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The Ice Plant Cometh: Lessons in Abiotic Stress Tolerance

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ABSTRACT

Mesembryanthemum crystallinum, the common ice plant, provides a model for the developmentally and environmentally inducible shift from C3 photosynthesis to Crassulacean acid metabolism. Study of its halophytic (salt tolerant) nature has also yielded crucial insights into abiotic stress biology. In addition, the ice plant serves as an anchor species for genomic studies in the order Caryophyllales in which very few species have been studied at the molecular genetic level. Large-scale expressed sequence tag projects allow comparisons of gene expression in different tissues, organs, and developmental states in stressed and unstressed plants during the life cycle. Microarray analyses pinpoint transcripts affected by abiotic stresses to functionally characterize the essential elements that constitute natural abiotic stress tolerance. *Mesembryanthemum* features advantages of a small genome, an emerging transformation system, a growing mutant collection, and the potential for bioremediation of salinized soil because of its ability to efficiently sequester salts into vegetative tissues. Disadvantages are the plant's potentially large size at flowering, a 4-month life cycle under growth chamber conditions, and extreme response plasticity to minute perturbations in the environment.

Key words: *Mesembryanthemum crystallinum*; Common ice plant; Abiotic stress tolerance; CAM pathway induction; Gene expression profile; Diurnal rhythm; Stress-induced transcripts; EST analysis

INTRODUCTION

Mesembryanthemum crystallinum L. (family: Aizoaceae, order: Caryophyllales), also termed the "common ice plant" and the "crystalline ice plant" has emerged as a model organism in plant molecular physiology (Adams and others 1998). The plant's rise to relative fame started with the serendipitous discovery of a stress-inducible switch from C_3 photosynthesis to Crassulacean acid metabolism (CAM) (Cushman and Bohnert 1999; Lüttge 1993; Winter and Smith 1996; Winter and von Willert 1972). The analysis of CAM and CAM induction remains a major strength, with increasing emphasis on molecular genetic and regulatory aspects of this alternative photosynthetic carbon fixation pathway (Taybi and Cushman 1999; Taybi and others 2000). A second major area of interest centers on the plant's extreme stress tolerance, particularly tolerance to high salinity. Within the last decade, the species has become nearly synonymous with halophytic stress responses modeled at the molecular level (Adams and others 1998; Barkla and others 1999, 2000; Nelson and others 1998, 1999; Vera-Estrella and others 1999). The ice plant is increasingly used to contrast its abiotic stress responses to stress-sensitive species (Mis-

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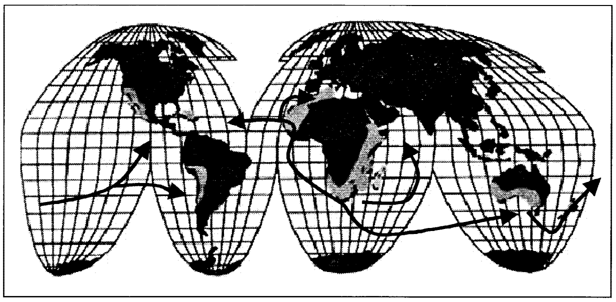


Figure 1. Distribution of *Mesembryanthemum crystallinum*. Originating from southern Africa, the plant seems to have been introduced along prevalent trading routes (arrows), possibly through seeds contaminating the ballast taken in by sailing ships. The present distribution is indicated by light color.

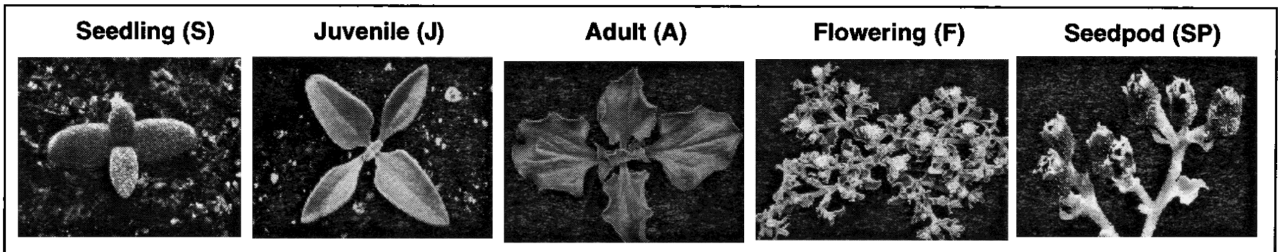
zalski and others 1998; Niewiadomska and others 1999). Recently, the plant gained the distinction of being the only noncrop plant for which large-scale expressed sequence tag (EST) sequencing and microarray analyses were deemed appropriate (Cushman and Bohnert 2000; Walbot 1999). Third, the ice plant has become a model system for molecular studies on the biosynthesis of betalains, a class of chromogenic compounds that replace anthocyanins as leaf, flower, and fruit pigments in most families of the Caryophyllales (Vogt and others 1999a, b). Progress in genetic analyses, transformation protocols (Ishimaru 1999), and efficient regeneration (Cushman and others 2000b) reveal the ice plant as the model of choice for exploring concepts and hypotheses of CAM regulation and mechanisms of stress tolerance among halophytes.

