VARIATION OF FREEZING HARDINESS IN CLOSE RELATIVES OF WHEAT *TRITICUM AESTIVUM* L. em THELL

By

Hoan T. Le

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To my parents, sisters, brothers and S. I. Seyadoussane.

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ABSTRACT

VARIATION OF FREEZING HARDINESS IN CLOSE RELATIVES OF WHEAT TRITICUM AESTIVUM L. em THELL.

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Significant levels of high and low intensity freezing tolerance similar to that of Triticum aestivum c. v. Winoka (ABD) were identified in a collection of 51 accessions of T. tauschii (D) and 35 accessions of T. turgidum var durum (AB). Leaf moisture studies indicated that within species, hardy accessions had lower moisture than non-hardy ones. Freezing hardiness was not related to size of crown, root, tiller, xylem and stele or number of roots or tillers of 5 hardy and non-hardy genotypes of T. aestivum and T. tauschii tested. Variation for hardiness from the identified accessions of the AB and D genomes was combined in a crossing project to produce interspecific hybrids. Crossability was more successful when T. tauschii accessions were used as the female parents. Embryo rescue was performed 11-19 days after pollination. The majority of plants were ready to be

transferred to the greenhouse after 13 to 31 days in the culture media. Fertility of the hybrids was restored by colchicine treatment. The hybrids were morphologically intermediate between the two parental species. Cytologically, hybrids at the F3 generation were a mixture of euploids and aneuploids. Laggards and micronuclei were observed frequently in meiosis. The hybrids were not hardier than the hardiest parents of either species. However, freezing hardiness of both parental species was expressed. In high intensity freezing, both additive and non-additive types of genetic action were observed while in low intensity freezing, non additive types of gene action were pronounced. Among 12 interspecific hybrids, two were identified as hardy as the T. aestivum c. v. Winoka at high intensity freezing.

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ABBREVIATIONS

ANOVA	Analysis Of Variance
CRS	Category Rating Scale
D	Durum parent
DNMRT	Duncan New Multiple Range Test
H	Hybrid plant
HC	Hybrid Combination
HIF	High Intensity Freezing
LIF	Low Intensity Freezing
LSD	Least Significant Difference
P	Probability
r	Correlation coefficient
T	*T. tauschii* parent
UCR	University of California, Riverside

INTRODUCTION

According to Gratius (1980), efforts to improve freezing hardiness of winter wheat Triticum aestivum L. em. Thell. (T. aestivum) have resulted in only a modest advance. Lack of usable genetic variability has been identified as the major cause for the slow progress (Gratius, 1980; Fowler and Gusta, 1979; Fowler et al., 1977).

Quisenberry and Reitz (1974) pointed out that prior to 1969, nearly all the new strains of hard red winter wheat grown in the Great Plains were developed from hybrids involving selected Crimean cultivars, introduced from Russia in the late 1800. Minhardi, one of the derivatives of the Crimean cultivars, was among the hardiest of cultivars tested in the period of 1920-1925. It still ranks among the hardiest cultivars in recent evaluations (Reitz and Salmon, 1959; Quisenberry and Reitz, 1974, Fowler and Gusta, 1979). Minhardi and its derivatives served as sources of winter hardiness for many of the breeding programs in the hard red winter wheat areas of the United States (Gratius, 1980). It is understandable that variability of cultivars derived from a common genetic source such as the Crimean cultivars introduced in the late 1800s is small.

If usable genetic variability is the limiting factor, the solution would be to increase genetic variability for the trait. The source of genetic variability could be found in the gene pool of cultivated wheats which may contain hardiness genes different from those existing in North America. However, with the international exchange of genetic resources, the world collection of cultivated wheats has been evaluated to a certain extent by wheat breeders and superior germplasm has not been found. This leaves another approach is to search for genetic variability for freezing hardiness in the related species of cultivated wheat.

It is useful to investigate the possibility of using the genetic resource of closely related species for the improvement of freezing hardiness in hexaploid wheat. This aspect has been exploited for traits controlled by major genes such as disease and insect resistance (Sharma and Gill, 1983). The possibility of increasing the genetic variability for a complex trait such as freezing resistance also exists since there is an indication that the genetic variability within the gene pool of the progenitor species is greater than that of the cultivated hexaploids. Several examples are cited as follows:

In 1977, Johnson and Waines stated that "the uniformity of the populations found by the UCR expeditions in the tetraploid T. dicoccoides points to its probable origin from one or a very few hybrids between its two parental diploid species. Such an origin suggests that initially, genetic

variability consisted of genes from only a few diploid individuals, and that it was isolated from the total gene resources of the wild diploid populations by the sterility of diploid x tetraploid crosses. Subsequent selection of the tetraploids under domestication further reduced their native genetic diversity" (UCR: University of California, Riverside). Johnson (1972) also compared the seed protein profiles of accessions of the T. aestivum (ABD) to that of the T. durum (AB) and T. tauschii (D) and of mixtures of accessions of AB and D genomes. He concluded that "the uniformity of pattern among all of the subspecies, primitive and cultivated, pointed to their origin essentially from one primary amphiploid ...little or none of the allelic polymorphism accumulated by the evolving diploid wheats could have been transmitted to their amphiploid derivatives... while the wild tetraploids, presumably originated from several primary amphiploids, the cultivated types initially comprising T. dicoccum clearly were selected from only one or two of these... the diploid wheats are the most variable in protein pattern, the wild tetraploids are intermediate and the hexaploid wheats are the least variable... thus, the endemic diploid wheats of the Near East may comprise a valuable resource of genes largely unrepresented in the cultivated hexaploids".

In another study, based on geographical distribution and growth habit of hexaploid, tetraploid and diploid wheats, Tsunewaki (1968) speculated that "common wheat

seems to have acquired its strong winter habit by receiving the most powerful winter habit genes, sg1 from Ae. squarossa. This resulted in common wheat being more readily adaptable to high latitudes than emmer wheat" (It should be noted that Japanese workers used Aegilops squarosa for the D genome species, however, according to the classification by Morris and Sears, 1967, this species name should be T. tauschii). Law and Jenkins (1970) wrote that "chromosomes 4D and 5D account for a major proportion of the difference in cold resistance between Chinese Spring and Cappelle Deprez". With this fact, they continued: "it is the introduction of the D genome which enables hexaploid wheats to expand and colonize the more Northern lattitudes whereas the tetraploids have been confined to regions with higher average temperatures". Other workers were more excited about the prospect of using the wild resources of wheat for the improvement of complex traits in hexaploid wheat. Feldman and Sears (1981) postulated that "it is possible that the hybridization of tetraploid wheat with diploid relatives from semiarid or arid regions would result in new crops that could improve currently cultivated wheats in drought resistance, salt tolerance or heat tolerance". Grafius (1980) was pessimistic about intraspecific gene transfers: "progress in cold hardiness from intraspecies crosses for wheat, barley and oat is quite likely to be slow...sources of genetic variation which hold the promise of dramatic increases in cold resistance are to be found in the wild relatives of

wheat and barley" and optimistically expressed that "a thorough investigation of the D genome might uncover new sources of hardiness for the 42 chromosome wheats".

All of the above suggestions and conclusions indicated that one might expect the gene pool of related species of wheat, especially the D genome to be a genetic resource for freezing hardiness. Genetic variation from the D genome could be transferred to the ABD genome of cultivated wheats by creating interspecific hybrids of the D and AB genome. Due to homologous pairing of chromosomes, gene transfers could occur readily between the interspecific hybrids and current cultivars. This thesis is an attempt to explore the possibility of ultilizing genetic resources from the closely related species of wheat for freezing resistance improvement. To do this, experiments were conducted to address three fundamental questions which must be answered before any practical use of this germplasm is made in a plant breeding program for the improvement of freezing hardiness. They are:

1/ Is there any variation for freezing hardiness within 51 accessions of T. tauschii (Coss.) Schmall. (D genome) and 35 acessions of T. turgidum L. var durum (AB genome)? These accessions were chosen from the existing germplasm collections based on their origin and/ or previous field data of other researchers. The durum wheat will be also screened for freezing resistance in order to select accessions that will be crossed with the D genome for the creation of a bridge

that will serve in genetic transfers between D and cultivated ABD genomes.

2/ If there is variation within accessions tested, what are the physiological, morphological and anatomical properties that freezing resistant accessions possess?

3/ Finally, how are freezing resistant characters expressed in the hexaploids synthesized from accessions of T. tauschii and T. turgidum var durum?

LITERATURE REVIEW

There are many good books and reviews on various aspects of the subject of winter hardiness, cold hardiness, freezing hardiness or in general, low temperature stress. Examples are Chandler (1954), Levitt (1956), Olien (1967), Mazur (1969), Weiser (1970), Levitt (1972), Burke et al. (1976), Levitt (1980), Olien and Smith (1980).

