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Freezing Injury and Root Development in Winter Cereals[1]

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ABSTRACT

Upon exposure to 2°C, the leaves and crowns of rye (*Secale cereale* L. cv 'Puma') and wheat (*Triticum aestivum* L. cv 'Norstar' and 'Cappelle') increased in cold hardiness, whereas little change in root cold hardiness was observed. Both root and shoot growth were severely reduced in cold-hardened Norstar wheat plants frozen to −11°C or lower and transplanted to soil. In contrast, shoot growth of plants grown in a nutrient agar medium and subjected to the same hardening and freezing conditions was not affected by freezing temperatures of −20°C while root growth was reduced at −15°C. Thus, it was apparent that lack of root development limited the ability of plants to survive freezing under natural conditions.

Generally, the temperatures at which 50% of the plants were killed as determined by the conductivity method were lower than those obtained by regrowth. A simple explanation for this difference is that the majority of cells in the crown are still alive while a small portion of the cells which are critical for regrowth are injured or killed.

Suspension cultures of Norstar wheat grown in B-5 liquid medium supplemented with 3 milligrams per liter of 2,4-dichlorophenoxyacetic acid could be cold hardened to the same levels as soil growth plants. These cultures produce roots when transferred to the same growth medium supplemented with a low rate of 2,4-dichlorophenoxyacetic acid (<1 milligram per liter). When frozen to −15°C regrowth of cultures was 50% of the control, whereas the percentage of calli with root development was reduced 50% in cultures frozen to −11°C. These results suggest that freezing affects root morphogenesis rather than just killing the cells responsible for root regeneration.

Evaluation of the cold hardiness of roots during the winter is difficult due to the frozen soil and the lack of reliable methods for assessing freezing damage. However, it is generally accepted that roots and other protected parts are less cold hardy than the aerial parts of the plant (5, 6, 9–11).

In woody species, it has been shown that cold hardening of roots is determined by genotype, soil temperature, and moisture (11). However, little information on root cold hardiness and development following freezing is available for winter cereals. It has been suggested that roots and the lower portions of the crown of cereals are more susceptible to freezing injury than the leaves and upper crown tissue (6, 7). However, because the crown is a highly heterogeneous structure different freezing processes can occur in areas less than 1 mm apart (8). This study was initiated to further detail the effects of freezing on root development of winter wheat and rye.

MATERIALS AND METHODS

Soil-Grown Plants. Seeds of rye (*Secale cereale* L. cv 'Puma') and wheat (*Triticum aestivum* L. cv 'Norstar' and 'Cappelle') were sown in 15-cm pots in vermiculite and maintained in a growth chamber at 20/15°C, day/night temperature with 14-h photoperiod for 3 to 4 weeks. These plants were supplied with half-strength Hoagland solution weekly. At the three- to four-leaf stage, the plants were transferred to a 2°C constant temperature with a 12-h photoperiod for cold hardening up to 2 weeks. A combination of cool white fluorescent and incandescent lighting (9:1) was used, and irradiance in the 400 to 700 nm range averaged 100 w m^{-2} as determined with a Li-Cor, Li-170 photometer. Harvested plants were thoroughly rinsed with distilled H_2O and separated into leaves, crowns, and roots. Leaves and roots were prepared as 1-cm sections from a composite of 60 plants/cultivar. Crowns were cut approximately 2 mm below and 8 mm above the apex for measuring ion leakage, or 5 cm above the apex for regrowth. Samples were wrapped in wet tissue for ice nucleation, held at −3°C for 16 h, and then cooled at 4.8°C/h as described by Gusta *et al.* (3). Three crowns, 10 leaf segments, and 20 root segments of each cultivar were used for each test temperature.

After freezing, crowns were transplanted into a soil mixture for regrowth as described by Gusta and Fowler (4). The fresh weight of shoots and roots was measured 3 weeks after the freeze test. Regrowth was expressed as a percentage of the nonfrozen control. Ion leakage was determined on samples immersed in 10 ml double-distilled H_2O, vacuum infiltrated and shaken for 1 h at 21°C. The conductivity (R_1) of the leachate was measured with a conductivity meter. To obtain total leachate, plant segments were frozen in a −70°C freezer for 1 h or longer, warmed up to 21°C and shaken for 1 h. The conductivity (R_2) of this leachate was determined again. The ratio of the two readings (R_1/R_2) was used as a measure of relative injury. The temperature which caused either 50% killing, as indicated by lack of regrowth, or 50% ion leakage, was defined as LT_{50}.[2] This experiment was repeated four times.

