Plant, Cell and Environment (2008) **31**, 11–38 doi: 10.1111/j.1365-3040.2007.01727.x

The effect of drought and heat stress on reproductive processes in cereals

BEÁTA BARNABÁS[1], KATALIN JÄGER[1] & ATTILA FEHÉR[2]

[1]*Agricultural Research Institute of the Hungarian Academy of Sciences, Brunszvik 2, H-2462 Martonvásár, Hungary and* [2]*Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Temesvári krt. 62, H-6726, Szeged, Hungary*

ABSTRACT

As the result of intensive research and breeding efforts over the last 20 years, the yield potential and yield quality of cereals have been greatly improved. Nowadays, yield safety has gained more importance because of the forecasted climatic changes. Drought and high temperature are especially considered as key stress factors with high potential impact on crop yield. Yield safety can only be improved if future breeding attempts will be based on the valuable new knowledge acquired on the processes determining plant development and its responses to stress. Plant stress responses are very complex. Interactions between plant structure, function and the environment need to be investigated at various phases of plant development at the organismal, cellular as well as molecular levels in order to obtain a full picture. The results achieved so far in this field indicate that various plant organs, in a definite hierarchy and in interaction with each other, are involved in determining crop yield under stress. Here we attempt to summarize the currently available information on cereal reproduction under drought and heat stress and to give an outlook towards potential strategies to improve yield safety in cereals.

Key-words: barley; elevated temperature; flowering; gamete development; limited water availability; maize; meiosis; grain filling; rice; wheat.

INTRODUCTION

During evolution, plants, as sessile organisms, have developed appropriate developmental strategies to ensure their survival and reproduction even under suboptimal conditions. Breeding efforts, however, placed yield rather than survival into the focus of crop, especially cereal, improvement. As high-yielding cereal cultivars became widespread in agriculture, yield safety gained more and more importance. Abiotic stresses such as extreme temperatures and low water availability frequently limit the growth and productivity of major crop species including cereals. High temperature is often accompanied with low water supply, so the primary aim of cereal breeding must be to develop cultivars tolerating both types of stresses (Tester & Bacic 2005).

Correspondence: B. Barnabás. Fax: 0036 22 569 576; e-mail: bea@mail.mgki.hu

Grain development begins with the process of double fertilization. It is perhaps superfluous to emphasize how important is that the processes leading to the development of the male and female gametes and those ensuring the fusion of the gametes and the development of the embryo and endosperm should take place undisturbed. The effects of various kinds of abiotic stress on these processes are different, but in all cases negative, and their influence always results in a decline in yield quantity (for reviews see Saini & Westgate 2000; Mahajan & Tuteja 2005). Breeding for specific cultivation conditions (life habitats) as well as for developmental synchrony further exacerbates the effects of adverse environmental conditions on the yield of modern cultivars. The process of grain filling, the accumulation of reserve nutrients in the developing and maturing grain, is also sensitive to environmental conditions strongly affecting final yield quantitatively and qualitatively as well (Yang & Zhang 2006). The success of cereal reproduction as well as the realization of the yield potential of a given cultivar, however, are dependent not only on the stress sensitivity of the reproductive and grain-filling stages but on overall plant growth and development. Efficient photosynthesis and stem reserve accumulation during the vegetative phase has a decisive role on the formation of generative organs and thus may directly affect final yield (Blum *et al.* 1994). Therefore, in order to improve yield safety in cereals, the whole developmental process, from grain to grain, needs to be considered and appropriate strategies may target several developmental stages (Triboï & Triboï-Blondel 2002).

GENERAL RESPONSE OF PLANTS TO DROUGHT AND HIGH TEMPERATURE

Plant responses to limited water supply

Drought is one of the major limitations to food production worldwide. As the world population continues to grow and water resources for crop production decline, the development of drought-tolerant cultivars and water-use-efficient crops is a global concern. Even the most productive agricultural regions experience short periods of drought within almost any year and occasional years with severe droughts.

Plants, as sessile organisms, evolved appropriate mechanisms to cope with temporary water limitations in order to ensure their survival and reproduction. Plant resistance to

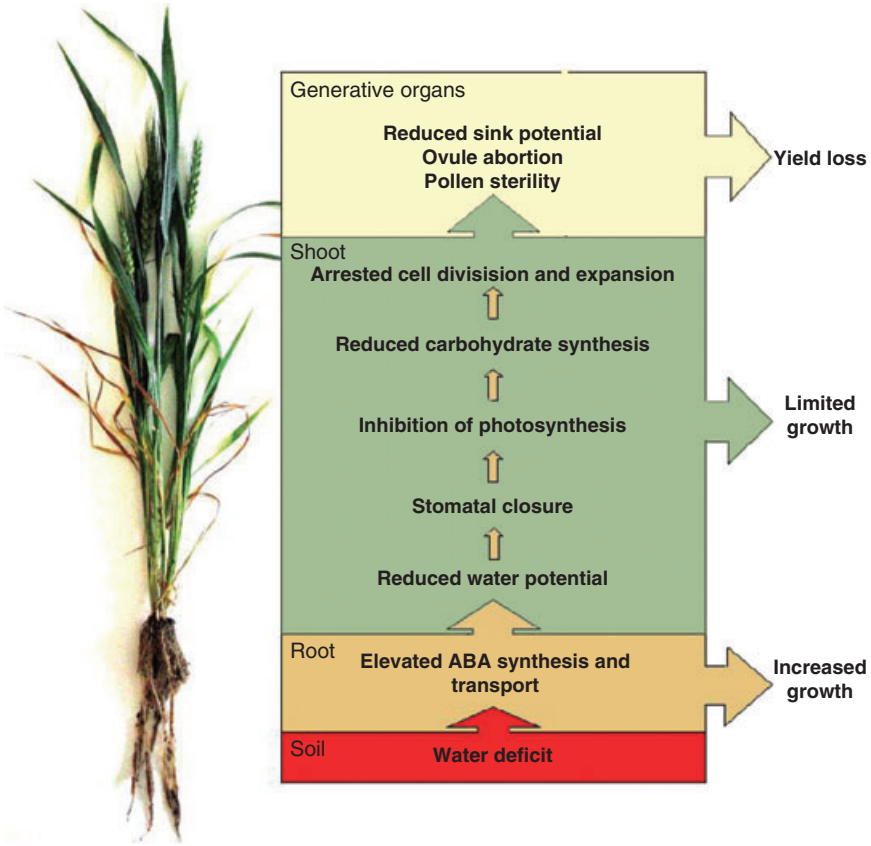

Figure 1. Drought-induced abscisic acid (ABA)-dependent plant responses.

drought can be subdivided into escape, avoidance and tolerance strategies (for a review, see Chaves, Maroco & Pereira 2003). Escape strategies may rely on successful reproduction before the onset of severe stress, by means of a short life cycle, a higher rate of growth or the efficient storage and use of reserves for seed production. Dehydration avoidance, that is, the maintenance of a high (favourable) plant water status during stress, may be the result of minimized water loss (e.g. caused by stomatal closure, trichomes, reduced leaf area, senescence of older leaves, etc.) or maximized water uptake (e.g. by increased root growth). Finally, tolerance to low water potential (the maintenance of plant function at limited water availability and/or the recovery of plant water status and plant function after stress) may involve osmotic adjustments, but may also be the result of rigid cell walls or small cells. Drought tolerance may also be associated with the efficient scavenging of reactive oxygen species (ROS) formed as a consequence of disturbed metabolism (Sairam & Saxena 2000).

Plants respond to drought stress at the molecular, cellular and physiological levels (Figs 1 & 2). The response depends on the species and genotype (Rampino *et al.* 2006), the length and severity of water loss (Araus *et al.* 2002; Bartels & Souer 2004), the age and stage of development (Zhu *et al.* 2005), the organ and cell type (Verdoy *et al.* 2004; Cominelli *et al.* 2005; Zhou, Wang & Jiao 2007) and the sub-cellular compartment (Battaglia *et al.* 2007). Figure 1 presents a simplified scheme of the responses of various plant organs

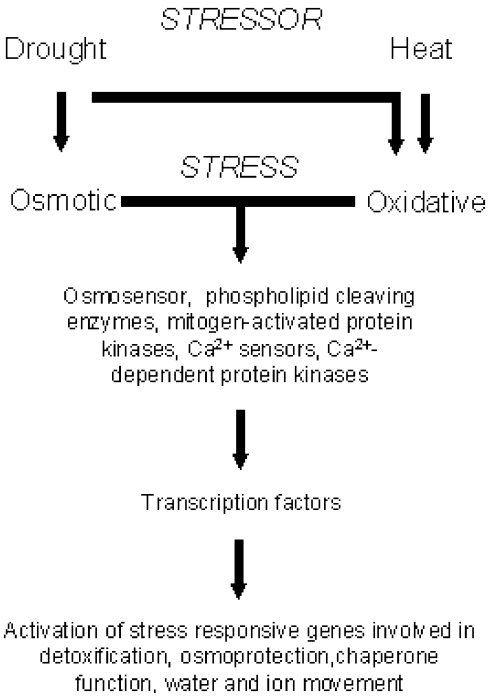

Figure 2. Mechanisms of cellular adaptation to water deficit and elevated temperature.

to limited water availability, highlighting the fact that one of the long-term signals operating during drought stress is the plant hormone abscisic acid (ABA) (Bray 2002). Increased concentrations of ABA in the root, induced by soil drying, may maintain root growth and increase root hydraulic conductivity. Both of these processes lead to an increase in water uptake and postpone the development of water deficit in the shoot. ABA is also transported in the xylem to the shoot, where it causes stomatal closure and reduces leaf expansion, thereby preventing the dehydration of leaf tissues. ABA seems to be involved in the mobilization of reserves under drought conditions (Yang *et al.* 2001a). Stress that reduces plant water status and photosynthesis during grain filling induces the conversion of stem reserves into soluble sugars and the mobilization of sugars into the grains (Blum 1998, 2005). Evidence has also shown that xylem-borne ABA can be transported to plant reproductive structures and influence their development, presumably by regulating the gene expression that controls cell division and carbohydrate metabolic enzyme activity under drought conditions (for a review, see Liu, Jensen & Andersen 2005). The ABA signal transduction pathway probably comprises a protein kinase/phosphatase cascade interacting with Ca^{2+} (Bray 2002, see also further).

The first step in switching on a specific response to an environmental signal, such as drought, is the perception of the signal by specific receptor(s). Plant responses to drought may rely on several mechanisms that sense water status, turgor, bound water, hormones (e.g. ABA), alteration in cell membranes, etc. (reviewed in Chaves *et al.* 2003). A transmembrane histidine kinase receptor (ATHK1) and associated proteins forming a potential 'osmosensor' have already been implicated in the perception of water deficit in *Arabidopsis* (Urao *et al.* 1999; Urao, Yamaguchi-Shinozaki & Shinozaki 2001). It is very likely, however, that there are further, yet unknown, mechanisms by which plant cells sense water deficiency (for a review, see Chaves *et al.* 2003). Water loss, if perceived by the cell, may trigger several cellular signal transduction pathways. In this way, physical stress can be converted into a biochemical response. In the light of the complexity of molecular, cellular and physiological responses to drought (see Figs. 1 & 2), it is not surprising that the signalling of water deficit is also a complex process in plants. A fairly generalized scheme is given in Fig. 2, listing some of the identified components of cellular adaptation to water deficit and elevated temperature. The stimulation of the 'osmosensor', and/or other drought-sensing mechanisms, may trigger signal transduction cascades involving protein phosphorylation/dephosphorylation mediated by kinases or phosphatases up-regulated by water stress (Bray 2002; Kaur & Gupta 2005; Mishra, Tuteja & Tuteja 2006). Changes in the cytoplasmic calcium concentration are likely to mediate the integration of different signalling pathways (Bray 2002; Kaur & Gupta 2005). Several Ca^{2+}-dependent protein kinases have already been implicated in water stress-related signalling (Bray 2002; Kaur & Gupta 2005; Klimecka & Muszynska 2007). Drought-activated kinase cascades finally result in the phosphorylation and activation of transcription factors regulating gene expression (Kaur & Gupta 2005; Nakashima & Yamaguchi-Shinozaki 2005). Genome-wide transcriptome analysis has identified hundreds of genes encoding transcription factors that are induced or repressed by environmental stresses (Chen & Zhu 2004). Chen *et al.* (2002) identified groups of transcription factors regulated (1) specifically by abiotic stress (class I) and (2) by both biotic and abiotic stresses (class II) in *Arabidopsis*. The expression patterns of these transcription factors are highly complex and suggest that stress tolerance and resistance are controlled at the transcriptional level by an extremely intricate gene regulatory network.

