Title

Chilling tolerance of cucumber (Cucumis sativus) seedling radicles is affected by radicle length, seedling vigor, and induced osmotic- and heat-shock proteins

Journal

Physiologia Plantarum, 124(4)

ISSN

0031-9317

Authors

Kang, H M
Park, K W
Saltveit, Mikal E

Publication Date

2005-08-01

Peer reviewed

Physiologia Plantarum 124: 485–492. 2005

Chilling tolerance of cucumber (*Cucumis sativus*) seedling radicles is affected by radicle length, seedling vigor, and induced osmotic- and heat-shock proteins

Ho-Min Kang[a], Kuen-Woo Park[b] and Mikal E. Saltveit[c,*]

[a]Department of Horticulture, Kangwon National University, Chunchon 200-701, Korea
[b]Division of Bioscience and Technology, College of Life and Environmental Sciences, Korea University, Seoul 136-701, Korea
[c]Mann Laboratory, University of California, One Shields Ave., Davis, CA 95616-8631, USA

Correspondence
*Corresponding author,
e-mail: mesaltveit@ucdavis.edu

Received 6 April 2005; revised 3 May 2005

doi: 10.1111/j.1399-3054.2005.00542.x

Cucumber seedling radicles decrease in chilling tolerance as they increase in length or decrease in vigor. The protein content of the apical 5 mm of the radicle decreased with decreases in chilling tolerance ($R^2 = 0.92$). This general reduction in protein content was reflected in a decrease of six dehydrin-like proteins with apparent molecular weights of 13.0, 15.0, 16.8, 23.0, 26.8, and 33.5 kDa. The disappearance of naturally occurring dehydrin-like proteins in cucumber seedling radicles as they elongate or lose vigor was correlated with a loss of chilling tolerance. Exposure to an osmotic (0.6 *M* mannitol) or heat (2 min at 45°C) stress enhanced chilling tolerance. The osmotic-shock treatment induced both chilling tolerance and the appearance or strengthening of dehydrin-like proteins previously present in radicles. The heat-shock treatment also induced high levels of chilling tolerance and protein(s) that reacted with a 23 and 70 kDa antibody. However, these heat-shock protein (HSPs) did not cross react with the probe for dehydrin-like proteins. When organized into high, medium, and low chilling tolerance groups, radicle that were chilling tolerant contained either the 13.0 and 16.8 kDa dehydrin-like proteins, or the 15.0 and 23.0 kDa dehydrin-like proteins, or the 23 or 70 kDa HSP.

Introduction

Plants native to tropical and subtropical climates suffer a physiological disorder termed chilling injury when exposed to non-freezing temperatures below approximately 12°C for an inductive period of time (Lyons 1973). Symptoms of chilling injury include reduced growth and photosynthetic capacity, tissue necrosis and vascular discoloration, abnormal ripening, and increased disease susceptibility. The chilling tolerance of sensitive plants varies among species and cultivars, and is affected by developmental and environmental factors. For example, seedling radicles increase in chilling sensitivity as they elongate (Rab and Saltveit 1996), while sublethal levels of certain abiotic stresses (e.g. salt stress, heat shock) increase chilling tolerance (Jennings and Saltveit 1994, Mangrich and Saltveit 2000). Osmotically stressing cucumber seedlings with 0.6 *M* mannitol induced chilling tolerance (Mangrich et al. 2005). A comparison of the chilling sensitivity of radicles as they elongated and after osmotic stress suggested that the loss of chilling tolerance was consistent with the progressive loss of a protective compound, possible through dilution as the radicles elongate.

Dehydrins are an immunologically distinct family of proteins [a subset of late-embryogensis-abundant proteins (LEA) proteins] that accumulate in plants during

Abbreviations – ABA, abscisic acid; HSP, heat-shock protein; LEA, late-embryogensis-abundant proteins.

the late stages of embrogensis, or in response to low temperature, abscisic acid application, or any of a number of environmental stresses that have a dehydration component, such as drought, salinity, or extra cellular freezing (Close 1997). Dehydrins can be produced under drought stress or as part of a developmental program activated during seed maturation (Chandler and Robertson 1994). They are temperature resistant and possess a conservative sequence that forms a putative amphiphilic α-helix domain, with the potential for both water binding and hydrophobic interaction (Dure 1993). Dehydrins have chaperone-like properties and stabilize an array of nuclear and cytoplasmic targets (Close 1996). Ismail et al. (1999b) reported that a specific dehydrin protein was associated with an increase in chilling tolerance during emergence of cowpea (*Vigna unguiculata* (L) Walp.) seedlings. Fu et al. (2000) made comparisons between the expression of dehydrin genes and several physiological parameters, especially those involving water relations in response to low temperatures.