Agar-Grown Plants. Norstar wheat seeds were surface-sterilized with 1% NaOCl for 15 min, rinsed four times with sterilized distilled H_2O, and transferred onto 0.7% agar medium in test tubes. The medium (pH 6.0) consisted of: $Ca(NO_3)_2 \cdot 4H_2O$, 500 mg/l; KNO_3, 125 mg/l; $MgSO_4 \cdot 7H_2O$, 125 mg/l; KH_2PO_4, 125mg/l; Fe (II) citrate, 10 mg/l; sucrose, 20 g/l; and 1 ml/l of trace element mixture (H_2SO_4, 0.5 ml; $MnSO_4 \cdot 4H_2O$, 3000 mg; $ZnSO_4 \cdot 7H_2O$, 500 mg; H_3BO_3, 500 mg; $CuSO_4 \cdot 5H_2O$, 25 mg; $Na_2MoO_4 \cdot 2H_2O$, 25 mg in 1 L H_2O).

Conditions were the same as for soil-grown plants. Two weeks after germination, aseptic plants were transferred to a growth chamber at 2°C constant temperature with 12-h photoperiod for 1 week. The controlled freeze test was essentially the same as for soil-grown plants. The crowns were cut approximately 5 cm above and 2 mm below the apex and frozen in a sterile container. After thawing, the plants were transferred to fresh media in test tubes for regrowth. The fresh weight of shoots and roots was measured 3 weeks after freezing. This experiment was repeated three times.

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[2] Abbreviation: LT_{50}, 50% killing temperature.

Suspension Cultures. The initiation, growth and hardening of Norstar wheat suspension cultures were as reported by Chen and Gusta (1). One-week-old aseptic cultures were hardened by shaking at 2°C for 1 week. The liquid medium was decanted and the cell aggregates were washed three times with autoclaved distilled H_2O. The cell aggregates were then placed onto a piece of wet filter paper in a covered aluminum weighing pan. Before transferring the cells, the wet filter paper and aluminum pans were autoclaved, frozen to −30°C, warmed up to −3°C, and maintained over an ice bath during transfer to ensure that the wet filter paper remained frozen. This served to initiate ice formation in the cells during the freeze test. After freezing, the samples were thawed at 4°C for 12 h and warmed to 21°C. Cell aggregates were transferred to Petri dishes containing an agar (0.7%) B-5 medium (1) with no 2,4-D. The cell aggregates, each approximately 1 mm in diameter, were placed in separate Petri dishes and maintained in a 20°C incubator in the dark. The growth of callus and roots from each cell aggregate was recorded 1 month after transferring. The experiment was repeated three times. In each replicate, three Petri dishes were used for each freezing temperature.

RESULTS

The cold hardiness of Puma rye and Norstar and Cappelle wheat crowns, subjected to a controlled freeze test, was evaluated by both regrowth and the conductivity method (Fig. 1). Upon subjection to 2°C, the crown and leaves of Puma rye and Norstar and Cappelle wheat plants rapidly cold hardened to different levels as determined by the conductivity methods (Fig. 1). The cold hardiness of roots showed little change during the hardening treatment and no differences among the three genotypes were observed. The inability of roots to cold harden in response to low temperature may explain why plants that look normal in early spring often die after a few weeks of warm weather. For example, winter-injured Puma rye plants collected in early spring from the field showed no signs of root regeneration, whereas noninjured plants had the ability to quickly regenerate functional roots (Fig. 2). In Saskatchewan, roots developed in the fall on winter wheat and winter rye are generally winter-killed even though there is no apparent sign of injury to the crown.

Generally, the LT_{50}s obtained by the conductivity method were lower than those obtained by regrowth. For example, at the 7th d of cold hardening, the LT_{50} of Puma rye crowns, as determined by regrowth and the conductivity method, was −20°C and −24°C, respectively.

