# The Agony of Choice: How Plants Balance Growth and Survival under Water-Limiting Conditions[1]

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When confronted with water limitation, plants actively reprogram their metabolism and growth. Recently, it has become clear that growing tissues show specific and highly dynamic responses to drought, which differ from the well-studied responses in mature tissues. Here, we provide an overview of recent advances in understanding shoot growth regulation in water-limiting conditions. Of special interest is the balance between maintained growth and competitiveness on the one hand and ensured survival on the other hand. A number of master regulators controlling this balance have been identified, such as DELLAs and APETALA2/ETHYLENE RESPONSE FACTOR-type transcription factors. The possibilities of engineering or breeding crops that maintain growth in periods of mild drought, while still being able to activate protective tolerance mechanisms, are discussed.

Due to their sessile lifestyle, plants are continuously exposed to changing environmental conditions that could potentially threaten survival. Therefore, complex mechanisms have evolved to accurately monitor the environment and very dynamically reprogram metabolism and growth. Water availability, which can be constrained by drought, salinity, or freezing, is one of the major factors limiting plant growth and development in agricultural settings (Boyer, 1982). For cereal crops, drought is the most important abiotic stress component reducing yield (Araus et al., 2002). A recent example is the extreme drought that affected 80% of cultivated land in the United States in 2012 and reduced yields of maize (*Zea mays*) by 27.5% and of soybean (*Glycine max*) by 10%, causing enormous economic damage (USDA, 2013). The effects of water limitation will likely worsen in the coming decades due to climate change and the growing scarcity of fresh water available for irrigation, mostly caused by urbanization and the depletion of aquifers, which are currently supplying water to grow food for at least 400 million people in India and China (Jury and Vaux, 2005; Pennisi, 2008). Although selection for high yield potential has also improved yields under water-limiting conditions, especially for mild to moderate drought, there still is a large "yield gap" that is difficult to tackle with classical phenotype-driven breeding (Cattivelli et al., 2008).

Given its importance for agriculture, the effects of drought on plant development have been extensively studied in the past decades. This has significantly contributed to our understanding of physiological and molecular responses to water limitation. In short, mechanisms for dealing with low water availability can be divided into two major categories: stress avoidance and stress tolerance (Verslues et al., 2006; Lawlor, 2013). The aim of stress avoidance mechanisms is to balance water uptake and water loss. Water uptake is enhanced by the accumulation of solutes to lower the tissue water potential and by improving root growth, and water loss through evaporation is limited by closing stomata, restricting shoot growth, and accelerating leaf senescence. Stress tolerance mechanisms are aimed at protecting against cellular damage when the stress becomes too severe and stress avoidance mechanisms are no longer sufficient. These mechanisms include detoxification of reactive oxygen species (ROS) and the accumulation of protective proteins, such as LATE EMBYOGENESIS ABUNDANT (LEA) proteins, and solutes such as Pro, which has a dual role as both osmolyte and osmoprotectant. Both avoidance and tolerance responses are mainly orchestrated by abscisic acid (ABA), although ABA-independent mechanisms involving DROUGHT-RESPONSIVE ELEMENT-BINDING (DREB)-type proteins play a role as well (for review, see Nakashima et al., 2009).

Inhibition of shoot growth, both directly through an active response and indirectly by stomatal closure, is an integral part of improving water balance and stress tolerance, aimed at ensuring plant survival by limiting water loss. However, if the stress is only temporary, limiting growth too extensively can lead to a competitive disadvantage and unnecessary yield losses; on the other hand, continued growth can threaten survival

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when water limitation turns out to be long and severe. Therefore, the balance between growth and survival is tightly regulated, and specific adaptations have evolved to allow growth under drought conditions (Fig. 1). The importance of this balance is illustrated by the finding that DREB2A, a pivotal regulator of water limitation responses, is tightly repressed in developing tissues by GROWTH-REGULATING FACTOR7, a member of a family of important leaf growth regulators (Kim et al., 2003), to avoid the highly detrimental effects of stress responses on growth (Kim et al., 2012).

While altering the expression of regulators of drought responses has often succeeded in enhancing drought tolerance, at least in laboratory conditions, this usually comes at the cost of growth inhibition, resulting in a significant yield penalty (for review, see Yang et al., 2010). Similarly, breeding for enhanced water use efficiency can lead to impaired plant productivity (Blum, 2009). Moreover, lines that show enhanced survival under severe stress do not exhibit improved growth under milder stress conditions, suggesting that both processes are regulated by different mechanisms (Skirycz et al., 2011c). In recent years, major advances have been made in the elucidation of mechanisms regulating shoot growth under water-limiting conditions, which is the subject of this review. We will focus on studies that profile growing shoot tissues subjected to controlled physiologically relevant stress levels, preferably combined with growth measurements. First, methods to study growth under stress conditions and general features of growth regulation will be discussed, followed by a detailed analysis of the effects of water limitation on cell proliferation and cell expansion, the two main processes driving plant growth. A brief section will then be devoted to stress tolerance mechanisms in growing leaves. Interestingly, common mechanisms have been identified that regulate both growth and tolerance, which will be highlighted in a separate section. Understanding how growth and survival are balanced is obviously of great agricultural importance, and in the final section, we discuss the practical perspectives of this line of research. This review will focus on Arabidopsis (*Arabidopsis thaliana*) and common crop species; extremely drought- or salt-tolerant species were not included, as these usually have very specific adaptations that cannot be generalized.