Some heat-shock proteins (HSPs) (e.g. 70 kDa) also serve as chaperons. Small HSPs (e.g. around 20 kDa) may contribute to cell survival by preventing protein aggregation during heat stress (Haslbeck 2002). The expression of small HSPs depends on development and growth cycle (Pauli et al. 1990), and they can accumulate during seed maturation (Wehmeyer and Vierling 2000).

High-vigor seedlings are more tolerant of environmental stresses than low-vigor seedling. Vigor is 'a physiological property determined by the genotype and modified by the environment, which governs the ability of seed to produce a seedling rapidly in soil and the extent to which that seed tolerates a range of environmental factor' (Perry 1992). Radicles of high-vigor seedlings grew to 20 mm in length in 36 h at 25°C, whereas it took 60 h for low-vigor seedling radicles to reach that length. Chilling at 2.5°C for 48 h inhibited the subsequent growth of high-vigor seedlings by 39%, whereas it inhibited the growth of low-vigor seedlings by 68% (Kang and Saltveit 2002).

Cucumber seedling radicles become more chilling sensitive as they elongated. Chilling cucumber seedlings with 20-mm long radicles for 48 h at 2.5°C inhibited subsequent growth by 36%, while it reduced the growth of 70-mm long radicles by 63% (Kang and Saltveit 2002).

The object of our present study was to compare patterns of constitutive and induced dehydrin-like and osmotic- and heat-shock-proteins in cucumber seedling radicles that displayed differences in chilling tolerance because of differences in length and vigor. Proteins were identified that were strongly correlated with induced chilling tolerance in cucumber radicles.

Materials and methods

Plant material

Cucumber (*Cucumis sativus* L.,cv. Poinsett 76-S) seeds were obtained from a local vendor. Cucumber seeds (2 g) were imbibed in 1 l aerated water overnight at approximately 20°C. Imbibed seeds were transferred to moist paper toweling overlying capillary cloth that was sandwiched between two 15 × 30 cm Plexiglas plates (6 mm thick) that were held together with rubber bands. The plates were held in a vertical position at 25°C in a humid, dark, ethylene-free atmosphere for about 48 h, or until the radicles were about 10 mm long.

Germinated seeds with 10 ± 1 mm long radicles were removed from the large Plexiglas sandwich and gently transferred to moist paper toweling overlying capillary cloth and sandwiched between 7 × 30 cm Plexiglas plates (3 mm thick) as before. Each smaller plate held six to seven seedlings and was treated as a unit of replication. The plates were positioned vertically in a 20 × 26 × 14 cm tall white translucent plastic tub and the top loosely covered with aluminum foil. The seedlings were either held at 25°C for the initial measurements of radicle growth, or chilled at 2.5°C in the dark before being moved to 25°C for the growth measurements. Seedlings were selected with 5, 10, and 45 mm long radicles. Seedling radicles were considered to be of high vigor if they grew to 10 mm in 24 h, medium vigor it they took 36 h to reach 10 mm, and low vigor if they did not reach 10 mm in length until 48 h at 25°C.

Measurement of chilling injury

The extent of chilling injury was measured as the subsequent linear growth of radicles after chilling (Rab and Saltveit 1996) by a method modified from that previously described (Jennings and Saltveit 1994). Radicle length was measured with a clear ruler to the nearest mm before and after treatment, after chilling, and periodically during growth at 25°C. In some experiments, the small plates were disassembled, and the radicles gently straightened before measurement. The growth measurements for each seedling were regressed over time, and the slope and correlation coefficient calculated.

Heat-shock and dehydration treatments

Some small plates of cucumber seedlings with 10 ± 1 mm long radicles were placed in a plastic bag and immersed in water at 25 or 45°C for 0–120 min for the heat-shock treatment. Bags were left open at the top, and the open weave of the capillary cloth allowed adequate ventilation

of the plates so that internal carbon dioxide levels did not exceed 0.1% (data not shown). The bagged plates were then held for 15 min in 20°C water before being removed from the bags and placed in plastic tubs lined with wet capillary cloth. Other plates were immersed in 0.6 *M* mannitol solution for 2 h for the dehydration treatment. The plates were raised and lowered every 10 min to facilitate movement of the osmoticum throughout the plate. Control plates were immersed in water at 25°C. After treatment with mannitol or water, the seedlings were removed from the plate, rinsed, and repositioned on freshly assembled plates.

