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# Seasonal and diurnal variations in gene expression in the desert legume *Retama raetam*

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## ABSTRACT

**Studying plants growing naturally within their habitat can contribute greatly to our understanding of molecular and biochemical processes involved in the response of plants to changes in environmental conditions. Seasonal and diurnal variations in the expression of several transcripts involved in the defence of plants against abiotic stresses, i.e. transcripts encoding dehydrins, heat shock proteins, and antioxidative enzymes, were followed in *Retama raetam* plants that grow naturally within an arid dune ecosystem. These were associated with changes in environmental parameters simultaneously recorded at the research sites. It was found that the expression pattern of some transcripts correlated with changes in environmental conditions in the different plants during the year. In contrast, the expression pattern of other transcripts appeared to be plant specific and may be associated with phenotypic variability (plasticity). Transcripts encoding different heat shock proteins were induced in a co-ordinated manner, suggesting that their corresponding products function as a chaperone network. Measurements of photosynthetic activity revealed a diurnal cycle in *R. raetam*. Photosynthesis was highest during early morning, declined toward the stressful midday hours and recovered at late afternoon. The present analysis suggests that *R. raetam* uses a combination of different avoidance strategies in co-ordination with active defence mechanisms to withstand the stressful conditions that prevail within the desert ecosystem.**

*Key-words*: *Retama raetam*; active oxygen; desert plants; drought; ecosystem; environmental stress; heat shock; photosynthesis; plasticity.

## INTRODUCTION

Plants show a remarkable ability to adapt and acclimate to different environments, as well as to changes in environmental conditions within a given ecosystem. The acclimation of plants to different habitats may require changes in plant morphology, physiology, development, growth pattern, reproductive timing, or even offspring developmental patterns. Moreover, a single plant genotype may produce different anatomical and physiological phenotypes within similar environments, a phenomenon known as phenotypic plasticity (Sultan 2000).

The majority of studies on the response of plants to changes in environmental conditions were performed under controlled laboratory conditions. However, in the field, the response of plants is affected by a combination of different environmental parameters, and might vary between different plants located within the same ecosystem. We previously reported that *Retama raetam* plants that naturally grow within an arid dune ecosystem use an acclimation strategy of partial plant dormancy to survive their extreme environment. To expand our analysis of these plants we conducted a study on the seasonal and diurnal changes in gene expression in naturally growing *R. raetam* plants within this ecosystem.

Different cellular mechanisms may be involved in the acclimation of plants to the desert environment. These may include drought-related proteins such as dehydrins (DHNs), molecular chaperones and heat shock proteins (HSPs), and antioxidative enzymes, such as catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD). These mechanisms were studied in laboratory organisms and mutants of bacteria, yeast, plants and animals. Analysis of some of these mechanisms in field-grown plants revealed that the behaviour of plants grown in the field may in some cases be different from that of plants grown in the laboratory. For example, the gene for the chloroplast HSP100/clpB, found to be induced by heat shock in laboratory-grown lima beans, was constitutively expressed in these plants under field conditions (Keeler *et al*. 2000).

Dehydrins (LEA; late-embryogenesis abundant, D11 family) are a group of plant proteins induced during environmental stresses associated with dehydration, salinity, or low temperatures and during seed maturation. It is generally believed that they enhance desiccation tolerance by stabilizing other proteins or membranes, required for survival and re-growth (Ingram & Bartels 1996). Most molecular chaperones, many of which being HSPs, can prevent

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heat-induced, irreversible aggregations in proteins (Vierling 1991; Guy & Li 1998; Miernyk 1999; Gurley 2000). Some chaperones can actively solubilize protein aggregates and refold them into stable native proteins (Glover & Lindquist 1998; Goloubinoff *et al*. 1999; Ben-Zvi & Goloubinoff 2001). In addition to being able to prevent protein aggregation and collaborate with other chaperones in the refolding of misfolded proteins, small HSPs have been involved in the protection of native proteins (Forreiter, Kirschner & Nover 1998) and membranes from heat-stress damage (Torok *et al*. 2001). Different subgroups of HSPs appear to act in a co-operative manner and form efficient chaperone networks (Veinger *et al*. 1998; Ben-Zvi & Goloubinoff 2001; Nover & Miernyk 2001). The removal of reactive oxygen intermediates (ROI) is essential for the survival of plants during environmental stress. Superoxide radicals produced by different electron carriers and proteins due to uncoupling of metabolic pathways during stress are mainly scavenged by SODs (Bowler *et al*. 1994). Hydrogen peroxide produced during photorespiration, through the action of SODs, or as a by-product of other cellular reactions during stress is scavenged by CAT and APX (Willekens *et al*. 1997; Asada 1999; Karpinski *et al*. 1999).

The combined action of these stress-response genes may be required for the defence of *R. raetam* plants against the harsh growth conditions that occur during the different seasons in the desert. To properly relate the changes in gene expression of the different stress-response genes to the environmental conditions and the metabolic state of plants we collected and recorded different environmental parameters within the research sites, as well as followed the expression of the $CO_2$-assimilating protein ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco).

## MATERIALS AND METHODS

### Plant material and sampling

All experiments were performed with plants that grow naturally within two research sites of the Minerva Arid Ecosystem Research Center (http://aerc.es.huji.ac.il/). Environmental parameters were collected, stored and analysed as described by Berkowicz, Blume & Yair (1995). For biochemical and molecular analysis stems were collected as described previously (Mittler *et al*. 2001) and immediately frozen in liquid nitrogen. The photosynthetic activity of plants was assayed with a portable LI-6400 apparatus (Li-Cor Inc., Lincoln, NE, USA) as described previously (Mittler *et al*. 2001).

### Analysis of gene expression

For the analysis of gene expression, plant tissues, frozen in liquid nitrogen, were ground to a fine powder with a mortar and a pestle, and protein and RNA were isolated and analysed by RNA and protein gel blots, as described by Mittler *et al*. (2001). RNA and protein gels were loaded based on equal amount of protein or RNA (Mittler & Zilinskas 1994; Mittler, Feng & Cohen 1998). RNA gel blots were hybridized with heterologous radiolabelled cDNA probes as described previously (Mittler *et al*. 2001) and quantified by exposing blots to a phosphoimager (Fuji BAS1000; Fuji, Kanagawa, Japan). In all cases the gene-specific signal was normalized to the level of ribosomal 18S RNA detected as described previously (Mittler & Zilinskas 1994). Protein extracts were prepared by grinding frozen plant tissue to a fine powder and dissolving the powder in the extraction buffer [100 mM Tris-HCl, pH 7, 1% sodium dodecyl sulphate (SDS), and 25 mM 2-mrecaptoethanol]. The extracts were then centrifuged for 10 min at $12\,000 \times g$ and the supernatant was subjected to SDS-polyacrylamide gel electrophoresis for staining, or to protein gel blots as previously described (Mittler *et al*. 2001). Protein-specific signals were normalized to the amount of total protein loaded on the gels. The following heterologous probes were used to detect *R. raetam* dehydrins, obtained from the Clemson University EST collection: cLEZ10D13, cLPT5E10, and cLED14O12.

