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ABSTRACT

Sugarbeet seedlings (*Beta vulgaris* L.) may be killed by Spring frost just as they emerge from the soil. Possible solutions to this problem were investigated under closely controlled laboratory and growth chamber conditions. The seedlings were germinated at different temperatures in contact with solutions containing both varying osmotic pressures and compounds known to promote cold-hardiness in other plants. Following germination, the seedlings were frozen in blocks of ice at different minimum temperatures and the degree of injury was noted.

The results indicated that two mechanisms were involved in seedling survival. One was related to the osmotic potential of the plant sap and the other to the amount of water in the sap that could be converted to ice without killing the seedlings. Seedling osmotic pressures could be increased through the solution in contact with the roots, while the tolerance for ice could be increased with cool temperatures during germination. These two factors had a strong positive interaction and could reduce the normal lethal temperature from -0.5 to -2.5 C. Growth regulators and other compounds that have been reported to increase cold-hardiness in other plants were not effective.

Additional index words: *Beta vulgaris* L., Frost, Osmotic potential, Freezing, Water relations.

COLD-HARDINESS in dormant perennials has been the subject of many studies. Cold injury to tender plants has had less attention and sugarbeet (*Beta vulgaris* L.) seedlings have received almost none, even though loss from untimely spring frost is an important economic problem.

The degree of plant injury by frost depends on a number of factors, i.e.; cooling and warming rates, relative humidity of air, cold-hardening of plant tissue, energy level of water in the plant, and of course, the minimum temperature reached by the plant tissue (10). In addition several chemicals have been reported to increase the tolerance of plant tissue to cold (12). The object of the work reported here was to test the effectiveness of some of these chemicals and to learn how beet seedlings respond to some of the physical factors that are known to affect frost tolerance.

MATERIALS AND METHODS

Controlled studies were conducted to develop methods for preventing extensive death from frost just as the beet seedlings emerge through the soil surface. Seeds were germinated on paper toweling in closed plastic pans in a constant temperature room (23 C) or in a temperature controlled box with a diurnal temperature cycle of 2 C for 5 hours, 15 C for 12 hours, and 3.5 hours for the temperature changes. The toweling was moistened with a saturated CaSO₄ solution in the case of controls or with solutions containing the desired level of test solutes made up from the saturated CaSO₄ control solution to form specific treatments.

When the overall length of the seedlings was 3 to 5 cm (4 to 7 days old at 23 C), 15 to 20 from each treatment were placed in separate aluminum foil pans. These pans were filled with a saturated CaSO₄ solution and placed in a freezing chamber in a constant temperature room. The freezing chamber was jacketed in water pumped from a precisely controlled water bath. The temperature was lowered to 0 C, the solution inoculated with ice, and the system cooled to a predetermined minimum temperature and held there for 1 hour before thawing.

The temperature of the blocks of ice which contained the seedlings was continually monitored and controlled to ± 0.05 C. Rates of warming and cooling were held to less than 4 C/hour. After thawing the seedlings were removed from the pans and placed on moist toweling in a closed chamber with high humidity and low light. After 2 or 3 days plant tissue killed by the freeze had darkened and it was not difficult to count the number of individual cotyledons, hypocotyls, and roots that survived.

Frost damage may also occur on beet seedlings after they have emerged from the soil, so this growth stage was also studied. Sandy loam soil was leached to remove nitrates. Pans were filled 12-cm deep with 13 kg of the soil and fertilized with 0.5 g of P. The soil was moistened with distilled water or with a solution containing the desired levels of salts. One-hundred beet seeds were planted in each pan and the pans were placed in a growth chamber. Each treatment was repeated in three pans so analysis of variance could be carried out on the results. The soil was watered daily to replace evaporative loss. The light intensity in the growth chamber was 0.58 μ einsteins/m² sec in the photosynthetically active wavelength range of 400 to 700 nm.

When the seedlings reached the desired size for freezing, they were thinned for uniformity. The lights were turned off and the temperature in the growth chamber lowered to a predetermined level. Ice in the seedlings was then nucleated with snow or freezing mist. The minimum temperature was maintained for at least 1 hour after all the plants were frozen. Cooling and warming were done slowly, requiring several hours on each side of the low temperature plateau. Two chambers were sometimes used to bring the seedlings to different levels of hardiness, but for any given set of comparisons all treatments were frozen in the same chamber at the same time.

