There are 3 research articles that I thought would be helpful for you as you designed your drought research experiment. Learning to read scientific papers is a very important skill but one that takes quite a bit of practice. In order to help you begin to gain mastery, I've taken excerpts from these papers and put in some explanation and guidance.

The articles can be found in the Google Drive.

> Lawlor DW. 2012 Genetic Engineering to improve plant performance under drought. Physiological evaluation of achievements. Journal of Experimental Botany
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> Verslues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Jhu J-K. 2006. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *The Plant Journal* 45, 523–539.
>
> Frank SJ. 2010. Plastidy and evolution in drought avoidance and escape in the annual plant *Brassica rapa.* New Phytologist (2011) 190: 249–257

How to Define Drought?

Drought is one of those terms that seems so obvious and self-explanatory that it isn't until we begin delving into the topic that we see the complexities involved in defining the term. Researchers in this field consider several factors:

- Drought Timing
- Drought Duration
- Drought Intensity

Here is an excerpt from Lawlor (2012).

Drought is defined in many ways, depending on a number of factors, for example country and affected process: see Wilhite (2005) for full discussion of drought, definitions, and consequences.In meteorological terms, drought is the deficiency in water supply (precipitation, i.e. rain, snow) compared with a measure of the supply, such as long-term annual rainfall. In the more agronomic and physiological literature, drought is the water deficit which impairs plant growth and yield compared with the supply required for maximum or optimum growth, etc. The concept is complicated, as a crop may absorb water from the soil or water table, even when rainfall is zero in an area where it is normally good, so there is substantial drought on the first definition but the crop has adequate water. The amount and timing of rainfall relative to evaporative demand are known for most geographical regions, as is soil water and rooting volume for particular crops; thus statistical methods are used to determine probabilities of drought, both timing and severity (Price *et al*., 2002). Both are very important in relation to developmental stages of plants, are well understood, and must be considered in any meaningful analysis of responses of plants (Witcombe *et al*., 2008) including GM (Genetic Modification) (Toenniessen *et al*., 2003). However, in the GM literature, there is little discussion of how **drought (timing, duration, and intensity) affects specific processes such as development and growth of**

vegetative (root and leaf) and reproductive [i.e. flowering, fertilization, seed set, filling, and maturation (Georges *et al*., 2009)] organs. In terms of crop production, all of these may be crucial. Drought is largely treated as a simple factor—cessation of watering—and focuses on generic changes in metabolic processes, with the implication that they will provide DR under all conditions.

Plant Response to Drought

There are several different pathways by which plants respond to some sort of water stress event- whether it be greater duration between rainfall, decreased rainfall, etc. These responses fall into two main categories

- Drought avoidance or escape
- Drought tolerance

As you read this excerpt, focus on how this information would be relevant for designing an experiment that tested the drought avoidance or tolerance of a particular genotype.

In this excerpt from Verslues (2006) elaborates on these responses: in order to understand these paragraphs, here are a few terms that you need to understand:

Ψ_w this is the symbol for the potential energy of any particular unit of water. This potential energy represents the potential for that water to do work. As you would expect, water moves from higher to lower potential energy.

g_s **Stomatal conductance, T**he stomata open and close to allow for the evaporation of water and the uptake of CO_2. The conductance determines the rate of water evaporation and is determined by many factors including the shape, location of stomata as well as the biochemistry of the leaf tissue,

Drought Avoidance:

To understand the responses of plants to low Ψ_w at the level of the organism and cell it is useful to consider the stress avoidance/stress tolerance terminology proposed by Levitt (1972), a modified version of which is presented in Figure 1. In most cases, the plant's first response is to avoid low Ψ_w .Tissue Ψ_w and water content are maintained close to the unstressed level by increasing water uptake or limiting water loss such that the rates of water loss and water uptake remain balanced. Such a balance is achieved in the **short term mainly by stomatal closure. In the longer term, changes in root and shoot growth, leading to an increased root/shoot ratio, tissue water storage capacity and cuticle thickness and water permeability are also of potential importance.** Of these, changes in root growth to maximize water uptake are of the greatest importance for crop plants. In the case of mild water stress or water stress of a limited duration, avoidance mechanisms by themselves can be sufficient to maintain plant performance (Kramer and Boyer, 1995). Under such conditions, modifications such as increased root growth or decreased stomatal conductance have the potential to increase crop productivity. **The tradeoff in this case is the lost photosynthesis caused by reduced stomatal CO2 uptake or a shift of resources into root growth at the expense of photosynthetic and reproductive tissue. Furthermore, these mechanisms for avoiding water loss do not themselves offer any protection from the effects of low Ψ_w if the stress becomes more severe and the plant is no longer able to maintain a balance between water uptake and loss. In cases where low Ψ_w cannot be avoided by altering water uptake and water loss, additional mechanisms become important in maintaining plant function**.