Experiments using micro-array technology have identified several genes that are induced by abiotic stresses, including drought, and these genes have been classified into two major groups. One group encodes products that directly protect plant cells against stresses, whereas the products of the other group regulate gene expression and signal transduction in abiotic stress responses (e.g. Chen *et al.* 2002; Seki *et al.* 2002; Shinozaki, Yamaguchi-Shinozaki & Seki 2003; Chen & Zhu 2004; Sreenivasulu, Sopory & Kavi Kishor 2007; Zhou *et al.* 2007).

Revealing complex stress-related transcriptional responses in appropriate transgenic plants or knockout mutants is a useful method to identify gene interactions and downstream elements. The transcript profiling (1300 genes) of *Arabidopsis* plants over-expressing the gene coding for dehydration-responsive element-binding protein 1a (DREB1a) identified 12 genes as cold- and drought-inducible target genes belonging to the DREB1 transcription factor family (Seki *et al.* 2001). The analysis of transgenic plants over-expressing DREB1 and DREB2 resulted in the identification of only eight genes in common and 14 genes that are probable targets of DREB2a (Sakuma *et al.* 2006). These latter consist of at least nine late embryogenesis abundant (LEA) proteins thought to confer dehydration tolerance. In the ABA-insensitive mutant abi1-1, the ABA regulation of about 84.5 and 6.9% of the identified genes was impaired or strongly diminished, respectively; however, 8.6% of the genes remained appropriately regulated (Hoth *et al.* 2002), indicating the involvement of both ABA-dependent and -independent regulatory systems for stress-responsive gene expression (Chaves *et al.* 2003; Nakashima & Yamaguchi-Shinozaki 2005). The accumulated information on transcription factors and the genes/promoters activated by them allowed the definition of drought-related transcription units or 'regulons' (Nakashima & Yamaguchi-Shinozaki 2005; Yamaguchi-Shinozaki & Shinozaki 2006; Shinozaki & Yamaguchi-Shinozaki 2007). It was established that changes in ABA-independent gene expression in response to drought are regulated by the DREB2, ZF-HD and NAC transcription factor regulons, while the AREB/ABF, DREB1/CBF4, MYC and MYB transcription factors are involved in the regulation of ABA-responsive genes under the same conditions (for details, see Nakashima & Yamaguchi-Shinozaki 2005).

The genes activated by drought include those involved in mechanisms to avoid water loss, protect the cellular machinery and repair damage (for reviews, see Ramanjulu & Bartels 2002; Shinozaki & Yamaguchi-Shinozaki 2007; Sreenivasulu *et al.* 2007). One response to plant water deficit is the synthesis of osmolytes (Yoshiba *et al.* 1997). Transport proteins, ion channels and carriers (Assmann & Haubrick 1996) also play an important role in water deficit avoidance or osmoregulation. Sugar transporters are thought to function by transporting sugars through plasma membranes and the tonoplast to adjust the osmotic pressure under stress conditions. Aquaporins, a family of membrane proteins that transport water, may be involved in controlling cellular water status in response to water deficit (Alexandersson *et al.* 2005). Many hydrophilic globular proteins accumulate in seeds during the maturation phase, when the seeds are developing desiccation tolerance. These LEA proteins (Shao, Liang & Shao 2005) are also expressed in vegetative organs during periods of water deficit. Many proteins involved in damage limitation or the removal of toxic compounds are also induced during water deficit. Several 'cold-regulated' (COR) and 'responsive-to-dehydration' (RD) genes encode hydrophilic polypeptides, which hypothetically play a role in protecting cells against low temperature and water deficit, such as drought or high-salinity stress conditions (Artus *et al.* 1996; Shinozaki & Yamaguchi-Shinozaki 1999). Ferritin may play a role in protecting cells from the oxidative damage caused by stress by sequestering the intracellular iron involved in the generation of various reactive hydroxyl radicals through a Fenton reaction (Bajaj *et al.* 1999). Genes encoding plant catalases are expected to play an important role in antioxidant defence in response to environmental and physiological oxidative stress (Scandalios 1990; Iwamoto *et al.* 1998). Fatty acid metabolism-related genes may participate in the repair of stress-induced damage in membranes, to regulate permeability to toxic ions and fluidity of the membrane (Torres-Schumann, Godoy & Pintor-Toro 1992; Holmberg & Bülow 1998).

Effect of elevated temperature on plants

Changes in the global climate, notably in regional spatial and temporal temperature patterns (Houghton, Jenkins & Ephraums 1990), are predicted to have important consequences for crop production (Parry 1990). Both plant growth and development are affected by temperature (Porter & Moot 1998).

The most significant factors for heat stress-related yield loss in cereals include the high-temperature-induced shortening of developmental phases, reduced light perception over the shortened life cycle and perturbation of the processes associated with carbon assimilation (transpiration, photosynthesis and respiration) (Stone 2001). Increased respiration requires greater carbon fixation for sustained growth and survival. Temperatures higher than 35 °C significantly decrease the activity of ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco), thereby limiting photosynthesis (Crafts-Brandner & Law 2000; Griffin, Ranney & Pharr 2004). Higher plants exposed to excess heat, at least 5 °C above their optimal growing conditions, exhibit a characteristic set of cellular and metabolic responses required for the plants to survive under the high-temperature conditions (Guy 1999). These effects include a decrease in the synthesis of normal proteins and the accelerated transcription and translation of heat shock proteins (HSPs) (Bray, Bailey-Serres & Weretilnyk 2000), the production of phytohormones (ABA) and antioxidants (Maestri *et al.* 2002), and changes in the organization of cellular structures, including organelles and the cytoskeleton, and membrane functions (Weis & Berry 1988).

Heat stress during vegetative growth causes many physiological and metabolic changes, including alterations in hormone homeostasis. Some of the heat-induced processes at the cell, organ and whole-plant levels may be hormone regulated; others may be the consequence of a new hormonal status, altered by heat stress (Hoffmann & Parsons 1991; Maestri *et al.* 2002). ABA is implicated in osmotic stress responses and mediates one of the intracellular dehydration signalling pathways (Davies & Jones 1991). In field conditions where water shortage and high-temperature stresses frequently occur simultaneously, ABA induction may also be an important component of thermotolerance (Gong, Li & Chen 1998). The effect of gibberellins on high-temperature tolerance is the reverse of that of ABA (Maestri *et al.* 2002). An inherently heat-resistant dwarf mutant of barley (*Hordeum vulgare*) with impaired gibberellin synthesis was made heat sensitive by the application of exogenous gibberellic acid, whereas the application of a gibberellin-antagonist compound conferred heat tolerance (Vettakkorumakankav *et al.* 1999). Overall, our understanding of phytohormone involvement in the thermotolerance of cereals is still far from complete.

One of the consequences of high temperature in plants is oxidative damage caused by the heat-induced imbalance of photosynthesis and respiration (Fitter & Hay 1987). Elevated temperatures may reduce the activities of antioxidant enzymes, as observed in maize (Gong *et al.* 1997). Accordingly, in a set of wheat (*Triticum aestivum*) genotypes, the capacity to acquire thermotolerance was correlated with higher activities of catalase and superoxide dismutase, higher ascorbic acid content and less oxidative damage (Sairam, Srivastava & Saxena 2000; Almeselmani *et al.* 2006).

High temperature causes modifications in membrane functions mainly because of the alteration of membrane fluidity. In plant cells, membrane–based processes such as photosynthesis and respiration are especially important. Three commonly used assays of heat tolerance in plants (Blum 1988) are related to the plasmalemma ('cell membrane stability' or CMS assay), the photosynthetic membranes (chlorophyll fluorescence assay) and the mitochondrial membranes (cell viability assay based on 2,3,5-triphenyl-tetrazolium chloride (TTC) reduction). In the chloroplasts, heat reduces the photochemical efficiency of photosystem II (PSII), the most heat-sensitive component in photosynthesis (Al-Khatib & Paulsen 1999). In the

mitochondrial electron transport chain, an NADH: ubiquinone oxidoreductase has been identified as one of the thermolabile components (Downs & Heckathorn l998). The contribution of saturated lipids and protein components to membrane function under high-temperature stress needs further study.

Heat stress results in the misfolding of newly synthesized proteins and the denaturation of existing proteins. Protein thermostability is believed to be provided in part by chaperones, a specific class of proteins capable of assisting other proteins in proper post-translational folding and in maintaining them in a functional state (Ellis 1990). Certain aspects of the response to heat stress have been highly conserved from bacteria to humans, including the induction of HSP synthesis. Chaperone functions, the prevention of denaturation or the re-folding of already denaturated proteins, were identified as one of the major functions of HSPs (Jagtap *et al.* 1998). The significance of HSPs in thermotolerance was first mooted on the basis of correlative evidence (for a review, see Vierling 1991; Klueva *et al.* 2001), but the involvement of several HSPs in the acquired thermotolerance of *Arabidopsis* is now well demonstrated (Burke, O'Mahony & Oliver 2000; Hong & Vierling 2000; Queitsch *et al.* 2000). Very little is known, however, on the possible involvement of HSPs in the thermotolerance of cereals (Cooper & Ho 1983; Marmiroli *et al.* 1986; Young *et al.* 2001). High-molecular-weight HSPs (HSP70, HSP90, HSP101) are characterized by a high level of sequence similarity within the plant kingdom. However, molecular diversity within families of high-molecular-weight HSPs suggest that even closely related members or alleles may vary in their specific functions. Furthermore, members of the same HSP family may function in different cell compartments (Boston, Viitanen & Vierling 1996; Maestri *et al.* 2002). HSP101 was shown to be a major component of thermotolerance in *Arabidopsis* (Hong & Vierling 2000; Queitsch *et al.* 2000). The homologous protein was isolated and cloned from wheat (Wells *et al.* 1998; Campbell *et al.* 2001). However, the regulation of HSP101 expression in cereals has not yet been studied in detail. Young *et al.* (2001) examined the expression of HSP101 during the development of maize and wheat following heat stress. The HSP101 protein was abundant in the developing tassel, ear and silk. A heat-induced increase in the HSP101 transcript level was accompanied by a corresponding increase in the amount of HSP101 protein in the vegetative and floral meristematic regions, fully expanded foliar leaves, the young ear and the roots. HSP101 transcript abundance increased in the heat-stressed tassel at anthesis without a similar increase in HSP101 protein. Alterations in the increase in HSP101 protein and mRNA levels during the development of other organs could also be detected. These observations suggest that HSP101 expression is regulated in an organ-specific manner during development, and that HSP101 accumulation is regulated at the RNA level as well as post-transcriptionally. Low-molecular-weight HSPs have been identified in the organelles and their association with membranes in response to heat stress was reported in pea (*Pisum sativum* L.) (Adamska & Kloppstech 1991), suggesting that HSPs play a role in protecting photosynthetic electron transport.

The heat tolerance of plants is a complex trait, most probably controlled by multiple genes. The comparative molecular biological analysis of heat-sensitive and heat-tolerant genotypes of *Festuca* (Zhang *et al.* 2005) revealed that heat-tolerant genotypes responded to stress by increasing the expression of genes participating in photosynthesis, protein synthesis and the preservation of cell status, and of those related to transcription factors. Heat stress transcription factors (HSFs) are the terminal components of signal transduction pathways mediating the activation of genes (including their own) responsive to heat stress. The sequencing of the *Arabidopsis* genome revealed the unique complexity of the plant HSF family, with 21 members belonging to three classes and 14 groups (Nover *et al.* 2001). Of the 24 000 monitored genes of *Arabidopsis*, 11% showed a significant effect in the case of heat-stress treatment (Busch, Wunderlich & Schöffl 2005). Many of these proved to be under the control of HSF-1a/-1b, as determined by the global transcriptional analysis of knockout mutants (Busch *et al.* 2005). Besides several HSP genes and other stress-related genes, HSFA-1a/1b-regulated genes were found to be involved in other functions, including protein biosynthesis and processing, signalling, metabolism and transport. Furthermore, all the steps in the pathway resulting in osmolyte accumulation were reported to be HSF and/or heat regulated (Busch *et al.* 2005). It is well documented that several steps in the heat response overlap with those involved in the response to various other forms of stress, such as drought and cold (Quinn 1988; Bray *et al.* 2000; Rizhsky, Liang & Mittler 2002; Swindell, Huebner & Weber 2007). Therefore, the investigation of the heat tolerance of the plants might provide useful information on general stress-tolerance mechanisms as well. The further understanding and potential manipulation of these mechanisms in cereals, either by transgenic approaches or by molecular breeding, will rely on further achievements in genomics, proteomics and metabolic profiling (Zhang *et al.* 2004).