Following a controlled freeze test, the regrowth of cold-hardened Norstar winter wheat plants transplanted to soil and to agar was compared (Fig. 3). In plants frozen to −15°C and transplanted to soil, both shoot and root growth were severely reduced. In plants transplanted to agar medium, the shoot growth of plants frozen to −20°C was not significantly different from the control. However, root growth was reduced at temperatures below −15°C as compared to the control (Fig. 4).

Although shoot growth of the plants was similar (Fig. 4), root growth was quite different. Plants frozen to −15°C developed roots which elongated and branched. Plants frozen to −18°C regenerated roots as long as the plants frozen to −15°C, but there was little or no apparent sign of branching. A very small, spindly root system developed from plants frozen to −20°C.

Suspension cultures of Norstar wheat readily differentiate roots when transferred to media containing a low level of 2.4-D (<1 mg/l) (Fig. 5). The growth of callus on agar medium was reduced by 50% after being frozen to −15°C, while root initiation was severely reduced at about −11°C. The percentage of callus with root initiation, maximum root number per callus, and callus size after 1 month regrowth are summarized in Table I. The differential response of growth and the capability of differentiation following freezing suggest that root initiation is more sensitive to freezing than cell division or growth.

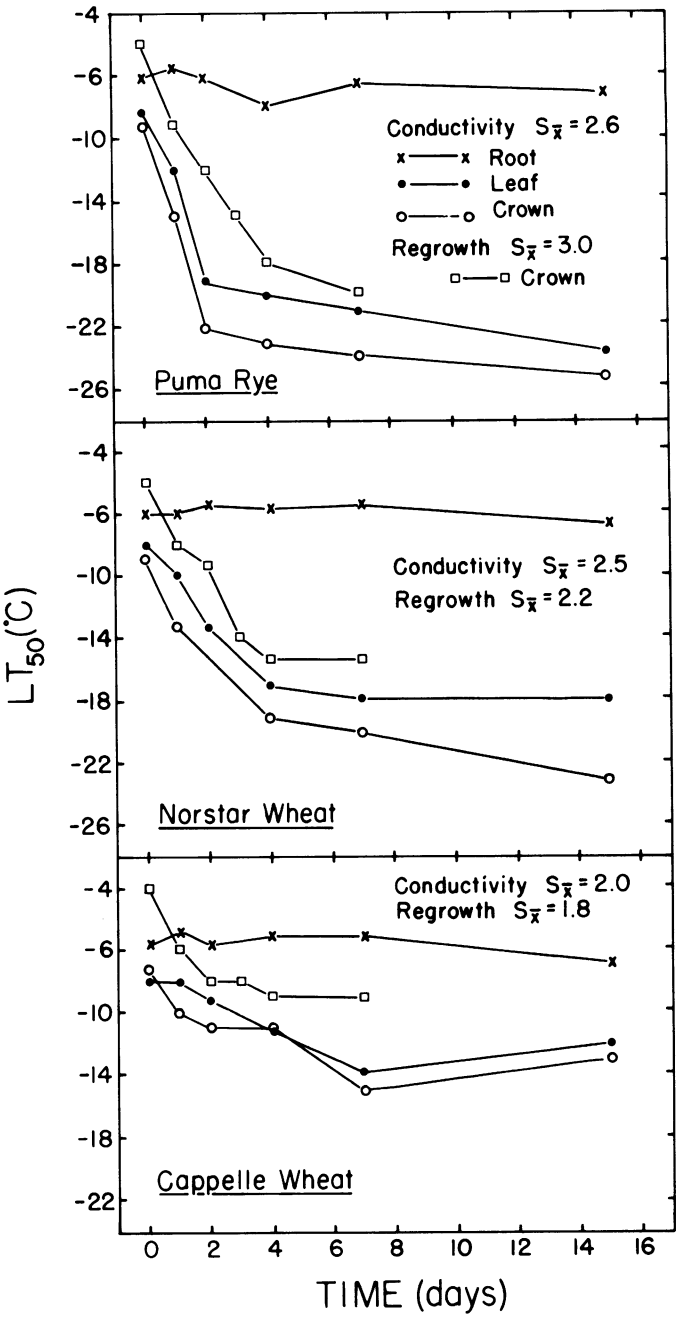

FIG. 1. Development of cold hardiness in leaves, crowns, and roots of rye and wheat plants hardened at 2°C constant temperature, as determined by the regrowth method for crowns and the conductivity method for leaves, crowns, and roots.

