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BRIEF COMMUNICATION

Induction of a novel boiling stable protein in response to desiccation and ABA treatments in *Sesbania sesban* var. *bicolor* leaves

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Abstract

Several polypeptides were induced in leaves of *Sesbania sesban* var. *bicolor* under water stress (desiccation). Among them, the SDS-PAGE resolved a few high molecular mass polypeptides along with one major of 66 kDa. After boiling the total protein fraction, some low molecular mass polypeptides (10 - 30 kDa) as well as the one of 66 kDa remained stable. The latter (66 kDa) polypeptide is also regulated by exogenous application of ABA, indicating its significant role in adaptation of sesban to drought.

Additional key words: drought-induced proteins, sesban, SDS-PAGE.

Plant productivity is greatly reduced by the adverse environmental conditions effecting the water status of plants. Although plant species vary in their sensitivity and response to decreased water potential caused by drought, low temperature or high salinity, it is believed that many plants cannot only perceive stress, but also signal as well as respond to it. Under stress, a wide variety of plant species express a common set of genes and resultant proteins (Skriver and Mundy 1990, Vilardell *et al*. 1990, Pinedo *et al.* 2000, Barathi *et al.* 2001, Sundar *et al.* 2003). Although functions of many of these genes have not yet been unequivocally assigned, based on their characteristics, it is likely that these proteins are formed in response to stress (McCue and Hanson 1990). Some proteins induced during drought are highly hydrophilic (like dehydrins) and remain stable even after boiling (Close *et al*. 1989, Jacobsen and Shaw 1989). Induction of 66 kDa boiling stable polypeptide has been reported in *Populus tremula* (Pelah *et al*. 1995) and several other species. A comparison of the sequences indicates conservation of lysine-rich regions (Mundy and Chua 1988, Vilardell *et al*. 1990). Conservation of these sequences in several plant species and the induction of dehydrins by desiccation indicate that these proteins serve to protect membranes and other cellular proteins in the event of water loss (Baker *et al*. 1988, Close *et al*. 1989, Close and Chandler 1990, Skriver and Mundy 1990).

Globally, a considerable land mass is lying barren because of the harsh climate conditions. Therefore, it is essential to develop some plants which can survive even under extreme stress. In the past, some investigations have been carried out in herbaceous plants on the tolerance of abiotic stress (Ingram and Bartels 1996). Likewise, characteristics of avoiding physiological drought by some commercially important woody plants have also been documented but only fragmentary information on the molecular basis of drought tolerance is available (Pelah *et al*. 1995). In view of the rapidly changing climatic conditions, understanding the genetic basis and molecular mechanisms of drought stress adaptation, as well as other stresses, are of paramount importance for long-lived forest trees. In the present

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Abbreviations: ABA - abscisic acid; EDTA - ethylenediaminetetraacetic acid; FM - fresh mass.

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investigation, analysis of drought response in *Sesbania sesban* var. *bicolor* was undertaken to elucidate the genetic control and physiological processes that determine its adaptability to stress conditions caused by desiccation as well as ABA treatment.

Leaves of *Sesbania sesban* var. *bicolor* were collected from plants growing in the Delhi University Botanical Garden. Under ambient laboratory conditions (25 ± 2 °C), they were desiccated for varying periods (16, 24, 48, 72 and 96 h) and rehydrated for the same duration. Control samples were maintained in water. At the end of the experiment, leaves were quickly frozen in liquid nitrogen and stored at -80 °C. For ABA treatment, leaves were dipped in 1 mM ABA solution for 4 h at room temperature and then transferred to water for another 4 h. The control leaves were maintained in water all along (8 h). At the end of the experiment, leaves were frozen in liquid nitrogen and stored at -80 ºC.

Leaflets with their petioles (2 g) were frozen in liquid nitrogen and homogenized in a chilled mortar and pestle in the extraction buffer (50 mM Tris HCl at pH 8, 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM dithiothreitol, 100 mM sucrose and 5 mM isoascorbate). The crude extract was centrifuged at 12 000 *g* for 20 min at 4 °C and the supernatant containing total proteins was stored at -20 °C (Vu *et al*. 1982). Proteins were estimated by the protocol of Peterson (1979). The supernatant containing 50 µg protein was precipitated with 4 volumes of cold acetone and incubated at -20 °C for 5 min and centrifuged at 12 000 *g* for 10 min. The pellet was dissolved in 0.025 cm^3 sample buffer (Laemmli 1970; 125 mM Tris HCl at pH 6.8, 40 g dm^{-3} sodium dodecyl sulphate, 100 g dm^{-3} β-mercaptoethanol, 200 g dm^{-3} glycerol and 0.04 g dm^{-3} bromophenol blue) and boiled for 2 min, promptly shifted to ice and centrifuged at 12 000 *g* for 5 min before loading. Boiling stable protein samples (150 µg) for SDS-PAGE were prepared by boiling total protein samples for 10 min and transferring them to ice for 5 min and finally, centrifugation at 10 000 *g* for 10 min. The supernatant containing the boiling stable proteins was processed for sample preparation as described above. Proteins were separated by SDS-PAGE. Different lanes were loaded with 50 µg of total proteins and 150 µg of boiling stable proteins and run at 25 mA for 2 h. Gels were stained with 2 g dm^{-3} (m/v) Coomassie blue R-250 in 400 g dm^{-3} methanol and 100 g dm^{-3} acetic acid for 3 h. They were destained for 2 h in 400 g dm^{-3} methanol containing 100 g dm^{-3} acetic acid. Gels were photographed and analysed in *Gel Documentation System 2000* (*Bio Rad*, Hercules, USA).