Definition

Freezing hardiness, as defined by Olien (1980) involves survival of plants after relief from stresses generated by water crystalization. Water in plant systems is in association with plant components. The redistribution of water with regard to state occurs until the system of water and ice is again at equilibrium. The conversion of liquid to ice is accompanied by an increase in the energy of water retention against which freezing acts. It is this transition that results in stress on the plant components associated with water (Olien, 1980).

Equilibrium and non equilibrium freezing

The types of stress that occur in plant tissues can be characterized by water transition patterns. Olien (1970) identified two main patterns of water redistribution: equi-

librium and non-equlibrium freezing patterns.

In equilibrium freezing, the process is reversible, free energy for initial crystal growth is small. As freezing progresses, heat of fusion is gradually released and freezing occurs at a moderate rate. The freezing point shifts as a function of liquid content. When ice and water are separated, vapor pressure equilibrium develops, water is drawn from protoplasts and freezes extracellularly. Severe loss of the cell water to ice in the intercellular space causes other secondary stresses. Some of the examples are: contraction of the cell to a critical size (Merryman, 1967), exceeding a maximum tolerable osmotic pressure (Meryman, 1970; Williams and Williams, 1976), reduction and/or precipitation of electrolytes may occur when solute concentration increases which may result in a pH change (Mazur, 1969), denaturation of protein due to a change in pH (Lea and Hawke, 1952), increase in salt concentration (Lovelock, 1953), formation of disulfide bonds (Levitt, 1962) and oxidation of sulfhydryl groups (Khan et al., 1968). Although low temperature does not inactivate all plant enzymes (Heber, 1968, Santarius, 1969 cited by Levitt, 1980), the overall metabolic activity may be disturbed if one or two key enzymes are inactivated. Photophosphorylation and electron transport are affected by freezing (Santarius, 1968 cited by Levitt, 1980).

Injury due to equilibrium freezing in hardened plants does not occur until protoplasts are severely contracted

from desiccation or when adhesion causes tearing of cell components (Olien and Smith, 1977). In the western plains of the United States, where the winter is cold and dry, equilibrium freezing seems to be a common cause of injury to herbaceous plants.

In non-equilibrium freezing, water remains in the liquid state at below freezing temperature ,i.e., supercools. The displacement of temperature from equilibrium is high (Olien, 1977). Free energy for crystal growth is a function of displacement from equilibrium that arises from supercooling before freezing is initiated or from rapid heat transfer as freezing progresses. There are two stages in non-equilibrium freezing. In the first stage, freezing progresses rapidly and latent heat is released immediately, causing a rise in temperature. Free energy from the first stage of freezing can cause ice crystals to grow into the protoplast (intracellularly). In the second stage of non-equilibrium freezing, the temperature of the system returns to that of the heat sink and water freezes more slowly with less energy. Temperature displacement in the second stage depends on thermal conductivity of the heat sink. In an efficient heat sink, freezing approaches an isothermal process with maximum displacement whereas in a poor heat sink, freezing approaches an adiabatic process and temperature rapidly approaches the equilibrium pattern. The displacement from equilibrium in the second stage is smaller than that in the first stage and energy per mole of water is low for ice

to grow through the plasmalemma but the total free energy is high because of the large number of water molecules involved. Non-equilibrium freezing causes histological disruption. The amount of water frozen is not a continuous function of temperature. Non-equilibrium freezing occurs in tissues with high moisture levels and in supercooled systems (Olien, 1967). Non-equilibrium freezing or high intensity freezing occurs to winter cereals in Michigan when the midwinter thaw causes high crown moisture due to water absorption, followed by low temperatures which cause the sudden freeze of the hydrated crowns. The most severe effect occurs when most of the soil remains frozen and only the plant crown thaws and refreezes (Olien ,1970). Olien et al. (1976) found that the size and location of ice crystals in crown wheat tissues are varietal characteristics. Ice crystal growth can cause physical disruption of tissues (Olien, 1968). Large and perfect ice crystals cause more damage than small imperfect ice crystals (Olien, 1977). Direct ice pressure injury occurs in the crown of winter cereals which suffer high intensity freezing (Olien, 1973, 1974).

In supercooled tissues ,i.e., xylem vessels of woody plants, the stress energies that develop from non-equilibrium freezing result in intracellular freezing. Intracellular freezing is nearly always lethal to the cells (Levitt, 1980). This may be due to ice crystals in the protoplasm which protrude into the plasma membrane. Enzyme destruction due to the break down of cellular compartment-

alization may aggravate the injuries. In nature, intracellular freezing occurs when the plant system is supercooled or in case where tissue temperature drops rapidly following a midday warming ,i.e., sunscald (Levitt, 1980).

The injuries due to freezing stresses, therefore, have an adverse effects at the molecular, biochemical, physiological and structural levels of stressed organisms.

Freezing resistance

To survive freezing stresses, plants evolved two strategies categorized as freezing avoidance and freezing tolerance (Levitt, 1980).

Freezing Avoidance-Supercooling-Freezing Tolerance and Hardening

Levitt (1980) suggested that species that survive via freezing avoidance are more evolutionarily advanced than those with freezing tolerance mechanism. Many spring annuals have evolved this strategy. They mature and have seeds before the cold season arrives. Seeds with their low moisture are very resistant to freezing and they survive well over the cold season.

Another form of freezing avoidance is supercooling of critical tissues. Many woody plants of the eastern deciduous forest species use this mechanism (Burke et al., 1976). For overwintering woody species and fruit trees that possess supercooling as a freezing resistance mechansim, there is evidence that supercooling changes occur during acclimation and tissue maturation (Wolpert, 1983). There is a reduction

in water content or there may be a reduction in the number of ice nuclei in the plant system (Burke et al., 1976). There is evidence that protoplasmic permeability increases during acclimation (McKenzie et al., 1974) which should allow water to move across the membrane more easily. Levitt (1966) also reported that hardier cells have a higher permeability to water than non-hardy cells. This may also be related to the reported increase in nonsaturated fatty acids in the membrane during hardening (Siminovitch et al, 1967). Changes in fatty acids and lipid content may be related to the phase transition of the membrane, and affect the permeability of the cell membrane. Low water content permits cells to remain supercooled and avoid intracellular freezing. Ice inoculation into the protoplast also depends on the properties of the protoplasmic membrane which acts as a barrier against ice. There have been reports on a correlation between lipid content and hardiness in black locust trees (Siminovitch et al. 1967), alfalfa (Grenier et al., 1975). An increase in phospholipids has been reported by Sakai and co-workers in black locust (Yoshida 1969; Yoshida and Sakai, 1973). For wood ray parenchyma cells of apple and hardwood trees, supercooling to temperatures as low as -38 ^{o}C to -47 ^{o}C is their protection mechanism against freezing kill (Quamme et al., 1973). The distribution of 49 native species of trees within the United States was shown to be correlated with the temperature of their exotherms occurring in fully hardened trees following supercooling (George et al., 1974;

George and Burke, 1976). The more northerly the distribution, the colder the temperature at which the exotherms occured. Supercooling confines tree species native to the eastern deciduous forest of North America to latitudes where minimum winter temperatures do not drop below -40°C. Pear and apple production areas are also limited by this minimum winter temperature where supercooling is maintained in these fruit crops (Quamme, 1976). Other fruit crops ,i.e., peach (Ashworth, 1982), grape (Pierquet et al., 1977), blueberry (Biermann et al., 1979) also share this property for freezing resistance. Their flower buds and xylem tissues remain deep supercooled. When minimum temperatures drop below the homogenous nucleation point of the sensitive tissues (flower buds and xylem parenchyma) at which exotherms occur, the plants are injured. It is self-explanatory that without ice formation, as in cases where supercooling occurs, freezing stress is avoided. However, if temperatures drop below the homogenous nucleation point, intracellular freezing occurs which is always lethal to the cell. However, some very hardy woody species do not have supercooling as a survival mechanism. The woody species Populus tremuloides which ranges into the arctic and subarctic regions where the temperature frequently falls below -40°C does not have a low temperature exotherms (Burke et al., 1976). This species has extracellular freezing as its survival mechanism.

Overwintering cereals harden as the temperature decreases in the fall. During the hardening period, there are

many physiological changes that help acclimated plants to modify the stress to make it less severe or to modify the system to be more resistant to stress (Olien, 1980). Photosynthesis and respiration are depressed by low temperature. However, in wheat, hardier cultivars have higher rates of photosynthesis at near freezing temperatures than less hardy cultivars (Barta and Hodges, 1970). There was evidence that respiration occurred via glycolysis and the Krebs cycle during initial hardening at 7 °C but shifted to the pentose phosphate pathway during the second stage of hardening at 2 °C. When plants were hardened at 2 °C, a 7 °C to 8 °C lower kill temperature was found (Khisamutdinova and Vasil'eva, 1970). A greater activity of flavin oxidases and a decline in activity of iron-containing oxidases were also involved in the shift.

