

Wild plant resources for studying molecular mechanisms of drought/strong light stress tolerance

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Abstract Drought is one of the major environmental factors restricting plant productivity worldwide. Under drought in the presence of strong light, plants are liable to be damaged by excessively-absorbed solar energy as well as dehydration of their tissues. Wild plants found in the arid zones are equipped with specialized mechanisms for either avoiding or tolerating drought, and successfully adapt to the harsh environments. Recent molecular studies on the wild plants have shed new lights on the unique features of their resistance mechanisms, which are markedly different from those found in the model and/or domesticated crop plants.

Key words: Wild plant, drought/strong light tolerance.

In nature, plants are exposed to various adverse environmental conditions, which restrict their productivity and even their survival. Water deficits are estimated to be the prime factor suppressing net primary production of the vegetation of the earth (Larcher 1995). This suppression is predominant in the arid and semi-arid zones between the latitudes 15° and 30°N and S, which occupies *ca.* 40% of the earth's land. Even in the temperate zone, water shortage is recognized as a prime factor limiting crop yield (Boyer 1982).

Water is an absolute requirement for plant growth and development. In C₃ plants, the amount of water lost by leaf transpiration through stomata is estimated to be 500 to 1000 times larger than that of CO₂ assimilated in the leaves on a molar basis (Larcher 1995; Yokota et al. 2006). Therefore, assimilation of carbon via photosynthesis incurs huge water costs in plants. Moreover, water transpiration from the leaves is important for lowering the leaf temperature (Nobel 1999). As soil water content decreases under drought in the presence of strong solar radiation, plants close their stomata, and photosynthetic CO₂ fixation is strongly suppressed (Cornic 2000). Under such conditions, excessively absorbed light energy that cannot be consumed through photosynthetic metabolism is liable to bring an increased risk of oxidative injury and photoinhibition (Price et al. 1989). Therefore, plants are

exposed to multiple physicochemical constraints under drought/high light stresses, ranging from excess light energy, dehydration and high temperature.

To adapt to the drought and strong light environments, terrestrial plants has developed various mechanisms in evolution. These adaptive mechanisms include those for stress-avoidance as well as stress-tolerant strategies, and are particularly manifest in wild plants inhabiting arid and semi-arid regions. In this mini-review, we would like to focus on recent physiological, biochemical and molecular studies on these plants, in which diverse strategies for coping with drought/strong light stresses have been elucidated.

Xerophytes as model systems for studying drought/strong light stress tolerance

Plants which survive in the environments with limited water resources are called xerophytes (*xero* means dry, *phyte* for plant), which include at least 20,000 economically-useful plants (Wickens 1998) and possess a number of mechanisms to adapt to dry habitats. Survival strategies employed by these plants are categorized into three groups (Larcher 1995); (i) drought-escaping strategy is observed typically in ephemeral plants, which germinate and complete their cycle of vegetative growth when water is available in the

short rainy season. Most plants of this kind survive dry seasons as inert, desiccation-tolerant forms such as seeds or dormant organs. (ii) Desiccation-avoidance strategy involves maintenance of the hydration status of plant tissues despite dryness of the surrounding air and soils. (iii) Desiccation-tolerant strategy represents the ability for tolerating severe desiccation of the plant tissues. Typical examples employing this strategy are observed in so called “resurrection plants”, which are characterized by the ability to tolerate nearly complete desiccation of their tissues, and by the surprisingly rapid recovery from their desiccation state to the photosynthesis-competent rehydration state upon resuming water supply (Gaff 1977). For example, *Craterostigma plantagineum* (Figure 1A) from South Africa is tolerant to severe desiccation in which 85% of the initial fresh weight of the leaves is lost, and its photosynthesis and other metabolisms are rapidly recovered to the original levels upon resuming irrigation (Bartels et al. 1990; Bartels and Salamini 2001).

