THE DEVELOPMENT OF SUPER-HARDY WINTER WHEAT CULTIVARS:
Identifying the Pieces of the Cold Hardiness Puzzle

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ABSTRACT

Several years of investigation of the genetics of cold hardiness in wheat, and attempts to improve the cold hardiness of this crop, have identified a number of 'pieces' in the cold hardiness puzzle. The cell cytoplasm does not appear to have any direct effect on cold hardiness, nor on the expression of nuclear genes affecting cold hardiness in wheat or its relatives. Cold hardiness appears to be controlled mainly, although not exclusively, by additive gene action. The most promising source of new genes for the improvement of cold hardiness in wheat appears to be from the rather distantly related wheatgrass group and rye. The chromosome doubled hybrids of rye and crested wheatgrass with wheat exhibited none of the superior cold hardiness found in these donor species. Three explanations were found which help to explain the expression of these alien genes in a wheat background: 1) specific wheat chromosomes were found to affect the expression of rye genes 2) the species contributing the greatest number of chromosomes in an interspecific cross has the greatest influence on cold hardiness 3) increasing the chromosome number, as when the chromosome number is doubled to make an interspecific hybrid fertile e.g. triticale, results in an increased cell size which gives the plant less cold tolerance than would be genetically expected. Small cell size was also found to be a factor related to cold hardiness within wheat cultivars. Methods for the improvement of cold hardiness in winter wheat are proposed based on these findings.

INTRODUCTION

In western Canada, greater cold hardiness is required to reduce the risk of winter kill in winter wheat (Triticum aestivum L.). However, the cold hardiness of winter wheat has improved little over the last 75 years (Fowler et al. 1983) suggesting that there is a lack of unexploited genetic variability for cold hardiness within the wheat gene pool. This observation has led to an investigation of cold hardiness and the mechanisms controlling its expression in wheat and in interspecific hybrids involving wheat and cold tolerant wheat relatives.

Synthetic hexaploid wheats, produced from selected progenitor species of common wheat, have demonstrated rather poor levels of cold hardiness (Limin and Fowler 1982) indicating that gains in cold hardiness through the utilization of genes from close relatives of wheat may be limited. The excellent cold hardiness of rye (Secale cereale L.) has led to the speculation that it may have considerable potential as a gene donor for the improvement of wheat cold hardiness or in the production of winter hardy triticale. It was also suggestd that the 'hardy' cell cytoplasm of rye might be used for the same purposes. However, attempts to exploit this potential (Dvorak and Fowler 1978) have demonstrated that the cold-hardiness genes of rye are poorly expressed in the presence of the wheat genome. More promising results have been reported in wheat - Agropyron (wheatgrass) hybrids (Gulyaev et al. 1973). Grafius (1981) reported that selected

derivatives of a wheat-wheatgrass cross produced in the 1950s had greater cold hardiness than rye, and Fedotova et al. (1978) reported that the addition of some wheatgrass chromosomes improved the cold-hardiness of wheat. However, no superior wheat varieties have been produced as a result of this research.

The above observations demonstrate the high degree of unpredictability of cold hardiness in progeny of interspecific crosses among species of the wheat relatives and demonstrates the need for a greater understanding of the mechanisms governing cold hardiness.

This paper will summarize the results of several years work conducted in Saskatoon. The experiments were designed to investigate the genetics of cold hardiness in wheat, potential gene donors, and the control of alien (donor species) cold hardiness gene expression when combined with wheat. Cold hardiness is expressed in terms of LT_{50} (temperature at which 50% of the population is killed by cold temperature stress.

RESULTS AND DISCUSSION

Cytoplasmic effects

Many changes are known to occur to cellular organelles of the cell cytoplasm during cold acclimation. Within the literature there are conflicting reports as to the importance of one 'type' of cytoplasm versus another. The 'type' of cytoplasm in a cross can be determined because the maternal parent supplies the cell cytoplasm, as well as one-half of the nuclear genes, while the male parent contributes only genetic material for the nucleus. Reciprocal wheat crosses produced by Brule-Babel (1987), having exactly the same genetic make-up but either the cell cytoplasm of one parent or the other, showed no difference in cold hardiness.

Even greater differences in cold hardiness exist between species than within wheat itself. The nucleus of very cold hardy 'Puma' rye was substituted into the cell cytoplasm of a nonhardy wheat relative, T. tauschii, which contribute the D genome to common wheat. The cytoplasm was found to have almost no effect on the cold hardiness expression of the rye nuclear genes (Table 1). The chromosome doubled wheat-rye hybrid, triticale, was also produced with either wheat or rye cytoplasm, the cold hardiness of both lines was the same (Limin and Fowler, 1984). Based on this evidence cell cytoplasm does not appear to have any direct effect on cold hardiness.

