Interrelations Between Photoperiod, Frost Hardiness and
Sulfhydryl Groups in Cabbage

Hubertus Kohn and J. Levitt
Botany Department, University of Missouri, Columbia

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Summary. Hardening of SD (8 and 12 hrs) and LD (18- and 24-hr photoperiods) cabbage plants in stages at temperatures starting with +5° and ending with −3° led to the following changes:

Soluble protein plus nonprotein N showed a net increase only in the SD plants. In both SD and LD plants, it decreased to a minimum toward the end of the first stage of hardening, increased to a maximum in the second stage. The degree of this change was proportional to the photoperiod. These changes were mainly due to the proteins.

As in previous investigations, SH content rose during the first 1 to 2 weeks, but only in the SD plants. This rise was primarily due to the protein fraction. After the first 1 to 2 weeks both SD and LD plants showed a decrease in SH content, most pronouncedly during the second stage of hardening.

Nonprotein SH content was very low and decreased during hardening in both SD and LD plants. Nonprotein SS increased during the first stage of hardening. Total nonprotein SH+2SS rose to a maximum during the first stage of hardening, paralleling both osmotic potential and hardiness. During the second stage the total decreased, in spite of the rise in hardness. These results held true for both SD and LD plants.

These results are all explainable on the basis of the SH=SS theory of frost resistance.

When cabbage plants are hardened in stages (1), by successively lowering the hardening temperature, the morphological characters, water contents, and osmotic potentials usually associated with hardiness are correlated only during the first stage. In the following investigation some chemical factors were investigated in the same way, to determine their relation to the stage of hardening, and whether any correlate at all stages.

Materials and Methods

In the earlier investigations, phosphate buffer was used, and measurements were usually made on the whole homogenate, because relatively little of the protein remained in the supernatant solution. This decreased the reproducibility of the results, due probably to the difficulty in maintaining the homogenate uniformly suspended. In the following investigation, borate buffer was used. This solubilized the major part of the proteins, enabling measurements on the supernatant solution instead of the whole homogenate. The results were therefore reproducible with a smaller error. Some SH and SS remains in the precipitate from the borate buffer, but it is very difficult to measure, because the precipitate cannot be resuspended uniformly.

The same plants were used as in the previous investigation (1), grown from seed in growth chambers under controlled conditions. The leaf blades for SH and SS measurements were taken from the same petioles that were used for osmotic potential determinations, so that these 2 sets of results are ideally comparable as far as sampling error is concerned. Two experiments were completed. The first with 4 photoperiods (8, 12, 18, 24 hrs), the second with only 2 (8, 18 hrs). Throughout the 12-hour plants are strictly speaking day neutral, for purposes of comparison they will be considered with the 8-hour plants as short day (SD) relative to the definitely long day (LD) 18- and 24-hour plants. Blending was done under N₂ in both experiments. Leaf blades (20 g) were blended for 3 15-second intervals in a Waring blender (equipped with razor blades), with 100 ml 0.045 M Na borate buffer (usually pH 8.3). The homogenate was then centrifuged for 10 minutes at 23,500 × g and the supernatant solution decanted. All the above was done in the cold at 1-3°. SH and SS were determined by the amperometric, argentimetric method (5) on 10 ml aliquots of the supernatant solution. Each measurement was made in duplicate, and the average variation was ±3% for the SH measurements, ±6% for the SS.

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measurements. Nonprotein SH and SS were determined after precipitating the proteins with 1 ml 40% trichloroacetic acid per 10 ml supernatant solution and centrifuging for 20 minutes at 23,500 × g. Preliminary tests proved that the addition of trichloroacetic acid had no effect on the SH and SS values. Protein values were calculated as the difference between the supernatant and nonprotein values.

The hardening was induced by lowering the temperature in stages, starting with 5° and ending with −3°. All the hardening above freezing occurred in the light and is arbitrarily called the first stage of hardening. The hardening at −3° occurred in the frozen state and is called the second stage of hardening. This is in conformity with Tumanov (7). This separation into stages should not be taken to imply a discontinuous hardening process.

**Results**

*Total Soluble N Content.* In both experiments the SD plants showed a marked increase in total soluble (i.e. supernatant) N, by the end of the hardening period. The LD plants either a slight decrease or no net change (fig 1,3). But the most striking change was the decrease during the later part of the first stage of hardening, followed by a sharp rise during the second stage of hardening; for these changes occurred in all photoperiods in both experiments. Furthermore, this mid-hardening change was proportional to the photoperiod, being only a leveling off in the 8-hour, a marked decrease in the 12-hour and a maximal decrease in the 18 and 24-hour plants.