The physiological change during hardening was also manifested in cytological change. Hydrolysis of starch to sugars occured early in hardening process of hardy cultivars. There was a complete disappearance of starch from the cells of winter hardy wheat Kharkov while for Selkirk, a non-hardy cultivar starch grains in chloroplasts were still observed after one month of hardening (Rochat and Therrien, 1975). There were also changes in numbers and/or sizes of mitochondria, endoplasmic reticulum and other cell organelles (Olien, 1980). Levitt (1956) indicated that sugars normally increase in the fall as plants harden, and decrease in the spring as they deharden. Such changes in sugar con-

tent occur in both woody and herbaceous perennials as well as in winter annuals in both cultivated and native plants (Markova, 1973). Increase in intracellular concentration of sugars during hardening was either due to hydrolysis of starch accumulated in the cells in the summer or due to an excess rate of photosynthesis over respiration in winter annuals (Levitt, 1966). In tea (Sugiyama and Simura, 1968), cabbage (Le Saint and Frotte, 1972), hardier tissues have higher sugar content than the non-hardy ones. Treatments that increase sugar content also increase hardiness and treatments that decrease the sugars level lower hardiness (Sakai, 1961) for mulberry but not for poplar (Sakai and Yoshida, 1968). The increase in hardiness by sugars was due to an osmotic effect or other metabolic effects. Sugars may be metabolized in the protoplasm at low, hardening temperatures into some unknown protective substances (Levitt, 1980). Levitt (1980) suggested effects of sugars to an avoidance of freeze dehydration. Without losing too much water, cell structure could be maintained during freezing which would result in less cell contraction. Winter wheat cells were severely compressed when frozen extracellularly. Hardened cells show maximum compression at -12^{o} C but the cells were square with a concave wall. The unhardened cells reached maximum compression at -7^{o} C to -8^{o} C with severely pinched portions. On thawing, the hardened cells were uninjured and smoothed out completely while the unhardened cells were injured and incompletely filled out (Salcheva and Samygin,

1963). Sugars also can act as a barrier between cell components, plasmalema and ice to prevent damages due to adhesion energies (Olien, 1980).

Lipid changes were also indicated during hardening. Low temperature also causes an increase in phospholipids in hardy wheat (Pankratova and Khokholova, 1977). There was an increase in osmiophillic globuli which indicated lipid changes in chloroplasts of the hardy Kharkov wheat (Rochat and Therrien, 1975). Hardening of wheat and rye also causes an increase in unsaturation of the fatty acids (Miller et al., 1974).

The production of protective substances during hardening help the plants to survive. However, not all tissues need equal protection. Survival of winter cereals depends upon survival of the crown and protection of these tissues is essential for survival of the whole plant. Olien (1965, 1967) has identified freezing inhibitors that slow down the process of high intensity freezing. This helps to reduce the abrupt destruction due to the explosion of ice formation in critical tissues. The freezing inhibitors from hardy genotypes also modify ice structures, which if perfect could cause serious damage. Interference of freezing inhibitors with ice formation processes resulted in imperfect and small ice crystals which cause lesser damage to the critical tissues. Shearman et al. (1973) reported a correlation in freezing inhibitors and hardiness of wheat cultivars.

Besides sugars and polysaccharides described above,

increase in soluble proteins has been associated with hardiness (Siminovitch et al., 1967). Glycoprotein has been reported to be accumulated in wheat and rye (Grzesiuk et al., 1974) and black locust seedling during hardening (Brown and Bixby, 1975).

Morphological and anatomical structures of hardy and non-hardy genotypes have also been investigated. Structure of stem internodes of wheat (Single, 1964; Patrick, 1972) with short tracheal elements and thickened transfer cells instead of bridging strands with open xylem, thus, allow water to be transferred to transpiring leaves but also serve effectively as a barrier for ice crystal growth. Bud scales of peach (Quamme, 1978), and azalea (George and Burke, 1977) act as a sink for withdrawal of water from bud primordia to reduce moisture. However, ice inoculation from bud scale to primordia is prevented due to no open xylem vessels to the bud primordia. With low moisture and no ice inoculation, the primordia could supercool to -38 ^{o}C to -40 ^{o}C. Glaucounous leaf surface of Eucalyptus urnigera do not allow water to wet the leaf, thus, preventing ice inoculation for supercooled plants at high altitudes as compared with the green, wettable leaves at low altitudes (Levitt, 1980). Woody species with high exotherms have diffuse porous while those with low exotherm have ring porous xylems (Burke et al., 1976).

Cell sizes were also investigated in relation to freezing resistance. Small cell sizes have been implicated in

freezing resistance (Levitt, 1956). Levitt (1980) indicated that the small cell size with greater specific surface reduce the strain per unit surface when the cell contracts during freezing. Weigand (1906) reported good correlation between smallness of cell size of twenty tree species and their ability to remain unfrozen at very low temperatures (-18 °C). Obviously, freezing avoidance is involved in these cases. Palisade cells of hardened cabbage were smaller than non-hardened (Rosa, 1921). Cortex of hardy wood tissue had smaller cells than non-hardy woody plants (Levitt and Scarth, 1936). Feeding sugars to cells to increase their freezing resistance resulted in reducing the size (Chandler, 1913). Bartulina, on the contrary, (as cited by Levitt, 1956) found that hardy wheats have larger cell sizes than non-hardy genotypes. Levitt (1956) indicated that hardy plants possess relatively small cells, though the converse is not necessarily true.

Selection for freezing resistance

Selection for freezing resistance of plants has long been evaluated based on field survival. This measurement represents winterhardiness which includes freezing hardiness, disease and insect resistance, winter habit, ability to detoxify toxins produced by freeze injured tissues and other characteristics and their interactions. Therefore, it is not clear which trait contributes to survival of a particular genotype in a certain year. The fluctuation of air and soil temperature from year to year is another

problem. A differential winter kill to separate the hardy from the non-hardy genotypes occurs only once every 10 years (Levitt, 1980) which does not allow a continuous and consistent screening. The development of artifical screening methods has resulted in a more precise technique for the evaluation of germplasm and selection of superior genotypes. The first artificial freeze test where plants were frozen in pots was made by Harvey (1918) . In winter cereals, survival of the crown and that of the meristematic tissues are most critical. Marshall (1965) evaluated freezing resistance of oat crowns in plastic bags. Warnes et al. (1970) screened winter barley crown for freezing resistance. With the understanding of various types of freezing stresses developed in tissues as a result of moisture differences (Olien, 1967), techniques were developed to evaluate freezing resistance of cereal crowns to various types of freezing stresses (Metcalf et al., 1970, Marshall et al., 1980). A high and a low intensity test were developed. In these high and low intensity freezing tests which correspond to non-equilibrium and equilibrium freezing, moisture of hardened crowns can be adjusted for the particular type of test.

Artificial freezing techniques provide information on freezing hardiness of germplasm within a short time and the exact types of freezing stress can be measured by manipulation of plant and freezing condition (Olien, 1967).

Besides the evaluation techniques based on field survi-

val or on survival after the artificial freezing tests, efforts have been made to determine specific plant chemicals that are associated with freezing tolerance. However, as a polygenic trait and each species or even each genotype may have different protection mechanisms, this search has not been paid off. The most efficient screening method for freezing evaluation is based on survival after the freezing test and the ultimate test of survival is in the field over the winter season.

For measurements of the limit of supercooling or freezing avoidance of species that use this strategy, temperatures at which exotherms of a critical tissue after the hardening stage occur can be measured (Burke et al, 1976). Distribution of species would depend on the minimum temperature of the region. This temperature must be higher than the temperature of exotherm of critical tissues. Thermal analysis, differential thermal analysis (DTA) and differential scanning calorimetry (DSC) have been used to measure plant exotherms (Burke et al., 1976). Nuclear magnetic resonance (Burke et al., 1976, Burke et al, 1975), electrical resistance, electrophoretic dye mobility and dye diffusion techniques (Olien, 1961, Dennis et al, 1972, Olien, 1974), ditolometry (Levitt, 1980) can be used to study freezing in plant system.

