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Living under a 'dormant' canopy: a molecular acclimation mechanism of the desert plant *Retama raetam*

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Summary

Desert plants are exposed to a combination of environmental stress conditions, including low water availability, extreme temperature fluctuations, high irradiance and nutrient deprivation. Studying desert plants within their natural habitat may therefore reveal novel mechanisms and strategies that enable plants to resist stressful conditions. We studied the acclimation of *Retama raetam*, an evergreen stem-assimilating desert plant, to growth within an arid dune ecosystem. *Retama raetam* contained two different populations of stems: those of the upper canopy, exposed to direct sunlight, and those of the lower canopy, protected from direct sunlight. During the dry season, stems of the upper canopy contained a very low level of a number of essential proteins, including the large and small subunits of rubisco, ascorbate peroxidase and the D1 subunit of the reaction centre of photosystem II. However, RNA encoding these proteins was present; cytosolic transcripts were associated with polysomes, while chloroplastic transcripts were not. Upon water application, as well as following the first rainfall of the season, these 'photosynthetically suppressed' stems recovered and accumulated essential proteins within 6–24 h. In contrast, stems of the lower canopy contained the essential proteins throughout the dry season. We suggest that *R. raetam* uses an acclimation strategy of 'partial plant dormancy' in order to survive the dry season. 'Dormancy', as evident by the post-transcriptional suppression of gene expression, as well as the suppression of photosynthesis, was induced specifically in stems of the upper canopy which protect the lower canopy by shading.

Keywords: desert plant, dormancy, drought, environmental stress, reactive oxygen, *Retama raetam*.

Introduction

To date, very little is known about the molecular mechanisms involved in the response of plants to changes in environmental conditions within their natural habitat. This knowledge is critical because in nature, unlike the controlled conditions used in the laboratory, a large number of different stresses may affect the plant simultaneously. For example, desert plants are exposed to a combination of extreme conditions that may change very rapidly within a few hours during the day, and more slowly, but not less extensively, between different seasons (Danin, 1996; McGinnies *et al.*, 1968). These may include high irradiance, low water availability, extreme fluctuations of temperature, high salt concentrations, and nutrient deprivation. Studying the response of desert plants to the combined effect of these stresses within their natural habitat may therefore unravel complex relations between known mechanisms, and possibly reveal novel mechanisms and strategies that enable plants to resist stressful conditions.

The two main strategies employed by desert plants to withstand the extreme environmental conditions of their natural habitat are avoidance, as with growth of winter annuals or resurrection plants only during the rainy season; and resistance, as in the survival of evergreens throughout the different seasons (Cushman *et al.*, 1989; Raven *et al.*, 1992). Although the anatomical and physiological adaptations of evergreen desert plants have been the subject of numerous studies, little is known about the molecular mechanisms that enable these plants to withstand harsh desert environments.

Retama raetam is a stem-assimilating, C_3, evergreen, desert legume common to arid ecosystems around the Mediterranean basin. It uses a number of anatomical and

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physiological adaptations that enable it to acclimate to and grow in a variety of arid environments (Fahn and Cutler, 1992; Mittler *et al.*, 1991; Streb *et al.*, 1997). We studied the acclimation of *R. raetam* plants that grow naturally within an arid dune ecosystem along the Israeli–Egyptian border (Berkowicz *et al.*, 1995). Meteorological conditions within our research sites were closely monitored by weather stations, and plants were systematically sampled. This approach enabled us to study the molecular responses of *R. raetam* plants, to compare them to other evergreens growing at the same sites, and to correlate them with changes in environmental conditions.

Water availability is perhaps the most limiting resource for the growth of desert plants. One of the most prominent effects of this stress on plant metabolism is thought to be the increased production of toxic reactive oxygen intermediates (ROI), such as superoxide radicals and H_2O_2 (Asada and Takahashi, 1987; Biehler and Fock, 1996). Drought-related physiological changes, such as decrease in leaf water content and the accompanying stomatal closure, result in limited CO_2 availability and the channelling of reducing equivalents from the photosynthetic apparatus to the production of ROI, rather than to CO_2 fixation (Krause and Cornic, 1987). A similar unbalanced metabolism is also thought to occur under other stresses such as chilling stress, which may be equivalent to the conditions that occur in the early daylight hours in the desert. During chilling stress, the decreased availability of CO_2 results from the inhibition of enzymes involved in CO_2 fixation at low temperature (Schoner and Krause, 1990). Production of H_2O_2 during drought stress may also result from the catalytic activity of glycolate oxidase in peroxisomes during photorespiration (Osmond, 1981). In order to study the molecular responses of desert plants to these stress conditions, we examined the expression of a number of anti-oxidative, photosynthetic, mitochondrial and housekeeping genes and proteins. These were tested and compared between different plants, different times of day, and different seasons.

Surprisingly, we found that during the dry season the level of a number of essential proteins in stems of *R. raetam* was dramatically suppressed. However, RNA encoding some of these proteins was present. This state of 'dormancy' was specifically induced in stems of the upper canopy which protected the lower canopy from direct sunlight. Following the first rainfall of the season, the low level of proteins in these stems was rapidly restored. We suggest that *R. raetam* uses a molecular acclimation strategy of 'partial plant dormancy' in order to survive the extreme conditions that occur in the desert during the dry season. Although it may not be similar to seed or bud dormancy, we refer to this state of suppressed gene expression as 'dormancy' because it may represent an intermediate level of the 'summer dormancy' used by many desert plants (however, one that is easy to exit from in a rapid and efficient manner).