Combined effect of water stress and high temperature on plants

Farmers and breeders have long known that it is often the simultaneous occurrence of several abiotic stresses, rather than a particular stress condition, that is most lethal to field crops. Recent studies (Rizhsky *et al.* 2004; Mittler 2006) have revealed that the molecular and metabolic responses of plants to a combination of two different abiotic stresses is unique and cannot be directly extrapolated from the response of plants to each of the different stresses individually. The combination of drought and heat stress represents an excellent example of two different abiotic stress conditions that occur in the field simultaneously (Moffat 2002; Shah & Paulsen 2003). However, relatively little is known about how their combination impacts plants. Of additional

concern is global climate change, which is expected to raise global temperatures, change the distribution of precipitation and intensify drought in arid and semiarid areas (Wigley & Raper 2001), leading to a reduction in grass productivity (Chaves *et al.* 2003; Bai *et al.* 2004). Several studies have examined the effects of a combination of drought and heat stress on the growth and productivity of maize, barley, sorghum and various grasses. It was found that a combination of drought and heat stress had a significantly greater detrimental effect on the growth and productivity of these crops compared with each of the different stresses applied individually (Heyne & Brunson 1940; Craufurd & Peacock 1993; Savin & Nicolas 1996; Wang & Huang 2004). In a recent study on the perennial grass *Leymus chinensis*, it was suggested that high temperature, combined with severe soil drought, might reduce the function of PSII, weaken nitrogen anabolism, strengthen protein catabolism and provoke lipid peroxidation (Xu & Zhou 2006). Different stresses might require conflicting or antagonistic responses. During heat stress, for example, plants open their stomata to cool their leaves by transpiration. However, if heat stress is combined with drought the plants are unable to open their stomata, so the leaf temperature remains higher (Rizhsky *et al.* 2002). Furthermore, a combination of drought and heat stress was found to alter plant metabolism in a novel manner compared with single stresses (Rizhsky *et al.* 2004).

The transcriptome analysis of *Arabidopsis* plants subjected to a combination of drought and heat stress revealed a new pattern of defence response, including the partial combination of two multi-gene defence pathways (i.e. to drought and heat stress) as well as 454 transcripts that were specifically expressed in plants during a combination of drought and heat stress (Rizhsky *et al.* 2004). Metabolic profiling revealed that plants subjected to a combination of drought and heat stresses accumulated sucrose and other sugars such as maltose and glucose. In contrast, proline, which accumulated in plants subjected to drought, did not accumulate in plants during a combination of drought and heat stresses. Heat stress was found to mitigate the toxicity of proline to cells, suggesting that during a combination of drought and heat stress sucrose replaced proline as the major osmoprotectant (Rizhsky *et al.* 2004). Based on physiological and molecular characterization, there were many similarities between the responses of *Arabidopsis* and *Nicotiana* (Rizhsky *et al.* 2002, 2004) to this stress combination, suggesting that this mode of defence response is conserved among different plant species.

Tolerance to a combination of different stress conditions, particularly those that mimic the field environment, should be the focus of future research programmes aimed at developing transgenic crops with enhanced tolerance to naturally occurring environmental conditions. At present, however, information on the combined effect of heat and drought stress on the reproductive development of cereals is rather limited, so it will only be discussed further at developmental stages where sufficient data are already available.

INFLUENCE OF WATER SHORTAGE AND ELEVATED TEMPERATURE DURING DISTINCT PHASES OF REPRODUCTIVE DEVELOPMENT – FROM FLOWER INITIATION TO FLOWERING

Flower initiation

Effect of water deficiency on the vegetative/generative transition

During their initiation and early development, the generative organs are well protected by vegetative tissues. Unless the stress is lethal, the reproductive cells/structures respond to unfavourable conditions indirectly, as mediated by the vegetative plant organs. For this reason, the stress sensitivity of reproductive processes and the biochemical and molecular background of stress responses can only be interpreted in association with the responses occurring in the vegetative organs. Depending on the cereal species and on the geographical location of plant cultivation, drought and heat stresses may occur during the phase of vegetative/generative transition in the shoot apical meristems. The appropriate matching of the pattern of inflorescence development and the time of flowering to the temporal variation in water availability is recognized as one of the most important traits conferring adaptation to drought (Bidinger, Mahalakshmi & Rao 1987; Passioura 1996). Although grain crops show sensitivity to drought during floral initiation and the pre-meiotic differentiation of floral parts (Barlow *et al.* 1977; Winkel, Renno & Payne 1997), the effects of drought on floral meristems are among the least understood aspects of crop reproductive development under water-limited conditions (Saini & Aspinall 1981).

In cereals, apical morphogenesis is sensitive to water deficit. Water stress during flower induction and inflorescence development leads to a delay in flowering (anthesis), or even to complete inhibition (Mahalakshmi & Bidinger 1985; Wopereis *et al.* 1996; Winkel *et al.* 1997). Mahalakshmi & Bidinger (1985) and Craufurd & Peacock (1993) reported a delay in flower initiation caused by water stress in *Pennisetum* and *Sorghum*. Very few studies have been done to determine the effects of drought on the process of floral induction in cereals per se, which is difficult to separate from post-induction floral development in many cases (Saini & Westgate 2000). Experiments on *Lolium temulentum*, a long-day plant with a simple single-cycle photoperiodic response, and *Pharbitis nil*, a single-cycle short-day plant, clearly show that flower induction is inhibited by water deficit, and an increase in the ABA level has been suggested to play a role in *L. temulentum* (King & Evans 1977).

High temperature and flower initiation

Losses in cereal yields can be attributed to heat stress-induced metabolic changes, to a decrease in the duration of the developmental phases of plants and the consequent reduction in light perception over the shortened life cycle, and to the perturbation of processes related to carbon assimilation (transpiration, photosynthesis and respiration),

all of which may lead to fewer and/or malformed and/or smaller organs (Takeoka *et al.* 1991; Stone 2001; Maestri *et al.* 2002).

Only a limited number of reports have been published on the effect of abiotic stresses, especially high temperature, on the transitional phase of development in cereal plants (Damptey & Aspinall 1976; Mahalakshmi & Bidinger 1985). Wheat plants have four to eight leaves on the main shoot when the growing apex changes from the vegetative to the reproductive stage. Temperatures above 30 °C during floret formation cause complete sterility (Owen 1971; Saini & Aspinall 1982). Rahman, Wilson & Aitken (1977) reported a positive correlation between the length of the vegetative phase and the number of spikelets per spike. Therefore, shortening the duration of the vegetative stage of the apex induces fewer spikelets per spike. The main effect of heat stress after/during floral initiation is observed on kernel number. The number of kernels per unit area decreases at a rate of 4% for each degree increase in mean temperature during the 30 d preceding anthesis (Fischer 1985). Considerable experimental effort has been devoted to examining carbohydrate availability for developing wheat florets as a major factor in the grain number (Mishra & Mohapatra 1987; Abbate, Andrade & Culot 1995; Demotes-Mainard & Jeuffroy 2004). Kirby (1988) was the first to suggest that the time of floret death corresponded with the period when the ear and stem were accumulating dry matter at their most rapid rate and that inadequate assimilate availability may be critical in the loss of florets. Nitrogen availability also seems to be essential at this stage of development (Fischer 1993). The duration and rate of spikelet production are controlled by temperature in maize (Otegui & Melón 1997) and genotypic effects exist for all these traits (Basetti & Westgate 1993). Floral abnormalities induced by heat stress (i.e. stamen hypoplasia and pistil hyperplasia), leading to spikelet sterility, represent significant problems in rice production (Takeoka *et al.* 1991).

Combined effect of drought and elevated temperature on vegetative/generative transition

The occurrence of simultaneous drought and heat stress during the early generative stages of cereal ontogeny used to be rare, so it attracted little attention. However, in the light of the increasingly frequent occurrence of early season temperature extremes in many areas, more research may be justified. The combined effect of these stresses is more typical of later stages of reproductive development in cereals (e.g. early seed formation and grain filling), as discussed further.

Ovary and embryo sac development

Effect of water deficiency on ovary and female gametophyte development

Although grain crops show sensitivity to drought during floral initiation and the pre-meiotic differentiation of floral parts (Barlow *et al.* 1977; Winkel *et al.* 1997), the most dramatic effects on yield have been recorded when stress coincides with the period bracketed by the onset of meiosis and early grain initiation (Westgate & Thomson Grant 1989; Saini 1997).

In wheat, pre-anthesis stem reserve accumulation is considered to be a significant factor affecting flower and grain development under stress conditions (for a review, see Blum *et al.* 1994; Blum 1998). When carbon assimilation during stem elongation is reduced by drought stress, the storage capacity in the stems also decreases. The authors of a recent review (Sinclair & Jamieson 2006) point out that the grain number in wheat is highly dependent on the environmental conditions prior to and during flower formation. They claim that grain number cannot be considered as a primary determinant of final yield, but as a consequence of nutrient resource accumulation. This view gives more significance to vegetative growth in the determination of final yield, emphasizing the importance of nitrogen availability for floret development. Limited nitrate uptake from the dehydrated soil and the decreased nitrate concentration in the xylem could lead to the alkalinization of the xylem sap, thus affecting ABA accumulation both in the leaves and the reproductive structures, resulting in reduced yield (review by Liu *et al.* 2005).

In maize, drought stress can cause a considerable delay in female organ development, while the male inflorescence is less affected. ABA may have a role in this process (Damptey, Coombe & Aspinall 1976; Blum 2000). An increase in ABA content in the generative organs is one of the factors suggested to play a role in yield reduction in response to drought stress (Westgate, Passioura & Munns 1996; Boyer & Westgate 2004; for a recent review, see Liu *et al.* 2005). The ABA concentration of the ovary was found to increase substantially as the result of severe, long-lasting stress prior to flowering compared with irrigated maize plants, but this difference disappeared by flowering (Ash *et al.* 2001). In spite of this, the authors did not exclude the possibility that ABA may play a role in the abortion of female flowers. Although increasing the ABA concentration without lowering the water supply did not induce low grain numbers in wheat (Dembinska, Lalonde & Saini 1992), in the case of low water supplies the ABA concentration increased in the developing floral organs of both maize and wheat (Ober *et al.* 1991; Westgate *et al.* 1996). High ABA levels in early reproductive structures caused by environmental stresses may inhibit cell division and impair floret and then seed development (Yang *et al.* 2001a).

Plant reproduction greatly depends on an adequate supply of photosynthetic products. Water shortage results in the inhibition of the photosynthesis process, and thus in the reduction in the nutrient supply to the generative organs. An insufficient supply can block the development of reproductive structures, causing abortion (Westgate & Boyer 1986; Mäkelä, McLaughlin & Boyer 2005). Disturbances in the carbohydrate metabolism of the ovary because of inhibited photosynthate influx during early development can be presumed to be responsible for these developmental

anomalies in maize (Zinselmeier, Lauer & Boyer 1995; for a review, see Boyer & Westgate 2004). Sucrose infusion was able to prevent kernel abortion under low-water conditions (Boyle, Boyer & Morgan 1991). Further investigations revealed that sucrose alone is capable of rescuing the ovaries (Zinselmeier *et al.* 1995) and that sucrose transport from the leaves to the ovaries may serve as a trigger to induce the molecular and developmental responses (for a review, see Boyer & Westgate 2004). Maize reproductive tissues contain starch reserves in the maternal tissues of the cob, pedicle and pericarp (Reed & Singletary 1989; Zinselmeier *et al.* 1995) and these reserves are thought to be remobilized to support reproductive growth when photosynthesis is inhibited (Zinselmeier, Jeong & Boyer 1999). During water deficit, photosynthesis may be inhibited for days, and the reserves become especially critical because respiration continues to demand substrates. Without a stream of substrates from photosynthesis, many cellular activities cannot continue. However, increasing evidence indicates that the reserve status of the cells might serve as a signal that affects gene expression (Boyer & McLaughlin 2007; for a review, see Rolland, Baena-Gonzalez & Sheen 2006). Koch (1996) and Sheen, Zhou & Jang (1999) have reviewed this area and identified several genes that appeared to change in expression when the sugar status of the cells changed. Schussler & Westgate (1994) found that a larger pool of reserves in maize plants was unable to maintain ovary growth during water deficit and that abortion continued in the female florets. This focused attention on the carbohydrate status of the ovary tissues themselves. Sucrose may have a dual role as substrate and signal (Boyer & McLaughlin 2007). Feeding sucrose to the stems during water deficit might cause the stream of substrates to resume, but might also signal to the developing reproductive structure that metabolism could proceed. The genes responding to the sugar signal would be those controlling the phenotype and preventing abortion. On the basis of recent studies by McLaughlin & Boyer (2004a) and Mäkela *et al.* (2005), reduction in the sucrose content of ovary cells is caused by the curtailing of sucrose delivery caused by a decrease in photosynthesis during water deficit. This is accompanied by the depletion of glucose, as starch is consumed in the ovary tissues. These possible signalling changes depend on the delivery and location of the sugars. Around anthesis, maize ovaries accumulate ~1 mg dry mass a day, mostly in the form of carbohydrate (Mäkela *et al.* 2005). The dry mass is delivered by the phloem as far as the pedicel, where the phloem terminates. In order to enter most of the ovary tissues, sucrose or its breakdown products must find their way without the help of phloem transport. McLaughlin & Boyer (2004a) found a large concentration of glucose in the upper pedicel tissues on the day of pollination, and these tissues also possess high acid invertase activity, which is restricted to the cell walls of the pedicel tissues. Therefore, the accumulating glucose appeared to be present mainly in the pedicel apoplast. The concentrated glucose created a steep gradient in the pedicel, extending downward into the nucellus, which had a low concentration. The gradient favoured passive glucose movement towards the embryo sac (Mäkela *et al.* 2005). During water deficit, Mäkela *et al.* (2005) fed the phloem-mobile dye carboxyfluorescein to the stems of maize and found less movement to the ovary than in controls supplied with water. This confirmed that less sugar was delivered by the phloem to the pedicel during water shortage. With less sucrose, starch was depleted and the glucose gradient disappeared.

