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Review:

Understanding plant responses to drought — from genes to the whole plant

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Abstract. In the last decade, our understanding of the processes underlying plant response to drought, at the molecular and whole-plant levels, has rapidly progressed. Here, we review that progress. We draw attention to the perception and signalling processes (chemical and hydraulic) of water deficits. Knowledge of these processes is essential for a holistic understanding of plant resistance to stress, which is needed to improve crop management and breeding techniques. Hundreds of genes that are induced under drought have been identified. A range of tools, from gene expression patterns to the use of transgenic plants, is being used to study the specific function of these genes and their role in plant acclimation or adaptation to water deficit. However, because plant responses to stress are complex, the functions of many of the genes are still unknown. Many of the traits that explain plant adaptation to drought — such as phenology, root size and depth, hydraulic conductivity and the storage of reserves — are associated with plant development and structure and are constitutive rather than stress induced. But a large part of plant resistance to drought is the ability to get rid of excess radiation, a concomitant stress under natural conditions. The nature of the mechanisms responsible for leaf photoprotection, especially those related to thermal dissipation, and oxidative stress are being actively researched. The new tools that operate at molecular, plant and ecosystem levels are revolutionising our understanding of plant response to drought, and our ability to monitor it. Techniques such as genome-wide tools, proteomics, stable isotopes and thermal or fluorescence imaging may allow the genotype–phenotype gap to be bridged, which is essential for faster progress in stress biology research.

Keywords: ABA, genes, imaging, isotopes, osmotic adjustment, photosynthesis, photoprotection, signalling, stomatal functioning, water stress.

Introduction

Research into the plant response to water stress is becoming increasingly important, as most climate-change scenarios suggest an increase in aridity in many areas of the globe (Petit *et al.* 1999). On a global basis, drought (assumed to be soil and/or atmospheric water deficits), in conjunction with coincident high temperature and radiation, poses the most important environmental constraints to plant survival and to crop productivity (Boyer 1982). Agriculture is a major user of water resources in many regions of the world. With increasing aridity and a growing population, water will

become an even scarcer commodity in the near future. Even though in viable agriculture severe water deficits should be a rare (but catastrophic) event (Passioura 2002), a better understanding of the effects of drought on plants is vital for improved management practices and breeding efforts in agriculture and for predicting the fate of natural vegetation under climate change.

Many works in this field were developed over the past couple of decades, covering subjects from plant strategies to control water status under drought (Schulze 1986a) to the physiological and biochemical processes underlying plant

Abbreviations used: ABRE, ABA-responsive element; ABRE BP, ABRE binding protein; ACC, 1-aminocyclopropane-1-carboxylic acid; AtHK1, *Arabidopsis thaliana* histidine kinase; ATHP, a phospho-relay intermediate; ATRR, a response regulator; C_a , carbon dioxide concentration of the air; C_i , intercellular carbon dioxide concentration; CDPK, Ca^{2+} -dependent kinases; DRE, dehydration-responsive element; DREBP, DRE binding protein; ERE, ethylene-responsive element; EREBP, ERE binding protein; EST, expressed sequence tag; HKT1, a high-affinity K^+ transporter; LEA, late embryogenesis abundant protein; MAPK, mitogen-activated protein kinase; NCED, 9-*cis*-epoxycarotenoid dioxygenase; ROS, reactive oxygen species; RWC, tissue relative water content; W_i , intrinsic water use efficiency; δ , isotope composition; Δ , isotope discrimination.

response to water deficits (Chaves 1991; Cornic and Massacci 1996) and oxidative stress (Foyer and Mullineaux 1994; Smirnov 1998). Progress was also made on the interpretation of the relationships between leaf structure and function (Valladares and Pearcy 1997) and on root morphology and stress tolerance (Jackson *et al.* 2000; Maggio *et al.* 2001), which is essential to the understanding of the competitive ability of plants to survive under different environments. But it was the research into the molecular processes involved in tolerance and resistance to stresses, including drought, that received special attention (Ingram and Bartels 1996; Bohnert and Sheveleva 1998). Nevertheless, there is still insufficient knowledge on the molecular-to-physiological mechanisms underlying plant responses within natural habitats, where complex interactions may occur as a result of the combination of different stresses (Mittler *et al.* 2001).

At present, hundreds of genes that are induced under drought have been identified, and a range of tools (gene expression patterns, transgenic plants, and so on) is being used to study their specific function and role in plant acclimation or adaptation to water deficits. Because plant responses to stress are complex, however, the functions of many of these genes are still unknown (Cushman and Bohnert 2000; Bray 2002). In contrast, not all stress-responsive genes are involved in cellular adaptive processes; some of them are simply involved in short-term deleterious responses. Many of the traits that explain plant adaptation to drought are those determining plant development and shape, such as phenology, the size and depth of the root system, xylem properties or the storage of reserves. These traits are mostly constitutive rather than stress-induced (Blum 1984; Passioura 2002).

Research on sensing and signalling mechanisms of water deficits (Bray 1997; Shinozaki and Yamaguchi-Shinozaki 1997) also registered dramatic progress. It became clear that signalling pathways constitute a complex network, interconnected at many levels (Knight and Knight 2001). This explains the so-called 'cross resistance' to different stresses and why plant responses to one particular stress can be elicited through several pathways (Bohnert and Sheveleva 1998). A revived interest in the hydraulic component of signalling is apparent (Jackson *et al.* 2000; Sperry *et al.* 2002), but we are still far from understanding where and how chemical and hydraulic signals are integrated to regulate plant response to dehydration (Comstock 2002).

The discovery of aquaporins was a major breakthrough in the understanding of water flow through living plant cells (Maurel and Chrispeels 2001; Tyerman *et al.* 2002). It is now acknowledged that they can increase water permeability of cellular membranes by 10–20-fold and that the regulation of their expression and activity is modulated by dehydration. For example, several genes that encode for aquaporins were shown to be upregulated in *Arabidopsis*

(Yamaguchi-Shinozaki *et al.* 1992) and in *Craterostigma plantagineum* in response to drought or ABA (Ramanjulu and Bartels 2002). A better understanding of the role of aquaporins in drought response and adaptation can be expected in the near future.

In nature, plants can either be subjected to slowly developing water shortage (within days to weeks or months) or face short-term water deficits (hours to days). In the case of slowly developing water deficits, plants can either escape dehydration by shortening their life cycle or optimise their resource gain in the long term through acclimation responses. In the case of rapid dehydration, plants react by minimising water loss or exhibiting metabolic protection (induced or constitutive) against the damaging effects of dehydration and co-developing oxidative stress (see Fig. 1). Fast and slow desiccation can have totally different results in terms of physiological response or adaptation (McDonald and Davies 1996), but the importance of time in shaping plant response may change dramatically according to genotype and environment.

In this review we summarise the progress made in the last decade on the understanding of plant responses to water deficits, bringing together information from the level of the whole plant to that of gene expression. This is a general overview written for stress biologists, necessarily incomplete, but we hope still useful. It also aims to encourage communication among molecular and whole-plant analyses and the many scientific areas involved in this complex field. We cover the general plant strategies that enable plant adaptation to drought, and pay special attention to carbon assimilation — because plants face the crucial dilemma 'lose water to fix carbon' — and to the perception and signalling of drought from the molecular to the whole-plant level. A short review is also made of new tools for monitoring and understanding the plant response to stress, ranging from molecular (genomics and proteomics tools) to more integrative techniques (e.g. stable isotopes, imaging). The latter are particularly important to aid progress in the understanding and testing of plant performance under natural conditions.

Successful strategies under drought stress — plasticity, acclimation, resistance

Classically, plant resistance to drought has been divided into escape, avoidance and tolerance strategies (Levitt 1972; Turner 1986). Nevertheless, these strategies are not mutually exclusive and, in practice, plants may combine a range of response types (Ludlow 1989). Plants that escape drought exhibit a high degree of developmental plasticity, being able to complete their life cycle before physiological water deficits occur. Escape strategies rely on successful reproduction before the onset of severe stress. This is important in arid regions, where native annuals may combine short life cycles with high rates of growth and gas exchange, using

maximum available resources while moisture in the soil lasts (Mooney *et al.* 1987; Maroco *et al.* 2000a). Improved reproductive success also includes better partitioning of assimilates to developing fruits. This is associated with the plant's ability to store reserves in some organs (stems and roots) and to mobilise them for fruit production, a response well documented in crop plants, such as cereals (Gebbing and Schnyder 1999; Bruce *et al.* 2002) and some legumes (Rodrigues *et al.* 1995; Chaves *et al.* 2002). This ability to mobilise reserves is increased in droughted plants (Rodrigues *et al.* 1995; Yang *et al.* 2001).

Plants can also endure drought conditions by avoiding tissue dehydration, while maintaining tissue water potential as high as possible, or by tolerating low tissue water potential. Dehydration avoidance is common to both annuals and perennials and is associated with a variety of adaptive traits. These involve (i) minimising water loss and (ii) maximising water uptake. Water loss is minimised by closing stomata; by reducing light absorbance through rolled leaves (Ehleringer and Cooper 1992), a dense trichome layer increasing reflectance (Larcher 2000), or steep leaf angles; or by decreasing canopy leaf area through reduced growth and shedding of older leaves. Water uptake is maximised by adjusting the allocation pattern, namely increasing investment in the roots (Jackson *et al.* 2000). Significant gains in crop productivity due to plant breeding

for semi-arid regions resulted from enhancements in rooting depth (Fisher and Turner 1978; Blum 1984). In addition, shedding of older leaves that contribute to water saving can be viewed as a recycling program within the plant, allowing the reallocation of nutrients stored in older leaves to the stem or younger leaves. During drought-induced senescence, some proteases are induced that are drought-specific and do not occur under natural aging, as is the case of some forms of cysteine proteases (Khanna-Chopra *et al.* 1999). Also, tolerant and susceptible species seem to differ in the degree of drought stimulation of endoproteolytic activity (aspartic protease), this stimulation being higher in susceptible than in tolerant species (Cruz de Carvalho *et al.* 2001).

