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Opinion

Drought Adaptation Mechanisms Should Guide Experimental Design

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The mechanism, or hypothesis, of how a plant might be adapted to drought should strongly influence experimental design. For instance, an experiment testing for water conservation should be distinct from a damage-tolerance evaluation. We define here four new, general mechanisms for plant adaptation to drought such that experiments can be more easily designed based upon the definitions. A series of experimental methods are suggested together with appropriate physiological measurements related to the drought adaptation mechanisms. The suggestion is made that the experimental manipulation should match the rate, length, and severity of soil water deficit (SWD) necessary to test the hypothesized type of drought adaptation mechanism.

Trends

Much work has been done recently on improving crop water conservation through physiological assays for stomatal closure at high evaporative demand.

Advances in metabolomics technology have allowed physiologists to assess plant response to water stress through new eyes.

Advances in high-throughput phenotyping for physiological responses to water stress offer great promise in coupling abiotic stress tolerance with plant breeding efforts.

Despite recent experimental developments, the concepts of how to define drought adaptation mechanisms and the experimental protocols for measuring these have had less attention. A new focus on the experimental methodology will be necessary to more precisely control water stress in plant biology, metabolomics, high-throughput phenotyping, and breeding experiments.

What are Drought, Stress, and Damage?

Drought may be defined from the perspective of precipitation, soil or plant water status, or availability of human water supply [1]. Accordingly, the ideal definition for comparative physiological studies would be independent of the characteristics of a particular plant but be related to the local environment. If drought is independent of the plant, then it can be uniformly applied to any genotype or species present in an environment. Thus, we define drought as a decrease in water inputs into an agro/ecosystem over time that is sufficient to result in SWD [2]. This definition encompasses many forms of drought such as rainfall anomalies, irrigation failure, and annual dry seasons. Whether drought affects a plant is determined by the plant characteristics and environment. SWD is the key variable that links soil water with plant physiology, and is defined as a decrease in the available soil water, in other words water losses are greater than inputs. Alternative definitions of drought are often based upon conditions where soil water availability limits plant uptake [3]. However, these definitions are not useful when comparing genotypes/species where SWD can vary due to differences plant leaf area, roots, and physiology. Thus, we have chosen to employ the specific term 'soil water deficit' instead of 'drought' in the definitions of drought **adaptation** (see Glossary).

Stress may be defined as a negative change in the physiology of a plant away from a reference state as a result of the action of an external stress factor or internal stress ([4–6] for discussion). In physics, stress refers to an external factor such as temperature while strain is the response of the material [7]. However, the physiology literature generally uses stress in reference to physiological responses [4]. Therefore, we prefer to define 'stress factors' as external and 'stresses' as physiological responses. Note that physiological responses to daily 'normal' variation in potential stress factors such as light and temperature should not be termed stress, and should be considered as part of the reference state [5]. Drought-induced damage is a negative stress that persists for some time after SWD has ceased. Damage may be recoverable or irrecoverable and, in response to these deviations from unstressed conditions, acclimation or adaptation occurs.

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New Definitions of Mechanisms for Dealing with Drought

General terms used as objectives or mechanisms in drought research are: drought or stress tolerance, drought resistance, etc. (Figure 1). These terms are poorly specific of a plant characteristic or the drought phenomenon; therefore, demonstrating that these mechanisms occur in a genotype/species is difficult. For instance, the manner in which ‘drought tolerance’ is typically applied in the literature could mean that a plant tolerates stress, tolerates damage, or avoids water stress, thereby tolerating drought.

The basic terminology provided by the highly cited Levitt [7,8]–drought escape, avoidance, and tolerance–has not been widely adopted, and only ‘drought tolerance’ is widely used (Figure 1). Other excellent reviews provide examples of drought adaptation [2,9–11], but in practice little link is generally made between the proposed adaptation and experimental manipulation. Interestingly, the literature associated with plant biology tends to focus on ‘stress tolerance’ rather than ‘water conservation’, the greater focus of ecology and crop-related literature (Figure 1).

Clearly, nuanced definitions of plant mechanistic response to drought will be necessary to drive directed approaches to experimental design. We propose here four terms representing a hierarchy of adaptation mechanisms (Figure 2, Key Figure; Figure 3, diagnostic graphs of mechanisms; see also Figure S1, a flow chart, in the supplemental information online). For instance, a plant may avoid SWD despite a lack of water inputs (termed SWD avoidance; Figure 2). Examples include plants that explore deeper soils [12], or have slow root growth, leaving water for later in the season [13,14], as well as plants that conserve water through lower leaf area or transpiration [15–17] or match **phenology** to the wet season [18].