## METHODS TO STUDY WATER LIMITATION

Much of our knowledge on the effects of water limitation comes from early studies exposing plants to severe dehydration, achieved, for instance, by cutting off leaves and leaving them to dry on the bench or by withholding water from plants for weeks until they show severe wilting. Alternatively, osmotic shock was used, realized by transferring plants to solutions containing high concentrations (more than 100 mM) of osmotica such as mannitol or polyethylene glycol (PEG). While these types of experiments have substantially increased our knowledge of stress physiology and molecular responses, they may not reflect physiological conditions that occur in the field (Verslues et al., 2006; Lawlor, 2013). Therefore, new methods have been developed. For short-term responses, in vitro systems in which plants are transferred to low levels of osmotica allow us to easily study very early responses (Skirycz et al., 2011a). While there is an ongoing debate about the relevance of osmotica (for review, see Verslues et al., 2006), their use avoids many of the problems associated with drought experiments on soil-grown plants (for review, see Lawlor, 2013). To address some of these problems, automated watering and phenotyping systems have been built, such as PHENOPSIS (Granier et al., 2006) and WIWAM (Skirycz et al., 2011c). These

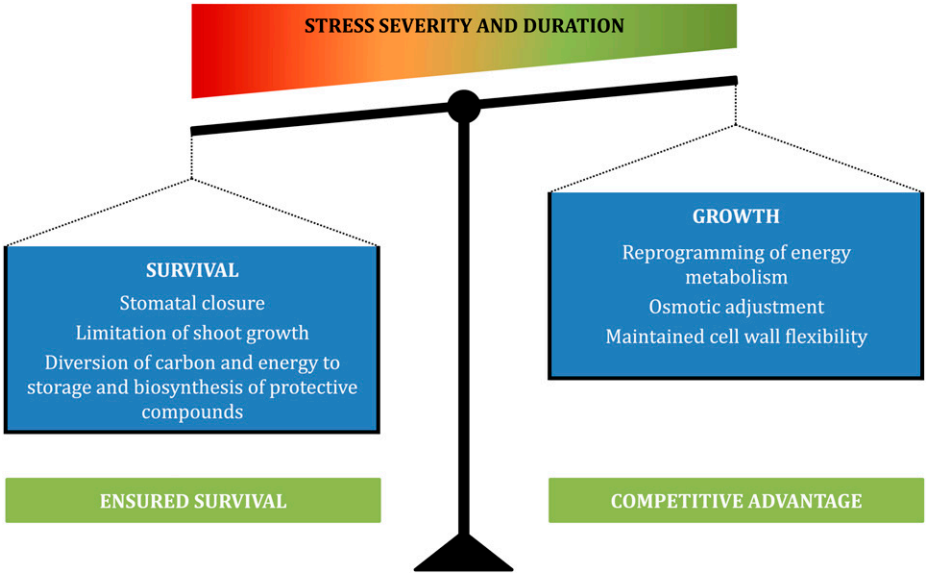


**Figure 1.** The balance between stress tolerance and maintained growth. In response to water limitation, stress avoidance and tolerance mechanisms are activated to ensure survival in case the stress is prolonged or becomes more severe, resulting in growth limitation and a potential competitive disadvantage. However, several adaptations allow plants to balance survival and continued growth depending on the stress level.

systems compensate for the amount of water lost through evaporation by regularly weighing individual pots and adding sufficient water, subjecting a large number of plants to controlled mild drought.

To study the effects of water limitation on growth, it is important to sample growing tissues as specifically as possible to avoid signal dilution due to the high developmental stage, tissue, and cell-type specificity of drought responses (Dinneny et al., 2008; Skirycz et al., 2010; Duan et al., 2013; Verelst et al., 2013). For instance, it has been shown that harvesting young seedlings for molecular analyses obscures effects on proliferating cells and mainly reveals responses of expanding cells (Skirycz et al., 2010). In addition to molecular analyses, growth should be accurately measured, preferably at the cellular level, to distinguish between effects on cell proliferation and cell expansion. Using growth and physiological parameters such as tissue water potential, the stress severity can be monitored, and in soil-drying experiments, this allows one to pinpoint when plants sense the stress and, thus, when tissue sampling is most informative (Bonhomme et al., 2012). In this way, correlating molecular changes to changes in growth becomes possible. Finally, it should be noted that comparing the stress sensitivity of different genotypes is not trivial, complicating the validation of the role of genes or processes through the analysis of mutant or transgenic lines; a recent excellent review by Lawlor (2013) is highly informative on this subject.

## THE EFFECTS OF LIMITED WATER AVAILABILITY ON GROWTH

Growth regulation, mainly aimed at limiting shoot growth and thereby the evaporation surface, is an integral part of the drought response of many plants. It has become clear that this is a very fast and actively regulated response that is not merely a consequence of altered hydraulics, as it cannot be abolished when xylem water potential is maintained (Nonami et al., 1997) and occurs in Arabidopsis, maize, and rice (*Oryza sativa*) even when the leaf water potential is not affected (Michelena and Boyer, 1982; Parent et al., 2010; Bonhomme et al., 2012). Growth is also much more sensitive to water limitation than photosynthesis, and as a consequence, carbohydrates often accumulate in stressed plants, showing that growth reduction is not the consequence of carbon deficit (for review, see Muller et al., 2011). To the contrary, growth is thought to be uncoupled from carbon availability under water-limiting conditions (Muller et al., 2011).

A striking feature that has emerged from many analyses is the highly dynamic and flexible nature of the growth response to water deficit. In many species, there is a fast and sharp decrease of leaf elongation rates, termed acute growth inhibition, followed by recovery to a new steady-state growth rate, referred to as acclimation (Skirycz and Inzé, 2010). Acclimation of growth can already occur within 20 to 30 min in wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) subjected to PEG or salt in hydroponic cultures (Veselov et al., 2002; Fricke et al., 2006). Finally, when the stress is relieved, growth rates can very quickly return to prestress levels (Chazen and Neumann, 1994; Ben-Haj-Salah and Tardieu, 1995; Veselov et al., 2002).

