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Short Communication

Protein Synthesis and Phospholipids in Soybean Axes in Response to Imbibitional Chilling[1]

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ABSTRACT

The responses of two cultivars of soybean (Merr.) to a chilling treatment (4 C for first hour of imbibition) were compared. The germination of cv. Biloxi was unaffected by the treatment, while the germination of cv. Fiskeby was reduced. The phospholipid fatty acids of dry axes of the two cultivars were very similar, and, thus, could not be correlated with their responses to chilling. The fatty acid composition of chilling-tolerant Biloxi did not change over a subsequent 23-hour warm incubation, but there was a marked reduction in the unsaturated fatty acids of chilling-sensitive Fiskeby after 12 hours, which may be a symptom of deterioration. Protein synthesis in both cultivars was reduced by the chilling treatment. Redrying of Biloxi axes up to 18 hours after the onset of imbibition had no effect on their germination upon rehydration. Germination of Fiskeby axes was reduced by redrying after 8 hours of imbibition. After 7 months of dry storage of intact seeds, the sensitivity of the axes to chilling was retested. Biloxi axes had become chilling-sensitive, while the germination of Fiskeby axes was reduced to zero by the chilling treatment. A hypothesis is presented that imbibitional chilling sensitivity is an indication of reduced vigor, axes with a high vigor can tolerate the stress, while those without cannot.

MATERIALS AND METHODS

Soybeans (*Glycine max* Merr. cv. Biloxi and cv. Fiskeby) were obtained from Agriculture Canada Experimental Research Station, Lethbridge, Alberta, Canada, and stored at room temperature and ambient RH (about 22 C, 25–30% RH). All tests were conducted on isolated axes. The axes were excised from dry seeds and stored at 22 C for up to 4 days before being used.

Germination was determined by incubating 10 axes in a 4.5-cm Petri dish with 2 ml distilled H_2O at 24 C for 72 h. Germination was considered to have taken place when an axis extended in a normal manner, axes which partially swelled and showed deterioration were scored as nongerminated. Axis growth was measured to 0.01 mm with a micrometer eyepiece on a dissection microscope.

Imbibitional chilling was administered by placing the axes in H_2O at 4 C for 1 h, followed by transfer to room temperature (about 22 C).

Superoxide dismutase was assayed on the basis of its ability to inhibit the photochemical reduction of nitro-blue tetrazolium, as detailed previously (14).

In vivo protein synthesis was determined by the incorporation of [^{3}H]leucine into a hot trichloroacetic acid-insoluble residue. Twenty axes were incubated for 60 min with 25 μCi of [^{3}H]leucine (New England Nuclear; 58 Ci/mmol) in 1 ml H_2O. Thereafter, the excess leucine was washed off, and the tissue was homogenized and extracted by the method of Bewley (1).

Phospholipid fatty acids were analyzed, as detailed previously (15), with minor modifications. After the total lipid sample was added to the silica gel column and washed with 1% acetic acid in chloroform, it was washed with acetone. It then was eluted with methanol as before (14).

RESULTS

Germination and Growth. Imbibitional chilling of Fiskeby axes reduced their germination to 21% from the 83% exhibited by nonchilled (22 C) controls, while imbibitional chilling of Biloxi axes was without effect. Both chilled and nonchilled axes germinated 100%.

Chilled and nonchilled axes exhibited very little growth during the first 24 h (Fig. 1). However, there was an initial increase in length associated with the first 2 h of imbibition of both cultivars, and this was slowed by the chilling treatment. After removal from the 1-h chilling treatment, the axes obtained a similar length to the unchilled controls within 2 h. The reason for the initial slow rate of increase by chilled axes is not known, but it may be related to the physical properties of water at 4 C and of the barriers through which the water must pass (6).

Phospholipid Content and Superoxide Dismutase Activity. The phospholipid fatty acids of dry axes of both soybean cultivars are similar, and they consist of palmitic, stearic, oleic, linoleic, and linolenic acids in proportions of 24%, 5%, 3%, 55%, and 13% of total, respectively. A chilling treatment of 1 h at the onset of imbibition did not result in any change to the proportionality of these fatty acids in the phospholipids of the chilling-intolerant Biloxi cultivar over a subsequent 23-h warm period. In the chilling-intolerant Fiskeby cultivar, no marked changes in phospholipid fatty acids occurred over the first 12-h warm period, following chilling the first h of imbibition; although, by 24 h, both linoleic and linolenic acid had declined to 20% and 4% of total phospholipid fatty acids, respectively; and palmitic, stearic, and oleic had increased to 52%, 11%, and 12% of total, respectively. Control axes of both cultivars, *i.e.* those imbibed at 22 C, did not exhibit any changes in proportionality of phospholipid fatty acids over the first 24 h from the start of imbibition.

