Review

Selection and Breeding of Suitable Crop Genotypes for Drought and Heat Periods in a Changing Climate: Which Morphological and Physiological Properties Should Be Considered?

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Abstract: Selection and breeding of genotypes with improved drought/heat tolerance become key issues in the course of global change with predicted increased frequency of droughts or heat waves. Several morphological and physiological plant traits must be considered. Rooting depth, root branching, nutrient acquisition, mycorrhization, nodulation in legumes and the release of nutrients, assimilates or phytohormones to the shoot are relevant in root systems. Xylem embolism and its repair after a drought, development of axillary buds and solute channeling via xylem (acropetal) and phloem (basipetal and acropetal) are key processes in the stem. The photosynthetically active biomass depends on leaf expansion and senescence. Cuticle thickness and properties, epicuticular waxes, stomatal regulation including responses to phytohormones, stomatal plugs and mesophyll resistance are involved in optimizing leaf water relations. Aquaporins, dehydrins, enzymes involved in the metabolism of compatible solutes (e.g., proline) and Rubisco activase are examples for proteins involved in heat or drought susceptibility. Assimilate redistribution from leaves to maturing fruits via the phloem influences yield quantity and quality. Proteomic analyses allow a deeper insight into the network of stress responses and may serve as a basis to identify suitable genotypes, although improved stress tolerance will have its price (often lowered productivity under optimal conditions).

Keywords: drought; heat; climate change; crop genotypes; morphology; physiology; stress susceptibility; assimilate allocation; yield

1. Introduction

Besides increasing atmospheric CO_2 concentration and higher average temperature, more frequent and/or more severe extreme climatic events including extended drought periods and superimposed heat waves as predicted by climate change models represent challenges for agriculture during the next decades [1–4]. Selection and breeding of suitable crop genotypes are key aspects in this context [5,6]. How should these genotypes behave? The superficial answer would be that they should be less susceptible to abiotic stresses, but a closer and critical look on specific functions and interactions on the whole plant level may help to evaluate the sustainable performance of crop species and varieties in a more comprehensive manner [6–8]. The overall performance of a species or a variety depends on the plant integral activity during the pre-stress phase, the stress period(s) and the recovery phase(s) [9]. Key aspects for the comparison of drought sensitivity in crop varieties are summarized in Figure 1.
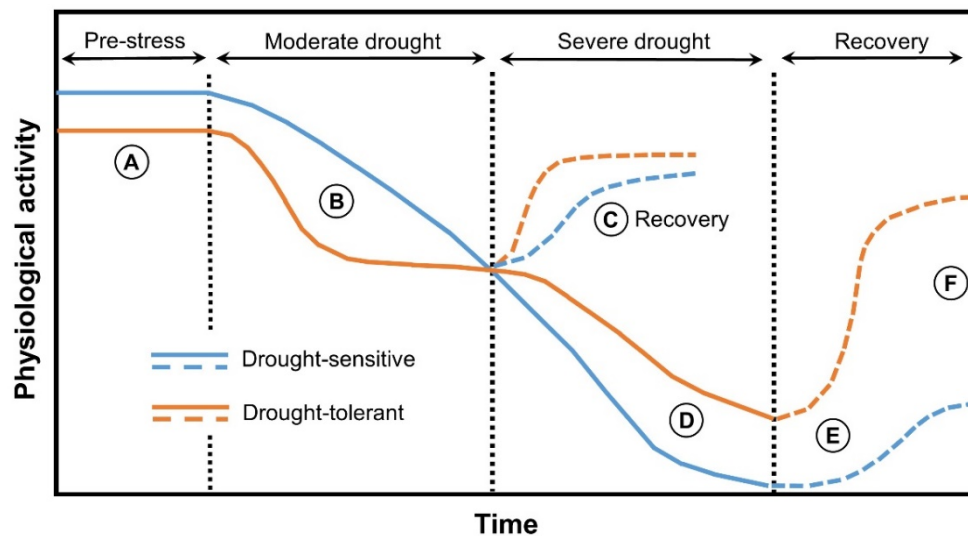
Figure 1. Schematic presentation of some key points for characterizing genotypes differing in drought tolerance. **A**: performance of non-stressed plants (pre-stress); **B**: performance under moderate drought; **C**: recovery from moderate drought; **D**: performance under severe drought; **E**: time course for recovery from severe drought; **F**: completeness of recovery from severe drought.

Performance during non-stress phases is often lower for drought-tolerant than for well yielding standard varieties (Figure 1A) [10]. The performance during moderate drought may decline more rapidly and then remain stable on a lower level in drought-tolerant genotypes, while standard varieties may decline more steadily (Figure 1B). The down-regulation of physiological activities in stress-tolerant varieties may improve the chances to survive, save resources (e.g., water during drought stress) and serve as a basis for rapid recovery after the stress phase [9]. On the other hand, less drought-tolerant varieties may be initially more productive during the stress period, but may then be more severely damaged and recovering less well than a more tolerant variety [9]. An important point is the velocity and degree of recovery after the stress period (Figure 1C). The low activity level reached after a severe drought (Figure 1D), the time course for physiological activities during a subsequent recovery phase (Figure 1E) and the completeness of recovery (Figure 1F) are important for genotype comparisons [11,12].