<u>Dehydration avoidance:</u>

When transpiration is minimized, as is likely to be the case when stomata are closed because of stress, the Ψ_w of the plant will equilibrate with that of the water source (in most cases this is the soil Ψ_w). Thus, when soil water content and Ψ_w are low, Ψ_w of the plant tissue must also decrease, either through water loss or by adjustments made by the plant to achieve a low Ψ_w while avoiding water loss. Such adjustments are termed 'dehydration avoidance' (Figure 1). **The main mechanisms of dehydration avoidance are accumulation of solutes and cell wall hardening.**

Lawlor (2012) repeats a lot of the information that you read above, but emphasizes a few key pieces of information: Notice how he emphasizes the timing of plant growth itself can be a form of drought escape. In his discussion of drought avoidance, he articulates the role of Leaf Area in water loss. The greater the LA the greater risk of water loss, but also reduced amount of photosynthesis:

(i) Drought escape (DE) is characterized by the timing and duration of growth (phenology) to coincide with water supply which is adequate for optimal production by adapted genotypes. Plants and crops are therefore unaffected by drought which may occur in the area at other times; that is, they 'escape'**. This is extremely important ecologically and in agronomy. In annual crops, such as cereals (but even in perennials), the growth cycle generally coincides with average climate/weather conditions,** for example vegetative growth exploits the rainy period in regions with pronounced wet and dry seasons and grain maturation occurs in the dry period.

(ii) Drought avoidance (DA) is shown by plants which grow in periods of drought but maintain water status, generally by the following methods. (a) By restricting water loss (transpiration) and conserving soil water, with a smaller LA and *g*s (*stomatal conductance)*. Here it should be emphasized that in agriculture (as also in natural vegetation) it is the LA per unit area of ground surface, the LAI, and its retention over a period, the LA duration, which are the important features determining water loss over a period. **Decreased LA and LAI may arise in the early stages of slowly developing drought by production of fewer, smaller leaves, and later, with more severe drought,**

by senescence of older ones.

Returning to the article by Verslues (2006), you can read about some of the mechanisms to reduce the impact of dehydration on the plant:

> <u>Dehydration tolerance</u>
> As lowΨ_w stress becomes more severe, it becomes increasingly difficult for the plant to avoid dehydration and mechanisms to tolerate reduced water content become important…... However, most mesophytic (*plants adapted to moderate water availability)* plants (including almost all crop plants) lack the ability to enter a dormant state to tolerate complete desiccation and thus cannot recover from a severe (approximately 50% or greater) decrease in water content. These plants instead attempt to tolerate lesser degrees of water loss while maintaining metabolic activity. Most of the **dehydration tolerance mechanisms studied to date function primarily to protect cellular structure from the effects of dehydration.**
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> Several types of protective proteins, most notably dehydrins and other late embryo genesis abundant (LEA) proteins, are well known to accumulate in response to decreases in tissue water content either in response to abiotic stress or during seed development (Close, 1997). Although the function of many dehydrins and LEA proteins is not fully understood, at least part of their function is to act as chaperones that **protect protein and membrane structure** (Bravo et al., 2003; Hara et al., 2001). Compatible solutes can also protect protein and membrane structure under dehydration (Hincha and Hagemann, 2004). **Another aspect of dehydration tolerance, and of tolerance to other abiotic and biotic stresses, is the control of the level of reactive oxygen species (ROS) or limitation of the damage caused by ROS. The** sources of ROS under stress, mechanisms of ROS detoxification and the role of ROS in stress signaling are all active areas of current research and have been extensively studied and reviewed (Apel and Hirt, 2004; op den Camp et al., 2003; Chen and Gallie, 2004; Corpas et al., 2001; Foyer and Noctor, 2003; Hung et al., 2005; Jiang and Zhang, 2003; Kwak et al., 2003; Laloi et al., 2004; Milla et al., 2003; Moller, 2001; Mori and Schroeder, 2004; Pastori and Foyer, 2002; Shin and Schachtman, 2004).
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> It should also not be assumed that stress avoidance and tolerance occur in a linear progression in time after the stress begins or in a linear progression from responses initiated by mild stress to those initiated by severe stress. For example, some decrease in water content and turgor is likely to be required to trigger accumulation of abscisic acid (ABA) (Creelman and Zeevaart, 1985; Pierce and Rashke, 1980) which then causes stomatal closure to prevent further decrease in water content. Also, dehydration tolerance mechanisms such as accumulation of dehydrin and LEA protein may be initiated before significant dehydration occurs as a way of preparing the plant for any further decrease in water content. Rather than attempting to classify the various stress responses at a molecular level**, a consideration of tolerance and avoidance mechanisms is most useful in clarifying the appropriate types of experiments, the interpretation of the data and the terminology used to establish the role of a particular molecular event in the plant's integrated response to low Ψ_w and other abiotic stresses**.