The underlying parameters of growth show great plasticity in their responses to water limitation. Both growth rate and duration can be affected, and the extent to which these parameters are impacted by mild drought was found to strongly depend on the accession or variety in Arabidopsis and sunflower (*Helianthus annuus*; Aguirrezabal et al., 2006; Pereyra-Irujo et al., 2008). As a result, a prolonged growth period can partially compensate for lower growth rates (Aguirrezabal et al., 2006; Skirycz et al., 2010; Baerenfaller et al., 2012). The contributions of cell proliferation and cell expansion to drought-induced growth inhibition were also shown to be accession specific in Arabidopsis and variety dependent in sunflower (Aguirrezabal et al., 2006; Pereyra-Irujo et al., 2008). Several observations suggest that this variety specificity also holds for maize: in cv DEA, both cell proliferation and cell expansion were reduced by mild drought (Tardieu et al., 2000), while in cv B73 and B104, only cell proliferation was affected (H. Nelissen and D. Inzé, unpublished data). The adjustment of growth to water availability is thus not only dynamic but also highly flexible, and different mechanisms have evolved or been selected through breeding.

## MOLECULAR RESPONSES OF GROWING LEAVES TO WATER LIMITATION

The existence of variety-specific responses suggests considerable genetic plasticity in the control of the growth response to water limitation; consequently, many quantitative trait loci for leaf elongation rate sensitivity to lower soil water potential were discovered using a maize recombinant inbred line population (Reymond et al., 2003). However, unraveling the precise molecular mechanisms controlling growth under water limitation requires specifically analyzing growing tissues, as drought responses have been shown to depend strongly on the developmental stage and the severity of stress (Dinneny et al., 2008; Skirycz et al., 2010; Baerenfaller et al., 2012; Verelst et al., 2013). Remarkably, most of the genes identified with a role in stress tolerance in mature tissues under severe stress conditions seem to have little effect on growth inhibition in mild drought conditions (Skirycz et al., 2011c). In recent years, several studies have been performed specifically on drought responses in growing tissues, revealing many general features.

Hormones have been shown to play an important role in adjusting growth to water availability. Transcript profiling of proliferating and expanding leaf tissue from Arabidopsis plants exposed to mild osmotic stress revealed a role for ethylene and GAs in acclimation to both short-term and long-term mild

drought stress (Skirycz et al., 2010, 2011a). This important role for GAs in growth regulation was corroborated by other studies that profiled leaf tissue at different developmental stages in *Brachypodium distachyon* and maize subjected to mild drought (Verelst et al., 2013; H. Nelissen and D. Inzé, unpublished data). Other hormones seem to be involved as well: mutants in jasmonate signaling showed altered growth under mild drought conditions in Arabidopsis (Harb et al., 2010), and phosphoproteome profiling of the maize leaf growth zone also revealed changes in proteins involved in ethylene and jasmonate signaling during drought and subsequent rewatering (Bonhomme et al., 2012). Pretreatment with salicylic acid conferred enhanced growth and stress tolerance in wheat exposed to osmotic stress (Kang et al., 2012). Auxin was found to play a role in growth regulation by osmotic stress in wheat and Arabidopsis (Veselov et al., 2002; Skirycz et al., 2010). Finally, the role of ABA, the canonical stress hormone, is confusing, but the current consensus suggests that ABA can both directly inhibit growth and indirectly stimulate growth by reducing ethylene biosynthesis and, in severe drought conditions, by activating aquaporin expression and opening and controlling hydraulic conductance (for review, see Tardieu et al., 2010; Wilkinson and Davies, 2010). Recent work on the effects of salt stress on root growth showed that the hormonal signals controlling growth are also organ and tissue specific, as ethylene mediates primary root growth inhibition, whereas the quiescence of lateral root growth is mediated by endodermal ABA signaling (Duan et al., 2013).

The dynamic response seen at the macroscopic level is also reflected at the molecular level: changes in the phosphoproteome of maize leaves can already be seen within 10 min of rewatering after moderate drought stress (Bonhomme et al., 2012), and gene expression changes occur in growing leaves within 1 h following the onset of osmotic stress (Skirycz et al., 2011a; Dubois et al., 2013). These rapid responses are especially impressive given the fact that water limitation is sensed by the roots and has to be signaled to the shoot (for review, see Skirycz and Inzé, 2010). Acute growth inhibition and acclimation likely involve different molecular processes, as the transcriptome of leaves from plants acclimated to drought was reported to be very different from previously identified short-term drought responses throughout leaf development (Baerenfaller et al., 2012), although in other transcriptome studies this disparity was not as pronounced (Skirycz et al., 2010, 2011a). In addition, in mature leaves, mild drought-induced transcriptome changes were also shown to exhibit substantial ecotype specificity in Arabidopsis (Des Marais et al., 2012); given the ecotype specificity of growth responses, this is most likely also the case in growing leaves.