Another aspect to be considered is that good overall performance of crop plants under stress does not necessarily coincide with yield stability [10]. Breeding for stress-tolerant crops should focus on acceptable yields under limited environmental conditions and not only on survival after stress [8]. Besides, combined response to drought and heat, which often co-occur or superimpose, could differ from adaptation to individual stresses [13–15]. For example, proline is accumulated under drought, but not under water stress combined with high temperature; strong activation of starch breakdown and malate metabolism in support to the mitochondrial respiration is typical for the combination of drought and heat but not for these stresses applied separately [14,15].

Several recent reviews have summarized the current knowledge of plant response to drought and high temperature stresses at morphological, physiological and phenological levels [16–21]. The aim of this review is to identify morphological and physiological properties that are important for drought and/or heat tolerance in crop plants and for sustainable crop production. Such key properties serve as a basis for the selection of suitable crops or genotypes on one hand and for breeding new varieties to be grown in a changing climate on the other hand. Special attention is paid on the search for suitable molecular markers for assisted selection and breeding, including some protein markers.
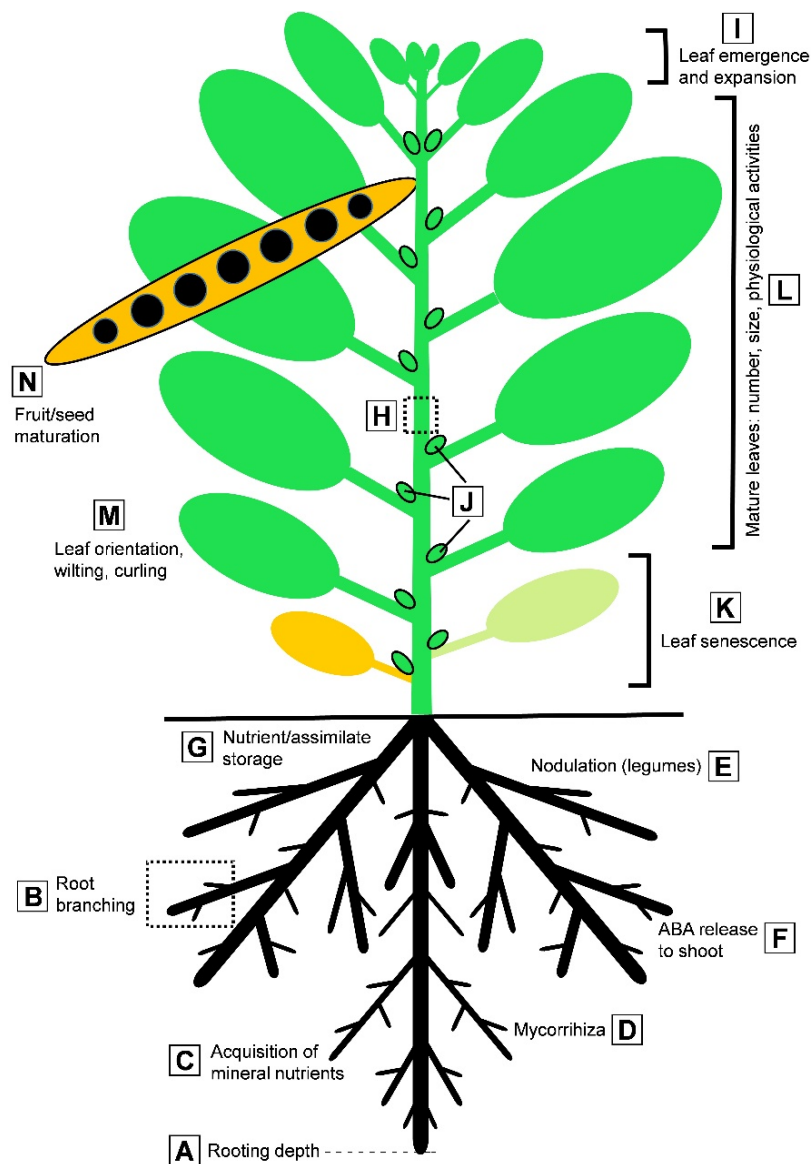
Figure 2. Morphological and physiological characteristics of annual crops relevant for their responses to drought. **A**: rooting depth (before and during stress period); **B**: root branching; **C**: uptake of mineral nutrients and release to the shoot; **D**: interactions with mycorrhiza; **E**: nodulation and nodule activity (in legumes); **F**: production of abscisic acid (ABA) and release to the shoot; **G**: storage of nutrients and assimilates in roots/rhizomes/bulbs; **H**: transport of nutrients/assimilates/phytohormones via xylem and phloem; **I**: emergence and expansion of new leaves; **J**: production of new leaves from axillary buds; **K**: senescence of older leaves; **L**: number, size and morphological/physiological properties of mature leaves; **M**: spatial orientation, curling and wilting of mature leaves; **N**: maturation of fruits and seeds.