The ovaries also displayed less cell wall-bound invertase activity when the plants were subjected to a water shortage (Zinselmeier *et al.* 1995). All the downstream metabolites were depleted by starch biosynthesis in the ovaries, which indicated that the loss of invertase formed a metabolic block in addition to the lack of sucrose delivery because of decreased photosynthesis (Zinselmeier *et al.* 1999). Other evidence for signalling comes from the response of invertase in the parent plant compared with the ovaries. Several authors (Pelleschi, Rocher & Prioul 1997; Kim *et al.* 2000a; Trouverie *et al.* 2003) reported that invertase activity increased in the leaves and roots of young maize plants during a water shortage, while Zinselmeier *et al.* (1999) found decreased activity in the ovaries at the time of anthesis. This contrast implies that the signal to abort development might be lacking in leaves and roots, but present in the ovaries. Beyond these biochemical and physiological approaches, there is now interest in possible changes in gene expression. Recent molecular studies (McLaughlin & Boyer 2004b; Boyer & McLaughlin 2007) revealed that several genes known to be involved in sucrose processing in young maize ovaries were down-regulated soon after water deficit began to affect the plants. These are the invertases (Incw1-4, Ivr1-2) and sucrose synthases (SS1, SS2), and their down-regulation was measured by comparing mRNA abundance in the ovaries after and before water shortage occurred. Because the down-regulation commenced before glucose disappeared from the ovaries, the signal for the genes may have been low sucrose (McLaughlin & Boyer 2004b). The gene coding for an inhibitor peptide for invertase (Zminh1) was unaltered in expression and Ivr1 was not expressed (McLaughlin & Boyer 2004b). Kim *et al.* (2000b) reported that Incw3 was also not expressed, and Cheng, Taliercio & Chourey (1996) found that Incw4 had little role to play at this stage of development in maize. Andersen *et al.* (2002) observed a strong correlation between the decreased activity of these forms of ovary invertase and the expression of their genes. McLaughlin & Boyer (2004b) found four genes involved in breaking down cell components. *RIP2*, the gene for the ribosome-inactivating protein, was strongly up-regulated 2 d after the down-regulation of the sucrose-processing genes, initiating senescence in the ovary tissues. Two days later, phospholipase D (*PLD1*) was up-regulated, suggesting that membrane breakdown may have been initiated. On the basis of these observations McLaughlin & Boyer (2004b) suggested that this action may initiate the irreversible loss of viability found during ovary abortion. The up-regulation of these putative senescence genes argues against the simple starvation hypothesis of Zinselmeier *et al.* (1999), who observed starch losses in

the ovaries of water-deficient plants, and first thought the ovaries had simply starved to death. The genome actively responded to the lowered availability of sugars. Feeding sucrose to the stems reversed these regulatory changes and abortion was partially prevented (McLaughlin & Boyer 2004b). Genes for two other enzymes, a bi-functional nuclease (Bfn1) and cysteine protease (CCP1), were down-regulated at about the time when sucrose-processing genes were down-regulated. These genes are thought to function in nucleotide and protein turnover during normal growth, which did not occur in aborting ovaries (McLaughlin & Boyer 2004b).

Taken together, it is difficult to identify which of the many expressed genes for sucrose processing might be considered candidates for controlling abortion, because all were down-regulated during water shortage. The multi-genic nature of the ovary response and the difficulty in identifying controlling genes were investigated by Zinselmeier *et al.* (2002), who used cDNA micro-arrays to measure differential gene expression in maize ovaries and pedicel tissues when water shortage occurred around the time of anthesis. More than 1500 genes representing 27 regulatory and metabolic pathways were investigated. Of these, 15–45 showed differential expression 4 d after moderate water shortage was imposed, with some genes down-regulated and others up-regulated. A greater number of genes were differentially expressed as the shortage intensified. In the case of floral abortion, it has recently proved possible to reverse the phenotype by physiological/biochemical means without altering the genetics of the organism (Boyer & McLaughlin 2007). During this functional reversion, only a few genes responded, thus identifying those likely to be involved in controlling the abortion phenotype. McLaughlin & Boyer (2004b) fed sucrose to maize stems during water shortage around the time of pollination in order to replace the sucrose missing because of inhibited photosynthesis and to cause the ovary phenotype to revert. The sucrose infusion prevented abortion in about two–thirds of the ovaries, and a large improvement in kernel number was observed at maturity. This functional reversion was specific for ovaries of water-deficient plants. McLaughlin & Boyer (2004b) used this reversal to reveal that only a few genes are likely to control the abortion genotype. In maize, key genes appear to be Incw2, coding for insoluble acidic invertases, and Ivr2, coding for soluble acidic invertase, both of which are involved in sucrose metabolism. In ovaries subjected to drought stress neither Incw2 nor Ivr2 were detected *in vivo* in the upper pedicel tissues or *in situ* in the nucellus, respectively, confirming the decline in the expression of invertase genes (McLaughlin & Boyer 2004b). Because the nucellus surrounds the embryo sac containing the egg cell, changes in Ivr2 expression might initiate or control sugar signals reaching the egg cells (Andersen *et al.* 2002). The invertases appeared to control normal sugar uptake by the ovaries. Their down-regulation depleted ovary sugar pools and resulted in an up-regulation of the genes for the ribosome-inactivating protein (*RIP2*) and phospholipase D (*PLD1*) (McLaughlin & Boyer 2004b). The latter changes appeared to initiate senescence, involving the degradation of cell membranes, causing irreversible abortion. McLaughlin & Boyer (2004b) also found a decrease in the glucose content of the upper pedicel about the time that starch disappeared from the vascular tissues of the pedicel during water deficit. These data indicate that the downturn in sucrose content of ovary cells, as a consequence of curtailed sucrose delivery caused by the decreases in photosynthesis during the water deficit, might be the first primary signal to the developing reproductive structures (Koch 1996; McLaughlin & Boyer 2004b). The second would be depletion of glucose as starch was consumed in the ovary tissues (McLaughlin & Boyer 2004a). On the basis of these findings, these genes have become targets for preventing abortion (Boyer & McLaughlin 2007).

It can be concluded that plants use a variety of mechanisms to regulate reproduction and maximize plant fitness and survival even under unfavourable conditions. It seems that plants adjust the expenditure of maternal resources during their ontogeny by regulating the number of flowers, and thus gametophytes, that advance further development. This agrees with the maternal resources hypothesis drawn up by Lloyd (1980). This strategy reduces plant fertility to conserve plant resources, which can then be shunted into processes that permit plants to acclimate to stressful environmental conditions. However, plant survival mechanisms may differ among plant species as regards the reproductive output under severe environmental conditions. Even when harshly stressed, *Arabidopsis* plants can allocate sufficient resources into the female generative organs to produce a few seeds, ensuring that the genetic line is continued (Sun, Hunt & Hauser 2004). In a recent review, Smith & Stitt (2007) discussed carbon allocation and flower/seed abortion in *Arabidopsis* as a consequence of 1–2 d of darkness, and suggested that this was an adaptive response aimed at conserving resources for older seeds and maintaining the meristem. Wheat can also sustain advanced embryo development even in a drought-sensitive genotype under combined stress involving high temperature and water deficit (Jäger, unpublished data). The new experimental techniques available in plant molecular biology will help to clarify the similarities and differences in the regulation of stress responses in various cereal species.

Despite the fact that a long period of intensive water deficiency during the differentiation of the female gametophyte generally caused severe structural and functional anomalies in the ovary, experience indicates that the female sexual generation has greater stress adaptability than the male one (Saini & Lalonde 1998). Moss & Downey (1971) found that water deficiency during maize embryo sac development caused severe anomalies in the structure of the female gametophyte. As a function of the extent and duration of drought stress, 15–45% of the ovules developed abnormally, while this figure was only 2.5% for irrigated plants. As a consequence of water deficiency, the stigma and style also elongated slowly (Westgate & Boyer 1985). Later studies confirmed the observation that the early development of the ovary was one of the most vulnerable

phases in reproduction (for a review, see Boyer & Westgate 2004).

Effect of elevated temperature on ovary and embryo sac development

There is a dearth of information on the effect of high temperature on female sexual generation. Chebotaru (1965) noted an increase in the number of antipodal cells in the maize embryo sac as a result of high temperature. Saini & Aspinall (1982) observed that a level of heat stress that caused male sterility in wheat had no damaging influence on the functions of female sexual generation, suggesting that the female gametophyte had greater heat stress tolerance. When the Australian wheat variety 'Gabo' was subjected to high temperature (30 °C) during meiosis, a third of the ovaries were found to exhibit abnormal development (Saini, Sedgley & Aspinall 1983). In some of the ovaries, no embryo sac was differentiated, and the cavity was filled due to the proliferation of the cell layers of the external integument or the encroachment of the internal integument. The occurrence of smaller embryo sacs with abnormal cell formation was also frequently observed. In many cases pollen tube growth was inhibited in the style and ovule, presumably because of the damage to signal transfer mechanisms. Seed setting decreased by an average of 21%. These authors drew attention to the fact that, although drought itself probably has a less drastic effect on the structure and functioning of the female sexual generation than on the male gametophyte, it may still cause severe yield losses when associated with high temperature (Saini *et al.* 1983).

Combined effect of water shortage and high temperature on ovary and female gametophyte development

Drought and high temperature together, 3–4 weeks prior to flowering, caused asynchrony in the tasselling and silking of maize, while the growth and receptivity of the style were also inhibited (Basetti & Westgate 1993). The number of kernels per ear did not increase when fresh pollen of unstressed plants was applied to late-appearing silks (Basetti & Westgate 1993; Otegui, Andrade & Suero 1995).

On the basis of the earlier discussion, it is clear that the structural and functional abnormalities occurring as a result of stresses in the processes leading to the development of the gametes have a serious influence on the success of fertilization because of the production of dysfunctional male or female gametophytes, even if fertilization takes place under optimal environmental conditions.

Pollen development

Effect of water deficiency on pollen development

Experience shows that during the reproductive development of plants two phases of ontogeny, meiosis and flowering, are particularly sensitive to environmental stresses such as cold, drought and heat (see reviews by Saini & Lalonde 1998; Saini & Westgate 2000; Boyer & Westgate 2004).