Molecular analyses have further uncovered reprogramming of the energy metabolism in growing leaves acclimated to stress. Proteome analysis of expanding cells acclimated to mild osmotic stress revealed that levels of enzymes involved in the Calvin cycle are decreased (Skirycz et al., 2011b), possibly due to feedback signaling in response to the accumulation of sugars in these leaves because of reduced demand from growth (Skirycz et al., 2010). Lower Calvin cycle activity results in less energy production and less $NADP^+$ regeneration, leading to overreduction of the photosynthetic electron transport chain and ROS production. While $NADP^+$ can be regenerated by redox homeostasis mechanisms in the chloroplast (Miller et al., 2010), this represents a substantial loss of energy. However, glycolysis and mitochondrial respiration are both up-regulated (Skirycz et al., 2011b), thereby using the excess of reducing units and sugars to produce energy for growth. Interestingly, also in proliferating tissues, mitochondria play a crucial role in maintaining metabolic homeostasis through the up-regulation of *ALTERNATIVE OXIDASE1A* (*AOX1A*) during acclimation to mild osmotic stress (Skirycz et al., 2010). Alternative oxidation allows energy production under stress conditions while preventing overreduction of the mitochondrial electron transport chain, which can lead to ROS formation (Arnholdt-Schmitt et al., 2006). Furthermore, it was shown that Pro, an important osmolyte and osmoprotectant in mature tissues, can be transported to growing tissues, where it is used as an energy source by Pro dehydrogenase, shuttling electrons directly into the mitochondrial transport chain (Sharma et al., 2011). This fits with increasing evidence that mitochondria play a crucial role in orchestrating stress responses (for review, see Jacoby et al., 2011).

As a final note, it should be mentioned that while many studies on stress-induced growth modulation focus on transcription factors (TFs), it is likely that there is also an epigenetic component to be considered here. Epigenetics are known to play a large role in the regulation of drought responses (for review, see Kim et al., 2010), partly explaining the large transcriptional reprogramming seen in response to stress. Consequently, the linker histone variant *H1-3* is strongly induced by moderate drought in growing tissues (Ascenzi and Gantt, 1999), and the chromatin-remodeling factor AtCHR12 mediates the moderate drought stress-induced arrest of stem growth in Arabidopsis (Mlynárová et al., 2007). Additionally, the ELONGATOR complex, which has histone acetyltransferase activity and in yeast (*Saccharomyces cerevisiae*) is involved in the adjustment of growth to environmental conditions, regulates stress-responsive gene expression and affects cell proliferation during leaf growth (Nelissen et al., 2005). Furthermore, microRNAs are differentially regulated by drought in proliferating and expanding leaf tissue from *B. distachyon* (Bertolini et al., 2013). An RNA-Seq study of proliferating maize leaf tissue also found evidence for substantial alternative splicing, although this was in response to severe drought (Kakumanu et al., 2012).

## MECHANISMS CONTROLLING CELL PROLIFERATION

Cell proliferation is driven by the activity of cyclin-dependent kinases (CDKs), which, as the name suggests, need to be associated with cyclins to be active

(for review, see De Veylder et al., 2007). Plants have many different CDK-cyclin modules (Van Leene et al., 2010), some of which have highly specific roles (Cruz-Ramírez et al., 2012). The activity of CDK-cyclin complexes is controlled by three major mechanisms: control of cyclin protein levels through degradation by complexes like the ANAPHASE-PROMOTING COMPLEX/CYCLOSOME (APC/C); activation or inhibition of the CDK-cyclin complexes by phosphorylation; and interaction of the complexes with inhibitory proteins, of which there are two main families, CYCLIN-DEPENDENT KINASE INHIBITOR (CKI)/KIP-RELATED PROTEIN (KRP)-type proteins and SIAMESE (SIM)/SIAMESE-RELATED-type proteins (for review, see Komaki and Sugimoto, 2012).

Different lines of evidence exist for an effect of drought on almost all components of the cell cycle machinery (Fig. 2, top left panel): the expression of many cyclins is down-regulated by salt stress (Burssens et al., 2000), mild osmotic stress causes down-regulation of APC/C repressors (Claeys et al., 2012), and both CKI/KRP- and SIM-type CDK inhibitors are induced by drought or salt stress (Pettkó-Szandtner et al., 2006; Peres et al., 2007). Besides the transcriptional response, there is also control at the posttranscriptional level. In growing *B. distachyon* leaves, moderate drought results in differential expression of microRNAs known to regulate cell proliferation and cell differentiation (Bertolini et al., 2013). CDKA activity is inhibited by mild osmotic stress in wheat (Schuppler et al., 1998) and Arabidopsis (Skirycz et al., 2011a) and by mild drought in maize (Granier et al., 2000). Also, many components of the mitotic machinery involved in cytokinesis showed differential phosphorylation upon rewatering after drought stress in maize (Bonhomme et al., 2012).

Recently, a pathway was established that connects mild osmotic stress to the cell cycle machinery, involving the hormones ethylene and GAs. This pathway starts with very rapid accumulation of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) and activation of ethylene responses within 1 h after stress onset, leading to posttranslational and reversible inhibition of CDKA, effectively halting the cell cycle (Skirycz et al., 2011a). Among the earliest transcripts induced by stress are *ERF5* and *ERF6*, a redundant pair of ethylene response factors. *ERF6* induction is highly specific for