DISCUSSION

Olien (7) reported that, because of the rigid structure and high water content of roots, non-equilibrium freezing occurs in the roots and lower crown at higher temperatures than in the leaves and upper crown. He also reported that in early spring, the injured roots preserved by low temperature still function. However, no data were presented to show that the roots were still functional. From our field observations in early spring, the crowns of severely injured plants often produced new shoot

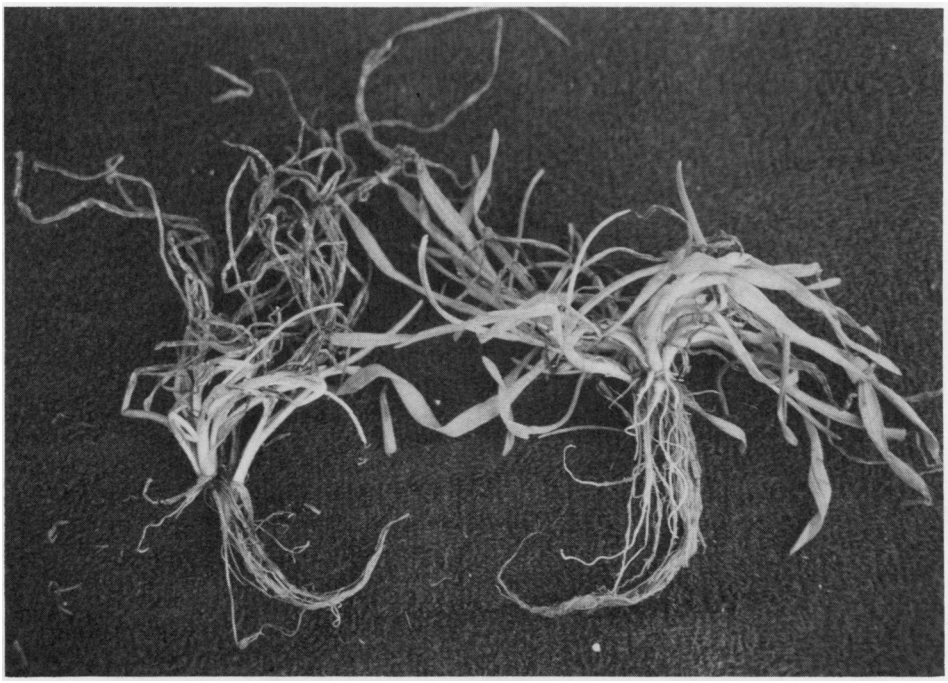

FIG. 2. Root regeneration in a winter-injured (left) and an uninjured (right) Puma rye plant in early spring.