ECOLOGY AND DEVELOPMENTAL PHYSIOLOGY

Mesembryanthemum is native to the Namibian desert in southern Africa. It has been introduced into western Australia, around the Mediterranean, along coasts of the western United States, Mexico, and Chile, and in the Caribbean, seemingly spread by maritime travel (Figure 1). The plant thrives in coastal areas under climatic conditions characterized by short, cool, moist winters and long, dry summers (Bloom and Troughton 1979; Vivrette and Muller 1977; Winter and Troughton 1978; Winter and others 1978).

Mesembryanthemum shows five distinct growth phases during its life cycle (that is, germinating seedlings, juvenile, adult, flowering, and seed-forming) (Figure 2) (see Adams and others 1998). The cold- and moderately salt-tolerant seedlings and juveniles display relatively high rates of vegetative growth by engaging in C_3 photosynthesis as long as soil moisture remains sufficient (Bloom and Troughton 1979; Winter and others 1978). Juvenile plants exhibit a determinate growth pattern consisting of typically seven pairs of broad, succulent primary leaves at a stunted axis (Adams and others 1998). With the onset of progressive drought, the plant switches from C_3 photosynthesis to CAM, which minimizes water loss and ensures reproductive success in the absence of rain and in saline soils (Winter 1985). CAM induction is accompanied by the transition into adulthood with the outgrowth of indeterminate side shoots bearing small secondary leaves and multiple flowers and seed capsules. CAM serves to prolong the period of net carbon gain, resulting in enhanced seed production (Winter and Ziegler 1992). A moderate amount of salinity (0.1–0.2 M NaCl) provides optimal growth. Up to 0.5 M NaCl is tolerated well and accelerates reproductive development. This concentration is well above seawater strength of approximately 0.43 M NaCl. Exposure to more than 0.75 M NaCl impairs growth and seed set (Kuznetsov and others 2000). During seed production, older portions of the plant progressively senesce, die off, and dry out. The developing seed capsules continue to sequester salt and produce viable seeds, whereas the skeleton of the plant provides merely a scaffold that holds the maturing capsules. Within the capsule, stratification occurs such that seeds at the top germinate immediately on imbibition. Seeds at the base of the capsule remain dormant for long times, presumably based on the continued expression of the transcriptional activator VP1 (Fukuhara and others 1999). Under natural conditions, the entire life cycle of the plant is completed within several months, depending on prevailing environmental conditions, but the cycle can be accelerated in growth chambers, for example, by extended photoperiods or high temperature (Cheng and Edwards 1991).

The stress-adaptive mechanisms of salt tolerance and CAM combine to provide a competitive edge in areas similar to the plant's native habitat: the ice plant can become a weed competing with indigenous species in coastal grasslands or with crops on marginal stress-prone agricultural lands. This competitive ability is based on an "osmotic interference" phenomenon by which accumulated inorganic salts are released into the soil (Vivrette and Muller 1977). Also, the leaching of salts from decaying plants seems to inhibit competitors while allowing ice plant seeds to germinate.



Features	S	J	A	F	SP
Photosynthesis	C3	C3	C3 → CAM	CAM	CAM
Leaves	succulent cotyledons	← large primary leaves	→ ← small secondary leaves	→	no leaves
NaCl tolerance level (mM)	150 mM	250 mM	500 mM	>500 mM	>500 mM
EBCs	—	present, inconspicuous	expanding	fully expanded	extreme size in seedpods
NaCl accumulation	—	minor	major	full capacity	EBC only
(Methyl-) Inositol accumulation	—	beginning	partitioning	fully partitioned	extreme in EBC
Betalaine production	—	at leaf tips, stress-dependent	at leaf tips, stress-dependent	at tips of leaves/fruits	at seed capsules
Development	sub-populations in dormancy	~ one leaf pair per week	6-7 leaf pairs, side shoots appear	extensive side shoot growth	only capsules are viable
Endopolyploidy	2C	2C - 16C	2C - 32C	2C - 64C	2C - 256C (256 C in EBC)

Figure 2. Growth phases of *Mesembryanthemum crystallinum* L. Representative plants at the five major growth phases: germinating (1 week), juvenile (3 weeks), adult (6 weeks), flowering (more than 8–9 weeks, salt-stressed, for example, by 500 mM NaCl), and during seed maturation when only seed capsules are still viable (16 weeks). For sizes, plant diameters are given: S (10–20 mm), J (3–5 cm), A (25–40 cm), F (30 cm–1 m), seed capsules are approximately 1.5 to 2.5 cm in length. Size is extremely variable. Under our growth chamber conditions, with plants in approximately 1 kg of soil, the weight of individual plants is 60–80 g after 5 to 6 weeks when side shoots appear. Plants under unlimited root growth conditions, meticulously watered, and kept at moderate temperature and light conditions may exceed 20 kg in weight. Characteristics of individual growth phases are outlined. See also Adams and others (1998) and Taybi and Cushman (1999).