Biochemical and molecular studies on *C. plantagineum* have offered interesting insights on how these resurrection plants survive extreme desiccation. Under the rehydration state, *C. plantagineum* accumulates unusual C₈ sugar 2-octulose up to 400 mg/g dry weight, which constitutes 90% of the soluble sugars

in the leaf cells (Bianchi et al. 1991). Water deficits induce conversion of 2-octulose to sucrose, which accumulates in large quantity in the desiccated leaves. Accumulation of sucrose has been commonly observed in many resurrection plants, which may confer desiccation tolerance by inducing vitrification of the cytosol, thereby stabilizing the cellular macromolecules and intracellular structure under desiccation (Ingram and Bartels 1996). Molecular studies revealed that global change in the gene expression patterns in the early phase of water deficits in the leaves of *C. plantagineum* (Ingram and Bartels 1996; Bartels and Salamini 2001). These stress-inducible factors include late embryogenesis-abundant (LEA) proteins, enzymes for sugar metabolism, transporters for sugars, and factors for transcriptional regulation and signal transduction, suggesting the occurrence of complex interplay between these factors for establishing dehydration tolerance in this plant.

An experimental system for genetic transformation is available in *C. plantagineum*, which enabled to isolate regulatory factors involved in the desiccation tolerance in this plant. The *CDT-1* gene isolated by activation tagging was shown to be essential for dehydration tolerance in this plant (Furini et al. 1997). *CDT-1* encodes a

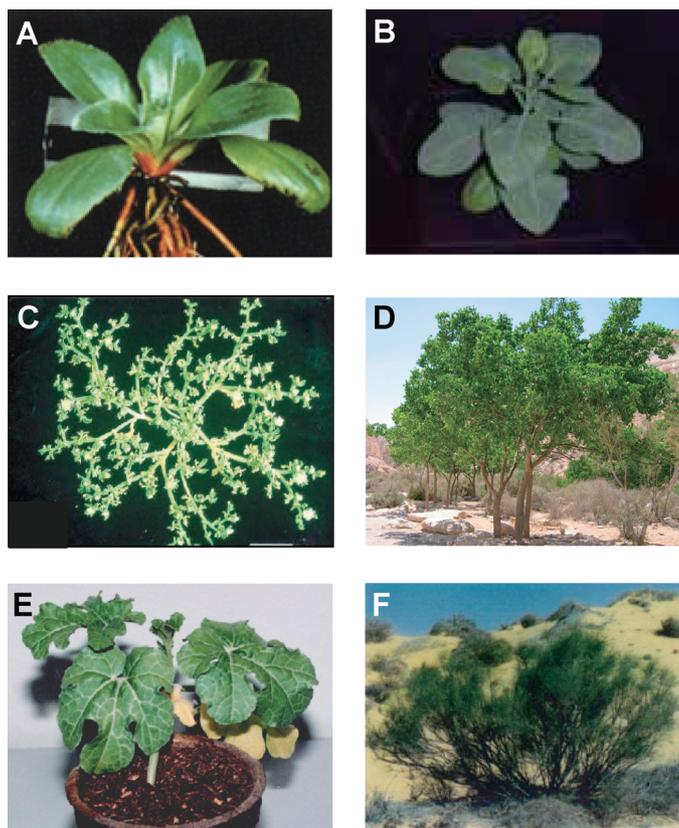


Figure 1. Wild plants with drought/strong light stress tolerance. (A) *C. plantagineum* (resurrection plant; adapted from Bartels et al. 1990). (B) *N. gularuca* (tree tobacco; Cameron et al. 2006). (C) *M. crystallinum* (ice plant; Adams et al. 1998). (D) *P. euphratica* (Brosche et al. 2005). (E) *C. lanatus* (wild watermelon). (F) *Retama raetam* (Mittler et al. 2001). Reprinted with permissions of *Planta* (A), *Plant Physiology* (B), *New Phytologist* (C) and *Plant Journal* (F).

regulatory RNA or short peptide involved in the activation of ABA signal transduction pathway. *CDT-2* gene isolated by a similar screening procedure showed high sequence similarity to *CDT-1*, suggesting that this *CDT1/2* family play an important role in the signal transduction for desiccation tolerance in this plant (Smith-Espinoza et al. 2005). No homologous genes have been found in the *Arabidopsis* genome, suggesting that this plant develops unique signal transduction system to adapt to the severe environmental conditions.

Mechanisms for maintaining water status

Minimizing loss of water from the plant tissues offers an obvious advantage to xerophytes, especially for the ones employing desiccation-avoidance strategy. Stomata in this kind of xerophytes are typically smaller in size than those found in mesophytes, and often hidden in the grooves on the lower side of a leaf with dense coverage of hairs, which effectively reduce water transpiration under dry air (Larcher 1995). QTL analyses in *Arabidopsis* also suggested importance of stomatal morphogenesis in water-use-efficiency of plants (Masle et al. 2005), which revealed that *ERECTA*, a gene for leucine-rich repeat receptor-like kinase, was a major determinant for water-use-efficiency of this plant via affecting stomatal density and epidermal cell morphogenesis.