A vapor pressure psychrometer was used to measure the osmotic potentials of the crushed seedlings and germination solutions. Measurements on sap from presumably identical seedlings were reproducible within ± 0.5 bar (19).

RESULTS AND DISCUSSION

Pre-emergence Conditions

The first 10 entries in Table 1 provide a comparison of the survival of sugarbeet seedlings, which were germinated in contact with various frost "protective" agents and then frozen in blocks of ice.

Small amounts of n-ds (n-decenylsuccinic acid) have been reported to decrease frost sensitivity of fruit blossoms by increasing cell water permeability (9). Abcissic acid (ABA) and tetra-butanol have also been reported to increase cell water permeability (5). Ethrel (1) has been used to increase winter-hardiness of fruit trees and CCC [(2-chloroethyl) trimethylammonium] may sometimes supplement the requirement of cool temperatures for hardening (7). The herbicide dalapon has been reported to increase freeze tolerance of sugarbeet seedlings (4) but its action may be linked to controlling the stability of supercooled water (2). Urea and DMSO (dimethyl sulfoxide) have also been reported to increase the freeze toler-

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Table 1. The effect of the external germinating solution on the temperature at which 50% or more of the sugarbeet seedling hypocotyls were killed. Roots were killed at slightly warmer temperatures and cotyledons at slightly lower temperatures than those shown for hypocotyls.

Germination Solution		Temperature causing at least 50% death					
Type of solutes*	Osmotic potential	-0.5	-1.0	-1.25	-1.5	-2.0	-2.5
	bars	°C					
Control, saturated CaSO ₄	-1		x				
N-de, 100 ppm	-1		x				
Ethrel, 50 or 100 ppm	-1		x				
CCC, 45 or 500 ppm	-1		x				
Dalapon, 7 or 35 ppm	-1		x				
ABA, 20 ppm	-1		x				
Tetra butanol	-4.5		x				
Urea	-3		x				
DMSO	-3			x			
CW200	-6				x		
Mannitol	-6		x				
KCl	-4.5			x			
NaC ₂ H ₃ O ₂	-4.5		x				
KCl	-8				x		
K ₂ SO ₄	-8		x				
NaH ₂ PO ₄	-8		x				
MuSO ₄	-8		x				
NaBr	-8				x		
CaCl ₂	-8				x		
KBr	-8				x		
KNO ₃	-8				x		
NaCl	-8				x		
DMSO	-12						x
DMSO +							
CW200	-6.5				x		
NaBr	-6.5				x		
KNO ₃	-6.5				x		
KNO ₃	-9.5					x	
KCl	-9.5					x	
NaBr	-13						x
KNO ₃	-13						x
CW200	-14						x

* When DMSO was mixed with salts, it accounted for approximately 1/2 of the total osmotic potential.

ance of tissue (8, 12). The GW200 (polyethylene glycol, molecular wt 200) was used to simulate the effects of certain polysaccharides which are known to be associated with cold-hardiness (15). Of all these, only seedlings germinated with the DMSO and GW200 were consistently more hardy than the control.

Olien (15) has shown that some polysaccharides in barley (*Hordeum vulgare* L.) are associated with cold-hardiness, possibly because they interfere with ice crystal growth, rendering them less harmful to the plant tissue. Observations of ice crystal growth in a dilute CW200 solution on a microscope slide also suggest a significant interference, causing the ice crystals to spread slowly with many feathered lobes similar to some of the photographs shown by Luyet (11). Since CW200 is taken up by plant roots, one might expect it to reduce internal ice crystal damage while plants absorbing a weak salt solution might be less resistant to freeze injury, as ice crystals (at least when growing in dilute salt solution on a microscope slide) tend to produce pointed spikes. However as shown in Table 1, dilute solutions of salts (i.e.; Cl, Br, and NO₃) were equally effective as CW200 in promoting frost tolerance in the seedlings.