Results

Characterization of gene expression in the upper and lower canopy of R. raetam *during the dry season*

Retama raetam plants (Figure 1a) contained two different populations of stems: those of the upper canopy, exposed to direct sunlight; and those of the lower canopy, protected from direct sunlight by the upper canopy (a reduction of 40–60% in light intensity). During the dry season about 85% of stems of the upper canopy contained a very low level of a number of essential proteins (referred to herein as 'dormant' stems). In contrast, almost 100% of stems of the lower canopy contained detectable levels of these proteins. About 1–5% of upper or lower stems were not viable and appeared to contain only degraded proteins, as determined by silver and coomassie staining of protein gels (not shown). Figure 1(b) shows a typical comparison between the protein profiles of 'dormant' stems from the upper canopy (U) and stems from the lower canopy (L). The differences observed in the level of proteins between the upper and lower canopy included soluble chloroplastic proteins such as the ribulose-1,5-bisphosphate carboxylase small (RbcS) and large (RbcL) subunits; chloroplastic membrane proteins from the photosynthetic apparatus (the D1 subunit of the photosynthetic centre II, D1; a subunit of the photosynthetic I complex, PsaH; and the cytochrome membrane protein b6f, B6f); and cytosolic proteins such as the defence enzyme ascorbate peroxidase (APX) and the structural protein tubulin. In contrast, the level of histone (H4) was similar in upper and lower canopy stems (Figure 1b), as well as the level of a chloroplastic RNA-binding protein involved in RNA metabolism (not shown). Additional proteins low in abundance in the upper canopy of *R. raetam* during the dry season included the chloroplastic heat-shock proteins (HSPs) HSP70 and HSP20, and Fe- and CuZn-superoxide dismutases (SODs; not shown).

In contrast to the differences observed in the level of proteins (Figure 1b), the amount of transcripts encoding some of these proteins was almost similar in 'dormant' upper stems and in lower stems (Figure 1c, left). 'Dormant' stems appeared to contain the RNA that encodes for some of the 'missing' proteins. To examine the mode of suppression of gene expression in these stems, we isolated polysomes from 'dormant' upper stems and from lower stems, and subjected RNA obtained from these to RNA gel blots. As shown in Figure 1(c) (right), cytosolic-located transcripts such as those encoding for APX and RbcS were found to associate with the polysomal fraction. In contrast, chloroplastic-located transcripts that

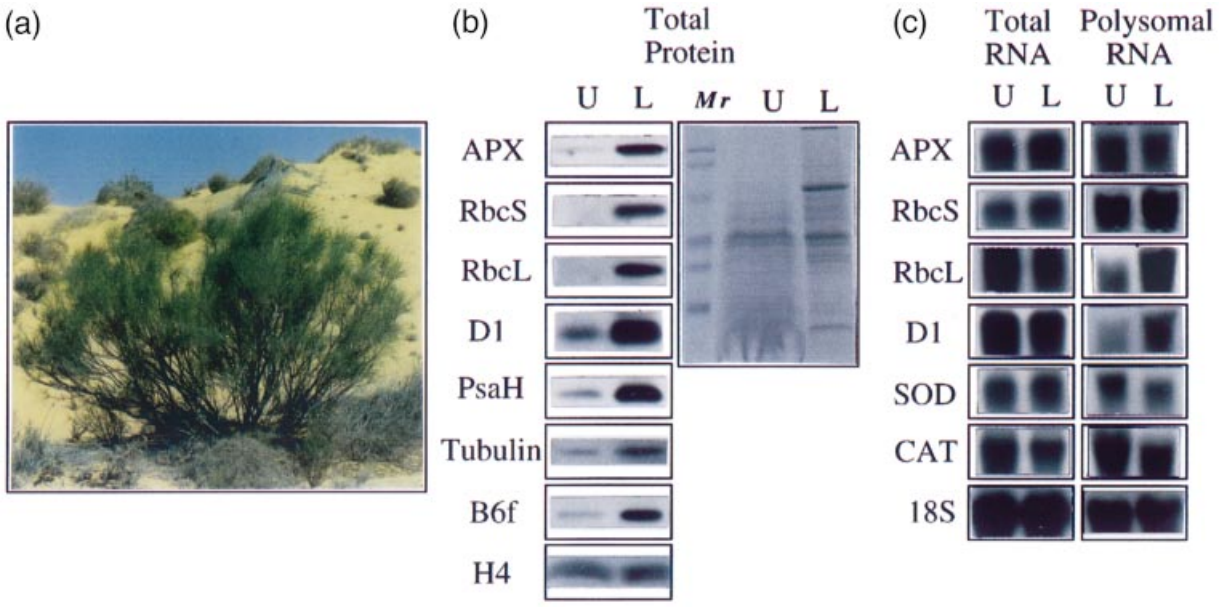

Figure 1. Characterization of gene expression in stems from the upper (U) and lower (L) canopy of *Retama raetam* during the dry season.
(a) An *R. raetam* plant 2.5 m high. Most stems seen here are at the perimeter of the plant and belong to the upper canopy.
(b) Protein gel blots (left), and a coomassie-stained protein gel (right), showing the decrease in level of a number of essential proteins in upper canopy stems.
(c) RNA gel blots of total RNA (left) and polysomal RNA (right) from upper and lower stems showing that RNA encoding for the 'missing' proteins (shown in b) is present in the upper stems. Analysis was performed on eight individual plants during the dry season (April–December), which followed a total of 29.4 mm of rain during the wet season, with similar results. Sampling of stems was performed between 10.00 am and 2.00 pm, with similar results. M_r, molecular weight marker (top to bottom in 1000): 102, 82, 48, 33, 28, 19.

