Plant-Water Relations (2)

How plants manage water deficit and why it matters

Photo credits: Mel Oliver; William M. Ciesla, Forest Health Management International; R.L. Croissant Bugwood.org
Outline

1. Water scarcity is a growing problem
2. Desiccation-tolerant plants and xerophytes
3. Plant responses to water deficit
   - Perception and signaling
   - Transcriptional responses
   - Effects on photosynthesis
   - Effects on growth and development
4. Towards water-optimized and drought-tolerant agriculture
   - Optimizing irrigation
   - Monitoring water stress
5. Breeding for drought-tolerance
   - Classical approaches
   - Candidate gene approaches
Key definitions and concepts

**Desiccation**: Extreme water loss, equilibration to the water potential of the air which is generally low

**Desiccation tolerance**: The ability to withstand and recover from desiccation

**Dehydration**: The process of water loss from a cell or system

**Water deficit**: Sub-optimal water status from the perspective of plant function

**Drought**: Lack of rainfall or water supply leading to water deficit

**Drought tolerance**: The ability to withstand suboptimal water availability through adaptations or acclimations (*not the same as desiccation tolerance*)

**Xerophyte**: A plant adapted to live in a low-water environment

**Mesophyte**: A plant without specialized adaptations to low-water or high-water environments

**Adaptation**: Long-term evolutionary process by which an organism becomes suited to its environment

**Acclimation**: Short-term physiological process by which an organism adjusts to changes in its environment
Water scarcity is a growing problem

Severe droughts are increasing in frequency and forcing up food prices

In 2013, a major drought affected large parts of the United States

In the past 40 years, Lake Chad has shrunk by 95% because of irrigation demands & climate change
Pressure on freshwater resources threatens security and biodiversity

The earth’s surface is 75% water, but most is not available to plants.
Water is both a renewable and a nonrenewable resource

Solar energy is a renewable resource, limited only by our ability to capture and harness it.

Rainwater and snowmelt are renewable.

Oil is a nonrenewable resource.

Aquifer-derived water is not.
The Ogallala (High Plains) aquifer is being depleted of its reserves.

Almost one third of the groundwater used for irrigation in the United States comes from the Ogallala aquifer (shown in color), but this water resource is running out.

Aquifers around the world are similarly running dry.
Agricultural yields in most countries are likely to decrease by 2050.
The growing population demands a more efficient use of water.

Understanding how plants manage water deficit is essential for meeting the needs of the growing (and growing in affluence) human population.

* total exceeds 100% because of multiple causes and cited for many emergencies

** includes internally displaced people

Source: FAO Water at a Glance
Some plants and plant tissues are desiccation-tolerant

Seeds and pollen – usually desiccation tolerant

Bryophytes (mosses, liverworts, hornworts) – many are desiccation tolerant

Vegetative tissues of vascular plants – usually not desiccation tolerant

Some vascular plants have acquired vegetative desiccation tolerance

Photo credit: Anne Wangalachi/CIMMYT; Mel Oliver; Dartmouth Electron Microscope Facility

Teaching Tools in Plant Biology
AN INNOVATION FROM THE PLANT CELL
Some plants are desiccation or drought tolerant, others not

**Desiccation tolerant**

- Many bryophytes
- A small number of vascular plants

**Desiccation sensitive**

**Xerophytes**

- Succulents and non-succulents

**Mesophytes**

Within families and species, a wide range of drought sensitivities are present

Drought tolerant  Drought sensitive

Plants fall in a continuum from desiccation tolerant to sensitive

<table>
<thead>
<tr>
<th>Desiccation-tolerant plants</th>
<th>Desiccation-sensitive plants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cellular protection and repair mechanisms</strong></td>
<td><strong>Use morphological, phenological and biochemical strategies to maintain constant cellular water potential and / or relative water content</strong></td>
</tr>
<tr>
<td>ψ fluctuates</td>
<td>ψ fairly constant</td>
</tr>
</tbody>
</table>

- Equilibrate their cellular water potential with the ambient water potential
- Tolerate desiccation through biochemical and structural adjustments