Thick cuticle layers are advantageous for suppressing water loss from the leaves (Nawrath 2006). In a tree tobacco *Nicotiana glauca* (Figure 1B) which inhabit in the arid region of South America, drought-induced deposition of cuticular wax was temporally correlated with the up-regulation of a gene for lipid transfer protein (Cameron et al. 2006), suggesting that the fortification of cuticle layer under stress is genetically controlled in this wild plant. Genetic studies in *Arabidopsis* and *Z. mays* have led to the identification of many genes involved in the biosynthesis of cutin and wax compounds (Nawrath 2006). For example, *shine* (*shn*) gain-of-function mutant isolated by activation-tagging was characterized by the increase in wax deposition in the cuticle layer, which conferred increased tolerance to drought in this mutant compared to wild type (Aharoni et al. 2004). The *SHN* gene encoded an AP2/EREBP-type transcriptional factor, which regulates wax biosynthetic genes.

Crassulacean acid metabolism (CAM) is known as one of the adaptive mechanisms for performing photosynthesis under water-limited environments (Ehleringer and Monson 1993). In CAM plants, stomata open at night, and atmospheric CO₂ is initially fixed as a C₄ acid, which is then stored in the vacuole of the photosynthetic cells. In the daytime, CO₂ is released by decarboxylation of the C₄ acid, which is then re-fixed by the operation of RuBisCO in the photosynthetic carbon

reduction cycle (Dodd et al. 2002). Water-use-efficiency in CAM plants is generally 3 to 10 times higher than those in C₃ plants (Larcher 1995).

CAM occurs in approximately 6% of the terrestrial angiosperms, and displays enormous species-specific variations concerning the metabolic scheme and its regulation (Dodd et al. 2002). One example is illustrated by the facultative CAM species, which operate C₃ photosynthesis at times of sufficient water supply but switch to CAM during periods of water limitation (Cushman and Borland 2002). In *Mesembryanthemum crystallinum* (Figure 1C), induction of CAM is accompanied with the up-regulation of the key enzymes such as phosphoenolpyruvate carboxylase and NADP-malic enzyme (Adams et al. 1998; Cushman and Bohnert 1999; Cushman and Borland 2002). Pharmacological studies have indicated that intracellular Ca²⁺ signal is implicated in the induction of these genes (Taybi and Cushman 1999). A gene for drought-induced Ca²⁺-dependent protein kinase McCPK1 was characterized in *M. crystallinum*, which undergoes stress-induced reversible change in its subcellular localization from the plasma membranes to nucleus and endoplasmic reticulum, suggesting the involvement of this protein in the Ca²⁺ signal transduction under stress (Chehab et al. 2004). To understand molecular basis of CAM induction process, integrative genomic approaches including large-scale EST analysis (Kore-eda et al. 2004) and DNA microarray (Cushman and Borland 2002) have been conducted in this plant.

Water storage as well as efficient water acquisition are the important traits for plants in the arid and semi-arid zones. Baobab (*Adansonia rubrostipa*) stores huge amount of water in the stem, and prepares for the long-term water deficits (Chapotin et al. 2006). Succulent plants such as *Opuntia acanthocarpa* and *Echinocereus engelmannii* accumulate massive amount of water-binding mucilage in their stem up to 35% of their dry weight, which serves as a large capacitor for water (Nobel 1992). *Populus euphratica* (Figure 1D) is a drought-tolerant woody species found in Central Asia and Middle East, which possesses developed root system for the access to deep water tables (Hukin et al. 2005). Comprehensive analyses including large-scale EST, DNA microarray and metabolome have been reported in this plant (Brosche et al. 2005).

Wild watermelon (*Citrullus lanatus*; Figure 1E) is a C₃ plant inhabiting in the Kalahari Desert in Africa (Yokota et al. 2002). Physiological study has shown that root development of this plant was significantly stimulated in the early phase of drought stress (Yoshimura et al. 2008). Proteome analysis in the root has revealed that drought stress induced a set of proteins related to root morphogenesis and carbon/nitrogen metabolisms in the early phase of the stress, whereas factors related to lignin

biosynthesis and molecular chaperones are up-regulated in the later phase (Yoshimura et al. 2008). These observations suggested that this xerophyte switches survival strategies during the progression of drought stress, from a strategy for acquiring new water resources to that for preserving tissue water, by regulating its root proteome in a temporally programmed manner.