The soluble proteins showed the same changes as the total supernatant fraction (fig 2,4) and were mainly responsible for these since they accounted for most of the supernatant N. But the nonproteins also showed similar changes (fig 1,3) though they were usually not as pronounced as in the proteins.

*SH Content.* In agreement with earlier results (5), the SD plants showed a rise in soluble (supernatant) SH content during the first 1 to 2 weeks of hardening, followed by a drop (table 1); but the LD plants failed to show the early rise. Similar results were obtained in the second experiment. Total SH content is, therefore another character

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**Fig. 1.** Nitrogen contents of supernatant solution and its nonprotein component during hardening of SD (8 and 12 hr) and LD (18 and 24 hr) cabbage plants.

**Fig. 2.** Contents of soluble protein N during hardening of SD (8 and 12 hr) and LD (18 and 24 hr) cabbage plants.
Table I. SH Contents of Supernatant Solution, Soluble Nonprotein and Soluble Protein Fractions in 8, 12, 18, and 24-hour Photoperiods.

<table>
<thead>
<tr>
<th>Date</th>
<th>Supernatant</th>
<th>Nonprotein</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov. 7</td>
<td>2.28</td>
<td>0.48</td>
<td>0.80</td>
</tr>
<tr>
<td>14</td>
<td>2.34</td>
<td>0.49</td>
<td>1.95</td>
</tr>
<tr>
<td>21</td>
<td>1.66</td>
<td>0.43</td>
<td>2.64</td>
</tr>
<tr>
<td>Dec. 5</td>
<td>1.89</td>
<td>0.28</td>
<td>1.74</td>
</tr>
<tr>
<td>12</td>
<td>1.86</td>
<td>0.19</td>
<td>1.85</td>
</tr>
<tr>
<td>19</td>
<td>1.66</td>
<td>0.07</td>
<td>2.19</td>
</tr>
</tbody>
</table>

Table II. SH Content of Supernatant Solution, Soluble Nonprotein, and Soluble Protein Fractions, in 8, 12, 18, and 24-hour Photoperiods

<table>
<thead>
<tr>
<th>Date</th>
<th>Supernatant</th>
<th>Nonprotein</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.69</td>
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<td>0.74</td>
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<tr>
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<td>0.58</td>
<td>0.43</td>
<td>0.83</td>
</tr>
<tr>
<td>21</td>
<td>0.53</td>
<td>0.41</td>
<td>0.70</td>
</tr>
<tr>
<td>Dec. 5</td>
<td>0.32</td>
<td>0.38</td>
<td>0.42</td>
</tr>
<tr>
<td>12</td>
<td>0.29</td>
<td>0.08</td>
<td>0.41</td>
</tr>
<tr>
<td>19</td>
<td>0.36</td>
<td>0.03</td>
<td>0.55</td>
</tr>
</tbody>
</table>

that is directly correlated with hardiness only at 1 stage of hardening and with 1 photoperiod. When expressed per unit N, the early rise in supernatant SH disappears even in the SD plants (table II). In all cases, the SH content /N decreased during the second stage of hardening.

The initial rise in the supernatant SH of the SD plants was due primarily to the protein fraction (table I). This rise in soluble protein SH was detectable in the 8-hour plants during the first week in both experiments even when expressed /N (table II, fig 5). But the more striking change was the subsequent decrease in soluble protein SH/N that occurred in both experiments, (primarily during the second stage of hardening in the first experiment) and in both SD and LD plants (table II, fig 5.6). The parallel with frost-killing temperature after the first 2 weeks of hardening is very obvious in the longer hardening period of the second experiment.

The nonprotein SH values were very low, and decreased during the hardening period at all photoperiods, whether expressed per g fresh weight or per

Fig. 3. Nitrogen contents of supernatant solution and its nonprotein component during more gradual hardening of SD (8 hr) and LD (18 hr) cabbage plants.

Fig. 4. Contents of soluble protein N during more gradual hardening of SD (8 hr) and LD (18 hr) cabbage plants.
N data show that a factor correlated with hardness under natural conditions is not necessarily correlated under artificial conditions. Since hardening, in nature, occurs during short days, it is not surprising that the SD plants show the same correlation between soluble protein (and to a lesser degree soluble nonprotein N) and hardness as has been reported in the literature (2). Yet hardening proceeds as well in the LD plants that do not show any net increase in either of these fractions during hardening.

At the onset of hardening, the LD plants had a much higher soluble protein and nonprotein N content than the SD plants, presumably because their higher carbohydrate content permitted them to convert all or nearly all the available inorganic N to the organic form. The protein increase during hardening in the case of the SD plants may therefore be explained by the carbohydrate deficiency before exposure to hardening conditions. This deficiency is demonstrated by the low dry matter content (7 % vs. 10 % in the LD plants) and osmotic potential (12 vs. 16 atm). As a result, the carbohydrate-dependent N absorption and amino acid and protein synthesis must have been limited. During hardening, on the other hand, carbohydrates accumulated (as shown by the rise in osmotic potential), leading presumably to amino acid and protein synthesis.

