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EFFECTS OF LOW TEMPERATURE ON WHEAT LEAF PROTEINS

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Key Word Index—*Triticum vulgare*; Gramineae; wheat; leaf; protein; esterases; cold hardiness; electrophoresis.

Abstract—Finnish wheat cultivars, winter wheat 'Vakka' and spring wheat 'Apu', grown for 9 days at 25° and then for 52 days at 2–4°, were called 'hardened'. Proteins and esterases of young leaves were resolved by polyacrylamide gel electrophoresis (PAGE), porosity gradient PAGE (poroPAGE), isoelectric focusing (PAGIF) between pH 3 and 9, and by Na-dodecylsulfate (SDS)-PAGE. Hardened and unhardened leaves have different protein patterns after PAGE, PAGIF, poroPAGE and SDS-PAGE, respectively, as well as for esterases after PAGE and PAGIF. The PAGIF patterns of esterases show distinct changes of two bands especially for hardened leaves of winter wneat, one appearing, the other one disappearing.

INTRODUCTION

Many plants are resistant to severe frosts, especially during the winter months. In late spring, during summer and in early fall, however, they may be killed by moderate frosts. Temperatures below 10° cause lethal biochemical dysfunction in sensitive plants, leading from initial to secondary injuries. Alden and Hermann [1] suggest that the initial causes are alteration of membrane structure or enzyme inactivation and they propose that the secondary effects are cessation of protoplasmic streaming, changes in physical characteristics of organelles, loss of selective permeability of cellular membranes and metabolic impairments. An increase in DNA and RNA before and during hardening may be the initial step [2]. Sugars [3], sugar alcohols [4], some amino acids [5] and some organic acid salts [6] have cryoprotective properties. Still more efficient are specific, soluble proteins [7, 8].

Rochat and Therrien [9] have reported that electropherograms of soluble leaf proteins of winter wheats after incorporation of L-[^{14}C]-leucine show the synthesis of two specific proteins during the cold hardening process in the hardier variety. Similarly, soluble proteins of intact chloroplasts isolated from spring wheat, winter wheat and freeze-resistant rye exhibit an extra band in electrophoresis after cold hardening only in the more cold-resistant rye and in winter wheat [10]. Seed proteins of heat-tolerant and heat-sensitive cultivars of Chinese cabbage could be easily distinguished by the relative intensities of two distinct protein bands after SDS-PAGE [11].

We investigated the effects of cold hardening during growth by electrophoretic separation of soluble leaf proteins of winter and spring wheat varieties. Our aim was to find characteristic bands in winter and spring wheat besides the general varietal differences as seen in cereal proteins (e.g. [12–14]) which could allow for faster screening for cold resistance.

Electrophoretic methods were conducted as given in ref. [15] (obtainable from the authors upon request). Further details are found in the legends of the figures and in the results.

RESULTS

The leaf proteins of cvs Apu and Vakka were resolved by PAGE into seven bands, one having temperature-dependent location with no differences between varieties at 25° or 2° (Fig. 1). Proteins were resolved by PAGIF (pH 3–9) which showed no differences between the two varieties, but patterns of hardened leaves show one strong band in the acidic range and three bands of lower intensity compared to unhardened leaves (Fig. 2). In poroPAGE the patterns are similar for both varieties, but in unhardened leaves there is a distinct shift to larger MWs in both varieties and some bands in the lower MW range disappeared. No typical band for the winter variety was seen (Fig. 5). SDS-PAGE of protomers in 5% PAA show only three slightly different bands in hardened leaves and no differences between varieties. In 10% PAA four bands were somewhat stronger in hardened leaves of both cultivars in Tris–borate buffer, pH 7.1, as well as in Tris–glycine buffer, pH 8.3 (Figs. 6, 7).