In the present investigation, the morphological appearance of leaves of *Sesbania* leaves changed within 16 h of desiccation treatment, *i.e*. they developed wrinkles, became dry, and turned yellowish green. The control leaves remained green and healthy. Detached leaves wilted rapidly under the ambient laboratory conditions, losing 65 - 68 % of their fresh mass during 16 - 24 h but their fresh mass remained constant thereafter. When leaves were rehydrated, they regained their fresh mass partially (Table 1). The leaves desiccated for 16 h showed a little increase in protein content compared to the control, but with further increase in time, the protein content decreased gradually. On rehydration, it decreased markedly (Table 2).

Table 1. Changes in fresh mass of *Sesbania sesban* detached leaves during dehydration and rehydration.

Time [h]	Mass loss [%]	Mass gain [%]
16	64.6 ± 0.7	80.0 ± 0.1
24	68.0 ± 0.1	84.5 ± 0.7
48	67.5 ± 0.7	90.5 ± 0.7
72	68.0 ± 0.1	94.5 ± 0.7
96	68.0 ± 0.1	95.5 ± 0.7

Table 2. Total protein content [mg g^{-1}(f.m.)] of desiccated and rehydrated leaves of *Sesbania sesban.*

Time [h]	Desiccated	Rehydrated
0	60.0 ± 0.5	60.0 ± 0.7
16	65.6 ± 0.1	29.6 ± 0.5
24	57.6 ± 0.1	28.0 ± 0.5
48	55.2 ± 0.7	26.4 ± 0.1
72	52.8 ± 0.3	18.4 ± 0.7
96	46.4 ± 0.7	14.4 ± 0.1

Gradual decrease in protein content as a result of dehydration, may be due to decrease in protein synthesis, as has been observed in pine callus cultures (Valluri *et al*. 1998) as well as in pea roots and maize mesocotyls (Bewley and Larsen 1980). Increase in proteolytic activity may cause protein breakdown as observed in drought-stressed *Phaseolus* and *Vigna* leaves (Roy-Macauley *et al*. 1992).

Rapid reduction in the total protein content was observed in ABA stressed samples, *i.e.* from 64.5 mg g^{-1}(f.m.) in control leaves to 48.4 mg g^{-1}(f.m.) in ABA stressed leaves. On rehydration for 4 h, total protein content increased to 58.4 mg g^{-1}(f.m.).

The SDS-PAGE analysis of control leaves showed 20 polypeptides with molecular masses 61, 58, 55, 52, 49, 44, 39, 38, 36, 33.34, 32.94, 31.96, 30.91, 30.37, 30, 28, 26, 25, 20 and 18 kDa (Fig. 1). Water stressed leaves not only retained all these 20 proteins but the intensities of their bands also increased considerably. In addition, six new polypeptides of molecular masses 198, 121, 66, 60, 54 and 40 kDa appeared in samples desiccated for 16, 24, 48, 72 and 96 h. The content of 20 kDa polypeptide

increased conspicuously when leaves were desiccated for 24 h. When the stressed leaves were rehydrated, all the stress-induced proteins disappeared (Fig. 1). Desiccation of leaves for 16 h is sufficient to induce the stress-related proteins after which the number of polypeptides did not change but their contents varied. The supernatants of the three leaf samples, *i.e.* control, stressed and rehydrated were boiled for 10 min to detect the boiling stable proteins. Six boiling stable proteins of 66, 28, 26, 25, 20 and 18 kDa were observed in desiccated samples. Out of these, polypeptides of molecular mass 28, 26, 25, 20 and 18 kDa were also present in control. Thus, polypeptide of 66 kDa is a water stress induced boiling stable protein (Fig. 2). Similar observations have also been earlier reported in *Brassica napus* (Reviron *et al.* 1992). In *Populus tremula* (Pelah *et al*. 1995) it accumulates rapidly within 4 h of dehydration.