Further knowledge of the regulation of these processes may allow development of plant breeding or genetic engineering strategies for the control of leaf senescence under drought. Leaf senescence, leading to short supply of sugars, is an important factor in fruit abortion under drought stress. Therefore, delayed-senescence phenotypes may be desirable in crops where yield is source-limited (Levy *et al.* 1997) and stem reserve storage and use is insufficient to support fruit growth under stress. However, in cereals, an interesting interaction was observed between water and nitrogen availability. Under non-limiting water, abundant nitrogen delayed senescence but reduced grain yield, whereas under controlled or mild soil drying, high nitrogen

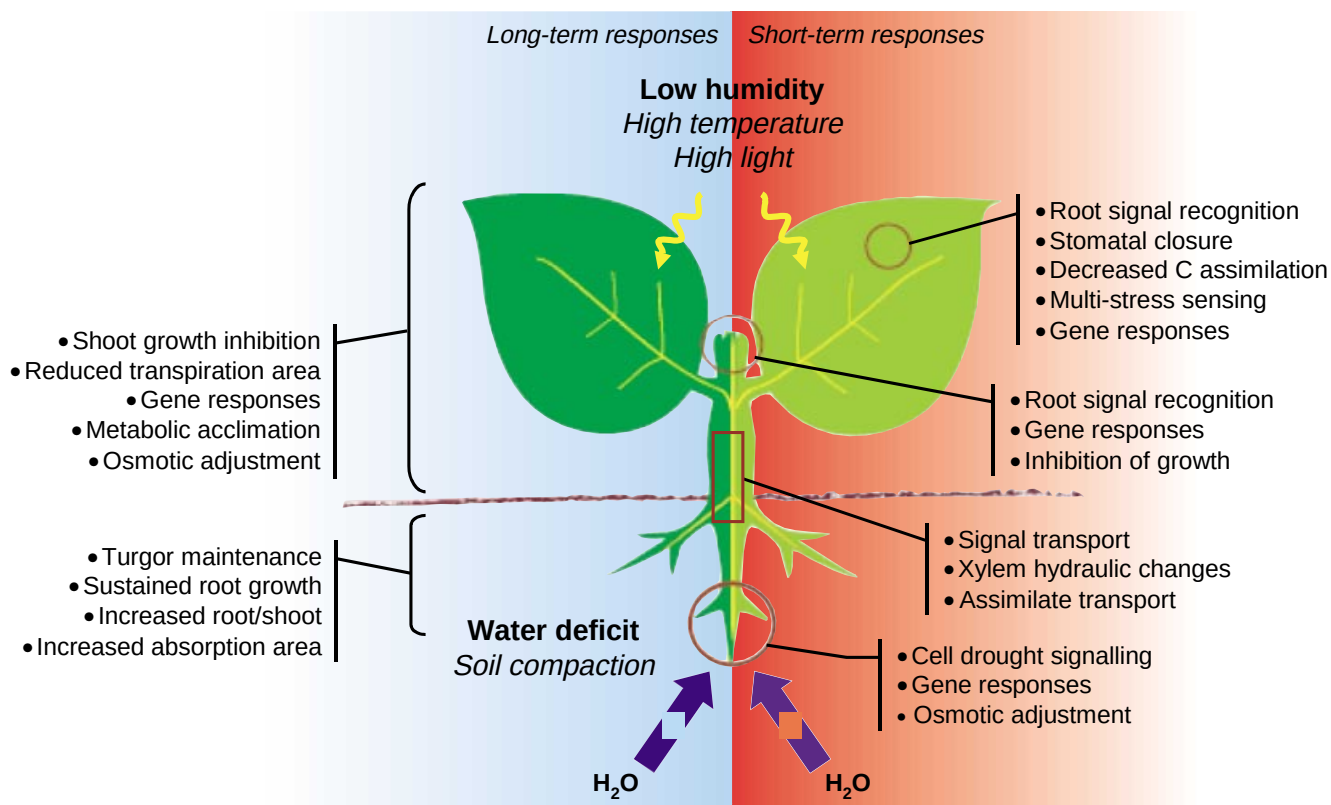


Fig. 1. Whole-plant responses to drought stress. Left, long-term or acclimation responses; right, short-term responses.

availability increased the use of stored carbohydrates and grain yield (Yang *et al.* 2000). Plant hormones and sugars are also involved in the regulation of shoot senescence. Elevated soluble sugars are known to repress the expression of photosynthesis-associated genes, in a process where hexokinases are involved in sugar sensing (Smeekens 1998). One important effect of the overexpression of hexokinase is accelerated senescence (Quirino *et al.* 2000).

Although water deficits lead to shedding of older leaves, the effects on younger leaves seem to be opposite. In fact, leaves that survive drought often show higher rates of photosynthesis (Ludlow and Ng 1974) and of Rubisco content per unit leaf area (David *et al.* 1998) than leaves of similar age in well-hydrated plants, in what can be described as 'suspension' of leaf aging. In contrast, leaves growing under drought usually reach maturity and become a carbon source at a smaller size than leaves grown in well-watered plants (Schurr *et al.* 2000).

Most of the drought-avoidance responses enable plant adjustment to low resources (water and minerals) (Lambers *et al.* 1998; Poorter and Nagel 2000). In fact, in addition to a restriction in available water, soil drying induces a decrease in nutrients, in particular nitrogen (but also calcium), with strong interactive effects on plant growth and function (McDonald and Davies 1996). This is an important area of research, requiring innovative methods to tease apart the effects caused by water deficits and by mineral nutrition under drought. Plant adjustments to the low resources in arid environments include altered leaf structure, increased proportions of assimilates allocated to roots (Poorter and Nagel 2000), slow growth rates and slow organ turnover rates (Bazzaz and Grace 1997). Whereas short-lived organs can be discarded in response to stress, long-lived organs must optimise their resource gain. This is the case of a change in leaf demography reported in the desert perennial species *Cryptantha flava*, whose leaf lifespan doubled under drought (Casper *et al.* 2001). This response results in lower turnover rates of leaves and therefore higher nutrient-use efficiency. This also means that plants can partially compensate for lower rates of carbon gain by investing less in new leaf construction.

However, long-lived leaves have to survive periods of hostile environment. This requires various protective mechanisms ranging from anatomical and morphological characteristics, such as sclerophylly, to resist extreme climatic events and herbivory (Turner 1994), to the biochemical mechanisms targeted at dissipating excess radiant energy (e.g. the xanthophyll cycle) (Demmig-Adams and Adams 1996; Garcia-Plazaola *et al.* 1997). Sclerophyllous leaves are normally small and thick, with low specific leaf area (SLA) and low nitrogen concentration. A decrease in SLA may also occur in response to drought in herbaceous leaves as a result of an increased investment in structural tissues,

allowing increased resistance to unfavourable environmental conditions (Maroco *et al.* 2000a).

Finally, tolerance to low tissue water potential may involve osmotic adjustment (Morgan 1984), more rigid cell walls, or smaller cells (Wilson *et al.* 1980). Many of the evergreen shrubs and trees in arid or semi-arid regions combine the high solute concentration in living cells (low osmotic potential) with sclerophylly and low photosynthetic capacity and stomatal conductance (Faria *et al.* 1998). However, this is not always the case, since some *Eucalyptus* and *Banksia* species, which have scleromorphic leaves, exhibit high maximal rates of photosynthesis per unit leaf area (Lambers *et al.* 1998). Small leaves are well adapted to the high light and high temperature that prevail in most arid regions, because their size permits greater sensible heat dissipation and an efficacious control of water loss by stomatal closure (Jarvis and McNaughton 1986).

Partial plant dormancy to survive the dry season is another tolerance strategy reported, for example in the evergreen legume *Retama raetam* (Mittler *et al.* 2001). In these plants, dormancy is evident by the repression of genes encoding photosynthetic proteins. After rain, proteins are synthesised within 6–24 h. The extreme desiccation tolerance is found in the angiosperm 'resurrection plants' as well as in some ferns, non-vascular plants, algae and lichens. The leaves of resurrection plants can equilibrate with air close to 0–2% (v/v) relative humidity and are still able to fully recover their physiological activity upon rehydration. Changes occurring rapidly at the messenger RNA (mRNA) and protein levels lead to the tolerant state (Ingram and Bartels 1996).

Leaf-level responses to drought — control of water status and carbon uptake

Stomatal control of gas exchange and water status of leaves: optimising water use or minimising damage?

Stomatal closure, together with leaf growth inhibition, are among the earliest responses to drought, protecting the plants from extensive water loss, which might result in cell dehydration, runaway xylem cavitation and death. This is a common response to water deficits that develop quickly or slowly, and may result from shoot or root dehydration (Schulze 1986a; Chaves 1991). The opening and closing of stomata result from the changes in turgor of guard cells relative to epidermal cells. Metabolic energy (e.g. from mesophyll photosynthesis) and changes in membrane permeability are involved as well. The detailed mechanisms of stomatal response to drought are not easy to rationalise because at any given moment stomata may be responding to a complex set of factors ranging from light intensity to CO₂ concentration in addition to leaf water status.

Studies in the late 1980s showed that stomata did close in response to drying soil even when shoot water status was

maintained at high turgor, either by experimental manipulation (Schurr *et al.* 1992) or by growing the plants with part of the root system in drying soil (split-root experiments) (Gowing *et al.* 1990). Further evidence indicated that stomatal closure is likely to be mediated by chemical signals travelling from the dehydrating roots to the shoots.

Other hormones besides ABA are likely to be involved in the regulation of stomatal aperture, either in isolation or acting in conjunction with ABA. Increased cytokinin concentration in the xylem decreases stomatal sensitivity to ABA and promotes stomatal opening directly (see the review by Wilkinson and Davies 2002). In grapevines subjected to partial root-zone drying, a decrease in root cytokinins (zeatin and zeatin riboside) has been observed, concomitantly with an increase in xylem ABA, bringing about a reduction in stomatal conductance (Stoll *et al.* 2000). Abscisic acid (ABA) was identified as one of the chemical signals involved in the regulation of stomatal functioning (Schulze 1986b; Davies and Zhang 1991). This regulation is not simple and involves both long-distance transport and modulation of ABA concentration at the guard cells to a given dose of the hormone (Wilkinson and Davies 2002). Among the factors implicated in this modulation are xylem sap and leaf tissue pH, which may increase in conditions of high evaporative demand such as high deficit in water vapour pressure of the air, high light intensity and high leaf temperature. An increase in pH could partly explain stomatal closure in the afternoon in well-watered plants, as discussed below. The complexity of plant response to ABA is apparent in the fact that selection for high capacity for ABA accumulation has yet to provide conclusive data that could help shape crop breeding for drought conditions (Pekic *et al.* 1995).

The evidence for stomatal regulation through root chemical signalling is not always easy to reconcile with the short-term response of stomata to hydraulic perturbations (either in supply of water, such as when xylem conductance changes, or in demand, such as low atmospheric humidity). Stomata respond directly to the rate of water loss from the leaf because of changes in evaporative demand, rather than to air humidity changes (Monteith 1995; Maroco *et al.* 1997). But evidence is accumulating that suggests that stomata respond to changes in the rate of water supply, through, for example, changes in xylem conductance (Salleo *et al.* 2000; Sperry 2000; Nardini *et al.* 2001). Buckley and Mott (2002) proposed that changes in turgor pressure in the leaf would translate into a signal that might lead to changes in guard-cell osmotic pressure and, consequently, in stomatal aperture in response to changes in water supply and demand. The nature of such rapid signalling is not yet understood. These responses seem to substantiate the idea that stomatal closure prevents the risk of a plant losing its water transport capability (Jones and Sutherland 1991), with stomata acting as pressure regulators that prevent xylem pressure from

runaway cavitation thresholds (Comstock and Mencuccini 1998; Jackson *et al.* 2000; Buckley and Mott 2002).

