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A COMPARISON OF FREEZE-INDUCED CARBOHYDRATE CHANGES IN WINTER BARLEY CROWNS

By

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ABSTRACT

A COMPARISON OF FREEZE-INDUCED
CARBOHYDRATE CHANGES
IN WINTER BARLEY CROWNS

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Past studies have shown that conversion of fructan to fructose and sucrose occurs in winter cereal crowns when they are frozen for 24 hours at -3C. Simple sugars reportedly increase in extracellular spaces and seem to provide resistance to adhesive stress due to freezing. Differences between rye and barley for freeze-induced conversion were reported. Differences within species may provide a basis for improvement of freezing resistance in that species. Four winter barley cultivars were grown in controlled conditions and frozen for 24 hours at -3C. Frozen crowns and an unfrozen control were extracted with ethanol and water. Carbohydrates were separated and quantified using HPLC, refractive index detection, and electronic peak area determination. Experimental variation was too high to show a significant interaction between cultivars and treatments but patterns in the variation indicate that under other conditions differences may be shown. Combining this component of winter hardiness with other freezing resistance mechanisms could provide a basis for improvement of overall hardiness in winter barley.

TO

MARIA and JESSE

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INTRODUCTION

Winter cereals, where adapted, are generally preferred over spring types (11) because winter cereals produce a higher yielding and better quality crop. A fall planting utilizes winter moisture and stored reserves by beginning spring regrowth when soil temperatures are warm enough. This promotes maturation before many summer diseases and insects reach damaging levels. By contrast, spring varieties cannot be planted until soil moisture levels allow entry into the field.

Freezing injury limits winter cereal production to areas where conditions are favorable; it also restricts production of less hardy winter cereals, such as barley and oats. For this reason, although winter wheat in 1983 accounted for 78% of the total wheat acreage in the U.S. (31), less than 1% of the barley acreage was winter habit in the state of Michigan.

Improving freezing resistance of winter cereals is, therefore, an important aspect of crop improvement. But, increasing winterhardiness as a whole is a broad, complex problem. Identifying individual freezing stresses and resistance mechanisms, then combining favorable characteristics through plant breeding would be a more

manageable approach.

CEREAL CROWNS

After germination in the fall, winter cereals establish a vegetative system of several tillers and secondary roots. Roots and leaves do not usually survive low winter temperatures, but spring recovery is not dependent on these organs. Provided crown meristems are not seriously damaged, and are capable of differentiating a functional root system, complete regeneration is possible. Cultivar hardiness will therefore, depend on the crown's withstanding individual freezing stresses as it overwinters.

A cereal crown consists of a densely packed transitional zone of differentiated intertwining vascular elements, surrounded by "a mantle of parenchymatous promeristem". This promeristem does not continuously differentiate, but has continuous meristematic potential (29). The promeristem includes axial meristems which differentiate into roots or leaves, and apical meristems which give rise to floral primordia after a vernalization period. Freezing barley crowns causes vascular disruption in the transitional zone of the inner and lower crown resulting in tissue death (29). The extent of injury depends on cultivar hardiness, extent of hardening before freezing, and

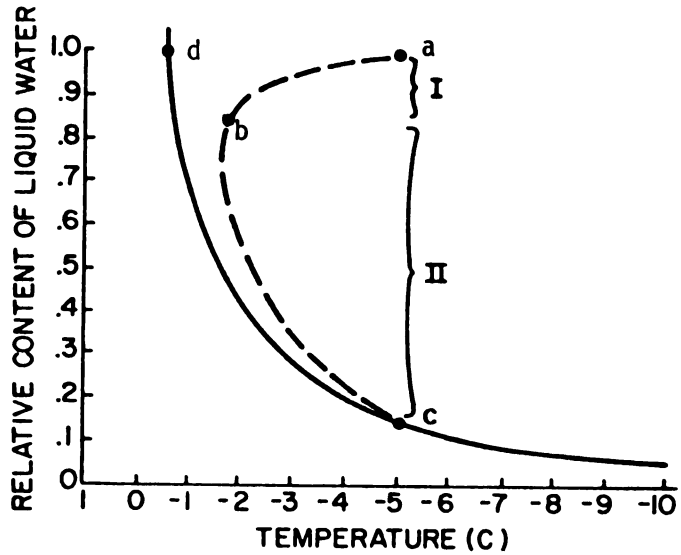
type of freezing stress. Uninjured, upper promeristems can generate leaves and roots which will be the basis for spring recovery (29).

FREEZING STRESS

Stresses resulting in tissue death involve water transitions and redistribution as crowns freeze. Two basic transistion patterns are followed as freezing progresses. First, there is a nonequilibrium pattern which involves "large displacements of temperature from the balanced state". Second, there is an equilibrium pattern caused by smaller temperature displacements which allow equilibration after each incremental decrease in temperature (15).

Nonequilibrium freezing:

Placing normally growing plants in freezing temperatures causes a disruption of the balanced state between plants and their environment. Plant liquid usually cools without freezing. As ice formation begins in extracellular spaces latent heat is released providing energy for ice crystal growth (15). The following diagram illustrates nonequilibrium freezing.



