Water Relations 2: How Plants Manage Water Deficit and Why It Matters (TTPB28) – Teaching Guide

Overview –

The availability of fresh water is possibly the greatest limitation to our ability to feed the growing human population (with 9 billion people forecast by 2050 and 11 billion by 2100). This *Teaching Tool* examines why water is so critical for plant growth and food production (in the form of seeds, but also whole fruits, leaves etc.), as well as the mechanisms that plants have evolved to deal with pervasive or transient water shortages. It also addresses how the understanding of plant adaptations and acclimations to water deficit can be applied towards improving food production when water is limiting.

Learning objectives

By the end of this lecture the student should be able to:

- Summarize how and why food production can be limited by the availability of fresh water
- Compare and contrast the ways that desiccation-tolerant plants, xerophytes and mesophytes are adapted to and acclimate to water deficit.
- Summarize the transcriptional, transpirational, photosynthetic and growth responses of plants to water deficit
- Evaluate strategies to increase crop production in the face of increasing water scarcity
- Summarize three methods that are used to diagnose water-stress and / or select for drought-tolerant plants
- Formulate a breeding strategy that will lead to drought-tolerant crop plants

Key terms and concepts

Desiccation: Extreme water loss, with loss of all free water from cytoplasm
Desiccation tolerance: The ability to withstand and recover from desiccation
Water deficit: A situation in which water demand exceeds supply
Drought: Lack of rainfall or water supply leading to water deficit
Drought tolerance: The ability to withstand suboptimal water availability through adaptations or acclimations
Xerophyte: A plant adapted to live in a low-water environment
Mesophyte: A plant without specialized adaptions 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

Study / exam questions (*understanding and comprehension*)

- Why is water scarcity a growing concern in terms of food production?
- True or False: Desiccation tolerance is a relatively recent evolutionary innovation for plants
- Explain two different strategies that help cells survive desiccation
- Why are recalcitrant seeds and pollen sometimes advantageous?

www.plantcell.org/cgi/doi/10.1105/tpc.114.tt0514

- Describe four adaptations that are often found in xerophytes that facilitate their ability to live in very dry environments, and explain the adaptive significance of each.
- True or False: During drought, leaf elongation is primarily decreased as a consequence of the carbohydrate deficiency that occurs with stomatal closure
- Propose the adaptive significance of each of the following drought responses:
    - Stomatal closure
    - Decreased leaf expansion
    - Maintenance of primary root elongation
    - Biosynthesis of compatible solutes
    - Activation of reactive-oxygen detoxifying systems
- Describe three ways that technology can contribute to more efficient use of irrigation water
- In your own words, explain why many plants can grow just as effectively even if the amount of water available to them is slightly decreased.
- What are two methods that can be used to measure a plant's rate of transpiration?
- What is stable isotope analysis and what does it measure?
- What is a phenotyping facility and what advantages do such facilities confer for data acquisition and analysis?
- How are systems biology and modeling approaches used in the study of plant responses to water deficit?

Discussion questions (*engagement and connections*)

- Desiccation tolerance is a trait found in some animals and plants as well as some microbes. Choose and investigate a desiccation-tolerant organism or tissue (animal, bryophyte, seed, spore, pollen, resurrection plant or other). What selective advantage does desiccation tolerance confer to it? How well understood are the signals that initiate desiccation tolerance? Compare the strategies of your organism to those presented for generic plants. How universal are the specific mechanisms for surviving desiccation?
- There is an ongoing discussion about the importance of hydraulic (physical) versus ABA (chemical) signals (or other signals) in mediating responses to water deficit. Starting with the references provided, investigate the evidence that supports hydraulic versus ABA as signals conveyed from roots in drying soil to the shoot. What is the evidence that supports hydraulic signals versus ABA in regulating stomatal aperture?
- Our current understanding is that the limitation of shoot growth under water deficit is not due to carbohydrate limitation, but instead to other regulatory influences. What evidence supports this model?
- The drought-sensitivity of seed production in cereals is a major concern for food production. Investigate the molecular mechanisms behind this effect.
- A farmer is trying to grow corn but has difficulty getting access to water for irrigation. Her region gets a lot of rain in the spring, so when she plants the seeds the soil is nice and moist, but no rain falls during the summer when the plants are growing. For the past few years, she has managed to grow tall, healthy looking plants, but although they flower, they set few seeds. What advice can you give this farmer to help her get a good grain harvest next year?