Plants that avoid SWD may have distinct physiological mechanisms from plants that encounter SWD but avoid physiological stress through **osmotic adjustment**, water storage in organs or root isolation from soil [19] (termed stress avoidance) (Figure 2). These mechanisms require specialized adaptations such as succulence [19] or are temporary because osmotic adjustment can only allow access to limited volumes of soil water [20].

Mechanisms that allow plants to tolerate SWD can include damage avoidance by preventing stresses from resulting in damage. Damage avoidance may also have a buffering effect whereby

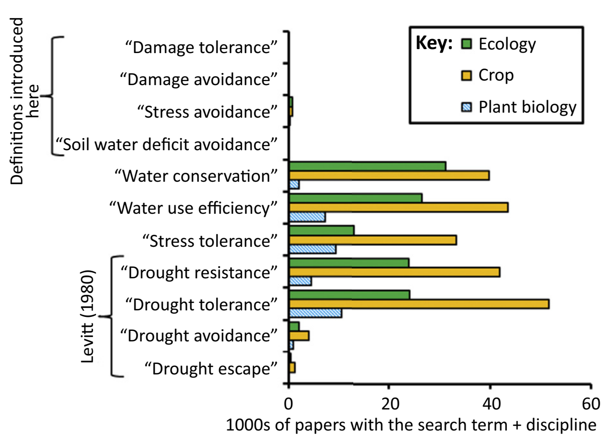

Figure 1. Use of Terms Related to Drought Adaptations in Scientific Literature by Discipline. The numbers of papers are results are from Google Scholar searches for the listed terms combined with +“drought” +“plant” and either +“plant biology”, +“crop”, or +“ecology”. Terms promoted by Levitt [7,8] and those proposed here are indicated. Citations and patents were excluded.

Glossary

Adaptation: a general term that technically specifies an evolved trait that affects plant performance, but refers more broadly to added traits in transgenics or traits bred in crops.
Evaporative gradient: the gradient in water concentration between the inside and outside of the leaf, the driving force for transpiration.
Hydraulic conductivity: the ability of soil or a plant component to transport water over a pressure gradient.
Matric potential: a component of the water potential of soils owing to adhesion of water to the soil particles.
Osmotic adjustment: accumulation of osmolytes to allow greater solute potentials in cells.
Phenology: the timing of plant growth.
Potential evapotranspiration (ET_o): the combined soil evaporation and transpiration of an unstressed crop.

Key Figure

A New Set Of Definitions for Mechanisms of Crop/Plant Drought Adaptation.

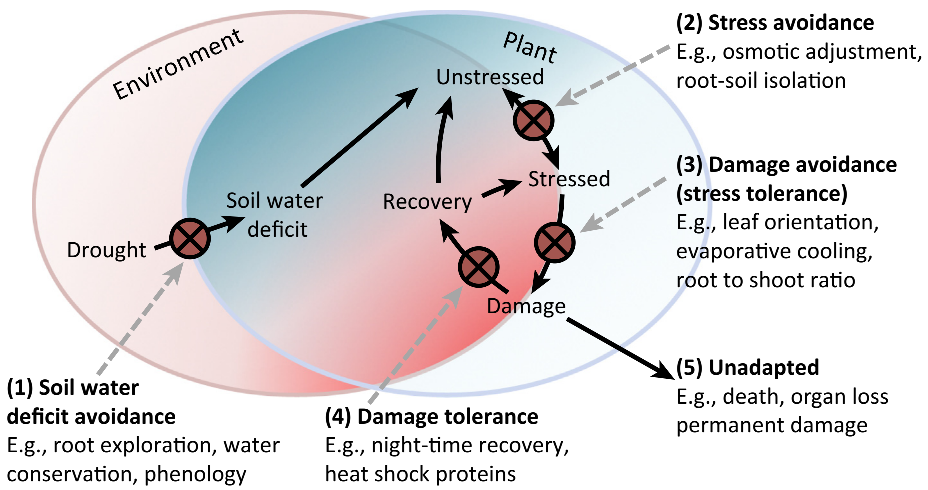

Figure 2. In this scheme, the response mechanisms of plants are a function of the interaction of the environment with physiology. Four new terms are proposed that relate to increasing levels of drought intensity and distinct physiological mechanisms underlying the adaptations. The four terms are not commonly used and thus avoid any confusion with current terms such as water use efficiency (WUE), drought tolerance, etc.

a given stress level leads to less damage (Figure 3). Examples include changing leaf orientation away from light [21], heat dissipation of excess absorbed energy by PSII [22], the Mehler reaction or photorespiration [23,24], and altered root to shoot ratios [25]. Damage avoidance is the preferred term because a similar term, stress tolerance, is commonly used in a general manner.