The important role that stomata play in the trade-off between water loss and CO₂ uptake is related to their extreme sensitivity to both environmental and internal physiological factors. For example, under field conditions a midday depression of stomatal conductance is an important regulatory response to control water loss, even under non-restricting water availability in the soil, and to optimise carbon gain in relation to water loss (Cowan and Farquhar 1977). The intensity of this depression varies with the air humidity, temperature and plant water status. The nature of the mechanisms underlying the midday depression of stomatal conductance has been the subject of much debate over the years (Schulze 1986b; Chaves 1991). Although stomata may close in response to the high evaporative demand at midday, stomatal sensitivity to changes in vapour pressure deficit varies during the day (e.g. Pereira *et al.* 1987; Correia *et al.* 1990). There is evidence that changes in xylem ABA concentrations or flow rates do not explain midday closure (Correia *et al.* 1990), but the increase in xylem sap pH during the day may cause such closure (Wilkinson and Davies 2002). However, decreased hydraulic conductance (e.g. increased xylem cavitation) during the period of intense transpiration may play a role, partly overriding the direct response of stomata to changes in evaporative demand of the air. Indications that circadian rhythms may be involved in the diurnal regulation of stomatal aperture by air humidity and leaf water status were also reported (Franks *et al.* 1997; Mencuccini *et al.* 2000). Other explanations for midday depression in leaf gas exchange include a response of the stomata to excessive solar radiation, either indirectly through a metabolic link with downregulated photosynthesis, directly triggered by the xanthophyll cycle in guard cells (Zeiger 2000) or by effects on leaf pH (Wilkinson and Davies 2002).

Species differences in maximum stomatal conductance may rely not only on the size and frequency of stomata, but also in the maximum xylem conductance, as derived from xylem anatomy (Aasamaa *et al.* 2001, 2002). It was suggested that endogenous ABA concentrations during growth might provide an explanation for those differences, as high ABA concentration was related to lower xylem conductance in some tree species.

Independently of the cause for stomatal closure, most plants tend to show an increase in water-use efficiency when water deficit is mild. This increase results from the non-linear relationship between carbon assimilation and stomatal conductance, that is, water loss being restricted before, and more intensely than, the inhibition of photosynthesis. These findings led to the hypothesis that plant evolution brought about an optimisation of carbon uptake *versus* water loss (Cowan and Farquhar 1977; Raven 2002). An analysis at a global scale seems to corroborate this

hypothesis, indicating the strong relationship between maximum stomatal conductance and leaf nitrogen concentration, and therefore photosynthetic capacity, in a range of vegetation types (Schulze *et al.* 1994).

Stomatal aperture also plays a role in leaf cooling through latent heat exchange. The positive effects of high stomatal aperture are apparent from the observation that stomatal conductance has increased over the years in parallel with agronomic yields in irrigated cotton and wheat selected for higher yields at high temperature (Lu *et al.* 1997). It was also shown that high stomatal conductance could be used as a selection trait for high yields in irrigated crops grown at high temperature.

Metabolic inhibition of photosynthesis — timing and importance

Over the last couple of years, interest has renewed in the relative importance and timing of the onset of metabolic *versus* stomatal limitations to photosynthesis (Cornic 2000; Flexas and Medrano 2002; Lawlor and Cornic 2002). This was mostly a result of the work by Tezara *et al.* (1999) reporting that ATP synthesis and thus ribulose-1,5-bisphosphate (RuBP) regeneration impair photosynthesis at mild water deficits. Other changes in plant metabolism also occur very early in response to tissue dehydration, presumably as a result of primary and secondary signalling processes, as reported in many molecular studies (Ingram and Bartels 1996) and as discussed below. However, what remains to be confirmed is to what extent decreased ATP synthesis and RuBP supply play a role in the impairment of photosynthesis of most species facing mild water stress under field conditions. The view that implicates a primary role for non-stomatal effects of water deficits on photosynthesis is in conflict with evidence suggesting that stomatal closure is the major cause for the decline of CO₂ uptake during mild stress. According to this evidence, the effects at the chloroplastic level would arise at tissue relative water content (RWC) below 70% (Kaiser 1987), although a decrease in mesophyll photosynthesis has been observed at earlier stages of leaf dehydration in older leaves and in plants subjected to sub- or supra-optimal temperatures (Chaves *et al.* 1992). During the onset of drought, stomatal conductance normally declines before photosynthesis, suggesting that the inhibition of photosynthesis under mild stress can be mostly explained by a restriction of CO₂ diffusion (Chaves 1991; Cornic 2000). This hypothesis is supported by experiments with *Primula palinuri*, where the removal of the lower leaf epidermis before dehydration did not cause any significant inhibition of photosynthesis (Dietz and Heber 1983). It is also supported by the full recovery of photosynthesis when leaves exhibiting RWC down to 60–70% were measured at saturating CO₂ concentration in the atmosphere (Quick *et al.* 1992).

Evidence suggesting that elevated CO₂ can overcome both stomatal and non-stomatal inhibition of photosynthesis under water deficits comes from the observed recovery of sucrose phosphate synthase activity in leaves of droughted plants after exposure to an increased concentration of CO₂ (Vassey and Sharkey 1989). Ort *et al.* (1994) also argued that intercellular CO₂ concentration (C_i) mediates a balanced interaction between stomatal and non-stomatal components in controlling photosynthetic capacity. Their hypothesis is based on a decrease (of *c.* 25%) observed in the rate of net photosynthesis of sunflowers following a 5-min treatment at low C_i (close to the CO₂ compensation point). Treatment with low CO₂ may cause an imbalance in triose-P *versus* RuBP regeneration, preventing recovery of photosynthesis when C_i is increased to normal levels. More recently, Meyer and Genty (1999) showed that the decrease in electron transport rates induced by dehydration and ABA treatment, which they argue is mediated by Rubisco deactivation, was almost reverted to the control rate under transient high CO₂ availability. These results suggest that the decline in the intercellular CO₂ after stomatal closure under prolonged water deficits may induce an adjustment (or downregulation) of photosynthetic machinery to match the available carbon substrate and the decreased growth. This is also consistent with the decrease in the activity of enzymes of the Calvin cycle observed when plants growing under field conditions are slowly subjected to a prolonged drought (Medrano *et al.* 1997; Maroco *et al.* 2002).

The maximal (light-saturated) stomatal conductance ($g_{\max}$) has been used in models to simulate plant response to drought and was suggested as an indicator of drought-induced inhibition of different photosynthetic subprocesses (Flexas and Medrano 2002). It must be pointed out, however, that under field conditions the intensity and duration of the midday depression of stomatal conductance, intensified under high light and high temperature stresses, may be more important than the 'maximal' stomatal conductance attained by the plants to predict the range and intensity of metabolic alterations (e.g. photoinhibition) observed in plants under drought.

The inhibition of net photosynthesis under water stress may result in part from lower diffusion of CO₂ across the leaf mesophyll (Flexas and Medrano 2002). The work by Tourneux and Peltier (1995), studying O₂ exchange using ¹⁸O₂ as a tracer, also concluded that water stress (RWC > 60%) induced a decrease in leaf conductance to CO₂ diffusion, which was overcome by high CO₂ concentration (2–4% CO₂). They suggested that the observed decrease in leaf conductance was due not only to stomatal restriction of CO₂ diffusion but also to a decrease in mesophyll conductance. This was supported by the decline in the estimated CO₂ concentration at the carboxylation site of Rubisco. Therefore, providing high CO₂ to the leaf may compensate for the increased resistance of the mesophyll under water

stress, leading to similar rates of photosynthesis in well-watered and water-stressed plants (Quick *et al.* 1992). Similarly, a longer time of exposure to elevated CO₂ was required in thick leaves of well-watered *Eucalyptus globulus* plants, as compared with thin leaves, in order to attain maximal rates of photosynthesis (Pereira *et al.* 1992).

Despite the evidence suggesting that stomata play a major role in the limitation of carbon assimilation under mild stress, results conflict in the literature, which was fully reviewed recently by Lawlor and Cornic (2002). These discrepancies, which are mainly related to the onset of metabolic changes induced by water deficits, may be explained by differences in the rate of imposition and severity of the stress, plant developmental stage and leaf age, species studied (showing different thresholds for metabolic inactivation), and the superimposition of other stresses (which can modulate the sensitivity of cellular processes to dehydration). Also, the approaches used to assess limitations may contribute to the variability in responses obtained, as is the case of the response curves of net CO₂ assimilation against intercellular CO₂ concentration ($A-C_i$ curves), which are generally plagued by errors in C_i determination, especially under fast-developing drought conditions. Under slowly developing drought in the field, a good agreement was shown between the results that were obtained by the $A-C_i$ responses and the biochemical evaluation of key photosynthetic enzymes, both suggesting that stomata and reduced biochemical capacity co-limited photosynthesis (Maroco *et al.* 2002).

How do plants get rid of excess light?

Under well-hydrated conditions, C₃ plants use a large fraction of absorbed light through photosynthesis and photorespiration (Maroco *et al.* 1998). This fraction decreases as photosynthetic photon flux density (PPFD) increases, even more so when drought is superimposed, although in some cases photorespiration was reported to increase in droughted plants (Wingler *et al.* 1999). To get rid of excess light, plants can either prevent its absorption, for example by adjusting leaf angles to the sun (heliotropism) (see review by Ehleringer and Forseth 1980) or by losing chlorophyll (Havaux and Tardy 1999), or they can divert absorbed light from photochemistry to other processes, such as thermal dissipation (Demmig-Adams and Adams 1996). Water availability plays a major role in modulating heliotropism, its deficit resulting in steeper midday leaf angles, or paraheliotropism. It was suggested that paraheliotropic leaf movements and photosynthetic characteristics evolved in a concerted way in species adapted to arid environments, so that water use efficiency is enhanced and photoinhibition is mitigated under conditions of water stress (Kao and Tsai 1998). Loss of turgor in wilting leaves is also an effective mechanism for decreasing light capture (Lawlor and Cornic 2002).

If plants are not successful in escaping excessive light by architectural or morphological photoprotection, it is their capacity to thermally dissipate absorbed light by a non-radiative process that plays a central role in leaf photoprotection under environmental stresses, such as drought. It has been shown that this process can dissipate 75% or more of the photons absorbed by the leaves (Niyogi 1999). Under these conditions, leaves experience a transient decrease of photochemical efficiency of PSII ($\Delta F/F_m$), called 'down-regulation of photochemistry', or they undergo photoinhibition, when a decline of leaf maximal photochemical efficiency (F_v/F_m), associated with D1 protein damage at the PSII complexes, is observed (Powles 1984; Osmond 1994). At the limit, photo-oxidative destruction of the photosynthetic apparatus may occur. As was highlighted by Ort (2001), until very recently photosynthetic capacity was thought to be a static characteristic dictated by the genotype and established during development. Now, it is acknowledged that the regulation of photosynthesis in response to the environment is highly dynamic and is modulated in the short term by thermal energy dissipation. This non-radiative energy dissipation, which occurs for example during the afternoon depression of PSII efficiency in leaves under strong light, is paralleled by an increase in the concentration of de-epoxidised xanthophyll cycle components, antheraxanthin and zeaxanthin, at the expense of violaxanthin (Demmig-Adams and Adams 1996; Havaux 1998). Although the xanthophyll cycle plays a primordial role in the thermal dissipation process, a large trans-thylakoid pH gradient in the chloroplast is simultaneously required, implying that other processes might be involved (Niyogi 1999). One hypothesis is that a conformational change (oligomerisation) of the light-harvesting complex LHCII, taking place as a result of zeaxanthin binding to LHCII, has a role in thermal dissipation (Ort 2001). However, recent data suggest that the xanthophyll cycle, in addition to its participation in the non-photochemical energy quenching, might have a direct antioxidant action by enhancing the tolerance of thylakoid membranes to lipid peroxidation (Niyogi 1999). Vitamin E (α -tocopherol), a constitutive component of the lipid matrix of thylakoid membranes, is also believed to have significant photoprotective and stabilising functions (Havaux 1998). Higher α -tocopherol contents occurred in drought-tolerant species than in susceptible ones (Munne-Bosch and Alegre 2000).

