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BRIEF COMMUNICATION

Stress-induced proteins in *Parthenium argentatum* leaves

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Abstract

We have analyzed the stress-associated proteins in a high-rubber-yielding guayule (*Parthenium argentatum* Gray cv. 11591) leaves. Protein profiles in leaf fractions, resolved by SDS-PAGE and visualized by Coomassie Brilliant Blue staining, were different under various stresses. Changes in 25, 34 and 74 kDa polypeptides were noticed in response to low night temperature treatment while 24, 40, 47 and 81 kDa proteins responded to low irradiance. 23, 50, 75 and 82 kDa proteins were altered in response to drought stress. Certain proteins may play a significant role in the acquisition of tolerance in parenchyma cells of guayule leaves and might be useful markers to study adaptation in guayule plants.

Additional key words: drought, guayule, irradiance, low night temperature, SDS-PAGE.

Guayule (*Parthenium argentatum* Gray) is a rubber producing shrub, tolerant to various environmental stresses such as drought, low night temperatures, shade, soil salinity and insect pests (Ramachandra Reddy and Das 1988, Backhaus 1998, Sundar and Ramachandra Reddy 2000, 2001). These environmental stresses result in significant morphological and metabolic changes in guayule, besides limiting the rubber yield and quality. Conspicuous changes in the protein profile of leaves under various stress treatments indicate that dehydrins, LEA and HSP class of proteins serve as markers in response to stress (Vierling 1991, Hernandez and Vierling 1993, Bohnert *et al.* 1995, Guy *et al.* 1998, Pinedo *et al.* 2000, Barathi *et al.* 2001). Since the principal adaptive response of the plant is repression of normal pattern of protein synthesis as well as synthesis of new set of proteins from newly transcribed mRNA, we sought to identify changes in proteins in guayule leaf tissues, following different abiotic stress treatments.

Guayule (*Parthenium argentatum* Gray cv. 11591) was grown in 30-cm pots under natural (12-h) photoperiod. The maximum irradiance (PAR, 400 - 700 nm) available at the top of the canopy was 1 600 μmol m^{-2} s^{-1} on a clear day. Daily average maximum and minimum air temperatures during the growth were 33 and 22 °C, respectively. Plants were well watered and periodically fertilized with Hoagland nutrient solution. Young and fully expanded leaves from three-year-old plants were used for the experiments.

Intact plants were subjected to different stresses separately in a controlled-environment growth cabinet (model *704A-2SDHFX, Labline*, Melrose Park, USA). The plants were subjected to low night temperature treatment (LNT) at 15 °C for 60 cycles (12-h daily; day temperature was 30 °C). Shading (LI) was provided by reducing the growth irradiance from 1500 to 450 μmol m^{-2} s^{-1}. Drought stress (DS) was imposed by supplying restricted amount of water. Measurement of leaf water potentials were made using psychrometer (*SKPM 1400, Skye Instruments, Powys, Wales, UK).*

Guayule leaves were cut into small pieces and homogenised in a buffer containing 50 mM Tris (pH 8.0), 50 mM NaCl, 2 mM EDTA, 5 mM $MgCl_2$, 1 mM dithiothreitol and 5 mM β-mercaptoethanol, 1 mM

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Abbreviations: DS - drought stress; HSP - heat shock proteins; LEA - late embryogenesis abundant protein; LNT - low night temperature; LI - low irradiance; SDS-PAGE - sodium dodecyl sulphate polyacrylamide gel electrophoresis.

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phenylmethyl sulfonyl fluoride (PMSF) and 0.5 % polyvinyl pyrrolidone-40 (PVP-40). The extract was passed through four layers of muslin cloth and centrifuged at 30 000 *g* for 15 min at 4 °C. The buffer-soluble proteins were precipitated using 8 volumes of chilled acetone (containing 10 mM β-mercaptoethanol). Precipitated proteins were solubilized as suggested by Laemmli (1970). Estimation of protein content was carried out following Coomassie blue dye binding assay of Bradford (1976). An aliquot of the protein extract was precipitated by adding equal volume of 20 % (m/v) trichloroacetic acid (TCA) for 2 h (4 °C) in order to minimize interference of chemicals such as Tris, 2-mercaptoethanol and SDS in development of colour by Bradford dye (Orr *et al.* 1988). TCA-insoluble protein pellet thus obtained after centrifugation (15 000 *g*, 5 min) was dissolved in 1 M NaOH (2 h, room temperature) and an aliquot of this was used for protein quantification.