### Cloning of a *R. raetam* DHN

A cDNA subtraction library was prepared using Clontech (Palo Alto, CA, USA) polymerase chain reaction (PCR)-select cDNA subtraction kit (K1804-1). The tester RNA was polyA RNA prepared from dormant plants (R1 January 2000), whereas the driver RNA was polyA RNA from non-dormant plants (R1 June 2000). Dormant and non-dormant stems were distinguished as previously described (Mittler *et al*. 2001). The library PCR fragments were cloned into a pGem-T Easy vector (Promega, Madison, WI, USA). Dot blots were prepared from the library clones, and hybridized with probes made from polyA RNA of dormant and non-dormant plants. Clones corresponding to RNA from dormant plants and not to RNA from non-dormant plants were chosen, confirmed by RNA blots, and sequenced.

## RESULTS

### Environmental conditions at the research sites

Two research sites located along the Israeli–Egyptian border (Berkowicz *et al*. 1995), were used for the study. Field 84 and Nizzana, were both stabilized desert dune ecosystems located 12 km apart. Figure 1 shows the daily changes in temperature, light intensity, and relative humidity (RH) in these sites recorded from November 1999 to November 2000. Light intensity is shown only for the Nizzana site whereas RH is shown only for Field 84. Rainfall, as well as total rain, for the sampling period is also shown. In both sites the rainy season began in December 1999 and ended in March 2000 and the next rainy season began at the end of October 2000. The total yearly amount of precipitation in both sites was within the designated 'arid deserts' definition as described by McGinnies, Goldman & Paylore (1968).

Daily changes in temperature within the sites recorded throughout the year were relatively drastic. These changes

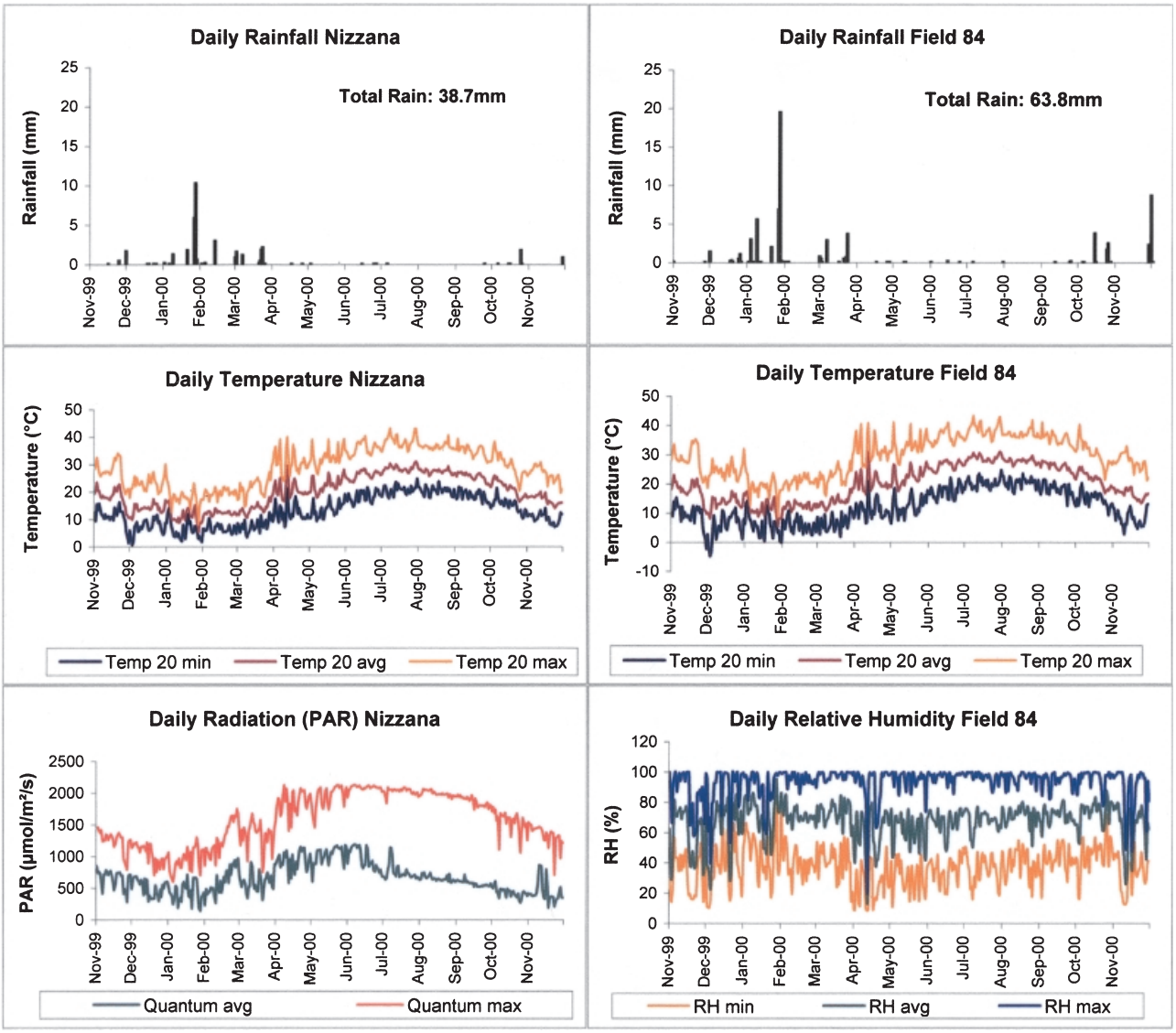


**Figure 1.** Environmental parameters recorded at the research sites. Environmental parameters recorded continuously at the two research sites from November 1999 to November 2000. Top: daily amounts of rain at the two sites (mm). Middle: measurements of temperature at 20 cm above soil level, displayed as daily minimum (min), average (avg), and maximum (max). Bottom left: daily measurements of maximum and average photosynthetic active radiation (PAR) at the Nizzana site. Bottom right: daily measurements of maximum, minimum and average relative humidity at the Field 84 site.

were apparent on two different scales: within a given day, i.e. the difference between the maximum and minimum temperature on a certain day, and between the different seasons, i.e. between the rainy season and the dry season. The amplitude of temperature change within a given day, about 20 °C, was almost constant throughout the different seasons. The maximum daily temperature during the rainy season was about 20 °C, whereas the maximum daily temperature during the dry season was about 40 °C. The lowest temperature recorded at night during the rainy season was –4 °C at Field 84.