www.plantcell.org/cgi/doi/10.1105/tpc.114.tt0514

- Peanut (aka groundnut, *Arachis hypogaea*) is an important protein-rich crop grown in tropical and sub-tropical regions, and most varieties are sensitive to drought. Investigate some of the strategies that are being used to improve drought-tolerance and to identify improved irrigation regimes for this important crop. Compare the various strategies in terms of their infrastructure and technology demands, financial costs and their relative risks and rewards. If you had $100,000 to apply towards researching drought-tolerant peanuts, how would you invest your money?
- *Seeds for Needs* (https://www.youtube.com/watch?v=Bdt5vpyJXeA) uses a "crowdsourcing" strategy to identify plant varieties that do well in various locations. Watch the video and then discuss the aims and outcomes of this approach.
- Compare and contrast the "forward" and "reverse" genetics approaches towards breeding drought tolerant plants. What are the arguments for and against each strategy? If you were going to get involved in this type of research, which strategy would you pursue and why?
- Investigate one of the many different plant varieties that have been developed using a "candidate gene" approach. What evidence led to the identification of this gene as potentially conferring drought tolerance? How was the gene introduced into an experimental plant system, and under what regulatory control (e.g., promoter)? What analyses were performed on the resulting transgenic plants, and how did the plants perform? Were there any drawbacks associated with the presence of the transgene?
- Read one of the articles cited under "Networks, systems, and modeling as avenues of research". In your own words, describe the methods introduced in the paper you read, and the way they are being used to address the questions of food production under water-limiting conditions.
- In what way does the Intergovernmental Panel on Climate Change (IPCC) suggest climate change will affect the availability of fresh water? (See http://www.ipcc-wg2.gov/AR5/.).
- Read the FAO report "Water at a Glance" and then summarize three low-tech water management techniques used in developing countries. (http://www.fao.org/nr/water/docs/waterataglance.pdf)

www.plantcell.org/cgi/doi/10.1105/tpc.114.tt0514

Lecture synopsis

The availability of fresh water is possibly the greatest limitation to our ability to feed the growing human population (9 billion people forecast by 2050 and 11 billion by 2100). This Teaching Tool examines why water is so critical for plant growth and particularly their production of food (primarily seeds, but also whole fruits, leaves etc.), as well as the mechanisms that plants have evolved to deal with pervasive or transient water shortages. It also addresses how the understanding of plant adaptations and acclimations to water stress can be applied towards improving food production when water is limiting.

INTRODUCTION (1 -3)

WATER SCARCITY IS A GROWING PROBLEM (4 – 10)

Growing plants need fresh water, which they can get from rainfall, surface water or water pumped up from underground reserves. Many underground aquifers are being depleted by removing water faster than it is replenished. Combined with droughts and changing rainfall patterns, this means that water scarcity is becoming an increasingly important concern for food production.

PLANTS HAVE EVOLVED SEVERAL STRATEGIES TO SURVIVE WATER DEFICIT (11 – 13)

Plants have two distinct strategies to deal with water deficit. Many bryophytes and a few vascular plants can tolerate desiccation, in which 80 – 90% or more of their cellular water is lost; these organisms passively conform to the environment. By contrast, most vascular plants maintain a relatively constant internal water status through efficient strategies to take up and retain water.

DESICCATION-TOLERANT PLANTS AND TISSUES (14 – 30)

Most bryophytes, most seeds and pollen, and a very small number of vascular plants are desiccation tolerant, and the strategies they use are very similar.

Bryophytes (16)

Bryophytes are mosses, liverworts and hornworts, and often described as non-vascular plants (although they do have some specialized conducting tissues). Most are desiccation tolerant and their water content fluctuates with the environment. Desiccation tolerance involves the stabilization of cellular structures, protection from reactive oxygen species, and repair during rehydration.

Seeds and pollen (17 – 19)

Seeds and pollen are specialized for dispersal, and in most plants they are able to survive desiccation. Orthodox seeds and pollen desiccate, whereas recalcitrant seeds and pollen do not. Desiccation is part of the normal developmental program that includes activation of developmentally-regulated desiccation response genes. Recalcitrant seeds and pollen avoid exposure to dry air through a variety of different modes of dispersal that for seeds include germination within the fruit or vivipary, and for pollen include minimizing the time or distance it is exposed to the air.