Given the overlapping functions of many low Ψ_w responses, it is perhaps not surprising that these responses are controlled by a complex regulatory network. This network responds to both external stimuli, such as loss of turgor or reduced water content, and internal stimuli, such as production of ROS, sugar sensing and various hormonal stimuli, that reflect the metabolic and developmental status of the plant (Verslues and Zhu, 2005).

Impacts of drought on plant

There are many different impacts of the water stress or drought on a plant with the most obvious being the survival of the plant. The drought could impact the plant's:

- Development
- Growth
- Leaf Area
- Stomatal conductance
- Photosynthesis

Here are some excerpts from the Lawlor 2012 article outlining these types of impacts:

Development
However, many GM studies are made during vegetative growth and few (Peleg *et al*., 2011) **address developmental and reproductive processes.** Timing, duration, and intensity of drought relative to those of development are particularly important determinants of yield (e.g. of cereal grain) and will be most important for evaluating GM crops in the field, where applying defined (with respect to timing, duration, and intensity) water deficits to analyze the effects on specific developmental processes of GM plants is complex and difficult. Sampling and measurements are required frequently during a single drying period (erratic watering or rainfall greatly complicates interpretation, necessitating rain-out shelters in many environments) using well-established field methods (e.g. Legg *et al*., 1979).

Growth
Decreased growth is apparent in many GM studies (Kasuga*et al*., 1999; Karaba *et al*., 2007; Nakashima *et al*., 2007; B.Z. Xiao *et al*., 2009; Lourenco *et al*., 2011), including vegetative and reproductive organs, so that plants produce less total dry matter and yield. Shoot architecture may be altered: ***Arabidopsis* may have more compact rosettes and** rice more erect, bunched culms.

Leaf area and structure
A significant number of GM plants have (or appear to have) less total LA with fewer leaves of smaller area. Laminae are often thicker, with smaller, more closely packed

mesophyll cells, and the number of stomata/ unit area increases and sometimes their structure is altered (Holmstrom *et al*., 1996; Goddijn *et al*., 1997; Fernandez *et al*., 2010).

Stomatal conductance (g_s)
However, in many GM studies, *g*s is often not well measured, although it appears to be frequently decreased (Belin, 2010). Water loss of detached leaves by weighing under conditions differing from those of growth is often presented, but is complicated by stomatal closure, changed conditions, and, above all, greatly compromised water status. Active stomata will close more than inactive stomata, so the method tends to underestimate water loss by WT (*wild type)* compared with GM plants, obscuring the cause of delayed stress onset**. Microscopic measurement of stomatal aperture (M.R. Li *et al*., 2011) may indicate responses but cannot substitute for *g*s in quantitative evaluations of water relations**

Photosynthesis
The value of large *A(photosynthetic rate)* and small *T* (*rates of transpiration)* with increasing biomass and WUE *(water use efficiency)* for growth is obvious providing that there are no adverse genotype.environmental interactions, such as poor control of leaf temperature or inadequate capacity for use of excess energy and prevention of ROS accumulation.

If potential *A (photosynthetic rate)* is large in bright light but *g*s (*stomatal conductance)* is small, **then CO2 supply may limit *A*. Energy capture by chlorophyll may exceed energy use, for example in CO2 assimilation, which increases generation of ROS with** consequent adverse effects on photosynthetic and other cellular mechanisms, for example photoinhibition of photosystems (Hideg *et al*., 2003; Demmig-Adams *et al*., 2006; Shi *et al*., 2007) and damage to ATP synthase (see Lawlor and Tezara, 2009). Protective and regulatory mechanisms altered in GM plants include those which increase energy dissipation and regulate the energy balance of cells (e.g. **the xanthophyll cycle in photosynthetic tissues)** and those preventing production of—or enhancing breakdown of—ROS (Hou *et al*., 2009; Melchiorre *et al*., 2009).