The RH at night was high, whereas the RH during the day was low and reached a minimum of 10%. Taking into account the little amount of rainfall at the two sites, the contribution of dew nights to the overall precipitation appeared important. This contribution was about 60 mm per year (Jacobs, Heusinkveld & Berkowicz 2000). As indicated by the annual RH measurements, events where RH was below dew point occurred during the dry season as well as during the rainy season. Maximum photosynthetic active radiation (PAR) during the dry season was very high and reached 2000 $\mu$mol m$^{-2}$ s$^{-1}$. In contrast, the maximum PAR during the rainy season was low and averaged 1000 $\mu$mol m$^{-2}$ s$^{-1}$.

The environmental conditions shown in Fig. 1 suggest that plants naturally growing within the sites may be subjected to a combination of different environmental stress conditions. These might vary from a temperature of 40 °C,

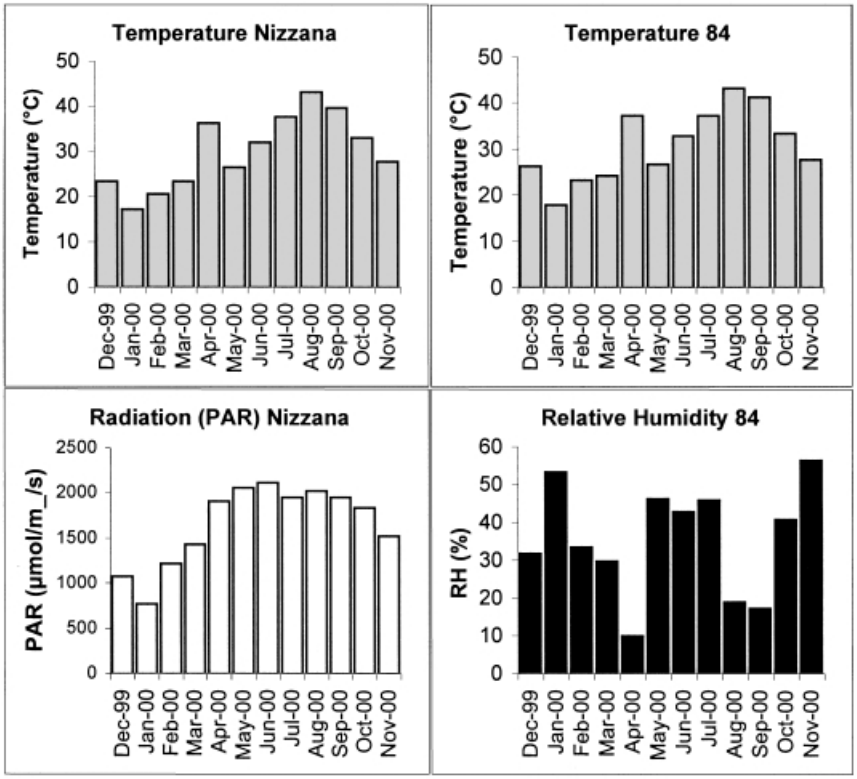


**Figure 2.** Environmental parameters recorded at the time of sampling. Top: measurements of temperature at 20 cm above soil level at Nizzana and Field 84 at the time of sampling. Bottom left: measurements of photosynthetic active radiation (PAR) at the Nizzana site at the time of sampling. Bottom right: measurements of relative humidity at the Field 84 site at the time of sampling.

a RH of 10%, and a light intensity of 2000 $\mu$mol $m^{-2}$ $s^{-1}$ around noon (1200 h) on a typical day of the dry season, to sub-zero temperature during the night in the rainy season. As we were mainly interested in changes in gene expression of heat-, drought- and oxidative stress-related genes, we sampled plants on a monthly base, at the beginning of each month, during the year, between 1200 and 1300 h. As shown in Fig. 2, temperature, PAR radiation, and RH at the times of sampling were in good correlation with the overall monthly parameters (at midday) shown in Fig. 1.

## Rubisco level in plant extracts from different months

Following a survey of a number of different plants during the two years prior to the sampled period (Mittler *et al*. 2001), we selected three different *R. raetam* plants, of similar size, overall appearance, and growth location (dune top) for our analysis: R1 (from the Nizzana site) and R2 and R3 (from Field 84). From each plant upper canopy shoots (50) were sampled randomly each month, pooled, and subjected to further analysis. The level of Rubisco detected in protein extracts by Western blot analysis, can be used as a good indication of plant photosynthetic activity in *R. raetam* plants (Mittler *et al*. 2001). We therefore measured the level of Rubisco (RbcL) in protein extracts obtained from plants during midday in the different months (Fig. 3). As shown in Fig. 3, the different plants selected for analysis had a different pattern of RbcL accumulation, suggesting that although they were of similar size and appearance, they were different in at least certain aspects of their metabolic activity during the year. Although the level of extractable Rubisco is reduced in *R. raetam* during midday, this reduction is not equivalent to the reduction of Rubisco in dormant tissues, and can be used as a good measure of plant photosynthetic activity (Mittler *et al*. 2001).

The analysis shown in Fig. 3, as well as the measurements shown in Figs 1 and 2, suggest that at least during the period studied the *R. raetam* plants selected for analysis had to cope with extreme environmental conditions between May and October when they appeared to be mostly non-dormant. This may require them to use active resistance mechanisms to withstand their extreme environment. To examine the involvement of active defence mechanisms and to correlate their expression with changes in environmental parameters and RbcL protein, we studied the changes in the expression pattern of three different groups of transcripts encoding DHNs, HSPs, and antioxidative enzymes. Because the pattern of RbcL accumulation appeared different between the plants studied, we present all following measurements of changes in transcript level individually for each plant and not as a mean and standard deviation for the different plants.

## Changes in the expression pattern of drought-related genes

We studied the expression pattern of two dehydrins, members of the LEA11 subfamily, and a LEA14 gene. As shown in Fig. 4, two dehydrins differing in their transcript size