(15)

Figure 1. Nonequilibrium freezing. Liquid water in gm/gm dry matter as a function of temperature. See text for explanation.

Nonequilibrium freezing can be further subdivided into two kinds of stress: 1.) intracellular freezing and 2.) extracellular ice.

Intracellular freezing: The solid line is the equilibrium transition pattern while the broken line represents nonequilibrium freezing. The distance between points a and d depict the amount of supercooling caused by initial temperature displacement from equilibrium. The temperature rise in stage I as freezing progresses from a to b is due to latent heat release. This provides

crystalization energy "that can cause ice crystals to grow from the outer free space into the protoplast" (15) usually resulting in cell death. The amount of energy available for crystal growth depends on the extent of supercooling below the protoplast freezing point (16).

Intracellular freezing resistance is provided by plasmalemma stabilization during hardening (mechanical resistance) and, freezing point depression through water loss, solute accumulation, and matric interactions (2,15).

Stage I of nonequilibrium freezing is an instance of high freezing intensity (high degree of supercooling) with low freezing capacity (small amount of liquid water). By contrast, stage II (b to c), consists of low freezing intensity (a small displacement from equilibrium) and high capacity (large amounts of liquid water). This causes a different kind of stress.

Extracellular Ice: As extracellular freezing progresses from b to c (fig.1) it results in large extracellular ice crystals. This can cause extensive disruption of intertwining vascular elements and meristems in crowns (17).

Protection involves the interaction of polymers, normally cell wall constituents, with growing ice crystals. Certain mucilaginous polymers inhibit ice crystal growth by forming films on crystal surfaces (17,21). Other polymers divert crystal growth to non-critical regions by inhibiting

initiation of freezing; this protects vital meristematic tissue (17,21). These polymers can be extracted and their inhibiting activity rated invitro. Rye (Secale cereale) polymers have greater freeze inhibiting activity than those of barley (Hordeum vulgare)(17,21).

Both types of nonequilibrium freezing stresses usually occur at temperatures around -5C. Equilibrium freezing stresses, however, take place at temperatures between -10C and -20C (15).

Equilibrium freezing:

While nonequilibrium stresses depend on large displacements from equilibrium and result in high crystalization energies, equilibrium stresses involve relatively small displacements and therefore smaller crystalization energies. As ice crystals grow during equilibrium freezing two other types of stress occur: 1.) Adhesion and 2.) desiccation.

Adhesion: Equilibrium freezing begins with a nonequilibrium freeze to provide crystalization energy for extracellular liquid. After initial crystalization, freezing progresses such that very small displacements from equilibrium occur. The advancing ice lattice reaching the cell wall competes with it for the intervening liquid. This causes adhesion between ice and the wall or wall and plasmalemma. It can be

a significant contribution to overall freezing stress (18,19,20).

As freezing progresses and intracellular desiccation removes water from the protoplast, adhesions can cause irreversible distortions of the plasmamembrane when it shrinks. This kind of damage is histologically different from other types of injury (18).

Resistance to adhesive damage is seemingly through adhesion inhibition by solutes which maintain a fluid barrier between ice and the plasmalemma. A dilute solute concentration in the extracellular spaces in winter cereals helps prevent the growth of disease-causing microorgansisms. This allows ice crystals to grow up to hydrophylic cell walls. When frozen, the cell reversively releases sugars outside the protoplast and maintains a fluid barrier neccessary for prevention of adhesions (14).

Minimization of adhesion allows freezing to progress to temperatures where intracellular desiccation occurs.

Protoplast Desiccation: Freeze-induced desiccation ensues when ice acts as a water accumulator. As freezing progresses, the vapor pressure of extracellular ice becomes lower than that of the protoplast and a gradient is established (8). The crystal grows at the expense of intracellular water and the protoplasm shrinks (8). When water is withdrawn to some critial level intracellular

injury results. Burke (2) Steponkus (30) and Levitt (8) discuss probable causes of desiccation injury such as, concentration of salts or ions, pH changes, disruption of protein function, and mechanical injury to the plasmallema.

Resistance to desiccation stress is probably through alterations of membrane proteins (9) and elevation of intracellular solutes. But, precise mechanisms are not well understood (9,34).

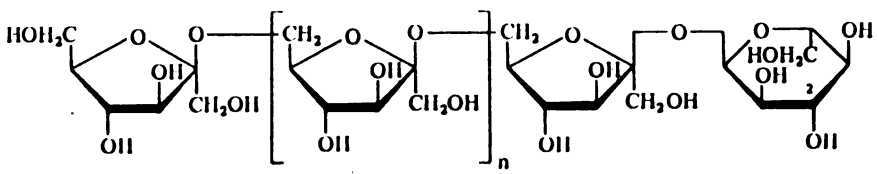
These four types of stress provide a basis for overall winter-hardiness improvement. The hardiness of winter cereals deficient in one or more resistance mechanisms may be increased if genetic variation for those mechanisms exist within that species and optimum combinations are made through breeding. Adhesive stress resistance through solute release from the protoplast is a mechanism for which variation exists between species (13). The storage polymer fructan is involved in this solute release.