2. Root Morphology and Physiology

Heat waves affect directly the shoot, while roots, being in direct contact with the drying soil, are the first organs that suffer from, sense and respond to drought stress, transmitting the stress signal to the whole plant. The water potential in the soil declines during a drought period, but there may be considerable differences in the vertical (depth) and in the horizontal (patchiness) direction (Figure 2) [22–24]. Since strong vertical gradients for soil water potential are very likely, rooting depth must be a key parameter (Figure 2A) [25–27]. However, from a comprehensive study including more than 40 species it became evident that rooting depth is not equally plastic [28]. Rooting depth in the

majority of these species was not significantly affected by soil drying, while in seven species it was increased and in five species even decreased in drying soil [28]. These findings indicate that caution is recommended when generalizing such results and that the different response may be relevant for species competition (e.g., in grasslands). It must be borne in mind that, depending on the actual weather during a drought period, minor quantities of water may be available in the top soil layer as a consequence of minor precipitation(s) or of dew condensation, while the water content in lower soil layers may not be improved by these events. Soil temperature near the surface depends on many factors (e.g., photon flux density, leaf area index, soil color, surface structure, air convection), which may influence heating by solar irradiation and heat dissipation. Therefore, interactions between various factors influence soil water availability as well as soil temperature pattern and finally influence the development of crop roots in a complex manner [29–32].

Root architecture depends on root branching as well as on the length of the various roots (Figure 2B) and is relevant for the exploration of soil regions with accessible water during a drought period [29,30]. Besides species-specific differences between crop plants grown under non-stress conditions, the plasticity of the root system in response to changing environment should be considered when breeding for stress tolerance [31,32]. Root architecture is affected by the soil water, temperature and nutrients, and additionally by soil microbial communities and microbial-plant interactions [32–37]. A good correlation between reduced lateral root branching and drought tolerance has been reported for maize [30,35], but the "rooting depth paradigm" is questioned recently [38]. A smaller number of longer lateral roots are found in maize genotypes with a good drought tolerance [30]. Since root branching is influenced by the availability of nitrogen [30,39,40], of phosphorus [40,41] and other nutrients in the soil [42], a "conflict of interest" in the acquisition of water and mineral nutrients may become relevant (especially when water is available in lower soil layers and limiting nutrients are left after fertilizer application mainly in the top layers).

Besides root architecture, physiological activities in roots including nutrient uptake, assimilation and xylem loading [43–46], release of phytohormones to the shoot [47–51] as well as root storage functions respond to heat and drought [52–56] (Figure 2C,F,G). Root hydraulic conductivity is another important parameter in this context [53]. The supply of shoots with N, P and K (based on the uptake into the roots, xylem loading in the roots and acropetal transport in the xylem) was found to be negatively influenced by drought in maize [45,46]. Possible mechanisms include the competition for phloem-borne carbon skeletons between root growth, nutrient uptake/assimilation and the loading into the xylem with an increased overall solute concentration and a decreased transport velocity. Nitrate uptake in maize is decreased during a drought period, but may increase again during a subsequent recovery phase [43]. Besides the activities of newly formed roots, older roots may recover to some extent after the stress period [43]. Considerable differences in nitrogen use efficiency were detected in tomato plants subjected to drought indicating that this plasticity might be relevant for selecting or breeding genotypes which are less sensitive to soil drying [44]. For the acquisition of potassium and other ions under drought stress it is important to distinguish between ion uptake into cortical cells mediated by specific translocator proteins and the subsequent loading of the ions into the xylem in the stele of stressed roots [57–60]. The two processes are affected differently by osmotic stress, a fact which must be borne in mind when evaluating responses of crop plants to drought [57–60]. An increased production of abscisic acid in roots and the release of this phytohormone via the xylem to the shoot where it is involved in the regulation of stomatal opening has been reported for several plant species [47,49,50]. Jasmonic acid may stimulate the production of abscisic acid in drought-stressed roots [49]. Transgenic *Agrostis stolonifera* plants with increased cytokinin synthesis have been found to be more drought-tolerant than control plants [48]. In these experiments, expression of isopentenyl transferase under control of the senescence-inducible SAG12 promoter increased cytokinin contents in roots and leaves, which resulted in less pronounced drought-induced senescence, functionally longer metabolic processes (including photosynthesis, respiration, amino acid metabolism and detoxification of reactive oxygen species) and improved

overall drought tolerance [48]. Therefore, modifications in cytokinin biosynthesis and signaling might be helpful for breeding more drought-tolerant crops. A down-regulation of ethylene biosynthesis was reported for *Medicago truncatula* under water deprivation [51]. From these findings it becomes evident that the whole hormonal network must be considered for the identification of genotypes performing well under drought stress. Carbohydrates and nitrogenous compounds in the roots of drought-stressed plants are important for drought survival and for the formation of new leaves and for physiological activities in the post-stress phase [13,52,53]. Vegetative storage proteins accumulating under drought in the taproot of alfalfa are identified as important players in the nitrogen dynamics in plants subjected to increased CO_2 and abiotic stresses [53].

