stages of development exhibited little or no injury. Selection of proper leaf tissue from tomato plants was required, since even different leaflets on a single leaf varied in sensitivity. If leaflets of similar maturity were selected, consistent injury development occurred on each of the disks cut from those leaflets. Plants such as sweet potato and aspen may prove to be easier to work with, since they possess simple leaves positioned alternately along the stem, and the entire leaf appears to be uniformly sensitive to injury.

Leaf disks cut from nearly fully expanded leaves or leaflets of eight other plant species (besides tomato) known to be susceptible to intumescence injury also were tested using this procedure. Of these eight, six developed typical intumescence injury. These species, along with the cultivars tested, are given in Table 2. The other two species tested, Solanum melongena 'Black Beauty' and Capsicum annuum 'California Wonder' and 'Yolo Wonder', showed no injury development. It is possible that in these species intumescence injury is exhibited at a different stage of tissue maturity than observed in the other species tested.

This investigation has resulted in the development of a procedure for consistently inducing intumescence injury on leaf disks. The small size of the system allows better use of growth chamber space and requires less plant material for each experiment. This procedure should prove to be a useful tool for investigations of factors regulating intumescence injury.

**Literature Cited**


**Freezing Tolerance and Rapid Cold Acclimation of Spinach**

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**Additional index words.** Spinacia oleracea, cold hardness, freezing tolerance, cold stress

**Abstract.** Freezing tolerance and rates of acclimation and deacclimation were determined for three Chinese spinach (Spinacia oleracea) cultivars ('Harbin', 'Beijing', and 'Shantung') and one American spinach cultivar ('Bloomsdale'). Detached leaf and whole plant freezing tolerances were compared during cold acclimation. Freezing tolerance of detached leaves was equivalent to the freezing tolerance of whole plants. Using detached leaves, freezing tolerance of the cultivars was determined after 0, 1, 3, 7, 14, and 21 days of cold acclimation at 5°C (day/night temperature) and then after 1, 3, and 7 days of deacclimation at 20°/17°C. All four cultivars acclimated and deacclimated very rapidly. Depending on the cultivar, a 2° to 4° increase in freezing tolerance was observed after 1 day of acclimation. The greatest change in freezing tolerance occurred in the first 3 days of acclimation. The rate of acclimation diminished dramatically after 3 days, and there was little change in freezing tolerance after 14 days of acclimation. Upon exposure to deacclimating temperatures, fully acclimated plants lost 3° to 5° freezing tolerance within 1 day. There was no further decline in freezing tolerance after the 3rd day of deacclimation.

Herbaceous plants have a limited capacity to withstand sub-zero temperatures during the growing season. However, some species acclimate extensively when subjected to cold-acclimating environments (i.e., low day/night temperatures) (8–12). Cold acclimation is important ecologically and agriculturally, particularly in temperate zones.

Spinach, a cool-season short-day, is grown in the fall, winter, and early spring. It can encounter both short or long periods of freezing temperatures (7, 12). Thus the capacity to acclimate is important for survival and productivity. Most cold acclimation studies focus on the ultimate freezing tolerance of the plant after relatively long-term acclimation (10, 11, 13). The rate of acclimation and the retention of a high level of hardiness during warm days in midwinter and early spring are equally important.
factors in the survival and productivity of a cool-season crop such as spinach.

Although spinach frequently has been used as a model system to study mechanisms of freezing injury, little information exists on acclimation characteristics of the whole plant. The objective of this study was to examine the rate of cold acclimation and deacclimation in four spinach cultivars. Both crowns and detached leaves were subjected to controlled freezing tests to measure changes in freezing tolerance during acclimation and deacclimation.

Materials and Methods

Freezing tolerances of crowns and detached leaves. Seeds of the spinach cultivars ‘Bloomsdale’, a savoy leaf type (Northrup King), and ‘Harbin’, a smooth leaf type (Beijing Vegetable Research Center, Beijing, China) were germinated in the dark on moist filter paper. The seedlings were transplanted into a surface (Acrilite, International Minerals and Chemical Co., Mundelien, Ill.)/sand mixture (by volume) in 3 × 12 cm seeding plug flats and watered daily with half-strength Hoagland’s solution. Plants were grown in controlled environment chambers at 20°/17°C, day/night (D/N) temperature and a 12-hr photoperiod with 350–400 μmol·s⁻¹·m⁻² PPF at plant height. After 4 weeks, the plants were acclimated at 5°/2°C D/N, 12-hr photoperiod, and allowed to grow for 4 weeks at the temperature and light conditions previously described. After 4 weeks, viability of plants was rated visually as live (green and growing) or dead (no growth).

The test temperature at which half of the plants were killed was designated as the plant LT₅₀. This experiment was replicated three times for ‘Bloomsdale’ and four times for ‘Harbin’, and the LT₅₀ of the leaves and the plants were compared.

