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# Electrophoretic Analysis of Seed Proteins of Heat-tolerant and Heat-sensitive Cultivars of Chinese Cabbage[1]

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*Additional index words.* gel electrophoresis, *Brassica campestris* (Pekinensis group)

*Abstract.* **SDS-Polyacrylamide gel electrophoresis of seed proteins of heat-tolerant and heat-sensitive cultivars of Chinese cabbage, *Brassica campestris* L. (Pekinensis group), resulted in clear, reproducible protein banding patterns. The heat-tolerant cultivars and the heat-sensitive cultivars could be easily distinguished by the relative intensities of the 2 distinct bands at the 75,000 and 85,000 molecular weight regions.**

Chinese cabbage (heading type) is considered to be a cool season crop. In the tropics this crop is usually grown during the winter months or in the highland. During the hot summer months in the lowland tropics, most cultivars do not grow well and form only very loose heads. Recently, a number of workers at the Asian Vegetable Research and Development Center, Taiwan (1) have attempted to identify and screen for heat-tolerant cultivars (i.e. the ability to form firm heads under hot, humid conditions) for the tropics. In this paper, we report a simple and rapid method for identifying the heat-tolerant cultivars of Chinese cabbage by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis.

To extract soluble proteins from the seeds of Chinese cabbages, we tested a number of extraction media, including the following: 1.) 30% DMSO (2); 2.) 0.5M NaCl in 0.05M sodium phosphate buffer, pH 7.2; 3.) 12.5mM Tris-glycine buffer, pH 8.3, containing 1% isoluble PVP (Polyvinylpyrrolidone) and 10% sucrose (3); and 4.) 0.1M Hepes (1-(N-2-hydroxyethylpiperazin-N'-yl) enthanesulfonic acid), pH 7.4, containing 1% PVP, 2% NaCl, 5mM dithiothreitol (4). We found that the last extraction medium gave the most consistent results.

The routine extraction method used was as follows: Dry seeds (0.3 g) were ground in 2.1 ml of the extraction medium with a pestle and mortar for 5 min at room temp. The resulting slurry was centrifuged at 15,000g at 4°C for 20 min. After centrifugation the supernatant fluid was removed and filtered through a Whatman No. 5 filter paper at 4°C. The filtrates were diluted (1:1) with dissociating solution containing 0.125M Tris-HCl (pH

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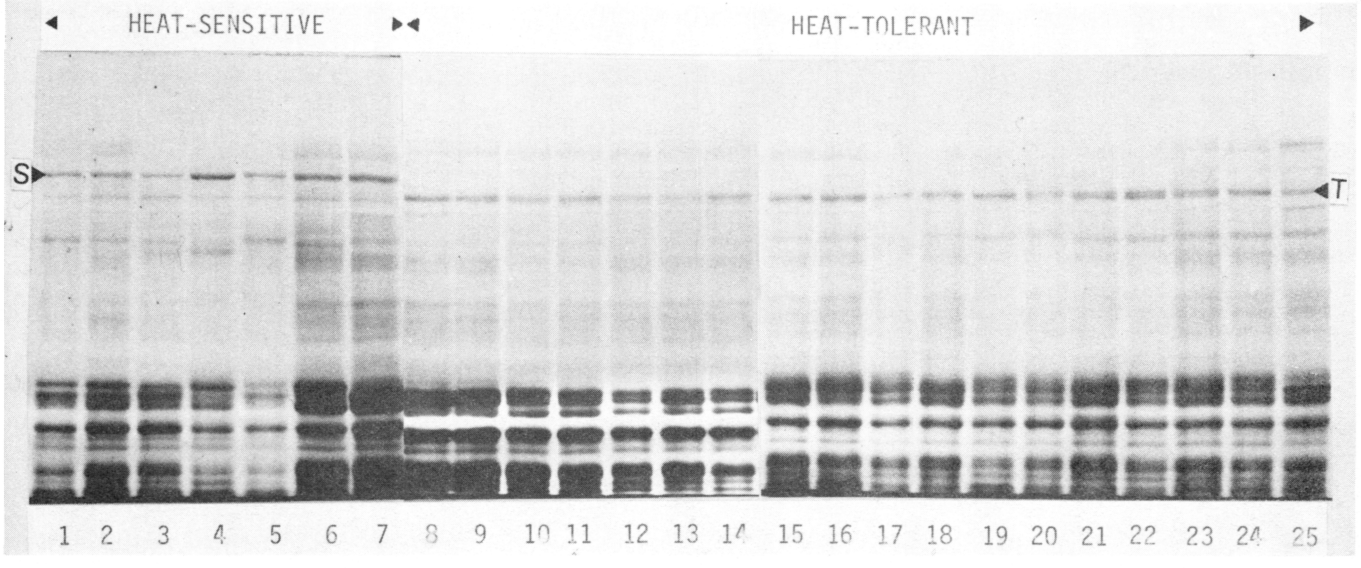