encode for RbcL and D1 (*psbA*) did not associate with polysomes. These findings suggested that the expression of some proteins (the cytosolic-translated APX and RbcS) is controlled by inhibition of translation at the level of elongation or termination, or by post-translational degradation, while the expression of other proteins (the chloroplastic-translated RbcL and D1) is controlled by restricting the interaction of their transcripts with ribosomes. We cannot, however, rule out the possibility that some of these differences may result from differences in our ability to isolate polysomes from the two cellular compartments. In the resurrection plant *Craterostigma plantagineum*, the expression of some members of the transketolase gene family was found to be controlled, during rehydration, by transcript association with polysomes (Bernacchia *et al.*, 1995). Additionally, regulating the expression of RbcS by controlling the translation of mRNA that is kept bound to polysomes was reported in *Amaranth* (Berry *et al.*, 1988).

Ultrastructural characterization of chloroplasts from upper and lower stems

Light and transmission electron microscopy of cell sections obtained from upper and lower stems of *R. raetam* revealed a similar number of chloroplasts in cells from these tissues (not shown). However, unlike chloroplasts from lower stems, which contained unstacked stroma lamellae and stacked grana lamellae (Figure 2b,d), those from 'dormant' stems (Figure 2a,c) contained mainly unstacked stroma lamellae and very few stacked grana lamellae. Chloroplasts from upper and lower stems contained numerous osmophilic droplets (Figure 2a,b). The appearance of mitochondria was similar in 'dormant' upper stems and in lower stems (not shown).

Recovery of dormant stems

The finding that cells from 'dormant' stems contained chloroplasts as well as RNA that encodes for some of the proteins that were very low in abundance (Figures 1 and 2) suggested that they may be able to recover and synthesize these proteins within a short time. To test this hypothesis, we excised upper canopy stems during the dry season, placed them in water, and sampled them for analysis by protein gel blots. As shown in Figure 3, 'dormant' stems from the upper canopy were able to recover and accumulate these proteins (APX, RbcL, RbcS, D1, PsaH and B6f). A time-course analysis (Figure 3a) revealed that many of these proteins were synthesized to their maximum level within 6–24 h. Using this method we were able to induce recovery in about 50% of the 'dormant' stems sampled

during the dry season, suggesting that 'dormant' stems may recover very rapidly during the wet season. Indeed, systematic sampling of *R. raetam* at the transition from the dry to the rainy season revealed that following the first rainfall of the season, almost 100% of 'dormant' stems of the upper canopy recovered and synthesized RbcL (Figure 3b). This finding suggested that stem 'dormancy' may play an important role in the adaptation of *R. raetam* to the desert ecosystem. The differences observed between the percentage of stem recovery obtained artificially during the dry season (about 50%), and the percentage of stem recovery obtained naturally following the first rainfall of the season (almost 100%), may indicate that during the transition between the different seasons, upper canopy stems may go through a certain metabolic change that primes them to undergo recovery immediately following the first rainfall of the season.

Changes in gene expression in 'non-dormant' upper canopy stems

Suppression of gene expression and entering into a state of 'suspended metabolism' is often used by certain microorganisms as a strategy to survive extreme environmental stress (Potts, 1999). It was therefore interesting to examine whether gene expression is also suppressed, at least partially, in 'non-dormant' upper canopy stems subjected to direct sunlight. Figure 4 shows the changes in RNA and protein in these stems at different times (4.30 am to 2.30 pm, every 2 h, 1–6) during a typical day of the dry season, characterized by drastic changes in environmental conditions. Especially notable were the midday time points (Figure 4a, upper panel, 4–6) in which the temperature and light intensity were high and the relative humidity was low. As shown in Figure 4(a), lower panel, the expression of the defence genes CuZn-SOD, APX and catalase (CAT) was upregulated during these hours, suggesting that the intracellular level of ROI may increase in plants during these time points. It is also possible that changes in the expression level of these genes reflect a specific diurnal rhythm.

Interestingly, two of the proteins found to be low in abundance in 'dormant' stems, RbcS and RbcL, were also downregulated at midday in the 'non-dormant' stems (Figure 4b; RbcS, 10.30 am to 12.30 pm, 4–5; and RbcL, 12.30 pm, 5). This finding, as well as the results shown in Figure 1, suggest that *R. raetam* may respond to stress by suppressing the expression level of particular proteins. However, the post-transcriptional suppression of gene expression in the 'non-dormant' stems was mainly that of RbcS and, to some degree, RbcL, while the suppression of gene expression in 'dormant' stems was more intense and included almost all the proteins examined. It is also possible that the level of RbcS and RbcL is dramatically decreased during the stressful hours, due to the induction of a specific proteolytic mechanism. A similar decrease in the level of RbcS and RbcL during the midday stressful hours was not observed in stems from the lower canopy (not shown; see also Figure 1b), suggesting that these stems were not subjected to a similar level of stress to the 'non-dormant' upper canopy stems.