Mycorrhization is highly important for the acquisition of phosphorus and other mineral nutrients (Figure 2D) [56,61–70]. These interactions between higher plants and fungi are sensitive to abiotic stresses on one hand and influence plant stress responses on the other hand [56,61,63–68]. Favorable effects of mycorrhiza in drought stressed plants have been reported for a series of plant species [66–68,70]. Interactions of higher plant with mycorrhiza (as well as root hairs) are not only relevant for the well-known acquisition of mineral nutrients, but may also play an important role in water fluxes from a drying soil into crop plants [62–70].

Legume plants possess the unique ability to fix atmospheric nitrogen via a symbiotic relationship with soil bacteria belonging to the genera *Rhizobium* (Figure 2E) [71]. This ability naturally enhances the nitrogen content in soils and reduces the need for nitrogen fertilizers, which makes their cultivation a sustainable practice in soil fertility maintenance and organic farming [72]. The legumes, such as soybean, common bean, broad bean, pea, chickpea and cowpea are widely cultivated as a valuable nutrition source [73,74] or offer a large potential for sustainable biofuel production [75]. However, the symbiotic relationships are highly vulnerable to extreme environmental conditions [76]. Drought is one of the major adverse factors suppressing symbiotic nitrogen fixation [77]. Water deprivation negatively affects both symbiotic partners and all stages of the establishment and functioning of symbiotic systems [64,78,79]. Most rhizobial symbionts have reduced viability and mobility under drought [80,81]. However, compared to host legume plants, rhizobial bacteria are more resistant and resilient to soil drought [82]. Host root growth and root hairs that are associated with rhizobial infection [83] are inhibited under exposure to water deprivation, which lead to a decrease in the number of infection threads and inhibition of nodulation [84]. At the next stages of the symbiotic partnership, drought can inhibit development of nodules, and triggers frequently premature nodule senescence. In general, the drought-induced inhibition of the rate of symbiotic nitrogen fixation could be related to several main factors: reduced carbon flux from the host plant leading to low ATP content, decreased shoot nitrogen demand, lower xylem translocation rate due to a decreased transpiration rate, drought-induced changes in nodule oxygen permeability resulting in low oxygen levels, decreased metabolic enzyme activity, and nitrogen feedback inhibition [64,85,86]. The latter is more pronounced in tropical ureide-exporting legumes [87,88], whereas temperate amide exporting legumes are generally more tolerant to drought than the ureide exporters [89]. This could be mainly attributed to the accumulation of ureides in nodules and shoots of drought-stressed plants [78,90,91]. Water deprivation causes oxidative stress in legumes, which leads to an extensive nodule damage and decreased nitrogen fixation [92]. It has been suggested that nodules with an increased antioxidant defense can have a higher drought tolerance [93–95]. Therefore, the performance of symbiotic systems under drought is a multifactorial trait, and different components of symbiotic relationships must be considered when breeding legumes with improved abiotic stress tolerance.

3. Stem Properties and Solute Allocation via Xylem and Phloem

A major function of the stem in annual crops is the solute transport between the root system and the aerial parts (Figure 2H). Inorganic nutrients (e.g., nitrate, sulfate, phosphate, cations), assimilates (e.g., amino acids or ureides deriving from assimilatory processes in roots) and phytohormones (e.g., abscisic acid representing a root-to-shoot signal) are transported with the transpiration stream in the

xylem to the aerial plant parts (preferentially to active leaves) [44–46,96]. Shabala *et al.* [96] nicely summarized in a recent review article the large series of drought effects on xylem sap composition and root-to-shoot signaling including chemical changes (e.g., compounds mentioned above), physical signals (e.g., electric or hydraulic effects) and waves (e.g., reactive oxygen species or calcium concentration) [96]. From these effects it became evident that the signals from drought-stressed roots to the shoot are complex and cannot be reduced to an altered concentration of one or two compounds [96]. Xylem embolism in drought-exposed plants and its repair are highly relevant for the transport of water and solutes from the roots to the various shoot parts [97–99]. Redistribution processes via the phloem are controlled by the source/sink network with leaves, roots and maturing fruits as major players. This network can be considerably disturbed by abiotic stresses [100–103]. Related to this transport functions is the capacity of stems and petioles to store solutes (especially carbohydrates and amino acids) [104,105]. An accumulation of solutes may be caused by the altered source/sink network under abiotic stress and may be important for a subsequent recovery phase [105]. From a study with isogenic lines of *Sorghum bicolor* subjected to abiotic stresses it became evident that stem reserves strongly influence grain filling [104]. Another important point during drought stress and recovery is the fate of the shoot apex (*i.e.*, reversible or irreversible damages) [100]. New leaves may be formed from axillary buds of previously stressed plants, resulting in different plant architecture than unstressed plants [100].

