

# Correlation between Cold- and Drought-Induced Frost Hardiness in Winter Wheat and Rye Varieties<sup>1</sup>

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## ABSTRACT

Exposure of six wheat (*Triticum aestivum* L.) and one rye (*Secale cereale* L.) cultivar to 40% relative humidity for 24 hours induced the same degree of freezing tolerance in seedling epicotyls as did cold conditioning for 4 weeks at 2°C.

Frost hardiness varietal relationships were the same in desiccation-stressed and cold-hardened seedlings. Drought stress could, therefore, be used as a rapid and simple method for inducing frost hardiness in seedling shoots in replacement of cold conditioning.

Desiccation stress is now recognized as able to induce freezing tolerance in winter wheat and rye (5, 11) and in other plants (2, 7, 9). However, the degree of freezing tolerance conferred by this treatment is not as great as is that by conventional cold-conditioning. Recently, it was shown that freezing tolerance can be increased by changing the conditions of the drought to a 24 h, 40% RH treatment of endosperm-dependent seedlings at room temperature (12), enabling us to obtain a high level of frost hardiness in seedling shoots of rye. Under these conditions, tissues do not reach equilibrium with respect to the imposed water stress, and, hence, different degrees of freezing tolerance are induced in different parts of the seedling, maximum freezing tolerance being induced in shoots. Questions arise as to whether freezing tolerance induced by desiccation is the same as that induced in the cold and if the same varietal relationship of freezing tolerance will be observed in desiccation-stressed shoots as is seen in the cold-hardened ones. If so, it will be possible to induce freezing tolerance in cereal varieties by drought instead of by cold, which will be much less demanding of time and equipment.

In this work, we report the degree of freezing tolerance induced by drought and cold in six varieties of wheat and one of rye and the correlation between the hardiness resulting from the two stresses.

## MATERIALS AND METHODS

**Plant Materials and Growing Conditions.** Seeds of six cultivars of wheat (*Triticum aestivum* L., cv. Marquis, Cappelle-Desprez, Kent, Fredrick, Rideau, and Kharkov) and rye (*Secale cereale* L., cv Puma) were surface-sterilized with 2.6% NaOCl for 3 min, imbibed at 21°C (to be unhardened or desiccation-stressed) or at 2°C (cold-hardened) for 6 h, placed on moist filter paper in the dark. Seeds were allowed to germinate for 2.0 to 2.5 days at 24°C

(to be unhardened or desiccation-stressed) or for 4 weeks at 2°C (to be cold-hardened). Seedlings were desiccation-stressed by placing them for 24 h at 21°C over H<sub>2</sub>SO<sub>4</sub> solution of 40% RH (12) and were reimbibed by immersion in tap water for 16 h.

**Frost Hardiness Evaluation.** Seedlings were surface-dried by blotting over filter paper and were frozen in groups of five in Petri dishes. Cooling rates were 1°C/h from 0 to –21°C, and 3°C/h from –21 to –30°C. For lower temperatures, the seedlings were transferred directly from –30°C to the desired temperature. Samples were seeded with ice crystals at –3°C to avoid supercooling. Frozen samples were thawed at 2°C for 1 h. Survival of shoots or of excised epicotyls was then assessed by the following methods: (a) vital staining with neutral red followed by observation of protoplasmic streaming; (b) detection of ability to plasmolyze and deplasmolyze in salt solution (6); (c) greening in White's solution (13) + 1% Agar-Agar after 2 days at room temperature or in White's solution + 0.2% sucrose after 2 weeks at 2°C (epicotyls developing into a leaf); (d) measuring the relative release of amino acids (10) and; (e) manually evaluating the relative turgidity (personal assessment on a scale of 1–10). All assessments were performed in duplicate.

**Water Content Determinations.** Seedlings imbibed in tap water were surface-dried by blotting with filter paper. Water content was determined by weighing the seedlings before and after drying them at 55°C in a vacuum oven for 48 h.

## RESULTS AND DISCUSSION

**Changes in Water Status.** When seedlings of winter wheat or rye were subjected to a desiccation stress of 24 h at 40% RH their moisture content decreased from 83% to 66%. After 16 h of reimbibition, they regained full turgidity. Their water content returned to 78.5% (Table I).