STRENGTHS

Much research with *Mesembryanthemum* in the past has focused on ecophysiologic aspects of its growth and development, yet the plant possesses desirable traits that contribute to its increasing popularity as a model for biochemical and molecular genetic analyses. The plant grows easily and rapidly from seed to produce abundant biomass suitable for biochemical work. The tissues contain negligible amounts of phenolic compounds or other inhibitory substances that could prevent the isolation of organelles, functional proteins, or intact nucleic acids of high purity. *Mesembryanthemum* was the first CAM plant from which photosynthetically active chloroplasts were obtained (Demmig and Winter 1983). Isolated mitochondria were also used to study the role of mitochondria in the CAM pathway (Winter and others 1986). Because of the ease with which relatively clean, intact organelles can be isolated from *Mesembryanthemum,* it was possible for the first time to conclusively study the intracellular localization of enzymes in a CAM plant (Winter and others 1982). Nuclei can be isolated with ease and have been used to study the underlying mechanisms controlling gene expression in response to stress (Cushman and others 1989; Cushman 1995). Abundant proteases in stressed plants may result in protein degradation during isolation (Forsthoefel and others 1998), necessitating the addition of protease inhibitor cocktails to the isolation media. The species is intrinsically resistant to insects and fungal-, bacterial-, or viral-based diseases. We have never had to resort to any special protection, be it in growth chamber, greenhouse, or the field, in more than 15 years of work.

Stress Tolerance Physiology and Biochemistry

Crassulacean acid metabolism. The ice plant is an important model for biochemical and physiologic studies of CAM. CAM, an alternative pathway of photosynthesis is present in approximately 7% of vascular plant species. CAM has evolved multiple times with most CAM species occupying arid regions (true deserts), semi-arid areas with seasonal water shortage (Mediterranean climates), or physiologically dry habitats (canopies of rain forests) (Winter and Smith 1996). Its hallmark is a diurnal fluctuation of carbon such that the initial fixation of CO_2 is accomplished by phosphoenolpyruvate carboxylase (PEPC) during the night when stomata are open. The fixed CO_2 is stored in vacuoles as malate, which is mobilized under light conditions behind closed stomata for final fixation by ribulose bisphosphate carboxylase/oxygenase (RUBISCO) (Cushman and Bohnert 1999). This CO_2 concentrating mechanism improves water use efficiency of CAM species up to fivefold relative to C_3 and C_4 species (Nobel 1996) and provides CAM plants with a competitive advantage in hot, dry climates. When grown at elevated atmospheric CO_2, some CAM species showed an average increase in biomass of 35% without the downward acclimatization of photosynthesis that is typically observed with many C3 species (Drennan and Nobel 2000). It may be that increased atmospheric CO_2 and the associated global warming caused by the burning of fossil fuels and deforestation will enhance the importance of selected CAM species in arid and semi-arid regions, which currently account for approximately one third of the earth's land area.

Because of agreeable biochemical traits, *Mesembryanthemum* is amenable to studies on enzyme regulation (Winter and others 1982; Li and Chollet 1994), metabolite transport and partitioning (Koreeda and Kanai 1997), and carbon flux (Paul and others 1993; A. Dodd and A. M. Borland, personal communication). Studies on the molecular genetic basis of CAM induction and signal transduction have focused almost exclusively on *Mesembryanthemum* (Cushman and Bohnert 1999). The degree or magnitude of CAM induction, as measured by PEPC or PPDK gene expression, protein abundance or activity, varies with leaf or plant age and growth conditions. Both environmental signals and developmental capacity appear to participate in controlling this pathway (Cheng and Edwards 1991; Cushman and others 1990; Herppich and Herppich 1997; Piepenbrock and others 1994; Schmitt and Piepenbrock 1992a).

The characterization of CAM-specific genes, gene families, and their expression in *Mesembryanthemum* has provided important insights into the molecular mechanisms that underlie the evolution and recruitment of genes important for CAM or specialized roles in various tissues within the plant (Cushman and Bohnert 1997, 1999; Cushman and others 2000a). Specific isoforms of a gene family are recruited to serve a CAM-specific role, whereas others maintain housekeeping functions. Alternatively, single genes may serve during both C_3 photosynthesis and CAM (Cushman and Bohnert 1999). The mechanisms by which CAM-, tissue- or cell-specific isogenes have evolved are not fully understood. The evolution of CAM, however, seems to have required gene duplications through recombination or transposon-induced translocation events followed by alterations in regulatory regions. The existence of gene families in plants for an increasing number of functions makes *Mesembryanthemum* an ideal model for understanding the mechanisms underlying gene recruitment in general (Cushman and Bohnert 1999).

CAM induction occurs by the transcriptional activation of genes in *Mesembryanthemum,* as demonstrated by nuclear run-on transcription assays and transient assays using promoter-reporter gene fusions (Cushman and others 1989; Schaeffer and others 1995). Transcriptional activation of CAM genes is based on the interaction of *cis*-acting DNA sequences with *trans*-acting factors that are responsible for salinity stress-responsive gene expression (Cushman and Bohnert 1992; Schaeffer and others 1995). Additional *cis*-elements are predicted to mediate light- and ABA-responsive gene expression (Cushman and Bohnert 1996; Schmitt and others 1996). Alterations in the activation state of PEPC during the 24-h CAM cycle, first recognized in *Mesembryanthemum* (Winter 1982), result from reversible phosphorylation by a stress-inducible protein kinase (Mc-PPCK1) that specifically targets PEPC (Li and Chollet 1994). Changes in kinase activity in both facultative and constitutive CAM species are the direct result of circadian regulation of transcript abundance of the PEPC kinase gene (Hartwell and others 1999; Taybi and others 2000).