Genetics of freezing hardiness

The genetics of winter hardiness, in which freezing hardiness is the principle component, has been investigated

for winter cereals. The trait is quantitative and has a strong genotype by environment interaction (Worzella, 1935, Eunus et al. 1962). Several components of winter hardiness were listed by Grafius (1980) as follows: 1/ winter habit, 2/ disease and insect resistance, 3/ resistance to different kinds of freezing stress and 4/ tolerance to mildly pathogenic fungi growing in injured tissues. The first trait is not complex as it involves only few genes (Reid, 1965). The second and last components are usually controlled by major genes. Freezing resistance is in itself a complex trait as many types of stresses develop during freezing (Olien, 1967). Each type of stress requires different protective systems (Olien, 1980). Winter hardiness of barley, a diploid species, was controlled by both dominant and reccessive genes which were in operation with additive effects of the genes (Eunus et al., 1962). For hexaploid wheat (Gullord, 1974), freezing hardiness is controlled by partial dominant genes which are mostly additive in their effects under controlled environmental conditions. There was a significant interaction of genotypes by freezing intensity. This has led to the hypothesis that different genetic systems may be involved in different types of freeze stresses of high and low intensity freezing (Gullord et al., 1975).

Progress in breeding for freezing hardiness in winter cereal has been slow in the last few decades (Grafius. 1980). The limiting factor has been identified as the lack of genetic variability within cultivated varieties in North

America (Fowler et al., 1977; Grafius, 1980). New usable variation is necessary if hardier cultivars are to be produced. The cultivated gene pool or the close relatives of cultivated wheat T. aestivum could serve as freezing resistance gene donors. However, a large collection of world genotypes of cultivated wheat has been screened and superior germplasm has not been found. This leaves genetic variation in the original gene pool ,i.e., in close relatives of wheat as a source that offers some hope.

Hexaploid wheat T. aestivum was formed through two events of polyploidy of three diploid species of the genus Triticum. The first event took place between the diploid T. monococcum L. (genome A) and an unknown species of the B genome to give rise to the tetraploid T. turgidum L. (genome AB). T. aestivum arose from the second polyploidy event which took place between the tetraploid AB genome and the diploid T. tauschii (genome D) (Kihara, 1944 cited by Kihara, 1982; McFadden and Sears, 1945). Based on morphological characteristics of the tetraploids and seed protein profiles of the diploids, tetraploids and hexaploids, Johnson (1972) and Johnson and Waines (1977) indicated that the formation of polyploid wheats involved specific accessions of the diploids and tetraploids. This leaves the polyploids with less genetic variation than that of the diploids. The gene pool of immediate relatives of the A, AB, and D genomes is, therefore, a valuable and available resource to be used for the improvement of cultivated

hexaploid wheat species (Feldman and Sears, 1981). This resource has been exploited for the improvement of major genes such as disease and insect resistance for almost a century (Sharma and Gill, 1983). For freezing hardiness, this resource could be ultilized as gene donors for the cultivated wheat. This prospect was reemphasized after the discovery of Law and co-workers (1970). Substituting chromosomes of the non-hardy T. aestivum cv. Chinese Spring with that of the hardy wheat Capelle Deprez, Law and Jenkins (1970) found that genes for freezing hardiness were located on chromosomes 7A, 4D and 5D. Cahalan and Law (1979) also found that chromosome 5A contained genes for cold tolerance. Furthermore, Fowler and co-workers evaluated freezing tolerance of accessions of the A, D, AB and AG genomes. Several accessions of the D and AB genomes possessed remarkable levels of freezing tolerance at low intensity (Fowler et al., 1977). Limin and Fowler (1981) studied hardiness of 161 accessions of the D, AB and A genomes. They reported that hardiness of the D genome approached that of the hardy T. aestivum cv. North Star. Fowler's group formed synthetic hexaploid wheats from the AB and D genomes. They did not obtain amphiploids that were hardier than the parental accessions. However, the F3 selection from a synthesized wheat of moderately hardy parental accessions of T. tauschii and T. turgidum had very high levels of freezing tolerance under conditions similar to low intensity freezing (Limin and Fowler, 1983). It would be interesting to inves-

tigate the possibility of ultilizing the close relatives of wheat for the improvement of freezing resistance of wheat under high intensity freezing.

MATERIALS AND METHODS.

I. FREEZING HARDINESS OF 51 ACCESSIONS OF T. TAUSCHII AND 35 ACCESSIONS OF T. TURGIDUM VAR DURUM

Freezing test.

Fifty-one accessions of T. tauschii and thirty-five accessions of T. turgidum var durum (from hereon T. turgidum var durum will be referred to as T. turgidum only) were screened for freezing hardiness, using the high intensity freeze (HIF) and low intensity freeze (LIF) tests (Metcalf et al., 1970, Gullord et al., 1975). These accessions were divided into three groups: two for T. tauschii and one for T. turgidum. The grouping of these accessions was due to the availability of adequate supply of seeds for the screening test and the limited space in the freezing chamber which could accommodate a maximum of 720 plants at a time. The experimental design was completely randomized.

Seed treatment- using a 20% solution of commercial bleach (Sodium hypochlorite 5%) for 3 min and rinsing 3 times with tap water- was applied for tests 13, 14, 15 and 16. For the remaining tests, the seeds were not treated. Seeds of different accessions were germinated in sterilized sand. Ten days after germination, uniform seedlings at the

one-leaf stage were transferred to a 11cm diameter steril ized clay pots filled with sand. Eight plants, one of which was a check- either Hudson barley or Genesee wheat- were planted in each pot in a completely randomized design. Hudson and Genesee were chosen as checks because they have been studied intensively and are good indicator lines for barley and wheat. The transplanted plants were grown for five weeks in a growth chamber with a temperature of 15 ^{o}C and 18 hours of light. Plants were then transferred to a hardening chamber for three weeks under continuous light at 2 ^{o}C. During the period of initial growth and hardening, plants were supplied with a modified Hoagland's solution as a source of nutrients every other day. On alternate days, tap water was used.

After hardening, the plants were trimmed of roots and leaves to about 3cm below the crown and 7cm above the crown and washed with cold water. Plant crowns from each pot were transferred into each of eight slotted donut-shaped sponges.

For the HIF test, the sponges and crowns were put into plastic lined peat pots and saturated with cold water. The pots were placed in a freezing chamber at 2 ^{o}C and the temperature was lowered 1 ^{o}C/hour until a temperature of -2 ^{o}C was reached. Ice was inoculated at -2 ^{o}C to prevent super-cooling. Crowns were held at this temperature for 24 hours to insure that all free water in the plants and sponges was frozen. The temperature was then lowered 2 ^{o}C/h until the test temperature was reached. For HIF tests, test temper-

atures ranged from -12 °C to -15 °C. The freezing chamber was shut off after crowns were held at the test temperature for 3 to 4 hours. The temperature rose slowly to room temperature at approximately 2 °C/h.

For the LIF test, the sponges and crowns were kept dry. The prepared crowns were left in the cold chamber at 2 °C for 24 hours so that moisture in the crowns might adjust evenly for the LIF test. The prepared crowns were then placed in the freezing chamber at 2 °C and the temperature lowered 1 °C/h until a temperature of -2 °C was reached. Supercooling was prevented by inoculation of the crowns with ice at this temperature. The temperature was then lowered 2 °C/h until the test temperature was reached. Test temperatures ranged from -14 °C to -17 °C for LIF tests. Plant crowns were kept at the test temperature for 3 to 4 hours. The freezing chamber was then shut off and the temperature allowed to rise slowly at the rate of 2 °C/h to room temperature.

After the freeze tests, the plants were trimmed of dead roots and leaves and replanted in sterilized sand filled flats and kept in the greenhouse. Conditions in the greenhouse during the winter and spring seasons were about 16 h light (with artificial light supplementing sunlight) and a temperature of 21 °C to 25 °C in the day time and 12 °C to 15 °C at night. Two weeks later, they were uprooted and evaluated on a rating scale from 0(dead), 1(one root), 2(2 roots), 3(3 or 4 roots), 4(more than 4 roots) and 5(no damage). This rating scale was later reviewed. Dr. Olien suggested the

consolidation of ratings 3, 4 and 5 into one rating of 3 only to eliminate errors that might arise due to difficulties encountered in differentiation of ratings 3, 4 and 5. Furthermore, a rating of 3 indicates full recovery and ratings 2 and 3 are fairly easy to distinguish.

These freezing tests were conducted in the Plant Science Greenhouse of Michigan State University in the Spring, Fall and Winter of 1981 and 1982. A total of 8 freeze tests (4 at LIF and 4 at HIF) was performed for T. tauschii and 6 freeze tests (3 at LIF and 3 at HIF) was performed for T. turgidum, using Hudson and Genesee as checks. From these 14 freeze tests, based on a category rating scale (CRS) described in the next paragraph, 4 of T. tauschii and 6 durum accessions which were always equal to or hardier than Genesee were chosen for comparisons with Winoka, a hardy accession of T. aestivum. Genesee was also used as a second check together with Winoka. Thirty-five to 45 plants were used for the tested accessions in these final comparisons using Winoka and Genesee checks.