**Induction of Frost Hardiness.** When subjected to a 24-h drought (40% RH), shoots of Puma rye hardened from –7°C to –14°C, as

Table I. Water Content of Seedling Shoots of Winter Wheat and Rye as Determined by Drying Samples in a Vacuum Oven

Cultivars	Unhardened	Desiccated	Desiccated + Reimbibed
	<i>g H<sub>2</sub>O/g fresh wt</i>		
Marquis	0.81	0.66	0.78
Cappelle-Desprez	0.81	0.65	0.79
Kent	0.84	0.67	0.78
Fredrick	0.83	0.65	0.79
Rideau	0.85	0.67	0.80
Kharkov	0.82	0.65	0.77
Puma	0.85	0.68	0.79
Mean	0.83	0.66	0.785

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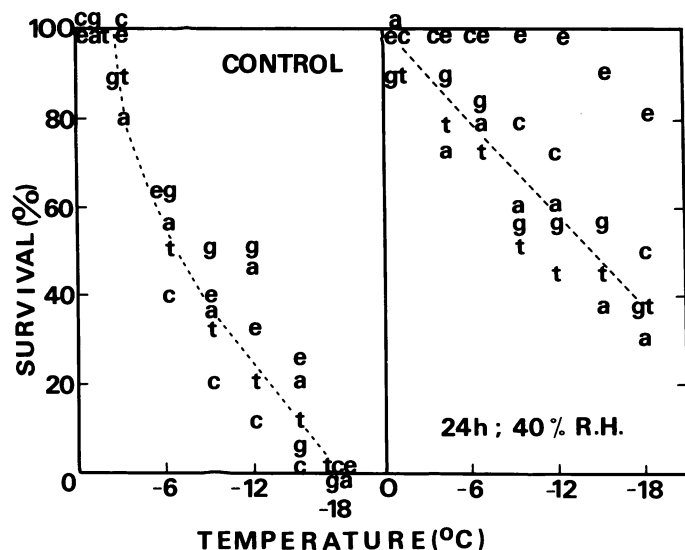


FIG. 1. Survival of plumules of Puma rye as measured by turgidity (T); amino acid release (A); growth in White's solution (G); and microscopic observation of uptake of neutral red, protoplasmic streaming, and salt plasmolysis and deplasmolysis in epicotyl (E) or coleoptile (C) tissues. Control, unhardened seedlings; 24-h, 40% RH, desiccation-stressed seedlings.

shown in Figure 1. The temperatures here are those at which 50% of the cells were killed ( $LD_{50}$ ). Three methods were used for survival assessments; greening of shoots in White's solution, manual measurement of relative turgidity, and amino-acid release were all in good agreement within each treatment. A fourth method, optical microscope observation of neutral red-stained epicotyl cells, gave different results in drought-stressed plants. Coleoptile cells showed the same  $LD_{50}$  as that in the whole shoot (coleoptile + epicotyl), but epicotyl cells hardened to a greater extent. As these tissues hardened to a different extent, the measured hardness of the intact whole shoot was limited by the greater cold susceptibility of the coleoptile. Inasmuch as expression of maximum hardness was being sought, epicotyl tissue was used as the material for further investigation. Microscopic observation, greening on Agar-Agar or in White's solution, and turgidity measurements were all in good agreement for hardened and unhardened epicotyls (Fig. 2), but amino-acid releases were at variance with the other results. The level of amino acids released from desiccation-stressed seedlings was similar to that from controls (unhardened), whereas, at any freezing temperature, their  $LD_{50}$ s differed greatly (results not shown).

Therefore, we assumed that amino-acid release was not closely indicative of the survival of epicotyl and resorted to other methods for assessing frost hardness. The  $LD_{50}$ s of epicotyls from unhardened, desiccation-stressed, and cold-hardened seedlings of Puma rye were  $-7^{\circ}\text{C}$ ,  $-67^{\circ}\text{C}$ , and  $-62^{\circ}\text{C}$ , respectively, with some epicotyls of desiccation-stressed seedlings surviving even in liquid  $\text{N}_2$  (Fig. 3).

**Relationship between Drought- and Cold-Induced Frost Resistance.** We measured the  $LD_{50}$  for the six varieties of wheat using the same methods we have used for assessing the  $LD_{50}$  of rye epicotyls. Epicotyls from less hardy varieties (Cappelle-Desprez and Kent) hardened less than did those from hardier varieties (Fredrick, Rideau, Kharkov, and Puma rye), while 'Marquis' spring wheat did not harden at all (Table II). An approximate linear relationship was observed between the frost hardness of epicotyl and whole seedlings.  $LD_{50}$ s values for whole seedlings were obtained from previous works (1, 3, 8).