Fig. 1. SDS-polyacrylamide gel electrophoretograms of seed proteins of Chinese cabbage stained with Coomassie Brilliant Blue. 1-7 are electrophoretograms of heat-sensitive cultivars: 1. 'Nagaoka King' (Rijk Zwan, F1 Hybrid); 2. 'Tip Top' (Rijk Zwan, FA Hybrid); 3. B21 (Kyoto No. 3 AVRDC); 4. Acc 61 (AVRDC); 5. 'Snow Mountain' (Takii, Japan; F1 Hybrid); 6. Acc 42 (AVRDC); 7. Acc 40 (AVRDC). 8-25 are electrophoretograms of heat-tolerant cultivars; 8. Acc 183; 9. Acc 177; 10. Acc 193; 11. Acc 23; 12. B129C; 13. B189C; 14. Acc 116; 15. Acc 171; 16. Acc 161; 17. Acc 152; 18. Acc 155; 19. Acc 115; 20. Acc 172; 21. Acc 140; 22. Acc 175; 23. Acc. 113; 24. Acc 130; 25. Acc 162. S = proteins with molecular wt of about 85,000 daltons. T = proteins with molecular wt of about 75,000 daltons.

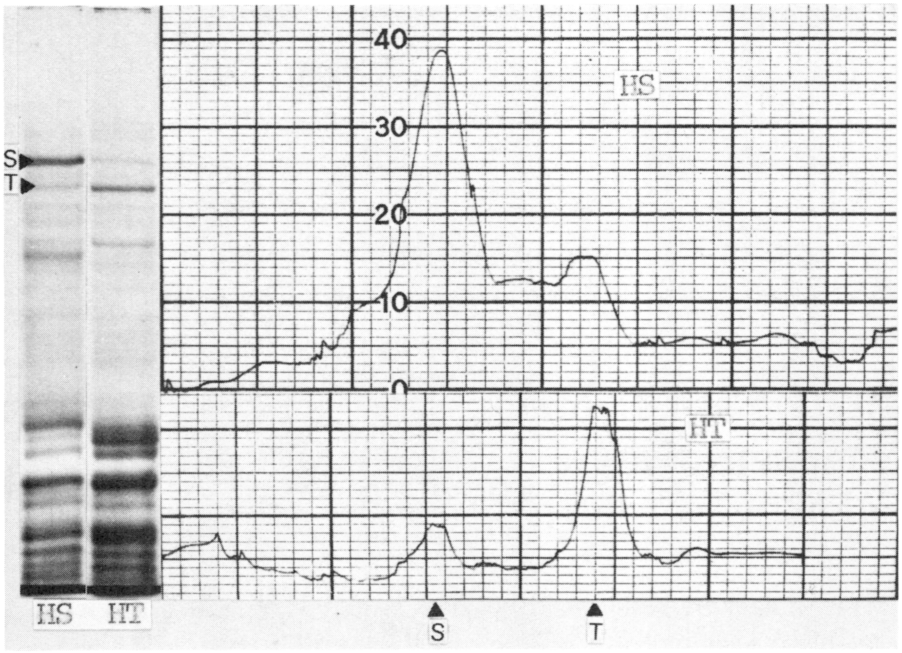


Fig. 2. Densitometric tracings of the 2 characteristic bands at the 85,000 (S) and 75,000 (T) molecular wt regions of a heat-sensitive (HS) (B21 AVRDC) and a heat-tolerant (HT) (B129C, AVRDC) cultivar.