4. Leaf Morphology and Physiology

The photosynthetic performance during heat and drought periods depends on morphological and physiological leaf properties as summarized in Figure 3 [100]. The thickness and the properties of the cuticle (Figure 3A) as well as the deposition of epicuticular waxes (Figure 3B) are relevant for the non-stomatal transpiration, while the regulation of stomatal opening (Figure 3C) and in some plants also the formation of a stomatal plug (Figure 3D) are key factors for the regulation of stomatal transpiration. CO_2 availability for photosynthesis in the mesophyll (Figure 3G) depends on stomatal and non-stomatal conductance (Figure 3C–E) [106].
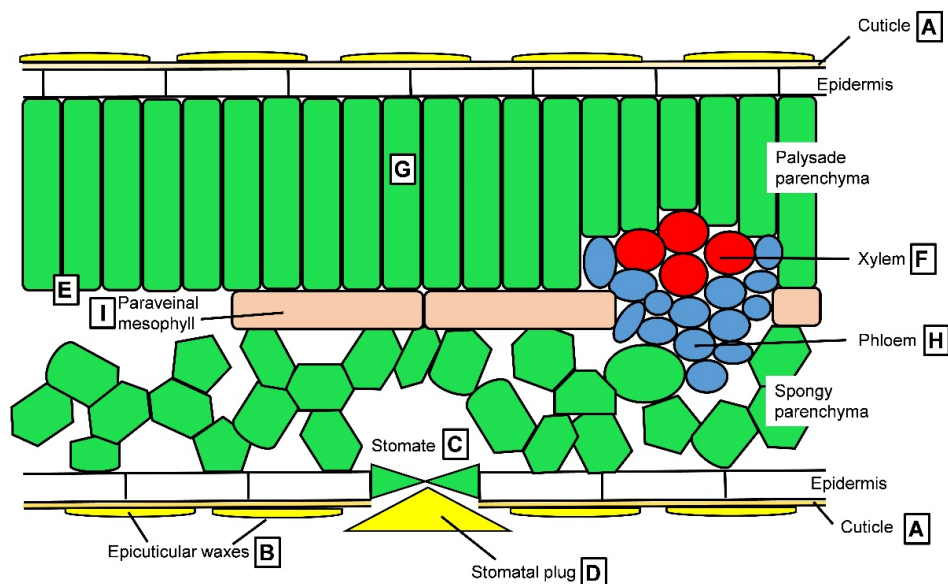

Figure 3. Morphological and physiological leaf characteristics relevant for the response to drought and heat. **A**: properties of the cuticle; **B**: deposition of epicuticular waxes; **C**: density, size and regulation of stomates; **D**: formation of stomatal plugs; **E**: mesophyll conductance; **F**: delivery of solutes via the xylem; **G**: metabolic properties of photosynthetically active cells; **H**: export of solutes via the phloem; **I**: deposition of vegetative storage proteins in the vacuoles of paraveinal mesophyll cells.

Water, nutrients and organic compounds including phytohormones (e.g., ABA) are transported from the roots to the leaves in the xylem (Figure 3F). Changes in these fluxes affect leaf morphology and functions in a complex manner and influence finally plant productivity and yield. Since heat and drought impacts on photosynthesis and leaf physiology were reviewed recently [6,7,100,103] these aspects are only briefly summarized here in Figure 3. Gas exchange between the atmosphere and the photosynthetically active mesophyll cells is a key aspect and depends on properties of the cuticle, stomatal conductance and mesophyll conductance [106]. Stomatal density and pore area (long-term adaptation, no longer influenced in fully expanded leaves) and the regulation of stomatal opening (reversible short-term adaptation) are highly important for the control of stomatal transpiration and water use efficiency in drought-stressed plants [107–110]. A reduced stomatal density in halophytes exposed to salt stress (compared to unstressed control plants) was reported by several groups [107–110] and such adaptations were also considered for selecting/breeding crop varieties with improved abiotic stress tolerance by "learning from halophytes" [109]. Besides the morphological adaptations in drought-exposed leaves, other important mechanisms are based on changes in the protein pattern (e.g., accumulation of chaperonins or increased activities of enzymes involved in the detoxification of reactive oxygen species or the production of compatible solutes [18,103]. Two aspects of abiotic stress impacts relevant for genotype selection and breeding are emphasized here and discussed in more detail: intactness of organelles (especially chloroplasts and mitochondria) and reactive oxygen species (production and detoxification).

