# Abiotic stress, the field environment and stress combination

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**Farmers and breeders have long known that often it is the simultaneous occurrence of several abiotic stresses, rather than a particular stress condition, that is most lethal to crops. Surprisingly, the co-occurrence of different stresses is rarely addressed by molecular biologists that study plant acclimation. Recent studies have revealed that the response 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 applied individually. Tolerance to a combination of different stress conditions, particularly those that mimic the field environment, should be the focus of future research programs aimed at developing transgenic crops and plants with enhanced tolerance to naturally occurring environmental conditions.**

## Abiotic stress research: a reality check

Abiotic stress conditions cause extensive losses to agricultural production worldwide [1,2]. Individually, stress conditions such as drought, salinity or heat have been the subject of intense research [2,3]. However, in the field, crops and other plants are routinely subjected to a combination of different abiotic stresses [4–7]. In drought-stricken areas, for example, many crops encounter a combination of drought and other stresses, such as heat or salinity [4,5]. Recent studies have revealed that the molecular and metabolic response of plants to a combination of drought and heat is unique and cannot be directly extrapolated from the response of plants to each of these different stresses applied individually [8–11]. Studies of simultaneous stress exposure in different plants are well documented in various agronomic and horticulture journals. In addition, tolerance to a combination of two different abiotic stresses is a well-known breeding target in corn and other crops [5–7]. Nevertheless, little is known about the molecular mechanisms underlying the acclimation of plants to a combination of two different stresses [10]. Because the majority of abiotic stress studies performed under controlled conditions in the laboratory do not reflect the actual conditions that occur in the field, a considerable gap might exist between the knowledge gained by these studies and the knowledge required to develop plants and crops with enhanced tolerance to field conditions. This gap might explain why some of the transgenic plants developed in the laboratory with enhanced tolerance to a particular biotic or abiotic stress condition failed to show enhanced tolerance when tested in the field [12–14]. A focus on molecular, physiological and metabolic aspects of stress combination is needed to bridge this gap and to facilitate the development of crops and plants with enhanced tolerance to field stress conditions.

## Tailoring a response to a particular stress situation

Plant acclimation to a particular abiotic stress condition requires a specific response that is tailored to the precise environmental conditions the plant encounters. Thus, molecular, biochemical and physiological processes set in motion by a specific stress condition might differ from those activated by a slightly different composition of environmental parameters. Illustrating this point are transcriptome profiling studies of plants subjected to different abiotic stress conditions: each different stress condition tested prompted a somewhat unique response, and little overlap in transcript expression could be found between the responses of plants to abiotic stress conditions such as heat, drought, cold, salt, high light or mechanical stress [10,15–18]. Although reactive oxygen species (ROS) are associated with many different biotic or abiotic stress conditions, different genes of the ROS gene network of *Arabidopsis* were found to respond differently to different stress treatments [19]. These findings suggest that each abiotic stress condition requires a unique acclimation response, tailored to the specific needs of the plant, and that a combination of two or more different stresses might require a response that is also unique.

In addition to the basic differences that exist between the acclimation responses of plants to different abiotic stress conditions [10,15–18], when combined, 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, plants would not be able to open their stomata and their leaf temperature would be higher [9]. Salinity or heavy metal stress might pose a similar problem to plants when combined with heat stress because enhanced transpiration could result in enhanced uptake of salt or heavy metals. Cold stress or drought, combined with high light conditions, result in enhanced production of ROS by the photosynthetic apparatus because these conditions limit the availability of $CO_2$ for the dark