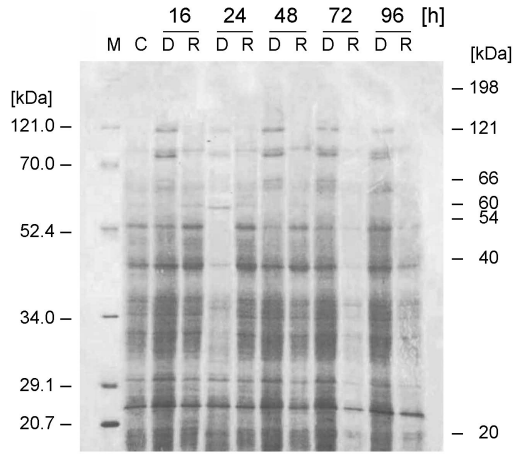

Fig. 1. SDS-PAGE of total proteins in *Sesbania sesban* leaves desiccated (D) for 16 to 96 h and rehydrated (R) for similar durations (C - control, M - marker).

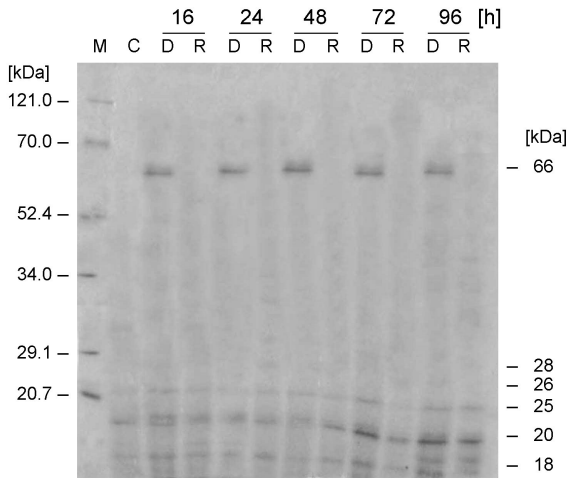

Fig. 2. SDS-PAGE of boiling stable proteins in *Sesbania sesban* leaves desiccated (D) for 16 to 96 h and rehydrated (R) for similar durations.

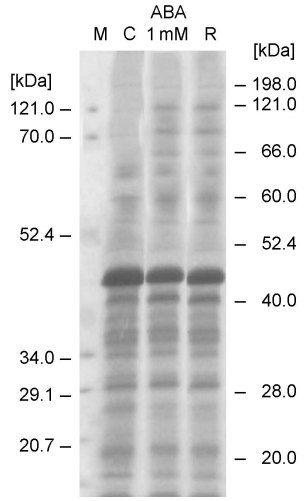

Fig. 3. SDS-PAGE of total proteins in *Sesbania sesban* leaves treated with 1 mM ABA for 4 h, control (C) and rehydrated (R) for 4 h.

Earlier, it has been reported that ABA content increases in tissues subjected to osmotic stress by dehydration, salt or cold and under these conditions, specific genes are expressed that can also be induced by ABA (*e.g.* Skriver and Mundy 1990). In the present study, the SDS-PAGE analysis of control leaves showed 15 polypeptides with molecular mass 198, 121, 60, 52.4, 40, 39, 34, 31, 28, 26, 25, 24, 20, 18 and 10 kDa. In addition, ABA treated samples induced one more of molecular mass 66 kDa. All the 16 proteins were also detected in rehydrated samples (Fig. 3). The 66, 28, 26, 25, 20, 18 and 10 kDa polypeptides remained stable even after boiling for 10 min. Of those, 28, 26, 25, 20, 18 and 10 kDa proteins were also present in the control. The

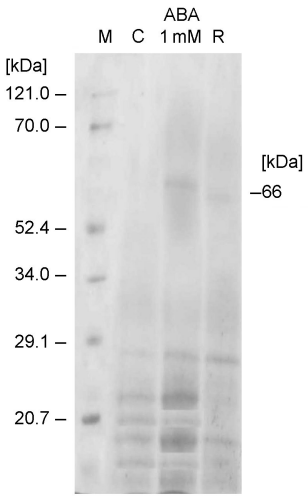

Fig. 4. SDS-PAGE of boiling stable proteins in *Sesbania sesban* leaves treated with 1 mM ABA for 4 h and rehydrated (R) for 4 h, along with control (C).

intensity of polypeptide bands with molecular mass 25 and 18 kDa increased in samples treated with 1 mM ABA (Fig. 4).

In the present study of *Sesbania sesban*, it has been revealed that a 66 kDa polypeptide was induced under water stress as well as the ABA treatment. The polypeptides of molecular mass 198, 121, 60 and 40 kDa induced during water stress (experiment conducted in spring, February 2001) developed even in control (unstressed) leaves during ABA treatment (experiment carried out in summer, June 1999). Thus, the leaf explants collected from *in vivo* raised plants at a gap of 15 months (in different seasons) revealed differences in protein profiles. Evidently, these four polypeptides (198, 121, 60 and 40 kDa) are regulated by adverse environmental changes. Further work on characterization of the novel 66 kDa boiling stable protein and its role in the physiology of explants is in progress.

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