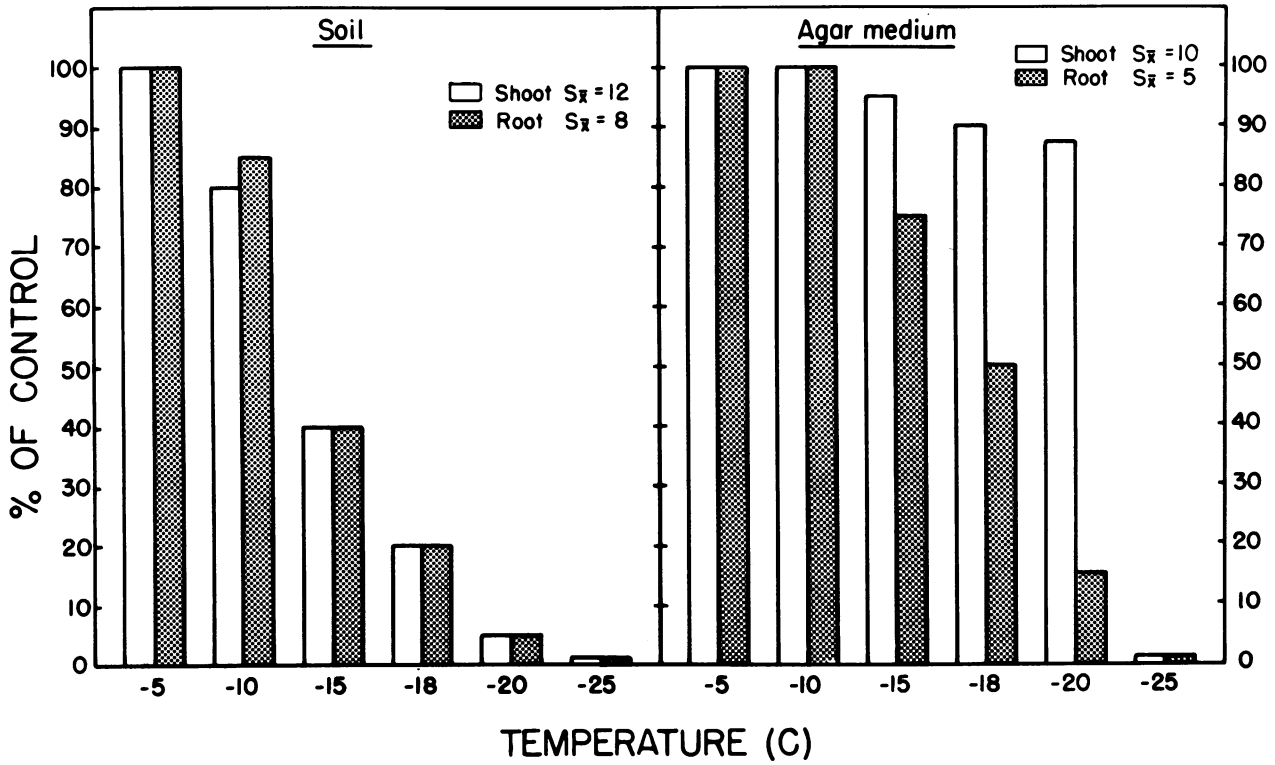

FIG. 3. Comparison of shoot and root regrowth after freezing of Norstar wheat plants transplanted to soil and to agar medium.

growth but, after a period of 2 to 3 weeks, died due to lack of root growth. Our hardening data (Fig. 1) also indicate that the roots were killed at temperatures below −8°C and that new roots are produced when plants are returned to conditions favorable for growth for both the winter rye and winter wheat. The soil temperature in Saskatchewan in winter is generally lower than −8°C at the lower base of the crown. The reason why roots were less hardy than leaves and crown is not clear, although this has been observed in many plant species (3, 9–11).

The LT_{50}s, as determined by regrowth, were always several degrees warmer than the values of the same genotype determined by the conductivity method (Fig. 1). Cloutier and Siminovitch (2) used several viability tests (neutral red vital staining, protoplasmic streaming, plasmolysis/deplasmolysis, and greening) and found that the epicotyls of cold-hardened Norstar wheat survived to −30°C whereas, by regrowth, crowns only survived to −13°C.