Esterases of leaves were separated into 5–9 bands by PAGE (Fig. 3). Two of them are characteristic for unhardened (—≺), one band for hardened leaves. PAGIF revealed 5–7 bands (pH 4–8) and 9–11 bands (pH 3–9) (Fig. 4). Two typical bands are found for spring wheat (–). The activity of esterases in hardened leaves of winter wheat is generally stronger compared to unhardened leaves of spring or winter wheat. One band (—≺) is stronger in winter than in spring wheat. A temperature dependency is seen in the basic part of the gel for winter wheat, where one band disappears after hardening (—≪), while the other band is appearing (Fig. 4). The mapping also shows that the hardened winter variety gives one distinct spot which

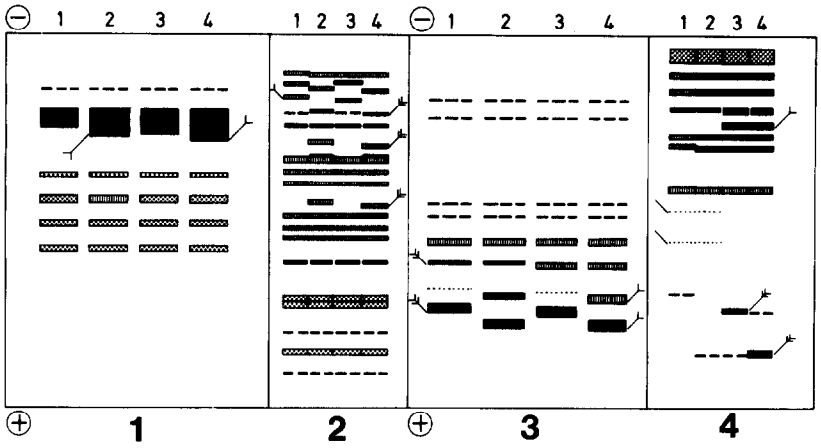

Fig. 1. PAGE of leaf proteins; from left to right: Apu 2°, Apu 25°, Vakka 2°, Vakka 25°. Gel: 5% Cyanogum, buffer 0.125 M Tris, 0.019 M borate, pH 8.9 (anode below).

Fig. 2. PAGIF of leaf proteins, samples as in Fig. 1. Gel: 6% PAA, 1% Servalyt, pH 3 (on top) to pH 9 (below), 1 hr at 100 V, 3 hr at 200 V.

Fig. 3. PAGE of leaf esterases. Samples and experimental conditions as in Fig. 1.

Fig. 4. PAGIF of leaf esterases, samples as in Fig. 1, experimental conditions as in Fig. 2.

is missing in the patterns of spring varieties or of unhardened winter wheat.

Malate dehydrogenase (MDH) of leaves was resolved by PAGE into 1–2 bands showing differences in migration rates; the two bands in hardened leaves of both varieties have the same mobilities, whereas the bands in unhardened leaves move slower, the spring variety having still two bands, but winter wheat only one band (Fig. 8).

DISCUSSION

The freezing process damages the cell by mechanical effects [17], or by dehydration with all its consequences such as a higher concentration of soluble substances, permeability changes, precipitation of salts and pH changes, reduction in cell volume and changes in structure of high MW substances [18]. Heber [19] showed that freezing changes the permeability of unprotected plasmic

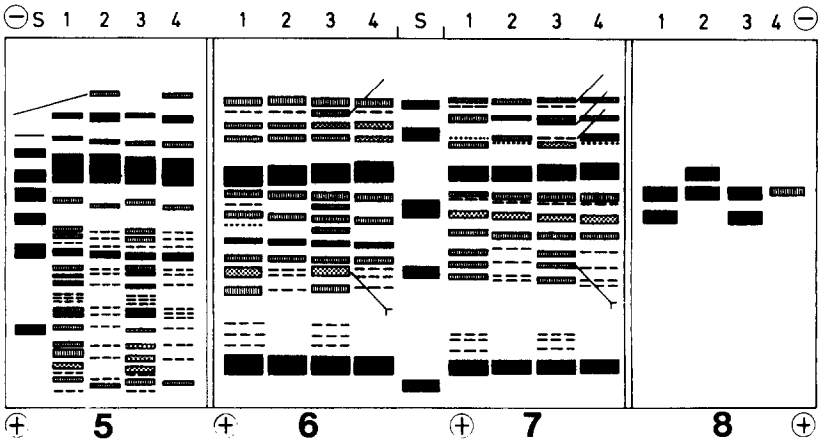

Fig. 5. PoroPAGE of leaf proteins. Samples and buffer as in Fig. 1, gel: 5–25% PAA. S: Standard albumin.

Fig. 6. SDS-PAGE of leaf proteins. Samples as in Fig. 1, gel: 10% PAA, buffer: 0.01 M Tris, 0.2 M borate, pH 7.1 (0.1% SDS), anode below.

Fig. 7. SDS-PAGE of leaf proteins, same samples, gel: 10% PAA, buffer: 0.025 M Tris, 0.129 M glycine, pH 8.3 (0.1% SDS).