Epicotyls from cold acclimated seedlings harden much more than do whole seedlings. Moreover, the degree of frost hardness

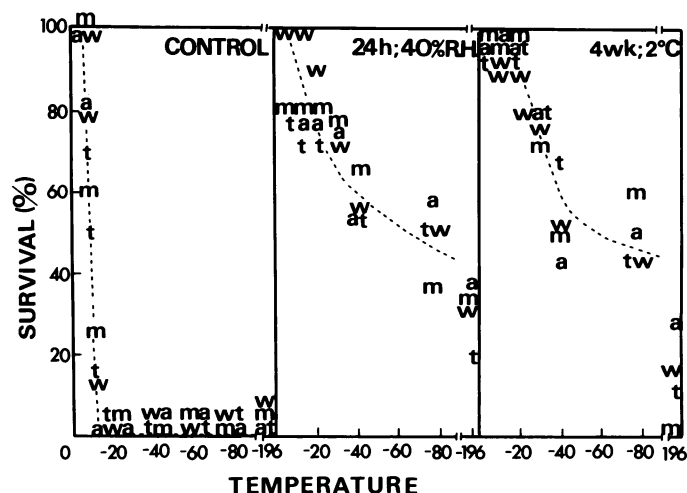


FIG. 2. Survival of epicotyl from seedlings of Puma rye as measured by turgidity (T); greening in White's solution (W) or on Agar-Agar + White's solution (A); and microscopic observation of uptake of neutral red, protoplasmic streaming, and salt plasmolysis and deplasmolysis (M). Control, unhardened seedlings; 24-h, 40% RH, desiccation-stressed seedlings; 4-week,  $2^{\circ}\text{C}$ , cold-hardened seedlings.

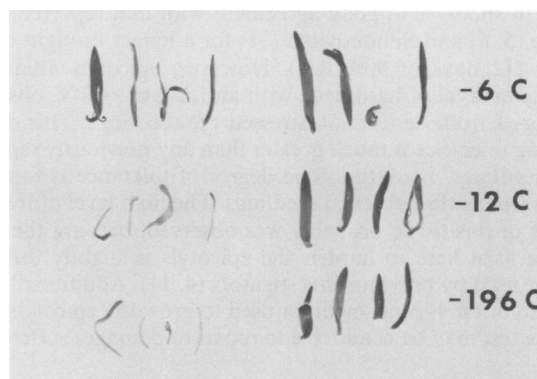


FIG. 3. Greening of epicotyls from seedlings of Puma rye on Agar-Agar + White's solution. Left, unhardened seedlings; right, 24-h, 40% RH, desiccation-stressed seedlings.

Table II. Killing Temperatures ( $LD_{50}$ ) of Epicotyls and Whole Seedlings of Wheat or Rye

Seedlings were exposed to different temperatures in freezing cabinet, then thawed slowly. Survival of epicotyls was assessed by greening in White's solution or on White's solution + Agar Agar, by microscopic observation and by turgidity.

Cultivars	Epicotyls			Whole seedlings (cold-hardened)
	Unhardened	Desiccation-stressed	Cold-hardened	
Marquis	-5	-5	-6	-6
Cappelle-Desprez	-6	-10	-7	-6.5
Kent	-4	-12	-10	-8
Fredrick	-7	-19	-20	-11
Rideau	-6	-23	-24	-11
Kharkov	-5	-40	-34	-14
Puma Rye	-7	-67	-62	-18

<sup>a</sup> Whole-seedling killing temperatures are reported from previous works (1, 3, 8).

is almost the same in epicotyls from cold- or desiccation-stressed seedlings, suggesting that the two kind of stresses cause the plant to harden to a similar degree of freezing resistance. The correlation between drought-induced and cold-induced frost hardiness is very strong among the seven cultivars chosen. The correlation coefficient was 0.99.

### CONCLUSIONS

Water content decreases associated with the 24-h desiccation stress at 40% RH are not completely compensated for by a 16-h imbibition in tap water. Nevertheless, the full turgor of the seedlings after imbibition indicates that most of the cells have deplasmolyzed. Previous authors have observed an increase in osmotic content of the cells of similarly stressed seedlings of Puma rye (12). Cells of winter wheat probably also behave the same way. Although an increase in osmoticum would certainly contribute to an increase of freezing tolerance of these cells, it would not be a sufficient increase to explain the high degree of freezing tolerance observed in some of the cultivars. It seems, instead, that the desiccation stress has triggered a mechanism of frost-hardening.