Analytical PAGE with SDS was performed using the method of Laemmli (1970). Leaf samples were solubilized in 2X-SDS sample buffer containing 62.5 mM Tris-HCl (pH 6.8), 50 g dm^{-3} SDS, 1 mM PMSF, 20 g dm^{-3} β-mercaptoethanol, 100 g dm^{-3} sucrose and 1 g dm^{-3} bromophenol blue and heated at 70 °C for 3 min. Aliquots of the denatured leaf samples in SDS sample buffer were then resolved by SDS-PAGE. The apparent molecular masses of proteins were estimated by comparison with the mobility of standard proteins. After electrophoresis, the gels were stained with Coomassie Brilliant Blue following standard protocol (Sambrook *et al.* 1989).

All biochemicals and enzymes were from *Sigma* (St. Louis, USA) and protein molecular mass markers were obtained from *Bangalore Genie Ltd.* (Bangalore, India). Reagents were purchased from commercial sources and were of analytical grade.

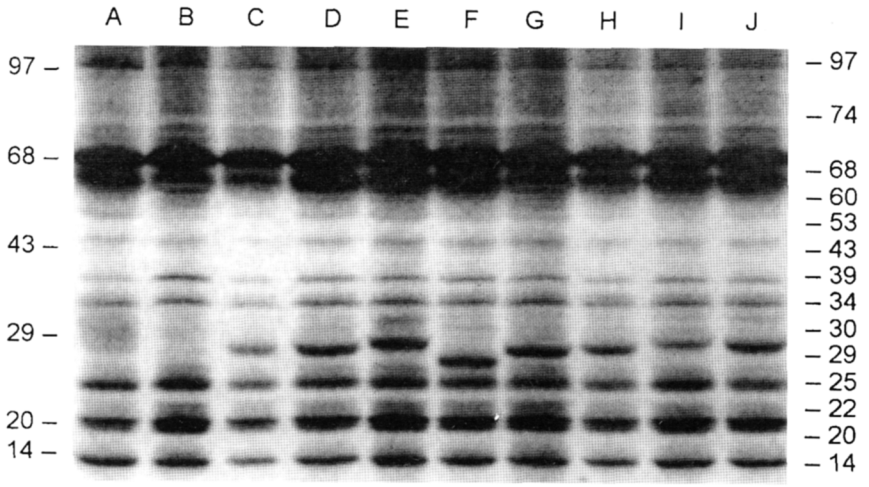

Fig. 1. SDS-PAGE protein profile of guayule leaf extracts. Guayule plants were subjected to different number cycles of low night temperature (15 °C). *Lanes* A - control, B - 10 cycles, C - 15 cycles, D - 20 cycles, E - 25 cycles, F - 30 cycles, G - 35 cycles, H - 40 cycles, I - 50 cycles, and J - 60 cycles. The day temperature was 30 °C.

Many new polypeptides were identified in guayule plants under different abiotic stresses like low night temperature (LNT), low irradiance (LI) and limited water supply (DS) (Figs. 1, 2, 3). It is likely that some of these new proteins help guayule to adapt to stressful environments. Several proteins isolated from higher plants (LEA, HSP, RAB) have already been shown to be very significant for stress tolerance (Singla and Grover 1994, Magnard *et al.* 1996, Guy *et al.* 1998). The effects of environmental stresses including low night temperature, low irradiance and drought on polypeptide composition and gene expression were summarized in a recent review (Shinozaki and Yamaguchi-Shinozaki 1999). The present study shows important changes in protein profiles in guayule in response to different stresses (Table 1). In response to LNT, changes were noticed with 7 polypeptides (Fig. 1). Increased content of 29, 60 and 68 kDa polypeptides and reduced content of 39 and 63 kDa polypeptides were observed with increasing duration of LNT treatment. The other proteins that changed due to the abiotic stress treatment include 25 and 56 kDa in response to LNT, 24, 40, 47 kDa to LI (Fig. 2) and 23, 50, 70 kDa in response to DS (Fig. 3). On the other hand, alterations in contents of certain specific polypeptides were found to be unique to given abiotic stress. For instance, 23, 50, 75 kDa polypeptides seemed to be induced in response to leaf water potential of -2.5, -3.0 or -3.5 MPa (DS), but the enhancement was too low to be interpreted as stress-inducible proteins.