Water deficit in the meiotic stage may reduce the grain set by 35–75% in various cultivars of self-pollinated wheat (Saini & Aspinall 1981) and rice (Sheoran & Saini 1996). Wheat, maize and rice plants can resist changes in the water status of the inflorescence prior to its emergence (Sheoran & Saini 1996; Westgate *et al.* 1996; Saini 1997) because of the limited transpiration within two or more enclosing leaf sheaths. Thus, the reduction in grain set in response to meiotic-stage water stress does not correlate with the water status of the reproductive structures. In the case of self-fertilized cereals such as wheat, drought stress results in increased pollen sterility (Saini, Sedgley & Aspinall 1984). The main cause of this is the development of sterile, dysfunctional pollen grains resulting from abnormalities in microsporogenesis and microgametogenesis. The developmental anatomy of stress-affected anthers gives some promising clues about the metabolic events that may be linked to the failure of pollen development (Saini 1997). Water deficiency disturbs photosynthetic processes in vegetative plant tissues, particularly in leaves, resulting in a reduction in the water-soluble carbohydrate level in the anthers and in the expression of the gene responsible for the synthesis of the acidic invertase enzyme (Saini 1997). In maize, water deficit during meiosis inhibits the further development of microspores or pollen grains, causing male sterility. The injury is not caused by desiccation of the reproductive tissues, but is an indirect consequence of water deficit in the vegetative organs, such as leaves (Saini 1997). Because of the disturbances in the carbohydrate metabolism, the internal pollen wall, the intine, which consists of pectocellulose, is unable to develop normally and insufficient amounts of reserve nutrients (starch) are stored in the cytoplasm of vegetative cells in the pollen grains (Dorian, Lalonde & Saini 1996; Sheoran & Saini 1996). Without starch to fuel pollen tube growth on the female florets, pollen tubes could not reach the ovule. These anomalies inhibit both the adherence of pollen grains to the surface of stigma papilla cells and normal pollen tube growth (Clément *et al.* 1994; Franchi *et al.* 1996). Recent research has confirmed the decisive role of acidic invertases in pollen sterility caused by water deficiency during male meiosis (Koonjul *et al.* 2005). Three invertase genes were isolated from a cDNA library prepared from wheat anthers, two of which (Ivr1, Ivr3) code for cell wall invertase and one (Ivr5) for vacuolar invertase. Data from a gel blot analysis on RNA samples derived from the generative organs of irrigated wheat plants indicated that the invertase isoforms were preferentially, but not exclusively, expressed in the anthers. Quantitative RT-PCR analysis demonstrated that water deficiency at meiosis selectively inhibited the transcription of the Ivr1 and Ivr5 genes, while it had no influence on Ivr3. The expression of the relevant genes was not detectable after irrigation. The cell-specific expression of

stress-sensitive genes was demonstrated by *in situ* hybridization (Koonjul *et al.* 2005).

Pollen sterility induced by drought in wheat shows common features with that induced by cold in rice: the accumulation of non-reducing sugars and the failure of starch accumulation in the pollen grains. These changes have been attributed to a reduction in the activity of vacuolar and cell wall invertases (Sheoran & Saini 1996; Koonjul *et al.* 2005; Olivier *et al.* 2005). Both stresses act specifically at the young microspore stage, when the tapetum is functioning at maximum capacity. Expression analysis of two rice cell wall (OSINV1,4) and one vacuolar (OSINV2) acid invertase genes showed that OSINV4 was anther-specific and down-regulated by cold treatment. OSINV4 was transiently expressed in the tapetum at the tetrad/young microspore stage and its down-regulation could cause a disruption in hexose production and starch formation in the pollen grains. Studies on the down-regulation of cell wall invertase genes by drought in various reproductive organs (maize ovules, wheat anthers, peduncles and anthers of rice) at different phases of reproductive development showed the pivotal role of these genes, as a class, in the rapid response to water deficit (Dorian *et al.* 1996; Sheoran & Saini 1996; Zinselmeier *et al.* 1999; McLaughlin & Boyer 2004a,b). However, it was not clear which of the multiple cell wall invertase genes found in these plants were expressed in each sink tissue and how many of them were down-regulated by stress. Andersen *et al.* (2002) and Ji *et al.* (2005) showed that maize Incw2, which is the ortholog of rice cell wall invertase OsCIN2, is mainly expressed in the ovaries after pollination and is repressed by drought. The same occurred with Ivr2, the soluble vacuolar invertase gene, which is the maize ortholog of the rice vacuolar invertase OsVIN2 gene, expressed throughout the pre- and post-pollination period. By contrast OsVIN2 was up-regulated by drought stress in rice flag leafs, panicles, anthers and peduncles. As was shown earlier in this review, a partial analysis of cell wall and vacuolar invertase gene expression in wheat anthers at meiosis was accomplished by Koonjul *et al.* (2005). From these observations it can be concluded that there are differences within and between the cell wall and vacuolar invertase sub-families in the drought responsiveness of individual genes, and there may be highly significant differences between crops in the responses of orthologous genes (Ji *et al.* 2005). However, the primary signal for a reduction in the expression of invertase genes might be the reduction in sucrose content, as suggested by Koch (1996) and McLaughlin & Boyer (2004b) in the case of maize ovaries. The recent molecular studies indicate that the failure of reproduction in wheat has a biochemical origin resembling that in maize, except that wheat loses pollen viability and maize loses ovary viability.

Effect of high temperature on pollen formation

The threshold for damage by high temperatures in reproductive organs is considerably lower than that in other organs. Pollen formation is one of the most heat-sensitive developmental stages in cereals (Saini & Aspinall 1982; Stone 2001). Pollen grain mitosis 1 and 2 are highly sensitive to elevated temperature both in wheat (Saini *et al.* 1984) and barley (Sakata *et al.* 2000). In wheat, two types of abnormal pollen development can be caused by high-temperature stress. The first is apparently caused by tapetal degradation during meiosis, when the microspores are not able to complete the first mitosis. They may have an exine but no cytoplasm, and may remain immature. In the second case, all the microspores complete the first mitotic division, but only a few of them are able to divide further to develop into normal tri-cellular pollen grains. The rest of the microspores remain immature and do not accumulate starch, so the anthers contain a mixture of fertile and sterile pollen grains (Saini *et al.* 1984). Barley plants are hypersensitive to high-temperature stress during panicle development and meiosis. In this species, high-temperature treatment for several days caused abnormal pollen development and complete sterility. Histological examinations and the serial analysis of gene expression showed that elevated temperatures (35 °C day/25 °C night) resulted in the transcriptional inhibition of genes that are active in the anthers under normal-temperature conditions (Abiko *et al.* 2005). The irreversibility of the inhibition correlated well with the duration of the high-temperature period and with the aborted development and differentiation of tapetum and pollen mother cells.

It has been proved that maize anthers at anthesis and mature pollen largely failed to respond to heat stress at the level of HSP101 protein or mRNA, indicating that HSP101 expression is not heat-inducible in these organs (Young *et al.* 2001). The observation that HSP101 protein is present at a moderate to low level in anthers and mature pollen is consistent with the finding that several other HSPs, such as HSP90, HSP70 and HSP60, and some low-molecular-weight HSPs are expressed during the early stages of pollen development (Marrs *et al.* 1993; Magnard, Vergne & Dumas 1996). Moreover, the failure of mature and germinating maize pollen to respond to heat stress has been observed for other HSPs (Hopf, Plesofsky-Vig & Brambl 1992; Magnard *et al.* 1996) and correlates with its pronounced loss of viability when exposed to elevated temperatures (Herrero & Johnson 1980; Mitchel & Petolino 1988; Dupuis & Dumas 1990). These observations suggest that the developmental expression of HSP101 and other HSPs in mature pollen is insufficient to meet the demand following heat stress.

Flowering and fertilization

Effect of drought stress on flowering and fertilization

For successful seed establishment, the pollen must remain viable and the stigma receptive, pollen tubes must grow properly and reach the ovules, double fertilization must be successful, and embryo and endosperm development should proceed normally. Some of these processes may be

severely compromised by the unfavourable environmental conditions frequently encountered by cereals in the field. In cereals, pollen viability and germination is one of the most stress-sensitive of these processes (Saini & Aspinall 1982; Stone 2001). In maize, low kernel numbers can rarely be ascribed to pollen sterility under water stress (Westgate & Boyer 1986; Schoper *et al.* 1987); instead, the genotype-dependent sensitivity of the pollen to high temperature could be observed (Schoper *et al.* 1987). Investigations by Barnabás (1985) showed that pollen grain viability in maize was closely and positively correlated with desiccation tolerance. In fact, maize pollen could sustain up to a limit of 80% water loss without detrimental changes in normal pollen functions. A close synchrony between pollen shed (anthesis) and silk emergence is required for high kernel set in maize and a negative relationship exists between final kernel number and the extent of the anthesis-silking interval (Westgate, Otegui & Andrade 2004). Anthesis and fertilization are particularly sensitive to drought in rice. Water stress during flowering may reduce the harvest index by as much as 60%, largely as a result of a reduction in grain set (Ekanayake, Steponkus & deDatta 1989; Garrity & O'Toole 1994). Among the events known to be drought-sensitive at flowering are panicle exsertion and anther dehiscence (O'Toole & Namuco 1983; Ekanayake, Steponkus & deDatta 1990). The failure of panicle exsertion alone accounts for approximately 25–30% of spikelet sterility because the unexserted spikelets cannot complete anthesis and shed pollen, even when development is otherwise normal (O'Toole & Namuco 1983). If an incomplete anther dehiscence occurred and only a few pollen grains could reach the stigma surface, the pollen grains appeared to cooperate rather than compete (Liu *et al.* 2006). Water deficiency, which may also be caused by dry winds during or immediately prior to rice flowering, leads to a substantial reduction in seed set. This can be attributed to the fact that the panicle is unable to free itself completely from the flag leaf, while the spikelets dry out or do not open at anthesis. The anthers may shrivel up, so that insufficient pollen is available for fertilization. These reproductive abnormalities may prevent fertilization completely (Ekanayake, DeDatta & Steponkus 1993). Grain abortion at the early stages following fertilization also accounts for part of the reduction in grain number in rice (O'Toole & Namuco 1983). In maize, abortion is highly dependent on the timing of water stress: low water availability before pollination resulted in abortion even if sufficient water was available at the time of pollination (Westgate & Boyer 1986). As neither the embryo nor the endosperm was present after the stress treatment prior to fertilization, these observations suggest that the ovaries themselves were affected deleteriously by water stress. According to our unpublished results on wheat (Jäger, unpublished data) it seems that water shortage (30% relative soil water content) around anthesis did not affect detrimentally the process of doubled fertilization or early embryo development unless the water shortage was lethal.

The detailed discussion of the stress dependence of reproductive development and fertilization outlined earlier may be further refined using genomic methods. Zinselmeier *et al.* (2002) examined the effect of a low-light environment and drought on the flowering of maize with the help of a DNA chip containing 384 genes representing four metabolic pathways. Shading the plants for 5 d in the early stages of flowering led to yield losses of 47–74% and caused changes in the expression of 23–26% of the detected genes. The activity of several genes involved in ABA signalling [ABA insensitive (ABI1), ABA and stress responsive (ASR1), glycine-rich RNA binding-ABA inducible protein] was found to increase, while that of genes coding for the enzymes of starch synthesis, such as G-6-P/Pi translocator, ADP-G pyrophosphorylase (AGPase) sh2 type, starch-branching enzyme (SBE) IIa and granule-bound starch synthase (GBSS) wx-like, declined. The expression of starch-degradation genes (α-amylase, β-amylase and starch phosphorylase) was unaffected by stress. These data suggest that the decrease in the starch pool in young maize ovaries induced by shading was a result of a decrease in starch biosynthesis regulated at the level of transcription and coordinated among multiple genes of this pathway (Zinselmeier *et al.* 2002).

Effect of heat stress on flowering and fertilization

High-temperature stress (>30 °C) from early meiosis to pollen maturity also has a damaging effect on the viability of pollen grains in wheat, resulting in a failure of fertilization, and thus in a reduction in seed set (Saini & Aspinall 1982). Increased temperature over the mid-anthesis period decreased the grain number per ear at maturity in spring wheat (Ferris *et al.* 1998), indicating the heat sensitivity of fertilization and grain setting. The heat sensitivity of the pollen might be explained by its inability to synthesize all the HSPs (Mascarenhas & Crone 1996). High-temperature stress at flowering reduces spikelet fertility in rice (*Oryza sativa* L.). Sterility is caused by poor anther dehiscence (caused by the tight closure of the locules) and low pollen production, and hence low number of germinating pollen grains on the stigma (Matsui, Omasa & Horie 2001; Matsui & Omasa 2002; Prasad *et al.* 2006). In maize, reduction in seed set occurs at temperatures higher than 38 °C mainly because of a reduction in pollen germination ability and pollen tube elongation (Dupuis & Dumas 1990; Stone 2001). Lateral ear heating (by 4.5 °C above the air temperature in the heated zone) prior to silk emergence reduced the kernel number per ear (Cárcova & Otegui 2001). Kernel number along the ear decreased from the heated side to the non-heated one. Ovaries in the non-heated zone started kernel formation as indicated by the presence of embryos, but their development was soon arrested. This can be attributed to a differential metabolic activity between the heated and non-heated ovaries during the early post-fertilization period, which may have promoted an uneven distribution of assimilates among ovaries of a similar position along the ear. Enhanced sink activity in the heated side altered assimilate partitioning within the ear and resulted in

increased kernel abortion in the non-heated side (Cárcova & Otegui 2001).