![Diagram showing cellular water potential fluctuations](image)

**Adaptations and acclimations to avoid desiccation**

- Death

![Diagram showing external and cellular water potential](image)
In a practical context: Plants and plant organs (e.g., seeds) equilibrated at water potentials below -100 MPa are considered desiccated.
Vegetative desiccation tolerance is the ancestral strategy

Desiccation tolerance (DT) is common in bryophytes (liverworts, mosses)

A small number of tracheophytes (vascular plants) called “resurrection plants” have reacquired vegetative desiccation tolerance

Note that most seeds and pollen retain desiccation tolerance

Bryophytes, “non-vascular” plants, are mostly desiccation tolerant

Liverworts (~7000 – 8500 species)  Mosses (~10,000 – 17,000 species)  Hornworts (~200 species)  Vascular plants

Green algae  Land plants

Most seeds are mostly desiccation tolerant, some are not

Wheat, corn, rice, beans and other human food staples produce “orthodox” seeds that desiccate and can be stored stably.

Orthodox seeds go through a desiccated state

Orthodox seeds

- Embryogenesis
- Seed reserve accumulation
- Acquisition of desiccation tolerance

Water content

Dry state, Dispersal

Germination

Recalcitrant seeds

- Embryogenesis
- Germination
- Seed reserve accumulation

Water content

Dispersal

Recalcitrant seeds disperse without the stability associated with desiccation

Most pollen desiccates for stability during dispersal

Most plants produce desiccated, orthodox pollen that can tolerate exposure to dry air during dispersal.

Some plants make recalcitrant pollen that does not desiccate and cannot tolerate prolonged exposure to dry air.

Some orchids package pollen inside a protective membrane, shown here attached to the head of a pollinator.

Some grasses live in dense groups so pollen disperses very short distances.

Squash (*Cucurbita pepo*) male flowers are each open for only a few hours.


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Some vascular plants are desiccation tolerant


Only about 0.2% of angiosperms are desiccation tolerant

Leaves and cells often fold or curl inwards during desiccation

Craterostigma pumilum, an angiosperm desiccation-tolerant “resurrection plant”

Upon desiccation,
• leaves curl in,
• purple anthocyanin photoprotective sunlight-absorbing pigments accumulate, and
• small molecules accumulate that stabilize cell integrity

Selaginella lepidophylla


Teaching Tools in Plant Biology
ideas to grow on

AN INNOVATION FROM THE PLANT CELL

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Cellular responses to desiccation

Removing all the water from a cell and retaining its viability is tremendously challenging

- Membranes and cell walls can break or adhere to each other
- Proteins can aggregate or denature
- Toxic reactive oxygen species can accumulate

Desiccation-tolerant cells must prevent irreversible damage

CW=cell wall, C= chloroplast, T= thylakoids, V= vacuole, L=lipid droplet

Desiccation may induce stabilization of membranes and proteins

- Small molecules and proteins help maintain proteins in their native forms.

**LEAs, intrinsically-disordered proteins, confer protection**

**I. Sequestering function**

LEA proteins (grey coil) accumulate during dehydration and protect cell structures.

**II. Membrane protection**

Late Embryo Abundant (LEA) proteins were first characterized in seeds during embryo maturation, but accumulate in vegetative tissues during dehydration.

**III. Protein/Enzyme protection**

The cell wall structure contributes to desiccation tolerance

Antioxidant systems are induced by dehydration stress

Light energy absorbed by chlorophyll creates excited electrons for photosynthesis

Dehydration stress interferes with photosynthesis, so the excess energy accumulates as reactive oxygen species

Reactive oxygen species (ROS)

- Oxygen: $O_2$
- Superoxide anion: $O_2^-$
- Peroxide: $O_2^{2-}$
- Hydrogen Peroxide: $H_2O_2$
- Hydroxyl radical: $\cdot OH$
- Hydroxyl ion: $OH^-$