Mesembryanthemum has also provided a model for posttranscriptional and translational control mechanisms involved in CAM and abiotic stress gene expression—conveniently induced experimentally. For example, rapid changes in Mc-PPCK1 mRNA abundance may be the result of transcript destabilization. Consistent with this suggestion is that the 3′-UTR of *McPpck*1 contains multiple, canonical downstream (DST) elements (GGA, ATAGATT, and GTA subdomain elements) invoked in mRNA instability in plants (Gutiérrez and others 1999). After CAM induction, *Ppc1* mRNA stability appears to be enhanced (Cushman and others 1990), whereas *RbcS* transcripts encoding the small subunit of RUBISCO decline during development and in response to salinity stress (DeRocher and Bohnert 1993). Accompanying CAM induction, changes in the population of RNA-binding proteins seem to stabilize specific transcripts, and alterations in the translational efficiency of mRNA populations are suggested (Cushman and Bohnert 1999). This aspect of the plant's suitability for understanding plant responses to the environment and the control of development has yet to be exploited fully, but we consider *Mesembryanthemum* an ideal model for studies on posttranscriptional control mechanisms.

Salinity stress, ion homeostasis, and partitioning. In addition to CAM, *Mesembryanthemum* has model character for studies targeting responses to abiotic stresses. It is most useful for focusing on immediate reactions to salt shock, because mature plants can be treated with 0.5 M NaCl in soil (or 0.4 M in aerated hydroponic tanks), resulting in only a transient negative effect on photosynthesis, water relations, and growth. Within 1 day, the plants recover.

The transition to life under stress is accompanied by dramatic changes in gene expression, which are synchronized with and responsible for new developmental patterns (Adams and others 1998; Cushman and Bohnert 1999). Many genes are transcriptionally upregulated by salt stress at all periods of development, such as *Inps1* and *Imt1,* encoding a *myo*-inositol phosphate synthase and a *myo*-inositol *O*-methyltransferase, respectively. Both are key enzymes leading to the production of compatible solutes (Nelson and others 1998). Although this response can occur during juvenile growth, juveniles have a limited capacity to transport sodium into vacuoles and to sequester salt in the specialized epidermal bladder cells (EBC) (Lüttge 1993). Growth in juvenile plants is arrested, possibly because the plants cannot grow side shoots into which sodium may be partitioned. In contrast, the CAM isoform of PEPC (gene *Ppc1*) and other CAM-related genes are not, or only marginally, inducible in juvenile plants. Instead, these genes become progressively transcriptionally inducible as juvenile leaves age and are constitutively expressed in mature leaves. The expression of many genes and individual members of gene families is related to developmental age and the severity of the environmental stress (see Adams and others 1998; Cushman and Bohnert 1999) such that from the responses tolerance mechanisms can be gauged in a developmental context.

Under strictly controlled conditions, the progression of development can provide insights into the coordination between growth phases and gene expression. In plants not maintained under such controlled conditions, however, quite variable results may arise. The most crucial parameters include light intensity, the size of the root system, the timing and duration of stress treatment (weeks after sowing), and rigorously maintained watering schedules. Controversies regarding gene expression and environmental conditions can be resolved by an understanding of the systems that are inducible at any given stage of development. As a consequence, we consider that the ice plant is, strictly speaking, not "facultative" in terms of either halophytism or CAM responses, but that both characters are the result of its genetic adaptation to the environment. The developmental plasticity within the overall progression through distinct stages of development is an asset of the system that can be exploited to probe how environmental conditions affect the expression of sets of genes. Understanding the growth cycle, gene expression profiles, and stress-adaptive measures in *Mesembryanthemum* may help identify genes suitable for manipulation in crop plants (Cushman and Bohnert 1999).

Mesembryanthemum is a sodium includer. After salt stress, sodium accumulates in a gradient from roots (about 70 mM) to the growing shoot apices (in excess of 1 M in EBC). The highest concentration is found in the EBC, trichomes whose basal section is enlarged (Adams and others 1998). Sodium is effectively partitioned to vacuoles especially in the EBC (Barkla and others 2000). The enhanced accumulation of Na^+ and pinitol in EBCs correlates with tonoplast Na^+/H^+ antiport and V-ATPase H^+-transport activities, which are highest in these cells (Barkla and others 2000). Sodium long distance transport seems to be based on sodium/inositol symporters that function in conjunction with sodium/proton antiporters (Nelson and others 1998; Nelson and others 1999; Chauhan and others 2000; Forsthoefel and others 2000). The isolation of genes encoding these transport functions make the ice plant a model for studying sodium partitioning to the vacuole of individual cells and for long distance transport from root to shoot.