The post-anthesis period in cereals is characterized by a more frequent occurrence of higher ambient temperatures in the field. Because in cereals pollination is not synchronous even within a single ear, it is difficult to distinguish the effect of continuous high temperature upon fertilization from that upon early embryo development (Stone 2001; Maestri *et al.* 2002). In contrast to anthers at anthesis and mature or germinating pollen, in which the developmental expression of HSP101 and other HSPs are insufficient to meet the demand following heat stress, the higher level of expression of HSP101 protein in silks prior to and following fertilization correlates with the higher degree of thermotolerance reported for these tissues in maize (Mitchel & Petolino 1988; Young *et al.* 2001). Young *et al.* (2001) could not observe a significant increase in HSP101 protein levels in heat-stressed maize embryo or endosperm at any developmental stage examined (12–48 days after pollination). This suggests that kernels are not significantly heat responsive with regard to HSP101 expression. No systematic analyses have yet been carried out in this field, so further research will be required to clarify the functions of the HSP genes expressed during sexual processes in cereals (Maestri *et al.* 2002).

Combined effect of drought and elevated temperature on flowering and fertilization

Drought and high temperature at flowering caused asynchrony in the tasselling and silking of maize. In the case of late-appearing silks, pollination with the pollen of untreated plants did not increase the kernel number (Basetti & Westgate 1993; Otegui *et al.* 1995). Nevertheless, fertilization was successful and zygotes were formed under these conditions, and the lack of seed setting could be attributed to the abortion of the zygote within 2–3 d, to the cessation of proembryo and endosperm development at the globular stage, and to the failure of seed-coat differentiation (Westgate & Boyer 1986).

GRAIN FILLING UNDER HEAT AND DROUGHT

Grain filling is the final stage of growth in cereals, where fertilized ovaries develop into caryopses. Its duration and rate determine the final grain weight, a key component of the total yield. High temperature and drought are the major stress factors during the maturation and ripening of cereals in many production areas. Periods of water limitation as well as of high temperature during grain development cause large yield losses in cereals. This reduction is mainly caused by a reduction in starch accumulation, because, in general, over 65% of cereal dry weight (DW) is accounted for by starch. The reduction in grain weight in response to drought or heat stress during the early periods of grain filling can mainly be attributed to the lower number of endosperm cells (Nicolas, Gleadow & Dalling 1985), while during the later stages stress results in the impairment of starch synthesis either because of the limited supply of assimilates for the grain (Blum 1998) or the direct effects on the synthetic processes in the grain (Yang *et al.* 2004b).

A considerable number of reports have been published on the effects of the environment on grain development in wheat (reviewed recently by Dupont & Altenbach 2003 and Yang & Zhang 2006), so this information will mainly be used as a guideline in discussing the previously mentioned effects of heat and drought during the grain-filling period of cereals.

The effect of water limitation and/or high temperature on grain development

The maximum amounts of starch and protein that accumulate in each grain depend on the number of endosperm cells, determined early in grain fill, and the final size of the cells, which is influenced by the rate and duration of grain fill (Egli 1998). The direct effect of both heat and drought on the division of endosperm cells is well documented (Commuri & Jones 1999; Setter & Flannigan 2001). In wheat plants subjected to 20 d of water deficit during the period of endosperm cell division, drought resulted in 30–40% lower endosperm cell number, and the number of small starch granules per cells was also reduced by 45% (Nicolas *et al.* 1985). In maize, the application of a high-temperature treatment (35 °C for 4 or 6 d) during the mitotic phase of the endosperm cell cycle (4, 6 and 8 d after pollination) was found to be more deleterious, inhibiting the entry of the mitotic cells into the endoreduplication cycle (Engelen-Eigles, Jones & Phillips 2001). In barley, similar heat stress (up to 35 °C during 5 d of the grain-filling period) was found to cause profound changes in the endosperm structure, including storage product degradation (Wallwork *et al.* 1998).

Grain filling is closely linked to whole-plant senescence and stem reserve utilization. Pre-anthesis assimilate reserves in the stems and sheaths of wheat and rice contribute to 10–40% of the final grain weight (for a review, see Yang & Zhang 2006). Water stress during the grain-filling period reduces photosynthesis, induces early senescence and shortens the grain-filling period, but increases the remobilization of assimilates from the straw to the grains (Gebbing & Schnyder 1999; Plaut *et al.* 2004; for a review, see Yang & Zhang 2006). In contrast to the duration, the rate of grain filling was hardly affected by water deficiency in many investigations (Brooks, Jenner & Aspinall 1982; Westgate 1994; Altenbach *et al.* 2003; Borrás, Westgate & Otegui 2003; for reviews, see Dupont & Altenbach 2003 and Yang & Zhang 2006). However, under controlled soil drying, when plants could sufficiently rehydrate during the night, enhanced whole-plant senescence and faster and better stem reserve remobilization could be observed (for a review, see Yang & Zhang 2006).

Chronic high temperature up to a mean of 27 °C (daytime maximum of 30 °C) during kernel filling has a similar effect to drought, with a significant reduction in the

duration in grain fill and only a marginal increase in its rate (Wardlaw 2002; for a review, see Dupont & Altenbach 2003). High temperature (37/17 °C) from anthesis to maturity caused a significant reduction in the starch accumulation period in developing wheat grains compared with plants grown under control (24/17 °C) conditions (Hurkman *et al.* 2003). When extremely high day and night temperatures were applied (37/28 °C), starch incorporation was completed 21 d earlier than in the control, while the starch granules were also larger than in the other two cases. An increased proportion of A-type starch granules and a decreased proportion of B granules, consistent with shorter starch accumulation, could be observed at high temperatures in wheat and barley (Bhullar & Jenner 1985; MacLeod & Duffus 1988; Hurkman *et al.* 2003; for a review, see Dupont & Altenbach 2003).

Temperature may also influence the rate of grain fill, depending on the wheat cultivar (for a review, see Dupont & Altenbach 2003). The increase in the grain-filling rate might be ascribed to enhanced enzyme activities and metabolic processes. As a result, the overall temporal program of grain development is accelerated and compressed at high temperatures. The increase in the rate of grain dry matter accumulation may even compensate for the decrease in its duration (for a review, see Dupont & Altenbach 2003). In maize, however, the application of heat stress (33.5/25 °C day/night) in the greenhouse from the 15th day after pollination until maturity resulted in a lengthening of the duration of grain filling, with a reduced kernel growth rate (Wilhelm *et al.* 1999). Differences in the effect of heat stress on the grains of wheat and maize may be related to the differential sensitivity of the enzymes involved in starch metabolism.

Effect of heat or drought stress on starch and protein synthesis in the grain

Starch accumulation in cereal grains is the result of complex enzymatic processes, with sucrose synthase (SuSase), AGPase, soluble starch synthase (SSS) and SBE as key players (Morell *et al.* 2001). High temperature was reported to reduce the activity of these enzymes in cereals (Keeling, Bacon & Holt 1993; Duke & Doehlert 1996; Wilhelm *et al.* 1999; Hurkman *et al.* 2003; Jiang, Dian & Wu 2003; Yamakawa *et al.* 2007). However, compared with all the other enzymes involved in endosperm starch synthesis, SSS and AGPase are the most thermosensitive, especially above 34 °C. Gene expression analysis in wheat revealed that high-temperature conditions caused a greater reduction in the transcript number for the starch synthase enzyme than for the other enzymes involved in starch biosynthesis (Hurkman *et al.* 2003). In maize, however, AGPase is considered to be the rate-limiting enzyme in starch synthesis under heat-stress conditions. Although a survey of the enzymes of sugar and starch metabolism extracted from developing maize endosperm revealed that AGPase and SSS were both sensitive to the high-temperature treatment, only AGPase exhibited reduced activity when the enzyme activities were adjusted with measured temperature coefficients (Wilhelm *et al.* 1999). In another study involving 17 enzymes in maize kernels developing at elevated temperatures, AGPase activity was found to be the most sensitive to high temperatures (Duke & Doehlert 1996). It was postulated that AGPase might have a faster turnover rate under heat-stress conditions compared with the other enzymes assayed (Duke & Doehlert 1996). The *in vitro* heat stability of the maize AGPase enzyme could be successfully increased by mutations stabilizing subunit interactions (Greene & Hannah 1998; Linebarger *et al.* 2005). Although the enhanced catalytic activity and heat stability of mutant AGPase forms have been observed in bacterial cells, further studies are required in order to prove that the new features of maize AGPase can be translated into increased starch synthesis in transgenic plants (Linebarger *et al.* 2005). Depending on its severity, water stress also results in a marked reduction in the sucrose and starch content in the grains. It was observed that while the cessation of grain growth in response to severe dehydration was primarily caused by reduced AGPase activity, at moderate water stress SSS was the enzyme that responded the earliest, and to the greatest extent and its decreased activity limited the growth of the grain (Ahmadi & Baker 2001). In contrast, in the case of mild water stress, allowing the recovery of the stressed plants during the night ('controlled soil drying'), the activities of SuSy, SSS and granule-bound SBE were substantially enhanced in rice and wheat grains (Yang *et al.* 2001b, 2003b, 2004b). The activity of AGPase was also increased by water stress, while that of GBSS and acidic invertase were less affected (Yang *et al.* 2001b, 2003b, 2004b). The ABA level in the grains correlated with the increased enzyme activities, and the manipulation of the ABA level indicated that ABA has a regulating role in this process. The activation of the key enzymes of sucrose-to-starch conversion resulted in increased sink activity, finally leading to an increased grain-filling rate under these specific conditions (for a review, see Yang & Zhang 2006).

Proteins are the most important components of wheat grains, governing end-use quality. Although the grain protein composition depends primarily on the genotype, it is also significantly affected by environmental factors (Triboï, Martre & Triboï-Blondel 2003 and references therein). The effects of temperature on storage protein composition are unclear and may vary with genotype (for a review, see Dupont & Altenbach 2003). Experimental results indicate that changes in the protein fraction composition under heat and drought stress are mainly caused by the altered quantity of total N accumulated during grain filling (total protein content; Triboï *et al.* 2003). In accordance, the post-anthesis application of N fertilizers reduced the effect of high temperature on the storage protein composition of wheat (for a review, see Dupont & Altenbach 2003). When wheat plants were raised at 24/17 °C with good nutrient and water supplies, transcripts of major gluten storage proteins could be detected 8 d after anthesis, while in plants grown at high temperature (37/17 °C) accumulation began earlier but continued for a shorter period

(Altenbach, Kothari & Lieu 2002), in agreement with the accelerated kernel development under high temperatures (see previous discussion).

The importance of stem reserves for grain filling under stress

The grain-filling rate in cereals is dependent on two main carbon resources: current assimilates from photosynthesis and reserve carbohydrates transported to the grain from vegetative tissues in leaves, stem and ear (Plaut *et al.* 2004; Yang & Zhang 2006). Heat stress, as well as limited water availability, can significantly impair photosynthesis (Harding, Guikema & Paulsen 1990a,b; Sharkey 2005; Subrahmanyam *et al.* 2006), reducing the amount of assimilates available to the grain. Stem reserve mobilization is an important process supporting grain filling under such conditions (Blum *et al.* 1994). Therefore, under stress conditions, stored carbohydrates may become the predominant source of transported materials, contributing as much as 75–100% to the grain yield (van Herwaarden *et al.* 1998). This phenomenon raised an interesting hypothesis about the potential competition for hydrolysed carbohydrates between the vegetative organs and the grain for the purposes of osmotic adjustment and starch synthesis, respectively (Plaut *et al.* 2004).