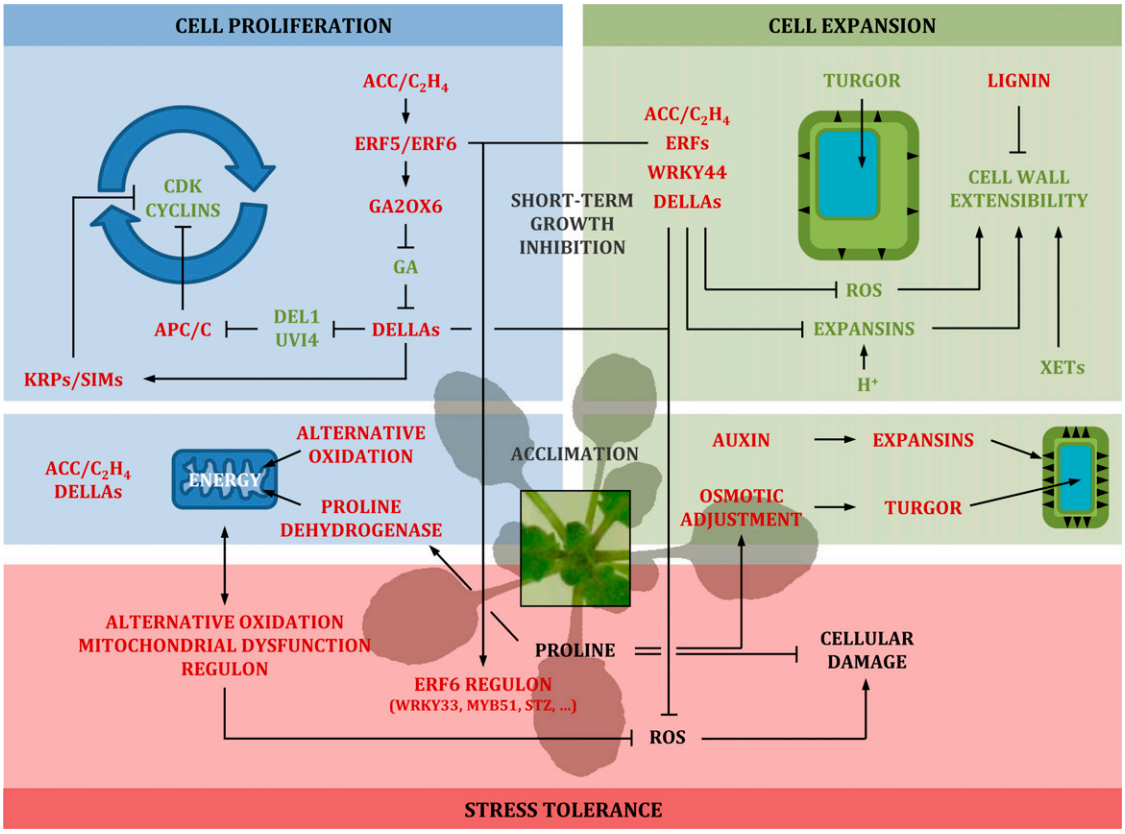


**Figure 2.** Mechanisms regulating growth and stress tolerance in developing leaves, and their interactions. In the top and middle panels, mechanisms regulating cell proliferation and expansion in short-term growth inhibition and more long-term acclimation to stress, respectively, are depicted. In the bottom panel, stress tolerance mechanisms that interact with growth-regulating mechanisms are shown. Font color indicates the direction of change by stress: red for up-regulation, green for down-regulation, and black for no change.

growing leaves; in mature leaves, its expression is repressed by osmotic stress (Dubois et al., 2013). ERF6 was shown to be posttranslationally activated through a mitogen-activated protein kinase cascade involving MPK3 and MPK6 in response to ACC treatment, oxidative stress, or infection with *Botrytis cinerea* (Meng et al., 2013; Wang et al., 2013), and this most likely also happens under osmotic stress. ERF6 then activates the expression of *GA2OX6*, encoding an enzyme that inactivates GAs and thereby causes DELLA stabilization (Dubois et al., 2013). Finally, DELLAs modulate APC/C activity by transcriptional repression of *DEL1* and *UVI4*, two important APC/C inhibitors, and thereby push cells into differentiation and early onset of endoreduplication, irreversibly abolishing their potential for proliferation (Claeys et al., 2012). GAs control the transition from cell proliferation to cell expansion in maize leaf development as well, both in control conditions (Nelissen et al., 2012) and during mild drought (H. Nelissen and D. Inzé, unpublished data), but the molecular mechanisms connecting DELLAs to the transition have not been elucidated yet in maize. In this respect, it is interesting that while Achard et al. (2009) demonstrated that DELLAs control the cell cycle by inducing the expression of *KRP2* and *SIM*-type inhibitors in young seedlings, we found no up-regulation of cell cycle inhibitors by DELLAs in proliferating leaves but, rather, an effect on APC/C regulators (Claeys et al., 2012). This suggests that cell cycle regulation by DELLAs may be a general theme, but the exact mechanism depends on the tissue and conditions.

In leaves of dicotyledonous plants, there is also a special type of cell proliferation in the stomatal lineage, which is controlled independently of the primary cell proliferation arrest front (Gonzalez et al., 2012). This lineage is based on the activity of meristemoids, proliferating cells that generate pavement cells to ensure accurate spacing of stomata, before finally differentiating into a pair of guard cells (for review, see Bergmann and Sack, 2007). The importance of the stomatal lineage is often underestimated in plant growth, but estimates suggest that 48% of pavement cells are the result of meristemoid divisions (Geisler et al., 2000). It is known that water limitation reduces the stomatal index in many species to reduce evaporation (for review, see Casson and Gray, 2008). Interestingly, under prolonged but stable mild osmotic stress, meristemoid activity is modulated in a highly elegant manner, leading to enhanced generation of pavement cells, and thus improved growth, while keeping the number of stomata, and thereby water loss through transpiration, low (Skirycz et al., 2011a). While the pathways involved in the generation of guard cells are well understood, little is known about the control of meristemoid divisions in leaf development (for review, see Gonzalez et al., 2012). ABA was recently shown to restrict entry into the meristemoid lineage, fitting the reduced number of stomata found in water-limiting conditions (Tanaka et al., 2013), but nothing is known about the control of the ratio of pavement cells to guard cells in the output of this lineage.

