

BRIEF COMMUNICATION

Effects of low temperature on winter wheat and cabbage leaves

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Contents of soluble proteins, proline and chlorophyll in winter wheat (*Triticum aestivum* cv. Doğu-88) and cabbage leaves (*Brassica oleracea* convar. *acephala*) during acclimation to low temperature were investigated. When both of the plants species were cold acclimated, soluble proteins, proline and chlorophyll contents were higher than in the controls (non-acclimated). Also protein patterns differed between the plants at control and cold conditions.

Additional key words: *Brassica oleracea*, chlorophyll, cold acclimation, proline, protein, *Triticum aestivum*.

Many plant species acquire increased freezing tolerance by exposure to a period of low, nonfreezing temperature. This process termed cold hardening, has been shown to involve changes in gene expression, including the synthesis of new polypeptides (Guy and Haskell 1987, Meza-Basso *et al.* 1986). Cold-induced proteins are proposed to play an important role in survival the plants at freezing temperatures (Matthias and Virginia 1989).

A cryoprotective function of proline, which accumulates during acclimatization, has been demonstrated (Bronman and Janson 1980, Hellegren and Li 1981). It plays a major role in osmoregulation and osmotolerance (Demir 2000). Moreover, proline has been shown to protect enzymes from inactivation by salinity, heat, chilling (Wright and Simon 1973, Demir and Kocaçalışkan 2001). Reduction of photosynthetic rate in the chilling-sensitive plants has been reported when temperature falls within the chilling range (McWilliam and Naylor 1967, Wright and Simon 1973). The failure to maintain photosynthesis was tough to be associated with the inability of plants to form chlorophyll at low temperature.

Although a considerable literature about cold acclimation exists, the mechanisms involved in the hardening process are not well understood (Graham and Patterson 1982, Steponkus 1982). This study aimed to determinate relationship among soluble protein, proline

and chlorophyll contents in winter wheat and cabbage leaves during cold acclimation.

Winter wheat (*Triticum aestivum* L. cv. Doğu-88) and cabbage (*Brassica oleracea* L. convar. *acephala*) seeds were grown for 45 d as a control on *Vermiculite* with Hoagland solution at 20/18 °C in a greenhouse with a photon flux density of 125 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a light/dark period of 16/8 h. Cold treatments were performed by moving the seedlings into a growth chamber (*Sanyo Co.*, Japan) (Table 1) according to Griffith (1993). Nonacclimated control plants were kept in the greenhouse. Samples from cold acclimated and nonacclimated plants were taken at 15 d intervals.

Soluble proteins were extracted from 0.5 g leaf tissue using a borate buffer (pH 8.5) containing 50 mM sodium borate, 50 mM ascorbic acid and 1 mM phenylmethylsulfonyl fluoride (Wisniewski *et al.* 1999). Soluble proteins contents were measured using the Bradford (1976) method with bovine serum albumin as the standard protein (*Sigma Chemical Co.*, Germany).

Polypeptides were separated by sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) on 15 % (m/v) polyacrylamide gel and stained with Coomassie brilliant blue R-250 according to Laemmli (1970).

Proline was extracted from 0.5 g of fresh leaf tissue by 10 cm^3 of 3 % sulfosalicylic acid, and estimated

spectrophotometrically (*Shimadzu UV 1208* spectrophotometer, Japan) following the ninhydrin method described by Bates *et al.* (1973) using pure proline (*Merck*, Germany) as a standard.

Chlorophyll was extracted with 80 % acetone. Absorbancies of the extracts were measured spectrophotometrically at 450, 645 and 663 nm. The formula of Arnon (1949) was used for the estimation of chlorophyll *a+b* contents.

Table 1. Growth conditions for cold acclimation.

Time [d]	Temperature [°C]	Photoperiod [h]
1 - 7	20/18	16
8 - 13	17/15	15
14 - 19	14/12	14
20 - 25	11/ 9	12
26 - 31	8/ 6	10
32 - 37	5/ 2	9
38 - 45	5/ 2	8

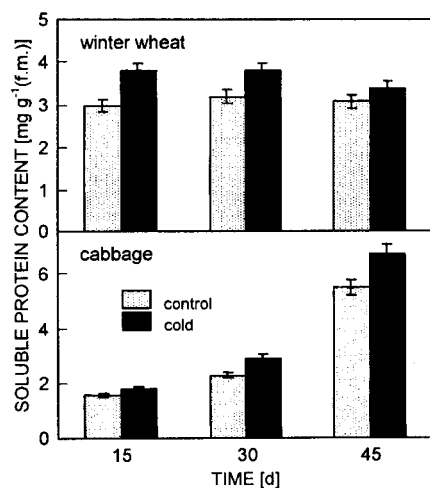


Fig. 1. Soluble proteins contents in winter wheat and cabbage leaves grown under control and cold conditions.

The contents of soluble proteins of leaves of winter wheat and cabbage grown for 15, 30 and 45 d at low temperature were higher than those in the control (Fig. 1). The electrophoresis showed changes in protein patterns in both plants, induced by cold conditions (Figs. 2, 3). Qualitative and quantitative differences in protein content between non-acclimated and cold-acclimated plants have been reported in various plants. For example, in winter rye and winter wheat the amount of soluble proteins increased after 4 weeks of cold acclimation at 2 °C (Cloutier 1983, 1984). More significant changes than observed in total soluble proteins occur in the concentration and composition of apoplastic proteins (Griffith *et al.* 1992, Atıcı and Nalbantoğlu 1999). However, some researchers demonstrated that a number

of proteins were strongly suppressed by cold (Matthias and Virginia 1989).