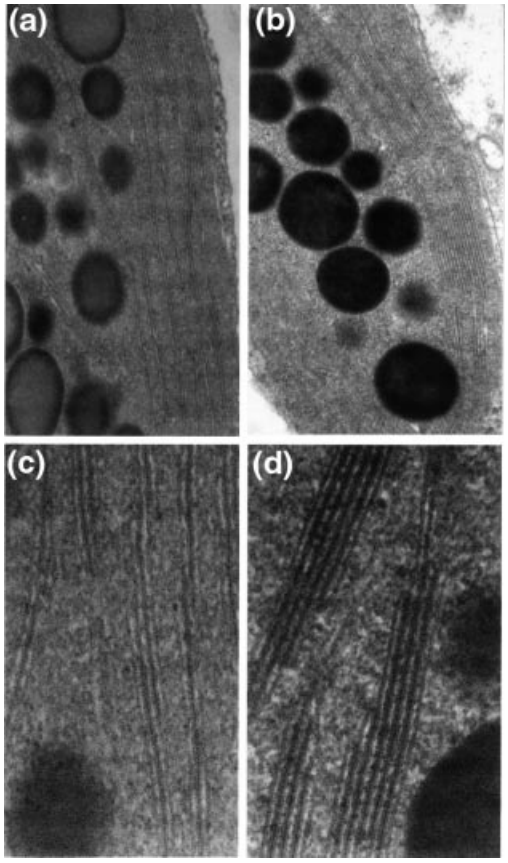

Figure 2. Ultrastructural characterization of chloroplasts from upper (a,c) and lower (b,d) stems sampled during the dry season.
Transmission electron microscopy images of chloroplasts from upper and lower stems are shown in ×33 000 (a,b) and ×100 000 (c,d) magnification. The amount of stacked grana lamellae is shown to be higher in chloroplasts from lower canopy stems (b,d). Osmophilic droplets can be seen in chloroplasts from upper and lower stems (a,b).

Characterization of gene expression in the upper and lower canopy of Anabasis articulata *during the dry season*

From an ecological point of view, it was interesting to examine whether the stem-assimilating, C_4 desert plant *Anabasis articulata*, which is an equally successful evergreen abundant in our research areas (Danin, 1996; Shomer-Ilan *et al.*, 1981), uses a similar molecular acclimation mechanism. A comparison between the levels of different proteins in the upper and lower canopy of this plant (60–90% reduction in light intensity at the lower

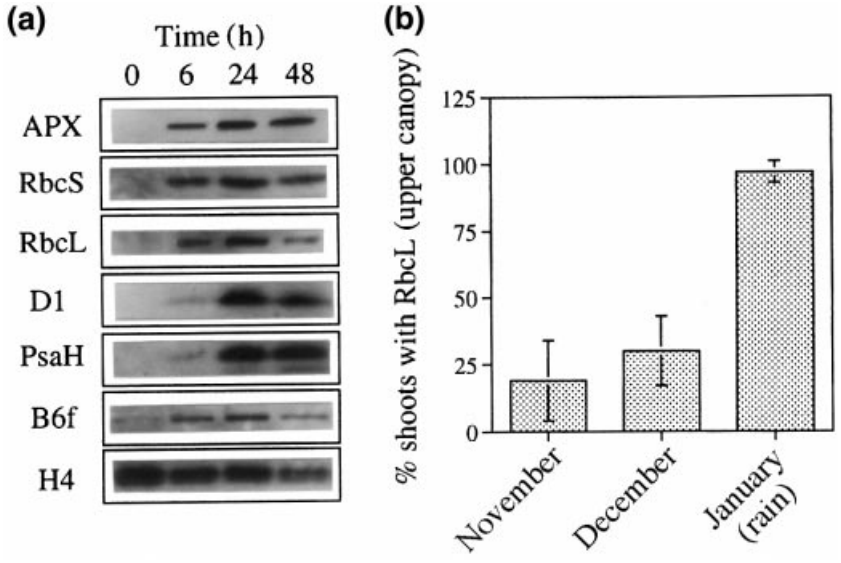

Figure 3. Recovery of upper canopy stems.
(a) Protein gel blots showing the recovery of excised 'dormant' upper canopy stems, sampled in August during the dry season. A representative example of a recovery assay performed with more than 50 individual stems from five different plants, with similar results.
(b) Recovery of 'dormant' stems on the first rainfall of the season. The amount of stems containing RbcL is presented as a percentage of total viable stems. The monthly averages for highest temperature (°C) and light intensity (PAR; μmol m^{-2} sec^{-1}) were: November, 29, 1258; December, 25, 1044; January, 19, 947. The total amount of precipitation prior to sampling in January was 12.7 mm. The statistical significance (*P* value, Student's *t*-test) between the number of stems of the upper canopy containing RbcL in January compared to December or November was ≤0.05.

canopy) during the dry season revealed that the level of the proteins examined was almost identical in these tissues (Figure 5), except for RbcS which was higher in upper stems. Moreover, when upper canopy stems of *A. articulata* were sampled at the same times of day as 'non-dormant' upper canopy stems of *R. raetam* (sampling was performed in parallel to the experiment shown in Figure 4), it was found that the level of RbcS or RbcL protein did not decrease in *A. articulata* stems during the stressful midday hours (Figure 5c). These findings suggested that different evergreen desert plants may use different molecular strategies for acclimation.