## MECHANISMS CONTROLLING CELL EXPANSION

Cell expansion in plants is essentially regulated by a combination of water uptake and expansion of the vacuole, on the one hand, and controlled loosening of the cell wall and deposition of new cell wall material, on the other hand. Loosening of the cell wall is mediated by the activity of expansins, which are mainly active at low pH (forming the basis of the so-called "acid growth" hypothesis), of xyloglucan endotransglucosylases/hydrolases (XETs), of pectin methylesterases, and of ROS (for review, see Cosgrove, 2005). The most important signals controlling the activity of these effectors are thought to be auxin and mechanical signals (Uyttewaal et al., 2010). The majority of these cell wall-modulating signals and effectors are modulated by water deficit (Fig. 2, top right panel).

It is likely that hydraulics play a role in cell expansion responses to water deficit. Osmotic adjustment, achieved by the accumulation of solutes to lower the cellular water potential and thereby facilitate water uptake, is seen after water deficit in barley leaves (Fricke et al., 2006) and maize leaves (Chazen and Neumann, 1994). This osmotic adjustment can occur specifically in the growth zone while at the same time being absent in the mature part of the leaf (Michelena and Boyer, 1982), again highlighting the specific responses of growing tissues to stress. However, from the aforementioned studies, it is clear that osmotic adjustment does not always correlate with enhanced cell expansion, indicating that there is also an active growth restriction, most likely targeting cell wall dynamics. Several observations confirm this theory. Chazen and Neumann (1994) showed cell wall hardening in PEG-treated maize leaves within minutes after stress onset. Cell wall extensibility decreased by drought stress in soybean, and this correlated with lower XET activity (Wu et al., 2005). Likewise, the expression of expansin genes in maize leaves correlates with growth dynamics in several environmental conditions, including drought (Muller et al., 2007). PEG treatment also leads to rapid cell wall alkalinization in the growth zone of the maize leaf, thereby counteracting the activity of expansins (Ehlert et al., 2011). In white clover (*Trifolium repens*), lignification was observed in leaves subjected to osmotic stress (Lee et al., 2007), and in the growth zone of maize leaves, drought increases the levels of enzymes involved in lignin formation (Riccardi et al., 1998). Thus, although different mechanisms are used in different species, the end result is always a fast hardening of the cell wall, thereby inhibiting cell expansion even with maintained turgor pressure.

However, during the acclimation response, cell walls become more flexible: in expanding Arabidopsis leaves that had acclimated to mild osmotic stress, cellulose synthesis was down-regulated, but genes involved in cell wall extensibility, such as expansins, were up-regulated, and levels of superoxide were significantly higher (Skirycz et al., 2010). The expression of expansin genes was also up-regulated in Arabidopsis plants exposed to

moderate drought (Harb et al., 2010). In tissues acclimated to a steady-state stress, a more extensible cell wall may improve growth under lower turgor pressure. Even more impressively, when Arabidopsis and sunflower plants were rewatered after they had apparently stopped growing, cell expansion was resumed, suggesting that the cell walls were kept in an extendable state (Lechner et al., 2008).

Not much is known about the signaling networks that relate water status to cell expansion. The accumulation of indole-3-acetic acid was demonstrated in barley leaves after osmotic stress onset (Veselov et al., 2002) and in leaves of maize plants exposed to salt stress (Veselov et al., 2008). This could aid in the acclimation of cell expansion to stress, as auxins are known to stimulate cell wall acidification, XET and expansin activity, and cell expansion (Cosgrove, 2005). Furthermore, MYB41 is thought to be part of a complex network regulating cell wall modification and cell expansion in response to abiotic stresses such as drought (Cominelli et al., 2008). Interestingly, some of the signals that regulate cell proliferation under water limitation, which were discussed in the previous part, may also play a role in the regulation of cell expansion. In expanding leaves of *B. distachyon*, drought consistently up-regulates *miR528*, the predicted target of which is thought to inhibit ethylene production (Bertolini et al., 2013). Transcriptome profiling of expanding Arabidopsis leaves acclimated to osmotic stress also points to a role for ethylene, along with GAs and auxin (Skirycz et al., 2010). Interestingly, *ERF6* overexpression strongly affects cell expansion as well and induces cell shape changes reminiscent of mannitol treatment, suggesting similar cell wall changes (Dubois et al., 2013). This could also be mediated by GAs, which are known to affect cell expansion (Achard et al., 2009). In accordance with this hypothesis, both *ERF6* and *GA2OX6* are induced within hours after stress onset in expanding leaf cells (M. Dubois and D. Inzé, personal communication), making a role for ERF6 and DELLAs highly likely. In the root, DELLAs also control cell elongation through the control of ROS, which contribute to cell wall extensibility (Achard et al., 2008b). Furthermore, overexpression of the stress-induced genes *BrERF4* (from *Brassica rapa*) and *WRKY44* in Arabidopsis conferred tolerance to salt and drought stress and specifically inhibited leaf cell expansion, most likely by affecting the expression of expansins, but had no effect on cell proliferation (Park et al., 2012). Thus, ethylene and GAs also play an orchestrating role in regulating cell expansion under water-limited conditions.

## STRESS TOLERANCE MECHANISMS IN GROWING TISSUES

While growing tissues actively reprogram their growth, they also activate tolerance mechanisms against cellular damage. Interestingly, genes traditionally associated with the response to water limitation, such as *DREB2A*, *RD29B*, *LEA*s, and ABA-related genes, are not induced or even repressed in growing tissues of plants subjected to mild osmotic stress, whereas they are induced in mature tissues at the same stress level (Skirycz et al., 2010, 2011a). However, in these studies, an enrichment of stress markers typically associated with biotic stress, such as WRKY and ERF TFs, mildew resistance locus proteins, and genes involved in the biosynthesis of indolic glucosinolates, was found (Skirycz et al., 2010, 2011a). A different study on young leaves of soil-grown Arabidopsis plants exposed to moderate drought stress found classical ABA-dominated water deprivation responses early after stress onset, but this response disappeared in acclimated leaves, at which point several "biotic" stress markers, such as *MYB51* and *WRKY33*, were induced (Harb et al., 2010). This suggests that tolerance mechanisms in growing leaves may be different from those in mature leaves.