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reaction, leaving oxygen as one of the main reductive products of photosynthesis [20]. Another example of antagonism between different abiotic stresses is drought and heavy metal stress, which exaggerate the effects of each other [21]. Because energy and resources are required for plant acclimation to abiotic stress conditions (e.g. for the synthesis of heat shock, or late embryogenesis abundant proteins), nutrient deprivation could pose a serious problem to plants attempting to cope with heat, cold or drought stress. Likewise, limited availability of key elements such as iron, copper, zinc or manganese, which are required for the function of different defense enzymes, such as superoxide dismutase or ascorbate peroxidase, could result in an enhanced oxidative stress in plants subjected to different abiotic stresses [22]. The acclimation of plants to a combination of different abiotic stresses would, therefore, require an appropriate response customized to each of the individual stress conditions involved, as well as tailored to the need to compensate or adjust for some of the antagonistic aspects of the stress combination.
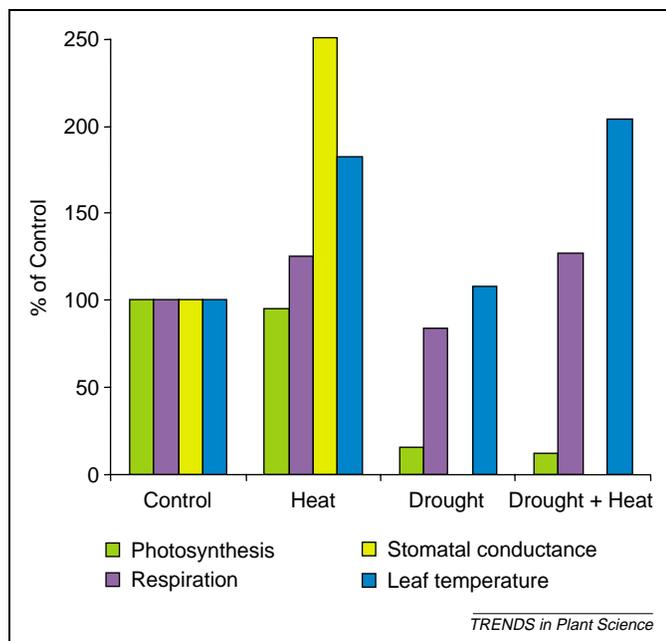
## Case study: drought and heat stress

Drought and heat stress represent an excellent example of two different abiotic stress conditions that occur in the field simultaneously [5–7]. Several studies have examined the effects of a combination of drought and heat stress on the growth and productivity of maize, barley, sorghum and different 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 plants and crops compared with each of the different stresses applied individually [5–7,23–27]. A sum of all major US weather disasters between 1980 and 2004 (excluding hurricanes, tornadoes and wildfires; Figure 1a) demonstrates the extent of the damage caused by a combination of drought and heat stress [compare the damage caused by drought to that caused by drought combined with a heat wave in Figure 1a, and compare the maps showing vegetation health in Figure 1b: August 1997 (no drought, no heat wave), August 2000 (drought combined with a heat wave) and August 2002 (drought without a heat wave)]. The vast agricultural areas
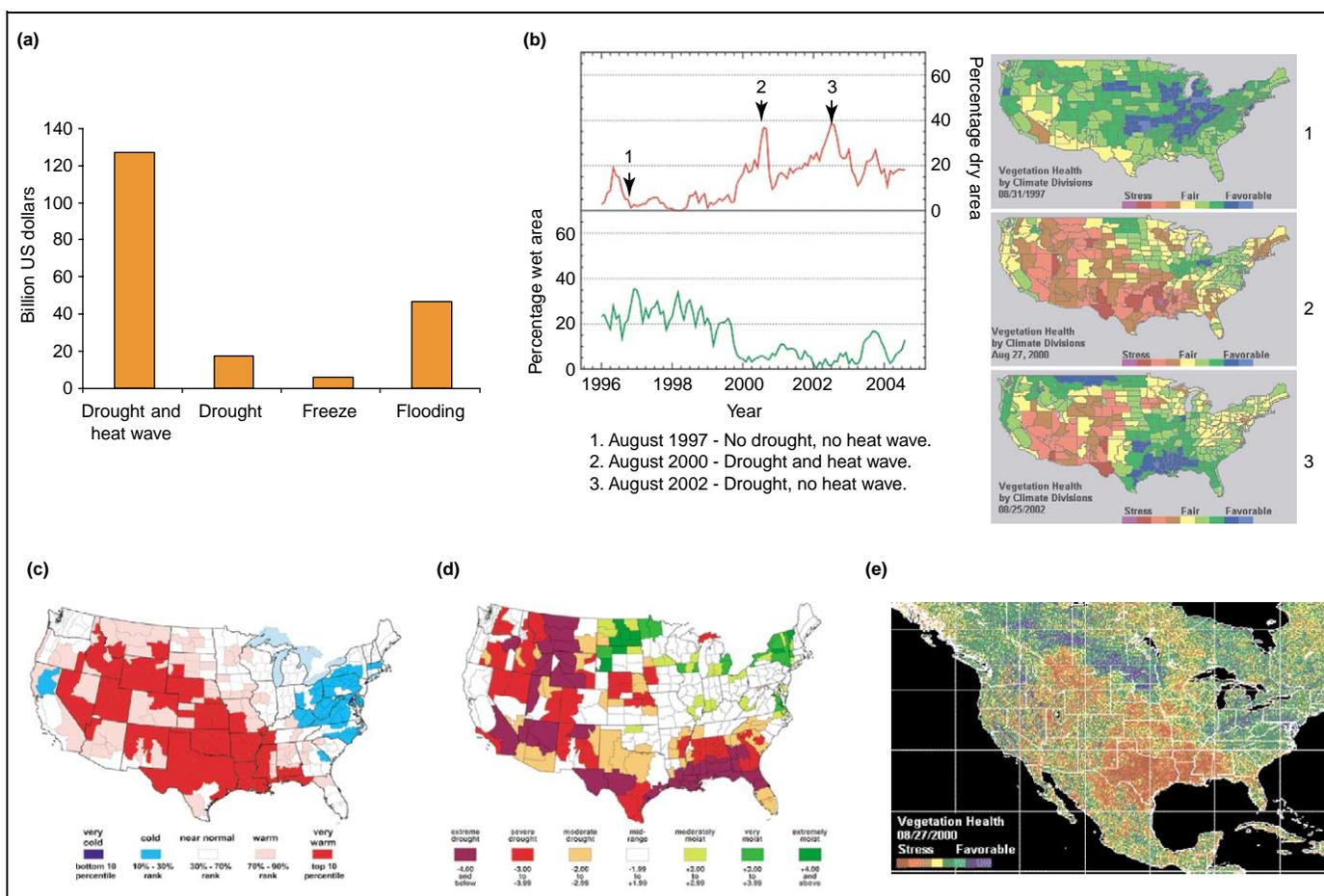