Dormant' stems of R. raetam *contain the mitochondrial protein cytochrome* c

The post-transcriptional suppression of many chloroplastic and cytosolic proteins in 'dormant' stems of *R. raetam* raised the question: what it the energy source for metabolic activities in these stems? To address this question we examined the cellular level of three different proteins involved in: (i) electron flow in the chloroplast via PSI (the ferredoxin-binding protein of PSI, PsaD); (ii) the pentose phosphate pathway (cytosolic glucose-6-phosphate dehydrogenase, G6PD); and (iii) respiration (cytochrome *c*, Cyt *c*). As shown in Figure 6, the level of Cyt *c* was not suppressed in 'dormant' upper canopy stems of *R. raetam* during the dry season. In contrast, the level of G6PD and PsaD was suppressed. It was therefore possible that 'dormant' stems of the upper canopy maintained a low level of metabolism via respiration, perhaps using sugars provided by the lower canopy. To test this possibility, we performed measurements of photosynthetic rates (CO_2 exchange in the light) of 'dormant' and 'non-dormant' stems in the field using a portable LI-6400 apparatus. Respiration rates were assayed by repeating the measurements in the absence of light. As shown in Table 1, 'dormant' upper canopy stems were found to have an overall negative photosynthetic rate that may be attributed to respiration. In contrast, 'non-dormant' stems were found to have an overall positive CO_2 exchange rate, indicative of active photosynthesis. In the dark, both 'dormant' and 'non-dormant' stems had similar values of negative CO_2 exchange rates, supporting the molecular data that showed similar levels of Cyt *c* protein (Figure 6), as well as the electron microscopy data which indicated intact mitochondria in 'dormant' stems (not shown). These findings supported the hypothesis that 'dormant' stems may use respiration as an energy source to maintain a steady level of metabolism.

Relative water content of 'dormant' and 'non-dormant' stems

In plants, suppression of photosynthetic activity may be triggered by a decrease in the water potential of plant tissue. To test whether the suppression of photosynthetic activity in 'dormant' stems was correlated with a decrease in the water potential of this tissue, we compared the relative water content (RWC) of 'dormant' and 'non-dormant' stems. As shown in Table 1, the RWC of 'dormant' stems was very low compared to that of 'non-dormant' stems. This finding suggested that a decrease in the water content of the 'dormant' stems may have triggered the suppression of photosynthetic activity. It is not, however, known why a proportion of the upper canopy stems (5–15%; Figure 3b) did not enter 'dormancy' and contained a high RWC similar to that of lower canopy stems.

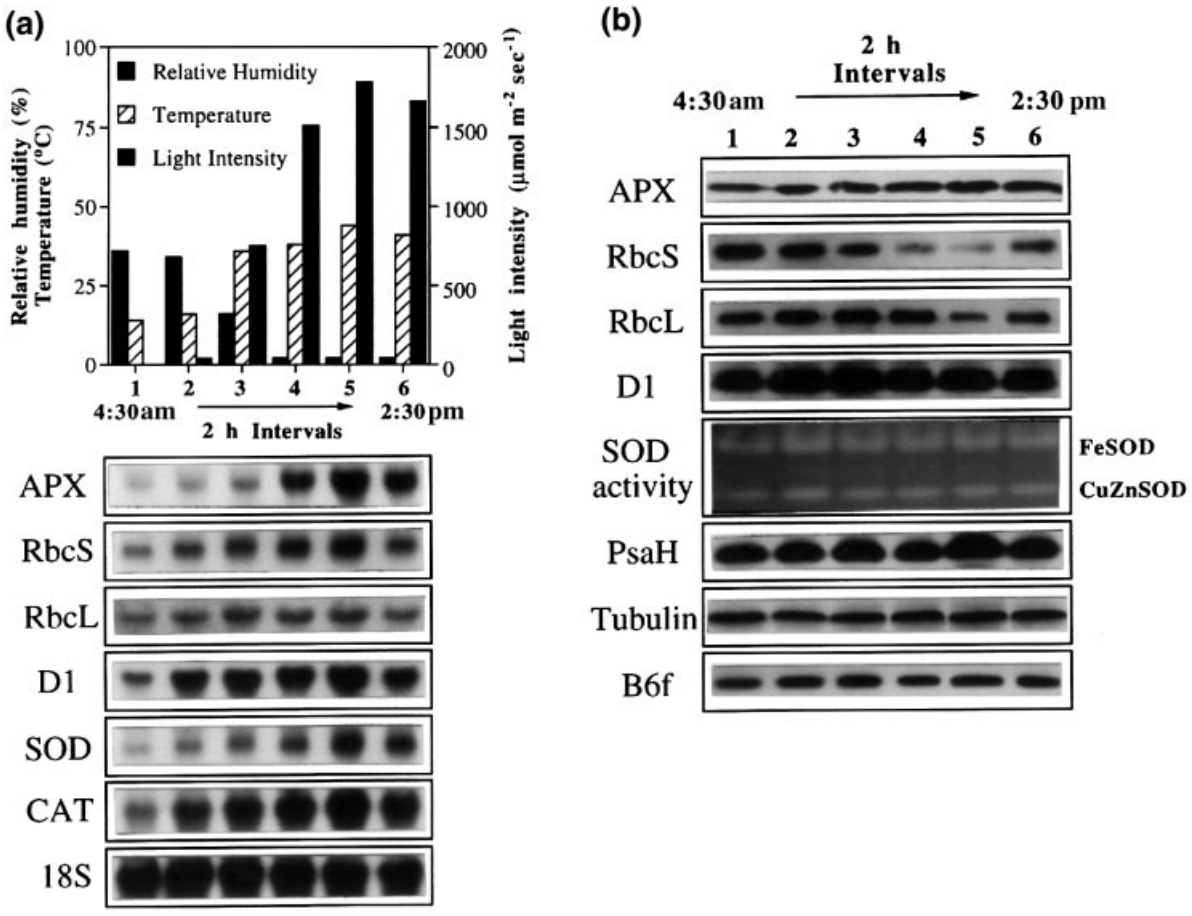

Figure 4. Hourly changes in gene expression in 'non-dormant' upper canopy stems of *Retama raetam* sampled during the dry season.
(a) Changes in environmental parameters (top) and steady-state level of various transcripts (bottom, RNA gel blots) during different times from 4.30 am to 2.30 pm (every 2 h, 1–6), showing that the upper canopy of *R. raetam* may be subjected to oxidative stress.
(b) Protein gel blots and an SOD activity gel performed with protein samples obtained at the times shown in (a). The steady-state level of RbcS and RbcL is shown to be suppressed at midday. Each time point was obtained by pooling 15–20 stems from the upper canopy of the same plant.