Osmolyte accumulation. Water deficit and salinity induce metabolism leading to the accumulation of osmolytes, methylated inositols (ononitol and pinitol), and proline. Their presence in high concentrations in the cytosol balances sodium accumulation in the vacuoles, where sodium may exceed molar concentration (Adams and others 1992). Although EBC are formed early in development, they remain appressed to the surface in unstressed plants. On salt stress, their volume increases dramatically (Adams and others 1998). Explaining the common name of the species, these cells reflect light and give the impression of being coated with ice crystals.

The accumulation of osmolytes is not unique to the ice plant (see Hasegawa and others 2000). The stress-enhanced induction of osmolyte synthesis, accompanied by enhanced carbon flux through glucose 6-phosphate toward inositol synthesis, provides a paradigm for stress sensing, signal transduction, and transcriptional induction of gene expression. The pathway can, however, only be understood in the context of coincident changes in plant morphology and anatomy and in a developmental context. Induction of *myo*-inositol synthesis may be a distinguishing factor between this halophyte and glycophytes with the latter reducing inositol synthesis in response to stress leading to reduced growth (Ishitani and others 1996).

In terms of economical importance, the ice plant certainly is not used as a crop. However, the plant's capacity to synthesize various inositols and their derivatives could become economically important. Because various inositols are of medical value, for example, by providing membrane anchors for surface receptors, the ice plant, after appropriate transgenic modification, could become a renewable resource for such compounds. At present, the development of the ice plant as a species for the removal of sodium from irrigated areas is being explored (Pantoja O, UNAM, Cuernavaca, Mexico; personal communication).

Water transport. The plant's ability for osmotic adjustment is a requisite for continued water uptake under stress. As all plants, the ice plant genome encodes a large number of water channel protein sequences (Kirch and others 2000). Often labeled aquaporins, at least some of these channels are also permeable for urea, glycerol, or other low-molecular-weight metabolites (Tyerman and others 1999). Regulation of water channel transcripts could be initiated by a disturbance of the osmotic potential as salt shock stresses the plants. In addition, amount and location of aquaporin proteins in membranes seem to be regulated; water deficit affects tonoplast- and plasma membrane–located aquaporins differently (Kirch and others 2000; R. Vera-Estrella and colleagues, unpublished). *Mesembryanthemum* is emerging as a model for the study of intracellular protein trafficking, a field that has recently received much attention (Jauh and others 1999). As osmolyte synthesis resumes in the ice plant, transcript levels return to normal, but additional regulation exists, including differential decline of either vacuolar or plasma membrane channels, depending on either osmotic or ionic stress (R. Vera-Estrella and colleagues, unpublished). Water channel synthesis, trafficking, and functioning under stress provide yet another system in which *Mesembryanthemum* can serve as a general plant model.

Plant growth regulators. The ice plant has contributed to defining roles of plant growth regulators as signaling mediators of CAM induction. Salinity and drought stress cause marked increases of endogenous abscisic acid (ABA) (Thomas and others 1992). Endogenous increases or exogenous application of ABA result in CAM induction (Dai and others 1994) by stimulating increased expression of key CAM enzymes (Chu and others 1990; Dai and others 1994; Forsthoefel and others 1995a, 1995b; Tsiantis and others 1996). Other plant growth regulators such as cytokinins either suppress or enhance PEPC expression, depending on the mode of application (Dai and others 1994; Peters and others 1997; Schmitt and Piepenbrock 1992b; Thomas and Bohnert 1993). Endogenous cytokinin levels are negatively correlated with *Ppc1* transcripts during dehydration stress, suggesting that cytokinins act as negative effectors in the expression of CAM (Peters and others 1997). Methyl jasmonate limits PEPC expression (Dai and others 1994; Schmitt and others 1996).

Signal Transduction

Signaling events that lead to CAM induction in the ice plant are known. Reductions in leaf water content and cell turgor pressure are thought to trigger expression of the CAM pathway (Winter and Gademann 1991). Split root experiments indicated that roots may perceive water stress and convey this information to leaves triggering a switch to CAM without a detectable reduction in leaf turgor (Eastmond and Ross 1997). However, water deficit in detached leaves can initiate CAM gene expression (Dai and others 1994; Peters and others 1997; Schmitt 1990), indicating that root-derived signals can be bypassed. Studies with detached ice plant leaves demonstrated a dependence on Ca^{2+} and the involvement of both protein phosphorylation and dephosphorylation events in signaling during CAM induction (Taybi and Cushman 1999). The ice plant has also been used to investigate the role of phytochrome in mediating enhancement of CAM induction (Cockburn and others 1996; see Cushman and Bohnert 1999).

In CAM plants, PEPC kinase activity is regulated by a circadian oscillator that controls the temporal separation of C_3 and C_4 carboxylation reactions by RUBISCO and PEPC, respectively, thereby avoiding futile cycling of CO_2 (Winter 1982; Nimmo 2000). Cloning of PEPC kinase from *Mesembryanthemum* and other CAM plants has provided unequivocal evidence that this kinase is responsible for regulating the allosteric properties of CAM-PEPC by reversible phosphorylation (Hartwell and others 1999; Taybi and others 2000). PEPC kinase expression is under the control of a circadian oscillator that dictates the circadian pattern of CO_2 fixation (Taybi and others 2000).