ROS image source: BioTek
Enzymatic and non-enzymatic antioxidants detoxify ROS

These systems can be up-regulated under water stress conditions

All plants respond to mild water deficit; DT plants take it further

Moderate water deficit (All plants)

- Osmolytes
- LEA proteins
- Dehydrins
- ROS-detoxification

Severe water deficit (Desiccation-tolerant plants)

- Cytoplasmic vitrification
- Increase in antioxidant systems
- Photopigment protection
- Vacuolar fragmentation
- Leaf shrinkage or folding

Synthesis of:

- Tortula ruralis is a highly desiccation-tolerant moss that has been used as a model system to understand desiccation tolerance

Desiccation Tolerance: Summary

Many bryophytes and a few vascular plants are desiccation tolerant. They use many strategies shared with non-desiccation tolerant plants, but take them to an extreme.

Selaginella lepidophylla

Xerophytes: Plants adapted to extremely dry environments

**Phenological**
Drought evaders: Grow and reproduce after rain, otherwise remain quiescent

**Morphological**
Deep roots, high xylem transport and/or tissue storage capacity, and tiny or absent leaves

**Anatomical**
Thick cuticle, rolled leaves, stomatal crypts

**Biochemical**
Crassulacean acid metabolism (CAM): stomata open at night to avoid water loss

Photo credits: Amrum; SAPS; James Henderson, Mary Williams
Similar features have evolved in disparate families

Anatomical traits frequently found in xerophytes:
- Succulent (water storing) leaves or stems
- Thick cuticle
- Sunken stomata

Cactus  
Euphorbia spp

Agave, Yucca, Aloe

Cacti

Euphorbia

Map of Life - "Succulent desert plants"
Plant responses to water deficit

Responses vary with extent of water deficit, rate of dehydration, and by genotype

The severity of water deficit affects plant responses

Rate of water deficit and genotype affect plant response and survival

**Sensitive species**

- Acclimation / Protection
- Injury / Repair

- Slow drying allows plants time to respond and avoid injury

**Resistant species**

- Acclimation / Protection
- Injury / Repair

- Drought-resistant species suffer less damage with the same extent of water deficit

Water deficit: Perception – signaling - responses

<table>
<thead>
<tr>
<th>Perception</th>
<th>Signaling</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Cell turgor</td>
<td>- Between cells:</td>
<td>- Short term:</td>
</tr>
<tr>
<td>- Membrane pressure</td>
<td>- ABA</td>
<td>- Decrease in stomatal conductance</td>
</tr>
<tr>
<td>- Osmotic content</td>
<td>- Ethylene</td>
<td>- Alterations in hydraulic conductivity</td>
</tr>
<tr>
<td>- Reactive oxygen</td>
<td>- Hydraulic signals</td>
<td>- Osmotic adjustments</td>
</tr>
<tr>
<td>- ? Other unknown</td>
<td>- Water potential</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Xylem pH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Other signals…</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Within cells:</strong></td>
<td><strong>Long term:</strong></td>
</tr>
<tr>
<td></td>
<td>- ABA</td>
<td>- Induction of drought-induced genes</td>
</tr>
<tr>
<td></td>
<td>- Reactive oxygen</td>
<td>- Changes in growth rate including root architecture</td>
</tr>
<tr>
<td></td>
<td>- Transcriptional cascades</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Other signals…</td>
<td></td>
</tr>
</tbody>
</table>

**Perception**

**Signaling**

**Responses**

- Decrease in stomatal conductance
- Alterations in hydraulic conductivity
- Osmotic adjustments
- Induction of drought-induced genes
- Changes in growth rate including root architecture
Water deficit normally occurs when efflux exceeds influx.

The site of perception can be cells in the leaf or root or both.

High evaporative demand

Soil water depletion
Water deficit may be perceived at the cell membrane

Several plasma-membrane localized pressure sensors in yeast have been identified. Similar proteins may be involved in sensing water deficit in plants.

