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Protein Comparisons of Non- and Cold-acclimated *Hibiscus syriacus* and *H. rosa-sinensis* Bark

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Short photoperiod and low temperature (5C) were used to induce cold acclimation of *Hibiscus syriacus* L., a deciduous, temperate woody species, and *H. rosa-sinensb* L., an evergreen, tropical woody species (Bailey Hortorium, 1976; Watkins and Sheehan, 1975). Protein extracts from acclimated and nonacclimated plants of the two species were compared by electrophoresis in an effort to detect changes in the protein content in response to low-temperature exposure.

One-year-old plants of a single, white (SW) flowered *H. syriacus* and *H. rosa-sinensis* 'President' were grown in 3.8-liter containers in a shadehoyse (47% light exclusion) under the natural temperature and photoperiod conditions of Gainesville, Fla. On 11 June, 10 uniform plants of both species were randomly placed in a controlled environment chamber. Chamber temperature was maintained at 5 ± 2C, 70% ± 5% RH with a 12-hr photoperiod consisting of 520 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PPFD. Control plants were kept in the shadehouse under ambient environmental conditions with a day/night range of 32 to 24C. All plants were watered and fertilized as needed. Stem samples were taken 5 weeks after the initiation of low-temperature treatment from acclimated and control plants and tested for freezing tolerance (Teets and Hummel, 1988; Teets et al., 1989). A 5-week photoperiod/temperature treatment was sufficient to promote changes in freezing tolerance of *H. syriacus* (Teets et al., 1989). Concomitantly, bark extracts of protein were prepared for gel electrophoresis.

Total protein was extracted (Schuster and Davies, 1983; Van Etten et al., 1979). Bark tissue (100 mg) was homogenized in 300 µ1 of extraction buffer (120 mM TrisHCl, pH 6.8; 50 mM ethylenediaminetetracetic acid; 100 mM KCl; 1 mM phenylmethylsufonylfluoride; 2% sodium dodecyl sulfate; 2% 2-mercaptoethanol) at 0C. An equal volume of 80% buffer-saturated phenol was mixed with the tissue extract and vortexed at maximum speed for 2 min. Phase separation was achieved by centrifugation for 5 min at 15,000× *g* at 4C. The phenol phase containing the protein was washed three times with equal volumes of extraction buffer. Protein was precipitated by the addition of five volumes of –20C methanol containing 100 mM ammonium acetate and stored overnight at -20C. After centrifugation, the protein pellet was washed three times with - 20C methanol containing ammonium acetate and once with -20C acetone and air dried. Protein was dissolved in sample buffer (120 mM Tris·HCl, pH 6.8; 10% glycerol; 1% sodium dodecyl sulfate; and 1% dithiolthreitol). Proteins (50 µg/lane) were separated according to methods described by Laemmli (1970) in 12% acrylamide gels and stained with Coomassie brilliant blue.

Hibiscus rosa-sinemis control plants and plants held 5 weeks at 5C failed to cold-acclimate and were killed by freezing at - 2C. *Hibiscus syriacus* SW nonacclimated control plants had a T_{k50} (temperature killing 50% of the samples) of -4C, while the plants held 5 weeks at 5C with a 12-hr photoperiod had cold-acclimated and were not injured by freezing at -16C. Separation of total protein by one-dimensional denaturing gel electrophoresis failed to resolve major differences between species or between nonacclimated and cold-acclimated hibiscus bark tissue (Fig. 1). Exposure of *H. syriacus* to temperatures that induce cold-acclimation did not cause the appearance or loss of proteins in the bark tissue. Only recently, with the use of two-dimensional gel analysis, have protein changes in response to cold acclimation been detected (Guy and Haskell, 1987). The lack of difference between the nonacclimated and cold-acclimated *H. syriacus* protein content does not rule out subtle low temperature-induced changes that might have been revealed by a more powerful gel electrophoresis technique than the one-dimensional system used in this study. The protein content of *H. rosa-sinensis* held at 5C for 5 weeks was much reduced, and this was evident by the absence of most of the protein bands present in the control tissue. The loss of protein provides evidence that extended low-temperature exposure was deleterious to *H. rosa-sinensis* (Teets et al., 1989).

Although obvious changes in bark proteins associated with freezing tolerance were not observed, two notable differences were found. Bark tissue of *H. rosa-sinensis* contained a protein of M_r 58,000 that was not observed in *H. syriacas.* In contrast, *H. syriacus* contained a major band at M_r 16,000 that was not apparent in *H. rosa-sinensis.* Otherwise, the profiles were identical in all respects and suggest a high degree of relatedness between the two species. The observation of only two protein band differences between the two species may be valuable to hibiscus breeders.

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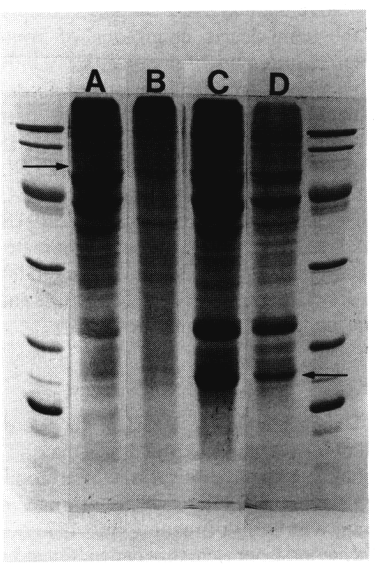

Fig. 1. Polypeptide profile of *Hibiscus* bark tissue separated in a 12% acrylamide gel and stained with Coomassie brilliant blue. *H. rosa-sinensis* nonacclimated (A) and cold-acclimated (B), *H. syriacus* nonacclimated (C) and cold-acclimated (D). Protein standards were phosphorylase *b* 92,000; bovine serum albumin 66,000; ovalbumin 45,000; carbonic anhydrase 31,000; trypsin inhibitor 20,500; and cytochrome c 14,000, Arrow adjacent to lane A denotes protein band present in *H. rosa-sinensis,* but missing in *H. syriacus.* Arrow adjacent to lane D denotes a protein band diminished by cold-acclimation treatment.