FRUCTAN

Unlike tropical and subtropical grasses which produce starch (a glucose polymer) as the main storage polysaccharide, temperate and cool zone grasses accumulate fructose polymers called fructans or fructosans (32). They

are produced not only in monocots but dicots as well and are distributed in stems, leaves, inflorescences, and seeds (12). In grass stem bases, at certain growth stages, fructan may constitute up to 45% of the dry weight (7).

Types: Three kinds of fructan, depending on glycosidic linkages, have been described. They are: inulin, levan (or phlein), and branched. Inulin occurs mainly in dicots and has 2-1, beta linkages between its fructofuranose residues. Levan is the principle monocot fructan and consists of 2-6, beta linkages. Branched types have been found in many monocots and have either a levan or inulin backbone with one or more short fructofuran branches (12). The structure of levan, the major fructan of winter cereals, is shown below.



(12)

Biosynthesis: Fructan (inulin) synthesis in Compositae and Liliaceae reportedly occurs through the concerted action of two enzymes. The first, sucrose-sucrose fructosyltransferase, forms a trisaccharide (glu-fru-fru) from two sucrose molecules (with a glucose released). The second, fructan-fructan fructosyltransferase, transfers a single terminal fructose residue from an oligosaccharide to the same carbon position on another molecule (3,5,24). One

oligosaccharide, therefore, grows at the expense of another. Fructan (levan) synthesis in grasses is less well understood but in Dactylis glomerata where trisaccharide intermediates do not accumulate, the polymer apparently grows by direct enzymatic transfer of fructose residues from sucrose (22,24). The remaining glucose is reportedly converted back to sucrose (22,24). Other grasses (Lolium temulentum, wheat, and barley) show a trisaccharide accumulation; fructan synthesis here is probably similar to inulin synthesis in Compositae (25,32). Pollock suggests that alternate biosynthetic mechanisms may be present in grasses depending on species and growth stage (25).

The average number of fructofuranose units attached to the terminal glucopyranose vary considerably depending on species and growing conditions but are from 260 in timothy (7) down to 10 in brome grass (12).

Storage: Environmental factors, affecting growth rates, have a significant influence on fructan accumulation. Grasses can store large quantities during slow growth periods, when assimilate production exceeds demand. This typically occurs in the fall with low temperatures and continuing photosynthesis (1,4,26). Fructan accumulation for four different grasses was highest between November and January (23).

Nutrient availability also affects fructan storage.

Archibold showed an inverse relationship of nitrogen and phosphorus levels to fructan amounts in barley and a positive relationship of potassium to fructan (1). Besides confirming the inverse nitrogen relationship, Westhafer (33) showed that fructan was the most responsive carbohydrate to nitrogen levels in Kentucky bluegrass stems.

Utilization: Mobilization of fructan reserves in grasses usually begins when active growth starts. For example, fructan decreases most rapidly in grasses when tillers begin spring growth (23). In addition, large fructan (inulin) decreases (with corresponding fructose and sucrose increases) occur in chicory (Cichorium intybus) within 3 days when normally growing plants are subjected to 2-6C temperatures (27).

Fructan conversion to fructose and sucrose is through hydrolysis by beta-fructofuranosidases. The molecule is reportedly hydrolysed stepwise from the fructose end (exo-action) until a sucrose remains (28). There is evidence for fructofuranosidase specificity since levan hydrolase will not hydrolize inulin (28).

Fructan and Adhesion Inhibition:

Rye and barley crowns undergo fructan conversion when hardened seedlings are frozen for 24 hours at -3C. Fructan decreases (13) while intercellular fructose and sucrose increase. Furthermore, this release to intercellular spaces

is reversible and recovery (to prefrozen levels) takes less than one hour (14).

Rye plants frozen directly to -12C do not convert fructan significantly and have a lower survival rate than those first incubated at -3C for 24 hours (13). In addition, Hudson barley converts fructan to a lesser extent and has a lower survival rate. Olien proposes that the protection seemingly offered by the intercellular solutes is due to adhesion inhibition (14).

The purpose of this research was to investigate fructan conversion in barley. If genetic differences can be found between cultivars for fructan conversion and solute release, a basis for improving the hardiness of winter barley may be established.

MATERIALS and METHODS

Plant material: The following winter cereals were used:

			C.I. Number
TRITICALE		BARLEY	
OAC Wintry		*Hudson Winter Tennessee	Breeder Selected 4633
WHEAT		Kearney	7580
Genesee	Breeder Selected	*Dictoo	Breeder Selected
		Wong	Breeder Selected
RYE		Reno	6561
*Rosen	Breeder Selected	*Khayyam	1117
S-6		*Durani	6316
Asterook			
Merkator			

*before and after freeze comparison.

Eight plants per 12.5 cm pot were grown from seed in washed, steam sterilized sand. They were raised in a growth chamber at 15C with 18 hour/day of light at 300 $Em^{-2}s^{-1}$ (80% white flourescent, 20% incandescent). After five weeks, they were transferred to a 1C hardening chamber with continuous light at 200 $Em^{-2}s^{-1}$ for 3 weeks. They were watered daily and fertilized 3 times weekly with a Hoaglands solution for the entire growth period.