Table 1. Cold hardiness expression of a rye nucleus in a wheat cytoplasm (alloplasmic rye).

Line	Genome	LT_{50}
Secale cereale cv. Puma	R	-24.5 a
Triticum tauschii	D	-12.0 b
Alloplasmic rye	R	-23.5 a

Genetics of cold-hardiness control in wheat

An extensive investigation into the genetics of cold hardiness in winter wheat (Brule-Babel, 1987) showed that it is a character controlled mainly by additive gene action, although evidence for at least one dominant cold-hardiness gene was found in spring by winter crosses but not in winter by winter crosses. Transgressive segregates with greater cold hardiness than either parent were most common in crosses between relatively nonhardy cultivars. Unfortunately, even with the best parents, no segregates were found to be more cold hardy than the hardiest cultivar. This suggests that our most hardy cultivars do, in fact, carry all of the best available genes for cold hardiness.

Cold hardiness in close relatives and ancestors of wheat

An investigation into cold hardiness of the near wheat relatives within the _Triticum_ genus found that none had greater cold hardiness than is present in a hardy cultivar such as Norstar (Limin and Fowler, 1985). Synthetic hexaploid wheat (AABBDD), combining hardy tetraploid (_T. durum_, AABB) and hardy _T. tauschii_ (DD) wheats, did not appear to demonstrate the additive type of gene expression found within the common hexaploid (AABBDD) winter wheats. None of the synthetic hexaploids produced had greater cold hardiness than the _T. durum_ parents and none therefore were as hardy as our best common winter wheats (Limin and Fowler 1982). Further testing of these synthetic hexaploids in crosses with hardy common winter wheats has indicated that they do not contain cold hardiness genes which can improve our current cultivars.

Expression of 'alien' cold-hardiness genes in wheat

It has long been known that some of the more distant wheat relatives, such as rye and some of the wheatgrasses, have a great deal of cold tolerance. The introduction of winter hardiness from these species was one of the original ideas behind the production of triticale and perennial wheat. Many of the wheatgrass species grown as forage crops in Saskatchewan were screened for cold hardiness and compared to the most hardy wheat and rye cultivars, Norstar and Puma respectively (Limin and Fowler, 1987). In the seedling stage of growth only crested wheatgrass was superior to Norstar. In established stands many wheat grasses were found to be similar in cold hardiness to Puma rye.

The cold hardiness genes of rye have been found to be poorly expressed in combination with both durum and common type wheats (triticale; Dvorak and Fowler, 1978; Limin et al., 1985). Crested wheatgrass genes have been found to be expressed in a similar manner as the rye genes when in combination with wheat (Limin and Fowler, 1988). In all instances the chromosome doubled hybrid of these hardy species with wheat had cold hardiness levels similar to the wheat parent (Table 2). In this form the excellent cold hardiness of these donor species does not appear to be expressed and based on this information these species would not appear to be exploitable for the cold hardiness improvement of wheat.

Table 2. Cold hardiness in chromosome doubled hybrids of rye and crested wheatgrass with wheat.

Line	Chromosome number	LT_{50}
Triticum aestivum cv. Chinese Spring wheat	42	-11
Secale cereale cv. Puma rye	14	-28
Agropyron cristatum crested wheatgrass	14	-29
Chinese Spring x Puma (triticale)	56	-11
Chinese Spring x Crested wheatgrass	56	-10

Specific wheat chromosomes affecting the expression of rye genes in triticale

A series of triticale lines have been produced in which individual pairs of wheat chromosomes were removed to investigate the possibility that some wheat chromosomes may carry genes which suppress the expression of rye cold-hardiness genes. Although several lines remain to be tested, at least one wheat chromosome was found to carry a gene or genes which suppressed the expression of rye cold hardiness genes. When this wheat chromosome was removed the resulting triticale line showed an increased level of cold hardiness. Although the cold hardiness of this line was greater than the normal triticale from which it was derived, the cold hardiness level was still poor indicating that this wheat chromosome is only partially responsible for the suppression of rye cold hardiness genes.