Resurrection plants (20 – 22)

Although most vascular plants have lost the ability to survive desiccation, a few species have regained it, and these plants are known as resurrection plants. These are adapted to their dry environment by losing water and "shutting down" metabolically. Along with the cellular responses described below, resurrection plants tend to curl or fold their leaves inward and often accumulate photoprotective purple pigments.

www.plantcell.org/cgi/doi/10.1105/tpc.114.tt0514

Cellular responses to desiccation (23 - 29)

To a first approximation, cellular desiccation responses are similar in all organisms, whether they are animal, fungus, prokaryote, bryophyte, seed, pollen or resurrection plant.

Stabilization of molecular structures (24 – 26)

When a cell desiccates and loses water, it loses much of its volume. If this water isn't replaced with an appropriate substance, the membranes, organelles and proteins can suffer irreparable damage, by denaturing, aggregating and fusing. Additional, cell walls can be damaged by the collapse of the cell, but orderly wall folding can preserve their integrity. As water is lost from the cytosol, it increases in viscosity until it becomes a solid, which can be a crystalline or glassy solid. Glassy solid are correlated with desiccation tolerance as they retain the ionic composition of the cytosol. Glass formation is associated with the accumulation of certain di- and oligo-saccharides and LEA proteins.

Roles of Late Embryo Abundant (LEA) proteins (25)

Late Embryo Abundant (LEA) proteins were first characterized in maturing seeds, but members of this large protein family also occur in other desiccating tissues including animal tissues. They seem to have various functions including contributing to cytosolic glass formation, acting as mechanical buffers, and stabilizing cellular components by directly binding to them.

Chemical detox (27 – 28)

Any kind of stress that interferes with metabolism tends to cause the accumulation of reactive oxygen species, and the response of most cells to these stresses is to accumulate enzymatic and non-enzymatic antioxidants.

Rate of dehydration and recovery can affect desiccation tolerance

Some bryophytes are constitutively desiccation tolerant and can survive fairly rapid rates of dehydration, but other many other desiccation-tolerant organisms can only survive a relatively slow rate of dehydration that enables them to active their protective mechanisms.

DROUGHT RESPONSES AND DROUGHT TOLERANCE (31 – 63)

For most vascular plants, desiccation is not an option; they must retain high cellular water content, by efficient uptake and retention of the water that is available. This can be achieved through the long-term adaptations that characterize xerophytes (dry plants) as well as short-term responses to water deficit.

XEROPHYTES: PLANTS ADAPTED TO EXTREMELY DRY ENVIRONMENTS (31 – 32)

Two adaptive strategies are often found in desert-dwelling plants. Desert ephemerals spend most of their lives as dormant seeds or bulbs and only sprout when water is available. Xerophytes are longer-lived plants that typically have reduced leaf area, water-storing leaves or stems, deep or broad roots etc. Crassulacean acid metabolism is also common, allowing plants to keep their stomata closed during the heat of the day. The similarity of many xerophytes can be attributed to convergence, as this harsh environment tends to select for a limited set of traits.

PLANT RESPONSES TO WATER DEFICIT (33 - 63)

Mesophytes are plants adapted to a generally moister environment than xerophytes. Nevertheless, they can respond to short-term periods of water deficit through acclimations that enhance water uptake or enhance water retention, or minimize water-deficit mediated damage.

Severity and timing of water deficit affect responses (33 – 35)

www.plantcell.org/cgi/doi/10.1105/tpc.114.tt0514

The responses of plants to water deficit are complex, and differ depending on the way and rate that it is experienced by the plant, the type of plant, and the age and developmental stage of the plant. Nevertheless, some responses are fairly well conserved and described here.

Perception of water deficit (36 – 38)

What is the molecular basis for water deficit sensing? This remains unknown, but it could involve changes in pressure or osmotic potential.

Water deficit signaling (39 – 40)

The experience of water deficit in one tissue is signalled to other tissues, possibly through hydraulic and chemical signals. The nature of hydraulic signals and responses are unknown. By contrast, the downstream responses to abscisic acid (ABA), a hormone produced in response to water deficit are well characterized and described in a separate Teaching Tool. ABA responses include transcriptional changes and stomatal closure.