A review of the literature (10, 13, 18) suggests that the osmotic potentials of plant sap may have been responsible for the differences shown in Table 1. Examples of these potentials from various germinating solutions are given in Table 2. The Cl, Br, and NO₃ were more effective than SO₄, PO₄, and mannitol because they are more readily absorbed by the seedlings. For example, seedlings germinated with 8-bar mannitol or K₂SO₄ solutions and then frozen in the mannitol

or K₂SO₄ solutions were not damaged by ice temperatures of -1 C. However, seedlings from the same group frozen at -1 C in the saturated CaSO₄ solution were killed, evidently because their osmotic potentials rose to about -3 bars soon after they were placed in the CaSO₄ solution. Initially, the osmotic potential of all seedlings' sap was about 1 bar lower than that of the germination solutions.

As ice crystals form in a solution the solutes are concentrated in the remaining unfrozen brine. The amount of ice relative to the amount of unfrozen brine is determined by the temperature and the amount of solute in the original solution. During freezing the vapor pressure of ice is lower than that of supercooled water, at any given temperature, so ice crystals continue to grow until they have removed enough liquid from the solution to raise the solute concentration to a point where its vapor pressure is as low as the vapor pressure of ice. Ice then stops forming and the system is in equilibrium (13).

This is an important concept, for it suggests that decreasing the osmotic potential of the plant sap may impart much more cold tolerance to plant tissue than is commonly supposed. Freezing point depression data for common solutions show that the freezing point is lowered about 1 C for each 12-bar decrease in osmotic potential. Thus ice begins to form in a solution with an osmotic potential of -3 bars at -0.25 C and at -0.75 C in one with an osmotic potential of -9 bars. If a plant is killed as soon as the first ice crystals form in its sap, the lethal temperature will be that determined solely by the freezing point depression from the osmotic potential. On the other hand if 80% of the plant's internal water can separate as ice before the plant is killed, the temperature may decrease well below the freezing point of the plant sap without causing cell damage. For example freezing 80% of a 3-bar solution decreases the osmotic potential to -15 bars and so requires a temperature of -1.25 C to form enough ice to kill the plant. If the initial osmotic potential of the plant sap were -9 bars, turning 80% of it to ice would lower the osmotic potential to -45 bars, requiring a temperature of -3.75 C to kill the plant. Thus for a plant that cannot tolerate ice, decreasing the osmotic

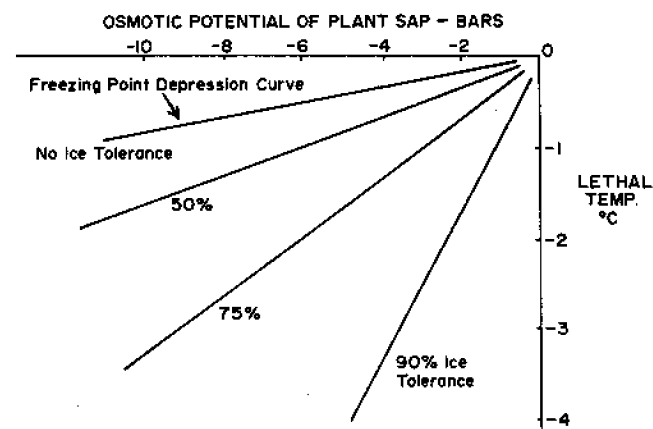


Fig. 1. The relation between the amount of ice a plant can tolerate, the osmotic potential of its sap before freezing, and the temperature at which death occurs.

Table 2. The effect of external germinating solution and temperature (W, 23 constant; C, 2 to 15 diurnal cycle) on the temperature at which at least 50% of the sugarbeet seedling hypocotyls were killed.

Type of solutes	Germination solution		Avg osmotic potential of sap bars	Temperature causing at least 50% death						
	Osmotic potential bars	Temperature conditions		-0.5	-1.0	-1.25	-1.5	-2.0	-2.5	-3.0
Control (saturated CaSO ₄)	-1	W	-2		x					
		C	-2.5			x				
KNO ₃	-4.5	W	-4				x			
		C	-4.5				x			
DMSO	-2	W	-3		x					
		C	-3			x				
KCl	-4.5	W	-4		x					
		C	-4							
KCl	-8	W	-10.5					x		
		C	-9.5					x		
DMSO	-7	W	-8						x	
		C	-10						x	
DMSO + KCl	-8	W	-9							x
		C	-9							x
KCl	-13	W	-14.5							x
		C	-15							x

Table 3. The effect of soil solution, air temperature, and plant size on the survival of sugarbeet seedlings.

