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Interrelations between Environmental Factors and Freezing Resistance of Cabbage Leaves

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WESLEY COX
Division of Biological Science, University of Missouri, Columbia, Missouri 65201

JACOB LEVITT[1]
Laboratory of Plant Hardiness, Department of Horticultural Science, University of Minnesota, St. Paul, Minnesota 55101

ABSTRACT

Rapid wilting of cabbage leaves (*Brassica capitata* L.), induced by excision of the shoot, induced as rapid and high a degree of freezing resistance as a similar period of hardening at low temperature. Maximum hardening in the leaf was generally associated with the maximum growth rate. On the other hand, exposure of the excised shoot to low temperature while immersed in aerated water failed to harden the plants. In the absence of light, abrupt wilting at room or low temperature induced little or no hardening. With the available equipment, which required the absence of light, freezing temperatures induced little or no hardening above that obtained by nonfreezing low temperature. In fact, the plant frozen at moderate temperatures showed a gradual but steady decrease in freezing resistance. Since these experiments were performed with plants grown in pots, and since they eventually became pot-bound, the results may not apply equally to field-grown plants.

It has long been known that low, nonfreezing temperatures (0-10 C) induce freezing resistance in plants capable of hardening, and that at least moderate light is necessary during the normal daylight hours of this low temperature exposure (4). A third environmental factor—water stress—has also been shown to induce some hardening, though usually far less than the low temperature treatments (4). The present reinvestigation of these three factors in more detail, now reveals that the importance of the water stress factor has been greatly underestimated, at least in the case of cabbage leaves.

MATERIALS AND METHODS

A full description of the methods used to grow the cabbage plants (*Brassica capitata* L.) and to measure their hardiness has been given earlier (1, 2). The same variety of cabbage (Badger Market) was used.

RESULTS

Water Stress. In previous experiments, the effect of water stress has been investigated simply by withholding water. The effect on the plant is slow, since it continues to absorb water at a gradually decreasing rate, as the water potential of the soil or other root medium decreases. Eventually the plant wilts. In the following experiments, wilting was induced much more quickly by an abrupt halt of all water absorption. This was accomplished by excising the shoot from the roots, just above the soil level, and supporting each excised plant in its normal upright position. All the leaves increased in freezing resistance, the maximum increase occurring in the youngest leaves (Table I). Seven days of this drought-hardening in the dark was fully as effective as 7 days of hardening at low temperature in the light (Table II).

As in previous experiments, maximum hardening at low temperature occurred in the leaf at the maximum growth rate (Table II). In the case of the excised plants, this was not true (Table I) probably because the leaf with the MGR[2] at the beginning of the experiment did not retain this state throughout the experiment. When hardened at 0 C, the leaf at the MGR usually remains at this stage for up to 1 week; but when kept at 10 C for 1 week, the MGR shifts to the second leaf above it. In the present experiment, at 25 C day/15 C night, even though the water supply from the roots is cut off, water and translocatable solutes move from the older leaves, which wilt first, to the younger leaves, which retain their turgor for some time and therefore continue to grow. At the time the leaves were tested for freezing resistance, the MGR had probably shifted—from leaf 14 to leaf 15 after 3 days and to leaf 16 after 5 days of drought hardening.

The effect on hardening of preventing a water loss was then investigated as follows. The shoots were excised from their roots, and were then submerged in double-distilled water in an upright position by attaching a 100-g weight to the base of the stem of each shoot. They were then exposed to normal hardening temperatures and light. During this time, the water was aerated continuously and replaced by fresh water at irregular intervals. In this case, weekly measurements for up to 7 weeks revealed no more than a 1 to 2° increase in hardiness, which occurred only in the youngest leaves (Table III). The submersion in water produced no observable injury to any of the leaves until the last week, by which time a progressive infiltration of the leaves had occurred. About six new leaves were formed during the 7-week period. This is the normal amount of growth to be expected at 5 C, though much less than is normally obtained in unhardened intact plants, which produce about 30 leaves within the same period at 25 C day/15 C night. The lack of hardening cannot, therefore, be ascribed to injury by immersion. In order to prevent any possible complication due to growth of microorganisms, the experiment was repeated, but the water was changed every other day. The results were essentially the same, a maximum hardening of 4° occurring in one young leaf, 2° in another, essentially none in the remaining leaves.

Low Temperature and Light. The above results point to the essentiality of plant dehydration for hardening even at normal hardening temperatures. It has long been known that the process

[1] To whom requests for reprints should be sent.

[2] Abbreviation: MGR: maximum growth rate.

Table I. *Effect of cutting off the water supply on hardiness of cabbage seedlings*

Average of four plants grown and held at 25 C day/15 C night and an 18-hr photoperiod was determined. Stems were cut just above the soil line and each plant was supported on an empty, dry 600-ml beaker.

	Freezing Temperature Producing 50% Killing				
Plant age (days)	48	49	51	53	55
Days cut from roots	0	1	3	5	7
			C		
Leaf No.:					
11	−2.5	−3.7	above −4.0	above −6.0	above −8.4
12	−2.3	−4.5	−4.0	−6.4	−8.0
13	−2.2	−3.2	−4.5	−7.6	−8.7
14 (MGR)	−2.3	−4.3	−4.5	−7.5	−9.4
15		−3.7	−10.0	−11.6	−13.2
16				−13.2	−14.6
17					−13.7
18					−10.3

Table II. *Hardening Obtained in Presence of All Three Hardening Factors*

Average of six 64-day-old cabbage plants hardened for 1 week at +5 C and 1000 ft-c of light was determined. No water was added during the hardening period. Original total leaf area 758 cm^2, total increase of 8%.