Fig. 8. PAGE of leaf malate dehydrogenases. Samples and conditions as in Fig. 1.

membranes. This uncouples photophosphorylation and leads to a reduction of ATP production [3].

An effect on soluble enzymes or nucleic acids was not found [3, 20–22]. Inorganic salts and other solutes toxic to membranes enhance the freezing inactivation of isolated membranes [23, 24].

Many plants are able to develop frost resistance, which is associated with numerous metabolic changes in the cells. Two protein factors isolated only from hardened leaves of spinach and winter wheat were highly active in preventing the inactivation of photophosphorylation of washed thylakoid membranes. The protection by these factors was 10–100 times better than protection by compounds of low MW, such as sucrose, glycerol or dimethyl sulfoxide [7]. Sucrose and other soluble sugars are often known to accumulate during hardening [17, 25]. Volger and Heber [8] isolated a number of soluble proteins with protective ability from leaves of winter-grown spinach and cabbage.

Lyons *et al.* [26] assume that a high ratio of unsaturated fatty acids prevents hydrocarbon chains of membrane lipids from crystallizing. A reduced activation energy of membrane-bound respiratory enzymes in sweet potato mitochondria at low temperatures has been associated with phase change in membrane lipids [27], but was now detected also for cold sensitive plants [28] making this assumption less likely.

Our results show PAGE patterns of proteins different in one band near the cathode among leaves grown at 2 and 25° (Fig. 1), which can be interpreted as a change of charge or a change of conformation. The results are supported by PAGIF (Fig. 2), where unhardened leaves show three bands with a stronger intensity in relation to the hardened leaves. These could be responsible for the change of migration of proteins of unhardened leaves by PAGE or by poroPAGE, which show one extra protein in unhardened leaves with a very high MW. It may be an indication of protein breakdown due to cold treatment (Fig. 5), since in hardened leaves it cannot be found. Also SDS-PAGE shows one stronger band (—<) with a lower MW (*ca* 30000) after cold treatment and indicates the shifting of proteins to smaller protomers (Fig. 7).

Rochat and Therrien [9] have reported that in wheat plants grown in the cold two new proteins are synthesized only in winter wheat as shown by SDS-PAGE. However, SDS-PAGE will deal with inactive protomers only, whereas poroPAGE is separating intact proteins. In diploid Finnish wheat varieties we could not find any new protein in hardened leaves from winter wheat, only some stronger bands in SDS-PAGE patterns. Also no typical protein band for a hardened winter variety was detected by poroPAGE. The reason may be due to the different pedigree compared to cvs Kharkov and Selkirk which they used.

We have also investigated a few enzymes. Almgard and Clapham [29] separated esterases from wheat leaves by starch gel electrophoresis (pH 3.2) distinguishing between spring cultivars (3 bands) and winter cultivars (1–2 bands). Our results after PAGE show more bands, but there is a similarity between spring and winter cultivars (Fig. 3). PAGIF patterns of spring wheat (hardened and unhardened) show two typical bands not visible in winter wheat (Fig. 4), but too faint for a clear distinction between winter and spring wheat. Growing in the cold activates esterases; this was pronounced for winter wheat and less for spring wheat (Figs. 3 and 4). MDHs show changes after cold treatment which can be a consequence of changes in conformation or a synthesis of new enzymes (Fig. 8).

EXPERIMENTAL

Spring wheat cv Apu and winter wheat cv Vakka were germinated and grown at 25° in Knopsch's medium (light–dark period 18:6 hr). After 9 days, the cvs were exposed to a temp. of 2–4° (light period 14 hr) for 52 days and called 'hardened plants'. Reference cvs ('unhardened') grown at 25° were harvested after 14 days when they had reached the same size as the former ones.

Extraction of proteins. Leaves (2 g) were cut and ground in a mortar for 4 min with 5 ml buffer (0.125 M Tris–borate, pH 8.9, containing 0.1% cysteine–HCl and 12.5% glucose (9), and with 0.4 ml sulfite soln (2 g Na_2SO_3 and 1.5 g $Na_2S_2O_5$ in 20 ml H_2O [15, 16] brei was passed through 2 layers of cheesecloth. The filtrate was centrifuged at 20000 *g* at 2° for 20 min. The supernatant was stored frozen. All operations were carried out at 4°.

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