Harvest: After 8 weeks, plants were at the three leaf stage with 3-4 tillers each. They were removed from pots, washed free of sand in ice water, and trimmed to four cm shoots and two cm roots. Plantlets were placed in slots cut in damp, circular, cellulose sponges with a pipe flange

assembly through the center to promote thermostabilization. Eight plants, from eight different pots, were used for each sample. In the comparison experiments plants were paired by size providing 2 identical sets, one for frozen and one for unfrozen (control) treatments. A randomized complete block design with 14 cultivars and four replications (separated over time, one week/rep) was used in the initial screening. A split plot design with four replications (separated according to crown size), two treatments (frozen and unfrozen), and four cultivars was used for the before and after freeze comparison.

Sponges containing plants were inoculated with ice, covered with plastic to help prevent desiccation, and placed in a freezer at -3C (-5C in the comparison experiment with 4 barley cultivars) for 24 hours. Thermocouples placed near crowns in the sponges were used to monitor tissue temperature. Actual time at equilibruim with the freezer was between 18 and 22 hours.

Sugar Extraction: After the treatments, plantlet roots were trimmed with a razor in a cold room at 6C. The transition zone was grated with a fine toothed hand grater and 1/2 cm of the stem was trimmed with a razor. The combined tissue was placed in a mortar containing 10 mls of 80% (vol./vol.) EtOH and mascerated for aproximately 2 min. with a motor driven mortar and pestle. The ground tissue was transfered

to a 100ml beaker and the mortar and pestle rinsed with 20mls 80% EtOH which was added to the beaker. The tissue was placed on a 70C water bath for 10 min. to inactivate enzymes, and shaken for 15 min on a gyratory shaker. The supernatant was transferred to an Erlenmeyer flask and the tissue extracted an additional two times with double distilled water (30 mls each) at 21C. A fourth water extraction revealed less than 5% additional carbohydrate. The combined supernatants were swirled by hand and a 25ml alaquat was transferred to a 50 ml graduated centrifuge tube. 400mg of mixed bed resin was added and the mixture was shaken for 10 min (to eliminate sulfate ions damaging to the separation column). Samples were centrifuged at 680G for 10 min. and 10mls of the supernatant decanted to a preweighed round bottom flask. They were taken to dryness under vacuum at 35C (the screening experiment was taken to dryness on a 50C water bath under forced air) and placed in a vacuum desiccator over P_2O_5 for approximately 12 hours. Samples were reweighed, brought to a concentration of 10 mg dry extract per ml double distilled water and passed through a .45 micron Millipore filter.

Chromatography: Cabohydrates were separated by high pressure liquid chromatography (HPLC) using a Bio-Rad Aminex HPX-87P Column heated to 85C. The mobile phase consisted of degassed HPLC grade water (Baker Chemical Co.) with a flow rate of .4

ml/min. A Micromeritics 771 Refractometer was used to detect carbohydrates and they were quantitated by co-chromatography with external standards. Rosen rye fructan, collected off the column and freeze-dried, was used as the fructan standard for all cultivars in the initial screening and fructan from Hudson barley for barley cultivars in the comparative study. Peak areas were determined by a Hewlett Packard 3390A integrator. Retention times in minutes were: fructan 9.2, sucrose 14.8, glucose 18.4, and fructose 25.8.

Data is presented as a percentage of total water soluable carbohydrate because using dry weight as a base caused erroneous results. This was because complete recovery of dried crown tissue and removal of sand was difficult. There was no significant difference in the total extractable carbohydrate per dry weight for treatments. The average total cabohydrate for each sample was approximately 353 mg/g dry weight. This varied slightly between cultivars and experiments. Percentages used in this study represent a proportion of this amount.

RESULTS and DISCUSSION

Confirmation of past findings:

The first part of this study was done to confirm differences in fructan conversion between species.

Treatment effects and species by treatment interaction were both highly significant (table 1) for all four sugars. Fructan decrease with sucrose and fructose increase was larger in Rosen rye than in Hudson barley (table 2). Glucose change in Rosen was not significant but was in Hudson. The glucose increase could be a result of sucrose hydrolysis from decompartmentation caused by freezing injury (13).

Table 1. Mean squares from analyses of variance for percentage carbohydrates in rye and barley.

		Mean squares			
Source	d.f.	Fructan	Sucrose	Glucose	Fructose
Replication	3	0.19	5.75	1.79	1.62
Species (S)	1	100.50**	135.14**	11.22*	25.00**
Error (a)	3	0.24	3.34	0.74	0.25
Treatment (T)	1	118.26**	43.23**	1.00**	10.89**
T x S	1	10.72**	11.06*	1.56**	1.56**
Error (b)	6	0.91	1.16	0.07	0.11

*,** = Significant at 2% and 1% level of probability, respectively.

Table 2. Mean percentages of total carbohydrates for Rosen rye and Hudson barley and differences between treatments[a].

	Species		
Sugars	Rye	Barley	LSD[b]
Fructan			
H	80.4	73.7	
HF	73.3	69.9	2.50%
D	-7.1	-3.8	
Sucrose			
H	11.3	18.8	
HF	16.3	20.4	2.80%
D	+5.0	+1.6	
Glucose			
H	4.5	5.5	
HF	4.3	6.6	.68%
D	-0.2	+1.1	
Fructose			
H	3.8	2.0	
HF	6.1	3.0	.88%
D	+2.3	+1.0	

H = Hardened plants (control)
HF = Hardened and then frozen.
D = Difference between H and HF.
a = Frozen 24 hours at -3C.
b = LSD at 1% probability for treatments.