Leaf No.	Freeze Killing Temperature	Growth
	C	*cm^2/week at 5 C*
9	−5.2	−1.3
10	−6.6	−1.4
11	−7.0	1.0
12	−8.6	7.4
13	−8.9	13.8
14	−9.3	18.4 (MGR)
15	−8.9	17.0
16	−5.7	8.6
17	−3.5	3.4
18	−3.0	2.2

Table III. *Effect of Submerging Cabbage Shoots in Water on Ability to Harden*

Average of three, 44-day-old cabbage seedlings was determined. Shoots were cut just above the soil line, submerged in double-distilled H_2O, and exposed to a 12-hr day of 700 to 800 ft-c at +5 C.

Days, Submerged	Freezing Temperatures Producing 50% Killing						
	0	7	13	20	28	40	48
Leaf No.:				*C*			
8	−1.8	−1.7	−1.3	−1.9	−1.9	−1.4	
9	−2.0	−1.8	−2.0	−1.7	−1.9	−2.1	−2.0
10	−1.8	−1.9	−1.7	−2.1	−2.0	−2.1	−2.0
11	−1.9	−1.9	−2.1	−2.2	−2.1	−2.1	−2.1
12	−1.9	−1.9	−2.2	−2.5	−2.3	−1.8	−2.2
13	−1.9	−2.0	−2.5	−2.9	−2.5	−2.5	−2.3
14	−1.9	−2.2	−3.0	−2.7	−2.7	−2.7	−2.5
15	−1.9	−2.0	−3.8	−3.4	−2.9	−2.4	−1.3
16	−1.9			−1.0	−2.7	−1.4	−1.0

can also occur when the leaves are at normal turgor, which is slightly below saturation, if the plants are exposed to hardening temperatures (0–10 C) and moderate light (4). If only light is omitted and the plants are exposed in the dark to the same kind of dehydration as was seen to induce up to 10° of hardening in the above experiments (Table I), only a few degrees of hardening occurred, even at +5 C (Table IV). This ineffectiveness of the water stress in the absence of light was shown even more conclusively by exposing the intact, potted plants to a hardening temperature (+5 C) and withholding water in the absence of light. At a relative humidity of 89%, which permits a gradual drying out of the plant, hardening was at no time detectable over a period of 36 days. Similar plants with the added factor of 700 to 800 ft-c of light (during a 12-hr day) hardened to from −8 to −16 C, depending on the leaf age.

Freezing Temperatures. Several plants have been shown to undergo their final, most extreme hardening at freezing temperatures (4). Cabbage also has been reported to attain its maximum hardening if cooled by stages to −3 C (3). In the present investigations, maximum hardening was produced at above freezing temperatures, except for a slight increase in the case of the leaves at or near the MGR, during a week at −3 to −6 C. With the available equipment, freezing temperatures could be maintained only in the dark. Under these conditions, plants hardened at above freezing temperatures showed no further hardening at very moderate freezing temperatures, for instance −0.6 C. If maintained at these moderate freezing temperatures, freezing resistance slowly decreased perhaps due to this absence of light and the freezing of the soil. Similarly, even the lowest nonfreezing temperatures were not as effective if the unhardened plants were transferred to them immediately. They were effective if preceded by 5 C. Hardening occurred even at 10 C, and as shown above, even at 15 to 25 C if exposed to a sufficiently severe water stress.

The length of time the plants were kept in the frozen state is also important. The plants did not maintain their maximum hardiness while frozen. Even if the hardened plants were kept frozen at temperatures well above their initial killing temperature (for instance at −5 C after hardening to −14 C) they were eventually killed within 35 to 50 days. These plants became potbound, and plants growing in the field under normal conditions may not show this relatively rapid response to the length of time frozen. These experiments were performed with the leaves outside the head—leaves which would probably senesce and die in the field during fall and winter after translocation of the major part of their reserves to the younger leaves which would normally survive.

DISCUSSION

It has long been known that drought-hardening by withholding water can induce some hardening to freezing (4). The degree of hardening reported was always much less than obtained by hardening at low (above freezing) temperatures. In the present investigation, for the first time, drought-hardening induced maximum freezing tolerance in cabbage leaves fully equal to that obtained by low temperature hardening. The explanation for this greater degree of hardening than in previous reports presumably is related to the greater speed of droughting. Perhaps this rapid

Table IV. *Hardening Effect of Dehydration at Hardening Temperature in Absence of Light*

Average of three 49-day-old cabbage shoots cut from their roots and exposed to +5 C in the dark at relative humidity 89% was determined.

Days, Submerged	Freezing Temperature Producing 50% Killing			
	0	4	12	20
Leaf No.:			*C*	
10	−2.2	−3.1	above −5.0	above −3.0
11	−2.3	−3.5	above −5.0	above −3.0
12	−2.4	−3.6	above −5.0	above −3.0
13	−2.4	−4.9	above −5.0	above −3.0
14	−2.4	−4.9	−5.0	−3.0
15	−2.4	−5.2	−7.6	−4.7

technique avoided the starvation effect of slow droughting, which would completely stop photosynthesis, although permitting maximum respiration for a much longer period. This starvation would prevent the attainment of maximum hardening. Whatever the true explanation, the ability of drought-hardening to induce maximum hardening to freezing favors the interpretation of freezing injury as a dehydration injury.

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