Five main changes during the hardening of cabbage need to be explained: 1) The decrease in soluble protein to a minimum during the first stage, which is proportional to daylength and is followed by a rise to a maximum in the second stage. 2) The lack of comparable changes in nonprotein N. 3) The increase in protein SH in the SD but not

Discussion

As in the case of the morphological, water content, and osmotic potential results (1), the soluble

mg N (tables I, II). The SS (and, therefore, the total SH + 2SS) values, on the other hand increased during the first stage of hardening (fig 7) in agreement with earlier results showing a rise in GSH oxidizing activity during the first part of the hardening period (3). In all photoperiods, total nonprotein SH + 2SS paralleled the osmotic potential during hardening (fig 7). There was a rise to a maximum at the end of 3 weeks, followed by a sharp drop during the second stage of hardening. Nonprotein SH + 2SS therefore also paralleled frost resistance during the first stage, but was a mirror image of it during the second stage of hardening. Similar results were obtained in the second experiment, the maximum again occurring at the end of 3 weeks even though the first stage of hardening was continued for a longer period.

Fig. 5. Protein SH contents per unit N and frost killing temperatures (F.K.T.) of SD (8 hr) plants during hardening.

Fig. 6. Protein SH contents per unit N and frost killing temperatures (F.K.T.) of LD (18 hr) plants during hardening.
in the LD plants, during the first 2 weeks of hardening. 4) The steady decrease in protein SH that follows the initial increase, and that parallels hardness from then on. 5) The rise in nonprotein SH + 2SS during the first stage of hardening in all photoperiods, followed by a decrease in the second stage.

Two of the above changes supply the key to the interpretation of all the hardness changes. One of these is the slight increase in protein SH during the first 1 to 2 weeks of hardening in the SD plants. This agrees with all the previous results (3). The other is the increase in nonprotein SH + 2SS during the first 3 weeks of hardening. Both results can be explained by a reduction of protein SS to SH followed by a slight unfolding, freeing the loose SH ends of the protein chains. These newly freed SH ends would then be accessible to proteolytic enzymes which are known to attack unfolded but not folded proteins, and which are themselves SH enzymes and therefore would be activated. The released, nonprotein SH would be quickly converted to SS, due to the high GSH oxidizing activity of hardened plants (3).

This proposed hydrolysis of proteins readily explains the marked drop in soluble protein during the first stage of hardening. The minimum soluble protein content occurs at precisely the time of maximum nonprotein SH + 2SS. The lack of an increase in nonprotein X during this time is explainable by translocation of amino acids and amides to other organs, or resynthesis to insoluble proteins, or both.

This interpretation appears to be a reversal of the previously published conclusion that the SH increase on hardening is an artifact (3). Actually, the contradiction is more apparent than real. In the LD plants no SH increase could be detected, presumably because the free ends were split off as rapidly as they were formed. In agreement with this, the LD plants showed a more marked decrease in protein than the SD plants. The present interpretation is, therefore, an extension of the earlier one. It is now suggested that a true rise in protein SH does occur during the first stage of hardening but that it is so rapidly followed by a removal of SH-containing amino acids, as to be all but undetectable. However, the high reduction potential that leads to conversion of SS to SH remains in the homogenate for a few minutes, and during this time prevents oxidation of protein SH to SS which therefore occurs more rapidly in the nonhardy than in the hardy tissues. The measured differences would then be mainly, if not solely, amplification artifacts.

From the point of view of the SH = SS hypothesis of hardness, the main significance of all these changes would be in bringing about the final change, the decrease in protein SH which was found to parallel hardness from the second week on. The rise in soluble protein during the second stage of hardening, when this decrease in protein SH is most marked, indicates that soluble proteins with little or no surface (i.e. unmasked) SH is being synthesized, probably at the expense of insoluble proteins which are simultaneously being broken down. This may be the significance of the long known increase in soluble proteins on hardening (4). Siminovich's recent results (6) are in agreement with the changes in soluble protein described above. In the parenchyma cells of the black locust, he found a decreased glycine incorporation into proteins to a minimum during the first stage of natural hardening, followed by a steep rise to a maximum at the end of the second stage. His soluble protein curves parallel these changes.

All the observed changes during hardening can therefore be interpreted as leading to the development of proteins incapable of forming intermolecular SS bonds on freezing.

**Literature Cited**