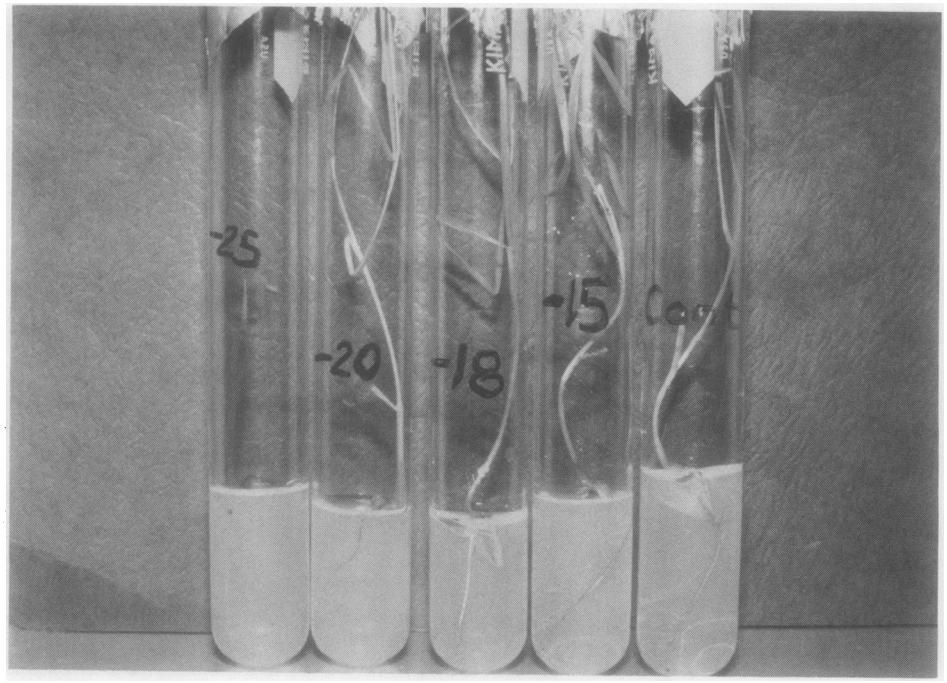

FIG. 4. Shoot regrowth of Norstar wheat plants grown in agar medium. From left to right, plants frozen to −25°C, −20°C, −18°C, −15°C, and control, respectively.

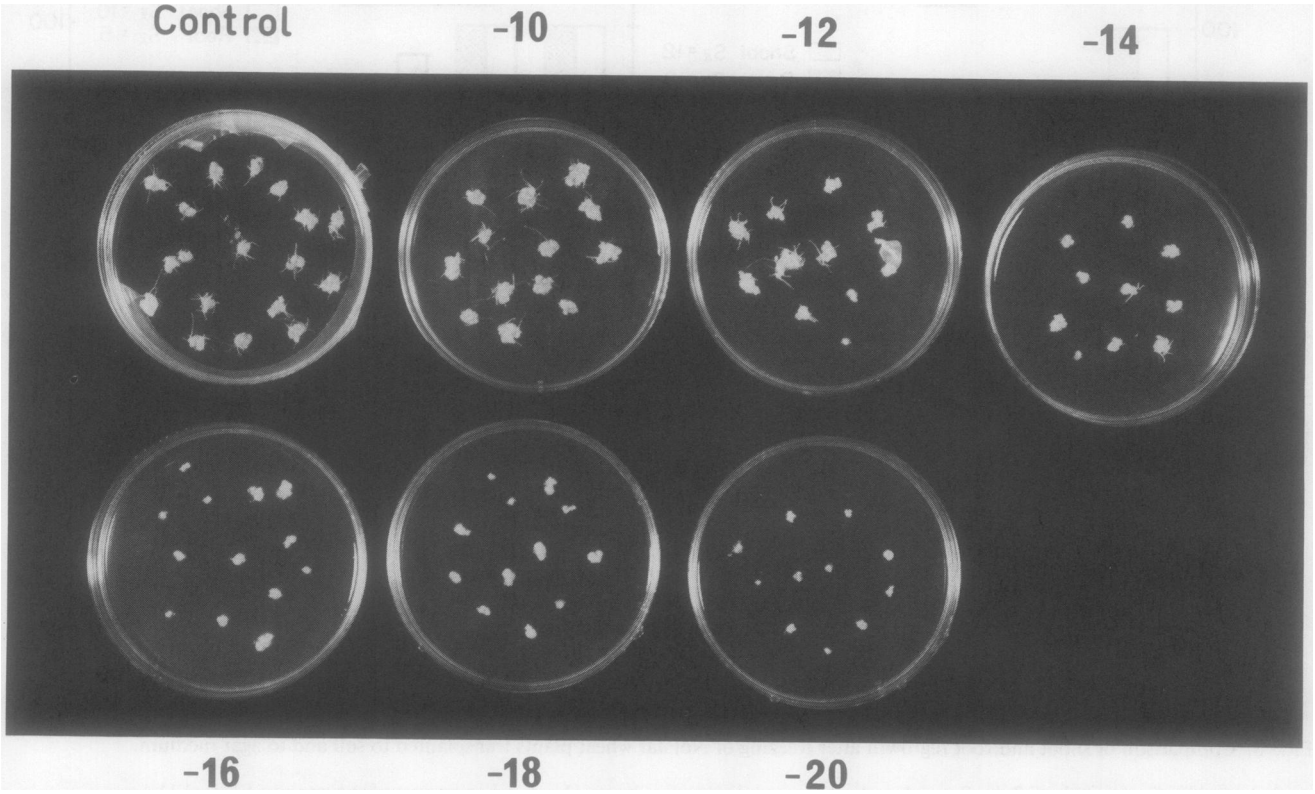

FIG. 5. Root differentiation in Norstar wheat cell aggregates in low 2,4-D medium. Cell aggregates were frozen to the temperatures indicated and then transferred onto agar-solidified B-5 medium with no 2,4-D for 1 month at 20°C in the dark.