Finally, plants may tolerate damage through recovery mechanisms (damage tolerance). On a daily timescale, recovery is important because night-time allows recovery of many processes. Refilling of embolized xylem vessels [26] and the growth of new conductive tissue [27] are also examples of damage tolerance.

The value of defining a putative drought adaptation mechanism before undertaking research is that this objective can be used to design experiments that control the factor affecting the mechanism of interest: an experiment could vary damage to assess damage tolerance, or it could vary water input to assess the ability of a plant to conserve water and avoid SWD.

Difficulties with New (and Old) Definitions of Drought Adaptations

The mechanism by which a plant is apparently adapted to drought may depend upon the particular physiological process considered. For instance, plants that avoid stress (negative water potentials) by isolating roots from the drying soil [19] would lead to an increase in another stress–stomatal limitations to photosynthesis. Similarly, changing leaf orientation avoids photosynthetic damage, but also decreases transpiration and affects SWD. Consequently, it is not appropriate to label a plant as having one adaptation mechanism because there are several, depending upon the physiological system of interest.

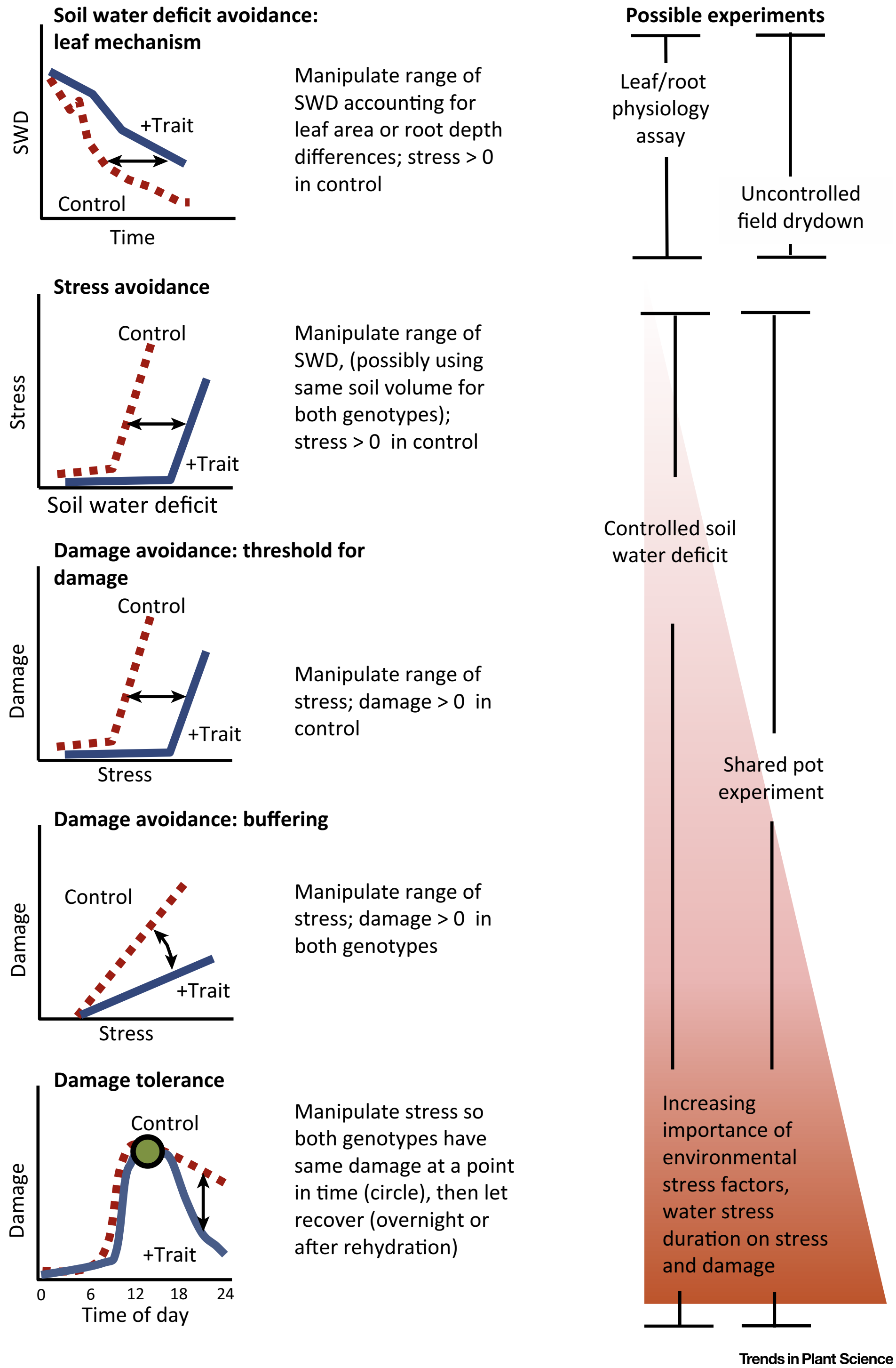

Figure 3. Suggested Diagnostic Experimental Manipulations for Testing for the Presence of a Specific Physiological Drought Adaptation Mechanism. Plants that have the hypothesized mechanism of adaptation to drought (+trait) are shown in comparison to a control plant. The *x* axis is the variable that should be controlled, and *y* axis the measured response variable. For an adequate experiment both variables need to be measured. Appropriate damage- or stress-related variables could be used in these tests, but note that many physiological variables report function, which is proportional to 1-stress or 1-damage.

Most mechanisms of drought adaptation lead to tradeoffs that ultimately cause a decrease in potential yield. Responses that result in stomatal closure, decreased leaf growth, or decreased light interception all decrease photosynthesis and growth. For instance, a greater root to shoot ratio may avoid negative water potentials (stress) but also limit the ability of the plant to capture light and carbon (leading to damage to yield).

Regardless of the adoption of these new definitions of drought adaptation, the main point of this article remains–drought experiments need to be explicitly designed with mechanisms of drought adaptation in mind. What follows are explanations of appropriate experiments and measurements for testing comparative drought adaptation mechanisms.