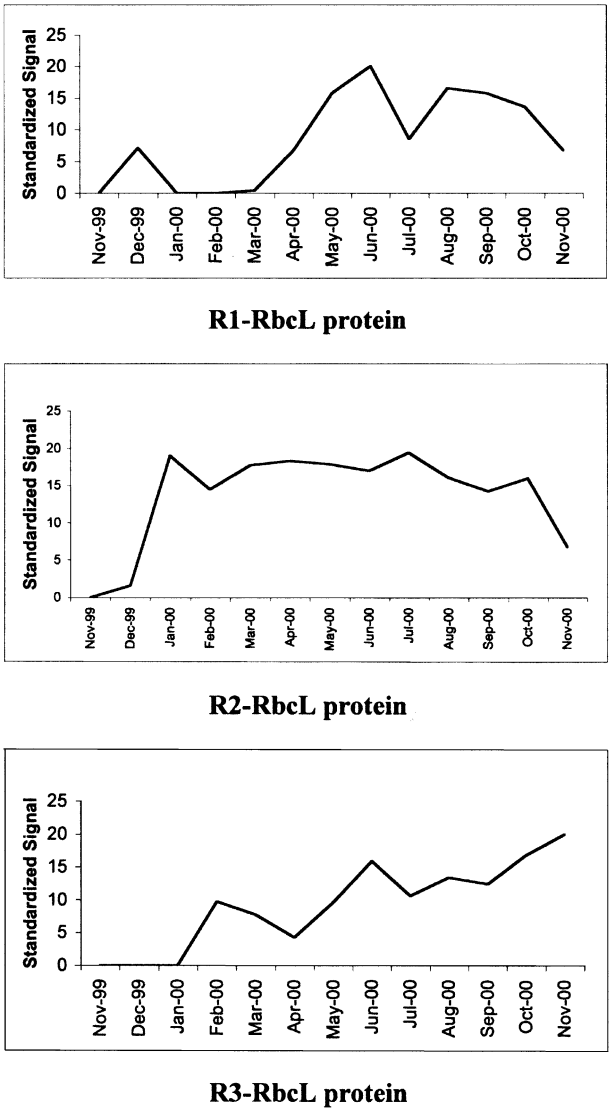


**Figure 3.** The level of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit protein (RbcL) in protein extracts obtained from the different *R. raetam* plants sampled. RbcL level was determined by protein blots in extracts obtained from the different plants as described in Materials and Methods.

could be detected by RNA gel blots in RNA obtained during the different months (there was a 40% similarity in amino acid sequence between the clones used to detect the two dehydrins; not shown). The small dehydrin transcript (approximately 700 bp; Fig. 4b) was termed DHN(A), whereas the large dehydrin transcript (approximately 1400 bp; Fig. 4b) was termed DHN(B). Interestingly, the expression pattern of DHN(A) was very different from that of DHN(B) in the different *R. raetam* plants during the year. As shown in Fig. 4a, the expression of DHN(A) was high in all plants between December 1999 and February 2000, and between October and November 2000. In contrast, the expression of DHN(B) appeared to be plant specific and somewhat correlated with the overall pattern of RbcL accumulation in the different plants (compare Fig. 4a and Fig. 3). In contrast to the changes in the expression of DHN(A) and DHN(B), the expression of LEA14 was relatively constant during the year (Fig. 4a). It should be noted that the complexity of expression as well as the composition of the LEA supergene family in *R. raetam* is not known.

## Cloning of a *R. raetam* DHN that may be associated with plant dormancy

To study the involvement of DHNs in the environmental tolerance of *R. raetam* plants we used a cDNA subtraction approach to clone an *R. raetam* DHN that may be expressed during plant dormancy. We used mRNA from dormant tissue (R1 at January) and from non-dormant tissue (R1 in June) for the subtractive cloning. As shown in Fig. 5a we cloned an *R. raetam* DHN (RDHN) that was mainly expressed between December 1999 and January 2000, and at November 2000 in all plants. The expression pattern of RDHN appeared similar to that of DHN(A) (Fig. 4), however, it was not induced as much as DHN(A) between October and November 2000. Based on sequence comparison, RDHN had little homology to DHN(A) or DHN(B) (less than 30% similarity; not shown). The transcript size of RDHN was also different from that of DHN(A) or DHN(B) (Figs 4b & 5b). As shown in Fig. 5c, the cloned RDHN (AY039800) is homologous to soybean and citrus DHNs induced in response to cold stress (Cai, Moore & Guy 1995; Takahashi & Shimosaka 1997), to a cowpea protein possibly involved in abscisic acid synthesis during drought stress (Iuchi *et al.* 2000), and to a grape berry DHN induced during ripening (Davies & Robinson 2000).

## Changes in the expression pattern of heat shock proteins

Figure 6 shows the changes in the expression pattern of three different transcripts encoding cytosolic HSPs: HSP-90, HSP-70, and HSP-18 (class I) during the season. For HSP probes we used *Arabidopsis* and tobacco cDNAs encoding the different HSPs. In all plants the expression pattern of the three HSPs appeared to be similar. Thus, HSPs were induced at midday during the hottest months of the year (July to September 2000; Figs 1 & 2), and HSP70 and HSP90 were also induced during April 2000 (a relatively exceptional month with respect to elevated temperatures and low RH; Figs 1 & 2). As described previously, and in agreement with the current observation, dormant tissue, with very low RbcL protein, did not contain elevated levels of HSP70 and HSP18 proteins (Mittler *et al.* 2001).

We have previously analysed daily changes in the expression pattern of ROI-removal enzymes and found that APX, CAT, and SOD were induced during midday (1030 to 1430 h; Mittler *et al.* 2001). As there was a large difference in temperature between day and night (Fig. 1) we studied the diurnal changes in the expression pattern of HSPs (sampling every 2 h) in Nizzana during September 2000. The expression of HSPs was correlated with environmental