Changes in fructan, sucrose, and fructose confirm the findings of Olien and are the basis for the remainder of the study. If differences exist for the magnitude of sugar change between species then differences may also exist within the same species.

Cultivar differences:

Screening: Fourteen winter cereals were screened by measuring carbohydrate levels after a freeze treatment. A fructan:fructose weight ratio was used to separate high and low converting lines. The results (figure 2) suggest ratio differences between some cultivars of different species, but provide no evidence for differences between barley cultivars.

Four cultivars: Two pairs of barley cultivars with the largest ratio differences (Durani and Khayyam-high, Hudson and Dictoo-low) were used in this experiment (Dictoo was selected instead of Winter Tennessee because of its hardiness). These were subjected to the same comparison test used previously for rye and barley.

A significant treatment effect (table 3) was found for all four sugars but a non-significant interaction for fructan, sucrose and glucose. While this confirms freeze-induced fructan conversion generally, it provides no evidence for a difference in the magnitude of fructan change between these four cultivars.

Figure 2. Mg fructan and fructose per gram dry weight of extract and their ratio for 14 winter cereals.

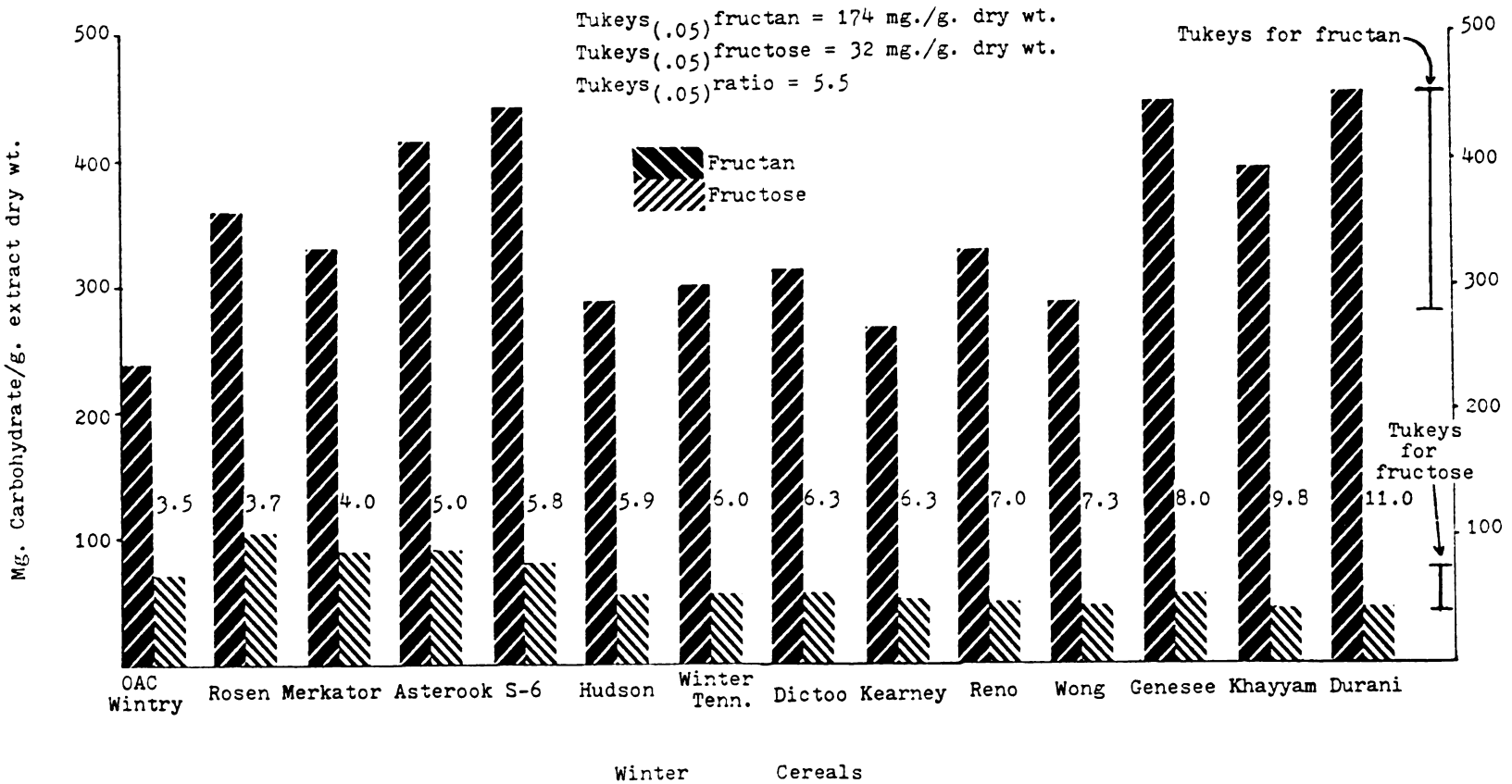


Table 3. Mean squares from analyses of variance for percentage carbohydrates in 4 barley cultivars.