Statistical analysis of data from the freezing tests

The Chi-square values estimated by Bartlett's test indicated that the variances for all accessions were not homogenous for almost all of the tests. The validity of a parametric test of significance, however, requires that the experimental errors be independent and normally distributed with a homogenous variance (Steel and Torie, 1980). No transformation brought about the homogeneity of the vari-

ances. There are two ways to solve this problem, one is the use of non-parametric statistics. The other- used in this study- is to set aside certain accessions. The remaining accessions are analysed, using parametric statistics with analysis of variance as usual, as homogeneity of variance was obtained for the remaining accessions. The error mean square estimated from the analysis of variance was used to obtain LSD values for comparisons of means of freezing resistance of different accessions, including those that were not included for the sake of homogeneity of variance. For these accessions, this was only an approximate test. Following the method described above in test 2, the two highest accession means of recovery rating (2.88 and 2.85) and in test 4, all means with values that were smaller than .30 were set aside and not used in the ANOVA for the calculation of the error mean square. The error mean square of other tests were estimated from ANOVA of accessions with mean values greater than .50. Furthermore, the plants for each accession in each test were grouped into 4 random groups. The mean of each group was treated as a replication in the analysis of variance.

The second assumption of normality of experimental errors for a valid ANOVA was also checked, using the Lilliefors test (Iman, 1982). The experimental errors (or residuals) were calculated from the freeze rating data of accessions used in the ANOVA and then were plotted on a Lilliefors bound paper to determine if deviations from norm-

ality were present (App. 1).

Category rating scale (CRS)

Inadequate seed supply, poor seed germination and limited space in the freezing chamber caused many accessions to be tested in separate experiments or even only once. It was, therefore, neccessary to have a system to compare these accessions even if they were not in the same test. As Genesee was used as a check in all tests, a category rating scale (CRS) based on a LSD test of recovery ratings with Genesee was designed:

1/ Hardier than Genesee in at least two severe tests (low temperatures).

2/ Hardier or equal to Genesee.

3/ Always equal to Genesee.

4/ Equal to or more tender than Genesee.

5/ Hardier or equal or less hardy than Genesee.

6/ Always more tender than Genesee.

It is necessary to take note of the test temperature before any judgment can be made about the classification of the accessions. For example, at a higher temperature, a hardy accession may have an equal rating to Genesee. As the temperature is lowered, the hardy line will be significantly different and hardier than the check. It must, therefore, be classified in CRS 1. Problems arise when an accession is hardier than Genesee at a lower temperature while at a higher temperature it is more tender than Genesee. This is where CRS 2, 4, 5 can be used to separate them from the rest

of the accessions.

Those that fall into CRS 1, 3 and 6 have clear cut differences in freezing hardiness to Genesee whereas those in CRS 2, 4 and 5 are those that have hardiness levels similar to that of Genesee. Conditions used in the tests failed to differentiate them clearly. More tests with more replications would help to differentiate hardiness levels of these fluctuating accessions.

II. THE NATURE OF FREEZING HARDINESS OF SELECTED ACCESSIONS OF *T. TAUSCHII* AND *T. TURGIDUM*

Leaf moisture determination

Selected accessions of *T. tauschii*, *T. turgidum*, *Hordeum vulgare* L. and *Secale cereale* L. with known levels of freezing hardiness were used for leaf moisture determination.

Plants were germinated, transplanted, grown and hardened in the same manner used for the freeze tests described in the previous section. The selected lines were divided into groups. Each group was composed of 6 accessions of both hardy and non-hardy lines. The first group consisted of *S. cereale* cv. Rosen, a very hardy line, *H. vulgare* cv. Dictoo, a hardy barley and 4 cultivars of *T. aestivum* with a range of freezing hardiness of fairly hardy (Frankenmuth and Augusta) and very hardy (Winoka and Kharkov). Two lines *T. aestivum* cv. Genesee, a moderately hardy wheat and *H. vulgare* cv. Hudson, a moderately hardy barley were grown together with

the 6 test plants of different genotypes in the same pot as checks. These lines with known levels of freezing resistance and moisture levels were used to verify the techniques of leaf moisture measurement for subsequent experiments where leaf moisture of selected accessions of T. tauschii and T. turgidum was to be assessed. The second group consisted of 6 T. tauschii accessions with 3 non-hardy lines TA1651, TK57-324, RL5257, two hardy lines TK91-455-1 and TK92-467-1 and one intermediate hardy KU2119. The third group was a mixture of both T. tauschii and T. turgidum: TK91-455-2 and TK93-471 were hardy, KU2113 was intermediately hardy and Ae1 was non-hardy. These four accessions belonged to T. tauschii. The other two accessions were of T. turgidum var durum cv. PI293918 a hardy line and cv. PI352457, a non-hardy spring type. The fourth group was of T. turgidum accessions. Two accessions were non-hardy: CI15296 and PI191380 while the other four accessions were of a very hardy type as PI344544 or moderately hardy as PI293422, PI345703 and PI326314. The fifth group came from a selection of T. tauschii of group 2 and 3: One non-hardy accession was TA1651, 3 hardy accessions TK91-455-1, TK91-455-2, TK93-471. The hardy varieties Winoka and Kharkov of T. aestivum were included in this group. Plants in each group were arranged in a completely randomized design with Hudson barley and Genesee as checks. Thus, each pot contained two checks and 6 test plants of different accessions. Twenty pots were used for each group but only fifteen with the most uniform plants were used for

the leaf moisture test. Twenty-four hours before the plants were brought to the laboratory, the plants in the hardening chamber were watered to insure their uniform uptake of available water. Tillers and old leaves were removed and only 2.5cm of a cylindrical portion of the youngest leaf, nearest to the crown was taken (Gullord, 1974). Three crowns from one accession, representing one replication, were put in a 7mm diameter vial and sealed with vaseline. Two to three replications were taken for each accession. Fresh weight was taken immediately and dry weight was recorded after the materials were dried for 24 hours at 60°C. Percentage of leaf moisture was calculated as:

100(Fresh weight-Dry weight)/Fresh weight.

Analysis of variance was carried out and mean comparisons made, using Duncan New Multiple Range Test.

Morphological and anatomical study of crown, tiller, root and xylem size of two accessions of T. aestivum and three accessions of T. tauschii.

Morphological and anatomical characteristics of five accessions with high and low freezing resistance were studied for the two species. Two accessions of T. aestivum cv. Genesee (moderately hardy) and cv. Winoka (very hardy) and three accessions of T. tauschii- TA1651 and RL5257 (tender lines) and TK91-455-2 (very hardy) were used.

Measurements were made on plants grown and hardened as previously described. Four plants were measured for each accession, using a Vernier Caliper. The diameter of 5

lateral roots that grew out from the side of the tillers was taken from each plant. Tiller diameter and crown diameter at the widest and narrowest crown region were also taken from each plant. Root and tiller numbers were also taken from each plant.

Xylem size was taken from the root of the largest tiller at 2mm from the crown. Freehand sectioning, using a razor blade was performed on materials fixed with FAA and preserved in 70% EtOH. The sections were stained with Fast Green, Safranin, or non-stained. Observations were made on pictures taken with a Nikkon microscope. A photograph of a haemacytometer was also taken in order to calibrate the size of xylem elements measured from a photograph to the actual measurement based on a known distance of the haemacytometer grids.

Analysis of variance was made on the root and tiller numbers, the means of root, tiller crown and xylem diameters.

III. INTERSPECIFIC HYBRIDIZATIONS OF T. TAUSCHII AND T. TURGIDUM

Hybrids and embryo culture

In the fall and winter of 1982, selected lines of T. tauschii and T. turgidum were planted every two weeks in order to have synchronization at the flowering stage for different accessions. After germination, these parental plants were vernalized for eight weeks at 4 ^{o}C and then transplanted and

kept in the greenhouse for use in the crossing project. The initial attempt was to complete all crosses for the 12x12 factorial design (Fig. 1). Reciprocal crosses were attempted to determine maternal effects. Emasculation was done prior to anthesis with pollination 48-72 hours later. Twelve to eighteen days after pollination, the immature seeds were brought to the laboratory. Seeds from each head were gently removed from the cut glume with a pair of forceps and placed in a small beaker (30-50ml) covered with cheese cloth, secured with a rubber band. The following steps were taken under aseptic conditions in a laminar hood: a/ Seeds were surface sterilized by dipping the seed containing beaker for 10-15 seconds into a Pyrex jar (100x50mm) which contained 95%EtOH, rinsing with sterilized distilled water and then soaking in a solution of 10% commercial bleach (Clorox) for 20 minutes. Finally, the seeds were rinsed with sterilized distilled water three times to prepare for the embryo extraction. b/ The seeds were then transferred to a sterilized Petri plate (Falcon 100x20). The seeds were dissected with sterile needles under a dissecting microscope sterilized with alcohol. The seed coat was gently removed in the area where the embryo was located and the embryo was teased out and placed on artificial media with the scutellum in contact with the media. The media used was LS media (App. 2) with no growth hormone (Linsmaier and Skoog, 1965). It was solidified with .8% agar and adjusted to pH 5.7 with KOH .1N prior to being autoclaved at 15 psi for 15 minutes. The