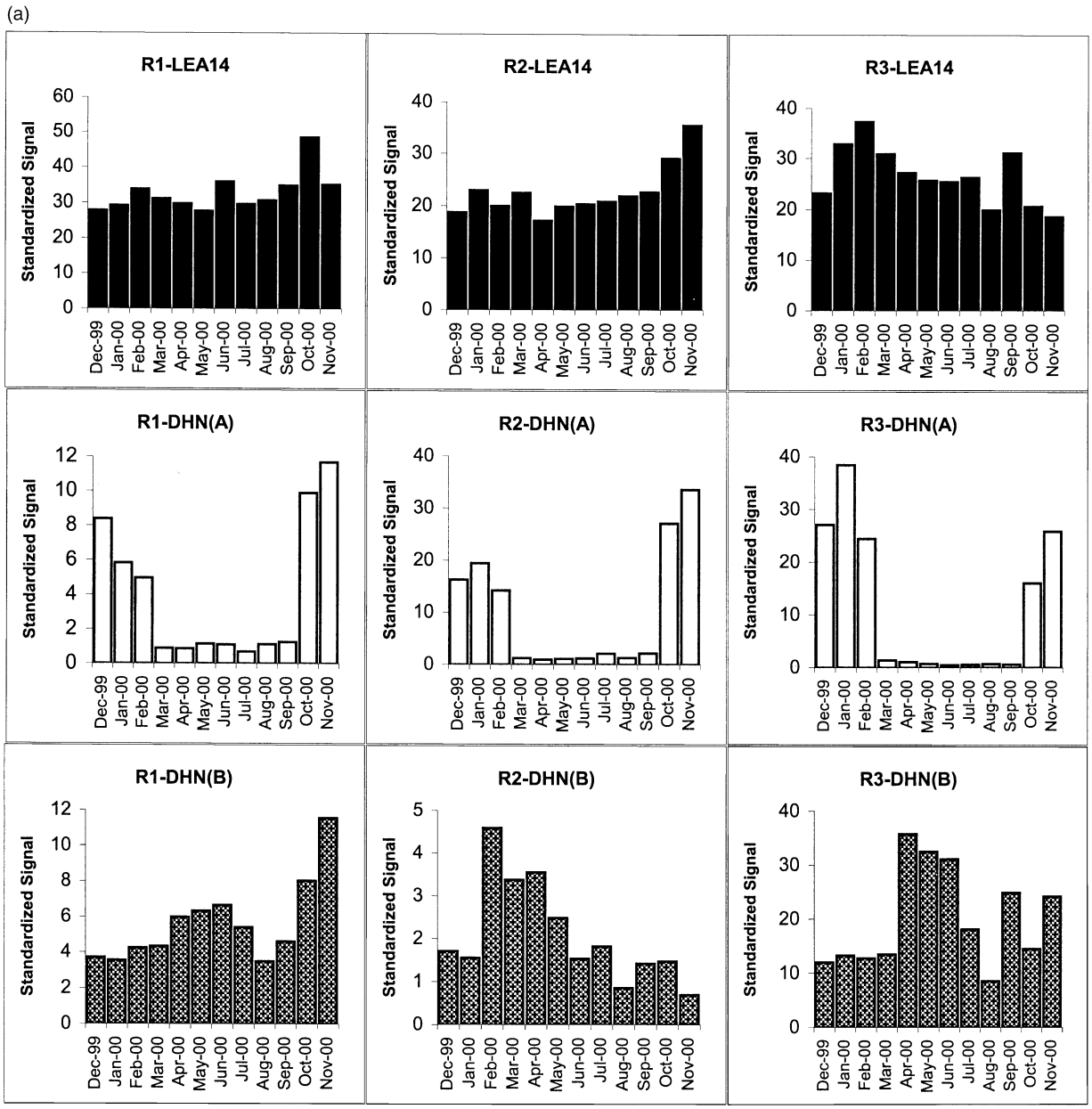


**Figure 4.** The expression pattern of transcripts encoding dehydrins (DHNs) at midday during the different months. (a) The expression level of LEA14, top, and two DHNs: DHN(A), middle, and DHN(B), bottom, is shown in the three different *R. raetam* plants. The expression of LEA14 and the two DHN transcripts was assayed by RNA blots, and standardized to the level of 18S rRNA. (b) RNA gel blot analysis showing the transcript size of the two DHNs. DHN(A) and DHN(B) were detected using heterologous probes as described in Materials and Methods.

parameters and the rate of photosynthesis. As shown in Fig. 7, all HSP transcripts studied, as well as cytosolic HSP100, were induced during the hottest hours of the day (1300 to 1500 h). Interestingly, during these hours photosynthesis was suppressed. The rate of photosynthesis was maximal early in the morning (0900 h), declined to a minimum at 1300 h, and reached another maximum at about 1700 h. This analysis was repeated four times on three different plants during September 2000 with similar results. The overall response of *R. raetam* plants to the midday stress hours may therefore be composed of induction of ROI-scavenging enzymes (Mittler *et al*. 2001), induction of an array of HSPs, and suppression of photosynthetic activity (Fig. 7).

## Seasonal changes in the expression of ROI-scavenging enzymes

We tested the expression pattern of two transcripts involved in $H_2O_2$ removal: ascorbate peroxidase (APX I), and catalase (CAT 1); a transcript involved in superoxide removal: superoxide dismutase (cytosolic Cu/ZnSOD); and

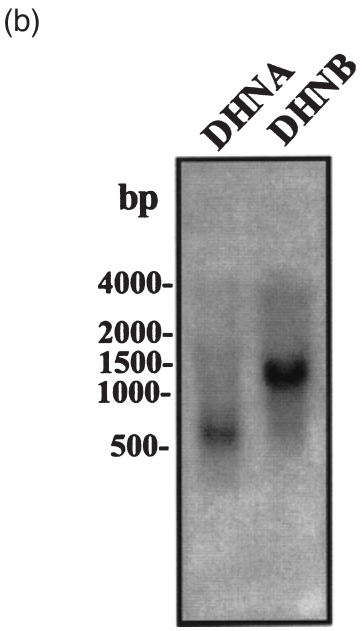


**Figure 4.** *Continued*

a transcript involved in the regeneration of reducing power within the Asada-Halliwell pathway: glutathione reductase (gr; cytosolic GR). Figure 8 shows the changes in the expression pattern of these transcripts at midday during the year. In general the expression pattern of these transcripts was different between the various plants during the year. The expression of SOD and GR appeared overall to change in a co-ordinated manner during the year within each of the different plants, whereas at least in R2 and R3, the expression of CAT and APX followed to some degree an inverse relation. The expression of ROI-removal enzymes during the year appeared therefore not to have a distinct overall pattern similar to that of DHNs or HSPs.

## DISCUSSION

We studied seasonal and diurnal variations in gene expression in plants that naturally grow within a stabilized arid dune ecosystem. To evaluate the expression of as many different genes as possible we sampled a small number of plants. In future studies a large pool of plants should be sampled and tested for the expression of only a selected