Under water stress, when the use of absorbed light in either photosynthesis or photorespiration and the thermal dissipation are not enough to cope with excess energy, the production of highly reactive molecules is exacerbated. These molecules, generated within the chloroplast, can cause oxidative damage to the photosynthetic apparatus (Foyer and Mullineaux 1994; Smirnov 1998; Niyogi 1999). Oxidative stress is a general term used to describe a state of damage caused by reactive oxygen species (ROS). These

oxygen species are hydrogen peroxide (H_2O_2), the superoxide ($\text{O}_2^{\bullet-}$) and hydroxyl ($\text{OH}^\bullet$) radicals and the singlet ($^1\text{O}_2$).

Antioxidant molecules and enzymes that are located in different cell compartments can scavenge ROS. These include the superoxide dismutases (SODs), which catalyse the dismutation of $\text{O}_2^{\bullet-}$ to H_2O_2 , catalases (CATs), responsible for removal of H_2O_2 , and the enzymes and metabolites of the ascorbate–glutathione cycle that are also involved in the removal of H_2O_2 (Foyer and Mullineaux 1994). Antioxidant capacity is greatly dependent on the severity of the stress as well as the species and the developmental stage. Acclimation to drought is generally associated with enhanced activity of the antioxidant enzymes, thus keeping ROS concentration relatively low (Smirnov 1998; Dat *et al.* 2000).

It must be pointed out that ROS can also serve as secondary messengers in the signalling for the activation of defence responses (Dat *et al.* 2000). This dual function of ROS, first described in responses to pathogens and recently demonstrated in several abiotic stresses, presumably plays an important role in acclimation processes (Dat *et al.* 2000).

Interestingly, water deficits can also elicit antagonistic or protective effects in relation to critical supra-optimal temperatures. In lupins (Chaves *et al.* 2002) and in various Solanaceae species (Havaux 1992), photosynthesis was significantly less inhibited by temperatures above 38–40°C in dehydrated plants than in well-watered plants. The nature of the protection of PSII against extreme heat stress in water-stressed plants is not yet clarified, one hypothesis being that membrane stability increases in dehydrated tissues.

Adaptation of CO_2 uptake mechanisms to drought — C_4 and CAM plants

One of the most intriguing plant metabolic adaptations to drought occurs in plants possessing C_4 or crassulacean acid metabolism (CAM) photosynthesis. In C_4 plants, a metabolic pump has evolved that concentrates CO_2 in the bundle sheath cells where Rubisco is located (Edwards *et al.* 2001). The CO_2 -fixation process is separated in mesophyll cells (where CO_2 is first accumulated into a C_4 acid) and the bundle sheath cells (where decarboxylation of the C_4 acids concentrates CO_2 , optimising its concentration at the Rubisco sites). While reduced CO_2 concentrations may have been the driving force for the evolution of C_4 plants (either *per se* or associated with drought stress) (Maroco *et al.* 2000b), this specialised photosynthesis led to greater water use efficiency and ecological success in arid environments. However, the sensitivity of the photosynthetic metabolism to water deficit in C_4 plants is similar to that in C_3 plants (Cornic and Massacci 1996).

CAM plants concentrate CO_2 during the night into C_4 acids and re-fix it to carbohydrates during the day, in a

process similar to C_4 plants, but separated temporally and not spatially as in C_4 plants (for a review see Cushman 2001). By switching the pattern of CO_2 uptake through the stomata to the night period and by fixing CO_2 into carbohydrates during the day (with closed stomata), these plants greatly increase their water use efficiency, improving their chances of survival in extremely arid environments. One of the most interesting plastic adaptations of photosynthesis to drought occurs in inducible CAM plants. These plants generally operate under a C_3 mode of photosynthesis, but once exposed to dehydration stresses (water, salt, low temperatures, and so on), they switch from the C_3 mode to the more water-use-efficient CAM photosynthesis. Drought stress induces a complex balance of gene expression and repression (see below and Cushman and Bohnert 1999) that results in the accumulation of a set of allosteric enzymes that will allow CAM photosynthesis to operate.

Sensing and signalling water deficits

Determining how plants sense water deficits is complex. Signals may be different for different processes (e.g. stomata may not respond to the same signal as cell expansion or leaf senescence). In addition, we have to identify the exact locations of signal production and sensing and the processes transducing the signal into a cell or tissue response. Finally, genes will be activated or de-activated and physiological changes will take place, leading to a reprogramming of plant development. The nature of the primary mediators of cellular processes — water status, turgor, bound water, hormones (e.g. ABA), alteration in cell membranes and others — are still under debate. It is also recognised that there is a considerable overlap between abiotic stress signalling pathways (partly through ROS production), with specificity to water stress present, for example, at the level of the initial stress perception (Knight and Knight 2001).

Signalling can either occur locally or at long distance. Root-to-shoot signalling requires that chemical compounds or physical signals travel through the plant in response to stress sensed in the roots. These signals may be either positive, in that something is added to the xylem flow, or negative, if something is taken away from (or not produced in) the xylem stream. If cells respond to changes in hydraulic pressure, then xylem cavitation itself may act as a hydraulic signal.

The relative importance of chemical and hydraulic signals for water stress has also been under debate for some time. This is most important in the case of the control of stomatal aperture and the regulation of transpiration, where both types of signals are likely to act (Comstock 2002). Changes in turgor that occur in cells because of transpirational water loss may directly influence cell expansion, but it is more a condition than a signal perceived by the cells, other factors being essential to determine cell expansion (see e.g. Proseus *et al.* 1999).

How do plant cells sense drought?

The first step in switching on a molecular response in response to an environmental signal (such as a water deficit) is its perception by specific receptors. Upon activation, these receptors initiate (or suppress) a cascade response to transmit the information through a signal-transducing pathway. Following early clues from yeast, where it was shown that initial perception of water deficit was mediated through a transmembrane histidine kinase that functions as an 'osmosensor' (Posas *et al.* 1996), Urao *et al.* (1999) were able to show that water deficit, followed by the resulting osmotic stress, triggered the expression of the putative osmosensor AtHTK1 in *Arabidopsis*. AtHK1 has a histidine kinase domain, a receiver domain and two transmembrane domains, and may be the first component 'sensing' changes in osmotic potential inside the cell and triggering the downstream signalling cascade that results in dehydration-induced gene expression (Urao *et al.* 1999). Consistent with this hypothesis, a high-affinity K⁺ transporter has been recently cloned in *Eucalyptus camaldulensis* (EcHKT) that shows some similarities to AtHK1 in sensing osmotic changes in solute concentration of the external medium (Liu *et al.* 2001). More recently, Urao *et al.* (2000) cloned three potential phospho-relay intermediates (ATHP1–3) and four response regulators (ATRR1–4) that may be involved in the step after osmosensing.

It is possible that during the development of water stress the interaction of cationic and anionic amphiphilic substances with plasma membranes results in changes in the physical state or protein–lipid interactions of membranes that relay osmosensing by cells. Such a mechanism was recently described in *Lactococcus lactis*, where activation of the osmoregulated ABC transporter OpuA was mediated by changes in membrane properties and protein–lipid interactions (Heide and Poolman 2000; see also Wood 1999). Most significantly, changes in the physical state of membranes may also regulate the activity of major integral membrane proteins, such as aquaporins, which are involved in the control of cell volume or turgor homeostasis (Tyerman *et al.* 2002). Gene expression for this protein family is affected by water and osmotic stresses (Kawasaki *et al.* 2001; Morillon and Chrispeels 2001; see also Tyerman *et al.* 2002 and references therein). However, from studies with *Mesembryanthemum crystallinum* (the common ice plant), it appears that post-transcriptional and/or post-translational factors may play a much larger role in water channel regulation than does gene expression (Kirch *et al.* 2000; Tyerman *et al.* 2002).

Signalling events and cell-mediated responses

Following the first perception of osmotic changes during water stress by osmosensor stimulation, the signal transduction cascade involves protein phosphorylation and

dephosphorylation mediated by several protein kinases and phosphatases whose genes have been shown to be upregulated by water stress (Luan 1998; Lee *et al.* 1999). Changes in cytoplasmic Ca²⁺ concentration are likely to mediate the integration of different signalling pathways. Some of the most abundant regulatory protein kinases involved in abiotic stress signalling are Ca²⁺ dependent (CDPK) (Knight and Knight 2001) and mitogen activated (MAPK) (Kutz and Burg 1998). Several MAPKs and CDPKs have been identified in plants subjected to water stress and shown to be involved in transducing the dehydration signals sensed at the plasma membrane to the nucleus (for a recent review see Ramanjulu and Bartels 2002).

After the first stress-recognition events, cell-to-organ drought-mediated responses diverge in different pathways according to the involvement or not of ABA. In the ABA-dependent pathway, the accumulation of ABA activates various stress-associated genes, some of them being recognised as stress adaptive. These gene products are either functional (such as aquaporins or the enzymes of osmo-protectant biosynthesis) or regulatory (such as protein kinases). The ABA-independent pathway so far identified is still poorly understood, but is known to be rapidly induced by water stress. Although the ABA-dependent and ABA-independent pathways are usually considered to function independently of each other, it is certainly possible that some 'cross-talk' exists between them, as suggested by Kizis *et al.* (2001).

ABA-mediated responses

Accumulation of ABA under water deficit may result from enhanced biosynthesis and/or a decrease in breakdown (Bray 1997). ABA is synthesised in the shoot and root in response to various stresses including drought, low temperature and hypoxia. ABA biosynthesis can be assumed to start at the epoxidation of zeaxanthin, by zeaxanthin epoxidase (ZEP) to form epoxyxanthophyll precursors (Audran *et al.* 1998; Taylor *et al.* 2000). While in leaves the steady-state levels of ZEP mRNA do not seem to be affected by dehydration, a sevenfold increase in expression was observed in roots after an 8-h dehydration period (Audran *et al.* 1998; Bray 2002). It appears that in leaves there is enough violaxanthin such that gene induction is not required for ABA biosynthesis, whereas in roots gene induction is essential to provide substrate for ABA biosynthesis (Bray 2002). Similar spatial expression patterns for these genes were also observed in tomatoes (Thompson *et al.* 2000).

The key regulatory step in ABA synthesis in both roots and leaves is probably catalysed by 9-*cis*-epoxycarotenoid dioxygenase (NCED), an enzyme that converts the epoxy-carotenoid precursor to xanthoxin in the plastids (Qin and Zeevaert 1999). Xanthoxin is then converted to ABA by two cytosolic enzymes via abscisic aldehyde. The *NCED* gene has been cloned in cowpeas, tomatoes, beans and maize, and

shown to have a promoter that is induced by drought both in leaves and roots (Qin and Zeevaart 1999; Iuchi *et al.* 2000; Taylor *et al.* 2000; Thompson *et al.* 2000), with evidence suggesting that regulation at the gene level is independent of ABA concentration (see Bray 2002 and references therein). Recently, the overexpression of the *Phaseolus vulgaris* *PvNCED1* gene in tobacco (Qin and Zeevaart 2002) as well as the overexpression of *AtNCED3* from *Arabidopsis* (Iuchi *et al.* 2001) demonstrated that ABA levels can be manipulated, through the overexpression of this key regulatory gene in ABA biosynthesis, to improve drought tolerance. The final step in ABA synthesis is catalysed by the abscisic aldehyde oxidase (AAO3) (Seo *et al.* 2000). In *Arabidopsis*, the *AAO3* mRNAs are upregulated by water stress but the enzyme levels remain unchanged (Seo *et al.* 2000; Taylor *et al.* 2000). Control of this enzyme seems to occur at the transcriptional and post-transcriptional level, but the precise functions of this regulation and the translational regulation remain uncertain (Bray 2002).