Rate of acclimation and deacclimation. ‘Bloomsdale’ and ‘Harbin’ and two additional smooth-leaved Chinese cultivars, ‘Beijing’ and ‘Shantung’, were used in this study. Seeds were planted in a 1 soil : 1 sand : 1 peat mixture (by volume) in 15-cm-diameter pots and fertilized with half-strength Hoagland’s solution. Plants were grown in controlled environment chambers as described. After 6 weeks, plants were acclimated at 5°/2°C D/N. Freezing tolerance of the leaves was measured after 0, 1, 3, 7, 14, and 21 days of acclimation. After 21 days of acclimation at 5°/2°C, plants were deacclimated at 20°/17°C D/N. The freezing tolerance of leaves was measured after 1, 3, and 7 days of deacclimation.

Freezing tolerance was determined by freezing detached leaves as described, with the following modifications. Three random, fully expanded leaves of similar age were wrapped in wet cheese cloth and placed in test tubes containing ice chips, then frozen at a rate of 2°C/hr. Tubes were removed from the temperature bath at 1° intervals between −2° and −20°, placed on ice, and then thawed at 4°C overnight. Electrolyte leakage was used to determine the leaf LT₅₀. Acclimation and deacclimation experiments were replicated three times for each cultivar.

Results

Freezing tolerances of whole plant and detached leaf. The leaf LT₅₀ had a high positive correlation with the whole plant LT₅₀, with r values of 0.79 and 0.94 for ‘Harbin’ and ‘Bloomsdale’, respectively. Regression analysis and t test (0.01 level) of the slope and x-intercepts showed no differences between the cultivars. Therefore, regression analysis was done on the mean leaf LT₅₀ and whole plant LT₅₀, pooling both cultivars and all days of acclimation with the whole plant LT₅₀ as the dependent variable (Fig. 1). The slope off the line was 0.94 and a t test

Fig. 1. Correlation of mean detached leaf and whole plant LT₅₀s determined at various times of acclimation at 5°/2°C D/N for ‘Harbin’ (●) and ‘Bloomsdale’ (●). The line and error bars represent the prediction equation (y = 0.95 × −0.33, r² = 0.86) and se of predicted values for whole plant freezing tolerance.
... showed the greatest decrease in freezing tolerance, losing 5°, while ‘Harbin’, ‘Beijing’, and ‘Bloomsdale’ lost 3° in the first day. By the 7th day, there was little further decrease in freezing tolerance occurring on the first day. ‘Beijing’, ‘Shantung’, and ‘Bloomsdale’, respectively. By the 7th day of acclimation, freezing tolerance increased 0.5° per day in all cultivars. Deacclimation proceeded rapidly at 20°/17°C D/N, reaching a maximum of −17°, −14°, −13°, and −13° in 21 days for ‘Harbin’, ‘Beijing’, ‘Shantung’, and ‘Bloomsdale’, respectively.

The greatest increase in freezing tolerance occurred in the first day of deacclimation, with smaller increments on successive days. There was a 4°, 3°, 4°, and 2°C increase in freezing tolerance after 1 day of cold acclimation for ‘Harbin’, ‘Beijing’, ‘Shantung’, and ‘Bloomsdale’, respectively. By the 7th day of acclimation, freezing tolerance increased 0.5° per day in all cultivars. Deacclimation proceeded rapidly at 20°/17°C in ‘Beijing’, ‘Shantung’, and ‘Bloomsdale’ (Fig. 3), with the greatest decrease in freezing tolerance occurring on the first day. ‘Beijing’ and ‘Bloomsdale’ deacclimated at the same rate. ‘Shantung’ showed the greatest decrease in freezing tolerance, losing 5°, while ‘Harbin’, ‘Beijing’, and ‘Bloomsdale’ lost 3° in the first day. By the 7th day, there was little further decrease in freezing tolerance and the cultivars retained about 2° greater freezing tolerance than before acclimation.

**Discussion**

The LT₅₀ of detached leaves and whole plants were highly correlated, with the regression analysis indicating a one-to-one relationship between them. Estrada et al. (6) found that results of a controlled freezing test of detached potato leaves correlated with whole plant response under field freezing conditions. Controlled freezing tests of winter wheat seedlings were highly correlated with field survival of older plants (1). In contrast, Rajashekar et al. (13) reported that leaves and crowns of turfgrass had different freezing tolerances. However, the results of this study indicate that the LT₅₀ of detached leaves can be used as a good predictor for whole plant response when comparing spinach cultivars. This technique is extremely useful as large numbers of plants can be screened in a shorter time period and fewer plants are needed than when using whole plants.

Freezing tolerance increased rapidly when plants were transferred from 20°/17°C to 5°/2°C and decreased rapidly when returned to the high temperature regime. While rapid rates of deacclimation have been reported (3, 8), little information exists on rapid acclimation. Dexter (5) indicated that some acclimation occurred in alfalfa roots after 4 days of acclimation at 0°C, but did not report the changes in freezing tolerance, so it is not possible to calculate rates of acclimation. Chen and Li (4) reported an increase of 2° freezing tolerance in S. commersonii after 4 days of cold acclimation. The rate of acclimation is much greater in spinach with a 2° to 4° increase in 1 day and a 4° to 8° total after 3 days of acclimation.