Western blot analysis

The expression of dehydrin-like proteins and HSPs was studied by homogenizing 5-mm cucumber radicle tips in sample buffer (40 m*M* HEPES, pH 6.8, 100 m*M* KCl, 5 m*M* KH_2PO_4, 2 m*M* EDTA, 1 m*M* $MgCl_2$). The suspension was centrifuged at 5000 × *g* for 15 min at 4°C. Heat-stable protein fractions were prepared as previously described (Close et al. 1989) by immersing extracts in a 100°C water-bath for 10 min, transferring to ice, then centrifuging in a microcentrifuge for 10 min at 4°C. Protein content was determined using BSA as a standard, according to the method of Bradford (1976). Equal amounts of the protein extract and 2 x Laemmli solutions [9% (w/w) SDS, 6% (v/v) β-mercaptoethanol, 10% (v/v) glycerol], were combined, and a trace amount of Bromophenol Blue dye in 0.196 *M* Tris/HCl (pH 6.7) was added.

A 20 µl sample of the extracts was electrophoresed through 10 (for HSP) and 14% SDS-PAGE (for dehydrins) and then transferred to Immobilon-P membranes (Millipore Corp., Bedford, MA) using a wet transfer system (Bio-Rad, Richmond, CA). Pre-stained protein standards (Bio-Rad) were run in each gel. The blots were blocked in Tris-buffered saline/Tween-20 (TBS-T containing 20 m*M* Tris base, pH 7.6, 137 m*M* NaCl, 0.1% Tween-20) supplemented with 5% skim milk for HSP or 3% gelatin for dehydrin-like proteins for 2 h. The blots were then incubated with diluted primary polycolonal antibodies against 23 and 70 kDa HSPs and dehydrin-like proteins for 1 h, and then with 1 : 10 000 diluted secondary antibodies of horseradish peroxidase-conjugated antirabbit IgG (Sigma, St Louis, MO) for 30 min at room temperature. After three washes of 5 min each, blots were treated with Enhanced Chemi-Luminescence (NEN life science products, Inc. Boston, MA) reagents, and the proteins were detected by autoradiography for 5 min with Kodak X-Omat film (Eastman Kodak, Rochester, NY). Quantification of the Western blot was performed by measuring the spot integrated density value of each band, corrected for film background, using an IS-1000 digital imaging system (Alpha Innotech Corporation, San Leandro, CA). The results are shown in figures as means ± SD of different blots per group.

Statistical design and analysis

Each experiment was repeated at least twice with similar results. All treatments were replicated at least four times within each experiment. Means and standard errors were calculated from pooled data. Gels were scanned, and the densities of each lane was normalized across experiments using common treatments to minimize variation among experiments. LSD values (5%) were calculated where appropriate.

Results and discussion

Changes in chilling tolerance with the various treatments

Radicle elongation after chilling (as a percent of non-chilled controls) decreased in a non-linear fashion as radicle length increased from 1 to 45 mm (Fig. 1). Seedlings chilled when their radicles were 1 mm long exhibited only a 7 ± 3% inhibition in subsequent radicle growth, while seedling chilled when their radicles were 45 mm long exhibited a 90 ± 5% inhibition in subsequent growth. Low-vigor seedlings with 10-mm long radicles exhibited greater injury (87% inhibition of

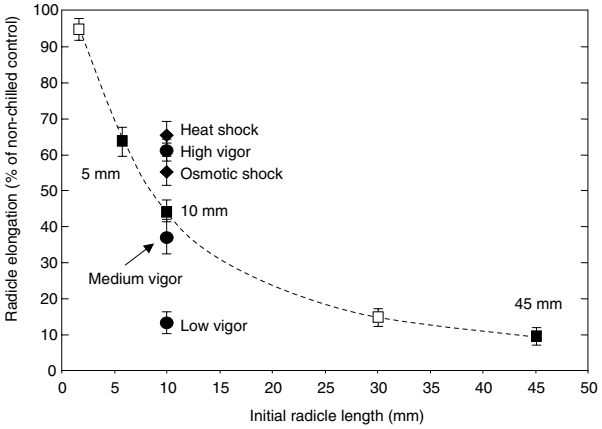

Fig. 1. Chilling-induced inhibition of elongation of cucumber radicles that were classified by length (0, 5, 10, 30, and 45 mm), vigor (high, medium, or low; i.e. 24, 36, or 48 h to grow 10 mm in length), and stressed (heat shocked at 45°C for 2 min, or osmotically stressed in 0.6 *M* mannitol for 2 h) before being chilled at 2.5°C for 72 h and then held at 25°C for 72 h. The vertical line associated with each mean represents the standard deviation about that mean. Radicles with solid symbols were used in subsequent experiments.