Unique mechanisms for high light tolerance

Photosynthetic apparatus in the thylakoid membranes in chloroplasts is one of the potential sites which are liable to be damaged under drought in the presence of high light. Under this excess light condition, some of the photosynthetic components in the chloroplasts tend to be over-reduced, which enhances the risks for photoinhibition (Anderson and Barber 1996). Survival strategies of plants against excess light stress are diverse, which include light avoidance by leaf movement or rolling (Larcher 1995), increase in the light reflection of the leaves by deposition of cuticle waxes (Cameron et al. 2006), shielding from the excess photons by anthocyanin accumulation (Gould 2004), thermal dissipation of excess light energy by non-photochemical quenching in photosystem II (Müller et al. 2001), and fortification of ROS scavenging system (Smirnoff 1993).

Retama raetam (Figure 1F) is a stem-assimilating C_3 xerophyte that thrives in the Mediterranean region. This species contains two different populations of stems in a single plant: although those of the lower canopy are shaded from direct sunlight and maintain capability for photosynthesis, the stems of the upper canopy are photosynthetically inert and establish tissue dormancy under drought conditions. Many of the essential proteins related to photosynthesis, such as RuBisCO, ascorbate peroxidase and D1 subunit of photosystem II are virtually absent in the upper stems (Mittler et al. 2001). However, mRNAs for these proteins are stored in association with polysomes in the cells, and are ready for the rapid synthesis of these proteins when resuming photosynthesis after rainfall. These observations suggest a presence of highly developed adaptive mechanisms in this plant, which protect the entire plant in the times of drought/high light stresses, and enable this plant to compete with other plant species for the limited water resources in the times of short rainy periods.

Another unique mechanism suggested in xerophytes involves a redox chain mediated by cytochrome b_{561} in the plasma membrane and ascorbate oxidase in the apoplasts (Nanasato et al. 2005). Cytochrome b_{561} catalyzes oxidation of ascorbate in the cytosol while regenerating it in the apoplasts, thereby mediating trans-membrane electron transfer from the cytosol to the apoplasts (Asard et al. 2001). Ascorbate oxidase, which catalyzes oxidation of ascorbate in the apoplast to yield

monodehydroascorbate and H_2O (Farver et al. 1994), accumulated abundantly in wild watermelon leaves in comparison to that in the domesticated watermelon (Nanasato et al. 2005). Protein abundance of cytochrome b_{561} increased in the leaf mesophyll cells of wild watermelon under drought stress (Nanasato et al. 2005), suggesting that the activation of this redox chain may contribute to the excess energy dissipation under drought in the presence of strong light.

Accumulation of compatible solutes

In response to water deficits, many plants synthesize and accumulate small organic compounds known as compatible solutes in the cells. Compatible solutes are a group of compounds which do not interfere with cellular metabolisms at high concentrations (Hare et al. 1998; Rontein et al. 2002). Compatible solutes are highly hydrophilic and include amino acids and their derivatives such as proline, glycine betaine and citrulline, and carbohydrates such as sucrose, trehalose, mannitol, pinitol, fructan and raffinose. It has been proposed that these compounds play a role in osmotic adjustment of the cells under water deficits. However, transgenic plants which accumulate them only at lower concentration often displayed significant resistance to stress (Tarczynski et al. 1993; Kishor et al. 1995), suggesting that these compatible solutes may have other functions in addition to the osmotic adjustment. A variety of protective roles have been suggested for the compatible solutes, such as stabilization of cellular proteins and membranes, storage of nitrogen and/or reducing equivalent, and detoxification of ROS (Smirnoff and Cumbes 1989; Hare et al. 1998; Rontein et al. 2002).

