Mechanisms of cold acclimation in winter rape plants

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Winter rape plants, as other herbaceous plants, do not develop a true dormancy during the autumn. Their tissues maintain the growth capability troughout winter season, despite the environmental conditions imposing the cessation of growth rate. Adaptation of plants to low temperature conditions consists, therefore, in two main phenomena: protection of cells against metabolic lesions that may occur as a result of daily fluctuations in temperature and a protection of cell constituents against frost effects, mainly against the frost–induced dehydration.

I. HARDENING REQUIREMENTS.

It has been shown (Sikorska and Kacperska-Palacz 1979) that acclimation of winter plants occurs in three stages, related to temperature conditions:

The first stage may be induced by lowering the temperature to 5°C or 2°C and results in the increase of frost tolerance by a few (4 or 5) degrees above the initial level which is –5°C for the winter rape leaves.

The second stage relies on the appearance of subfreezing temperature (0°C to –2°C or –3°C) and does not occur under field conditions if minimum air temperature does not fall below 0°C. This stage may result in the achievement of the maximum frost tolerance by the turgid tissue if the temperature conditions foregoing the hardening process were favourable for plant growth.

The third stage of frost hardening may be coincident with the second one and depends on the occurrence of prolonged frost which induce cell dehydration. It may be assumed that the other factors which decrease a water potential of a tissue will also bring about similar effects.

Shortening of photoperiod during the first stage of hardening was found to decrease the level of frost tolerance from T_{k50} = –10°C to T_{k50} = –7.8°C (Kacperska-Palacz and Wciślińska 1972 a). The observation points to the photosynthetic role of light in the first stage of hardening. On the other hand, experiments performed on the young winter rape seedlings (Kacperska-Palacz et al 1975) indicate that light may act in the process of hardening through its morphogenetic influence. Phytochrome was demonstrated to be involed in the regulation both tissue elongation and frost hardening: the light–induced inhibition of hypocotyl growth corresponded to the increased frost tolerance of seedlings (Kacperska-Palacz

et al 1975). It was also shown that all the factors which inhibit the expansion of cells such as growth retardants (Kacperska-Palacz and Długokęcka 1971), water stress (unpublished data) increase a frost tolerance of a tissue for a few degrees. Thus, it is suggested that the first stage of frost hardening of winter rape plants is related to the cessation of growth. Inhibition of cell expansion brings about the decreased tissue hydration (Kacperska-Palacz and Wciślińska 1972 a). This may in turn, limit the probability of intracellular freezing and increase frost tolerance in the absence of the other hardening factors. On the other hand, changes in a hormonal balance, observed in the overwintering rape leaves (Forycka et al 1978) may also result in a modification of metabolic pathways, e.g. of photosynthetic carbon metabilism (Kacperska-Palacz et al 1981).

II. METABOLIC CHANGES.

During the first stage of hardening several metabolic events were registered for winter rape plants. Some of them, e.g. accumulation of reducing sugars (Kacperska-Palacz and Wcislinska 1972 a) or accumulation of water–soluble proteins (Kacperska-Palacz and Wcislinska 1972 a and b) seem to be related to metabolic shift brought about by growth cessation, not necessarily by low temperature itself. For example, it has been demonstrated that the light – –induced inhibition of the winter rape hypocotyl growth was coincident with an accumulation of soluble proteins in the seedling tissue (Kacperska-Palacz et al 1975). Application of CCC (chlorocholino chloride) into winter rape plants also increased the protein content in the leaves, even at higher temperature (Kacperska-Palacz and Wciślińska 1972 b). On the other hand, several metabolic changes seem to be a specific response of the plant tissue to lowering the environmental temperature. The specific water soluble protein fractions of low molecular weight, impoverished in proline and methionine residues and localised in the soluble phase of the cell have been observed to accumulate in the winter rape leaves under the cold treatment (Kacperska-Palacz et al 1977 b, Kacperska-Palacz and Wciślińska 1972 b). The increase of ATP level in winter rape leaves treated with cold for a few days (Sobczyk and Kacperska-Palacz 1978) was also demonstrated not to depend on the inhibition of a tissue growth (Sobczyk 1982). The increased unsaturation of phospholipids as well as a modification of phospholipid composition in the cold-treated rape plants was also shown (Smoleńska and Kuiper 1977). The former effect seems to be due both to the cold promoted biosynthesis of linolenic acid and to the cold inhibited phospholipid turnover (Smoleńska-Sym and Kacperska 1983). The cold –promoted synthesis of specific phospholipids: phosphatidyl choline and phosphatidyl ethanolamine was also noted in the winter rape leaves (Smoleńska-Sym and Kacperska 1983). However, the data obtained by other authors for other plants (e.g. Huitema et al 1982) indicate that the water stress may also affect the phospholipid content and composition in a similar way as the low temperature does. It may be suggested that there is a specific „key" point in a cell metabolism which is affected by different stresses, such as a low temperature, water deficit, salt stress. It seems that this is a reducing power of a tissue which increases under stress conditions: the high accessibility of $NADH^+$ or $NADPH^+$ promotes these metabolic pathways the operation of which depends on the availability of these cofactors. Some confirmation of that hypothesis came from our studies on the influence of cold on