subsequent radicle elongation) than did medium-vigor seedlings (64%), while high-vigor seedlings were least affected (39%). Subjecting seedlings with 10-mm long radicles to an osmotic shock (0.6 *M* mannitol for 2 h) before chilling produced levels of tolerance (44% inhibition of radicle elongation compared to non-chilled control) similar to high-vigor seedlings (39%), whereas heat-shocked seedlings (45°C for 2 min) exhibited the greatest level of chilling tolerance (35%). Because high-vigor seedlings had levels of chilling tolerance similar to those induced in medium-vigor seedlings by osmotic- and heat-shock treatments, we examined the occurrence of dehydrin-like proteins and HSPs in the apical 5 mm of cucumber radicles of different length and vigor, or subjected to osmotic or heat shock.

Chilling tolerance and protein content

The soluble protein content of 5-mm radicle tips exhibited a pattern similar to that of chilling tolerance (Fig. 2). The shortest radicles and the highest vigor radicles had the highest protein content. The osmotic- and heat-shock treatments increased both chilling tolerance and protein content. The relation between protein content (mg g^{-1} FW) of the apical 5-mm radicle tip and subsequent radicle growth was linear (Fig. 3), and is given by the equation: Radicle elongation as percentage of non-chilled control = (10.6 × protein content) −97.3 with an R2 of 0.92. Kang and Saltveit (2001, 2002) reported that higher protein content was related to chilling tolerance in cucumber and rice radicles. Higher protein content may reflect a higher capacity for metabolic activity that would assist in the recovery from chilling injury. However, the apical 5 mm, protein dense meristematic tissue is more chilling sensitive than the mature, highly vacuolated cells more than 5 mm from the apex of the cucumber radicles (Rab and Saltveit 1996). The higher levels of certain antioxidant enzymes and proteins with radical scavenging activity in chilling tolerant radicles could account for some of the higher protein content and greater chilling tolerance (Kang and Saltveit 2001, 2002).

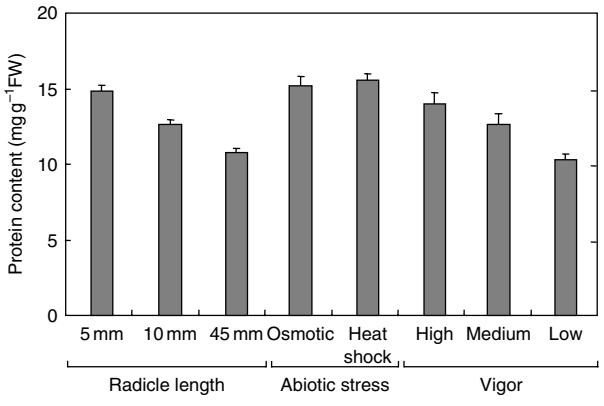

Fig. 2. Soluble protein content of cucumber radicles that were classified by length (5, 15, 45 mm), by abiotic stress (heat shocked at 45°C for 2 min, or osmotically stressed in 0.6 *M* mannitol for 2 h), or by vigor (high, medium, or low; i.e. 24, 36, or 48 h to grow 10 mm in length). Soluble protein content was measured after samples were heated to 100°C for 10 min. The vertical line associated with each bar represents the standard deviation about that mean.

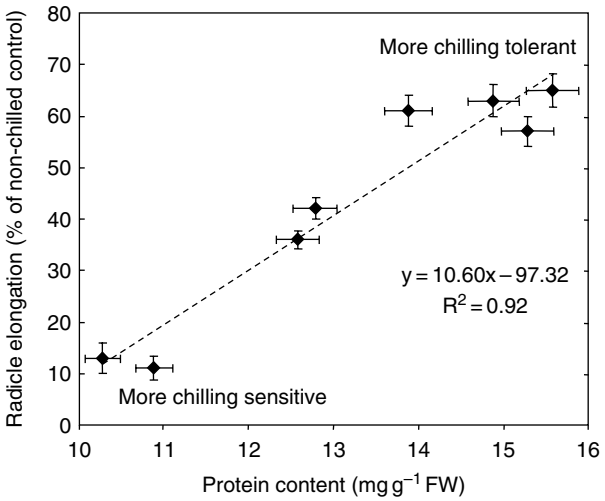

Fig. 3. Relationship between the soluble protein content of the apical 5 mm of seedling radicles and the chilling-induced inhibition of radicle elongation of seedlings subjected to the treatments described in Figs 1 and 2. The vertical and horizontal lines associated with each mean represents the standard deviation about that mean.