Plasticity vs. Natural Selection

You have a good understanding of natural selection and adaptation for greater survival and reproductive success in a given environment. Even though there is natural selection for a particular genotype, it can have different phenotypes based on what environmental conditions the individual is exposed to. This range of phenotypes is called **phenotypic plasticity.** We're all familiar with this phenomenon in humans. An individual has a specific genotype for musculature, but the environmental conditions that person is exposed to (how much exercise, the type of exercise etc) will shape the expression of that genetic tendency.

Be careful that you don't confuse plasticity with evolution and adaptation. Plasticity is a change in how the genes are being expressed under specific environmental conditions (with good nutrition a person can reach their genetically maximum height). Evolution is the result of natural selection for different genotypes (natural selection for short stature in certain rainforest habitats). However, the degree of plasticity in an individual could be the result of natural selection, and differences among populations of plants in regards to plasticity may be adaptive. In other words, since plants can't move, under particular conditions there may be an adaptive advantage to a high degree of plasticity.

> From Franks (2010)
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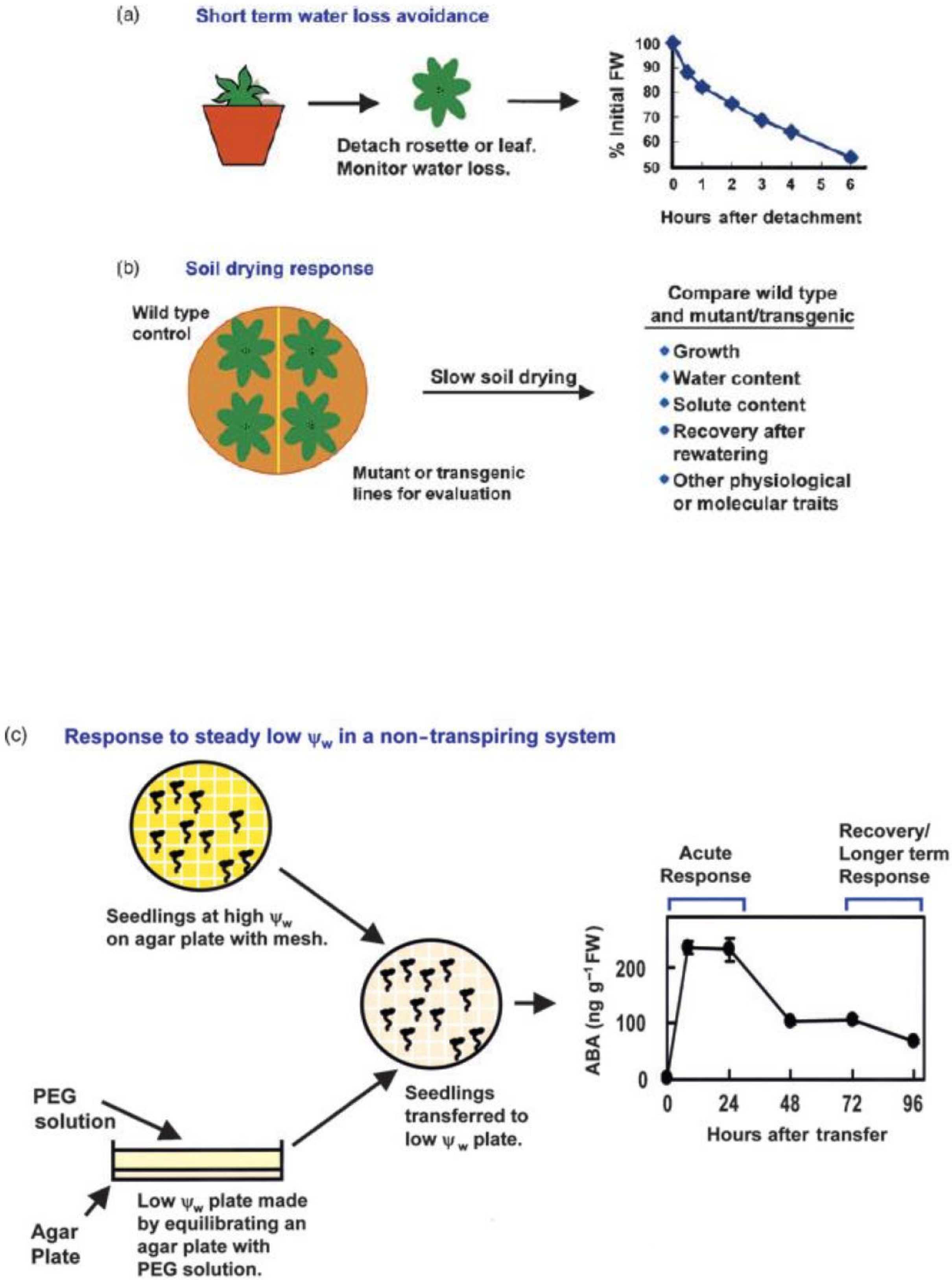
> To anticipate how plants will respond to changes in climatic conditions, such as drought,it is important to understand dehydration physiology, as well as how drought responses are shaped by natural selection (Heschel et al., 2004; Ludwig et al., 2004; Sherrard & Maherali, 2006; Agrawal et al., 2008; Wu et al., 2010). Plants can cope with drought either through escape or avoidance (Ludlow, 1989). **….Although plants could potentially escape and avoid drought, theory and previous findings suggest that there are likely to be trade-offs between these strategies (Bazzaz, 1979; McKay et al., 2003; Heschel & Riginos, 2005). The reason for this is that a trait that allows for greater drought avoidance, such as high WUE (*water use efficiency)*, may reduce the rate of growth and development and constrain or prevent drought escape**. Drought can also potentially cause either plastic or evolutionary changes in avoidance or escape. With plasticity, the expression of the phenotype is shaped by environmental conditions (Via et al., 1995; Schlichting & Pigliucci, 1998**). A plastic response to drought would mean that the plants alter their phenotype by increasing avoidance or escape traits in drought relative to nondrought conditions** (Mal & Lovett-Doust, 2005; Caruso, 2006). By contrast, drought could also act as an agent of selection and cause genetically based evolutionary changes in avoidance or escape (Fox, 1990; Ludwig et al., 2004