		Mean squares			
Source	d.f.	Fructan	Sucrose	Glucose	Fructose
Replication	3	8.65	4.21	0.41	0.12
Cultivar (C)	3	247.25**	159.32**	8.43**	0.99**
Error (a)	9	1.69	1.79	0.06	0.02
Treatment (T)	1	55.65**	0.45ns	8.61**	26.64**
T x C	3	1.10ns	0.11ns	0.05ns	0.31**
Error (b)	12	1.18	0.54	0.13	0.04

** = Significant at 1% level of probability.
ns = not significantly different at 5% level.

The significant cultivar by treatment interaction for fructose may have been a function of sucrose hydrolysis. Fructan hydrolysis results in sucrose (as well as fructose) increase (13,14,27,28). Hydrolysis of sucrose (possibly injury induced, since these cultivars were frozen at a lower temperature) from converted fructan could explain the lack of evidence for sucrose change and the significant increase in glucose.

Highly significant cultivar differences were found for all four carbohydrates. Fructan differences may be related to partitioning as discussed in the next section. Winter hardiness has been related to accumulation of simple sugars during hardening; with a few exceptions, hardier varieties of a species accumulate more sugars than non-hardy ones (8). In this study, the hardier cultivar, Dictoo, shows about 10%

more simple sugars than the less hardy cultivars, Khayyam and Durani. Rosen rye's lower sugar accumulation compared to Hudson barley's at first seems to contradict this observation. But, Rosen's greater ability to convert fructan and release sugars when incubated probably provides more protection from freezing stress than Hudson's higher sugar content.

Table 4. Mean percentages of total carbohydrates for 4 barley cultivars and differences between treatments[a].

	Cultivars			
Sugars	Hudson	Dictoo	Khayyam	Durani
Fructan				
H	75.6	71.3	81.8	82.8
HF	72.1	68.9	78.9	81.8
D	-3.5	-2.4	-2.9	-1.7
Sucrose				
H	19.0	23.8	15.2	13.9
HF	19.0	23.5	15.0	13.4
D	0.0	-.3	-.2	-.5
Glucose				
H	4.4	3.7	2.2	2.5
HF	5.5	4.8	3.4	3.2
D	+1.1	+1.1	+1.2	+.7
Fructose				
H	1.1	1.3	0.9	0.7
HF	3.5	2.8	2.7	2.3
D	+2.4	+1.5	+1.8	+1.6

H = Hardened plants (control)
HF = Hardened and then frozen.
D = Difference between H and HF.
a = Frozen 24 hours at -5C.

Patterns in variability:

Experimental variability was too high in this study to find genetic differences in fructan conversion between the four barley cultivars. However, patterns in the variability suggest that under other experimental conditions differences might be shown.

Freeze-induced fructan hydrolysis is probably complete within 24 hours (Olien unpublished). With relatively high and consistent starting amounts the same proportion of the total fructan is always converted. This suggests a genetically specified equilibrium point for total fructan conversion and/or fructan partioning into available and unavailable amounts.

This study suggests that partitioning may be occurring. In figure 3 the vertical line for Dictoo (% fructan converted) could be viewed as an extension of the abscissa (the scale is the same for both axes). So the percentage converted (ordinant) plus the percentage post-freeze levels (abscissa) equals the initial starting amount. For example, Dictoo's highest intial fructan level (a) was approximately 74% (4.8% + 68.9%) and the lowest was 69.5% (.6% + 68.9%). With or without partitioning one would expect different fructan levels depending on environmental conditions. Without partitioning, however, all the fructan would be available for conversion and similar proportions would probably be converted regardless of initial amounts. The