	A	B	C	D	E	F	G	H	I	J	K	L	
M													M
N													N
O													O
P													P
Q													Q
R													R
S													S
T													T
U													U
V													V
X													X
Y													Y
	A	B	C	D	E	F	G	H	I	J	K	L	

Fig. 1. A 12X12 factorial design cross of T. tauschii and T. turgidum var durum

T. tauschii	T. turgidum var durum
A. TK91-455-1 ++	M. PI293918 ++
B. TK91-455-2 ++	N. PI344544 ++
C. TK92-467-1 ++	O. PI345703 ++
D. TK93-471 ++	P. PI404584 ++
E. KU2133 +	Q. PI293422 +
F. PI428563 +	R. PI326314 +
G. KU2119 +	S. PI372431 +
H. KU2113 +	T. PI191380 -
I. TK57-324 -	U. PI383357 -
J. Ae1 -	V . PI352457 -
K. TA1651 -	X . CI11246 -
L. RL5257	Y . CI15296

++: Very hardy

+ : Moderately hardy

- : Non-hardy

media was then poured on a Petri plate (Falcon 60x15mm). Many embryos from one genotype could be placed on one plate. The plates with embryos were incubated at room temperature with 16 hours of light and 8 hours dark from a fluorescent lamp at 60mEm-1s-1. These conditions were found suitable for embryo growth. When the plantlets with roots were about 4-5cm in height, they were transferred to an autoclaved mixture of 1:1:1 of soil: sand: vermiculite in the greenhouse. The agar was washed off the roots by sterilized water. A plastic bag was used to cover the potted plants to maintain a saturated humidity condition around the young plant for two weeks. Humidity was reduced gradually by holes punched in the bag. Later in Spring 1982, two plastic tents (.4x1m) were built, using flats and bamboo sticks as frames. Several water dishes were kept inside the tent to maintain a saturated humidity. One tent was used for the newly transferred plants with the plastic doors tightly closed whereas the other was used for older plants with the plastic doors slightly opened so that air could circulate. This practice was found satisfactory for plant growth and helpful in making watering easier.

The goal to complete the 12x12 factorial crossing design was not fulfilled due to limited resources. The crossing and embryo culture project was stopped after more than 30 interspecific hybrids were obtained.

Colchicine treatment of the interspecific hybrids of T. tauschii and T. turgidum.

The interspecific hybrids developed via embryo rescue were expected to be triploid and sterile. Thus, the colchicine treatment was employed in order to induce chromosome doubling in the hope that fertility would be restored. The triploid plants were allowed to grow to the tillering stage. Cloning of plants was performed by dividing the crown into groups of 2-3 tillers each and replanted again in pots. After the plants recovered, at about 2 weeks after cloning, they were subjected to colchicine treatment. Four methods of treatment were employed for the first two batches of plants to determine the most satisfactory method for inducing chromosome doubling: the first was to introduce the chemical through a tiller, using .4% colchicine on cotton, wrapped around a cut tiller (Cauderon and Saigne, 1961) and the other three via the root methods, using different concentrations of colchicine with and without DMSO: a/ .2% colchicine (Gill, pers. com.), b/ .05% colchicine+1.5%DMSO (Winkle and Kimber, 1976) and c/ .1% colchicine+2%DMSO +.3%Tween+1%GA3 (Thiebaut and Kasha, 1978). For the root methods, plants were uprooted and washed free of soil. The roots were trimmed to about 2cm below the crown. Plants were put into a beaker and the colchicine was added until the level of the solution was about 1cm above the crown. Plants were allowed to take up the solution actively for 5 hours from 12 noon to 5 p.m. under summer greenhouse conditions where strong sun-

light and high temperature occurred (maximumm temperature was about 35°C). Plants were then rinsed of the excess colchicine under running water for 3 min. and replanted into sterilized flats filled with an autoclaved mixture of 1:1 soil and vermiculite. The flat with colchicine treated plants was wrapped with a plastic bag and placed under the bench inside the growth chamber at 15°C for one week. The plastic bag was then removed and the flat put on the bench in the growth chamber. When fully recovered, plants were transferred to pots and vernalized in a cold chamber at 2°C and continuous light for eight weeks. After vernalization, plants were transferred to the greenhouse and were ready for the reproductive stage of growth.

Chromosome number determination

Seeds harvested from F1 clones derived from one single hybrid embryo were bulked. These seeds were used to produce F2 plants. In the fall of 1983 and winter of 1984, 30 hybrid seeds harvested from F2 plants of each combination were germinated and grown under a controlled environment of 15°C and 18 hours light (6am-12midnight) and 6 hours dark (12midnight-6am). Ten days later, 20 plants were used for the freezing tests while the remaining plants, at least 5 for each hybrid were maintained for root tip collection. Roots from one combination were collected at 9.30am and put in a small vial filled with ice water. The vial was placed in an ice box, jammed with ice and the box put in a cold chamber at 2°C for 24-28 hours. The roots were then fixed with

Farmer's solution (1:3 acetic: alcohol) for 24 hours at 2°C. Roots were then stored in 70% ethanol. Root tips from a hexaploid wheat T. aestivum cv. Genesee and a cultivar of T. turgidum cv. CI15296 and two accessions of T. tauschii KU2133 and TK57-324 and several dihaploid clones not treated with colchicine but grown under similar conditions were also collected, fixed and treated for a control study.

Root tips of the hybrids and control cultivars and accessions were hydrolysed in 1N HCl for 10 min. at 60°C. They were then treated with pectinase 5% for 90min. in a water bath at 37°C. For T. tauschii accessions, however, the enzyme treatment lasted only 60min. Root tips (.5 to 1mm) of two or three of the enzyme digested roots were gently macerated in one or two drops of aceto-carmine (see App. 2 for preparation) with the blunt end of a dissecting needle on a slide. All visible debris was removed. A cover slip was placed on the slide, at an angle of 45° to avoid bubbles. The slide then was flamed over an alcohol lamp until it was warm on the back of the hand. Boiling was avoided. The slide was then squashed between layers of paper towel and observed under a Zeiss phase contrast microscope. If the cells were broken, care was exercised in order that the roots were macerated more gently. If the cell was not flat enough, it could be squashed again with more pressure from the edge of the palm.

Feulgen staining was also used to stain some hybrids (see App. 2 for preparation). After the enzyme treatment,

roots were stained in the dark with Feulgen stain for 60 min.. The root tips were then macerated in one or two drops of 45% acetic acid. Any visible debris was removed, a cover slip applied and the slide squashed. In both cases, whether aceto-carmine or Feulgen stains was used, 45% acetic acid was added to the edge of the cover slip if the slide started to dry out. A photograph was taken immediately when an unbroken cell with well-spread chromosomes was found. A Cannon FT camera, assembled on the microscope was used for photo taking, using Kodak film Panatomic-X ASA 32. Pictures were taken mostly with the 40x and 100x (with oil immersion) oculars. If the slide was to be stored for several days, it was sealed with sticky wax at the edge of the cover slip and kept in the refrigerator. At least 10 cells were photographed for each hybrid. Chromosome counts were carefully made from the photographs.

For meiotic chromosome behavior, only two hybrid combinations RL5257XP1293918 and TK92-467XPI191380 of the F2 generation were observed. Young, immature inflorescences were collected at the early boot stage. They were fixed for 24 hours in Farmers' solution and stored in 70% EtOH. Anthers were teased from the glume and placed on a small drop of aceto-carmine on a slide. Under a dissecting microscope, the anther was split transversely. Using the sharp end of a dissecting needle, the two halves of the split anther were pressed gently so that all of the pollen mother cells (PMC) were released into the drop of stain. The PMC were then

macerated with the sharp end of the dissecting needle. A cover slip was applied and photographs taken at various stages of meiosis.

IV. FREEZING RESISTANCE OF THE COLCHICINE TREATED INTERSPECIFIC HYBRIDS OF T. TAUSCHII AND T. TURGIDUM

Seeds of the colchicine treated interspecific hybrids were grown in the greenhouse for seed increase. In the winter of 1984, there were enough seeds of 12 hybrids for four freeze tests with 2 HIF and 2 LIF tests. The two HIF tests and two LIF tests were combined for analysis.

The experiment was conducted as a 12x3 factorial in a split plot design with whole plots arranged in a randomized complete block with twenty replications. The first factor was the hybrid combination (HC) with 12 levels. The second factor was the type of plant within the combination. Levels were: 1/ the T. turgidum or durum parent (D) 2/ the T. tauschii parent (T). and 3/ the hybrid plant of the respective parents (H).