**Figure 1.** The effects of a combination of drought and heat stress on US agriculture. **(a)** Total of all US weather disasters costing US$1 billion or more between 1980 and 2004 (excluding hurricanes, tornadoes and wildfires). Total damage was normalized to the 2002 US dollar value (http://www.ncdc.noaa.gov/oa/reports/billionz.html). **(b)** Vegetation health maps for three different times (end of August in 1997, 2000 and 2002) showing the effect of the combination of drought and heat (August 2000) on plant health. Left panel: percentage of area dry or wet between 1996 and 2004 in the USA; during 2000 and 2002 drought conditions were enhanced. Right panels: vegetation health maps corresponding to the times indicated by arrows in the left panel. Time point 1 (August 1997): no drought stress, no heat wave. Time point 2 (August 2000): a combination of drought and heat wave. Time point 3 (August 2002): drought no heat wave. **(c)** Temperature index map, **(d)** long-term drought – Palmer map, and **(e)** a satellite image of vegetation health for August 2000, a summer that included a drought and a heat wave causing damage of more than US$4.2 billion to US agriculture. Maps, statistical data and satellite images were obtained from the National Climatic Data Center (http://www.ncdc.noaa.gov). Data presented in Figure 1 should not be interpreted as scientific evidence for the effects of stress combination. Please see text and references below for controlled scientific experiments documenting the effects of stress combination on plant and crop performance.

potentially affected by a combination of drought and heat stress can be extrapolated from weather maps and satellite images of the USA during August 2000, a month in which the co-occurrence of drought and heat stress caused damage costing more than US$4.2 billion (Figure 1c). The data shown in Figure 1 support the findings described above [5–7,23–27], and vividly demonstrate the impact that drought and heat stress have on agriculture when combined.

Physiological characterization of plants subjected to drought, heat stress or a combination of drought and heat stress reveals that the stress combination has several unique aspects, combining high respiration with low photosynthesis, closed stomata and high leaf temperature (Figure 2) [9]. Starch breakdown coupled with energy production in the mitochondria might, therefore, play a key role in plant metabolism during a combination of drought and heat stress [9,10]. Transcriptome profiling studies of plants subjected to drought, heat stress or a combination of drought and heat stress support the physiological analysis of plants (Figure 2) [9,10] and suggest that the stress combination requires a unique acclimation response involving > 770 transcripts that are not altered by drought or heat stress (Figure 3) [10]. Similar changes in metabolite accumulation were also found, with several unique metabolites, mainly sugars, accumulating specifically during the stress combination (Figure 3) [10]. By contrast, the level of proline, thought to be important for plant protection during drought stress [2,3], is strongly suppressed during a combination of drought and heat stress [10]. The profiling experiments summarized in Figure 3 further illustrate that the acclimation responses of plants to heat or drought stress
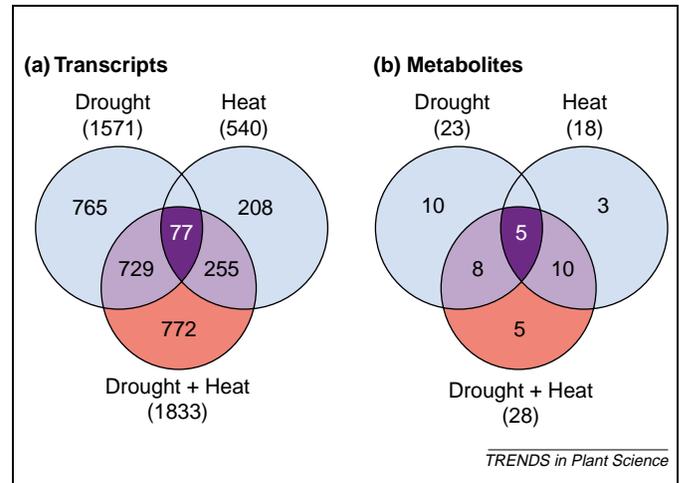