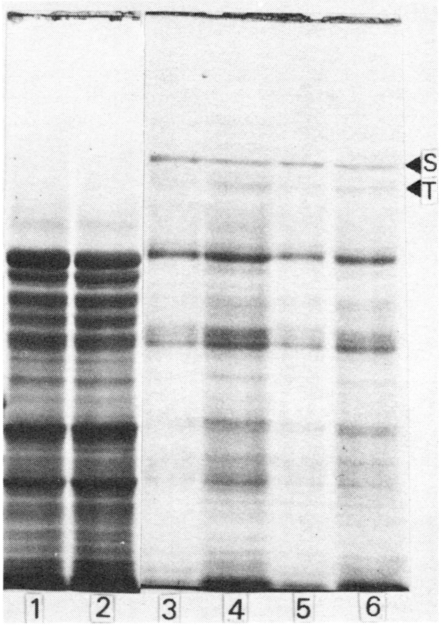


Fig. 3. 1 and 2 are electrophoretograms from the cotyledon extracts. 1 = Heat-sensitive cultivar (B21 (Kyoto No. 3 AVRDC); 2 = Heat-tolerant cultivar (B129C AVRDC) 3-6 are electrophoretograms from the hypocotyl extracts. 3 and 4 are from B21 and 5 and 6 from B129C. 1, 2, 4, 6 were extracted using 0.1M Hepes, pH 7.4, 1% PVP, 2% NaCl and 5 mM DTT; 3 and 5 were extracted with 12.5 mM Tris-glycine, pH 8.3, 1% PVP and 10% sucrose. S = protein region with Mol. wt ≈ 85,000 daltons; T = protein region with Mol. Wt ≈ 75,000 daltons.

6.8), 2% SDS, 2% 2-mercaptoethanol, 20% glycerol, and 0.004% bromphenol blue. After boiling for 2 min, 10 $\mu$l were applied to each gel slot of 10% acrylamide/SDS slab gel and electrophoresed according to Epstein, Waterston and Brenner (5). The gel apparatus used was constructed as described by Reid and Bieleski (3). Proteins after separation were stained with Coomassie Brilliant Blue, and the molecular wt of the proteins were estimated by relative mobilities upon the same 10% acrylamide/SDS slab gel with marker proteins of known molecular weights.

We have obtained protein profiles from the seed extracts of 7 heat-sensitive and 18 heat-tolerant cultivars of Chinese cabbage by using the methods of SDS-polyacrylamide gel electrophoresis (Fig. 1). Most seed samples were obtained from the Asian Vegetable Research and Development center (AVRDC), Taiwan, with some from Takii, Japan, and some from Rijk Zwaan, The Netherlands.

The major proteins (probably mainly storage proteins) of the 25 cultivars examined were very similar. But in the high molecular wt range of 75,000 to 85,000 daltons, the banding patterns of the heat-tolerant and heat-sensitive cultivars indicated marked differences in the electrophoretograms (Fig. 1). The heat-tolerant cultivars possessed a very prominent and intense band at the 75,000 region. This band is comparatively indistinct in the heat-sensitive cultivars, which show instead an intense band at the 85,000 region. This 85,000 band is very faint in the heat-tolerant cultivars. Differences in intensity between these 2 bands are illustrated in the densitometric tracings in Fig. 2.

Extracts obtained from the hypocotyls and cotyledons of 6-day-old seedlings of heat-tolerant and heat-sensitive cultivars (Fig. 3). did not show any marked differences and were of no use in separating the heat-tolerant from the heat-sensitive cultivars as were the seed extracts. Seed extracts have several additional practical advantages over the cotyledon or hypocotyl extracts: 1.) the seed extracts were more convenient screening materials, 2.) the seed extracts could be kept for about 2-3 weeks in the dissociating buffer, at 4°C, without marked deterioration, and 3.) the banding patterns of the seed extracts were very reproducible. The method outlined in this paper should be useful for mass-screening heat-tolerance in the Chinese cabbage.

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