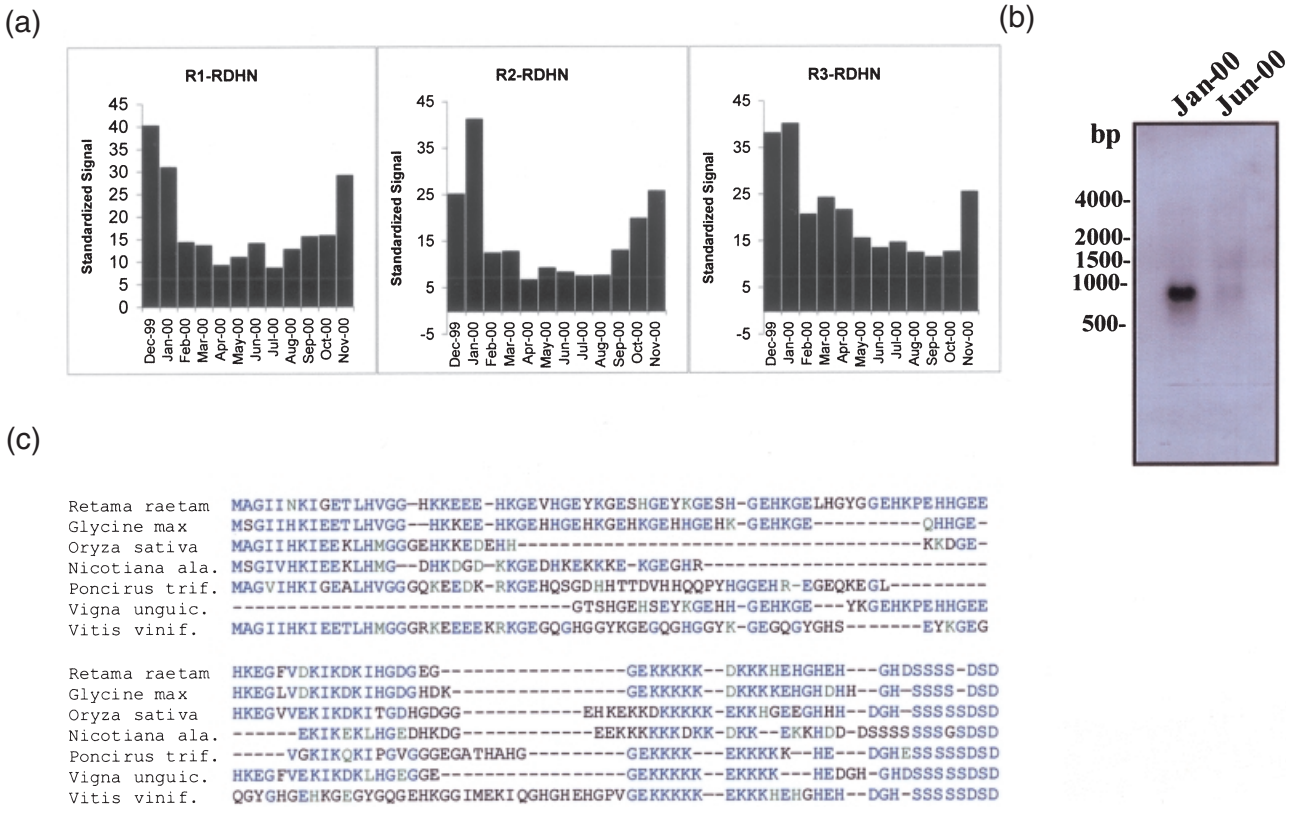


**Figure 5.** Cloning of a cDNA encoding an *R. raetam* DHN (RDHN). (a) The expression pattern of RDHN in the three different *R. raetam* plants sampled at midday during the different months. The expression level of RDHN was determined by RNA blots and standardized to the level of 18S rRNA. (b) An RNA gel blot showing the transcript size as well as the expression of RDHN in samples obtained from plant R1 at January 2000 (Jan – 00), and June 2000 (Jun – 00). (c) Sequence alignments of RDHN and other plant DHNs (*R. raetam* – AY039800; *Glycine max* – AB000129; *Oryza sativa* – AF010584; *Nicotiana alata* – AJ277643; *Poncirus trifoliate* – L39005; *Vigna unguiculata* – BAB11933; *Vitis vinifera* – CAB85631).

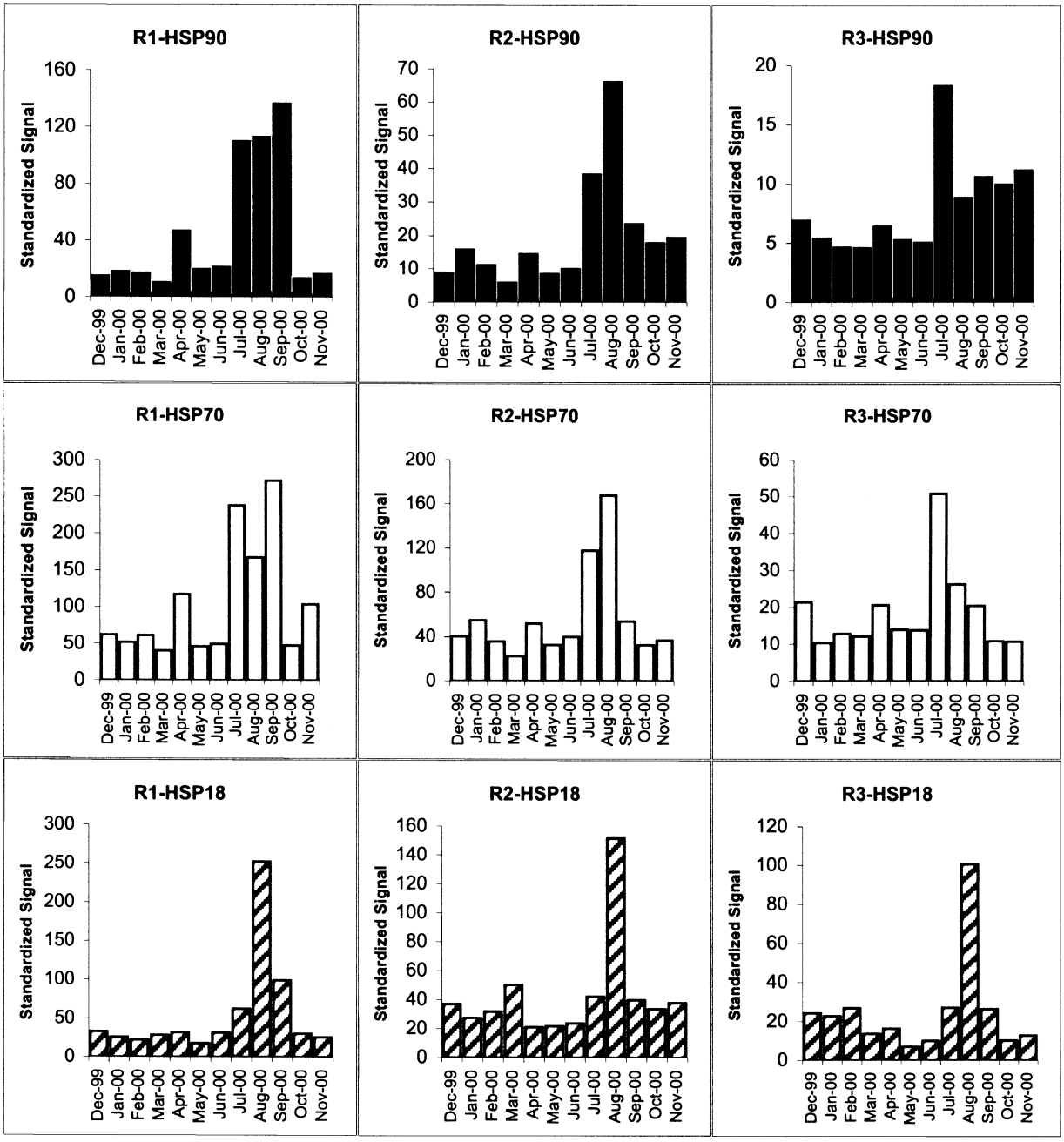


**Figure 6.** The expression pattern of three transcripts encoding cytosolic heat shock proteins (HSPs) at midday during the different months. The expression level of the three cytosolic HSPs: HSP90 – top, HSP70 – middle, and HSP18 – bottom, is shown in the three different *R. raetam* plants. The expression level of the three HSPs transcripts was assayed by RNA blots, and standardized to the level of 18S rRNA.

