

**ASP53, a 53 kDa cupin-containing protein from *Acacia erioloba* seeds that protects proteins against thermal denaturation.**

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*Acacia erioloba*, the Camel Thorn tree, grows in the semi-arid areas of Southern Africa. Since the seeds are exposed to extremely harsh environmental conditions including ground temperatures exceeding 70 aC prior to germination, the presence of unusual LEA-like proteins was investigated. ASP 53, a 53 kDa heat soluble protein was identified as the most abundant seed protein present. Atypical of LEA proteins was the high hydrophobic amino acid content and the presence of defined secondary structure. ASP 53 was rapidly degraded during germination and this coincided with the loss of desiccation tolerance in germinating seedlings. Immunocytochemistry demonstrated the presence of ASP 53 in the vacuoles and cell walls in mature seeds with the cell wall content decreasing upon germination. ASP 53 was found to inhibit all three stages of protein thermal denaturation. ASP 53 significantly decreased the rate of loss of alcohol dehydrogenase activity at 55 XC, decreased the rate of temperature-dependent loss of secondary structure of haemoglobin and completely inhibited the temperature-dependent aggregation of egg white protein. Since ASP 53 was not amenable to Edman degradation, the sequences of two tryptic peptides were obtained by PSD MALDI-TOF mass spectrometry allowing the preparation of a degenerate primer. The ASP 53 gene sequence was determined by RT-PCR and 5' RACE. Homology studies identified two cupin motifs, and only seed storage proteins exhibited close sequence similarity.