ABA induces the expression of several genes, mediated through ABA-responsive *cis*- and *trans*-acting factors and protein kinases or phosphatases interacting with Ca^{2+} (Luan 1998; Bowler and Fluhr 2000), in a signalling cascade that was recently reviewed (Bray 2002). Early work has shown that ABA can induce signalling cascades from inside or outside of the cell (Allan and Trewavas 1994), and multiple ABA receptors have been proposed (Walker-Simmons *et al.* 1997). Several genes that are upregulated under drought conditions contain a conserved ABA-responsive element (ABRE) in their promoter regions (Uno *et al.* 2000). Several *cis*-acting basic leucine zipper (bZIP) transcription factors (ABRE1–3) have been cloned in *Arabidopsis*, and their transcription is upregulated by drought and salt stress in a mechanism probably based on the increase of osmotic potential generally associated with these stresses. As discussed above, one of the main effects of ABA at the leaf level is leaf stomatal closure (see also the recent review by Wilkinson and Davies 2002). However, the origin of ABA that controls stomatal closure is now under debate. Recently, work by Holbrook *et al.* (2002) with grafted tomato plants constructed from the ABA-deficient tomato mutants *sitiens* and *flacca* and their near-isogenic wild-type parents, demonstrated that stomatal closure in response to soil drying can occur in the absence of leaf water deficits and may not require ABA production by the roots. ABA effects on stomatal closure may be mediated by a chemical signal originated in the roots (other than ABA itself) that may lead to changes in apoplastic ABA levels in the leaves, which may then cause stomatal closure. In leaves, the induction of stomatal closure by apoplastic ABA under water stress is mediated by phospholipase D activity and by the hyperpolarisation of membrane Ca^{2+} channels and tonoplast K^{+} channels of the guard cells (MacRobbie 2000).

Roots play a primordial role in sensing soil water deficits (Sauter *et al.* 2001; Wilkinson and Davies 2002). Sustained root growth under moderated levels of water stress — in opposition to inhibition of shoot growth observed in the early phases of drought — results from rapid osmotic adjustment that allows for partial turgor recovery and maintenance of the ability to loosen cell walls (Hsiao and Xu 2000). This ability of roots to maintain growth under mild water stress is correlated with ABA accumulation. At low water potentials, maize mutants deficient in ABA production maintained better shoot growth than root growth as compared with wild-type plants (Sharp *et al.* 1994; for a recent review see Wilkinson and Davies 2002). Recent work with maize primary roots suggests that endogenous ABA accumulation under drought restricts ethylene production, preventing the ethylene-induced growth inhibition (see also Sharp 2002). However, maintenance of apical root growth at low water potentials in maize was also correlated with an increase of expansin transcripts and expansin activity, which are independent of ABA content (Wu *et al.* 2001). These results suggest that, in addition to the ABA-mediated maintenance of root growth, an ABA-independent mechanism must also be in place.

In contrast to roots, leaf growth is severely inhibited at the onset of drought. Leaf cell expansion during water stress is regulated by changes in pH, and inhibition of growth is mediated by a rapid decrease in extensibility of expanding leaf cell walls (Hsiao and Xu 2000). These changes are associated with the inhibition of wall acidification, which has been hypothesised to occur owing to the inactivation of plasma membrane H^{+} -ATPases under water stress (Bogoslavsky and Neumann 1998). Cell wall peroxidases have also been proposed to have a potentially causal role in restricting leaf cell expansion during drought (Bacon *et al.* 1997).

Local expression of expansins induces the entire process of leaf development and modifies leaf shape (Pien *et al.* 2001). However, and in contrast to roots, it is not clear how water stress affects expansin accumulation in leaf meristems. An increase in xylem sap pH may act as a drought signal to reduce leaf expansion via an ABA-mediated mechanism, as found with ABA-deficient tomato and barley mutants (Bacon *et al.* 1998). In addition, work with ABA-deficient mutants of tomato suggests that maintenance of leaf expansion requires endogenous ABA (within a regulatory pathway acting independently of plant water status). Impairment of leaf growth in these mutants caused by ABA deficiency was in part attributable to ethylene (Sharp *et al.* 2000).

In a recent review Sharp (2002) proposes that the important role of ABA in the control of shoot and root growth under water stress is not a direct one, but rather results from the limitation of ethylene production, which inhibits growth. Insufficient ABA accumulation in shoots

allows ethylene to inhibit shoot growth, whereas in roots higher accumulation of ABA prevents ethylene-mediated inhibition of growth. Translocation of ABA from roots to shoots may be viewed as a strategy of the plant to prevent the inhibitory effects of ethylene over shoot growth in conjunction with the effects on stoma closure and maintenance of turgor (Sharp and LeNoble 2002).

Non-ABA-mediated responses

Several genes are induced by cell dehydration in ABA-deficient and ABA-insensitive mutants, suggesting that such genes do not require ABA for expression (Shinozaki and Yamaguchi-Shinozaki 1997; Luan 1998). ABA-independent genes have a conserved dehydration responsive element (DRE, with a TACCGACAT consensus) in their promoters that is involved in gene regulation by interaction with an ABA-independent signalling cascade. In *Arabidopsis*, two groups of transcription factors that bind to the *cis*-acting DRE have been cloned and characterised (Knight and Knight 2001). The *cbf1* (or *dreb1*) and *cbf2* (or *dreb2*) genes encode structurally different proteins that bind to DRE but are induced by different abiotic stresses. Low temperatures and water deficits induce *cbf1* transcription (Stockinger *et al.* 1997), while *dreb2* transcription is induced by drought and osmotic stress (Liu *et al.* 1998). Two isoforms of DREB2 that show differential tissue specificity have been cloned. *dreb2A* is expressed only in roots in response to salinity, while *dreb2B* is expressed in stems and roots in response to drought (Nakashima *et al.* 2000). The DRE promoter element seems to act as a cross-point where different signalling pathways triggered by abiotic stress converge to integrate information and plant responses to multiple stresses (Knight and Knight 2001).

Sharing of ABA-dependent and ABA-independent pathways may also occur downstream of the first stress recognition and signalling events, and/or a gene may contain both and DRE and ABRE elements in its promoter. For example, in *Arabidopsis* the regulation of the *rd29A* gene (which contains a DRE and an ABRE) is independent of ABA in the first hours of dehydration, but becomes dependent on ABA in later stages of expression (Shinozaki and Yamaguchi-Shinozaki 2000). Similarly, *dreb1* has a conserved motif that is also found in the ethylene-responsive element (ERE) of the ethylene-responsive gene family (Stockinger *et al.* 1997; Shinozaki and Yamaguchi-Shinozaki 2000).

Other mechanisms

Membrane lipids have also been postulated to 'sense' osmotic stress (due to either water stress or cold stress) by changing their fluidity. These changes lead to cytoskeleton reorganisation and affect ion and water channels (Wood 1999; Heide and Poolman 2000; Knight and Knight 2001). This mechanism involves phospholipase C and D as well as phosphatidyl-4,5-phosphate 5-kinase (PIP5) (Mikami *et al.*

1998). Frank *et al.* (2000) cloned two phospholipase D genes (*CpPLD1* and 2) that are involved in the early events of dehydration in the resurrection plant *Craterostigma plantagineum*. While *CpPLD1* was constitutively expressed, *CpPLD2* was induced specifically by dehydration and abscisic acid. Evidence that phosphatidic acid (the product of phospholipase C and D activity) acts as a second stress messenger mediated by ABA was reviewed recently, but the processes involving lipid metabolism in response to water stress remain largely unknown (Munnik 2001; Ramanjulu and Bartels 2002).

In the past, ethylene was mostly studied in connection with hypoxia, for example during soil flooding (for a review see Grichko and Glick 2001). Early suggestions for a role of ethylene during drought were controversial (Elklund *et al.* 1992; Feng and Barker 1992; Michelozzi *et al.* 1995). However, recent evidence (Sharp and LeNoble 2002) suggests that ABA influences growth by mediating ethylene effects in plant organs. Ethylene-response factors (ERFs) have been cloned in *Arabidopsis* and tobacco and shown to mediate ethylene-induced signalling cascades (Solano *et al.* 1998). In *Arabidopsis*, Fujimoto *et al.* (2000) cloned a set of transcriptional activators and repressors (*AtERF1*–5) that bind to the ERE to modulate gene expression mediated by the GCC-box (a consensus region in the promoter of several genes). Further analysis showed that the *AtERF4* gene was induced by dehydration stresses (including cold, salt and drought), while *AtERF3* was moderately induced by drought and salinity in processes apparently independent of ABA and ethylene (Fujimoto *et al.* 2000). One of the most interesting results from this work is that *AtERF3* and *AERF4* can act as transcriptional repressors in *Arabidopsis* leaves, while *AtERF1*, 2 and 5 (whose transcription is induced by ethylene) may activate a specific subset of stress-response genes. The expression of the *AtERF* gene family in response to abiotic stresses appears to be controlled by both ethylene-independent and ethylene-dependent signalling pathways. It must also be noted that ethylene and/or ethylene precursors are also translocated from the roots to the shoot. Several lines of experimental work (for a review see Jackson 1997) showed that 1-aminocyclopropane-1-carboxylic acid (ACC, the immediate precursor of ethylene) does act as a root-to-shoot stress signal. While it is clear that the interaction of ABA and ethylene signalling cascades determine how plant organs respond to drought, this remains an area requiring much more research to elucidate the molecular mechanisms involved.

The sensing-to-signalling processes described above are summarised in Fig. 2.