During the transition from C_3 to CAM, stomatal behavior is reversed relative to stomatal rhythms in C_3 and C_4 plants. *Mesembryanthemum* served as a model for understanding the basis of this "inverse" stomatal behavior. In well-watered plants performing C_3 photosynthesis, blue light is more effective than red light in stimulating stomatal opening. In plants performing CAM, however, illumination with red and/or blue light or treatment with fusicoccin fails to stimulate stomatal opening, suggesting that the blue- and red-light photoreceptors become inactivated or are bypassed during CAM (Mawson and Zaugg 1994). In contrast, ABA-induced stomatal closure was maintained. Inactivation of guard-cell photoreceptors may allow other factors, such as ABA or changes in CO_2 concentration to dictate stomatal movements (Mawson and Zaugg 1994). Although the nature of the inactivated photoreceptors remains uncertain, recent work showed that CAM induction abolishes both white- and blue-light-stimulated stomatal opening and light-dependent zeaxanthin formation (Tallman and others 1997). Inhibition of light-dependent zeaxanthin formation in guard-cell chloroplasts may be a component regulating the shift from diurnal to nocturnal stomatal opening during CAM induction. In summary, the ice plant has been critical to our understanding of the unique control of stomatal behavior in CAM plants.

Functional Genomics

A small genome with developmentally regulated polyploidy. Ice plant nuclei show ploidy levels ranging from 2N to 256N (DeRocher and others 1990), seemingly associated with cell type, development, and stress progression. Ploidy levels of 2N are invariably found in guard cells and phloem companion cells, and ploidies of 8N to 16N are common in mesophyll cells (DeRocher J, Bohnert HJ, unpublished). In the large EBC extreme polyploidy up to 256 N is observed. Yet unexplored is the observation of changes in ploidy connected to stress, which may also be under developmental control. During development, and after stress, ploidy in many cells increases, accompanied by wholesale changes in methylation of genomic DNA (Burjanov J, Cushman JC, Bohnert HJ, unpublished).

The genome size of the ice plant is approximately 370 Mb in nine chromosomes (2N = 18) (Adams and others 1998), or approximately 2.5 times that of *Arabidopsis* (approximately 145 Mb) and slightly smaller than the genome of rice. The analysis of some 30 kb of ice plant genomic DNA indicated that the size increase, relative to *Arabidopsis,* is based on intergenic regions that are approximately twice as long as they are in *Arabidopsis* (DeRocher and Bohnert, 1993; Meyer and others 1990). The small-size genome contributes to the attractiveness of the ice plant model.

cDNA and EST collections. At present, more than 30 cDNA libraries exist in our laboratories alone. They cover the entire life cycle, from seedling to adult and flowering stages, as well as different tissues such as roots, shoots, leaves, flowers, and seed capsules and different stress treatments. Finally, cDNA libraries for EBCs and for meristematic tissues in salt-stressed plants exist. Random collection and sequencing of more than 10,000 clones provided a number of surprises. First, expression profiles indicate significant differences in abundant transcripts in stressed and unstressed roots, shoots, and leaves. Only approximately 20% of the most abundant transcripts in unstressed leaves are also found in stressed leaves. Equally surprising is the number of transcripts from libraries of stressed ice plants that are not represented in the databases compiled for other plants. Indeed, there may be several thousand transcripts reserved for the stressed state that are underrepresented in EST collections from unstressed plants. On the basis of EST expression profiles, being established at present through microarray analyses, connecting the developmental progression of the ice plant's lifespan with specific gene expression patterns will soon be possible (see: http://www.biochem.arizona.edu/bohnert/index.html).

Gene mining in *Mesembryanthemum* indicates a number of transcripts absent or yet to be found in the mainstream plant models. Can we expect the presence of "special" genes for functions specific for a halophyte in *Mesembryanthemum?* It is presently difficult to draw conclusions because any statement made today may be superseded by next week's release of DNA sequences. The number of "novel" transcripts in cDNAs from stressed ice plants, that is, mRNAs that have not (yet) been found in *Arabidopsis,* rice, corn or tomato is much higher in libraries from stressed compared with unstressed plants (Cushman and others, 1999). We consider several possible explanations. These transcripts could encode ice plant–specific genes; an extrapolation from data in the ongoing EST sequencing projects may place this number at not more than 1,000 genes. What we expect are a few genes, possibly only a few dozen, in metabolism that distinguish the ice plant from glycophytes. We expect such genes to participate in carbon metabolism, cell wall structure, methylation reactions, pigment biosynthesis, and stress defenses. Additional genes may contribute to the function of unique signal transduction chains that evolved during adaptation to stressful environments. In essence, we expect that the ice plant is using the ubiquitous signal conduits and, beyond that, duplicated signal transduction chains that are modified for response ranges beyond those present in stress-sensitive species. The presently large number of "novel" ESTs, sequences not known from *Arabidopsis,* in the *Mesembryanthemum* database is rationalized by several explanations. It may indicate a larger than expected number of truly novel proteins, but we also expect that EST sequencing errors, the limited length of these sequences, and the evolutionary distance between *Mesembryanthemum* and the other plant models contribute to the inability to assign homologues for these sequences. We anticipate that gene family expansions have occurred to meet the requirements of adaptive strategies such as CAM and enhanced capacities for functions such as proton pumping, ion sequestration, and stress defenses.