Water shortage affects growth and development of crop plants at multiple levels of biological organization [111]. Depending on drought intensity, multiple abnormalities occur in cellular organelles and structures [112,113]. Under drought, the leaf mesophyll cells contain chloroplasts and mitochondria of irregular shape and size [114–117]. The chloroplast ultrastructure is compromised, manifested by swollen granal compartments, disrupted chloroplast membranes, accumulation of plastoglobuli and a reduced size/lack of starch grains [116,117]. Drought-affected mitochondria are mostly enlarged, often devoid of cristae, and possess large electron-transparent areas [116]. The damages on subcellular level, in part, depend on the level of drought tolerance of the crop genotypes. Leaf cell organelles in the drought-tolerant genotypes are better preserved, whereas the chloroplast and mitochondrial structure of the sensitive cultivars is severely disorganized, which leads to organelle dysfunction. All the visual structural alterations can be easily converted into very informative numerical data by a quantitative morphometric analysis [26,116,117]. Therefore, the intactness of subcellular structures could be considered as an additional trait, contributing to crop improvement and could serve as a reliable stress marker in the selection of sustainable crop genotypes.

Production of short-lived activated oxygen (ROS—reactive oxygen species: singlet oxygen, superoxide anion, hydroperoxide radical, hydrogen peroxide, hydroxyl radical) and keeping them at safe local steady state level is indispensable for normal metabolic processes in every plant tissue [118–120]. Major sources of ROS are the electron transport chains in chloroplasts and mitochondria and photorespiration in peroxisomes; local active production and utilization of ROS also occurs in the apoplast [120]. The steady state level of ROS usually corresponds to tissue metabolic activity, depends on the subcellular compartment, and normally is strictly controlled over space and time. Plants possess double enzymatic and non-enzymatic ROS scavenging systems, represented by several protective enzymes: superoxide dismutases, catalases, various peroxidases including unspecific, ascorbate and glutathione peroxidases, glutathione reductase and others, as well as by low-molecular metabolites among which the most important are ascorbic acid, glutathione, carotenoids, tocopherols [118–120]. These systems successfully cooperate, for example in the ascorbate—glutathione cycle which is key ROS detoxifying system in the cytosol and organelles.

Many environmental constraints lead to disbalance in metabolism and overproduction of ROS. The early transient increase of ROS serves as a signal for an unfavorable change [119–121]. Prolonged or/and severe abiotic stress leads to development of secondary oxidative stress and mobilization of the enzymatic and non-enzymatic defense systems as shown in Table 1. Excessive ROS formation

could damage cell structures, lipids, proteins and nucleic acids, and ultimately could lead to cell death [119]. Different species and tissues have particularities regarding development of oxidative stress and the major detoxifying players. For example, grass species are reported to be less sensitive to oxidative stress during drought and warming compared to legume species [122]. Upregulation only for catalase is reported in heat stressed roots [123] while concerted increase in the activities of several antioxidative enzymes is found in stressed leaves [124,125]. Control of ROS accumulation and detoxification are key processes during abiotic stress phases, which play critical role for yield stability [10]. As ROS protection is common mechanism mobilized in many abiotic stresses including drought [126–129], heat [10,115,125,130,131] and their combination [122,132], ROS scavenging enzymes and compounds are good candidates for enhanced protection to multiple stress situations. Higher tolerance to drought and/or heat is associated with concerted up-regulation of key detoxifying enzymes in several crop species/varieties leading to better ROS protection along with stability of key metabolic processes like photosynthesis [132] and maintenance of the alternative mitochondrial respiration [123]. Field drought causes an increased oxidative stress during the grain filling period, especially to drought sensitive wheat varieties [127]. The upregulation of the total antioxidant capacity during grain development in heat tolerant wheat genotypes is linked to delayed senescence and better nutrient reserves mobilization [125]. A principal role of the cytosolic ascorbate peroxidase is established for acclimation to combined drought and heat stress [15]. In Table 1 are listed certain leaf proteins involved in the responses to drought and heat with relation to stress tolerance, among which are several antioxidant enzymes such as superoxide dismutase isoforms and ascorbate peroxidase. Enzymes related to ROS protection are systematically found to be upregulated under abiotic stresses in proteomics studies, which are briefly commented below.

Table 1. Leaf proteins involved in the responses to drought and/or heat.

Protein	Proposed Function	References
Aquaporins	H_2O transport through membranes	[133]
Dehydrins	Stabilization of macromolecules	[134–136]
Chaperonin-60 β	Stabilization of macromolecules	[137]
Heat shock proteins	Stabilization of cell constituents	[138–140]
Cu/Zn Superoxide dismutase	Detoxification of reactive oxygen species	[124,141]
Mn Superoxide dismutase	Detoxification of reactive oxygen species	[124]
Fe Superoxide dismutase	Detoxification of reactive oxygen species	[124]
Ascorbate peroxidase	Detoxification of reactive oxygen species	[124,142]
Catalase	Detoxification of reactive oxygen species	[124]
P5CS [1]	Accumulation of proline	[143]
Rubisco activase	Activation of Rubisco (Calvin cycle)	[6,144]

[1] Delta-1-pyrroline-5-carboxylate synthetase (P5CS).