Water availability is the limiting growth factor that triggers 'partial plant dormancy'

Because a number of different environmental stress conditions may cause *R. raetam* plants to enter 'dormancy', we examined how changing one parameter, water availability, will effect the entry of plants into this state. As shown in Figure 7(a), upper canopy stems of naturally growing *R. raetam* plants that were artificially watered in a continuous manner between February (end of the rainy season) and September (dry season) did not enter 'dormancy'. In contrast, upper canopy stems of unwatered plants that grew in the same areas (25–50 m distance between the watered and unwatered plants) and were subjected to the same environmental conditions (temperature, light intensity, humidity and soil conditions) entered 'dormancy' (Figure 7b). These findings strongly suggest that water availability is the major limiting environmental factor for the growth of *R. raetam* plants within this ecosystem.

Discussion

Our findings suggest that *R. raetam* uses a molecular acclimation mechanism of suppressed gene expression in order to enter a state of 'dormancy'. This mechanism is activated in the majority of stems of the upper canopy, exposed to direct sunlight (Figure 1). In contrast, stems of the lower canopy, protected from direct sunlight, and a small proportion of stems from the upper canopy, do not enter this state. These may maintain a higher degree of metabolism as well as conducting photosynthesis, which enables the plant to survive the dry season. Following the first rainfall of the season, the suppression of gene expression is removed and the plant is capable of using all its tissues to achieve maximal growth and productivity

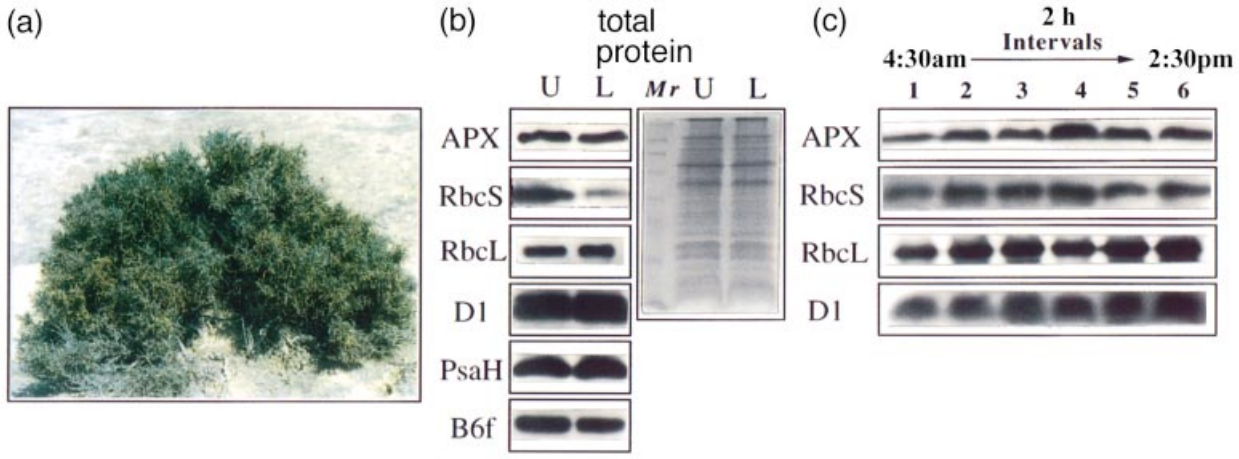

Figure 5. Characterization of gene expression in stems of the C_4 desert plant *Anabasis articulata*.
(a) An *A. articulata* plant, 0.75 m high. Most stems seen here belong to the upper canopy.
(b) Protein gel blots (left) and a coomassie-stained protein gel (right), showing that in contrast to *Retama raetam* (Figure 1), *A. articulata* did not contain stems with suppressed gene expression at its upper canopy during the dry season. Analysis was performed on three individual plants during the dry season (August, as described in Figure 1), between 10.00 am and 2.00 pm, with similar results.
(c) Protein gel-blot analysis of upper canopy stems of *A. articulata* obtained at the same times shown in Figure 4(a) (4.30 am to 2.30 pm; every 2 h, 1–6). The steady-state level of RbcS and RbcL is shown not to be suppressed at midday. Each time point was obtained by pooling 15–20 stems from the upper canopy of the same plant. M_r (top to bottom in 1000s): 98, 64, 50, 36, 30, 16, 6.