**Figure 3**. Unique molecular characteristics of drought and heat stress combination. Venn diagrams showing the overlap between **(a)** transcripts and **(b)** metabolites (enhanced or suppressed) during drought or heat stress, or a combination of drought and heat stress. The total number of transcripts or metabolites is indicated in parentheses. Data was obtained from [10].

are different and only a small overlap in transcript expression has been found between these responses.

Overall, the example of drought and heat combination underlines the potential severity of a stress combination, as well as its unique physiological, molecular and biochemical aspects. The results presented in Figures 1–3 strongly argue for a need to develop dedicated research programs aimed at enhancing the tolerance of plants and crops to a combination of different abiotic stresses.

## Regulatory aspects of stress combination: a key to enhancing tolerance?

Enhancing plant tolerance to biotic or abiotic stress conditions by activating a stress-response signal transduction pathway in transgenic plants is a powerful and promising approach [3,28–30]. It is logical to assume that the simultaneous exposure of a plant to different abiotic stress conditions will result in the co-activation of different stress-response pathways. These might have a synergistic or antagonistic effect on each other. In addition, dedicated pathways specific for the particular stress combination might be activated [10]. Several examples of synergistic or antagonistic relationships between different pathways exist. Heat stress was found to silence the UV-B response of parsley [31], whereas ozone induces the UV-B and/or pathogen responses of some other plants [32]. The phenomenon of cross-hardening, which reflects some of the synergistic relationships among different stresses, has been reviewed in several papers (e.g. [33]).

Cross-talk between co-activated pathways is likely to be mediated at different levels. These could include integration between different networks of transcription factors and mitogen-activated protein kinase (MAPK) cascades [34,35], cross-talk mediated by different stress hormones such as ethylene, jasmonic acid and abscisic acid [36], cross-talk mediated by calcium and/or ROS signaling [19,33], and cross-talk between different receptors and signaling complexes [37]. Although evidence



**Figure 2**. Unique physiological characteristics of drought and heat stress combination. A combination of drought and heat stress is shown to be different from drought or heat stress by having a unique combination of physiological parameters. Data was obtained from [9,10].

exists for stress-mediated cross-talk at the level of MAPKs and hormone signaling [34–36], much remains to be studied, particularly if we wish to use specific signal transduction components as a molecular leverage to enhance the tolerance of plants and crops to a combination of different stresses. Ethylene was recently shown to play a central role in the response of *Arabidopsis* to a combination of heat and osmotic stress, and expression of the transcriptional co-activator MBF1c in *Arabidopsis* was found to enhance the tolerance of transgenic plants to heat and osmotic stress by partially activating or perturbing the ethylene-response signal transduction pathway [11].

## Summary and conclusions: the 'stress matrix'

The extent of damage caused to agriculture by a combination of two different stresses (Figure 1) underscores the need to develop crops and plants with enhanced tolerance to a combination of different abiotic stresses. Drawing upon the limited physiological, molecular and metabolic studies performed with plants that were simultaneously subjected to two different abiotic stresses (Figures 2–3) [5–11,23–27], it is not sufficient to study each of the individual stresses separately. The stress combination should be regarded as a new state of abiotic stress in plants that requires a new defense or acclimation response. It should be studied in the laboratory or the field by simultaneously exposing plants to different abiotic stresses [10]. In addition, transgenic plants with enhanced tolerance to biotic or abiotic stress conditions should be tested for their tolerance to a combination of different stresses before they are introduced into field trials.