5. Reproductive Structures and Yield Formation

The maturation of fruits and seeds under abiotic stresses is highly relevant for a series of the world most important crops including rice, wheat, maize and soybean [145–148]. The translocation of nutrients and assimilates via xylem and phloem to the reproductive structures (Figure 2H), the remobilization of leaf constituents (Figure 2K) and processes in the maturing fruits including the deposition of storage compounds in the seeds (Figure 2N) are relevant in this context and depend on water availability [146–148] and ambient temperature [145,147,148]. Landraces with a high genetic diversity may serve as a helpful basis for breeding crop genotypes with suitable properties in the course of climate change (e.g., stable yields, high quality of harvested products) [148]. Genetic variability in the response to abiotic stresses such as heat and drought were reported for several crop plants indicating that there might still be a potential for further breeding [146–148]. Since drought and heat occur often simultaneously, the combined effects of these abiotic stresses are of special relevance [147,148].

6. Proteomics in Search of Molecular Markers for Assisted Selection and Breeding

The potential of proteomics has been increasingly exploited in search of suitable protein markers for assisted selection and breeding, thus complementing the widely used genomic tools [149–152]. Proteins are direct effectors in the processes related to cell structure and function, as well as in adaptation to the changing environment—underlying the so called phenotypic plasticity [152]. Cell protein composition is highly dynamic and much closer to the plant phenotype than the transcript profiling. Protein and transcript profiles do not necessary correspond to each other due to the complexity of regulation of gene expression (at genetic, transcriptional, translational, and post-translational levels). Plant proteomics has benefited from the technological advances in the field and from the development of databases with partially or fully sequenced plant genomes and expressed sequence tags, necessary for correct protein identification; comprehensive proteome maps of major crops have been established [131,153]. Both gel-based and chromatography-based approaches are applied in plant proteomics as they are complementary. Two-dimensional electrophoresis combined with mass spectrometry detects relatively more abundant proteins like key metabolic enzymes, thus providing essential information about changes in the main metabolic pathways and biological processes affected by the stress, usually in good correlation with metabolomics data; moreover, isoforms and posttranslational modifications of a given protein can be established [153]. Deeper proteome coverage especially for less abundant proteins (signaling, transporters, *etc.*) is reached by the second generation shotgun proteomics [153–155]. Knowledge about dynamic changes of crop proteomes in response to abiotic stresses is regularly reviewed [131,150,152,153,156]. For that reason, our attention is mainly focused on recent proteomic reports addressing drought, heat and combined stress.

Recent proteomic studies on drought response of crops encompass different plant species, such as wheat [138,157], rice [158,159], barley [160], brassica [154], legumes [141,161]. Different types of tissues are studied: roots [141,154,159], root nodules [161], leaves [138,160], roots and leaves compared at seedling stage [157], roots, flag leaves and spikelets at reproductive stage [151]. As the high temperature is particularly detrimental during the reproductive stage, besides the studies on leaf proteome under heat stress in rice [113,162], wheat [163] radish [142], alfalfa [164] and stromal proteins in agave [165], special attention is paid to the reproductive phase [166], source-sink interactions at grain filling [167], grain development and composition [155,160] in cereals, and protein composition of soybean seeds [168] formed under unfavorable temperature conditions. Relatively few proteomic studies deal with combined drought and heat stress [169–171]. Current efforts are directed at elucidating the drought adaptations in roots and leaves, the response to heat at reproductive developmental stage, as well as at the early signaling events captured after 4–8 h of drought [154,157], heat [147,163] or combined [169] treatments, including changes in phosphorylation of specific proteins [157,169]. As signal transduction associated, 14-3-3 proteins and calreticulin-like proteins were upregulated in tolerant wheat cultivar under drought stress [157]. In roots of transgenic rice, overexpressing DREB1A, a novel protein containing ricin B lectin domain was found to be highly accumulated, probably with potential role in breeding for drought tolerance [159]. Proteomic findings support and further develop the concept about key mechanisms affected by drought and heat stress and point at some potential protein markers for assisted selection.

7. Conclusions and Outlook

Genetic variability is a prerequisite for breeding plants with an improved heat or drought tolerance [6–9,145]. Modern techniques including genetic engineering [172] and high-throughput phenotyping [173] may facilitate the production/selection of suitable genotypes. From the facts and concepts reviewed in this paper, it becomes evident that the susceptibility to abiotic stresses is complex and incudes morphological and physiological traits [174,175]. Several important traits for drought and heat tolerance in crop plants are summarized in Table 2. Stress responses can be reversible after the stress period (e.g., leaf orientation, stomatal opening, activation status of Rubisco), partially reversible (e.g., rooting depth, activity of the shoot apical meristem) or irreversible (e.g., senescence of a leaf or

a root, deposition of compounds on the leaf surface). In the case of partially reversible and irreversible responses, the effect as such may be irreversible, but it may be partially or fully compensated after the stress period (e.g., xylem functionality, photosynthetically active biomass).