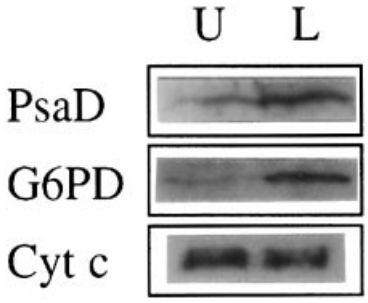

Figure 6. Upper canopy stems of *Retama raetam* contain the mitochondrial protein cytochrome *c*.
Protein gel blots showing that 'dormant' upper stems of *R. raetam* (U) contain the mitochondrial protein cytochrome *c*. In contrast, the expression level of the ferredoxin-binding subunit of PSI, PsaD, and the pentose phosphate pathway enzyme G6PD is suppressed. The level of proteins in the upper 'dormant' stems (U) is compared to that in lower stems (L). Protein extracts from upper and lower stems were obtained as described in Figure 1.

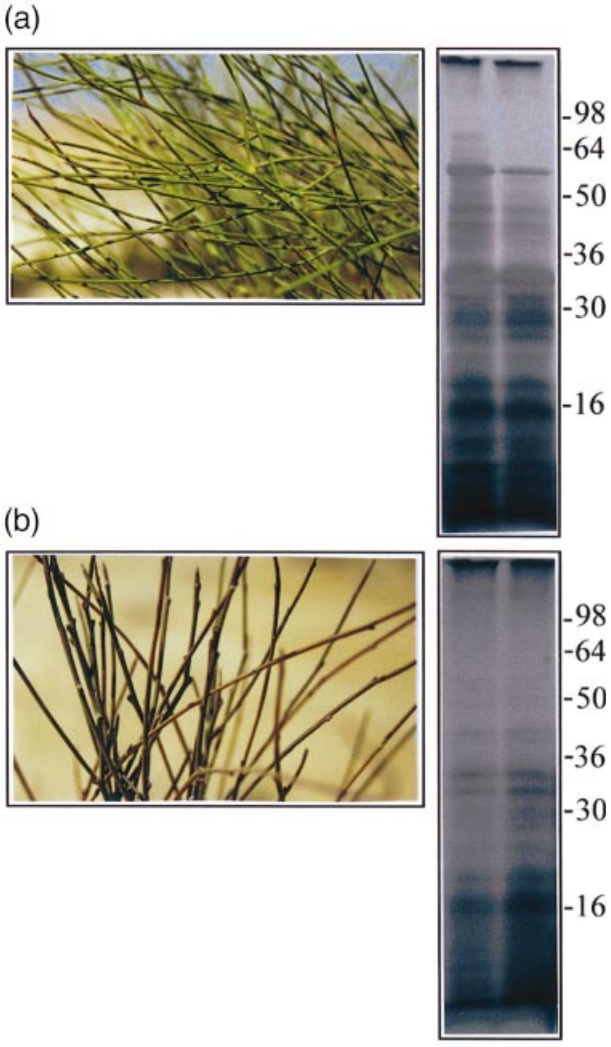

Figure 7. Upper canopy stems of watered *Retama raetam* plants do not enter summer 'dormancy'.
(a) Stems from the upper canopy of a watered *R. raetam* plant (left), and a silver-stained protein gel of protein extracts obtained from these stems (right), showing that they did not enter dormancy (Figure 1).
(b) Upper canopy stems of an unwatered *R. raetam* plant (left), and a silver-stained protein gel of protein extracts obtained from these stems (right), showing that they did enter summer 'dormancy' (Figure 1). Watered plants were watered from the end of the rainy season (February) to the middle of the dry season in a continuous manner using drippers. Watered and unwatered plants naturally growing within the arid ecosystem were subjected to the same environmental conditions. Plants were sampled for analysis in September. Size of protein molecular weight markers is given in kDa.

Table 1. Measurements of photosynthesis, respiration and relative water content in 'dormant' and 'non-dormant' stems during the dry season

	Dormant	Non-dormant
Net CO_2 exchange in the light[a] (μmol CO_2 cm^{-2} sec^{-1})[c]	–1.21 (0.32)[b]	7.31 (0.81)
Net CO_2 exchange in the dark (μmol CO_2 cm^{-2} sec^{-1})[c]	–2.78 (0.33)	– 2.67 (0.45)
Relative water content (%)[d]	34.4 (4.1)	80.1 (2.0)

Measurements were performed on stems from different plants naturally growing within the research site during the dry season (September) between 1.00 and 3.00 pm. Stem 'dormancy' was determined as described in Experimental procedures.
[a] 1500 μm m^{-2} sec^{-1}.
[b] Standard deviation in parentheses.
[c] n = 10 (each a pool of 10 stems).
[d] n = 5 (each a pool of 10 stems).

during the rainy season (Figure 3). This strategy, which enables *R. raetam* to recover very rapidly following rainfall, may be critical for plant acclimation to the desert ecosystem because the amount of available resources within this system is very limited, and there is harsh competition among different plant species during the rainy season.

The mode of suppression of gene expression in the upper canopy of *R. raetam*, that is, post-transcriptionally, as well as the presence of chloroplasts, albeit without a fully organized photosynthetic membrane, enables *R. raetam* to recover very rapidly upon rainfall. Nevertheless, maintaining a high level of RNA in cells, as well as a high number of chloroplasts, during the dry season may have an energetic price for the plant. It is possible that the advantages of this strategy, maximal recovery and growth rates early during the wet season, outweigh this energetic cost. The energy required for maintaining 'dormant' stems may be supplied by respiration as indicated by the presence of Cyt *c* (Figure 6); the relatively intact appearance of mitochondria in the electron microscopy analysis (not shown); and the overall emission (negative CO_2 exchange rates) of CO_2 from 'dormant' stems in the light (Table 1).