group of genes identified by our study. Meteorological parameters continuously recorded at the research sites provided a reference to the environmental conditions encountered by plants. We found that the expression pattern of some transcripts correlated with changes in environmental parameters and was similar between the different plants during the year, whereas the expression pattern of other transcripts appeared to be plant specific and may be associated with phenotypic variability (plasticity) between the plants. Most notably, dehydrins [RDHN and DHN(A)] and HSPs appeared to be expressed in all plants in a similar pattern during the year (Figs 4–6). Their expression did not seem to be linked to the changes in extractable RbcL protein in the different plants during the year (Fig. 3). Thus, R1 and R2, which had very different patterns of RbcL accumulation, expressed DHN(A), RDHN and HSP18 in a similar manner. The expression of DHNs and HSPs may therefore be correlated mainly with changes in environ-

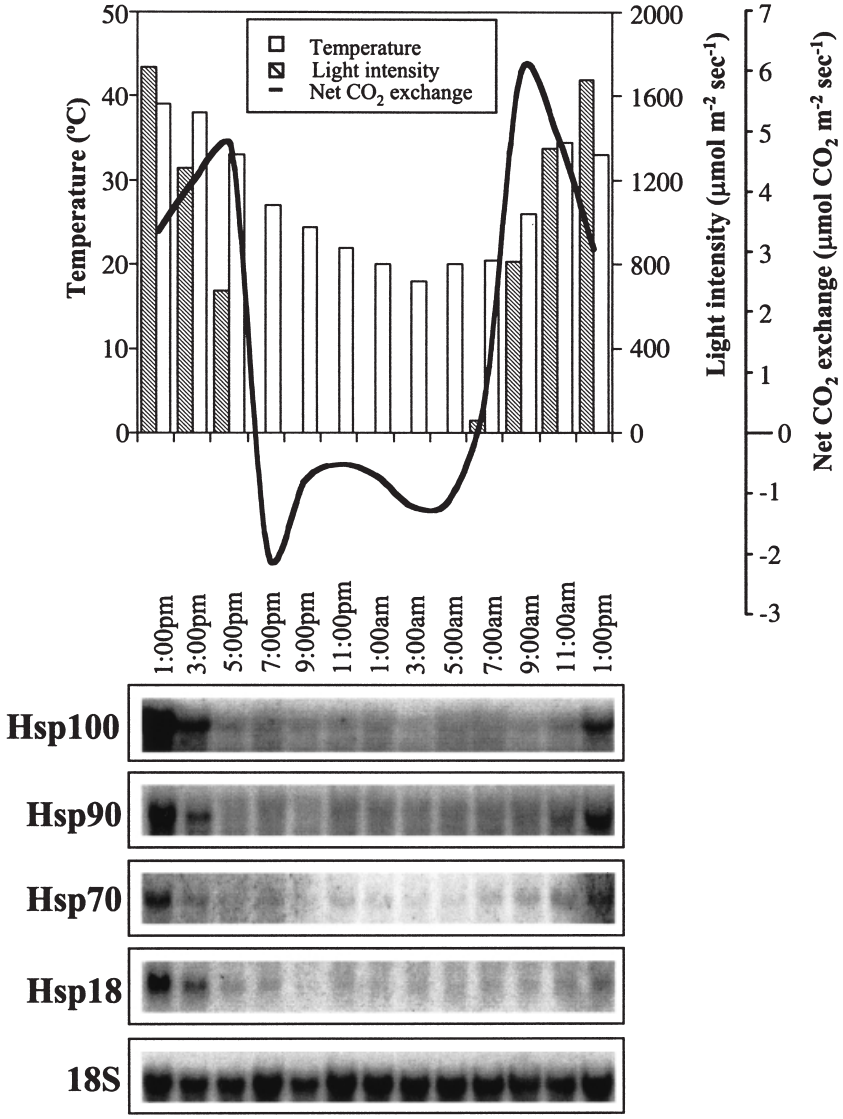


**Figure 7.** Changes in the expression of transcripts encoding HSPs and in $CO_2$ assimilation during daytime and night. Top: a graph showing the changes in temperature and light intensity, and the changes in the rate of $CO_2$ assimilation, as recorded every two hours from 1300 h of one day to 1300 h of the next day (at the beginning of September 2000). Bottom: RNA gel blot analysis of the expression pattern of four different transcripts encoding cytosolic HSPs (HSP100, 90, 70, and 18) in samples obtained at the different times as described above. RNA gel blots were first hybridized with the different HSPs probes and then with a probe for 18S rRNA. This experiment was repeated four times on three different plants with similar results.

mental parameters (Fig. 1), and not to the same extent with the level of RbcL protein that may reflect changes in metabolic activity during the year (Fig. 3). On the other hand, the expression pattern of the ROI-removal enzymes APX, SOD, GR, and to a lesser degree CAT (Fig. 8), and the expression of DHN(B) (Fig. 4) appeared to be plant specific and was different between the different plants during the year. Expanding the current analysis to include more gene families and further studies into the signal transduction pathways involved in their regulation may shed some light on the complex relations between gene expression, phenotypic plasticity, and the acclimation of plants to different habitats.

Transcripts encoding HSPs appeared to be induced in a co-ordinated manner (Figs 6 & 7). The induction of HSPs mainly during July to September and during April (Fig. 6) may suggest that the threshold temperature for their induction in this particular desert plant is about 40 °C. The co-ordinated expression pattern (induction and decline) of transcripts encoding different HSPs (Fig. 7) may also suggest that their corresponding products function together as a chaperone network to alleviate the impact of temperature stress. Thus, small HSPs may bind to and stabilize membranes, proteins, and protein complexes, and assist HSP70 in the refolding of partially unfolded proteins by preventing protein aggregation (Veinger *et al*. 1998; Bukau, Schmid & Buchner 1999). HSP100 and HSP70 may work together to disaggregate protein aggregates (Goloubinoff *et al*. 1999), and HSP90 may be involved in stress protection as well as stress signalling (Reddy *et al*. 1998; Pratt, Krishna & Olsen 2001).