Table 2. Important traits involved in drought and/or heat responses of crop plants.

Trait	Relevance for Abiotic Stress Response	References
Rooting depth	Access to more suitable soil regions [p,c]	[25–29]
Root branching	Access to more suitable soil regions [p,c]	[29–37]
Nutrient uptake into roots	Acquisition of mineral nutrients [r,c]	[44–46,59]
Xylem loading in roots	Transfer of nutrients to the shoot [r,c]	[57–60]
Nutrient assimilation in roots	Acquisition of mineral nutrients [r,c]	[44–46]
Mycorrhization	Acquisition of mineral nutrients/water [r,c]	[56,61–70]
Nodulation in legumes	Symbiotic nitrogen fixation [p,c]	[76–95]
Storage functions in roots	Stress survival and recovery [p,c]	[13,52,53]
Release of nutrients to shoot	Supply of aerial parts with nutrients [p,c]	[44–46]
Phytohormone release to shoot	Root-to-shoot signaling [r]	[47–51]
Root senescence	Root architecture and functions [p,c]	[43]
Xylem-to-phloem transfer	Solute channeling to leaves and fruits [r]	[176]
Xylem embolism and repair	Acropetal flux of water and solutes [p]	[97–99]
Storage of reserves in the stem	Accumulation of reserves for recovery [r]	[104,105]
Shoot apical meristem activity	Shoot architecture and performance [p,c]	[103]
Development of axillary buds	Shoot architecture and performance [p,c]	[103]
Leaf expansion (final size)	Shoot architecture and performance [i,c]	[103]
Leaf orientation	Light interception [r]	[103]
Leaf senescence	Loss of assimilatory capacity [i,c]	[103]
Leaf surface (wax deposition)	Reduction of non-stomatal transpiration [i]	[177,178]
Density and size of stomates	Stomatal transpiration [i]	[107–110]
Stomatal regulation	Reversible control of stomatal transpiration [r]	[106,179]
Formation of stomatal plug	Reduction of stomatal transpiration [i]	[180,181]
Mesophyll conductance	CO_2 diffusion inside the leaf [i]	[106]
Vegetative storage proteins	Intermediate storage of mobilized nitrogen [r]	[182]
Intactness of organelles	Functionality of plastids and mitochondria [p]	[26,112–117]
Photosystems	Light energy conversion to ATP/NADPH [r]	[132]
Rubisco activase	Activation of Rubisco (Calvin cycle) [p,c]	[103,144]
Detoxification of ROS	Protection of cell constituents/metabolism [r]	[119–132]
Respiration in leaves	Maintenance of basic cellular functions	[9]
Compatible solutes	Protection of cell constituents/metabolism [r]	[143]
Transcription factors	Regulation of gene expression under stress [r]	[183–185]
Dehydrin pattern	Protection of cell constituents [r]	[134–136]
Aquaporins	Water/CO_2 transport across membranes [p]	[133]
Chaperonins	Protection of enzymes [r]	[137–140]
Cytokinin levels/effects	Regulation of metabolism and senescence [r]	[48]
Proteolytic activities	Intracellular protein degradation [p,c]	[127]
Solute transport to fruits	Yield formation [p,c]	[145–148]
Seed maturation/composition	Yield quantity and quality in seed crops [p,c]	[5,160,168]

[r] reversible after stress period; [p] partially reversible; [i] irreversible; [c] can be compensated after stress phase.

There might be some defined properties, which are directly advantageous for stress-exposed plants (e.g., heat tolerance of Rubisco activase, root and shoot architecture or properties of the leaf surface) [6,144], but in most cases a complex regulatory network including signal perception and transduction must be considered [154]. Although anatomical and physiological properties of the root system are not easily accessible in field experiments, root growth, activity and response to abiotic stresses must be borne in mind for identifying suitable genotypes [27,141,174]. Besides basic characteristics of unstressed plants, especially stress-inducible adaptations in gene expression, protein pattern and physiological properties are important for the stress susceptibility of a genotype [113,162,163]. Heat waves and drought periods occur often simultaneously and should therefore also be addressed in combination in selection procedures [169–171].

Several environmental factors are relevant for agronomic practices (e.g., altitude, soil properties, nutrient availability) and can interfere with the responses of crop plants to heat or drought. Such interactions are relevant for selecting genotypes with suitable properties. Therefore it cannot be expected to find "the suitable drought- or heat-tolerant genotype", but there might be a collection of varieties for various environments. Since genotypes must be selected before the growing season (with or without severe stress phases), experiences from preceding years may serve as a basis. Since more frequent and more severe extreme events including droughts and heat waves must be expected in the course of global change, breeding of crop plants with an improved performance during and after stress periods becomes a key determinant [9–11,141,157,175].

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Abbreviations

The following abbreviations are used in this manuscript:

P5CS	D-1pyrroline-5-carboxylate synthetase
ROS	Reactive oxygen species

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