Biochemical responses at the cellular level — osmotic adjustment

Cellular responses to water deficit include loss of turgor, changes in plasma membrane fluidity and composition,

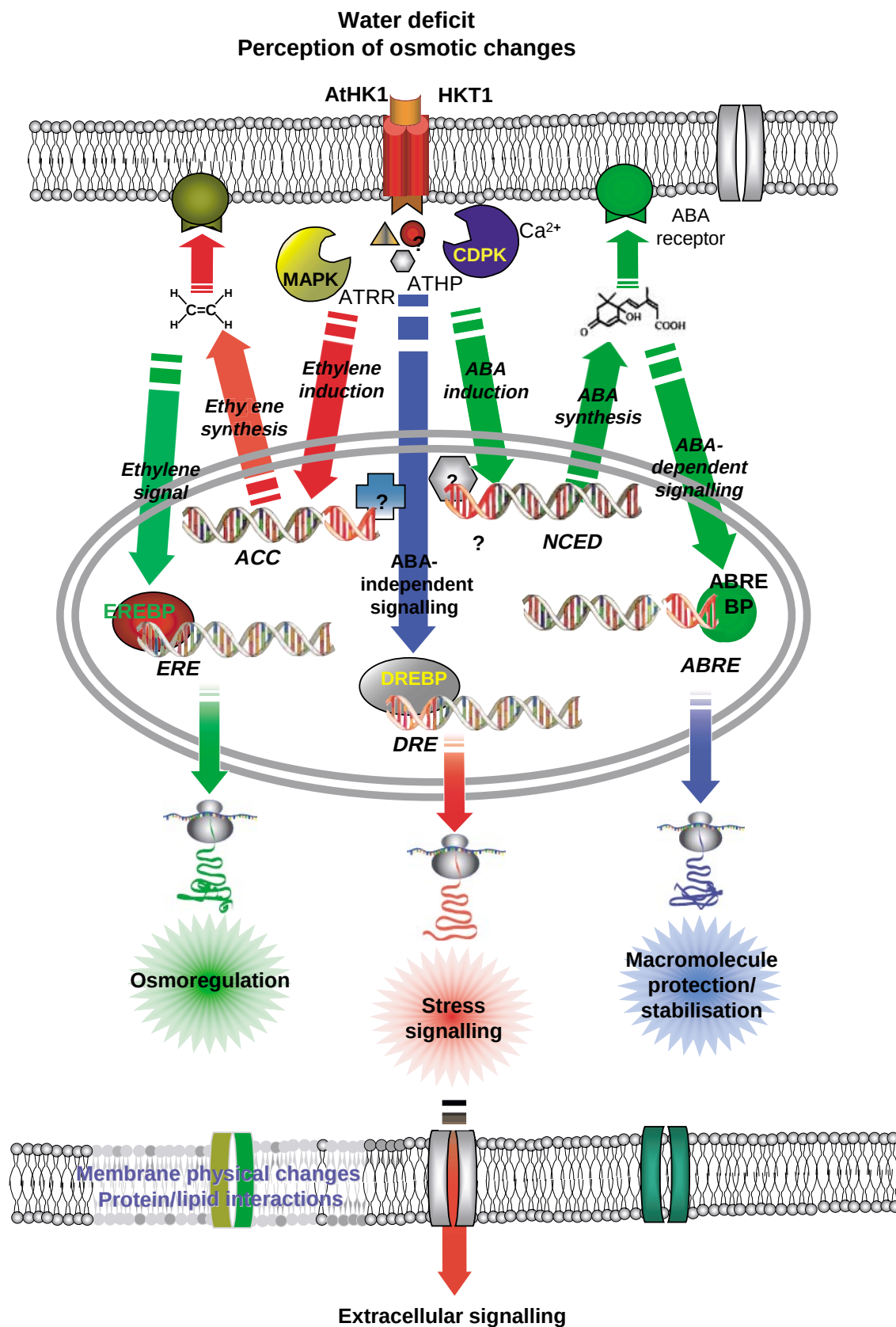


Fig. 2. Sensing, signalling and cell-level responses to drought stress (see text for abbreviations).

changes in water activity and/or solute concentration, and protein–protein and protein–lipid interactions (Bray 1997; Heide and Poolman 2000). Following the early activation of a multigene stress response, several metabolites that play important roles in stabilising enzyme complexes, protecting membranes, and ensuring the osmotic adjustment required to maintain turgor, are synthesised in response to drought.

Osmotic adjustment has been considered one of the crucial processes in plant adaptation to drought, because it sustains tissue metabolic activity and enables regrowth upon rewetting but varies greatly among genotypes (e.g. it is more important in rice or wheat than in maize) (Morgan 1984). However, in terms of crop yield there are not many field studies showing a consistent benefit from osmotic adjustment (e.g. Quarrie *et al.* 1999), presumably because turgor maintenance in cells is often associated with slow growth (see Serraj and Sinclair 2002 for a review). Nevertheless, osmotic adjustment is important in roots, enabling its sustained growth under decreasing water availability in the soil. Osmotic adjustment is normally a slow process (at least in shoots) and is triggered above a certain threshold of cell water deficit. The osmotic compounds synthesised include proteins and amino acids (like proline, aspartic acid and glutamic acid) (Samuel *et al.* 2000; Hamilton and Heckathorn 2001), methylated quaternary ammonium compounds (e.g. glycine betaine and alanine betaine) (Rathinasabapathi *et al.* 2001; Sakamoto and Murata 2002), hydrophilic proteins (e.g. late embryogenesis abundant, LEA), carbohydrates (like fructans and sucrose) (Vijn and Smeekeens 1999) and cyclitols (e.g. D-pinitol, mannitol) (Anderson and Kohorn 2001). In addition to decreasing cell osmotic potential, thus allowing the maintenance of water absorption and cell turgor under water deficit, these solutes may protect the cell membrane and the metabolic machinery under dehydration. Compatible solutes exert their protective activity by interacting with water molecules, rather than by interacting with proteins. Sequestration of water molecules, favouring the reduction of solvent–protein/membrane interaction, can lead to the stabilisation of protein complexes and membranes (Bohnert *et al.* 1995). It should be pointed out that osmoprotection mechanisms are probably not functional until severe dehydration occurs, with the implication that osmotic adjustment may be critical to survival rather than to increase plant growth and crop yield under drought.

One of the most studied compatible solutes is the amino acid proline. In plants, proline is synthesised in the cytosol and mitochondria from glutamate via Δ^1 -pyrroline-5-carboxylate (P5C) by two successive reductions catalysed by P5C synthetase (PC5S) and P5C reductase (PC5R), respectively (Hare *et al.* 1999). Genes encoding these enzymes have been cloned in several plant species, and the expression of P5C was shown to be upregulated by water and osmotic stress involving both ABA-dependent and ABA-independent signalling cascades (for a review see Hare *et al.* 1999).

In addition, there is evidence suggesting that accumulation of products of proline synthesis and catabolism (namely glutamine and P5C) can selectively increase the expression of several stress-regulated genes in rice (Iyer and Caplan 1998). Accumulation of high proline content in cells has been associated with prevention of protein denaturation, preservation of enzyme structure and activity (Rajendrakumar *et al.* 1994; Samuel *et al.* 2000), and protection of membranes from damage by ROS produced under drought and high-light conditions (Saradhi *et al.* 1995; Hamilton and Heckathorn 2001). These effects of proline accumulation may be even more important than its role in osmotic adjustment (Hare *et al.* 1999). Catabolism of proline, catalysed by P5C dehydrogenase (P5CDH), is repressed by drought stress, and recent work suggests that inactivation of P5CDH can result in the accumulation of P5C to toxic levels, the promotion of ROS formation and the facilitation of hypersensitive reactions under pathogen attack. It is apparent that P5C may also serve as a regulator of cellular stress responses (Deuschle *et al.* 2001).

Glycine betaine is an amphoteric quaternary methylated ammonium compound, which under physiological conditions carries no charge and is able to stabilise the structure and activity of many molecules. Protective effects of glycine betaine result either from direct interaction with macromolecules or through the formation of hydration shells around the macromolecular complexes, creating a favourable thermodynamic status to prevent unfolding and denaturation (Papageorgiou and Murata 1995). Glycine betaine was reported to stabilise Rubisco conformation under high salt concentration (both *in vivo* and *in vitro*), to stimulate the repair of the PSII complex during photo-inhibition, and to protect membranes from lipid peroxidation (see Sakamoto and Murata 2002 and references therein). An interesting feature of glycine betaine is that some plant species, like spinach and barley, accumulate large amounts of this compound under several abiotic stresses (drought, low temperature, salt stress, among others) while other species (e.g. *Arabidopsis* and tobacco) do not synthesise this compatible solute (Sakamoto and Murata 2000). Glycine betaine is synthesised in the stroma through a two-step oxidation of choline to betaine aldehyde, catalysed by choline monooxygenase (CMO), and from betaine aldehyde to glycine betaine, catalysed by betaine aldehyde dehydrogenase (BADH) (McNeil *et al.* 1999). Synthesis of glycine betaine can be induced by stress, and the expression of CMO and BADH genes are driven both by drought and salt stress in leaves and roots of different species (Wood *et al.* 1996; Russell *et al.* 1998; Nakamura *et al.* 2001). Inducers of glycine betaine synthesis include NaCl and other inorganic salts (KCl, MgCl, LiCl, Na₂SO₄), H₂O₂, ABA and salicylic acid, with antagonistic effects of free Ca²⁺ (Jagendorf and Takabe 2001). However, the regulatory mechanisms at the gene expression level remain poorly

understood, especially concerning the mechanisms of stress perception leading to changes in its expression and the signal transduction pathways involved (Jagendorf and Takabe 2001). In contrast, there have been multiple attempts to genetically engineer abiotic stress tolerance in plants by overexpressing the enzymes involved in glycine betaine biosynthesis derived from plants or prokaryotic micro-organisms (McNeil *et al.* 1999; Sakamoto and Murata 2001; recently reviewed in Sakamoto and Murata 2002). The success of transgenic plants under field water-stress conditions has, however, been dim (Serraj and Sinclair 2002).

Several hydrophilic globular proteins accumulate in vegetative organs in response to drought. These proteins have been characterised initially in maturing seeds and are designated 'late embryogenesis abundant'. The functions of LEA proteins are multiple as they are involved not only in seed maturation and desiccation but also in vegetative organs, where they can stabilise enzyme complexes and membrane structures and promote ion sequestration (Close 1996; Garay-Arroyo *et al.* 2000). Messenger RNA levels of dehydrins (the D11 class of LEA proteins) increase during progressive drought in sunflowers (Cellier *et al.* 1998), wheat (Labhili *et al.* 1995) and poplars (Pelah *et al.* 1997). In sunflowers, transcription of two dehydrin genes (*HaDhn1* and *HaDhn2*) under drought showed an ABA-dependent diurnal fluctuation, with peak mRNA content occurring at midday for severely stressed plants (Cellier *et al.* 2000). Dehydrins have a highly conserved lysine-rich domain, which is predicted to be involved in hydrophobic interactions and water exclusion, leading to stabilisation of proteins and macromolecular complexes (Close 1996). It is clear that dehydrins are involved in a range of responses to abiotic stress that share a dehydration component (e.g. low temperature and salt stress). The mechanisms by which dehydrin synthesis is enhanced and its functions *in vivo* remain poorly understood (Cellier *et al.* 2000).

Carbohydrates have different functions in plants (from energy storage to signalling), and plants use several sugar-based strategies to adapt to environmental stresses (Anderson and Kohorn 2001). Although the understanding of the mechanisms of sugar signal transduction and sugar regulation of gene expression is still incomplete (Smeekens 1998), it seems that sugars favour the expression of genes in connection with biosynthesis and storage of reserves (including starch) and repress those genes associated with photosynthesis and reserve mobilisation (Ho *et al.* 2001). Hexokinase plays a role in the mechanism by which plants sense carbohydrate status and adjust resource allocation accordingly (Paul *et al.* 2001). Recently, coordinated regulation of gene expression by sugar-starvation and environmental-stress signals was reported, indicating that both signal types are perceived by the same receptor or that the signal pathways converge downstream (Ho *et al.* 2001). Examples of this coordinated response are the induction of

hydrolytic enzymes, such as α -amylases or invertases, and the associated expression of stress-related proteins, which have been observed in water-stressed plants (Pinheiro *et al.* 2001). This also suggests convergence of the nutritional and environmental signal transduction pathways. In general, soluble sugar content tends to be maintained in the leaves of droughted plants, in spite of lower rates of carbon assimilation. This is achieved at the expense of starch, which drastically declines (Chaves 1991). This response favours osmoregulation, enhances desiccation tolerance and allows metabolic activity to be regained more quickly in young leaves. Sugar-induced pathways interact with other signal transduction pathways, including those that involve ethylene, phenylalanine ammonia lyase, ABA and other hormones (Smeekens 1998).