Previous work has suggested that cultivars with savoy leaf types have a greater freezing tolerance than smooth leaf types (2). In this study, the smooth leaf types were as hardy or more hardy than the savoy type. The Chinese cultivar Harbin showed a more rapid response and greater increase in freezing tolerance during acclimation, and maintained a greater amount of freezing tolerance during deacclimation than the other cultivars. This cultivar therefore might provide useful germplasm for breeding freezing-tolerant spinach.

The rapid response of spinach to temperature changes in its environment is important in a herbaceous crop where low temperature (and not short days) is the primary factor for initiating the processes of cold acclimation (10). It is of particular importance in spinach, which is grown from fall to spring at a variety of latitudes and may be subjected to sudden as well as long periods of freezing temperature during its growth. The capacity to acclimate rapidly also offsets the potential hazard of rapid deacclimation of spinach during exposure to warm temperatures.

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Variation in Glucosinolates in Oriental Brassica Vegetables

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Abstract. The glucosinolates (GSs) were estimated in the normally eaten portions of 72 cultivars of Oriental brassica vegetables including mustard greens (Brassica juncea L.), Chinese kale (B. oleracea L. Alboflabra Group Bail.), Chinese cabbage (B. rapa L. Pekinesis Group Bail.), pak choy (B. rapa Chiniscus Group Bail.), tendergreen (B. rapa Perviridis Group Bail.), turnip (B. rapa L. Rapifera Group Bail., B. narinosa Bail., and B. nipposinica Bail.). Variation in GS profiles was complex. There was variation in percentages of major GSs and total GS among B. juncea, B. oleracea, and the combination B. rapa plus narinosa and nipposinica and among four subspecific groups of rapa plus the two species closely related to rapa: narinosa and nipposinica. B. juncea had distinctively high proportions of allyl-GS, ranging from 81% to 94%, whereas B. oleracea had distinctively high proportions of 4-methylsulfinylbutyl-GS, ranging from 9% to 68%. Differences in GS profiles among the rapa groups, narinosa and nipposinica, were less distinctive. Cultivars of pak choy from China differed in percentages of three minor GSs from cultivars from Japan and elsewhere. There was also variation among cultivars of Chinese kale and between turnip foliage and roots.

Vegetable brassicas including Chinese cabbage, pak choy, turnip, tendergreen, B. narinosa, B. nipposinica, mustard greens, and Chinese flowering kale long have been important in the diet of East Asians (10, 25, 27), and they are increasing in popularity in the United States and Europe (8). Important in crucifers are the biologically active thioglucosides known as glucosinolates (GSs), the chemistry of which has been extensively reviewed (18, 20). The hydrolytic products of GSs have been implicated in mustard flavor (11), insect attraction (6), disease susceptibility (1), toxicity to animals (13), and antitumorogenesis (26).

As part of a comprehensive study to provide information on levels and variation of GSs in cruciferous vegetables (2, 3, 5, 19), we have analyzed the GS contents in the edible parts of 72 cultivars or lines of mustard greens, Chinese flowering kale, B. narinosa, B. nipposinica, and vegetables in four subspecific groups of B. rapa.

Materials and Methods

Seeds of 72 cultivars or breeding lines of oriental brassica vegetables were provided by seed companies, institutions, or individuals (Table 1). Seeds were sown in plastic pots containing 1 coarse sand: 1 compost: 1 peatmoss (by volume), and plants were grown in the greenhouse at 24°C and fertilized weekly with a 10N-10P-10K fertilizer. Twenty-one days after seeding, plants were transplanted to the field, which consisted of a Columbia silt-loam (fine, loamy, mixed, mesic, typic, haplaquolls) with a 10N-10P-10K fertilizer. Twenty-one days after seeding, plants were transplanted to the field, which consisted of a Columbia silt-loam (fine, loamy, mixed, mesic, typic, haplaquolls) at the Univ. of Wisconsin agricultural experiment station, Madison. The Chinese flowering kale was grown in Plainfield loamy-sand (mixed, mesic, typic, udipsamments) at the Univ. of Wisconsin agricultural experiment station. Hancock. Chinese cabbage cultivars from China were sown in August and harvested in Nov. 1980; Chinese flowering kale was sown in June and harvested in Aug. 1981; the remainder of the vegetables were sown in June and harvested in July 1980. Two or three plants of each cultivar were chilled, harvested, and shipped to the USDA Northern Regional Research Center in Peoria, Ill., where they were stored at 4°C for up to 3 weeks before extraction. Storage of plants in the refrigerator had no effect on GS levels.

The plant parts sampled were those normally eaten (Table 1). For Chinese kale, samples consisted of 100 g of fresh tissue...