Transcriptional response to water deficit (41 – 42)

Because of the well-developed tools, transcriptional responses are some of the best characterized responses to water deficit. Some are mediated by ABA, and others not. A suite of transcription factors are activated and they in turn activate target genes. Amongst those induced are genes involved in metabolic and signaling pathways, osmotic adjustment, hydraulic conductance and cellular protections.

Drought effects on stomatal closure (43 – 46)

In most plants, a decrease in stomatal conductance is an easily observed and rapid response to ABA or water deficit, and the mechanisms are well characterized and involve changes in activity of membrane-localized ion channels, pumps and transporters.

Drought effects on photosynthesis (47 – 49)

Closing the guard cells is an important acclimation to water deficit because it conserves water by lowering the rate of transpiration, but it also lowers the plant’s ability to take up CO_2, which limits the rate of photosynthesis. The relationship between transpiration and photosynthesis is not linear though, and some stomatal closure can cut down on transpiration without affecting photosynthetic rate. More severe water shortages, leading to more severe stomatal closure, does cause a restriction in photosynthetic carbon assimilation which leads to a depletion in the energy available to the plant.

Water deficit effects on growth and development (50 – 61)

Effects on leaf growth (52 – 56)

Given the impact of water limitation on photosynthetic rate, it is not surprising that water deficit can lead to a reduction in plant growth rate. However, it is interesting to note that leaf expansion stops at milder water deficit than the arrest of photosynthesis, indicating that it is not a consequence of decreased energy for growth, but instead due to other factors. These can include mechanical or hydraulic limitation (not enough water to force cell expansion), or be mediated by hormonal signals restricting cell proliferation or expansion.

Root responses to water deficit (57 – 59)

By contrast to the situation observed for leaves, the rate of elongation of the primary root is usually maintained under water deficit, a response that is mediated by ABA. Obviously, by maintaining root elongation, the plant increases its chances of reaching deep water reserves. .

Water deficit effects on reproductive development (60 – 61)

A plant’s developmental stage affects how it is affected by water deficit, and for some cereals the reproductive phase is very sensitive to water deficit.

www.plantcell.org/cgi/doi/10.1105/tpc.114.tt0514

TOWARDS WATER-OPTIMIZED, DROUGHT-TOLERANT AGRICULTURE (63 - 101)

Preventing water deficit through optimized irrigation (64 – 69)

Soil moisture sensors and remote monitoring of rainfall (64 – 65)

One of the best strategies for using water more efficiently is to only use irrigation water when it is needed. This can be enhanced through technologies like soil moisture sensors, and better weather forecasting.

Optimizing irrigation (66 – 67)

The way that water is applied can be improved, through things like drip or micro-irrigation strategies, and by using variable rate irrigation that provides the right amount of water to each part of the field.

Deficit irrigation practices (68)

Deficit irrigation or partial root-zone drying methods are proving effective in some regions. The idea is that if some of the plant root system experiences water deficit, the available water will be used more efficiently (through decreased transpiration) without limiting growth.

Diagnosing water deficit by monitoring plant physiology (70 - 85)

The optimal time to provide water to a plant is before it experiences a growth-limiting water stress, but how can we determine when a plant needs water? Can these methods be applied during breeding strategies?

Quantifying photosynthetic rate and efficiency

Chlorophyll fluorescence is a non-invasive method for measuring photosynthetic efficiency, but it really is only useful for indicating late-stages of drought stress. Using gas exchange measurements, the rate of CO_2 uptake can be measured at the plant or field scale.

Quantifying transpiration

Several methods can be used to measure transpiration, including the weighing of a sealed pot and thermal imaging (transpiration removes heat with the phase change of water from liquid to gas, so transpiring plants are cooler). These methods can be used in high-throughput phenotyping platforms as well as remote larger scale sensing.

Quantifying water use efficiency

Water use efficiency (WUE) is a useful parameter to measure as it compares the amount of water used by the plant to the amount of biomass or CO2 accumulated. This can be measured at field-scales or the level of individual plants through several methods.