Experimental Design Based upon Mechanisms

The experiments highlighted in Box 1 are often used as comparative tests of drought adaptations. In general, the following rule can be applied: an experiment should match the degree of manipulation of SWD, stress, and damage to the nature of the mechanism being tested. For instance, testing of genotypes for SWD avoidance versus stress avoidance mechanisms should be approached differently. The initial term can imply that two genotypes differ in how fast available water is used, in other words that genotypes access separate volumes of soil and thus some are able to conserve water for future use. The appropriate pot or field experiment for this type of investigation would separate the genotypes in pots, or in the field by border rows of the same genotype, to prevent competition. In natural ecosystems competition may be appropriate. The metric of success should be the resulting conservation of accessible soil water. Alternative mechanisms of SWD avoidance include differences in root exploration and phenology, and should be tested in other manners (e.g., Figure S1).

Stress avoidance implies that, for the same SWD, one genotype would not show stress responses. The appropriate manipulation is to equalize soil moisture contents between genotypes. Thus, controlled SWDs through the use of shared pots, close field plantings, or regulated dry-downs appear to be more appropriate experiments (Box 1). These experimental designs account for differences in growth and leaf area affecting dry-down rates, but are comparatively rarely employed (especially shared pots). While controlled SWD experiments allow comparison of genotypes at similar stress levels, they largely preclude comparisons of SWD avoidance because SWD is equalized.

Should SWD, Stress, and Damage Be Measured in an Environmental Context?

Variation of soil moisture can typically be used to generate the stress and damage levels necessary to test if genotypes avoid damage or recover differentially (Box 1 and Figure 3). However, the development of SWD, stress, damage, and recovery are functions of the plant and environment. For instance, plant water status is determined by the combination of water availability (supply) and physiology, phenology, and the leaf-to-air **evaporative gradient** (demand). Thus, a distinction could be made between experiments that investigate the function of a particular mechanism versus experiments that test how that mechanism performs in an environmental context. Studies of mechanism can be performed in more controlled environments such that environmental variation independent of drought is minimized, small differences are clearly detectable, and the experiment is repeatable.