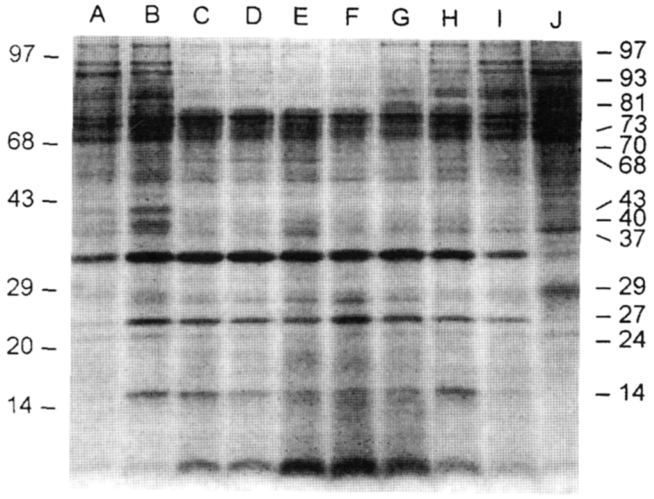

Fig. 2. SDS-PAGE of soluble proteins in leaf extracts of guayule plants subjected to low irradiance (450 μmol m^{-2} s^{-1}). *Lanes* A - control, B - 10 cycles, C - 20 cycles, D - 30 cycles, E - 40 cycles, F - 45 cycles, G - 50 cycles, H - 55 cycles, I - 50 cycles, and J - 60 cycles of LI.

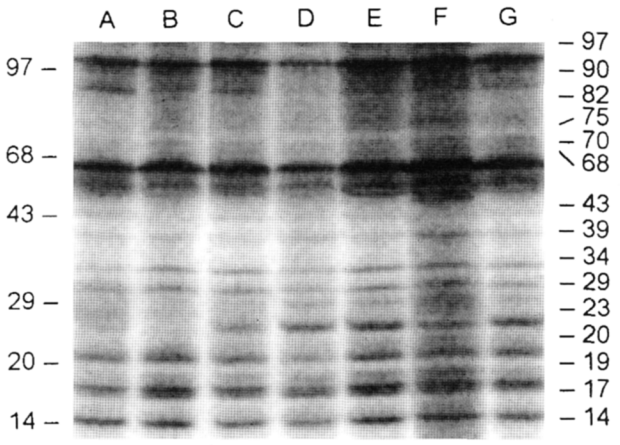

Fig. 3. SDS-PAGE of soluble proteins obtained from guayule leaf extracts from progressively drought stressed (DS) guayule plants. *Lanes* A - control, B - leaf water potential -0.1 MPa, C - -1.5 MPa, D - -2.0 MPa, E - -2.5 MPa, F - -3.0 MPa and G - -3.5 MPa.

Table 1. Changes in polypeptide profile modulated by increasing durations of LNT, LI and DS (* - proteins either induced or accumulated, ^ - proteins either decreased or present in lower quantities).

Stress	Protein [kD]
Low night temperature	20*, 25, 29, 30, 34, 39^, 53^, 68*, 74
Low irradiance	24, 40^, 43^, 47, 81*, 93
Drought	23*, 39^, 43, 50, 70, 75, 82*

In summary, we have identified certain proteins in guayule leaves induced in response to different abiotic stresses, which suggest that these changes should have special adaptive significance during different growth stages of guayule. Precise biochemical identification and characterization of some of these proteins will help evaluating them as marker proteins for studying their unique tolerance mechanism and the protective role for guayule crop improvement.

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