Water deficit signaling

Drought responses are mediated by ABA and hydraulic processes

Water deficit → Cellular dehydration → Turgor sensor

ABA biosynthetic enzymes → ABA

Altered ion channel activity → Stomatal closure and inhibition of opening

Transcription factor activation → Cellular defenses and osmotic adjustment

Altered hydraulic conductance

Growth (cell division and expansion)

Arrows indicate interactions, whether positive or negative

Abscisic acid accumulation is a rapid response to water deficit

ABA levels are tightly controlled by synthesis, conjugation and degradation

Abscisic acid (ABA)

Synthesis induced by water deficit

Reversible inactivation by conjugation

Irreversible hydrolysis and inactivation

Transcriptional responses to water deficit are well characterized.

The drought response is complex and pleiotropic

Hundreds of studies have revealed 1000s of genes involved in drought responses

Some are regulated by ABA, others are ABA-independent

Many different transcription factors are involved

Many of the targets are involved in cellular protection or conserving water

Water deficit induces stomatal closure & decreased photosynthesis

In this study of maize growing in a pot, stomatal resistance and ABA levels increased after three days without irrigation.

When a leaf is detached, the rate of transpiration decreases over time, indicating stomatal closure.


A decrease in stomatal conductance $g_s$ is a rapid response to water deficit.

Guard cells respond to ABA produced locally and distally, and to a change in leaf water potential.
ABA alters ion channel activities to promote stomatal closure

Ion flux and water flow into the guard cell causes stomata to open.

ABA promotes stomatal closure.
Plants that cannot close their stomata lose internal cellular pressure (turgor) and wilt.

ABA-deficient tomato

ABA-deficient
Arabidopsis

ABA-insensitive
Arabidopsis

Water deficit can affect CO$_2$ concentration in the chloroplast

$C_a$ is [CO$_2$] ambient

$C_i$ is [CO$_2$] inside the leaf

$C_c$ is [CO$_2$] inside the chloroplast

$g_s$ is stomatal conductance of CO$_2$ (from outside the leaf to inside the leaf air spaces)

$g_m$ is mesophyll conductance of CO$_2$ (from air spaces into chloroplasts)
Water deficit can affect photosynthetic carbon assimilation

\( A \) is the rate of photosynthetic carbon assimilation. \( A \) depends on the amounts of enzyme and substrates.

Under ideal conditions, \( A \) depends on \([\text{CO}_2]\) inside the chloroplast (where the enzyme is located).
Water deficit can but does not have to affect photosynthesis

In a plant with wide-open stomata, the rate of transpiration may be more than necessary for the maximum photosynthetic rate.

In a plant with tightly closed stomata, CO₂ might limit photosynthetic carbon assimilation.

There is an ideal point at which the exchange of CO₂ and H₂O is optimal.
Water deficit affects growth rate

Water deficit often reduces crop growth and yield (shown here – wheat)

<table>
<thead>
<tr>
<th>Water content: Percent of control</th>
<th>Well watered</th>
<th>Water deficit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Psi_w$ (MPa)</td>
<td>100%</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>-0.03</td>
<td>-0.20</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td>-0.81</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>-1.60</td>
</tr>
</tbody>
</table>

As water become scarce, the plant’s resources are preferentially allocated to the root

What controls growth under water deficit?

What limits shoot and leaf growth?
- Decrease in photosynthetic rate?
- ABA signaling?
- Hydraulic effects on cell elongation?

How is root growth maintained relative to shoot growth under water deficit?
Water limitation interferes with leaf expansion and function

**Zea mays**

<table>
<thead>
<tr>
<th>Well watered</th>
<th>Water withheld for 10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully expanded, green</td>
<td>Reduced leaf area, leaf margins curled to conserve water, yellowing</td>
</tr>
</tbody>
</table>

Reduced leaf elongation is often not due to a decrease in photosynthesis.

Leaf enlargement is more sensitive to mild water deficit than photosynthesis; therefore, factors other than availability of photosynthate must limit leaf growth.