Methods of drought

As you can see from the figure below, there are several different mechanisms of inducing drought. We are focusing on soil drying. Here is a nice summary of the issues from the Verslues 2006 article. This will be helpful in designing your drought experiment.

> Soil drying experiments using pot-grown plants are typically done by removing the water supply and measuring some aspect(s) of plant growth, survival and water status after a fixed period of soil drying. Such soil drying experiments can at first seem quite straightforward but often turn out to be one of the most difficult types of experiment to interpret. This is because **the severity of stress experienced by the plant is not determined directly by the investigator but rather by the plant itself based on the rate at which it depletes the available soil water. This can lead to confusion if the severity of the stress is not quantified by measuring leaf or soil Ψ_w or if steps are not taken to ensure that the genotype of interest is exposed to the same severity of stress as a wild-type control.** An example of one of the complexities of soil drying experiments is the evaluation of mutants or transgenic plants with decreased stomatal conductance or decreased growth and leaf area. When water is withheld and the condition

of the plants assessed after a given time, plants that have reduced stomatal conductance or reduced leaf area can be expected to deplete soil water more slowly (avoidance of low Ψ_w) and may exhibit delayed wilting compared with wild-type plants. Such delayed wilting has been used to label such plants as stress or drought tolerant when instead the **transgenic plant has avoided low-Ψ_w stress by using the available water more slowly**. In general, to establish whether a particular genetic modification leads to tolerance of low Ψ_w, it must be shown that the stress response under study differs in plants exposed to the same severity of stress (sameΨ_w) and that this difference leads to a desirable change in phenotype. A better-defined use of the term 'tolerance', as well as other terms related to the lowΨ_w response, could do much to clarify the literature on this topic. These difficulties can be overcome in two ways. **The first is by quantification of leaf and/or soil Ψ_w during the drying cycle.** This can be combined with control of humidity levels or partial rewatering of some plants to ensure that the comparisons of stress response are made only between plants exposed to the same Ψ_w (see for example: Sharp et al., 2000; Thompson et al., 2004). Partial rewatering can also be used to extend the time for which the plants are exposed to low Ψ_w, thus allowing physiological and molecular responses to low Ψ_w be examined in more detail. These experiments are particularly relevant to more detailed evaluation of crop species (Sharp et al., 2000; Thompson et al., 2004) and numerous other studies where parameters such as osmotic adjustment and leaf growth have been evaluated in a number of crop species (see for example Babu et al., 1999; Puliga et al., 1996). In the case of Arabidopsis, however, repeated measurements of leaf or soil Ψ_w during the drying cycle are laborious and require a quantity of material that may be difficult to obtain. **For genetic studies, where a mutant or transgenic plant is being compared with a wild type, the easiest way to ensure a valid comparison while avoiding extensive measurements of Ψ_w is to grow the wild-type plant in the same pot as the genotype under evaluation (Figure 4b).** Thus the roots of both genotypes will grow into the same soil and be exposed to the same Ψ_w even if one genotype uses water more quickly than the other. This approach can be combined with measurement of soil □w at the end of the drying cycle to quantify the final severity of the stress.



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term responses can be seen after 72 or 96 h or longer exposure to low ψ_w.