However, drought in the context of the natural environment exists alongside multi-dimensional variation in environmental factors. Thus, it is unlikely that drought mechanisms studied in the absence of other environmental stress factors will be easily extendable to a functional context. In contextual studies, the level of drought should be considered relative to variation in other environmental conditions (temperatures, evaporative demand, light) and the length of exposure

Box 1. Examples of Comparative Drought Experiment Designs

General Experiments

Natural Dry-Downs. Unmanipulated SWD experiments require measurements of SWD, stress, or damage so that genotypes/species can be compared.

SWD Avoidance

Uncontrolled Soil Dry-Down. Genotypes are grown in separate soil volumes, watering is ceased, and monitoring of SWD and plant water status is performed until stress occurs. Experiments could account for different leaf areas, pot dry-down rates matched to field data [56], and, in the field, many row plots limiting competition with adjacent plots [61].

Deficit Irrigation. The application of irrigation based upon supplying a percentage of the **potential evapotranspiration** (ET_o); however, the actual ET is likely to vary with genotype. Thus, it is difficult to test other mechanisms of drought adaptation without measurements of SWD, stress, and damage.

Root Depth. Altered patterns of water extraction and root length density with depth can be monitored with rhizotron tubes, deep pots, or tube experiments [62,63].

Stress-Avoidance Experiments

Shared Pot/Soil Volume. By growing genotypes in the same pot or field soil volume they are exposed to similar SWDs [64,65]. Above-ground competition can be limited by choosing an appropriate control genotype (e.g., short, non-climbing) or by limiting the duration of growth and providing a growth barrier. In the field, small plot sizes (one row, or interspersed planting) and probably controlled depth irrigation (e.g., drip) are necessary to ensure that the genotypes share the same soil volume, but root depth variation and competition for light are complications in the field.

Regulated Pot Water Deficit. Regulation of the rate of SWD can be achieved through other mechanisms including daily watering of pots to maintain similar soil water contents/weights between treatments [56]. Dry-down rates can be equalized by planting grass in pots to equalize leaf area/transpiration per pot [66]. Small pots relative to leaf area may lead to undesirable, large daily fluctuations in soil water content.

Damage-Avoidance or Damage-Tolerance Experiments

Osmotic Stress. Membrane-impermeable compounds (e.g., polyethylene glycol) can be used to establish levels of osmotic stress that are equivalent to SWDs, but have the potential to cause hypoxia as a result of high viscosity and toxic impurities, and require measurements of osmotic potential. Alternatively, membrane permeable osmolytes (e.g., NaCl) can be used, with additional salinity/ionic effects [67].

Other. The controlled dry-down experiments described above may be suitably adapted to vary stress or damage, and natural dry-downs or other experiments may achieve variation in measured stress and damage, with loss of control.

to drought (pots, days; versus field, weeks) [28]. While daily variation in environment may be minimal for controlled environments, common-garden and field experiments are likely to have different temperature and light conditions for stages of SWD progression. Thus, environmental variation may confound experiments where genotypes reach high stress intensity on different days, and therefore controlled experimental designs listed in Box 1 may be preferable in variable environments. If genotypes have the same SWD while exposed to stress factors, and sufficient physiological variables are measured, then stress avoidance, damage avoidance, and damage tolerance can likely be distinguished. Damage is more likely to occur in field experiments where development of stress can take weeks and environmental conditions are more severe. Thus, experiments testing for stress and damage should expose plants to environmental variation, and match the rate of SWD imposition and duration to what the plant would experience in a sustained field dry-down.

Measures of Soil Water, Stress, and Damage

The list of variables that measure stress and damage is likely infinite; we simply point out literature dealing with typical variables used in ecophysiology [29–31].

Soil Water

Soil moisture is the key factor that is manipulated in many drought experiments, but three variables are necessary to represent moisture: soil water content, **matric potential,** and **hydraulic conductivity**, and these are dependent upon soil type [29,31]. Matric potential typically limits water extraction, although soil water content is the easiest to measure and control (e.g., by weighing). Typical media used in pot experiments, coarse-textured or organic-based soils, make it difficult to control moisture. In such soils, soil hydraulic conductivity can limit transpiration at even moderate matric potentials [32]. Finer-textured soils can lead to hypoxia, particularly in shallow pots [33], and pots have problems with wetting, nutrients, and salinity [2,10]. Soil mixtures, fritted clay, deep pots, and draining wicks may allow pot experiments with soils that are more realistic of natural dry-downs [34,35].