Transformation and regeneration. Transgenic hairy-root cultures, callus tissue, and cell suspensions from *Mesembryanthemum* have been recovered after transformation by *Agrobacterium rhizogenes* and *A. tumefaciens,* respectively (Andolfatto and others 1994; Ishimaru 1999). Efficient regeneration systems for *Mesembryanthemum* (see Cushman and others 2000b) provide an experimental platform for future transgenic analysis aimed at dissecting stress tolerance mechanisms. *In planta* transformation trials using nontissue culture approaches (Clough and Bent 1998) are underway using *Mesembryanthemum* (S Agarie, P Tobin, JC Cushman, unpublished). If successful, these will dramatically reduce cost and labor associated with traditional transformation procedures, while increasing the efficiency of the transformation process. Provided adequate efficiency, which appears possible given the ice plant's susceptibility to *Agrobacterium* transformation in tissue culture, it should be feasible to produce large populations of T-DNA insertion/activation tagged mutants in *Mesembryanthemum.* Success could also prove useful for targeted gene replacement. Such advancements will improve the attractiveness of the ice plant for functional genomics studies addressing specialized traits such as CAM and halophytism.

Mutant collections. Aside from having a small genome, the ice plant has other desirable features that make it an attractive genetic model. Stressed plants produce large quantities of small seeds, and the plant is self-fertile, whereas out-crossing is easily accomplished. Relative to models such as *Arabidopsis,* the ice plant is larger with slower growth. Compared with other CAM or halophytic models, however, the ice plant is fast growing. Furthermore, it is possible to accelerate the normal life cycle of *Mesembryanthemum* from 4 to 5 months to approximately 7 weeks with a prolonged photoperiod (Cheng and Edwards 1991). An extended photoperiod miniaturizes the plants—an important consideration when conducting genetic screening. Mutant collections have been established in *Mesembryanthemum* from fast neutron irradiated or chemically treated (EMS, ethylmethane sulfonate) plants (Adams and others 1998). Selection schemes for the isolation of plants defective in CAM or sensitive to salinity stress have been developed (Cushman and others 2000a).

PRESENT WEAKNESSES OF THE MODEL

An Efficient Transformation System

Although *Agrobacterium*-based transformation of root and callus tissue have been reported (Andolfatto and others 1994; Ishimaru 1999; Cushman JC, unpublished), an efficient procedure for recovering transgenic plants has been lacking. Only recently has an efficient system, based on somatic embryo formation, emerged for the regeneration of plants (Cushman and others 2000b). Improving the efficiency of transformation, preferably using a floral dipping approach comparable to that in *Arabidopsis,* will support future systematic studies of gene function.

Hyperplasticity of Stress Responses

Hyperplasticity of stress responsiveness can be a problem for reproducible physiologic studies. It seems a trivial point, but when working on stress responses or CAM induction, taking good care of the plants is essential to obtain reproducible results. This requires growth facilities in which the duration, spectral composition and intensity of lighting, soil composition and amount, nutrient provision, water relations, and developmental progression are stringently controlled. This may explain why other models, such as *Kalanchoe daigremontiana,* which lack comparable plasticity, are sometimes preferred for studying CAM biochemistry and physiology. In most species of *Kalanchoe,* CAM is little affected by the environment. However, *Kalanchoe* lacks even the beginning of a genetic system and includes few molecular genetic resources.

Plant Size

Plant size can be a problem. For 2 to 3 weeks after germination, ice plants can be grown in Petri dishes. As soon as the oldest primary leaves have reached approximately 3 cm in length, an explosive growth phase begins. *Mesembryanthemum* adapts its size to nutrient availability such that single plants may accumulate biomass of 50 g up to 20 kg. One way to reduce size has been explored (Cheng and Edwards 1991). Long photoperiods or continuous light, low nutrient amounts, and limited root volume induce CAM in juvenile plants and lead to stress symptoms, miniaturize plants and promote early flowering. It should be possible to find ecotypical variations, select for small size, or screen for dwarfism in mutant collections to overcome the inconvenience of large size.

Long Life Cycle

Relative to other plant model systems, *Arabidopsis* in particular, the 4 to 5 months from seed to seed seems long. Attempts should be directed at accelerating the plant's life cycle, identifying more rapidly cycling variants, or searching for more suitable ecotypes.