We face many challenges in our attempts to develop transgenic plants with enhanced tolerance to a stress combination. Tolerance to a combination of different stresses is likely to be a complex trait involving multiple pathways and cross-talk between different sensors and signal transduction pathways. Mapping genes essential for tolerance to a combination of abiotic stresses could, therefore, be costly and pose a technical challenge requiring multiple controls. In addition, resistance to a combination of different stresses could be genetically linked to suppressed growth or yield of plants. However, some stress combinations have the advantage of enhancing lethality [25]. Genetic screens of mutant or inbred lines could, therefore, be turned into selection for survivors with enhanced tolerance, and identified genes could be tested in transgenic plants.

What stress combinations should we study? Figure 4 summarizes many of the stress combinations that could have a significant impact on agricultural production (The 'Stress Matrix'). Perhaps the most studied interactions presented in Figure 4 are those between different abiotic stresses and pests or pathogens (i.e. biotic stress). In some instances it has been reported that a particular abiotic stress condition, such as ozone stress, enhances the tolerance of plants to pathogen attack [38,39]. However, in most cases, prolonged exposure of plants to abiotic stress conditions, such as drought or nutrient deprivation, results in the weakening of plant defenses and enhanced susceptibility to pests or pathogens [32,40]. In contrast to
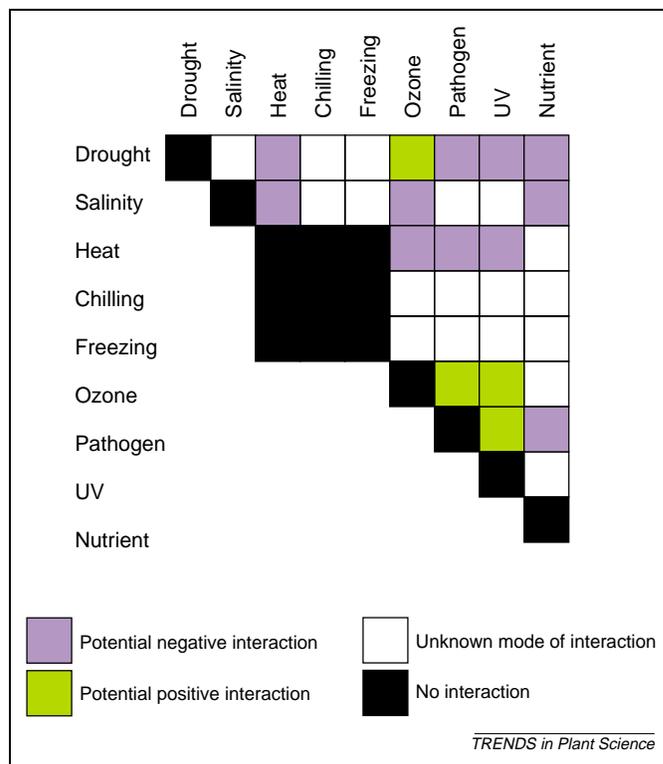
**Figure 4**. Agriculturally important stress combinations ('The Stress Matrix'). Different combinations of biotic and abiotic stresses are presented in the form of a matrix to demonstrate potential interactions that can have important implications for agriculture. Different interactions are color coded to indicate potential negative [i.e. enhanced damage or lethality owing to the stress combination (purple)] or potential positive [i.e. cross-protection owing to the stress combination (green)] effects of the stress combination on plant health. However, the potential effects of stress combination could vary depending on the relative level of each of the different stresses combined (e.g. acute versus low) and the type of plant or pathogen involved. Data to generate the matrix was obtained from [4–10,21–23,25–27,31–33,35,36,38–45].

the biotic–abiotic axis, most of the different abiotic stress combinations presented in Figure 4 have received almost no attention. The experience of farmers and breeders should be used as a valuable guide and resource to molecular biologists trying to address a specific stress combination that is pertinent to their crop of interest or region. The data presented in, for example, Figure 1, combined with different reports available from websites such as http://www.usda.gov/wps/portal/usdahome indicate that major US crops, including corn and soybean, are particularly vulnerable to a combination of drought and heat stress. By contrast, reports from northern countries such as Sweden or Canada identify a combination of cold stress and high light as having a major rate-limiting affect on agriculture.

Perhaps the most important guideline for studying abiotic stress combination is to address it as if it is a new state of abiotic stress in plants and not simply the sum of two different stresses. To develop transgenic crops with enhanced tolerance to field conditions, researchers need to widen their area of study to include stress combination.

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