Strikingly, in the three aforementioned studies on growing Arabidopsis leaves, the oxidative stress response was much less pronounced than in mature leaves, which showed accumulation of Pro, flavonoids, and LEA proteins. As the stress level was the same, this likely reflects developmental stage specificity rather than the low stress severity (Skirycz et al., 2010). However, dividing cells are especially sensitive to damage from ROS, and ROS also function as regulators of cell division and differentiation (Schippers et al., 2012), suggesting that there must be mechanisms regulating the redox status in growing tissues. A proteome analysis of expanding leaf cells subjected to mild osmotic stress indeed revealed higher protein levels of redox components such as glutathione *S*-transferases and ascorbate peroxidase (Skirycz et al., 2011b). Interestingly, the extensive reprogramming of mitochondrial metabolism (see above), involving the up-regulation of *AOX1A* and the mitochondrial dysfunction regulon (Van Aken et al., 2007), also has an important function in maintaining ROS homeostasis and thereby limiting cellular damage (Giraud et al., 2008; Skirycz et al., 2010). Mechanisms to prevent and deal with oxidative stress may thus also be somewhat different in growing leaves compared with mature leaves.

## COREGULATION OF GROWTH AND TOLERANCE

As it is crucial for plants to balance, on the one hand, ensured survival through growth quiescence and tolerance mechanisms and, on the other hand, maintained competitiveness through continued growth, there is extensive coregulation of both processes. Here, we highlight four common mechanisms: DELLAs, AP2/ERF-type TFs, Pro, and mitochondrial metabolism reprogramming (Fig. 2, bottom panel).

DELLA proteins, an important class of negative regulators of GA signaling, were shown to be crucial integrators controlling growth and survival in response to various stresses, such as low temperature and high salinity (Achard et al., 2006). DELLA stabilization following severe salt stress results in the activation of many genes that protect cells from cellular

damage, such as ROS-inactivating enzymes, and it was proposed that lowering ROS levels both enhances stress tolerance and limits cell expansion and thereby root growth (Achard et al., 2008b). Consequently, quadruple DELLA mutants, lacking the four major DELLAs, are less tolerant to severe salt stress when survival is scored but show less growth inhibition (Achard et al., 2006). *GASA14* was recently suggested to be a downstream mediator of DELLAs in tolerance and growth regulation control through ROS; it is a GA-regulated gene that stimulates cell expansion and induces tolerance to severe abiotic stress by limiting ROS accumulation, potentially because the protein exhibits redox activity (Sun et al., 2013).

However, there is a level of regulation upstream of DELLAs that suggests that stress tolerance and growth responses can be uncoupled. As mentioned before, ERF6 stimulates the inactivation of GAs by 2-oxidation and thereby induces the stabilization of DELLAs, which inhibit cell proliferation and expansion (Dubois et al., 2013). However, ERF6 also activates stress tolerance genes such as *WRKY33*, *MYB51*, and *STZ*, and this is independent of DELLAs (Dubois et al., 2013). Additionally, ERF6 was also shown to provide a protective role against oxidative stress (Wang et al., 2013) and biotic stress (Meng et al., 2013). For cold stress, a similar pathway was established in which CBF1 is the functional equivalent of ERF6, leading to DELLA-dependent growth inhibition by up-regulation of *GA2OX3* and *GA2OX6* and DELLA-independent stress tolerance (Achard et al., 2008a). Similarly, in response to high salinity, DDF1 directly activates the transcription of *GA2OX7*, leading to a decrease in GA levels and subsequent growth inhibition, and stress tolerance genes such as *RD29A* (Magome et al., 2008). Finally, when *AtDREB1A*, a master regulator of drought tolerance, is overexpressed in soybean, up-regulation of *GA2OX4* leads to a drop in GA levels and subsequent growth inhibition, which can be reversed by GA application (Suo et al., 2012). All these observations point to a common mechanism in which stress-specific AP2/ERF-type TFs induce GA inactivation to regulate growth and independently activate stress tolerance genes.

A very different and surprising form of interplay between tolerance and growth is mediated by Pro. Pro accumulates in response to many abiotic stresses and acts as an osmolyte, osmoprotectant, regulator of redox balance, and signaling molecule (for review, see Szabados and Savouré, 2010). Recently, Pro was shown to be transported to growing tissues to act as an energy source to support both root and shoot growth in Arabidopsis, as Pro catabolism directly transfers electrons to the mitochondrial electron transport chain (Sharma et al., 2011). This fits the observation that an increased production or exogenous application of Pro results in higher stress tolerance and maintained growth under abiotic stress conditions (for review, see Ashraf and Foolad, 2007).

Finally, the role of mitochondria in regulating stress responses, as discussed previously, is also dual: alternative oxidation supplies energy for growth while maintaining redox homeostasis and thereby preventing the formation of ROS. Accordingly, plants overexpressing *AOX1A* showed less growth inhibition when subjected to mild drought (Skirycz et al., 2010), while plants lacking functional AOX1A were more sensitive to combined drought and heat (Giraud et al., 2008). CDKE1 was recently shown to have a role in mitochondrial retrograde signaling and AOX1a activation in response to oxidative and cold stress and was proposed to integrate environmental signals and act as a switch between growth and tolerance (Ng et al., 2013). Furthermore, the Arabidopsis TF WRKY15 regulates both cell expansion and osmotic stress tolerance through control of the mitochondrial stress response (Vanderauwera et al., 2012).