Chilling tolerance and dehydrin-like protein levels

Proteins isolated from cucumber radicles exhibited six major bands of dehydrin-like protein that had apparent molecular masses of 13.0, 15.0, 16.8, 23.0, 26.8, and 33.5 kDa (Table 1). Except for the 13.0 kDa protein, the dehydrin-like proteins were expressed more strongly in shorter radicles than in longer ones, and the bands became less distinct as the radicles grew from 5 to 45 mm in length. A 2 min 0.6 *M* osmotic shock to 10-mm long radicles induced the appearance of bands at 13.0 and 16.8 kDa, and strengthened all the other bands, except 15.0 kDa which decreased in intensity. The predominant band at 15.0 kDa in 5 mm radicles decreased 82% as radicles grew to 45 mm in length and lost 83% of their chilling tolerance. However, the intensity of this band decreased 30% in osmotic stressed 10 mm radicles, while the osmotic shock increased their chilling tolerance by 36%. Similarly, the heat-shock treatment did not significantly increase the

Table 1. Protein content, level of chilling-induced inhibition of radicle elongation, and relative band density of dehydrin-line proteins extracted from the apical 5 mm of cucumber radicles. Cucumber radicles were classified by length (0, 5, 10, or 45 mm), vigor (high, medium, or low; i.e. 24, 36, or 48 h to grow 10 mm in length), and stress (heat shocked at 45°C for 2 min, or osmotically shocked in 0.6 *M* mannitol for 2 h) before being chilled at 2.5°C for 72 h and then held at 25°C for 72 h. Equal amounts of protein (50 μg) were loaded in each well and subjected to SDS-PAGE. The resolved polypeptides were electro-transferred and proteins immuno-revealed using dehydrin-like proteins antibodies. The density of the lanes were quantified and normalized across similar treatments in different experiments.

			Molecular weight					
Treatment	Protein (mg g^{-1})	Radicle elongation (% control)	13.0	15.0	16.8	23.0	26.8	33.5
Heat shock	15.6	65	39	34	28	49	60	41
5 mm	14.9	63	25	94	56	107	115	49
High vigor	13.9	61	41	121	48	82	92	40
Osmotic shock	15.3	57	113	32	82	43	104	46
10 mm	12.8	42	26	45	36	37	67	37
Medium vigor	12.6	36	35	38	21	42	53	40
Low vigor	10.3	13	19	27	18	27	37	30
45 mm	10.9	11	17	16	21	17	30	43
LSD 5%	1.5	7.4	14.1	12.7	21.2	19.6	9.8	11.2

intensity of the 15.0 kDa band over that in the 10 mm radicles, but it did increase their chilling tolerance by 35%.

A comparable pattern was found in cucumber seedling radicles of differing vigor (Table 1). The apical 5 mm portion of high-vigor 10 mm seedling radicles contained dehydrin-like proteins that produced bands with apparent molecular masses similar to those seen in 5 mm radicles; 15.0, 23.0, and 26.8 kDa. The 2 min 0.6 *M* osmotic shock of medium-vigor radicles again induced the appearance of two bands at 13.0 and 16.8 kDa, and strengthened all the other bands, except 15.0 which decreased in intensity. The osmotic shock did not significantly alter the intensity of the band among the medium-vigor, low-vigor, and shocked medium-vigor radicles (32 ± 5.5). However, the osmotic shock did significantly increase chilling tolerance 37% over the medium-vigor radicles. Heat shock had a similar effect; it did not increase the intensity of the 15.0 kDa band, but it did increase chilling tolerance by 45%.

The five bands found in these cucumber seedling radicles are similar in number and apparent molecular mass to the five LEA proteins identified in four plant species (i.e. cotton, carrot, barley, and rape) that ranged in size from roughly 14.5 to over 60 kDa and had regions of sequence homology (Dure et al. 1989). Major dehydrin-like protein bands that were detected in mature seeds of barley and radish decreased during germination and seedling growth (Close et al. 1993). The loss of dehydrin-like proteins during seedling growth and their induction by dehydration during maturation may be a common feature of these proteins; at least for the 25 kDa barley and the 20 kDa radish proteins. LEA mRNAs and the proteins which they encode reached maximum levels in dry seed and disappeared rapidly after germination (Raynal et al. 1989).