Growth restriction may be independent of photosynthesis, in the short term

Water deficit’s effect on leaf expansion may be independent from those of photosynthesis (although smaller leaves mean less area for photosynthesis)

Water deficit responses are specific to developmental stage and age

The developmental state of the leaf / cell affects its responses to water deficit

Red = proliferating (dividing) tissues
Green = expanding tissues
White = mature tissues

Summary of tissue-specific responses to water deficit and signals involved

ABA = abscisic acid
GA = gibberellin
ACC = 1-aminocyclopropane-1-carboxylic (ethylene precursor)
AOX = alternative oxidase
KRP & SIM = cell cycle regulators

Plants have to balance their growth and survival responses

Adaptations of roots to water deficit depend on severity and genotype

Well watered

Drought stress response

Preferential primary root elongation

Preferential lateral root proliferation in deep soil

Production of short-lived, drought-induced lateral roots

Root hair elongation

Increase in number or diameter or xylem elements

Increase in hydraulic conductance, through expression of aquaporins or changes in exodermis and endodermis

Growing deeper is an adaptive response and under genetic control

Soil moisture in the upper soil zones is depleted during drought

A gene that promotes deeper rooting (enhanced gravitropic response confers drought protection)

Maintained root elongation to reach deep water is mediated by ABA

Fluridone, an ABA synthesis inhibitor, inhibits primary root elongation at low water potentials

ABA may act by suppressing the accumulation of growth-inhibitory reactive oxygen and ethylene

Reproductive development can be particularly drought sensitive

The developmental stage of a plant experiencing water deficit affects the outcomes.

In wheat and rice, seed (kernel) number is highly sensitive just before anthesis (pollen maturation).

Drought at the wrong time can drastically reduce seed yield

Plant responses to water deficit affect many processes. Plant responses to water deficit are pleiotropic, complex and context dependent, but typically include an increase in root growth, decrease in shoot growth, and decrease in transpiration and photosynthesis.
Towards water-optimized, drought-tolerant agricultural plants

New technologies are making it possible to prevent, diagnose and ultimately eliminate the harmful effects of water deficit.

**Prevent:** Monitor the weather and soil moisture, use water wisely

**Diagnose:** Monitor plant stress responses

**Breed tolerance:** Genetic strategies to confer drought tolerance

Image source: USDA
Prevention: Information can guide planting and watering schedules

Soil moisture sensors use a variety of technologies and can indicate moisture at deep as well as shallow depths.

Radios and cell phones inform farmers about weather forecasts so they can decide when to plant or irrigate.

Satellites can monitor soil moisture and rainfall.

Photo credit: Francesco Fiondella; NASA / JPL

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The VegDRI integrates satellite-based observations of vegetative conditions, climate data and other information to indicate drought level.

A false-color thermal image of several fields. Blue and green colored fields are cooler due to evaporative transpiration, indicating greater stomatal aperture and less water stress.

US National Drought Mitigation Center; NASA Earth Observatory
Prevention: Less wasteful irrigation strategies

Overhead watering allows wasteful evaporation from the spray and the bare soil.

Drip irrigation minimizes wasteful evaporation.
Variable rate irrigation can support good yields with less water use

By adjusting the rotation speed of the irrigation arm, different amounts of water can be delivered to different sectors.

By adjusting the rotation speed and flow from sprinklers, zones can each have a customized water application.

Soil or plant information can be sent to the irrigation system for real-time irrigation optimization.

Prevention: Partial root-zone drying can conserve water

In PRD irrigation, the plants are watered on alternating sides, so each half of the root system alternates between wet and dry.

PRD often results in equivalent harvests, even if the leaf size is smaller.

Hypothesis: The wet roots hydrate the shoot, the dry roots send a signal that causes partial stomatal closing and water conservation.