Additionally, several genes were identified that regulate both growth and tolerance to stress, with potential for independent regulation. KUP-type $K^+$ transporters are induced by different stresses with an osmotic component and specifically inhibit cell expansion while enhancing drought tolerance (Osakabe et al., 2013). The kinase NEK6, which is induced by ACC and severe salt stress, negatively regulates ethylene production and signaling and stimulates growth by enhancing the expression of the cyclins *CYCB1;1* and *CYCA3;1* while also inducing stress tolerance (Zhang et al., 2011). In rice, RSS1, a monocot-specific protein that is specifically expressed in proliferating cells and the stability of which is controlled by APC/C, is important for maintenance of the shoot meristem under abiotic stress conditions but is also thought to control stress tolerance responses, as its loss-of-function mutation results in the up-regulation of genes responsive to salt, drought, and cold (Ogawa et al., 2011).

The examples of coupled stress tolerance and growth modulation described here show that a flexible network of genes and processes controls the balance of survival and growth. DELLAs and KUP-type $K^+$ transporters activate stress tolerance at the cost of growth inhibition, as is often seen. However, in order to maintain growth, other mechanisms allow more flexibility. AP2/ERF-type TFs, such as ERF6, represent nodes in the network where growth inhibition and stress tolerance diverge. At the same time, there are factors that both promote stress tolerance and maintain growth, such as Pro, the reprogramming of mitochondrial metabolism, NEK6, and RSS1. Understanding how these nodes, and additional ones that are yet to be discovered, function dynamically in the network controlling growth and survival is one of the major challenges of abiotic stress research, which holds great promise for the engineering or breeding of drought-tolerant plants.

## PERSPECTIVES FOR ENHANCING DROUGHT PERFORMANCE IN THE FIELD

After decades of research on how plants respond and adapt to drought, many interesting leads have

been identified, but little of this knowledge has been translated to the field (for review, see Deikman et al., 2012). One reason that was brought forward to explain this discrepancy centers around the observation that often artificial and too severe stress assays are used, which bear little relation to physiological conditions (Lawlor, 2013). Therefore, the severity, duration, and developmental timing at which stress occurs should be carefully controlled. Moreover, the use of noninvasive high-throughput phenotyping allows one to directly analyze growth and physiological parameters during water limitation, which may be a better measure than scoring survival under very severe stress (Skirycz et al., 2011c; Deikman et al., 2012). Also in classical breeding, precise and proper phenotyping is currently seen as one of the most limiting factors in the generation of drought-tolerant crops, as this is a quantitative trait in which single genes or quantitative trait loci usually have subtle effects that are strongly dependent on the genetic background and show strong environment interactions (Cattivelli et al., 2008; Araus et al., 2012). A final complication comes from the fact that, in the field, different stresses are often experienced simultaneously, and a recent report suggests that responses to combinations of stresses cannot easily be predicted from single stress responses (Rasmussen et al., 2013). Indeed, transcriptome and metabolome responses to combined heat and severe drought, two stresses that commonly occur together in agricultural conditions, were previously found to be very different from responses to either stress alone (Rizhsky et al., 2004).

Despite these shortcomings, the first generation of targeted drought-tolerant crops is coming to the field. In recent years, a number of drought-tolerant maize varieties have been released, such as Syngenta's Agrisure Artesian and Pioneer's Optimum AquaMax hybrids, achieved through advanced molecular breeding based on knowledge gained by fundamental research into drought responses (Tollefson, 2011). The first drought-tolerant genetically modified crop, Monsanto's DroughtGard maize, is set for release in 2013. It is a transgenic hybrid line expressing *CspB*, an RNA chaperone isolated from *Bacillus subtilis*, which was shown to enhance productivity during drought without yield penalty under well-watered conditions, although the exact mechanism is unknown (Castiglioni et al., 2008). An interesting aspect of this variety is that it was specifically developed to tackle moderate drought in the western Great Plains of the United States, and this highlights a growing insight in the field: most likely, there is no magic bullet that will offer generic tolerance to water limitation, necessitating the development of specific solutions for specific situations (Tardieu, 2012).

It is our belief that enhancing growth with limited water can be beneficial in areas that experience mild drought spells, especially during vegetative growth, as was recently argued for $C_4$ plants such as maize (Lopes et al., 2011). Likewise, for temperate cereals, enhanced shoot growth is seen as a contributing factor in breeding for higher yields under water-limiting conditions, as this minimizes moisture evaporation from the soil and is associated with enhanced root growth and, therefore, better water uptake (Richards et al., 2010). Factors controlling both growth and tolerance mechanisms, like those discussed in this review, have a large potential for the engineering of continued growth in mild drought conditions, as this allows one to deactivate growth inhibition while maintaining a certain level of protection against damage. However, this approach may exacerbate the problems during severe drought (Tardieu, 2012), during which lack of $CO_2$ due to the closure of stomata, inhibition of photosynthesis, and reduced turgor will passively limit growth. In this case, different strategies have to be used to endure the stress as long as it occurs and, in the meantime, limit evaporation and cellular damage as much as possible. This is reminiscent of submergence tolerance in rice, where two basic strategies are used upon flooding: very rapid growth to bring the leaves back into the air above the water surface, useful in instances of shallow prolonged flooding, and completely shutting down growth and metabolism, which improves survival during short but deep floods (Bailey-Serres et al., 2012). This knowledge has greatly contributed to the engineering of flood-tolerant rice, yet both strategies are detrimental when used in the wrong conditions. The agony of choice is thus not limited to plants but also extends to us.

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