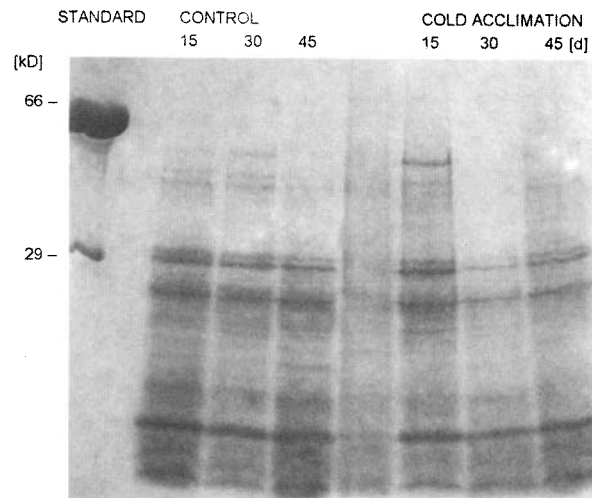


Fig. 2. SDS-polyacrylamide-gel electrophoresis of soluble protein in winter wheat leaves grown under control and cold conditions for 15, 30 and 45 d.

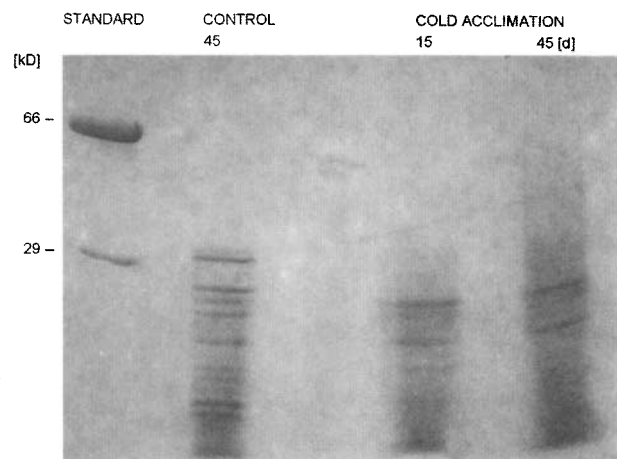


Fig. 3. SDS-polyacrylamide-gel electrophoresis of soluble protein in cabbage leaves grown under control and cold conditions for 15, 30 and 45 d.

The proline content at cold conditions was mostly higher than that in the controls at both winter wheat and cabbage leaves (except in non-acclimated winter wheat at 15 d) (Fig. 4). Similarly, some researches showed that proline content increased at beginning of winter (Öztürk and Szaniawski 1981). Maize mutant callus with high free proline content survived longer exposure to 4 °C than callus with low free proline content (Abromeit *et al.* 1992). Cold hardening was found related to increase in proline amount in barley cultivars (Demir and Kocaçalışkan 2001).

Chlorophyll contents in leaves decreased during growth of cabbage under control conditions. Chlorophyll contents during cold acclimation were lower at 15 d but

did not differ significantly from control at 30 and 45 d. However, chlorophyll contents at winter wheat leaves were higher during cold acclimation than in the controls

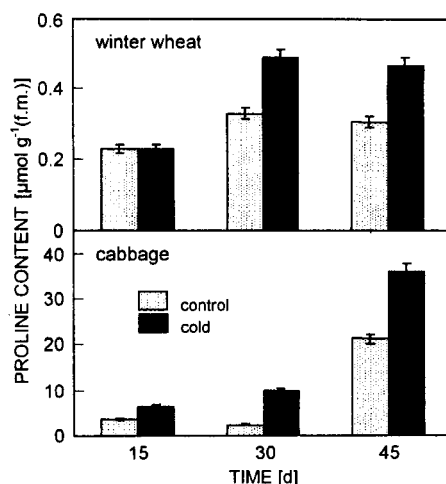


Fig. 4. Proline contents in winter wheat and cabbage leaves grown under control and cold conditions.

(Fig. 5). This difference in chlorophyll contents between plants may be result of higher tolerance of winter wheat than of cabbage to cold.

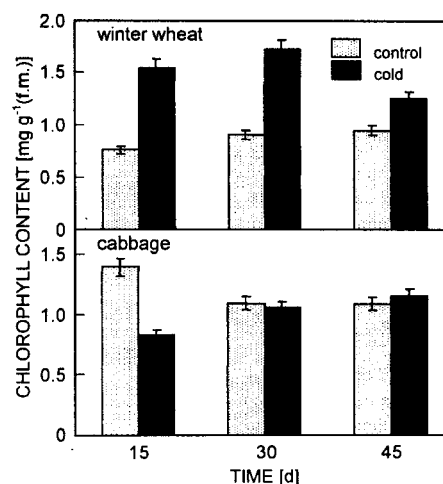


Fig. 5. Total chlorophyll contents in winter wheat and cabbage leaves grown under control and cold conditions.

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