Stable isotope analysis as a proxy for WUE

Stable isotope analysis can be used to determine WUE, and is based on the assimilation of ^{13}C versus ^{12}C. The enzyme Rubisco that fixes carbon in C_3 plants preferentially fixes ^{12}C, so as stomatal conductance decreases, the concentration of ^{13}C in the intercellular pool increases. Therefore, the isotope differential ($\delta\ ^{13}C$) reflects stomatal conductance.

Phenotyping platforms

Automated greenhouses and phenotyping platforms can increase the precision by which phenotypic measurements are made, and are becoming increasingly important for breeding purposes. Robotic systems move plants in pots through weighting and imaging stations to record growth, water use, and other parameters. Field-level phenomics are also possible using specialized wheeled vehicles and flying blimps or drones.

BREEDING FOR DROUGHT TOLERANCE (86 - 101)

Can insights into how plants respond to water stress be applied towards improved drought tolerance in crop plants? Can studies on model organisms be translated and applied towards the crop plants that feed us?

www.plantcell.org/cgi/doi/10.1105/tpc.114.tt0514

Classical approaches (forward genetics) (86 – 93)

The classical or forward genetics approach depends on the desired traits being present in the starting population. For some crops, it may be necessary to extend the breeding population to include older varieties and to explore the genetic diversity of wild relatives. For any breeding strategy, it is also important to know what you are selecting for. Is the ability to survive extreme desiccation going to be a good selection criterion if you want to breed plants that produce a bit more grain under mild water deficit? Finally, any potentially beneficial trait under drought may have a negative consequence under other conditions.

Candidate gene approaches (94 – 97)

The other approach starts with genes identified as associated with water-deficit responses or tolerance, and modify them to try to enhance drought tolerance. A lot of different genes have been tested, and many show promise in controlled or laboratory settings. At this point, only one gene, a bacterial RNA chaperon, has made it to commercial distribution.

Networks, systems and modeling as avenues of research (98 – 100)

New types of analysis are being applied to plant breeding and management programs. These include network and systems analyses and modeling approaches. Their benefit lies in their ability to identify key hubs in large amounts of data, and also to run experiments virtually to identify those with greatest promise.

FUTURE CHALLENGES (101 – 103)

Agriculture is by far the largest user of fresh water resources and the demand for water will increase with increasing human population and standard of living. Water limitations and accompanying food shortages are expected to contribute to significant human suffering and ecosystem degradation in coming years. The study of plant-water relations is one of the most complex and important topics in plant biology and also one that is progressing most rapidly.

www.plantcell.org/cgi/doi/10.1105/tpc.114.tt0514

Slide concepts

Slides	***Table of contents – concepts***
1	**How plants manage water deficit and why it matters**
2	Outline
3	Key definitions and concepts
4	**Water scarcity is a growing problem**
5	Pressure on freshwater resources threatens security and biodiversity
6	The earth's surface is 75% water, but most is not available to plants
7	Water is both a renewable and a nonrenewable resource
8	The Ogallala (High Plains) aquifer is being depleted of its reserves
9	Agricultural yields in most countries are likely to decrease by 2050
10	The growing population demands a more efficient use of water
11	**Some plants and plant tissues are desiccation-tolerant**
12	Some plants are desiccation or drought tolerant, others not
13	Plants fall in a continuum from desiccation tolerant to sensitive
14	**Desiccation-tolerant plants**
15	Vegetative desiccation tolerance is the ancestral strategy
16	Bryophytes, "non-vascular" plants, are mostly desiccation tolerant
17	Most seeds are desiccation tolerant (orthodox), some not (recalcitrant)
18	Orthodox seeds go through a desiccated state
19	Most pollen desiccates for stability during dispersal
20	Some vascular plants are desiccation tolerant
21	Only about 0.2% of angiosperms are desiccation tolerant
22	Leaves and cells often fold or curl inwards during desiccation
23	Cellular responses to desiccation
24	Desiccation may induce stabilization of membranes and proteins
25	LEAs, intrinsically-disordered proteins, confer protection
26	The cell wall structure contributes to desiccation tolerance
27	Antioxidant systems are induced by dehydration stress
28	Enzymatic and non-enzymatic antioxidants detoxify ROS
29	All plants respond to mild water deficit; DT plants take it further
30	**Desiccation Tolerance: Summary**
31	**Xerophytes: Plants adapted to extremely dry environments**
32	Similar features have evolved in disparate families
33	**Plant responses to water deficit**
34	The severity of water deficit affects plant responses
35	Rate of water deficit and genotype affect plant response and survival
36	Water deficit: Perception – signaling - responses
37	Water deficit occurs when efflux exceeds influx
38	Water deficit may be perceived at the cell membrane
39	Water deficit signaling
40	Abscisic acid accumulation is a rapid response to water deficit
41	Transcriptional responses to water deficit are well characterized
42	The drought response is complex and pleiotropic