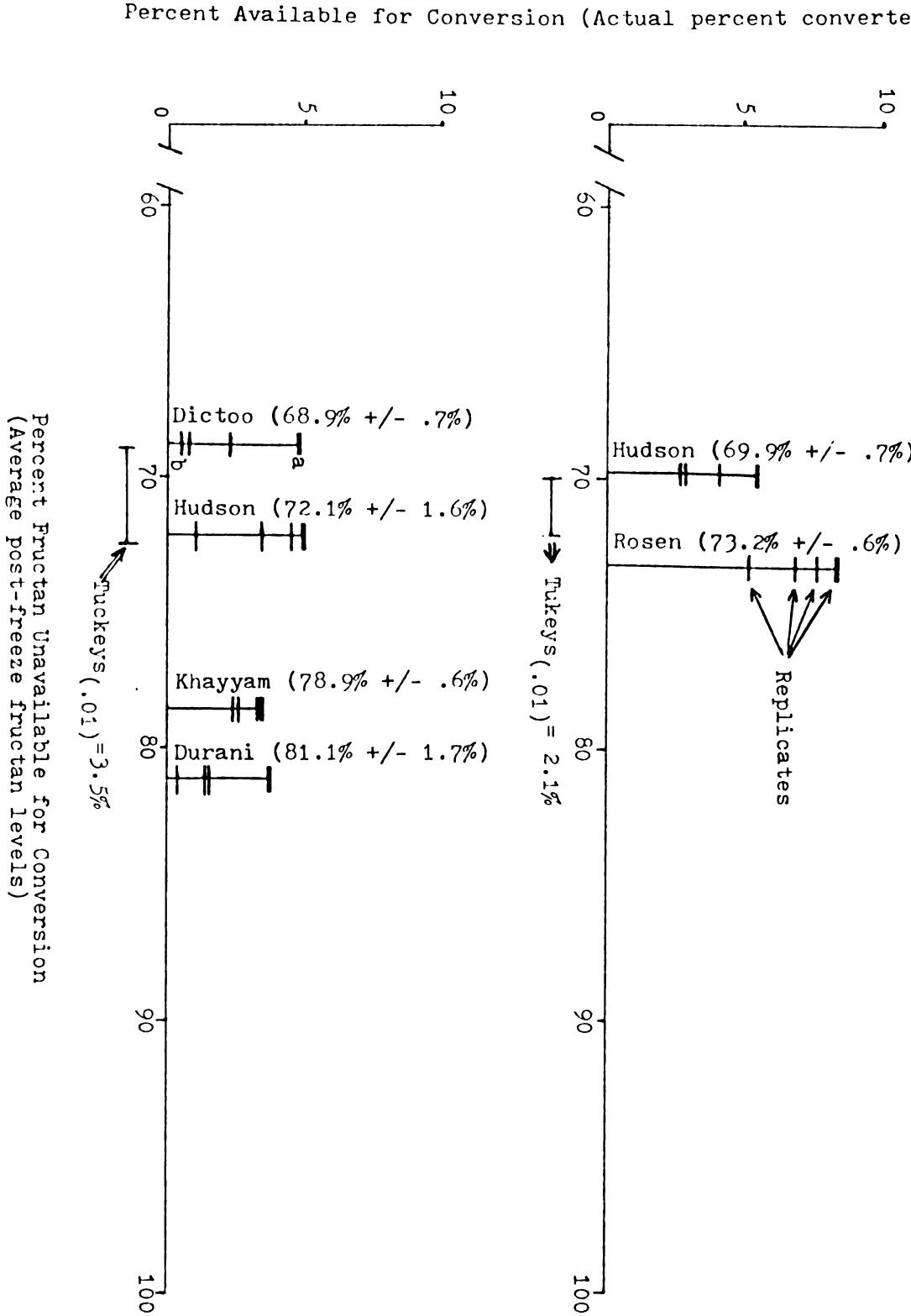


Figure 3. Percentage fructan before and after freezing and percentage converted, showing partitioning into available and unavailable amounts.

replicate with higher initial fructan, however, converted more (4.8%) than the low one (.6% converted). While Dictoo provides the best example, this general pattern was observed in all four barley cultivars and Rosen rye.

Unavailable fructan may be located in the vacuole; the majority of fructan is probably stored there (5,12,32). The small amounts in the cytoplasm or cell wall region may be available for conversion. If hydrolysing enzymes are bound to the plasmalemma, available fructan could easily be converted and the monomers transported outside the protoplast.

If fructan composes 70-80 percent of the water soluble carbohydrates then the remaining 20-30 percent are the simple sugars fructose, glucose, and sucrose. These sugars might also be released from the protoplast when crowns are incubated. Changes in intercellular sugars could, therefore, be considerably higher than the relatively small changes in total carbohydrates shown here. It will be important to know how much total sugar (free and converted) is exported because presumably only exported sugar can inhibit adhesions.

If breeding experiments are to be successful, variability must be minimized to the extent that the fructan conversion of a cross may be distinguished from its parents. This will allow heritability studies and enable evaluation

of adhesion resistance when high fructan conversion is incorporated into a cultivar.

Highly significant differences were found between Rosen and Hudson as well as between Dictoo-Hudson and Khayyam-Durani for fructan levels after freezing (table 3 and figure 3). If this represents unavailable fructan and these levels are genetically determined then accumulated fructan in cultivars should always be converted to this amount relative to each other. If this is true, then inducing fructan accumulation to highest possible levels during hardening may reduce conversion variablity. Initially, it was assumed that large, unstressed plants would convert fructan to a greater extent than smaller, stressed ones. But, smaller plants not only accumualted more fructan, they also converted fructan to a greater extent than large, healthy ones. Reducing the growth rate of plants while maintaining high photosynthetic activity may, therefore, induce higher and more consistent fructan accumulation with a consequent reduction of experimental variability.

Future: A technique to screen greater numbers of cultivars should be devised if a large scale breeding program is to be implemented. Post freeze quantification of fructan hydrolases might allow easier selection of high converting lines. This will be effective providing the

hydrolases correlate with amount of sugars exported.

Greater genetic variation may exist in cultivars from other species such as rye and wheat, providing a potential for increasing winter hardiness.

CONCLUSION

The aim of this study was to identify genetic differences in barley for freeze induced fructan conversion. Past studies have shown that fructan conversion results in higher extracellular solutes and is important in minimizing freezing stress. This study has confirmed genetic differences for conversion between species but gives no evidence for differences within the four barley cultivars tested. Patterns in the variation which seem to suggest fructan partitioning may provide a basis for minimizing evironmental variability and allow identification of genetic differences in winter barley.