Loss of unsaturated fatty acids from the phospholipids of the axes of the Fiskeby cultivar between 12 and 24 h could not be correlated with a decline in superoxide dismutase. Activity of this

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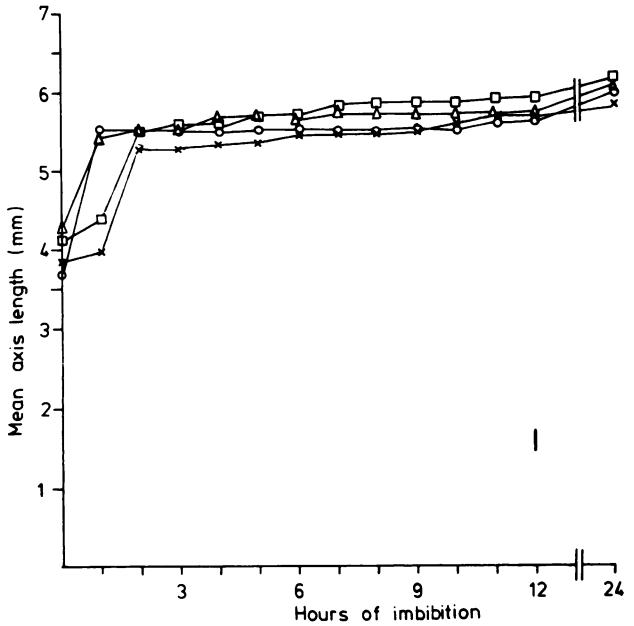

FIG. 1. Axis length at various times after the start of imbibition. Axes were either chilled for the first h of imbibition and then transferred to 22 C, or they were imbibed at 22 C. (×), Fiskeby chilled; (○), Fiskeby nonchilled; (□), Biloxi chilled; (△), Biloxi nonchilled. The *bar* represents the maximum SE of any sample.

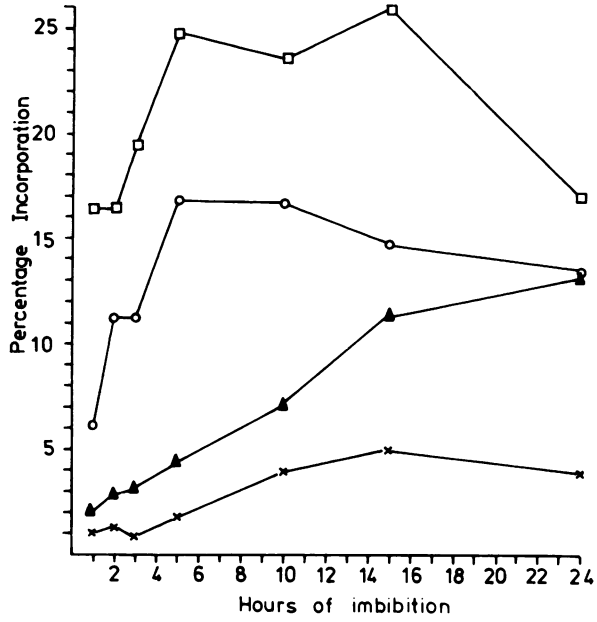

FIG. 2. The incorporation of [^{3}H]leucine into protein by excised axes of soybean, expressed as a percentage of the amount taken up by the axes. Axes were incubated with the radioactive compound for 1 h from the time shown. (×), Fiskeby chilled; (▲), Fiskeby nonchilled; (○), Biloxi chilled; (□), Biloxi nonchilled. Typical total uptake values (for feeding at 3 h) were ×, 453 450 dpm; ▲ 488 600 dpm; ○, 567 550 dpm; □, 610 400 dpm.

enzyme did not change appreciably in the imbibed axes of either cultivar over 24 h from the start of imbibition, whether or not they had been chilled initially (data not presented).

Protein Synthesis. Isolated axes of Biloxi incorporated more leucine into protein than did those of Fiskeby, whether they had been chilled or not. This could be due to differences in the rate of synthesis or differences in the size of the endogenous leucine pools. Chilling reduced the incorporation in axes of both cultivars, even though the former is tolerant of this treatment and the latter is not (Fig. 2).

Chilling and Desiccation Injury. To ascertain whether excised axes could tolerate a different stress, nonchilled axes were dried back at various times after imbibition but before any extension growth had taken place. Redrying of Biloxi axes up to 18 h after the onset of imbibition had no effect on their germination upon rehydration. Germination of Fiskeby axes was reduced to 20 to 30% by redrying after 8 h from imbibition (Fig. 3).

Seven months after the major portion of this work was performed, the susceptibility of the two cultivars to chilling injury was retested. (Whole seeds had been stored at 22 C and approximately 25% RH). Germination of nonchilled Fiskeby axes was reduced to 23%, while nonchilled axes germinated. Unchilled Biloxi germinated 100%, but chilling reduced the germination to 60%. At this time, the phospholipid fatty acids of dry axes were reanalyzed, those of Biloxi were similar to dry axes of unaged soybeans, while those of Fiskeby showed a 5 to 6% reduction in unsaturated C-18 fatty acids.