Stress

Stomatal conductance provides an easy, integrated measure of physiological response of plants to SWD. However, environmental variation needs to be accounted for [17], or standardized as stomata respond to light and evaporative demand. Plant transpiration is easily measured in pots by weighing [36,37]. Inter-day variation in environment, particularly evaporative demand, can be accounted for using the normalized transpiration ratio to fraction of transpirable soil water [38] or plant available water [39] protocols. Leaf transpiration measured in gas exchange chambers is not representative of leaves before measurement, and stomatal conductance is the preferred measure [40]. Dark-adapted maximum photochemical efficiency of PSII (F_v/F_m) is a useful indicator of past stress in leaves and acclimation. Low F_v/F_m can represent damage avoidance due to associated upregulation of excess energy dissipation processes [22]. F_v/F_m depression is sustained and may decrease photosynthetic rates and thus also represent damage. Leaf relative water contents can be measured to represent drought stress [41], but have some technical problems [42,43]. Stem and leaf water potentials are used for measurements of plant stress using several established, rapid techniques [44–48]. Coding of wilting stages are useful rapid measurements [44,49]. Leaf orientation/rolling and compensatory growth may indicate stress- and damage-avoidance responses [21], but decrease photosynthesis and transpiration. Leaf/canopy temperature is determined by the energy balance–a function of weather and plant characteristics including stomatal conductance [50]. Consequently, canopy temperature depression relative to air is often used as a proxy for severe stomatal closure while removing effects of air temperature. Other environmental factors can be accounted for using the temperature-based crop water-stress index [51,52]. Leaf elongation, internode expansion, growth, and other turgor-mediated processes are also representative of stress [53]. However, sustained turgor effects on growth may be considered as damage. Changes in respiration, protein synthesis, and nutrient uptake all may represent a SWD response (stress) or lead to long-term negative effects (damage).

Damage

Photosynthesis is a vital component of yield/growth potential but is difficult to assign as stress or damage because CO_2 exchange results from many processes: carboxylation, photorespiration, and respiration, as well as stomatal, diffusional, and metabolic limitations [54]. However, determination of sustained biochemical limitations [55] could be used as an indication of damage [56]. Lower photosynthesis also decreases available carbohydrates, which may be unrecoverable, and thus is a form of damage. PSII quantum efficiency and estimated electron transport rate are similarly affected by many processes, and in particular vary greatly with light intensity [57,58]. Chlorophyll bleaching and loss of membrane integrity are other examples of damage [59]. Low stem and leaf hydraulic conductance can limit recovery of leaves after re-watering [60]. Leaf senescence and reproductive failure represent damage as an unrecoverable opportunity cost. All physiological damages are likely to have effects on growth expressed as productivity, biomass, relative growth rate, and yield.

Concluding Remarks

How many drought papers have you read that simply withhold water from plants regardless of the mechanism being tested? We contend here that manipulations used to impose drought should match the hypothesized mechanisms of adaptation, and give clear examples of such experimental protocols. We also redefine drought adaptation mechanisms such that they are more specific of physiology and relate more easily to experimental design. The most important suggestion is that the chosen experimental design matches the level of drought intensity, speed of stress development, and duration specified by the hypothesized mechanism. Future research could focus on improving experimental protocols to investigate drought adaptations, particularly in the field (see Outstanding Questions).

Acknowledgments

Theodore Hsiao is thanked for constructive comments. M.E.G was supported by the United States Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA), Hatch project 1001480.

Appendix A Supplemental information

Supplemental information associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tplants.2016.03.003.

Outstanding Questions

Natural drought is usually associated with interactive environmental factors: how do interactive stress factors such as solar radiation, high temperature, and high evaporative demand affect drought responses?

How do extreme events, for example heatwaves, affect drought responses?

How does plant/root/leaf plasticity affect drought response and recovery?

How does the rate of drought imposition and the length of water stress affect recovery?

What are the primary limitations upon recovery from drought–loss of hydraulic conductance, biochemical signaling effects constricting stomata, photosynthetic damage, lack of carbohydrate reserves for recovery, loss of functional leaf area, or loss of potential fillable seeds?

What experimental protocols allow greater control of SWD in pots and particularly in field experiments?

What protocols equalize SWD between pots or plots such that differences in stress or damage tolerance can be tested?

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