The main stored stem carbohydrates are starch in the case of rice and fructan in the case of wheat. For the mobilization of these stored carbohydrates under dry soil conditions, rapid hydrolysis is required and it was shown that starch- and fructan-hydrolysing activities, respectively, were substantially increased under these conditions both in rice and wheat (for a review, see Yang & Zhang 2006). The effect of temperature stress, however, was not manifested as a limitation of sucrose to the spike in wheat (for a review, see Dupont & Altenbach 2003). The mobilization of total non-structural carbohydrates from the stem was enhanced at high temperatures during wheat grain filling (Plaut *et al.* 2004; Tahir & Nakata 2005). In contrast, the limited assimilate supply to the grain was suggested to be the main factor limiting grain weight under high-temperature stress in rice (Kobata & Uemuki 2004).

As plant hormones, ABA and cytokinins play major roles in regulating the link between senescence and assimilate remobilization as observed in both wheat and rice (Ahmadi & Baker 1999; Yang *et al.* 2001a, 2002, 2003a, 2004a; for a recent review, see Yang & Zhang 2006). In rice, water stress substantially increased ABA but reduced the zeatin and zeatin riboside concentrations in the root exudates and leaves (Yang *et al.* 2002). It was observed that the rate of photosynthesis and the chlorophyll content of the flag leaf (as parameters of senescence) were negatively correlated with ABA and positively with cytokinin levels. In both the leaves and stems, ABA, but not cytokinins (Z + ZR), was significantly and positively correlated with the remobilization of pre-stored carbon, and this remobilization was enhanced by low concentrations (below 0.001 M) of exogenous ABA (Ahmadi & Baker 1999; Yang *et al.* 2003a). High ABA concentrations (0.1 M), however, reduced the transport of sucrose into the grains and lowered the starch synthesis ability of intact grains, resulting in low grain weight in wheat (Ahmadi & Baker 1999).

The combined effect of high temperature and water limitation on the development of the cereal grain

Despite the fact that drought and heat stress often coincide under field conditions and interact during the grain-filling period of cereals, there is only limited information on the combined effect of these stress factors on kernel development. The combination of high temperature and drought reduced the duration of grain fill more than either treatment alone in wheat (Nicolas, Gleadow & Dalling 1984; Altenbach *et al.* 2003; Shah & Paulsen 2003). Interactions between the two stresses were pronounced, and the consequences of drought on all physiological and developmental parameters (e.g. maximum water content, fresh weight, DW, temporal pattern of development) were more severe at high temperature than at low temperature (Altenbach *et al.* 2003). The synergistic interactions indicated that the productivity of wheat is reduced considerably more by the combined stresses than by either stress alone, and that much of the effect is exerted on photosynthetic processes (Shah & Paulsen 2003). However, the combined effects of heat and drought are not necessarily additive ones. For example, in the case of the kernel DW at maturity, high temperature reduced the effect of post-anthesis drought in wheat (Wardlaw 2002). It was suggested that where high temperature and drought occur concurrently after anthesis, a degree of drought escape may be associated with chronic high temperature because of the reduction in the duration of kernel filling, even though the rate of water use may be enhanced by high temperature (Wardlaw 2002). On the other hand, under certain circumstances, limited water availability may promote nutrient remobilization from the leaves and stems and increase the rate of grain filling (for a review, see Yang & Zhang 2006), thus compensating for its shortened duration.

MODERN APPROACHES TO THE IMPROVEMENT OF YIELD SAFETY IN CEREALS UNDER TEMPERATURE AND/OR WATER STRESS

Environmental stresses have a great impact on the yield of cereal crops. As detailed earlier, the effect of drought and/or heat stress on yield is highly complex and involves processes as diverse as stem reserve accumulation, gametogenesis, fertilization, embryogenesis, and endosperm and grain development. Our present knowledge on these processes and on their mutual interactions is still scant, especially if the potential impacts of environmental factors also have to be considered. The application of modern research tools to reveal the complex molecular networks behind the

observed physiological and developmental responses in higher plants, including cereals, has only recently begun.

The overall potential and drawback of modern genetic and genomic approaches in cereal improvement and in deciphering the regulatory mechanisms of abiotic stress tolerance in plants have recently been thoroughly reviewed (Snape *et al.* 2005; Langridge, Paltridge & Fincher 2006; Varshney, Hoisington & Tyagi 2006; Sreenivasulu *et al.* 2007). Therefore, only a few examples directly related to the subject of the present review are discussed further in detail.

Use of molecular markers

Good genetic maps based on molecular marker technologies are now available for major cereal species (for reviews, see Snape *et al.* 2005; Langridge *et al.* 2006). Many of the traits determining abiotic stress tolerance and the quality and quantity of yield are controlled by a large number of genes, which have only minor individual effects but which act together (quantitative trait loci, QTL). In crop species with large, complex genomes, QTL analysis is an important tool in the identification of genetic markers to assist breeding efforts. This approach is complicated in wheat because of the polyploid nature of the genome and the low levels of polymorphism, but is straightforward in rice, maize and barley (Snape *et al.* 2005). The strong synteny observed between the genetic maps of cereals, however, may help transfer the knowledge gained for rice or barley to wheat.

Studies on the abiotic stress tolerance of cereals include the extensive analysis of QTLs linked to the field evaluation of stress tolerance (for a recent review, see Langridge *et al.* 2006). Despite extensive efforts, it seems to be very difficult to identify QTLs linked to grain yield and yield components with a high consistency in diverse environments (Bruce, Edmeades & Barker 2002 and references therein). However, Root-ABA1, a major QTL consistently affecting root architecture, leaf ABA concentration, grain yield, and other agronomic traits in maize under both well-watered and water-stressed conditions was recently identified, demonstrating the usefulness of this experimental approach (Giuliani *et al.* 2005; Landi *et al.* 2007).

Genomic regions associated with grain yield and its components under drought stress have been identified in rice by Lanceras *et al.* (2004). In another study, the genetic bases of traits representing source, sink and transport tissues and their relationship to yield have been investigated by QTL analysis in rice (Cui *et al.* 2003). Correlating genetic information with physiological and morphological traits related to high yield and/or drought tolerance will allow the development of new varieties with improved yield safety under water-limited conditions using molecular marker-assisted breeding.

This technology may also be useful to survey drought and heat tolerance in various genotypes, including land races and wild relatives of cereals. In addition, the comparison of QTLs linked to stress tolerance in various cereals may help identify common loci or genes linked, for example, to drought tolerance (Langridge *et al.* 2006).

Functional genomics

Expression profiling may help identify the key molecular events underlying stress tolerance and grain development, as well as their interactions. The number of cereal expressed sequence tag (EST) sequences available in public databases is continuously increasing. The cDNA libraries used to generate these ESTs represent various tissues and growth conditions, but yield- and stress-related libraries dominate. ESTs are especially important in wheat genomics, where whole-genome sequencing is unlikely to be completed in the near future because of the size and complexity of the genome. Recently, Houde *et al.* (2006) reported that the digital expression analysis of EST sequences combined with gene annotation (annotation of 29 556 different sequences) resulted in the identification of several pathways associated with abiotic stress resistance in wheat.

The number of macro- and micro-array platforms available for cereal research is also increasing. Zhu *et al.* (2003) hybridized a DNA chip containing around 21 000 genes, representing approximately half the rice genome, with cDNA probes characteristic of the successive stages of grain filling. They then examined the expression patterns of known genes that could potentially be involved in the process (e.g. genes linked to carbohydrate and fatty acid metabolisms). The expression of 98 of the 491 selected genes exhibited a correlation with grain filling. Based on the expression patterns of these genes, a further 171 genes were found with similar regulation, suggesting that they might also be involved in grain filling. Another methodological refinement led to the identification of a further 28 similar genes (Anderson *et al.* 2003), which means that at least 297 genes in the rice genome are currently thought to be related to grain filling. The knowledge of the rice genome sequence has allowed the promoter regions that control these genes to be identified and for common elements in these regions to be discovered. These in turn led to the discovery of nine transcription factors that regulate the expression of the genes (Zhu *et al.* 2003).

Recently, Yamakawa *et al.* (2007) elucidated the effect of high temperature on grain filling during the milky stage of rice. The down-regulation of several starch synthesis genes (especially GBSS and SBE) and the up-regulation of the starch-consuming α-amylase were observed and verified. The reduction in the transcription of the genes coding for AGPase small subunits, but not those coding for SSS isoforms, could also be observed in rice caryopses treated at high temperature. The observed changes in transcription level corresponded with the biochemical differences observed between the starch grains formed under normal and high temperatures, namely, with reduced amylase content, amylopectin side chain elongation and decreased grain size.

The effect of withholding water from maize for 5–9 d after pollination was studied by cDNA micro-array in endosperm and placenta/pedicel tissues by Yu & Setter (2003). There was a huge difference in the response of the two types of tissues: 89% of the 79 transcripts affected

were up-regulated in the pedicel, while 82% of the 56 transcriptionally altered genes were down-regulated in the endosperm. The sets of genes affected were also different: in the first case the level of transcription of stress-related genes (e.g. HSP and chaperon genes) increased, while in the endosperm genes related to cell division, growth and cell wall degradation were down-regulated. In agreement with this observation, water deficit during the first few days after pollination was found to inhibit endosperm division, which is closely correlated with kernel size at maturity in both maize (Ober *et al.* 1991) and wheat (Nicolas *et al.* 1985).

Zinselmeier *et al.* (2002) analysed the expression of 1502 maize genes in response to moderate or severe water deficiency induced immediately prior to or 2–4 d after pollination, and changes were monitored in the gene expression patterns of the silk and the grain. Over all the treatments, changes were observed in the expression of 179 of the 1502 genes. A large majority of these genes also showed changes in the leaves of water-deficient plants, indicating the general nature of the stress response (Zinselmeier *et al.* 2002). Interestingly, drought caused no decline in starch synthesis genes, in contrast to the response to shading (Zinselmeier *et al*. 2002 and see previous discussion).

Micro-array profiling of the drought stress response of whole plants or vegetative organs has been conducted in many species including cereals (Ozturk *et al.* 2002; Rabbani *et al.* 2003; Buchanan *et al.* 2005; Mohammadi, Kav & Deyholos 2007). Considering the important role of vegetative tissues in supplying nutrients to the developing reproductive organs, these studies may also significantly contribute to improving yield safety under water shortage conditions. In an interesting approach, Xue *et al.* (2006a) investigated gene expression differences in the progeny of wheat lines with high and low transpiration efficiency (TE). TE and high water-use efficiency (WUE) are known to be associated with higher yield under water-limited conditions. The expression profiling of 16 000 unique wheat genes allowed the identification of 93 genes that showed differential expression in progeny lines with contrasting TE levels. One-fifth of these genes was also responsive to drought. Furthermore, several potential growth-related regulatory genes were also identified as being correlatively expressed with high TE. The further analysis of these genes, associated with high biomass and high yield production, in the case of limited water availability during the vegetative period, may provide useful markers for future breeding efforts.

As the fertilization process takes place deep within the female tissues, considerable advances in the isolation of female gametes, micromanipulation and *in vitro* cell culture techniques were required to make the cells of the female sexual generation and the zygote accessible for molecular biological analysis (Barnabás *et al.* 2001). Now, improvements in molecular techniques have made it possible to isolate mRNA from a single cell in order to study the gene expression pattern in isolated egg cells and zygotes of cereals with the aid of polymerase chain reaction (Brandt *et al.* 2002; Okamoto *et al.* 2005) as well as by genomic (Brandt *et al.* 2002; Sprunck *et al.* 2005) and proteomic (Okamoto *et al.* 2004) methods.

Proteomics, metabolomics

Investigating the effect of drought and/or heat stress on protein composition might also be an important step towards understanding the link between environmental factors and plant development.

Proteomic studies in cereals can be based on rice as a model species (for reviews, see Agrawal & Rakwal 2005; Agrawal *et al.* 2006; Komatsu & Yano 2006). A proteomic analysis of drought- and salt-stressed rice plants found that around 3000 proteins could be detected in a single gel and over 1000 could be quantified (Salekdeh *et al.* 2002). Forty-two proteins were found to change their abundance or position in response to the treatments. The effect of salt stress on young rice panicles has been investigated by the same authors (Dooki *et al.* 2006). The proteomic analysis of rice leaf sheaths during drought stress identified 10 up-regulated and two down-regulated proteins. One of the drought-responsive proteins identified was an actin depolymerizing factor also present at high levels in the leaves of non-stressed drought-resistant cultivars (Ali & Komatsu 2006).

Proteomic analysis has been started in all major cereal species in addition to rice. Proteomic reference maps have been compiled for maize (Méchin *et al.* 2004) and wheat (Vensel *et al.* 2005) endosperm and for barley grain (Finnie *et al.* 2002) during the processes of grain filling and maturation.