Hydroxyl radicals are one of the most toxic agents among known ROS, which react with various molecules in the cells at diffusion-limited rates, and leads to the dysfunction of the cellular activities. The *in vitro* studies have shown that efficiency of compatible solutes for detoxifying hydroxyl radicals is higher in polyol such as mannitol compared to proline and glycine betaine (Smirnoff and Cumbes 1989). The *in vivo* role of compatible solutes as ROS scavenger has been analyzed using transgenic plants in which polyol metabolism was modified. Transgenic tobacco in which *E. coli* mannitol-1-phosphate dehydrogenase was targeted to chloroplasts by the addition of chloroplast transit peptide has been generated (Shen et al. 1997). Mannitol accumulated at an estimated concentration of up to 100 mM in the chloroplast of the transgenic plant, which showed increased tolerance to photooxidative treatment such as an application of methyl viologen (Shen et al. 1997). Therefore, it has been suggested that accumulation of polyol is one effective strategy to protect plant cells from photooxidative damages under drought in the presence of

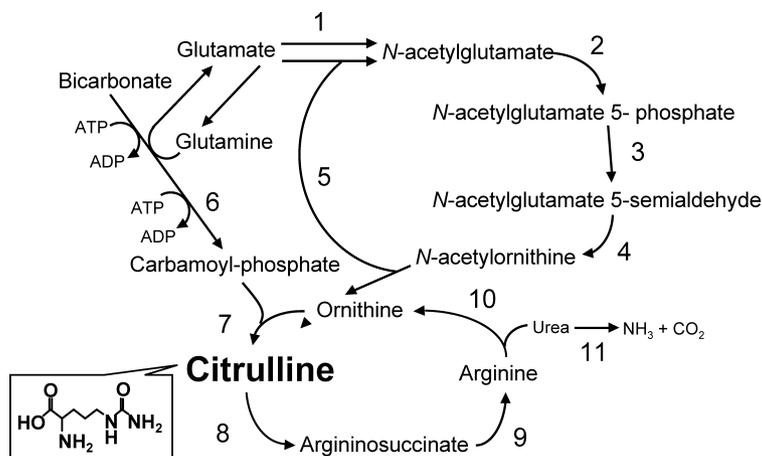


Figure 2. Pathways of citrulline metabolisms. Enzymes that catalyze the indicated reactions are: 1, *N*-acetylglutamate synthase; 2, *N*-acetylglutamate kinase; 3, *N*-acetylglutamate 5-phosphate reductase; 4, *N*-acetylorithine aminotransferase; 5, glutamate *N*-acetyltransferase; 6, carbamoylphosphate synthetase; 7, ornithine carbamoyltransferase; 8, argininosuccinate synthetase; 9, argininosuccinate lyase; 10, arginase; 11, urease. Molecular structure of citrulline is shown in inset.

strong light.

In the leaves of wild watermelon, citrulline accumulates up to $25 \mu\text{mol g}^{-1}$ FW during drought in the presence of high light (Kawasaki et al. 2000). Second-order rate constant of the reaction between citrulline and hydroxyl radicals was shown to be $3.9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, demonstrating that citrulline is one of the most efficient scavengers for hydroxyl radicals among known compatible solutes (Akashi et al. 2001). These observations suggested that hydroxyl radicals generated in the cells are efficiently decomposed *in vivo*, with a calculated half time of approximately 0.9 ns. Citrulline is an intermediate compound in the arginine biosynthetic pathway, in which 11 different enzymes are involved in its metabolism (Slocum RD 2005; Figure 2). Recent study has indicated the intricate regulatory network in the citrulline metabolism of this plant under drought and high light stress (Takahara et al. 2005).

Wild plant genetic resources—Perspectives

Terrestrial plants are overwhelmingly diversified not only for their morphology and life forms, but also for their responses to any kind of external stimuli. For example, there is a significant difference in the transcriptional regulation during drought stress between wild plants and domesticated/model plants. *M. crystallinum* showed drought-induction of a gene for an enzyme, *myo*-inositol 1-phosphate synthase, which involves in the committing step of the biosynthetic pathway for a compatible solute pinitol (Ishitani et al. 1996). However, homologous gene in *Arabidopsis* was not induced during drought. A similar phenomenon has been observed in many other wild plants such as wild watermelon (Akashi et al. 2004), *Lycopersicon pennellii* (Mittova et al. 2002), *Tortula ruralis* (Oliver et al. 2004) and *Xerophyta viscosa*

(Garwe et al. 2003). Integrative analyses on the physiological, biochemical and molecular responses of wild plants under adverse environments will be an important step in unraveling the potentials of plant genetic resources on earth. Information from these studies is expected to reveal a wealth of genes that would be useful in molecular breeding of crop plants in the future.

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