APPENDIX

Calculations for Evaluating the Effect of Solute Release on Adhesion Inhibition

Provided certain assumptions are made, one could use data from this study to calculate the thickness of the fluid barrier (in water diameters) between ice and solute releasing protoplasts. One may then speculate whether adhesions could indeed be inhibited. It must be remembered, however, that the calculated barrier is an average thickness and could vary considerably with actual protoplasts. While the calculations may suggest a high degree of "protection" from adhesion, the barrier at some locations on the protoplast may be too thin to protect the cell. Adhesion at this point could result in protoplast damage and/or cell death.

Morrison (1959. Can. Jor. of Chem. 37:1379-1390) calculated that 3.2g of liquid water constituted a monolayer on 100g of cellulose. Olien (19) found 19.2g liquid water per 100g cellulose at equilibrium with ice (no net freezing or melting) at -10C and 48.0g liquid water per 100g cellulose at 0C. This means that 6 water diameters exist between ice and cellulose at -10C (causing strong adhesions) while 15 diameters exist at 0C with no adhesions (19). If released solutes can maintain a liquid water diameter greater than this, adhesion will be prevented.

Assumptions:

1. The cellulose systems above are an accurate model of adhesion and fluid barrier effects on crown protoplasts.
2. Crown cells are sperical and uniform in size with a radius of .01 mm.
3. The crown is a cylinder with a radius 4mm and height 10mm.
4. All converted sugars are released outside the protoplast. This represents 7.3% and 3.7% of total carbohydrate for Rosen rye and Hudson barley, respectively (table 2).
5. All sugars from converted fructan have the same inhibiting effect as sucrose.
6. Only 1/3 of the crown consists of cells capable of sugar release when incubated (personal obsevation).
7. A water monolayer is 3 A thick (Morrison. 1959).

Calculations for the surface area of sugar releasing protoplasts (p).

Volume of one crown:

$\pi(4mm)^2 \times (10mm) = 503mm^3/crown$

Volume of one p:

$4/3\pi(.01mm)^3 = 4.2X10^{-6}mm^3/p$

Total number of p in one crown:

$$\frac{503mm^3}{4.2X10^{-6}mm^3} = 1.2X10^8 \times 1/3 = 3.6X10^6 \text{ p}$$

Surface area of one p:

$4\pi(.01mm)^2 = 1.26X10^{-3}mm^2/p$

Total protoplast surface area per crown:

$(1.26X10^{-3}mm^2/p) \times (3.6X10^6)\ p = 4.5X10^4mm^2$

Calculations for the amount of sugar/crown released from protoplasts.

Average dry wt per crown:

Rosen

.656g dry wt./8 crowns = .082g dry wt./crown

Hudson

.522g dry wt./8 crowns = .065g dry wt./crown

Average (3 reps) total carbohydrate (c) (fructan + sucrose + glucose + fructose) per g dry wt. (dried pulp + dried extract):

Rosen = .313g

Hudson = .392g

Total c per crown

Rosen

(.082g dry wt./crown) X (.313g c /g dry wt.) = .026g c/crown

Hudson

(.065g dry wt./crown) X (.392g c /g dry wt.) = .026g c/crown

Total sugar per crown exported from the protoplast:

Rosen

.026g c/crown X .071* = .00185g sugar/crown

Hudson

.026g c/crown X .038* = .00097g sugar/crown

*proportion of total fructan converted from table 2.

Calculations for the thickness of the protoplast fluid barrier.

A sucrose concentration of 1.4mg/ml. will prevent net freezing of water at -11C (Weismann, V. O., 1938. Protoplasma 31:27-56). This value will be used to calculate the volume of water which would be prevented from freezing at -11C by released sugars. From the water volume the thickness of the fluid barrier, in water diameters, will be calculated.

Total volume of water kept unfrozen at -11C by released sugar

Rosen

$$\frac{1.4\text{g sucrose}}{1000\ \text{mm}^3} = \frac{.00185\text{g sugar/crown}}{X\ \text{mm}^3} \qquad X = 1.3\text{mm}^3\text{/crown}$$

Hudson

$$\frac{1.4\text{g sucrose}}{1000\ \text{mm}^3} = \frac{.00097\text{g sugar/crown}}{X\ \text{mm}^3} \qquad X = .7\text{mm}^3\text{/crown}$$

Thickness of fluid layer

Rosen

$$\frac{1.3\text{mm}^3\text{/crown}}{4.5\text{X}10^4\text{mm}^2\ \text{surface area/crown}} = 2.9\text{X}10^{-5}\text{mm}$$

Hudson

$$\frac{.7\text{mm}^3\text{/crown}}{4.5\text{X}10^4\text{mm}^2\ \text{surface area/crown}} = 1.6\text{X}10^{-5}\text{mm}$$

Number of water diameters

Rosen	Hudson
$\frac{290\ A}{3\ A}$ = 97 diameters	$\frac{160\ A}{3\ A}$ = 53 diameters

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These calculations suggest that with the assumptions stated the amount of sugar from converted fructan on average exceeds the thickness of the layer necessary for adhesion inhibition.

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