www.plantcell.org/cgi/doi/10.1105/tpc.114.tt0514

43	Water deficit induces stomatal closure & decreased photosynthesis
44	A decrease in stomatal conductance g_s is a rapid response to water deficit
45	ABA alters ion channel activities to promote stomatal closure
46	Plants that cannot close their stomata lose internal cellular pressure (turgor) and wilt
47	Water deficit can affect CO_2 concentration in the chloroplast
48	Water deficit can affect photosynthetic carbon assimilation
49	Water deficit can but does not have to affect photosynthesis
50	Water deficit affects growth rate
51	What controls growth under water deficit?
52	Water limitation interferes with leaf expansion and function
53	Decreasing leaf elongation is not due to a decrease in photosynthesis
54	Growth restriction may be independent of photosynthesis, in the short term
55	Water deficit responses are specific to developmental stage and age
56	Plants have to balance their growth and survival responses
57	Adaptations of roots to water deficit depend on severity and genotype
58	Growing deeper is an adaptive response and under genetic control
59	Enhanced root elongation to reach deep water is mediated by ABA
60	Reproductive development can be particularly drought sensitive
61	Drought at the wrong time can drastically reduce seed yield
62	**Summary: Plant responses to water deficit affect many processes**
63	**Towards water-optimized, drought-tolerant agricultural plants**
64	Prevention: Information can guide planting and watering schedules
65	Prevention: Forecasting droughts
66	Prevention: Less wasteful irrigation strategies
67	Variable rate irrigation can support good yields with less water use
68	Prevention: Partial root-zone drying can conserve water
69	An ideal climate-smart agricultural landscape of the future
70	Diagnosing plant physiological activities and drought stress effects
71	Physiological parameters can be monitored in the lab or field
72	Quantifying photosynthetic operating efficiency by fluorescence
73	Quantifying photosynthetic operating efficiency by fluorescence
74	Quantifying photosynthesis by gas exchange measurements
75	Transpiration can be measured as water transpired or heat lost
76	Water use efficiency (WUE) is the ratio of CO_2 assimilated to H_2O transpired
77	WUE can be measured by non-invasive imaging methods
78	WUE can be determined by stable isotope analysis
79	If stomata are closed, Rubisco discrimination tends to enrich the internal [CO_2] in $^{13}CO_2$
80	Isotope discrimination indicates water use efficiency in C_3 plants
81	Large phenotyping facilities can be shared by many users
82	Multispectral imaging provides information about plant status
83	3D imaging of single plants or fields can contribute to phenotyping
84	Breeding programs depend on high-throughput phenomics tools
85	Scaling up: Field-wide surveys for breeding programs
86	**Breeding approaches for drought-tolerant plants**

www.plantcell.org/cgi/doi/10.1105/tpc.114.tt0514

87	Translational research: From model systems to real-world complexity
88	The goal of most breeding programs is high yield, even with water deficit
89	For success, drought-tolerant traits have to be in the breeding population
90	Components of yield under drought stress: WU, WUE and HI
91	Different “drought tolerance” traits have different consequences
92	Under the “right scenario”, nearly *any* trait can confer tolerance
93	**Breeding for drought tolerance: Candidate gene approach**
94	The regulatory pipeline for genetically engineered plants is long
95	Biotech plants with increased drought tolerance are growing
96	Many genes show promising results for drought tolerance
97	Modeling approaches contribute to plant breeding and production
98	The modeling – testing iterative cycle informs breeding
99	Modeling allows experiments to be carried out virtually
100	**Summary: Strategies for water-optimized, drought-tolerant plants**
101	**Future directions: Translating from model systems into crop plants**
102	Water scarcity may be one of the major threats to human security
103	How will California’s agriculture be affected by drought in 2014?

www.plantcell.org/cgi/doi/10.1105/tpc.114.tt0514