4 true-leaf stage frozen at -5.5C*		2 true-leaf stage frozen at -3.5C†		Cotyledon and 2-leaf stages frozen at -1.5C‡	
Treatment	Survival [§]	Treatment	Survival [§]	Treatment	Survival [§]
	%		%		%
Control	6 a	Control	31 a	Unhardened control, 2-leaf	21 a
Control, N and K	30 b	Control, N and K	35 a	Unhardened KNO ₃ , -2 bars, 2-leaf	6 b
KNO ₃ , -2 bars	21 b	KNO ₃ , -2 bars	25 a	Hardened control, cotyl.	97 c
NH ₄ NO ₃ , -2 bars	28 b	NH ₄ NO ₃ , -2 bars	30 a	Hardened KNO ₃ , -2 bars, cotyl.	90 c
Pretreated	22 b	Control, rapid thaw	7 b	Unhardened control, cotyl.	6 b
CaCl ₂ + N and K, -2 bars	60 c	CaCl ₂ + N and K, -2 bars	29 a	Unhardened KNO ₃ , -2 bars, cotyl.	13 ba

* "Pretreated" seeds soaked in 1 M NH₄NO₃ before planting; N and K indicated fertilization with KNO₃. † "Hardened" germinated with 5 to 20 C diurnal cycles, and "unhardened" at 20 C constant temperature. Unhardened 2 true-leaf and hardened control cotyledon stages were same age, while hardened control and unhardened cotyledon were the same physiological stages. ‡ Like letters following the means indicate they were not different at the 95% confidence level.

potential of the plant sap 6 bars only decreases the lethal point 0.5 C, but decreasing the sap 6 bars in a plant that can tolerate 80% ice decreases its lethal point 3 C.

Using this concept one can plot the family of theoretical plant temperature survival curves shown in Fig. 1. These curves illustrate the important interaction between internal plant ice tolerance and the osmotic potential of the plant sap before freezing. The concept also lends itself to the interpretation of the results summarized in Table 1.

The DMSO, being relatively nontoxic, was an excellent agent for decreasing seedling osmotic potentials. Other solutions, in addition to those listed in Table 1, were tested too but ions such as C₂H₃O₂, NH₄, Li, and I were all too toxic at concentrations high enough to be effective.

Other trials were also conducted in which varying amounts of n-ds, CCC, ethrel, and ABA were mixed with the germinating solutions of KCl, KNO₃, CW200, and DMSO. No consistent improvement in cold tolerance resulted from these additions nor did spraying these agents on the seedlings a day or even a few hours before freezing have any measureable effect. Spraying DMSO on seedlings a few hours before freezing did have a small effect, but much less than germinating them in a DMSO solution. Use of high concentrations of CCC (2,000 ppm) in the germinating solution did tend to increase cold survival, however the effect appeared to be primarily of an osmotic nature.

It is well known that cool temperatures promote hardening in some plants (10). Table 2 illustrates the effect of a 2 to 15 C diurnal temperature cycle during

germination on seedling freeze tolerance compared to a constant 23 C temperature during germination. The cool temperatures lowered the lethal freeze levels of the seedlings 0.25 to 0.50 C. The mechanism involved is not known. The cool germinated seedlings were of course, older than the warm germinated ones since the comparisons were made when the seedlings were approximately the same size.

When the results in Table 2 are compared to the curves in Fig. 1, it appears that the plants germinated at 23 C could tolerate less than 50% of the water in their hypocotyls as ice, while the seedlings germinated with the 2 to 15 C cycle could tolerate more than 50% of their water as ice. This is in general agreement with the work of Samygin and Livshin (17) and it is important, for the minimum survival temperature decreases rapidly as the percentage of ice tolerance increases, particularly at lower osmotic potentials (Fig. 1).

It is possible that the amount of ice the plant tissue will tolerate depends in part on the osmotic potential of its sap but the data now available are not complete enough to decide this point. It was noted in preliminary experiments using an approach similar to Olien's (16) that the formation of ice was responsible for most of the injury to very small sugarbeet seedlings, rather than the low water potentials and temperatures *per se*.