IMPROVING THE ICE PLANT MODEL

cDNA and EST Collections

Even with more than 30 cDNA libraries for different cells, tissues, stages in development, and different stresses, we think that additional libraries are justified. From these cDNA libraries expression profiles in the form of EST sequences, that is, single pass sequencing from either the 5′ or 3′ ends of the cDNA, should be generated. Current collections do not encompass the entire life cycle of the ice plant nor do they cover enough experimental conditions. cDNA libraries for senescent leaves and roots, for the last stages of seed capsule maturation, for embryo development, and for germination and breaking of dormancy are missing. Also lacking are libraries representing different stages of floral and meristem development and EBC development. Stress conditions, such as cold, drought, or high light conditions are not yet represented. Libraries for the transition from C3 photosynthesis to CAM and the four major phases of CAM likely to contain transcripts with nocturnal or circadian expression patterns are not yet established. We already know about a surprising diversity even of abundant transcripts in the comparison of stressed versus unstressed primary leaves and in the unique transcript population of EBCs. Thus, more detailed comparisons of different developmental stages, tissues, and stress conditions are essential for our understanding of plant development and stress responses.

Sequencing the Ice Plant Genome

Technological breakthroughs make it reasonable to envision the complete sequencing of the ice plant genome. What would we gain from the *Mesembryanthemum* sequence? Apart from a more accurate determination of the total size of the genome, barely more than twice *Arabidopsis,* such an analysis would produce a genomic sequence for an anchor species in the Caryophyllales for comparative genomics studies. Most genome studies have focused on the major crop species of the Cruciferae, Poaceae, and Solanaceae (Gale and Devos 1998). In contrast, very few Caryophyllales species are the subject of genomic mapping or sequencing efforts. Complete sequence information for the ice plant will be important not only for sorting out evolutionary relationships and relationships of gene organization within the Caryophyllales but also for improving our understanding of gene function. Comparisons of related gene families require that relationships among related gene family members be resolved into both orthologs and paralogs. Identification of orthologs—genes or corresponding gene products in different species that evolved from a common ancestor—using clustering analysis is critical to obtain reliable predictions of gene function. In essence, this requires complete genome sequences (Tatusov and others 1997). Defining clusters of orthologous groups will also be important in discerning gene functions common to all plants from specialized gene functions that have evolved in response to selection pressures such as severe environmental stress conditions. In summary, the sequence of the ice plant genome will provide clues about the evolution of redundant gene function in plants, the adjustment in the size of gene families for specialized metabolic and signal transduction pathways, and the development of stress-coping strategies important to many species in the order Caryophyllales, which evolved to colonize environments characterized by water deficit, salinity, or extreme temperature.

TAXONOMIC CONTEXT

The Caryophyllales are an order with families such as the Aizoaceae, Amaranthaceae, Cactaceae, Chenopodiaceae, Caryophyllaceae, Phytolaccaceae, and Portulacaceae. Most families include abundant species adapted to extreme climatic and abiotic stress conditions. The order includes few crop species but includes a number of important ornamental species. Crop species, all of which are of minor economical importance, include spinach, grain amaranth, buckwheat (in the closely related order Polygonales), Opuntias, sugar beet, and other species in the beet family. Ornamentals such as amaranth, carnation, bougainvillea, species of *Mesembryanthemum* and *Lithops* ssp., and cacti are of horticultural value, mainly as ornamentals or ground cover in arid environments. Some species studied for biochemical specialties, such as the biosynthesis of betalaines, are valued for the stunning color variations these pigments provide to horticultural species (Vogt and others 1999b). We view the scarcity of economically important species in this order as a strong point favoring the ice plant model, because preliminary gene mining indicates the presence of novel twists or extensions to general pathway biochemistry, gene regulation, and physiologic mechanisms of growth, development, and stress tolerance. It is this tolerance for which many species in the order Caryophyllales are known and for which *Mesembryanthemum* provides an ideal model.

CONCLUDING REMARKS

Mesembryanthemum's initial rise to prominence as a model in plant physiologic research has contributed significantly to our understanding of carbon metabolism, in particular to the metabolic events leading to CAM. The availability of mutant collections and a versatile and facile transformation procedure will enhance future physiologic studies. Within the last decade, the plant has been adopted as a tool by an increasing number of laboratories interested in plant responses to environmental, abiotic stresses. In part, the growing interest in this species is based on the realization that abiotic stresses, even more than biotic stresses, are the main factors limiting crop productivity. By contrasting stress responses of *Mesembryanthemum* to those of stress-sensitive models, such as *Arabidopsis,* we have begun to realize that most of the "biochemical hardware" is present in all plants and could potentially be used to provide protection. The difference between stress-sensitive and stress-tolerant species, it seems, lies mainly in the "software" that controls how stress signals are perceived, transduced, and how adaptive processes are controlled within the plant. In sensitive species, appropriate stress adaptive responses are either absent, misdirected, or improperly engaged or used (Cushman and Bohnert 2000). In addition, *Mesembryanthemum* seems to be distinguished from salt-sensitive species by a small number of genes for specific stress-defusing pathways and a larger number of genes for ubiquitous functions, which are duplicated to become induced by stress-specific signal transduction circuits. By working with models such as the ice plant, we are convinced that we will learn about plant reactions that are not observed or experimentally reproduced in traditional glycophytic models. We hope that a larger section of the plant community will work with *Mesembryanthemum* and that this will enhance our knowledge of plant behavior under stress conditions. With most of the tools soundly in place to make the ice plant an important and widely used model, we are confident that those still missing are only a short time away.

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