The treatments and radicle attributes were grouped into those that produced high levels of chilling tolerance ($60 \pm 3\%$ of radicle elongation compared to the non-chilled control; 5-mm, high vigor and osmotic shock), medium levels of chilling tolerance ($39 \pm 3\%$; 10 mm, medium vigor) and low levels of chilling tolerance ($12 \pm 2\%$; 45 mm, low vigor) (Fig. 4). There were small differences in the density of the various dehydrin-like protein bands within the medium- and low-chilling tolerance groups (as reflected by their low-standard deviations). In contrast, the high-chilling tolerant groups exhibited large differences. It appears that radicles will exhibit chilling tolerance if they contain relative high levels of either the 13.0 and 16.8 kDa dehydrin-like proteins (osmotic shocked), or the 15.0 and 23.0 kDa dehydrin-like proteins (5 mm, high vigor) (Table 1). It appears that similar levels of chilling tolerance can be produced by different combinations of constitutive or induced dehydrin-like proteins.

Chilling tolerance and HSPs

HSPs (e.g. 23 and 70 kDa, HSP) have a chaperon function (Wehmeyer and Vierling 2000). However, in this study, there was no difference in the intensity of the 70 kDa HSP band among the non-heat-shock treatments (Fig. 5). Immunoblots with a 70 kDa HSP antibody showed no significant difference in band density (8.5 ± 2.8 relative levels) among different radicle lengths, different seedling vigor, or exposure to the osmotic-shock treatment. However, the

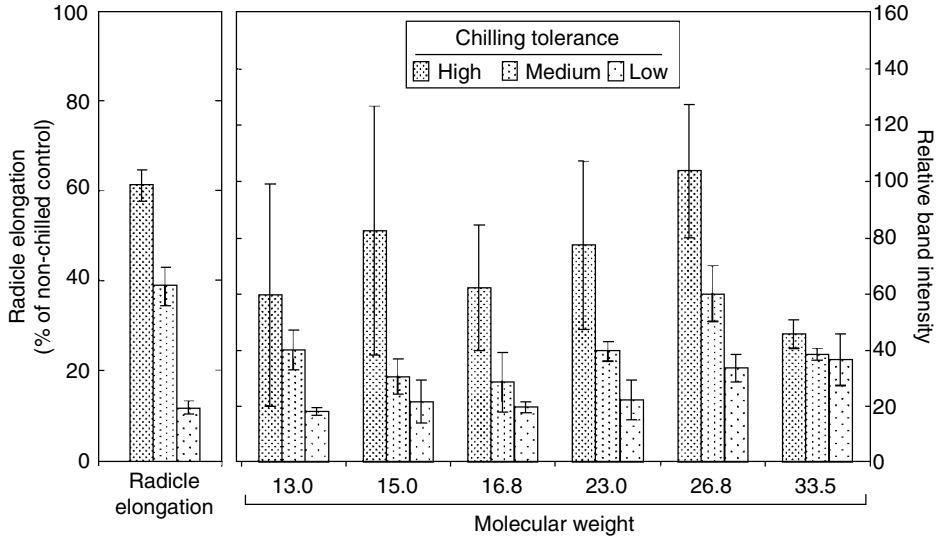

Fig. 4. Level of chilling-induced inhibition of radicle elongation, and relative band density of dehydrin-line proteins extracted from cucumber radicles. Cucumber radicles that were classified by length (0, 5, 10, or 45 mm), vigor (high, medium, or low; i.e. 24, 36, or 48 h to grow 10 mm in length), and stressed (heat shocked at 45°C for 2 min, or osmotically stressed in 0.6 *M* mannitol for 2 h) before being chilled at 2.5°C for 72 h and then held at 25°C for 72 h. Equal amounts of protein (50 μg) were loaded in each well and subjected to SDS-PAGE. The resolved polypeptides were electro-transferred and proteins immuno-revealed using dehydrin-like proteins antibodies. The density of the lanes were quantified and normalized across experiments. The vertical line associated with each bar represents the standard deviation about that mean.

heat-shock treatment induced a significant 7.3-fold increase in the density of the 70 kDa band along with the highest level of chilling tolerance (65% of radicle growth).