photosynthetic carbon metabolism in winter rape leaves (Maciejewska et al 1974, Sosińska et al 1977). It appeared that the incorporation of ^{14}C into alanine, asparate, glutamate, malate as well as to proline was increased in the cold–grown winter rape plants, despite the inhibition of total $^{14}CO_2$ assimilation and ^{14}C incorporation into carbohydrates in the same material. A high accessibility of reduced nucleotides may also slow down the catabolism (peroxidation?) of polyenoic acids and help in the maintenance of lipids on the high unsaturation level.

In several studies on the winter rape plants we found that the first stage of plant adaptation to cold is composed of two phases: the reaction phase when cold may induce disturbances in enzymatic activities (Sobczyk and Kacperska-Palacz 1980) or in some metabolic pathways, e.g. in glycolic acid path (Sosinska et al 1977), and the restitution phase when the cold–induced disturbances are overcome and a new metabolic balance is established (Sobczyk and Kacperska-Palacz 1980, Sosińska et al 1977). By the end of the restitution phase the enzyme and membrane stability is improved (Sobczyk et al 1980, Sikorska and Kacperska-Palacz 1980, respectively). It has been shown that during the first stage of hardening the membranes of winter rape cells reach their maximum fluidity (Sikorska et al 1981), unlike the wheat Miranovskaja 808 which continously increased membrane fluidity during the whole hardening period (Vigh et al 1979).

The second stage of frost hardening of winter rape plants seems to rely both on the physical and chamical modifications of the membrane structure, induced by subzero temperature (Sikorska and Kacperska-Palacz 1980, Sikorska and Kacperska 1982). It was found that at $-2^{o}C$ the membranes of the winter rape seedlings hardened to the stage I underwent a thermotropic phase transition connected with phase separation (Sikorska et al 1981). On the other hand, in the leaves slightly injured by frost (i.e. a small permeability increase) the transient decrease of phosphatidyl choline (PC) was correlated with a pronounced increase of phosphatidyl glycerol (PG) level (Sikorska and Kacperska-Palacz 1980). The further studies showed that freezing and thawing activates phospholipase D in the rape cells and may induce a shift in the enzyme activity from phosphatidyl hydrolase to transferase, depending on the degree of cell membranes' alterations (Sikorska and Kacperska 1982). It seems plausible that the freezing induced initiation of membrane phase separation and the presence of such alcohols as glycerol and ethanolamine promotes transferase activity of phospholipase D and formation of phosphatidyl glycerol. That phospholipid may be further used for a resynthesis of PC. Therefore, hardening effect of a slight frost during the second stage of hardening of winter rape plants seems to rely on the activation of a recovery system in the cell (with phosphatidyl transferase taking part in the phospholipid turnover) and on putting into operation mechanism(s) that allow cell structure remodelling. A high content of phosphatidyl choline and phosphatidyl ethanolamine have been shown to play an important role in the maintenance of membrane fluidity over a broad range of temperature. The formation of membranes with the reduced ratio of free sterols to phospholipids, but with the increased cholesterol content (Sikorska and Farkas 1982) during the second stage of hardening may also result in changes in the molecular architecture and physiological functions of the membranes.

III. CONCLUSIONS.

Taking into account the results obtained so far, one may propose that adaptation of winter rape plants to low temperature consists in two types of mechanisms:

1) the mechanisms that protect a cell against deleterious effects of frost: intracellular ice formation and the freezing–induced dehydration of the cell components. Changes in the physical and chemical properties of membranes as well as the increased stability of the enzymes are the main factors which permit the maintenance of unaltered cell structure upon freezing and thawing. They are also of importance for the tolerance of desiccation stress (Kacperska-Palacz and Egierszdorff 1972). A high fluidity of the membranes under cold conditions and the transient phospholipid degradation caused by a slight frost facilitate the water flow into intercellular spaces and protect cells against intracellular freezing.
2) mechanisms that allow plants to function at low but positive ($> 0^{o}C$) temperature. The non–specific increase in linolenic acid occurring both in the leaves and in the roots, whereas the frost tolerance increased in the leaves only seems to be factor important for membrane functioning at cold (Smoleńska and Kuiper 1977). However, the increased unsaturation of membrane lipids brings about the increased water permeability of membranes and, in turn, may expose a tissue to the secondary strain of dehydration under conditions of a limited supply of water from a soil. It seems that an accumulation of water–soluble proteins and of osmotically active substances (e.g. sugars) may ensure the maintenance of positive water balance in a cell. These mechanisms, assuring the abundance of water for life processes in cells, operate parallel to the frost hardening mechanisms and may partially counteract their effects by increasing the amount of freezable water in a tissue of herbaceous plants.

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