In several plant species, polyols (reduced forms of aldose and ketose sugars) and cyclitols accumulate in leaves in response to water stress (Noiraud *et al.* 2000). Mannitol and sorbitol are the most frequent polyols found in plants. In the cytoplasm, mannitol is synthesised from fructose-6-P (Rumpho *et al.* 1984) and sorbitol from glucose-6-P. Accumulation of these two polyols under drought may account for up to 80% of the total solutes involved in the osmotic adjustment process of some species (e.g. peach and celery) (Lo Bianco *et al.* 2000). Mannitol works as an osmoprotectant and protects thiol-regulated enzymes (e.g. phosphoribulokinase) against inactivation by hydroxyl radicals, which are abundant during the oxidative stress processes associated with water stress (Shen *et al.* 1997). In addition, mannitol serves as an osmoticum, accumulating in the vacuoles. In celery, the vacuole pool accounts for up to 80% of the total mannitol content (Keller and Matile 1989). Accumulation of mannitol under osmotic stress (salt- and/or water-stress driven) seems to be attributable to a reduction in the catabolism of mannitol in green tissues (Stoop *et al.* 1996). The key enzyme in mannitol catabolism is NAD-dependent mannitol dehydrogenase (MTH), which shows decreased activity in celery leaves exposed to high salinity, due to reduced amounts of MTH protein (Stoop *et al.* 1995) and MTH transcripts (Williamson *et al.* 1995). There have been some attempts to improve stress resistance by promoting polyol accumulation in transgenic plants. However, the results did not clarify their role as osmoprotectants in transgenic plants. In some cases, the accumulation of polyols in transgenic plants can even have negative effects on growth and productivity (see e.g. Karakas *et al.* 1997; Sheveleva *et al.* 1998).

Methodological advances in evaluating plant responses to drought

Traditional evaluation of plant responses to drought has relied mainly on leaf-to-atmosphere gas exchange, growth analysis and *in vitro* determination of enzymes, metabolites, and gene expression patterns. However, in the last few years,

methodological advances in robotics and miniaturisation are revolutionising the way plant responses to stress are studied and understood. In addition to genomics and proteomics, which give insight into the molecular mechanisms underlying plant response to stress, integrated tools such as stable isotopes and thermal or fluorescence imaging can support the interdisciplinary study of plant responses to stress within their natural habitat.

Genome-wide tools

The creation of high-throughput DNA-sequencing methods and the completion of model plant genome-sequencing projects (*Arabidopsis* was the first, and very recently draft sequences of the rice genome for both *indica* (Yu *et al.* 2002) and *japonica* (Goff *et al.* 2002) subspecies were published) has enabled investigations into global transcriptome responses to drought, as well as many other stresses and developmental stages. One of the most promising and currently used tools in stress genomics studies is DNA microarray technology (DNA chips). Briefly, a systemised collection of thousands of complementary DNA (cDNA) gene probes [generally derived from collections of expressed sequence tags (ESTs), although other DNA sources can be used] is spotted onto a glass slide by high-accuracy printing robots. Fluorescence-labelled cDNAs generated from a control sample and a stressed sample are mixed in equal amounts and competitively hybridised to the probes in the microarray slide. The relative amount of hybridisation is quantified with a high-resolution scanner (Brown and Botstein 1999). Using such an approach, Maroco *et al.* (1999) studied the relative changes of key regulatory genes for the induction of CAM metabolism in *Mesembryanthemum crystallinum*, as well as 60 functionally unknown ESTs from *M. crystallinum* leaves. During CAM induction by drought or salt stress, there are coordinated changes in gene expression leading to CAM metabolism. CAM-specific genes are strongly upregulated, followed by genes related to glycolysis, starch synthesis and vacuolar transport. In contrast, genes coding for enzymes involved in the Calvin cycle, photorespiration and photochemistry are downregulated. One of the advantages of this technique is that there is no need to have prior knowledge of the gene's identity or function to study its expression patterns. In the study just described, it was possible to identify 60 cDNA clones that were strongly up- or down-regulated during CAM induction, which were not identified or characterised previously (J. Maroco, H. Bohnert and J. C. Cushman, unpublished results) (see Fig. 3). This microarray-based approach has been successfully used with other plant species to monitor the expression pattern of thousands of genes during exposure to drought, cold and salt stresses (see e.g. Kawasaki *et al.* 2001; Seki *et al.* 2001).

Proteomics tools

One of the major drawbacks of genome-wide tools is that alteration of gene expression does not necessarily lead to a functional response, as post-transcriptional and/or post-translational events may prevent a gene response from being translated into a functional protein response. To evaluate the effects of stress on the proteome, researchers have traditionally used two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) to separate proteins according to their isoelectric point (the first dimension) and molecular weight (the second dimension). A drawback of this technique is its low reproducibility and the large degree of protein diversity, which requires several extraction and separation steps. It is also time consuming and the loading capacity and detection in 2D-PAGE gels is limited. The shortcomings of 2D gel analysis have encouraged the development of new techniques to address the requirement of a high-throughput proteome analysis in the post-genomic era. Washburn and Yates (2000) reviewed several promising combinations of multi-dimensional chromatographic protein separation coupled with mass spectrometry techniques that will allow proteome studies in the quest for function. Printing a variety of protein ligands to microarrays can also provide a high-throughput solution for function determination (MacBeath and Schreiber 2000). The spotted ligands, or capture agents, can be thousands of specific antibodies, protein-protein interaction partners or protein ligands. In order to identify proteins in mixtures, they can be labelled with different fluorescent tags, specific proteins being then identified by their positional coding or by further mass spectrometry where ligands are unknown (for a recent review see Zhou *et al.* 2001). Using such a 'proteomic' approach, Riccardi *et al.* (1998) studied the quantitative variation of hundreds of proteins in maize exposed to progressive water deficit. In addition to several proteins that were previously known to be involved in drought responses (e.g. ABA-responsive proteins), they identified new classes of drought-responsive proteins that covered processes from basic metabolism (e.g. glycolysis) to membrane lignification metabolism (Riccardi *et al.* 1998) (for a review on proteomic applications to drought and plant physiology studies see Thielllement *et al.* 1999).

Stable isotopes

Stable isotopes became a very powerful tool to study plant responses to the environment, from the cellular to the ecosystem level. Differences in physical and chemical properties between heavy (e.g. ^{13}C or ^{18}O) and lighter isotopes (e.g. the more common ^{12}C or ^{16}O) result in so-called isotopic effects. The use of natural abundances of stable isotopes in plant science began in the middle 1900s when it was acknowledged that plant material was depleted in ^{13}C in relation to CO_2 in the air. Later, it was discovered that C_4 and CAM plants discriminated less against the

heavier carbon isotope than did C_3 plants. This was followed in the early 1980s by the detailed description and modelling of ^{13}C fractionation during C_3 photosynthesis (O'Leary and Osmond 1980; Farquhar *et al.* 1982). Later, other elements such as oxygen ($^{18}O/^{16}O$) and hydrogen (D/H) were added to the studies of plant water relations. Theoretical develop-

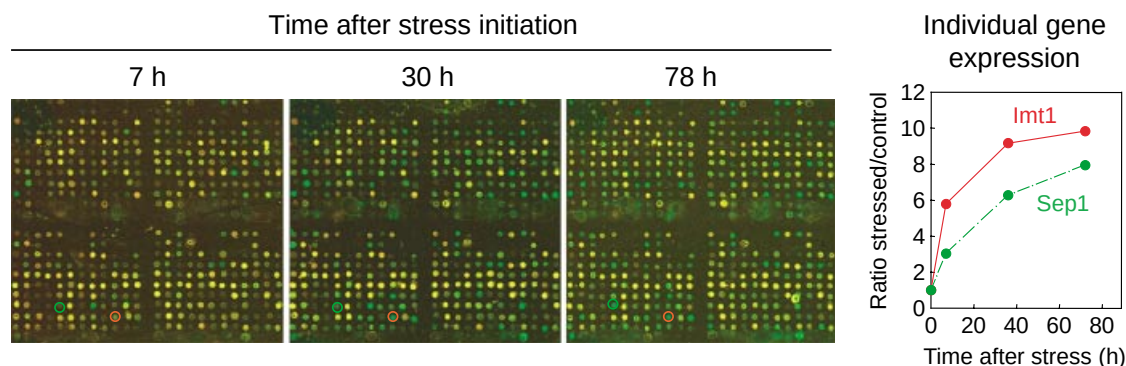
ments and improvements in mass spectrometry led to an exponential growth in the use of stable isotopes, for which theory and practice were compiled in several works, such as the one by Griffiths (1998).

The isotopic composition of a sample is measured relative to a standard as $\delta_s = (R_s - R_{st})/R_{st}$, where R_s and R_{st}

A Stress treatment



B Microarray analysis of individual gene expression



C Clustered patterns of gene expression

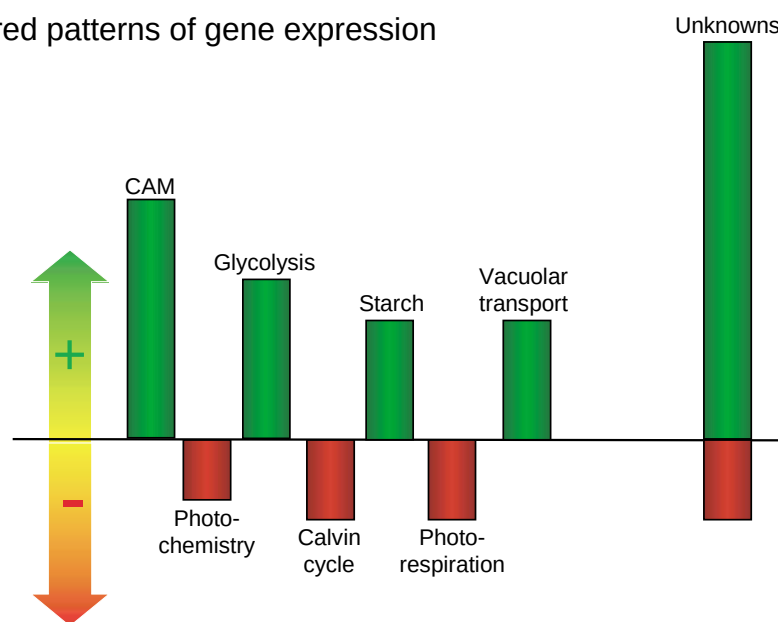


Fig. 3. Whole-genome stress responses in the halophyte *Mesembryanthemum crystallinum*. (A), Stress imposition. (B), Microarray profiling of 384 selected EST clones at different times from stress initiation. (C), Clustered patterns of gene expression into key metabolic pathways during drought or salt stress.

are the isotopic abundances of sample and standard, respectively. As plants discriminate against the heavier isotopes, discrimination is $\Delta = (\alpha - 1)$, where $\alpha = R_a/R_b$, and R_a and R_b are the isotopic abundances of, for example, carbon in atmospheric CO_2 and carbon in the plant, respectively (Farquhar *et al.* 1982). Carbon-13 has been widely used to study water use efficiency in plants. According to the model developed by Farquhar *et al.* (1982) for carbon isotopic fractionation during C_3 photosynthesis, $\Delta^{13}\text{C}$ is related to gas exchange by the ratio between the intercellular and the atmospheric CO_2 partial pressures, C_i/C_a . A wealth of works has been published showing the robustness of the relationship between $\Delta^{13}\text{C}$ and intrinsic water use efficiency (W_i), that is, carbon assimilation divided by stomatal conductance, which is also a function of C_i/C_a . Early use of $\Delta^{13}\text{C}$ in crop breeding for improved water use efficiency faced some limitations because a lower $\Delta^{13}\text{C}$ (higher W_i) may result either from higher rates of carbon assimilation or from stomatal closure and lower productivity (Farquhar *et al.* 1989). Nevertheless, the carbon isotopic composition of biomass is basically determined by water availability and stomatal limitation (and is more positive at drier sites or with increased stomatal limitation) (e.g. Stewart *et al.* 1995; Schulze *et al.* 1998; Bowling *et al.* 2002; Fessenden and Ehleringer 2002). This was used at the ecological level, for example to distinguish functional groups of trees in tropical rainforests according to their access to water (Bonal *et al.* 2000), to compare water use by communities with different biodiversity (Caldeira *et al.* 2001), to assess hydraulic limitations in forests (Fessenden and Ehleringer 2002), and to assess the past water use efficiency by measuring the $\delta^{13}\text{C}$ of tree rings (e.g. Duquesnay *et al.* 1998; Switsur and Waterhouse 1998).