In contrast to the 70 kDa HSP, there were significant differences among these tissues when probed with an antibody to 23 kDa HSP. Proteins isolated from 5-mm long and high-vigor radicles produced far denser bands (64 and 38% of maximum) than did proteins isolated from the other treatments (5.4 ± 2.5%) (Fig. 5). Osmotically stressed radicles also had elevated levels of 23 kDa HSP (18%), but the highest level was induced by the heat-shock treatment (100%). However, even though levels of the 23 kDa HSP varied significantly among these radicles, the chilling tolerance of the osmotically stressed 10-mm long radicles (band intensity of 18% and chilling tolerance of 57% of non-chilled control) was similar to that of the heat-shocked radicles (100 and 65%), 5-mm long radicles (64 and 63%) and the high-vigor 10-mm long radicles (38 and 61%) (Table 1, Fig. 5). A positive relationship has been reported between the expression of small HSPs and chilling tolerance (Garcia-Ranea et al. 2002, Haslbeck 2002). These small HSPs are thought to have a chaperone effect protecting some proteins that may be damaged at low temperatures. Our results expand on these findings by showing that similar levels of chilling tolerance can result from the presence (whether constitutive or induced) of a number of dehydrin-like or HSPs.

The 23 kDa protein(s) induced by osmotic stress (Fig. 4) and heat stress (Fig. 5) may be only similar in molecular

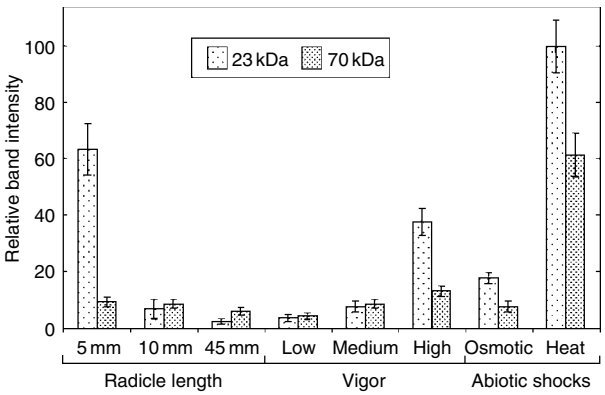

Fig. 5. Immunoblot analysis of two heat-shock protein (HSP) proteins. Equal amounts of protein (50 μg) were loaded in each well and subjected to SDS-PAGE. The resolved polypeptides were electro-transferred and proteins immuno-revealed using 23 and 70 kDa HSP antibodies. Cucumber radicles that were classified by length (0, 5, 10, or 45 mm), vigor (high, medium, or low; i.e. 24, 36, or 48 h to grow 10 mm in length), and stressed (heat shocked at 45°C for 2 min, or osmotically stressed in 0.6 *M* mannitol for 2 h) before being chilled at 2.5°C for 72 h and then held at 25°C for 72 h. The density of the lanes were quantified and normalized across experiments. The vertical line associated with each bar represents the standard deviation about that mean.

weight and not necessarily the same protein. The 23 kDa HSP was strongly correlated with chilling tolerance, but the dehydrin-like 23 kDa protein was not strongly associated with increased chilling tolerance induced by either the osmotic- or heat-shock treatments (Table 1).

Conclusion

Radicles exhibiting high levels of chilling tolerance either contained the 13.0 and 16.8 kDa dehydrin-like proteins, or the 15.0 and 23.0 kDa dehydrin-like proteins, or the 23 or 70 kDa HSP. Additional HSPs may be involved in acquired chilling tolerance, but we did not probe for general heat-shock like proteins as we did for dehydrin-like proteins. Another dehydrin-like protein (33.5 kDa) does not appear to be involved in the chilling tolerance of cucumber seedling radicles. The presence of specific dehydrin-like proteins has been strongly related with chilling tolerance (Ismail et al. 1999a, b). Our results show that the disappearance of naturally occurring dehydrin-like proteins in cucumber seedling radicles, as they elongate or loose vigor, are correlated with a loss of chilling tolerance. An osmotic stress induced both chilling tolerance and the appearance or strengthening of dehydrin-like proteins previously present in radicles. A heat-shock treatment also induced high levels of chilling tolerance, and protein(s) that reacted with a 23 kDa antibody. This heat-shock-induced protein may be the dehydrin-like 23 kDa protein naturally present in 5 mm or high-vigor seedling radicles, but was not inducible by either the osmotic or heat shocks.