F3.11 An ideal climate-smart agricultural landscape of the future would enable farmers to use new technologies and techniques to maximize yields and allow land managers to protect natural systems, with natural habitats integrated into agriculturally productive landscapes.
Diagnosing plant physiological activities and drought stress effects

What would a plant “tricorder”* measure?
• Plant water potential
• Photosynthetic gas exchange (CO$_2$ assimilation, O$_2$ evolution)
• Chlorophyll fluorescence (indicates efficiency of photosystem II)
• Transpiration (H$_2$O release, change in temperature)
• Water use efficiency (ratio of CO$_2$ assimilated to H$_2$O transpired)
• Stress-responses: hormones, transcripts, metabolites
• Growth rate
• Growth direction and orientation

*In the TV series Star Trek, a tricorder is a handheld scanning and analysis diagnostic device
Physiological parameters can be monitored in the lab or field.
Quantifying photosynthetic operating efficiency by fluorescence

The light energy absorbed by chlorophyll has three fates: photosynthesis, dissipation as heat, or re-emission as longer wavelength light (fluorescence).

It is possible to determine the photosynthetic rate *indirectly* by measurements of fluorescence.

- **Fluorescence**: Easy to measure
- **Photosynthesis**: Hard to measure
- **Heat**: Can be accounted for with appropriate controls
Quantifying photosynthetic operating efficiency by fluorescence

1. Block photosynthesis and measure maximal fluorescence ($F_{m}'$)

Fluorescence

$F_{m}'$

Chl*

Photosynthesis

All of the energy is emitted as fluorescence

2. Unblock photosynthesis and measure fluorescence ($F'$)

Fluorescence

$F'$

Chl*

Photosynthesis

The energy not emitted as fluorescence is used for photosynthesis

The difference, normalized to $F_{m}'$, indicates the photosynthetic operating efficiency

\[
\frac{F_{m}' - F'}{F_{m}'} = A
\]

This value can be used to calculate $A$, the CO$_2$ assimilation rate

The energy not emitted as fluorescence is used for photosynthesis

This value can be used to calculate $A$, the CO$_2$ assimilation rate
Gas exchange in a leaf can be measured in a closed or open (flow through) system.

[CO$_2$] is measured by infrared gas analysis (IRGA) and [O$_2$] by an oxygen electrode.
Transpiration can be measured as water transpired or heat lost.

The concentration of water in a sealed leaf chamber can be monitored in a flow-through or closed system.

Transpirational water loss can be measured by weight loss from a plant growing in a sealed pot.

Thermal imaging: transpiration requires heat energy and cools plants.

Top row: Wild-type Arabidopsis.

Bottom two rows: Mutants (ost1-1 and ost1-2) defective in stomatal closure. More transpiration = cooler plants.

Water use efficiency is the ratio of CO$_2$ assimilated to H$_2$O transpired.

WUE values are typically on the order of 1 mol CO$_2$ fixed to 100 - 400 mol H$_2$O transpired (for C$_3$ plants).

WUE = \[ \frac{\text{Crop yield (kg)}}{\text{Water consumption (kg)}} \]

On a larger scale, WUE can be measured as yield relative to water consumed.
WUE can be measured by non-invasive imaging methods

Instantaneous WUE can be calculated from simultaneous measurements of chlorophyll fluorescence (CO₂ assimilation) and temperature (transpiration)

WUE can be determined by stable isotope analysis

Approximately 1.1% of the Earth’s carbon has an extra neutron, making it larger and more massive. The $^{13}$C isotope is stable (non-radioactive) (unlike the radioactive $^{14}$C isotope)

The carbon-fixing enzyme Rubisco preferentially uses $^{12}$CO$_2$ as a substrate

Ribulose-1,5-bisphosphate $+$ $^{12}$CO$_2$ $\rightarrow$ 2 x 3-phosphoglycerate
If stomata are closed, Rubisco discrimination tends to enrich the internal \([\text{CO}_2] \) in \(^{13}\text{CO}_2\).

**Stomata open:**

\([\text{CO}_2]_{\text{inside}} \) high, Mostly \(^{12}\text{CO}_2\) assimilated

**Stomata closed:**

\([\text{CO}_2]_{\text{inside}} \) low, Relatively more \(^{13}\text{CO}_2\) assimilated

The isotope differential \((\delta \ ^{13}\text{C})\) reflects stomatal conductance.