The strong correlation between stem 'dormancy' and low RWC suggest that water availability is the key environmental growth factor which induces stem 'dormancy' (Table 1). By controlling the availability of water, we were able to show that the lack of water, and not the extreme temperatures, excess light, or low relative humidity which occur during the dry season, is the limiting environmental factor that triggers the plant to enter 'dormancy' (Figure 7).

The induction of a state of dormancy is a known defence mechanism of some plants, such as woody plants which enter dormancy during winter (Taiz and Zeiger, 1998). However, unlike bud dormancy, the type of 'dormancy' used by *R. raetam* is not induced in all parts of the plant; it is induced only in stems of the upper canopy which protect the lower canopy. In addition, recovering from this 'dormancy' is a very rapid process.

Interestingly, *A. articulata,* an evergreen stem-assimilating desert plant (but a C_4 plant), did not appear to use a similar 'dormant' strategy (Figure 5). This finding may suggest that, physiologically, *A. articulata* is not subjected to the same level of stress as *R. raetam*. It is possible that the additional anatomical and physiological adaptations of a C_4 plant better protect it from the stressful conditions in the desert ecosystem (Edwards and Walker, 1983). Our findings may provide an example of the advantages of C_4 metabolism over C_3 physiology under natural stress conditions, particularly water deficit.

'Non-dormant' stems of the upper canopy were found to respond to changes in environmental conditions (Figure 4). This response was characterized by an increase in the steady-state level of transcripts encoding the anti-oxidative enzymes SOD, APX and CAT, and a decrease in the steady-state level of RbcS and RbcL. These changes may point to a complex response of plants to stress. Three different cellular compartments may be involved in this response: the chloroplast, in which RbcS and RbcL level were suppressed; the cytosol, in which the APX and SOD isozymes tested were induced; and the peroxisomes, in which CAT is located. In controlled laboratory studies the expression level of RbcS was reported to be suppressed in response to different biotic or abiotic stresses (Conklin and Last, 1995; Mittler *et al.*, 1998). In addition, a strong link between excess light stress in the chloroplast and the expression level of cytosolic isozymes of APX was reported (Karpinski *et al.*, 1997; Karpinski *et al.*, 1999). Furthermore, the importance of the peroxisomal-located catalase in defending cells from ROI was clearly demonstrated with transgenic plants (Willekens *et al.*, 1997). Here we show that all these different aspects of the plant's response to environmental stress, tested in the laboratory, can be found in naturally growing plants that respond to naturally occurring stresses within their natural habitat.

We have found a new stress response strategy: 'partial plant dormancy'. It appears that, in response to prolonged exposure to extreme conditions, the upper part of a C_3 desert plant such as *R. raetam* enters a state of 'dormancy' that may protect the entire plant during stress. It would be interesting to determine whether other C_3 plants subjected to long-term stress periods use a similar defence strategy.

Experimental procedures

Plant material and sampling

All experiments were performed with plants that grow naturally within two research sites of the Minerva Arid Ecosystem Research

Center (Berkowicz *et al.*, 1995; http://aerc.es.huji.ac.il/). Environmental parameters were collected, stored and analysed as described by Berkowicz *et al.* (1995). The upper canopy of plants was defined as all aerial parts subjected to direct sunlight (typically accounting for ≈80–90% of the total number of stems in an *R. raetam* plant). The lower canopy was defined as all aerial parts of the plant shaded by the upper canopy. For biochemical and molecular analysis, stems were collected and immediately frozen in liquid nitrogen. 'Dormant' and 'non-dormant' stems were distinguished based on the level of RbcL as shown in Figure 1(b). For the analysis of artificially induced stem recovery, individual stems from the upper canopy were excised, divided into equal sized portions using a razor blade, and placed in water at 24°C under light (250 μmol m^{-2} sec^{-1}). At different times, portions obtained from the same stem were selected randomly and frozen in liquid nitrogen. Naturally occurring stem recovery was assayed by randomly sampling 45 stems from the upper canopy of five individual plants at the same time of day, and assaying these by protein gels for the level of RbcL. Photosynthetic activity of stems was measured in the field with a Licor LI-6400 apparatus using the following measuring cell (6 cm^2) parameters: 26°C, 1500 μmol photons m^{-2} sec^{-1}, and an air flow of 300 μl sec^{-1}. The relative water content of stems was determined as described by Mittler and Zilinskas (1994).

Analysis of gene expression

For the analysis of gene expression, tissues were sampled and immediately frozen in liquid nitrogen. Plant tissue was ground to a fine powder with a mortar and a pestle, and protein, RNA and polysomes were isolated and analysed by activity gels and RNA and protein gel blots, as described by Mittler and Zilinskas (1994). RNA and protein gels were loaded based on equal amounts of protein or RNA (Mittler and Zilinskas, 1994; Mittler *et al.*, 1998).

Transmission electron microscopy

Cross-sections ≈1 mm wide, obtained from upper and lower canopy stems of *R. raetam* plants during the dry season, were fixed for 12 h in 2.5% glutaraldehyde, 0.1 M sodium phosphate buffer pH 7.0, washed in 0.1 M sodium phosphate buffer, and post-fixed for 2 h with 1% osmium tetroxide, 0.1 M sodium phosphate buffer pH 7.0. Samples were then washed, dehydrated in a graded ethanol series, and embedded in epon resin. Thin sections stained with toluidine blue were examined by light microscopy, and ultrathin sections obtained with a diamond knife were stained with uranyl acetate and lead citrate (Mittler *et al.*, 1997). These were observed and photographed in a Jeol 100CX transmission electron microscope.

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