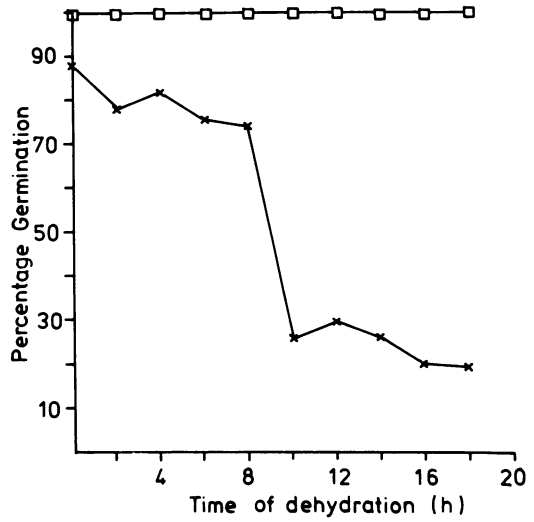

FIG. 3. The effect of drying back on germination of soybean axes. Axes were redried at various times after imbibition and imbibed once more, and germination was scored 72 h later. (×), Fiskeby; (□), Biloxi.

DISCUSSION

Simon (12) suggested that chilling during imbibition interferes with the reorientation of the seed membrane components, from the purported 'hexagonal' configuration found in dry seeds to the lamellar configuration formed as membranes become hydrated (11, 13). This is supported by the observation that an increase in the water content of the 'dry' seed reduced the sensitivity to imbibitional chilling (2), presumably because the reorientation process is complete before the onset of chilling. Moreover, it has been suggested that phospholipid fatty acids of chilling-tolerant plants are generally more unsaturated than those of intolerant plants (4), and, thus, their phospholipids are in the liquid crystalline state at chilling temperatures. This imparts a fluidity to membranes which facilitates reorientation at low temperatures. If phospholipids have a highly saturated fatty acid composition, then they will be in the crystalline state at chilling temperatures. Hence, reorientation might be less because of the reduced mobility of the phospholipid fatty acid chains.

It seems that the above suggestions may not apply in the response of soybean axes to chilling stress (7). We detected no differences in the composition of the phospholipid fatty acids from the axes of the chilling-intolerant Fiskeby cultivar and the chilling-tolerant Biloxi cultivar. Also, the phospholipid fatty acids of the

Biloxi axes did not become more unsaturated in response to the chilling treatment, unlike the changes reported for chilling-tolerant beans and peas (5). Likewise, the fatty acids of Biloxi did not change during the development of chilling sensitivity. However, it is perhaps overly simplistic to expect any change in membrane fluidity to be reflected in its fatty acid composition alone. Sterols (9), polar head-group identity, and protein components all may have an effect on the fluidity of a bilayer. For example, Vigh *et al.* (15) showed that 'hardening' of rye and wheat plants results in reduced membrane microviscosities, which could not be ascribed to changes in fatty acid composition alone. Recently, even the suggestion that there is reorientation of membrane due to stress (in this case, desiccation stress) has been questioned (8). Twenty-four h after the onset of the 1-h chilling treatment there was a marked reduction in the level of unsaturated fatty acids of the phospholipid fraction from the axes of the Fiskeby cultivar. This may be a symptom of deterioration.

Protein synthesis was reduced considerably in the axes of both cultivars by the chilling treatment, and, yet, germination of the Biloxi cultivar was unimpeded. The fact that both chilling-tolerant and chilling-intolerant axes showed reduced protein synthesis emphasizes the necessity for comparing tolerant with nontolerant individuals when studying the effect of a physiological stress.

As batches of seeds age during storage, they often exhibit a reduction in vigor (3). In the course of this study, we found that Fiskeby axes were unable to withstand desiccation stress at a time when Biloxi axes were able to do so. It is possible that the chilling sensitivity exhibited by the Fiskeby axes is merely another indication that this cultivar was less vigorous and less able to tolerate any stress. The observation that axes of the Biloxi cultivar developed chilling sensitivity during 7 months of dry storage of the seeds may be a reflection of the deterioration of the axes during storage, which is not detectable by germination tests at warm temperatures. Hence, a seed may have a natural 'competence' to germinate and to produce a seedling. When conditions are favorable, even seeds with a low competence will germinate. But if a stress is applied, only those with a high competence will germinate and produce vigorous seedlings; those with a competence below the threshold imposed by the stress will deteriorate. It is also evident that the loss of vigor of the Fiskeby cultivar occurred without any marked loss of unsaturated fatty acids, and, hence, can occur in the absence of lipid oxidation, as has been suggested previously (10).

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