Acknowledgements – We are grateful to Professor C. B. Hong, Institute for Molecular Biology and Genetics, Seoul National University for the kind gift of HSP, and to Professor T. J. Close, Department of Botany and Plant Science, University of California, Riverside for the kind gift of dehydrin-like protein antibodies. This project was supported in part by a Korea University Grant.

References

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254

Chandler P, Robertson M (1994) Gene expression regulated by abscisic acid. Annu Rev Plant Physiol Plant Mol Biol 45: 113–141

Close TJ (1996) Dehydrins: Emergence of a biochemical role of a family of plant dehydration proteins. Physiol Plant 97: 795–803

Close TJ (1997) Dehydrins: a commonalty in the response of plants to dehydration and low temperature. Physiologia Plantarum 100: 291–296

Close TJ, Fenton RD, Moonan F (1993) A view of plant dehydrins using antibodies specific to the carboxy terminal peptide. Plant Mol Biol 23: 279–286

Close TJ, Kortt A, Chandler P (1989) A cDNA-based comparison of dehydration-induced proteins (dehydrins) in barley and corn. Plant Mol Biol 13: 95–108

Dure L (1993) Structural motifs in LEA proteins. In: Close TJ, Bray EA (eds) Plant responses to cellular dehydration during environmental stress. American Society of Plant Physiologists, Rockville, pp 91–103

Dure L, Crouch M, Harada J, Ho T-HD, Mundy J, Quatrano R, Thomas T, Sung ZR (1989) Common amino acid sequence domains among the LEA proteins of higher plants. Plant Mol Biol 12: 475–486

Fu P, Wilen RW, Wu G-H, Robertson AJ, Gusta LV (2000) Dehydrin gene expression and leaf water potential differs between spring and winter cereals during cold acclimation. J Plant Physiol 156: 394–400

Garcia-Ranea J, Mirey G, Camonis J, Valencia A (2002) p23 and HSP20/alpha-crystallin proteins define a conserved sequence domain present in other eukaryotic protein families. FEBS Lett 529: 162–167

Haslbeck M (2002) sHsps and their role in the chaperone network. Cell Mol Life Sci 59: 1649–1657

Ismail AM, Hall AE, Close TJ (1999a) Allelic variation of a dehydrin gene cosegregates with chilling tolerance during seedling emergence. Proc Nat Acad Sci USA 96: 13566–13570

Ismail AM, Hall AE, Close TJ (1999b) Purification and partial characterization of a dehydrin involved in chilling tolerance during seedling emergence of cowpea. Plant Physiol 120: 237–244

Jennings P, Saltveit ME (1994) Temperature and chemical shocks induce chilling tolerance in germinating cucumis sativus (cv. Poinsett 76) seeds. Physiol Plant 91: 703–707

Kang H-M, Saltveit ME (2001) Activity of enzymatic antioxidant defense systems in chilled and heat shocked cucumber seedling radicles. Physiol Plant 113: 548–556

Kang H-M, Saltveit ME (2002) Effect of chilling on antioxidant enzymes and DPPH-radical scavenging activity of high- and low-vigour cucumber seedling radicles. Plant Cell Environ 25: 1233–1238

Lyons JM (1973) Chilling injury in plants. Annu Rev Plant Physiol 24: 445–466

Mangrich ME, Martinez-Font RT, Saltveit ME (2005) Radicle length and osmotic stress affect the chilling sensitivity of cucumber (Cucumis sativus, L., cv. Poinsett 76) radicles. Crop Science. (In press)

Mangrich ME, Saltveit ME (2000) Effect of chilling, heat shock, and vigor on the growth of cucumber (Cucumis sativus) Radicles Physiol Plant 109: 137–142

Pauli D, Tonka CH, Tissieres A, Arrigo AP (1990) Tissue-specific exprssion of the heat shock protein HSP27 during

Drosophila melanogaster development. J Cell Biol 111: 817–820
Perry DA (1992) Seed vigor and field establishment. Hort Abstracts 42: 334–342
Rab A, Saltveit ME (1996) Differential chilling sensitivity in cucumber (Cucumis sativus) Seedlings Physiol Plant 96: 375–382
Raynal M, Depigny D, Cooke R, Delseny M (1989) Characterization of a radish nuclear gene expressed during seed late development. Plant Physiol 91: 829–836
Wehmeyer N, Vierling E (2000) The expression of small heat shock proteins in seeds responds to discrete developmental signals and suggests a general protective role in desiccation tolerance. Plant Physiol 122: 1099–1108

Edited by C. Guy