The seeds were treated with Vitavax 200 (1:7 v/v) as suggested by Dr. Olien (see Appendix 3). The treated seeds were germinated in a sterilized sand filled flat. After 10 days, germinated plants were transplanted into autoclaved sand filled pots. Eight plants were grown in each pot with two checks and 3 plant types for each H C. T. aestivum cv. Genesee, a moderately hardy and cv. winoka, a very hardy cultivar were used as checks. This arrangement gave a more

precise comparison of freezing resistance of the hybrid and its parents by removing the large pot to pot variation (Olien, personal communication). Growth and hardening conditions were described in previous section. The test temperatures were -12.2 °C and -13.3 °C for the two HIF tests and -14.4 °C for the two LIF tests. Standard high and low intensity freeze tests were used (Gullord et al., 1975). Freezing resistance was measured based on a root regrowth rating scale of 0: dead, 1: 1 or 2 roots, 2: 2 to 3 roots and 3: more than 3 roots (non damage). Data collected were analysed and comparisons were made, using Duncan's New Multiple Range Tests (Steel and Torie, 1980).

RESULTS AND DISCUSSIONS

I. FREEZING HARDINESS OF 51 ACCESSIONS T. TAUSCHII AND 35 ACCESSIONS T. TURGIDUM

The method used for the statistical analysis of the data described was found satisfactory to meet the requirements for the analysis of variance. Homogeneity of variance was restored by putting aside accessions with a too high or too low mean rating for freezing resistance. The Lilliefors graph of residual values indicated that the slight deviation from normality at the intermediate range (App. 1 and Fig. 6) was of no concern.

Results of the first 14 primary freeze tests in table 1 indicated that variation for freezing resistance at high intensity freeze test (HIF) and low intensity freeze test (LIF) exist within the accessions of T. tauschii and T. durum. Fourteen accessions of T. tauschii and 7 accessions of T. turgidum were in category rating scale (CRS) 1, 2 or 3 at either HIF or LIF or both. (Table 1). These were the most interesting accessions as they were hardier or at least as hardy as Genesee. They may contain a moderate number of freezing resistant genes whose effects could be higher or at least comparable to Genesee. These accessions could serve as

Table 1. Origin and freezing hardiness of T. tauschii and T. turgidum var durum based on category rating relative to that of Genesee check.

T. tauschii				T. turgidum(D)			
Accession	CRS(a) HIF(c)	CRS(a) LIF(c)	Origin	Accession	CRS (a) HIF(c)	CRS (a) LIF(c)	Origin
1. WIS2086 (S)	3(a)(b)	6(a)(b)	Afghan.	1. C111245 (S)	6(a)	6(a)	Yugosl.
2. TK93-471	1	1	Turkey	2. C111246	4	4	"
3. TK92-467-1	1	1	"	3. C115296	4	6	Afghan.
4. TK91-455-1	1	2	"	4. C115304	4	3	"
5. TK91-455-2	2	3	"	5. C115386 (S)	6	6	"
6. TK91-454	2	3	"	6. PI191380	3(b)	6(b)	
7. TK75-405	2	3	"	7. PI295422	2	2	USSR
8. TK75-400	1	(N)	"	8. PI293918	1	1	"
9. TK64-354	2	3	"	9. PI326314	3	3	"
10.TK61-312	3	3	"	10.PI344544	5	1	"
11.TK57-324	3	3	"	11.PI345705	5	1	"
12.TK57-322	3	3	"	12.PI352371(S)	6	6	"
13.TK57-318	4	3	"	13.PI352372(S)	6	6	"
14.TK57-317	3	2	"	14.PI352380	6	6	Turkey
15.TA1642	4	4	Iran	15.PI352402(S)	6	6	"
16.TA1644	4	4	"	16.PI352440	6	4	USSR
17.TA1645	6	4	"	17.PI352450	6	6	France
18.TA1647	4	3(b)	"	18.PI352451(S)	6	6	"
19.TA1651	4	3	"	19.PI352452	6	6	"
20.RL5257	4	4	Unknown	20.PI352453	6	6	"
21.RL5363	5	4	"	21.PI352454	6	6	"
22.PI220326	4	3	Afghan.	22.PI352455(S)	4	6	"
23.PI220641	4	3(b)	"	23.PI352456	4	6	"
24.PI220642 (S)	4	4	"	24.PI352457(S)	6	6	"
25.PI276985	4	4	Unknown	25.PI372430	4	3	USSR
26.PI317392	4	4	Afghan.	26.PI372431	2	2	"
27.PI317394 (S)	4	4	"	27.PI372432	4	5	"
28.PI428563	3(b)	(N)	Unknown	28.PI383357	4	6	Turkey
29.PI431598	4	4	"	29.PI383359	4	6	"
30.PI431599	4	3	"	30.PI404583	3	3	USSR
31.PI431600	4	4	"	31.PI404584	2	1	"
32.PI431601	4	3(b)	"	32.PI418199	6	6	"
33.KU20-9	6(b)	5(b)	"	33.PI428688	2	1	"
34.KU2010	4	4	"	34.PI428689	4	2	"
35.KU2017	3	3	Pakistan	35.PI428690	6	6	"
36.KU2021	4	4	"				
37.KU2066	4	3(b)	Afghan.				
38.KU2071	4	(N)	"				
39.KU2085 (S)	6(b)	6	"				
40.KU2110	4	4	Unknown				
41.KU2113	3	3(b)	"				
42.KU2119	3	3	"				
43.KU2122	5	2	"				
44.KU2133	3(b)	(N)	Turkey				
45.KU2829A	4	4	USSR				
46.KU2832	3(b)	4	"				
47.AE1	4	4	Unknown				
48.AE3	4	1	"				
49.9364	4	(N)	"				
50.9435	4	3(b)	"				
51.9436	4	4	"				

(a): C R S: Category Rating Scale for freezing hardiness (also see materials and methods):
1: hardier than Genesee in at least 2 severe tests with Genesee as check
2: hardier or equal to Genesee "
3: always equal to Genesee "
4: equal or more tender than Genesee "
5: hardier and/or equal and/or less hardy than Genesee ""
6: always less hardy than Genesee
(b): based on only one test
(c): HIF: High intensity freezing; LIF: low intensity freezing
(D): T. turgidum var durum
(N): Not available
(S): Spring habit.

potential donors of variability for freezing hardiness.

The remaining accessions were confirmed non-hardy (CRS6) or in the border line of non-hardy (CRS4) or only as hardy as Genesee at one level of intensity. These accessions might be of interest if they possess specific combining ability for freezing hardiness at a high level.

There was a significant positive correlation between HIF and LIF results. Correlation coefficients of means (Mean ratings tabulated in App. 5, 6, 7 and 8) of HIF and LIF tests were .714 ($P < .001$) and .635 ($P < .005$) for T. tauschii and .858 ($P < .0$) for T. turgidum. There were 5 cases where a jump of 2 or more steps in CRS occurred. Accessions KU2122 of T. tauschii and PI344544 and PI345703 of T. turgidum with CRS of 5 for HIF and 2 for LIF. AE3 of T. tauschii had HIF CRS of 4 and LIF CRS of 1. PI428689 of T. turgidum had HIF at CRS 4 and LIF at 2. This indicates that there was an interaction of accession by freezing intensity for these accessions. However, the general trend in this study was for T. tauschii and T. turgidum accessions to be hardy in both HIF or LIF or non-hardy in both.

Two accessions RL5257 and RL5263 were reported hardy under freezing conditions similar to that of LIF (Fowler et al., 1977). However, results tabulated in Table 1 indicated that their hardiness only approached that of Genesee.

Since this was the first time that accessions of both species were planted for freezing evaluation, their growth habit was also observed. Accessions with a spring habit were

identified in both species (Table 1). Their freezing hardiness was not impressive since with a spring habit, plants changed into a reproductive stage during hardening and lost their hardiness (Smith and Olien, 1980). However, the hardiest among the A genome accessions tested by Limin and Fowler (1981) was a spring type.

Accessions that have a CRS of 1, 2 or 3 were selected for the final test for a comparison with T. aestivum cv. Winoka. Table 2 shows that there were significant differences among accessions tested for both HIF and LIF of the selected lines for each species.

T. aestivum cv. Winoka was the hardiest in both HIF and LIF tests. T. tauschii accession TK91-455-1 was as hardy as Winoka in both the HIF and LIF. All accessions tested, except KU2122, were hardier than Genesee in the HIF. Accession TK91-455-2 was found as hardy as Winoka in the LIF test but was not as hardy in the HIF test. The reverse is true for TK92-467-1. In HIF, TK91-455-1, TK91-455-2 and TK92-467-1 were not significantly different. All accessions tested were hardier than Genesee at the LIF test at -17C. The T. tauschii accessions tested possessed hardiness at HIF and also at LIF. Interestingly, accessions TK91-455-1, TK91-455-2, TK92-467-1 and TK93-471 were collected from 3 nearby locations in Kars, Eastern Turkey (Metzger, 1983; personal communication). Phenotypically, these accessions were similar. They had very fine stems, small leaves, high tillering with prostrate growth habit and a long vernalization period.