A 24-h drought (40% RH) does not harden whole seedlings as well as does a cold-conditioning of 4 weeks, because equilibrium with the imposed desiccating water potential is not reached in all parts of the seedling (12). Nevertheless, the level of hardiness attained in shoots is in good agreement with that reported by de la Roche (5, 6) and Simonovitch (11) for a longer drought of less intensity (12 days of 90% RH). However, epicotyls attained a much higher level of hardiness, with an  $LD_{50}$  of  $-67^{\circ}\text{C}$  observed for epicotyls from desiccation-stressed rye seedlings. This degree of freezing tolerance is much greater than any previously reported for rye seedlings. Also, the same degree of tolerance is found in epicotyls from cold-hardened seedlings. The high level of freezing tolerance in this tissue probably was observed, because the technique we used here to harden the epicotyls is slightly different from that used by previous investigators (4, 11). Additionally, the White's solution + Agar medium used to grow the epicotyls after the freeze test may be conducive to repair of damages suffered on freezing.

A similar degree of freezing tolerance can be induced in epicotyls either by a 24-h drought at  $21^{\circ}\text{C}$  or by a 4-week growth at  $2^{\circ}\text{C}$ . Almost the same degree of frost hardiness is expressed by each cultivar, whether induced by cold or by drought. This observation suggests that drought stress could be a promising method for rapidly cold-hardening wheat or rye seedling epicotyls for determining the relative frost hardiness of different wheat or

rye cultivars. For example, if we plot the killing temperatures of epicotyls from desiccation-stressed seedlings *versus* the killing temperatures of cold-hardened whole seedlings, we will observe the following relationship between the two: the least hardy cultivar (*i.e.* Marquis) epicotyls do not harden at all, but harder cultivar epicotyls harden about 4 times more than does the rest of the seedling. Therefore, our measurements of epicotyl frost hardiness greatly enhance the relative differences in frost hardiness of these cultivars, so that it enables discrimination to be made between small but definite differences in hardiness.

Calibration with a larger number of cultivars of known relative frost hardiness would be necessary, however, in order to increase the confidence level of the correlation between desiccation-stressed and cold-hardened seedling frost hardiness. Development of such a method should save much time and equipment in evaluating relative frost hardiness of different cultivars of wheat, rye, or other plants.

### LITERATURE CITED

- ANDREWS CJ, MK POMEROY 1977 Mitochondrial activity and ethanol accumulation in ice encased winter cereal seedlings. *Plant Physiol* 59: 1174-1178
- CHEN HH, PH LI 1978 Interactions of low temperature, water stress, and short days in the induction of stem frost hardiness in red osier dogwood. *Plant Physiol* 62: 833-835
- DE LA ROCHE IA, MK POMEROY, CJ ANDREWS 1975 Changes in fatty acid composition in wheat cultivars of contrasting hardiness. *Cryobiology* 12: 506-512
- DE LA ROCHE IA 1978 Development of freezing tolerance in wheat without change in lipid unsaturation. *Acta Hort* 81: 85-89
- DE LA ROCHE IA 1979 Mechanism of thermal tolerance. In LS Underwood, LL Tieszen, AB Callahan, GE Folk, eds, *Comparative Mechanisms of Cold Adaptation*. Academic Press, New York, pp 235-237
- DE LA ROCHE IA 1979 Increase in linolenic acid is not a prerequisite for development of freezing tolerance in wheat. *Plant Physiol* 63: 5-8
- PARSONS LR, PH LI 1978 Effects of moderate and severe water stress on frost hardiness of red osier dogwood. *Hortic Sci* 13: 376
- POMEROY MK, CJ ANDREWS 1975 Effect of temperature on respiration of mitochondria and shoot segments from cold-hardened and non-hardened wheat and rye seedlings. *Plant Physiol* 56: 703-706
- ROSA JT 1921 Investigations on the hardening process in vegetable plants. *Miss Agr For Expt Stn Bull* 48: 1-97
- SIMINOVITCH D, H THERRIEN, F GFELLER, B RHEAUME 1964 The quantitative estimation of frost injury and resistance in black locust, alfalfa and wheat tissues by determination of amino acids and other ninhydrin reacting substances released after thawing. *Can J Bot* 42: 637-649
- SIMINOVITCH D 1977 Induction of cold hardiness and plasmolysis injury resistance in coleoptiles and epicotyls of winter rye seedlings in the dark at the room temperature by desiccation. *Plant Physiol* 59: 4
- SIMINOVITCH D, Y CLOUTIER 1981 Twenty-four-hour induction of freezing and drought tolerance in plumules of winter rye seedlings by desiccation stress at room temperature in the dark. *Plant Physiol* 69: 250-255
- WHITE PR 1943 *A Handbook of Plant Tissue Culture*. Jacques Cattell Press, Lancaster, PA, p 103