect of these factors results in a drought-tolerant phenotype of the *shn* mutant. A single mutation can thus have multiple effects affecting leaf gas exchange. Another example of epidermal wax load modification resulting in increased drought tolerance is the overexpression of the ABA and drought-inducible AP2 transcription factor WXP1 (wax production) in alfalfa, leading to higher wax accumulation, with a minor growth retardation as a side-effect.

5. MONITORING OF DROUGHT STRESS RESPONSES

The stomatal pathway represents the major route for gas exchange, whereas the remaining part of the leaf surface (98 to 99,8% of its area) represents only a fraction of the total transpiration (10 to 100 times lower) since it is covered by a waxy cuticula (Nobel, 1991). The mechanisms described above have largely been discovered and characterized using techniques that reveal stomatal functioning (Merta et al., 2001) (see Table 1 for an overview).

5.1. Monitoring at the Lab Scale

An indication of modified water relations in a mutant plant is generally given by a wilty or withered phenotype (Kacira et al., 2002). Confirmation thereof is obtained by weight loss measurements, either using potted plants (with covered soil or substrate), detached leaves or shoots (Aharoni et al., 2004; Pandey and Assmann, 2004; Ruggiero et al., 2004). Integrative weight loss measurements over time, covering either complete dark or light periods allow to discriminate between stomatal and cuticular transpiration. However, this difference in transpiration between light and dark is more easily obtained by measuring changes in the humidity of air circulated over the leaf in a semi-closed measuring system. Direct assessment of leaf gas-exchange provides a real time, higher resolution measurement of the actual water loss (and CO_2 uptake) (Lasceve et al., 1997). Reduced transpiration can be monitored by porometric measurements, during which a small leaf region is enclosed in a cuvette for a measuring time of under 1 minute (Ahlfors et al., 2004). The use of multiple-cuvette systems enclosing leaves (or complete plants) yields time-courses allowing to compare the characteristics of different plants (Dodd et al., 2004). The high time-resolution also reveals differences in response to changing environmental factors, such as air humidity (Hosy et al., 2003).

Single cuvette portable systems are limited to short intermittent measurements on a batch of plants. This approach is labor intensive, and suffers from the lack

Table 1. Overview of the measuring techniques to reveal changes in stomatal control.
The time resolution of weight loss measurements is at the hour level in detached leaves due to accelerated water loss; for intact plants differences can be revealed with day resolution. Gas exchange measurements need an equilibration time for the air continuously circulated over a leaf enclosed in a measuring cuvette. Clamping of the leaf can influence leaf physiology, especially for longer time measurements (*)

Measuring technique	Measured parameter	Stomatal closure response	Destructive/ invasive	Time resolution
Weight loss	Amount of water evaporated	Decrease	– / –	Hours to days
Gas exchange	Change in water content of air	Decrease	– / *	Seconds to minutes
Thermography	Leaf temperature	Increase	– / –	Real-time
Carbon isotope discrimination	Discrimination of ^{13}C over ^{12}C	Decrease	+ / +	Integrative over growth period

of reproducible positioning of the measuring cuvette. Longer-term leaf clamping inevitably affects leaf physiology (e.g. by shading). Thermal imaging overcomes these limitations and monitors evaporation at the leaf surface non-invasively, in real time. Importantly measurements should be carried out in stable environmental conditions (Chaerle and Van Der Straeten, 2000). In addition, thermal imaging can also visualize the temperature of detached leaves, offering an alternative to integrative water measurement by weighing (Mustilli et al., 2002). Thermography has the additional benefit of visualizing heterogeneity in response of leaves. This might not be needed for field applications, where an average temperature measurement using a non-imaging infrared thermometer will be sufficient to monitor the temporal evolution of leaf temperature (under a developing stress). Light intensity, known to positively regulate stomatal aperture, was reported to influence drought stress detection by thermal imaging in Chrysanthemum (Blom-Zandstra and Metselaar, 2006). An approach to directly quantify stomatal conductance from thermal imaging data was recently proposed, and will provide the means to directly correlate temperature measurements to the above-described gas-exchange measurements (Leinonen et al., 2006). Furthermore, the reflectance of plant leaves also depends on water content. Changes in water status can be revealed by near infrared imaging, since in this spectral region, water absorbs part of the radiation (Peñuelas and Filella, 1998). This technique is mostly used in remote sensing applications, but has the potential to be used in a multi-sensor setup at the laboratory scale.

An integrative measurement of yield over a whole growing season can be obtained by the destructive carbon isotope discrimination technique at the end of the growth period (DELTA technique, see www.csiro.au). The heavier ^{13}C isotope containing CO_2 is discriminated against during fixation in the substomatal cavities. Upon stomatal closure, discrimination of the two isotopes becomes less likely, and values closer to zero are obtained for the delta (Δ) parameter. This parameter negatively correlates with transpiration efficiency and thus water use efficiency (WUE). The *ERECTA (ER)* gene, encoding a receptor-like kinase (RLK) from Arabidopsis was shown to confer increased WUE (Masle et al., 2005). Arabidopsis plants homozygous for *erecta* mutant alleles (Ler, Col*er105*, Col*er2*) had a higher Δ, higher transpiration and a higher stomatal density, compared to homozygous *ERECTA* plants, harboring the functional *ER* alleles. Using the delta screening approach, high yielding wheat cultivars were obtained (Condon et al., 2004). An important technical advance in the study of water relations is the possibility to measure water transport in-planta using magnetic resonance imaging (Windt et al., 2006). This allows to visualize changes in phloem and xylem transport, which also influence the water status of the plant.