The picture may be somewhat more complex than depicted because the carbon isotopic composition of plant tissues results not only from the discrimination during photosynthesis but also from fractionation during respiration and the biosynthesis of certain compounds (Brugnoli and Farquhar 2000). The measurement of $\delta^{13}\text{C}$ online during gas exchange coupled with the isotopic composition of metabolite pools does improve the perception of processes occurring during drought. Drought changes the isotopic signatures of primary compounds but also changes the metabolite pool size and fluxes that, in turn, will change fractionation during respiration (Duranceau *et al.* 1999; Ghashghaie *et al.* 2001). The nature and importance of fractionation during respiration remains an active field of research.

Oxygen-18 and deuterium in water can be used to trace water movements in the soil–plant–atmosphere continuum. Evaporation leaves behind water enriched in the heavier isotope. Therefore, during transpiration, water in the leaves is enriched in the heavier isotopes, but xylem water must carry the signature of soil water. The source of water for plant transpiration can be studied by comparing the isotopic

signatures of water in the soil, groundwater and xylem (Dawson *et al.* 1998; Yakir 1998). This method has proven useful with woody vegetation that may have deep root systems. In the ecological community, different plants may be using different sources of water, thus bypassing competition for water resources and changing plant–plant interactions and tolerance to drought (Dawson *et al.* 1998; Yakir 1998). To unravel the stratification of water sources may provide an invaluable tool to better understand vegetation dynamics.

The combined study of ^{13}C and ^{18}O in plant tissues may provide information pertinent to separating stomatal and non-stomatal effects when changes in water use efficiency occur. As mentioned above, a lower $\delta^{13}\text{C}$ (higher W_i) may result either from higher rates of carbon assimilation or from stomatal closure and lower productivity. However, the ^{18}O signature of the biomass reflects transpiration rates, as there is no further discrimination for the element during photosynthesis. As CO_2 inside the leaf equilibrates with water in the tissues, it acquires its ^{18}O signal leading to a positive shift in $\delta^{18}\text{O}$ (which may reach 29‰). After photosynthetic CO_2 assimilation, the ^{18}O signal of water is transferred to plant tissues (Farquhar *et al.* 1998; Yakir 1998). As a consequence, the oxygen isotopic composition of plant tissues is correlated only with stomatal conductance. Comparisons of ^{18}O and ^{13}C isotopic compositions can thus aid our understanding of plant response to stress because the effects of stomatal conductance and photosynthetic capacity on changes in water use efficiency can be separated (Farquhar *et al.* 1998; Barbour *et al.* 2000).

Thermal and fluorescence imaging

The early diagnosis of stress in plants has been hampered in the past by the need for destructive sampling or intensive *in situ* measurements. Accurate, non-intrusive methods were scarce. Current imaging techniques, with an increased capacity for data processing and analysis, allow presymptomatic monitoring of changes in the physiological state of plants in a non-destructive way (Buschmann and Lichtenthaler 1998; Chaerle and Van Der Straeten 2000). They also provide spatiotemporal localisation of plant responses to stress, which is essential for a better understanding of the dynamics of plant performance under field conditions. The new imaging technologies include thermal imaging, fluorescence imaging and multi- or hyperspectral reflectance imaging. The combinations of these new tools further enhance their diagnostic power. Until recently, the imaging technologies were confined to controlled conditions. Recent technological advances have made it possible to extend their use to the field (Jones *et al.* 2002). Because they are fast they can speed the screening of large numbers of phenotypes, becoming increasingly important in crop breeding programs, crop management techniques (e.g. field irrigation scheduling) or in supporting models that simulate the impact of global climate change on natural and agroecosystems.

Thermography detects differences in surface temperature by measuring spatially emitted infrared radiation. Computer software transforms the radiation data into thermal images, temperatures being shown as predefined colours (Chaerle and Van Der Straeten 2000). The temperature of leaves and canopies is dependent on the rate of transpiration and so can be used as an indicator of stomatal conductance and stress intensity. This technique has a clear advantage over direct and punctual measurements of stomatal conductance, because it reveals the spatial heterogeneity of the response over a leaf or a canopy. This can provide highly valuable information for the understanding of plant responses to stress. This technique has been applied to quantitative studies of plant response to infections, including the identification of the 'hypersensitive response' before visual symptoms became apparent (Chaerle and Van Der Straeten 2000). It has also been used to screen for spatial variation in stomatal apertures to identify mutants with altered stomatal function (Merlot *et al.* 2001, 2002). Moreover, it allows many measurements, essential for screening a large number of genotypes, for example.

The applicability of the thermographic approach has been limited by difficulties in obtaining temperature data that are truly representative of the crop (Jones 1999). This problem can be avoided under controlled conditions by the use of thermal imagers with associated image analysis software. However, the application of thermal imaging to the study of stomatal behaviour or other stress responses in the field is more difficult, because of the variation in environmental conditions (Jones 1999; Jones *et al.* 2002). In a recent paper by Jones *et al.* (2002), field-portable thermal imaging was used to characterise the frequency distribution of temperature in a sparse grapevine canopy and to establish a thermal index that is proportional to stomatal conductance. In the near future, the establishment of correlations between gas exchange and thermographic data in various vegetation types will allow the use of thermography for satellite or airborne remote sensing, to study plant functioning in response to the environment.

The measurement of chlorophyll fluorescence by modulated fluorometers provides a powerful probe of photosynthetic functioning in response to various stresses, including drought (Schreiber 1986). The recent development of fluorescence imaging has greatly extended the potential of this approach for understanding processes and screening diagnoses. It offers even greater potential when combined with reflectance or thermal imagery. Imaging of chlorophyll fluorescence taken during continuous illumination and/or during intense light pulses saturating for photosynthesis has been already widely used to visualise and analyse non-uniform leaf photosynthesis in response to various abiotic and biotic stresses (Osmond *et al.* 1999). Fluorescence imaging allows the computation of quantitative maps of photosynthetic electron transport activity over the leaf, thus

providing a detailed spatial analysis of stress-induced inhibition of photosynthesis (Meyer and Genty 1999). This has been successfully used to screen and identify mutants with altered PSII photochemistry and xanthophyll cycle (Niyogi *et al.* 1998; Li *et al.* 2000). Recently, imaging of leaf chlorophyll fluorescence also provided the means for a thorough investigation into the basis of photosynthesis inhibition by pathogen attack (Meyer *et al.* 2001).

In addition to chlorophyll fluorescence, leaves display blue and green fluorescence images when illuminated by UV radiation. Multispectral fluorescence images and the ratios between the spectral bands are also being used as fluorescence signatures in the study of plant response to stress (Lichtenthaler 1997). The blue–green fluorescence signature was proposed as an indicator of bound (cell wall) and soluble (vacuolar) cinnamic acids and flavonoids accumulating in leaf cells.

A critical point in these technologies is the need for calibration and validation, using classical physiological analysis of gas exchange and water relations.

Concluding remarks

Great progress in the understanding of individual processes and genes implicated in plant response to drought has been made over the past decade. Nevertheless, a better integration of the molecular and cellular information with whole-plant responses is still needed. Molecular approaches have, however, often been biased towards the short term (Passioura 2002). Unravelling the molecular mechanisms underlying plant responses in their natural habitat is also critical, because in nature, unlike under controlled experimental conditions, various stresses can act in combination. Therefore, novel mechanisms of resistance to stress may occur, such as those apparent in cross-resistance responses. These are great challenges for future work in functional genomics and 'metabolomics' applied to plant stress biology. Studying mutants with altered responses to water deficit is an important approach for the further understanding of the molecular basis of plant responses to drought stress, because it allows us to identify genes important for the expression of whole-plant responses underlying drought adaptation.

Although the role of ABA signalling in plant response to drought has received considerable attention, current knowledge has proven insufficient for the design of effective transgenic strategies to modify ABA metabolism and improve plant resistance to drought. The same applies to transformation strategies related to improving accumulation of compatible solutes. In some experiments, important tolerance gains have been observed, while in others negative effects over plant growth and reproduction were observed. These results confirm the need for increased efforts to relate molecular information to whole-plant processes. Despite this, modulation of stomatal conductance based on present knowledge of long-distance chemical signalling in response

to soil water deficits has already been successfully exploited in agriculture to improve the regulation of water use, plant growth and yield (Davies *et al.* 2002). The interaction of hydraulic and chemical signals and their relative role under field conditions seems to be a critical point in our understanding and modelling of stomatal functioning.

It is also recognised that many of the traits that explain plant adaptation to drought are constitutive and not stress-induced, such as deep rooting and shoot reserves. New molecular and ecological research suggests that roots are the weakest link along the hydraulic flow path (Jackson *et al.* 2000). Studies of root physiology and key genes that regulate water transport, complementing traditional studies of shoot physiology and stomatal control of water loss, are fundamental for the understanding of plant water balance under drought.

Besides escape strategies, which rely mostly on successful reproduction before the onset of severe water deficits, plants adapted to arid and semi-arid environments sacrifice carbon gain for water conservation and photoprotection, both structurally through shoot architecture and physiologically through energy dissipation. It is now well established that the non-radiative dissipation of excess energy is a major process in plant photoprotection under stress conditions (representing up to 80% of total absorbed light). The mechanisms governing light dissipation have become progressively better understood in recent years, including the role of the xanthophyll cycle. Still, intense research is underway to clarify the mechanism of interaction between de-epoxidised carotenoids and H^+ in facilitating energy dissipation.

The development of new tools for monitoring and understanding plant response to stress, which range from the molecular (genomics, proteomics tools) to the plant and ecosystem levels (isotopes, imaging), will allow faster progress in the comprehension of plant performance in diverse environments and in bridging the phenotype–genotype gap.

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