The different LT_{50} values obtained by regrowth and other viability tests, thus, must be real. Therefore, a simple explanation is that the majority of cells in the crown are still alive, while a small portion of the cells, critical for regrowth, were injured or killed. Studies with winter barley (*Hordeum vulgare* L. emend. Lam.) indicate that, although the roots and lower part of the crown may be killed by freezing, these tissues are not critical for recovery as long as the meristem is alive (6–8). This implies that the cells in a crown do not contribute equally to survival of that crown. The conductivity method only provides information as to what

Table I. *Effect of Sub-Zero Temperatures on Root Development and Callus Growth of Norstar Wheat Cultures*

Suspension cultures were hardened at 2°C for 1 week and frozen as described in "Materials and Methods." Cell aggregates about 1 mm in diameter were transplanted to Petri dishes containing B-5 medium in 0.7% agar with no 2,4-D. Root initiation and callus growth were recorded 1 month after transferring. LT_{50}s, as evaluated by regrowth and by the capacity to initiate root in callus, were −15°C and −11°C, respectively.

Temperature	Root Initiation	Root No./Callus	Callus Regrowth[a]
°C	% callus		
0	85 ± 15[b]	11 ± 5	10
−5	70 ± 13	11 ± 3	10
−10	61 ± 11	11 ± 4	9
−12	47 ± 12	11 ± 3	8
−14	17 ± 10	2 ± 2	6
−16	0	0	5
−18	0	0	4
−20	0	0	2
−22	0	0	1

[a] Increase in callus diameter from original size after 1 month. 10 = 10-fold growth; 1 = no growth.

[b] SE.

percentage of ions are lost from the tissue following a controlled freeze test. Thus, if only a small number of cells in the crown are critical for survival, this method may not accurately predict the killing temperature of the genotype.

There was a reduction in shoot and root growth in wheat plants frozen from −10 to −20°C when transplanted to soil. The reduction in shoot growth was probably due to the effect of the lower temperatures on root regeneration. The growth of shoots of similarly frozen plants transplanted to agar was uniform from 0 to −20°C, whereas root growth was reduced in plants frozen to −15°C (Figs. 3 and 4). Thus, it appears that the shoot apical meristem in Norstar wheat plants was not severely injured when frozen to −20°C. Plants in agar in test tubes were grown under sterile conditions, with high humidity and a supply of readily available nutrients, whereas plants transplanted to soil have a less favorable environment. Shoot growth would then be restricted due to less water and nutrient uptake by the injured roots. No shoot or root growth was evident in plants frozen lower than −22°C in either soil or nutrient media.

These results suggest that root initiation is more sensitive to freezing than shoot regrowth. This may account for the difference in the temperature for LT_{50} when regrowth was compared to the conductivity method (Fig. 1) and other viability tests (2). Results from freezing cell cultures indicate that the lack of root initiation in the crowns is not solely due to killing of the cells responsible for root initiation. Cell cultures of Norstar wheat grown in a low 2,4-D medium readily generate roots. As shown in Figure 5 and Table I, the differential response of callus growth and root initiation strongly suggests that root development is more sensitive to freezing than cell regrowth. It appears that freezing causes cells to lose their capacity for root morphogenesis at higher temperatures than required to kill cells or to stop cell division.

In conclusion, roots of winter wheat and winter rye have a limited capacity for cold hardening, relative to the crowns and shoots. Therefore, regeneration of a new root system becomes critical for recovery in the spring. At the LT_{50} temperature determined by regrowth, the cells responsible for root initiation are impaired, while the majority of crown cells are still undamaged as judged by viability tests. The lack of root morphogenesis in frozen cells may be due to a specific lesion, rather than death of the cells.

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