Isotope discrimination indicates water use efficiency in C₃ plants

As the stomata are more closed, \([\text{CO}_2]_i / [\text{CO}_2]_a\) decreases, relative abundance of \(^{13}\text{CO}_2\) increases, and the carbon isotope discrimination decreases.

Note that in C₄ plants there is no additional isotope discrimination because PEPCase (the CO₂-fixing enzyme in C₄ plants) does not discriminate between stable isotopes.

\(^{13}\text{CO}_2\) diffuses less effectively through the stomatal pores, so inside the leaf there is a 4.4% non-enzymatic isotope discrimination.

Large phenotyping facilities can be shared by many users

Automated greenhouse

Imaging chamber

See a video here

Educational resources here

Australian Plant Phenomics Facility

Donald Danforth Plant Science Center
See video here
Multispectral imaging provides information about plant status

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Color</th>
<th>Reflectance</th>
<th>Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>440 nm</td>
<td>Blue</td>
<td>Much reflected</td>
<td>Much absorbed by chlorophyll</td>
</tr>
<tr>
<td>550 nm</td>
<td>Green</td>
<td></td>
<td></td>
</tr>
<tr>
<td>660 nm</td>
<td>Red</td>
<td></td>
<td></td>
</tr>
<tr>
<td>770 nm</td>
<td>Near infrared</td>
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</tr>
</tbody>
</table>

NDVI is a normalized indicator of chlorophyll absorption

\[
NDVI = \frac{R_{NIR} - R_R}{R_{NIR} + R_R}
\]

**3D imaging of single plants or fields can contribute to phenotyping**

Breeding programs depend on high-throughput phenomics tools

Successful breeding programs, whether using forward or reverse genetics, require accurate, preferably high-throughput, phenomics tools.

Scaling up: Field-wide surveys for breeding programs

The phenomobile carries equipment to measure:
- leaf greenness and ground cover
- canopy temperature
- volume (biomass) of plants, plant height and plant density
- crop chemical composition

Breeding approaches for drought-tolerant plants

Reverse Genetics:
Identify drought-tolerance genes and alter expression levels in plants

Forward Genetics:
Classical approach, select for drought tolerance based on phenotype

Two different inbred maize varieties showing different responses to water deficit

Translational research: From model systems to real-world complexity

If you find a solution that works in a model organism in a controlled environment, will it work in a vastly more complex environment?

The same problem occurs when translating lab mice studies to humans with all their diversity and complexity.

The goal of most breeding programs is high yield, even with water deficit.

For example, these two cultivated types of peanut (*Arachis hypogaea*) are very different in their response to drought. The drought-tolerant variety produces plenty of peanuts, while the drought-sensitive variety produces almost none.

Drought tolerant

Drought sensitive

Peanuts are legumes whose fruits grow underground.

Photos by Paxton Payton and Franz Eugen Köhler. Köhler's *Medizinal-Pflanzen* see also NOAA.
For success, drought-tolerant traits have to be in the breeding population.

Many ancestral traits are not represented in modern varieties, but can still be found in germplasm collections.

Components of yield under drought stress: WU, WUE and HI

Yield under drought is the product of water uptake (WU), water use efficiency (WUE), and harvest index (conversion of biomass to grain)

Different “drought tolerance” traits have different consequences

<table>
<thead>
<tr>
<th>Extensive root system</th>
<th>Decreased stomatal aperture / leaf area</th>
<th>Cellular protectants</th>
<th>Early reproduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benefits</td>
<td>Decreases transpiration</td>
<td>Avoids cell death under severe drought</td>
<td>Avoids late season water deficit</td>
</tr>
<tr>
<td>Increases water uptake</td>
<td>Decreased photosynthesis and biomass accumulation</td>
<td>Reduced growth rate, energy diverted to protection</td>
<td>Shortened growing season</td>
</tr>
</tbody>
</table>

**Benefits**
- Increases water uptake

**Drawbacks**
- Biomass sequestered in root not available for shoot / reproduction
- Decreased photosynthesis and biomass accumulation
- Reduced growth rate, energy diverted to protection
- Shortened growing season