Chromosome dosage and cell size

A series of interspecific hybrids and chromosome doubled hybrids were made between wheat, rye, and several of the wheatgrass species. Analysis of the results from these interspecific combinations suggested two possible explanations for the cold-hardiness levels observed: 1) an effect of chromosome (gene) dosage and 2) an effect of cell size due to chromosome number (small cell size has been associated with cold hardiness).

Interspecific hybrids between wheat and intermediate wheatgrass were intermediate in cold hardiness between both parents. In this combination, where both species contribute an equal number of chromosomes to the hybrid, the cold hardiness expression is at the level that would be expected from crossing two wheat cultivars. When a species such as tall wheatgrass was crossed with a nonhardy wheat, the hybrid was closer in cold hardiness to the hardy wheatgrass parent which has a very large chromosome number.

An increase in chromosome number is believed to often result in a larger cell size. Measurements of cell size revealed that triticales produced from either durum or common wheat have cell sizes significantly greater than either parent. Wheat-rye hybrids, though nearly genetically identical, have

one-half the chromosome number of triticale. a smaller cell size. and greater cold hardiness (Table 3). Unfortunately, in interspecific hybrids produced from parents of unequal chromosome number, it is impossible to separate the effects due to cell size and gene dosage because both are dependent on chromosome number.

Table 3. Cold hardiness and cell size in wheat-rye hybrids and amphiploids.

Line	Chromosome number	LT_{50}	Cell size (microns)
Triticum aestivum			
cv. Chinese Spring	42	-11	80.94
Secale cereale			
cv. Puma	14	-28	57.56
Triticale (CS X P)	56	-11	95.33
F_1 hybrid	28	-15	73.74

Within-cultivar variation for cell size

The effect that cell size appeared to be having on the expression of cold hardiness in interspecific combinations, suggested that this could also be a factor partially responsible for variation between cultivars. Cell size (based on leaf guard cell length) in several cultivars known to vary over a range of cold hardiness levels, was compared to their cold tolerance based on actual field survival data (FSI) (Table 4). The results suggest that within the common wheats, cell size is one of the genetically controlled factors affecting cold hardiness and is one of the reasons for differences in cold hardiness between cultivars. This data clearly reinforces the relationship between small cell size and cold hardiness.

Table 4. The relationship between winter hardiness (based on field survival index (FSI) and cell size (length of leaf guard cells) in cultivars selected over a range of cold hardiness levels. Note that cultivars are ranked from most cold hardy (largest FSI) to least cold hardy.

Cultivar	FSI	Cell size (microns)
Ulianovka	530	78.06
Norstar	514	77.34
Cheyenne	445	80.58
Redwin	425	82.38
Yorkstar	360	83.10
Capelle	306	83.28
Chinese Spring*		87.05
Manitou*		97.30

*No field survival data available on spring wheats.

SUMMARY

Cold-hardiness genes in wheat appear to behave in a mainly additive manner, although some dominance-recessive relationships are known to exist. The cell cytoplasm does not appear to play any direct role in cold tolerance. Small cell size, however, does appear to be associated with cold hardiness. Although the very closely related wheat relatives do not appear to hold much potential for the improvement of cold hardiness in wheat, species such as rye and some of the wheatgrasses are very cold hardy. The results of the research reported here suggest some reasons why the cold hardiness genes from species such as rye are so poorly expressed in combination with wheat. There appear to be some genes in wheat which suppress the expression of rye cold hardiness genes in triticale. There also appears to be an effect of chromosome dosage which will influence the cold hardiness expression of a interspecific hybrid combination. Therefore, the species with the largest chromosome number will tend to have the greatest influence on cold hardiness in the hybrid. In addition to these direct genetic effects, any operation that increases the chromosome number will probably also increase cell size, and thereby reduce the cold hardiness of the plant and mask the effects of the introduced cold-hardiness genes.

Since interspecific hybrids often confound the effects of chromosome dosage and cell size, they are probably of little use in estimating the potential of a gene donor. Once a cold hardy gene donor is selected its potential could be tested by adding individual pairs of chromosomes to wheat. Chromosome addition lines such as this would avoid the problem of chromosome dosage and cell size effects found in interspecific hybrids since the dosage of that particular chromosome would be known and cell size increases would be small. In this way individual chromosomes from the donor species could be selected based on their ability to improve the cold hardiness of wheat. Since wheat chromosomes, which have been found to suppress the expression of rye cold hardiness genes in triticale, are also known to carry wheat cold hardiness genes, it is possible that substitution of the donor species chromosome for such a wheat chromosome would both introduce superior cold hardiness genes and remove wheat genes which might suppress the alien cold hardiness gene expression.

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