The effect of heat stress on the grain of hexaploid wheat has been thoroughly studied at the protein level (Majoul *et al.* 2003, 2004). The down-regulation of several proteins involved in the starch metabolism and the induction of HSPs were reported (Majoul *et al.* 2004). As regards the effect of drought on the wheat grain proteome, Hajheidari *et al.* (2007) reported the detection of 121 proteins exhibiting significant changes in response to the stress, of which 57 could be identified. Two-thirds of the identified proteins turned out to be thioredoxin targets, revealing the link between drought and oxidative stresses. In addition, the contrasting protein changes observed in susceptible and tolerant genotypes allowed the identification of potential markers or regulators of drought tolerance.

In maize, changes in the protein complement have been monitored under progressive water deficit (Riccardi *et al.* 1998). This study allowed the identification of several genes/proteins involved in the drought response. The high genetic variability observed at the proteome level for the drought response in maize (de Vienne *et al.* 1999) allowed the identification of the *Asr1* (ABA/water-stress/ripening-related1) gene as a candidate for genetic improvement (Jeanneau *et al.* 2002, see also further).

Metabolomic research on cereals has also recently begun (Sato *et al.* 2004) and may, in the future, provide valuable information, for instance, on the sugar and amino acid metabolism in the vegetative and reproductive organs of

cereals under various environmental conditions (Langridge *et al.* 2006).

Genetic engineering

Genetic modification allows the introduction of isolated individual genes into the cereal germplasm and offers a variety of opportunities to increase environmental stress tolerance. The major cereal species are all amenable to the technologies of genetic modification (for recent reviews, see Shrawat & Lörz 2006; Vasil 2007). Although there is still a political debate on the intensive use of genetically modified plants in agriculture, especially in Europe, there is a continuous increase in transgenic crop production worldwide, including many varieties of genetically modified maize resistant to herbicide and/or pathogens. As knowledge on the molecular networks underlying abiotic stress responses in plants, especially in *Arabidopsis*, continuously increases (for a recent summary, see Zhang, Creelman & Zhu 2004; Nakashima & Yamaguchi-Shinozaki 2005), more and more candidate genes will be identified with the potential to improve stress tolerance using transgenesis. In addition to model species such as *Arabidopsis*, several candidate genes have also been identified in cereals themselves through the application of modern genomic approaches (for a recent review, see Shinozaki & Yamaguchi-Shinozaki 2007).

For example, the maize gene *Asr1*, coding for a putative transcription regulator, has been identified as an ABA- and drought-regulated gene using genomic and proteomic approaches (see previous discussion). Transgenic maize lines over-expressing the *Asr1* gene showed an increase in foliar senescence under drought conditions. The ASR1 protein is hypothesized to function in the re-routing of the metabolism from source to sink and in re-allocating carbohydrates, and maybe other key compounds, to sink tissues, finally leading to the premature senescence of the source organs (Jeanneau *et al.* 2002). In a parallel study, the same authors (Jeanneau *et al.* 2002) developed transgenic lines expressing a *Sorghum* C4 phosphoenolpyruvate carboxylase (C4-PEPC). Transgenic lines with increased C4-PEPC activity exhibited increased WUE accompanied by a DW increase under moderate drought conditions. It was hypothesized that the elevated WUE values displayed by the over-expressing line may be caused by the improved ability of the plants to fix CO_2 under moderate drought conditions when gas conductance is reduced.

The over-expression of an NAC-type transcription factor (SNAC1) in rice significantly enhanced drought resistance, with 22–34% higher seed setting as compared with the controls under severe drought conditions at the reproductive stage in the field (Hu *et al.* 2006). The transgenic line showed increased ABA sensitivity, enhanced stomatal closure and slower water loss. The SNAC1 transcription factor was also shown to up-regulate the expression of a large number of stress-regulated genes in the transgenic plants. Other stress-related transcription factors, belonging to the DREB family have also been found to increase drought tolerance in cereals (Oh *et al.* 2005; Wang *et al.* 2006).

The field evaluation of transgenic wheat plants ectopically expressing the barley *HVA1* gene coding for a LEA protein revealed increased drought tolerance, with higher relative water content in the leaves and higher yield under moderate drought conditions (Sivamani *et al.* 2000; Bahieldin *et al.* 2005). The over-expression of a similar gene in rice also resulted in improved drought tolerance in the field (Xiao *et al.* 2007).

Further strategies used to successfully improve drought or heat tolerance in transgenic cereals included the over-expression of kinases or phosphatases involved in the stress signalling cascade (Saijo *et al.* 2000; Shou, Bordallo & Wang 2004; Xu *et al.* 2007), osmoprotectants (Garg *et al.* 2002; Abebe *et al.* 2003; Jang, Oh & Seo 2003; Capell, Bassie & Christou 2004; Quan *et al.* 2004; Shirasawa *et al.* 2006) or other stress-related genes (Shi *et al.* 2001; Katiyar-Agarwal, Agarwal & Grover 2003).

Most of these transgenic strategies have an indirect effect on reproductive development by ensuring normal plant growth and metabolism under adverse environmental conditions. However, as discussed earlier, photosynthesis, CO_2 assimilation, carbon and nitrogen metabolism, and nutrient transport during vegetative and reproductive development all have very important roles in the development of the reproductive organs under both normal and stress conditions. In addition to these general stress-resistance traits, which might also be successfully used to ensure high yield under water limitation and/or temperature stress, the genetic modification of more specific pathways associated with reproduction and seed development can be expected in the future.

STRATEGIC CONSIDERATIONS AND FUTURE PERSPECTIVES

The complexity of both cereal reproduction and plant stress responses makes it difficult to construct a simple model of ways in which successful reproductive development and high yield can be achieved under water-limited and/or high-temperature conditions. Both specific and more general approaches are conceivable, targeting various aspects of plant development and stress responses. However, where the safety of the final yield is concerned, all the breeding or genetic manipulation approaches used in cereals have to converge finally at flowering and/or grain development. Therefore, the better understanding of these developmental processes is of utmost importance for the future, and modern genomic approaches may help considerably in this respect.

However, cereal yield is not only dependent on the success of the reproductive processes themselves, but is indirectly determined by overall plant growth and development as well. This is well supported by the breeding of modern cultivars with reduced plant height (most of the cereal species) or tassel size (maize), with concomitant

improvement in assimilate availability for the growing spike, thus enhancing kernel number (for a review, see Otegui & Slafer 2004).

Environmental stresses have a great impact on the reproductive development of cereal crops and, consequently, on the final yield. The effect of drought and/or heat stress on yield is highly complex and involves processes as diverse as nutrient assimilation and supply to reproductive organs, stem reserve accumulation, gametogenesis, fertilization, embryogenesis, and endosperm and grain development (see previous discussion). Despite the fact that yield can be affected at any time from sowing to grain maturity, it is generally accepted that only certain periods of the whole growing season are critical for the determination of the final yield in cereals (Cakir 2004; Otegui & Slafer 2004). These critical periods are around flowering, when the number of grains per land area is determined, and during the grain-filling stage, when the average grain weight is determined (Otegui & Slafer 2004). Flowering is considered to be the most critical stage, as the cereal yield is much more closely related to the number of grains per land area than to the average weight of the seeds. As the yield is primarily determined by resource availability and the number of grains is adjusted in the plant to match the resource-defined yield level (Sinclair & Jamieson 2006), strategies to improve yield should be based on improved resource accumulation or translocation from the vegetative to the reproductive organs prior to or during floret formation, especially under unfavourable environmental conditions.

The special strategies, however, may differ between species. For example, as discussed by Otegui & Slafer (2004), grain number in wheat, a cleistogamous species, is primarily determined by the number of fertile florets, as most fertile florets become grain after anthesis, while in maize, a monoecious crop, the critical step is grain set, which is dependent on the success of fertilization. Therefore, strategies to further increase the yield potential of wheat may be based on increasing the stem elongation phase (stem reserve accumulation), while a high level of synchrony in the emergence of the silks could be the primary target of breeding efforts in maize (Otegui & Slafer 2004). These traits could result in improved yield under moderate stress conditions as well.

It would also be of great importance for modern research techniques to allow the detailed investigation of the link between resource availability and floret formation. As more and more is known about the molecular background of flower formation in cereals (Bommert *et al.* 2005), especially in rice (Yano *et al.* 2001; Kater, Dreni & Colombo 2006), suitable molecular markers can be expected to be available to address the question of how the nutrient supply, especially that of nitrogen (Oscarson 2000), affects flowering and fertility. In addition, the manipulation of the regulation of flowering time might also have considerable significance in order to avoid the most stressful periods of the growing season (Tewolde, Fernandez & Erickson 2006).

The overall development of the plant and, consequently, its yield potential may be affected by prolonged stress conditions during vegetative growth, and the low efficiency of stem reserve accumulation during this period may seriously affect subsequent reproductive development. Stem reserves may serve, for example, as a supply of carbohydrates for grain filling under conditions where photosynthesis is inhibited. Increasing the efficiency of photosynthesis (Horton 2000; Long *et al.* 2006), nitrogen metabolism (Donnison *et al.* 2007) or WUE (Blum 2005; Xue *et al.* 2006b) under stress conditions during the vegetative growth period of cereals may serve as a means to increase reserve accumulation and to ensure the proper development of reproductive organs.

The ability to accumulate a large pool of reserves in the stem, however, is often inversely correlated with grain yield potential in modern cultivars (Blum 1998). High stem reserves are often correlated with enhanced leaf senescence and, hence, with increased nitrogen export from the leaves to the grains. It is therefore hypothesized that non-senescence ('stay green'), as a sustained supply of current assimilates, and stem reserve utilization might be mutually exclusive strategies to support grain filling under stress (Blum 1998), making the proper control of senescence in source tissues of utmost importance under both normal and unfavourable conditions. The identification of cereal QTLs (Xu *et al.* 2000; Abdelkhalik *et al.* 2005; Harris *et al.* 2007) or genes (Lee *et al.* 2001; He *et al.* 2005; Gregersen & Holm 2007) associated with leaf senescence is in progress and may provide the necessary tools for genetic modifications.

Besides the importance of stress tolerance during vegetative growth, the stress sensitivity of the reproductive phase deserves more attention, especially in the case of short, but extreme, stress conditions during the reproductive phase, more particularly during fertilization and early grain filling. It is clear that drought and/or heat induce structural, physiological and molecular abnormalities in the processes leading to the development of the gametes and have a serious influence on the success of fertilization because of the production of dysfunctional male and female gametophytes, even if fertilization itself takes place under optimum environmental conditions. The grain-filling period is also of considerable importance in determining yield and although more and more is known about the stress sensitivity of the enzymatic processes required for starch accumulation, we are still far from having the complete picture. The genomic and proteomic approaches may help identify the most sensitive molecular processes during reproductive development, making them amenable to genetic improvement. In addition, the improvement of drought tolerance by increased osmoprotection (Santamaria, Ludlow & Fukai 1990; Tambussi, Nogués & Araus 2005; for a review, see Vasil 2007) or that of heat tolerance via the expression of HSPs (Katiyar-Agarwal *et al.* 2003) may provide suitable strategies to protect reproductive organs from short-term environmental extremes.

Modern genetic and genomic tools linked to physiological and field experiments will provide more and more experimental details about the link between the development of the cereal plant and its environment. This will allow

Vegetative development	Reproductive development
Enhanced root growth Increased water uptake Increased water-use efficiency Protection of the photosynthetic apparatus Increased stem reserve pool Regulation of senescence Enhanced stem reserve mobilization	Early, synchronized heading and flowering to avoid stressful periods Favourable morphological features for inflorescences Increased sink capacity and accelerated grain filling

Figure 3. Potential physiological/developmental targets of breeding approaches in order to increase yield safety of cereals under drought and high-temperature stress.

the identification of a growing number of molecular markers associated with complex developmental and stress-related traits, as well as that of individual candidate genes with a significant potential impact on the development and/or stress tolerance of transgenic plants. These emerging approaches can be expected to have a substantial influence on cereal improvement, including the drought and temperature tolerance of reproductive processes, in the coming decades. Potential targets of genetic approaches aimed at improving drought and/or heat tolerance in cereals are summarized in Fig. 3.

ACKNOWLEDGMENTS

The authors are thankful for the support of the grants 'Búzakalász' National Office for Research and Technology, Republic of Hungary (NKTH) 4-064/04 and Economic Competitiveness Operational Programme, Republic of Hungary (GVOP)-0522/3.1.

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Received 11 February 2007; received in revised form 6 June 2007; accepted for publication 20 August 2007