Suppression of photosynthetic activity during the stressful midday hours (Fig. 7) may be viewed as an avoidance strategy used by *R. raetam*. The photosynthetic apparatus is considered to be one of the major cellular sources of ROI production during stress (Asada & Takahashi 1987; Asada 1999). We previously found that during the midday stressful hours (1030 to 1430 h) the level of Rubisco is reduced,

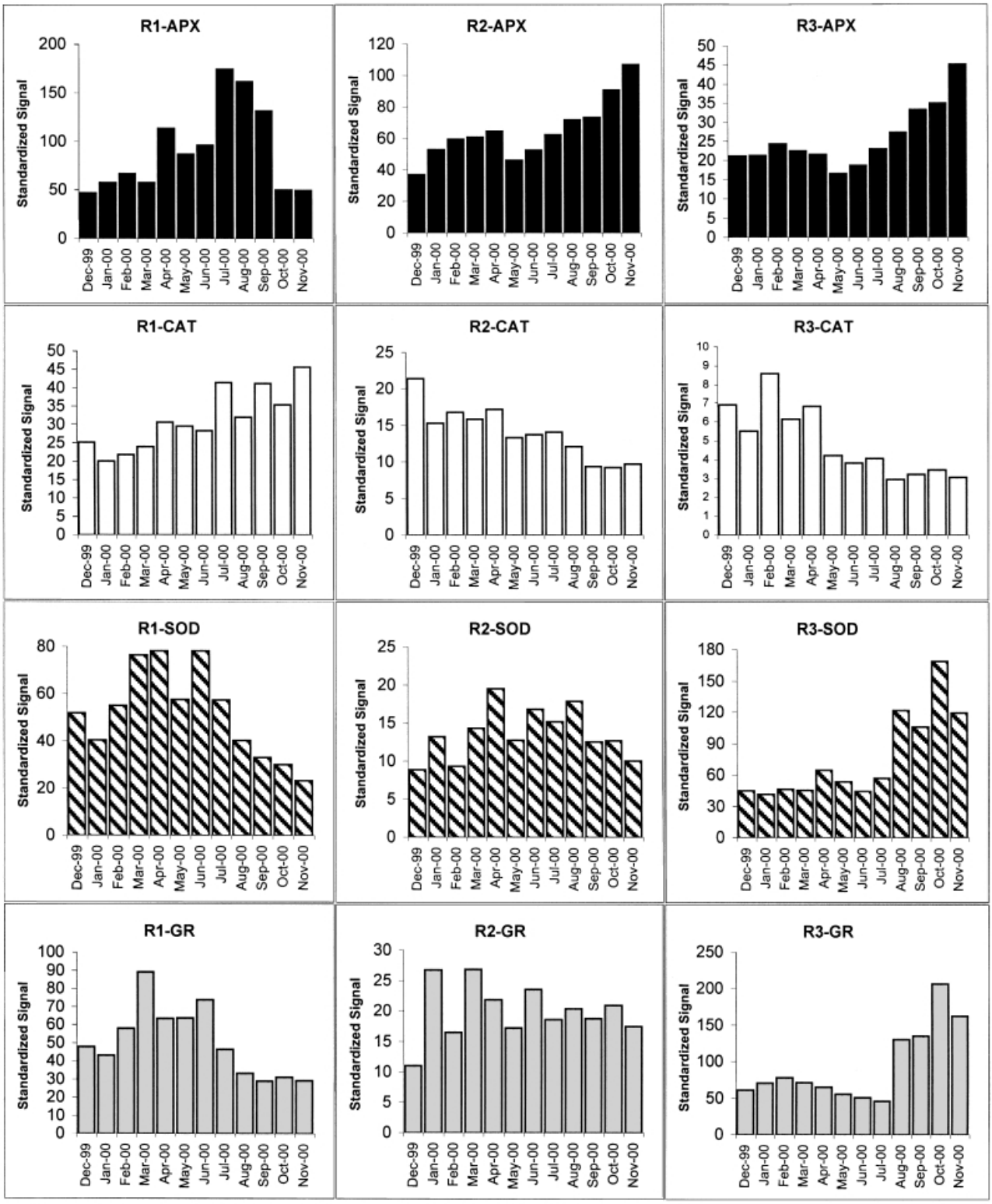


**Figure 8.** The expression pattern of four transcripts encoding ROI-detoxifying enzymes (APX, CAT, SOD, and GR) at midday during the different months. The expression level of four transcripts encoding the ROI-removal enzymes: cytosolic APXI (APX), peroxisomal CAT1 (CAT), cytosolic CuZnSOD (SOD), and cytosolic GR (GR) is shown in the three different *R. raetam* plants. The expression level of the four ROI-detoxifying transcripts was assayed by RNA blots, and standardized to the level of 18S rRNA.

although the steady-state level of transcripts encoding the large and small subunits of Rubisco is unchanged or induced (Mittler *et al*. 2001). The reduction in Rubisco protein was accompanied by an induction of transcripts encoding the ROI-scavenging enzymes APX, CAT, and SOD (Mittler *et al*. 2001). Here we show that the expression of at least four different transcripts encoding cytosolic HSPs is also induced during these hours (Fig. 7). Measurements of photosynthetic activity during the different hours of the day indicated that photosynthesis is indeed suppressed (Fig. 7). Our findings suggest an interesting diurnal cycle in a desert plant (at least during the dry season): The majority of photosynthetic activity is carried out during the morning hours (between 0700 and 1000 h) and again during the afternoon hours (between 1500 and 1700 h). During the midday hours, i.e. between 1100 and 1500 h, when environmental conditions are the harshest, photosynthesis is suppressed and transcripts encoding defence enzymes such as HSPs and ROI-removal enzymes are induced. A bimodal pattern of photosynthetic activity was reported to occur in field-grown *Prosopis chilensis* trees (Ortiz *et al*. 1995), suggesting that other $C_3$ plants use a similar avoidance strategy under field conditions.

We previously reported that *R. raetam* plants exit dormancy very rapidly following rainfall (Mittler *et al*. 2001); however, based on our year round analysis (Fig. 3), it appears as if in some plants this response may be transient and the plant will re-enter dormancy (see R1 in Fig. 3). Moreover, not all plants may exit dormancy upon the first rainfall of the season (compare R2 and R3 in Fig. 3). It therefore appears that the environmental/developmental decision of when to exit dormancy is much more complex than simply the availability of water, as previously suggested (Mittler *et al*. 2001). It is possible that the plasticity observed in this response, i.e. the timing of exiting from dormancy, is important for plant survival within the desert ecosystem, similar to the importance of the plasticity observed in the cycles of seed germination in certain desert plants (Koller 1969).

We cloned a cDNA encoding an *R. raetam* DHN that may be associated with plant dormancy (Fig. 5). We are in the process of cloning additional *R. raetam* cDNAs involved in dormancy or active stress resistance (Pnueli *et al*. 2002). We are using a subtraction cloning strategy as described above (Fig. 5) with mRNA from dormant and non-dormant tissues, as well as with mRNA from tissues sampled when the environmental conditions were mild or harsh (Figs 1 & 2).

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