Adapted from Tardieu, F. (2012). Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. J. Exp. Bot. 63: 25-31; Photo credits: Michael Thompson.
Under the “right scenario”, nearly any trait can confer tolerance

<table>
<thead>
<tr>
<th>Trait</th>
<th>Scenario for maximum positive effect</th>
<th>Scenario for maximum negative effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extensive / deep root system</td>
<td>Deep water available (enhanced uptake)</td>
<td>Low availability of water / nutrients in deep layers</td>
</tr>
<tr>
<td>Decreased stomatal aperture / leaf area</td>
<td>Severe drought (less transpiration uses less water)</td>
<td>Favorable year (loss of potential biomass)</td>
</tr>
<tr>
<td>Cellular protectants</td>
<td>Very severe drought (avoid death)</td>
<td>Could lead to reduced growth rate in non-extreme conditions</td>
</tr>
<tr>
<td>Early reproduction</td>
<td>Very dry year, late season drought (ensure reproduction)</td>
<td>Favorable year (loss of potential biomass)</td>
</tr>
</tbody>
</table>

Breeding for drought tolerance: Candidate gene approach

Based on our understanding of plant responses to desiccation and water limitation, hundreds of different candidate genes have been analyzed for drought-tolerance effects.

Osmotic adjustment (mannitol, proline, glycine betaine etc.)

Transport proteins (e.g., vacuolar Na⁺/K⁺ antiporter, aquaporins)

Protective proteins (chaperones, dehydrins)

Signaling molecules and hormones, hormone responses

Transcription factors

Genetically-engineered plants are subject to regulatory controls

Plants made using genetic engineering methods have additional regulatory hurdles to cross.

Biotech plants w/ increased drought tolerance are now appearing

A gene encoding a bacterial RNA chaperone increases yields in plants that experience late-season drought

After extensive testing, the first plants carrying a transgene for drought tolerance were launched in the US in 2013 and have been approved for use in China

Many genes show promising results for drought tolerance


Modeling approaches contribute to plant breeding and production

Models can test lots of scenarios “virtually” to identify those worth testing “in the real world”

**Types of questions models can investigate:**
- Which genotype will work best for my environment?
- Should I grow a cover or companion crop?
- When should I plant?
- Should I spend my money on water or fertilizers?
- Which combinations of alleles will work best for my needs?
- Which genes should I start introducing now in anticipation of future climate changes?

Diagram showing various simulated breeding paths from low yield (blue) to high yield (red)

The modeling – testing iterative cycle informs breeding

Observation
Abstraction

Modeling

Trait Understanding & Modeling
Physiological basis of adaptation
Represent as meta-processes

Germplasm
Genetic mapping of control parameters

Performance Landscape
Environmental Characterization
Simulation and Phenotype prediction

Breeding Program
Search strategy applied to performance landscape

Analyses
Prediction

Testing
Experimentation
Selection

Modeling allows experiments to be carried out virtually.

Historical data about QTL effects on leaf growth and water sensitivity were fed into a model that indicated the expected effects of each on yield during various drought-stress scenarios.

Summary: Strategies for water-optimized, drought-tolerant plants

Research is key to advancing:

• Irrigation management
• Plant stress monitoring
• Phenotyping methods
• High throughput phenotyping platforms
• –omics methods
• Breeding strategies and tools
• Modeling plant responses to water deficit
• Modeling gene x environment interactions

Image source: USDA
Excellent model systems are available for learning how *cells* respond to *desiccation* and how *plants* respond to *drought*, but translating that understanding into *drought-tolerant food production* is not easy.
Water scarcity may be one of the major threats to human security

Source: Comprehensive Assessment of Water Management in Agriculture, 2007
How will California’s agriculture be affected by drought in 2014?

At the time of this article’s publication, California was facing the prospect of its worst drought in 500 years. These photos show the snow accumulated in January 2013 vs January 2014.

How will governments, growers and consumers respond to rising food prices and food shortages as a consequence of this drought?