5.2. Field Scale Monitoring

Thermal imaging can visualize early crop responses to water limitation from the plant level to the field scale. However, to be useful under field conditions at an early

stage of plant development (before canopy closure), the image parts corresponding to leaf area need to be isolated selectively by dedicated software (Luquet et al., 2003).

To exploit thermography as a monitoring tool, water stress levels are expressed on a reference scale (0–1) by various Water Stress Indexes. The most basic approach is taking into account the temperature difference between canopy and air (T_{canopy} -T_{air}). Parameters based on these temperature measurements, such as Crop Water Stress Index (CWSI) and Water Deficit Index (WDI) are used to assess the water status of field plots (http://www.uswcl.ars.ag.gov/epd/remsen/irrweb/thindex.htm) (Jones, 2004a; Jones, 2004b). Leaf water potential (LWP) is determined by the osmotic status of the leaf, and can be measured on leaf discs using a vapor pressure osmometer (Verslues and Bray, 2004). Even though LWP is not always directly related to stomatal conductance (g_s), a correlation with the CWSI parameter was found (Cohen et al., 2005).

For early monitoring applications to be effective, it is important to take into account that non-isohydric plants do not display a change in stomatal conductance (and hence leaf temperature) upon early drought stress. As another complicating factor, crop water status at the field scale is characterized by spatial variability, due to soil characteristics, crop canopy variability, and inter-plant variability. This heterogeneity has to be discriminated against the effects of hydric stress. Approaches using normalized CWSI values that take into account crop canopy characteristics show great promise for making irrigation practices more efficient (Jones, 2004b).

Leaf temperature measurements are also amenable to simulation. The development of modeling approaches with virtual plants, allows to grow 'virtual crops' under different conditions and to assess their predicted responses (Tardieu, 2003). Especially in agronomically important crops, a longer generation time puts a limit on the development of new improved cultivars. Targeting the most promising approaches as revealed by the simulations allows to speed up crop breeding. Modeling specifically applied to the guard cell system regulatory network can also help predicting the effect of manipulations and guide the experimental approach (Li et al., 2006). Given the complexity of guard cell regulation, combining the available knowledge on interactions and regulations into a dynamic model can help to define missing links and to test new hypotheses. Predictive tools can therefore further advances to targeted improvement of water use.

5.3. Screening Applications

Using screening under controlled conditions, altered responses to drought stress among a batch of cultivars or within a mutated population can be pinpointed using thermal imaging. The isolation of the barley 'cool' mutant was the first example of a successful thermal screen (Raskin and Ladyman, 1988). Analogous screens have been carried out in Arabidopsis to isolate mutants with aberrant leaf temperature, shown to carry a mutation in kinases or phosphatases that regulate stomatal aperture (see above ABI and OST) (Merlot et al., 2002; Mustilli et al., 2002).

Using thermography to reveal stomatal responses upon a steep drop in air humidity, OST1 was subsequently revealed to be also implicated in the stomatal closure upon low air relative humidity (or low vapor pressure deficit VPD) (Xie et al., 2006).

As a consequence of a leaf temperature increase, assimilation can be directly affected. A limitation of photosynthesis is however predominantly caused by diffusion limitation (Flexas et al., 2006). Thus (drought) stress induced stomatal closure will limit crop yield. Therefore, screening for plants that have 'mild' reactions to a developing stress might be beneficial to increase yields.

The leaf temperature screening approach can also be carried out with infrared thermometers at the field plot scale. This technique was effectively used to screen Brassica genotypes for drought tolerance under decreasing soil moisture conditions (Singh et al., 1985).

6. ROUTES TO YIELD ENHANCEMENT

Constitutive expression of genes involved in the response to stress is of great benefit in applied research since it often results in strong phenotypes. However this approach mostly leads to a considerable growth penalty (Liu et al., 1998). The use of inducible promoter constructs can alleviate these adverse consequences (Chini et al., 2004; Umezawa et al., 2006). In some cases however, constitutive expression enhances yield significantly under stressed conditions without growth inhibition in optimal circumstances. As an example, overexpression of a NAC (NAM, ATAF, and CUC) transcription factor resulted in higher drought resistance both in the vegetative and in the reproductive stage of rice (Hu et al., 2006). Conversely, knockout of single genes in T-DNA insertion mutants (in general loss-of-function mutants) can remain without phenotype under normal growth conditions, yet confer a drought resistance phenotype, as exemplified by the *gcr1* mutant (G-Protein Coupled Receptor, GCR1) which is hypersensitive to ABA (Pandey and Assmann, 2004). GCR1 could thus be a key factor in engineering plant resistance to drought stress.

The use of modeling techniques together with the increasing genetic information available from whole genome sequencing efforts (achieved for Arabidopsis, Oryza, and Populus), micro-array gene-expression datasets and associated tools to extract signaling network information (Zimmermann et al., 2005), and expressed sequence tag (ESTs) databases (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html) (Rudd, 2003) will provide the means needed to further increase crop yield in a world faced with an increased pressure on the available resources.

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