Postemergence Conditions

The effects of salt in the root environment of seedlings after they emerge into light is quite different than during the initial germination stage. The seed-

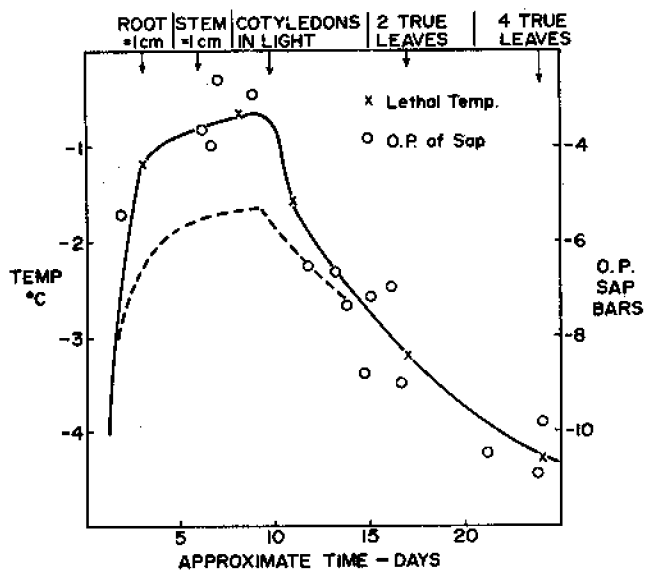


Fig. 2. The relationship between osmotic potential of sugarbeet seedling's sap, its physiological age, and the 50% survival temperature. The solid line and data points are for "normal" conditions and the dashed curve is an example of how the survival curve can be altered by decreasing the osmotic potential around the seedling's root.

lings can only tolerate about -2 bars osmotic potential in the external solution rather than the -8 bars or so that can be applied during germination.

To illustrate some of the differences, results from three growth chamber studies are summarized in Table 3. The first section shows the effects of various salts in the soil on frost survival of beet seedlings. The control seedlings growing in the low fertility sand had a statistically significant lower survival rate than did those grown in the other treatments. These plants were smaller and chlorotic, indicating N deficiency. The survival at high levels of nitrate salts was no better than where the soil had received KNO_3 equivalent to 50 kg N/ha at a 15-cm depth (the N and K control). The high level of nitrate salts (i.e., the 2-bar solution of KNO_3 was equivalent to about 800 kg N/ha 15 cm.

Plants grown in the 2-bar CaCl_2 solution fertilized with N and K showed a statistically significant better survival than those of all other treatments, which might be directly attributed to an osmotic effect. On the other hand the CaCl_2 was no more effective than other treatments on smaller seedlings as shown in section 2 of Table 3. In this case the unfertilized check was as hardy as the others, because the residual N in the soil and the seed were still adequate to support normal photosynthesis leading to the decrease in osmotic potential that follows emergence (Fig. 2).

The only treatment in section 2 of Table 3 statistically different from the others was the control in which the seedlings were allowed to thaw quickly at room temperature. Rapid thawing has been noted before as a cause of tissue injury (6, 13, 14) in various plant materials that were frozen slowly. Several explanations have been proposed but one possibility, which has not received any study, is the relatively rapid transport of water due to thermal gradients (3). Even a small thermal gradient in a tiny capillary con-

taining an ice-liquid interface can create large pressure gradients in the liquid phase resulting in flow of water toward the colder regions and rapid ice crystal growth. Rapid freezing or thawing may cause micro-thermal differences in the tissue at temperatures near the freezing point where this type of liquid transfer occurs so quickly.

Section 3 in Table 3 illustrates the effect of cool night temperatures during germination on the hardening of beet seedlings. The results are in agreement with those in Table 2, however in this case the cool night seedlings are compared with warm germinated plants of both the same size and the same age.

Practical Aspects

Figure 2 summarizes the results presented in this paper. Sugarbeet seedlings growing under normal springtime conditions with some cold nights will have a 50% frost survival curve similar to that shown by the solid line. The osmotic potential of the plant sap is correlated with survival and may be scaled to the same curve. The osmotic potential of germinating seedlings can be increased under controlled conditions so that the survival curve is modified as shown by the dashed line. This could have important consequences because most beet seedlings are killed by frost just as they are emerging through the soil surface. Lowering their lethal temperature by 1 C might often save them because even during a hard freeze the temperature just below the soil surface is buffered by the release of latent heat from freezing soil water.

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