Table 2. Freezing resistance at high and low intensity levels of selected T. tauschii and T. turgidum var durum accessions.

T. tauschii Accession	HIF(A) Test 15a Temp.-15° C Hardiness	LIF(A) Test 16a Temp.-17° C Rating(B)	T.turgidum(D) Accession	HIF(A) Test 15b Temp.-15° C Hardiness	LIF(A) Test 16b Temp.-17° C Rating(B)
WINOKA	2.48(M)	2.73(M)	WINOKA	2.52(M)	2.73(M)
TK91-455-1	2.11	2.54	P1283918	1.10	2.25
TK92-467-1	2.05	1.90	P1326514	1.05	2.19
TK91-455-2	1.96	2.41	P1293422	1.00	1.93
P1428563	1.55	1.76	P1344544	.82	2.37
TK95-471	1.20	(N)	P1404584	.81	1.97
GENESEE	.55	.12	GENESEE	.45	.38
KU2122	.55	(N)	P1345703	.35	1.93
Error M.S.	.28	.22		.24	.18
LSD .05 min.	.50	.47		.46	.40
max.	.55	.48		.48	.44

(A): HIF: high intensity freezing test
LIF: low intensity freezing test

(B): Based on a rating scale of root regrowth 0: dead, 1: one or two roots, 2: two or three roots, 3: more than 3 roots.

(D): T. turgidum var durum

(M): Means of 7 to 9 replications, each replication consists of 5 plants.

(N): not available.

They could belong to the same population with similar genes for freezing resistance as these lines exhibited high levels of hardiness in most of the freeze tests.

For T. turgidum, table 2 shows that at HIF, all selected accessions were inferior to Winoka. Three accessions PI293918, PI326214 and PI293422 were hardier than Genesee. The other accessions PI344544, PI404584 and PI345703 were comparable to Genesee. At LIF, freezing resistance of PI344544 was comparable to that of Winoka. The remaining lines were inferior to the hardy check but they were all hardier than Genesee. Four accessions of the durums were comparable. They were PI344544, PI293918, PI326314 and PI404584. The durum wheats, in this test, showed a higher level of freezing resistance in the LIF than in the HIF test. According to Feldman and Sears (1981), durum wheats were grown in the low rainfall area, and they may be well-adapted to drought conditions which enable them to survive the freeze dehydration stress under low intensity freezing. As pointed out by Levitt (1980), tolerance to drought stress and freeze dehydration stress are correlated. High levels of freezing resistance of T. turgidum were an indication that the AB accessions could also serve as potential gene donors of freezing hardiness to hexaploid wheats.

The finding that some of the D genome accessions have a high level of HIF was of particular interest for breeding freezing hardiness under Michigan conditions where winter kill of cereals is of the HIF type. The finding also

supported findings of other genetic studies where 4D and 5D were found to contain freezing resistant genes (Law and Jenkins, 1970). Furthermore, the results of this study also suggest the possibility of using the AB genomes for the improvement of freezing hardiness in hexaploid wheat. In this study and that of Fowler et al. (1977), some of the AB genome accessions possess a remarkable level of hardiness, especially in LIF. Recombination of genomes AB and D of these accessions may generate more variability which could be ultilized in the improvement of freezing resistance in cultivated varieties of T. aestivum.

II. THE NATURE OF FREEZING RESISTANCE OF SELECTED ACCESSIONS OF T. TAUSCHII AND T. TURGIDUM

Leaf moisture studies

Results of the leaf moisture studies indicated that within species, there was a relationship between leaf moisture and freezing resistance (Table 3). This result agreed with that reported previously where high moisture was negatively correlated with freezing hardiness of artificially freeze tested plants (Metcalf et al. 1970, Olien, 1967; Gullord, 1974; Gusta and Fowler, 1976) and field plants (Nass, 1983).

The hardy T. aestivum cv. Winoka and Kharkov had the lowest moistures and were significantly different and lower from that of Genesee and Frankenmuth. Augusta was only slightly hardier than Frankenmuth and its leaf moisture was

Table 3. Leaf moisture of selected T. tauschii, T. turgidum var durum, T. aestivum, H. vulgare and S. cereale accessions.

	Test1&2	Test3&4	Test5&6	Test7&8	Test9&10
Accession	Percentage of leaf moisture				
Kharkov (C)++	75.64a(A)				77.26ab(A)
Frankemuth (C)+	78.59cd				
Winoka (C)++	75.70ab				77.06a
Genesee (C)+	78.73d	80.54cd(A)	79.79cde(A)	80.35ab(A)	80.21c
Augusta (C)+	77.23bc				
Rosen (R)++	78.80d				
Dictoo (B)++	75.88ab				
Hudson (B)-	77.60cd	80.08bcd	79.64cde	80.55a	79.59bc
TA1651 T-		80.93cd			82.49d
TK92-467-1 (T)++		78.03a			76.98a
KU2119 (T)+		79.94bcd			
TK91-455-1 (T)++		78.45ab			
RL5257 (T)-		81.82d			
TK57-324 (T)-		79.45abc			
TK93-471++			76.25a		78.26ab
Ae1 (T)-			80.04de		
TK91-455-2 (T)++			78.54cd		78.81abc
P1293918 (D)++			78.49bcd		
P1352457 (D)-			80.42e		
KU2133 (T)+			77.85b		
P1326314 (D)+				79.96bc	
P1293422 (D)+				77.63d	
C115296 (D)-				81.53a	
P1344544 (D)++				78.96bc	
P1345703 (D)+				78.88c	
P1191380 (D)-				81.50a	

- Non-hardy accessions.
+ Moderately hardy accessions
++ Very hardy accessions.
(A): Treatments in the same column with one or more of the same letters are not significantly different at the .05 level, using DNMRT.
(B): H. vulgare accessions (barley).
(C): T. aestivum accessions (common wheat).
(D): T. turgidum var durum accessions (durum).
(R): S. cereale accessions (rye).
(T): T. tauschii accessions.

comparable to Frankenmuth and signiiicantly different from that of Genesee, a more tender line (Tests 1&2, Iable 3).

The hardy barley H. vulgare cv. Dictoo had signiiicantly lower leaf moisture than that of the tender Hudson.

For T. tauschii accessions tested, the hardy TK92-467-1 and TK91-455-1 had leaf moisture which was significantly different from the two tender lines TA1651 and RL5257 (tests 3&4, table 3). The tender Ae1 had signiiicantly higher leaf moisture than tne hardy TK93-471 and intermediate hardy KU2133 (Iest 5&6, Table 3). Leaf moisture of the tender TA1651 was also signiiicantly higher than that of the hardy TK92-467-1, TK91-455-2, TK93-471 and KU2113 of T. tauschii (Tests 9&10, Table 3). Leaf moisture of the hardy TK92-467-1 and TK93-471 were comparable to that of the hardy Winoka and Kharkov of T. aestivum while that of the non-hardy TA1651 was higher (tests 9&10, Iable 3). There were two exceptions, that of TK57-324 and TK91-455-1 (tests 3&4) and Ae1 and TK91-455-2 (Iests 5&6, Table 3) where leaf moisture of the tender (TK57-324 and Ae1) were not significantly different at the .05 level irom the hardy accessions (TK91-455-1 and TK91-455-2)

For T. turgidum, the hardy PI293918 had lower leaf moisture than that of the non-hardy PI352457 (Tests 5&6, Table 3). The tender cultivars CI15296 and PI191380 had the highest moisture. Leaf moisture of the hardy PI293422, PI326314, PI344544 and PI345703 were signiiicantly different from that of CI15296 and PI191380.

The relationship between moisture and hardiness did not hold true when comparisons were made between species. For example, leaf moisture of Rosen rye was higher than that of Winoka and Kharkov, yet Rosen was much hardier than Kharkov. In all tests, moisture of Hudson barley and Genesee wheat were comparable, but Genesee was hardier than Hudson. Dictoo had a moisture level comparative to Kharkov and Winoka, however, the two wheat cultivars were much hardier than Dictoo. This suggested that other factors which must be species specific were involved in freezing resistance. Results of Table 3 also showed that the three hardy durum cultivars PI293918, PI344544 and PI345705 had moisture levels comparable to the moderately hardy Genesee check. At the same moisture levels, some durums are hardier than common wheat. This also suggests that freezing hardiness is complex and hardiness is not just a matter of regulation of water content. Other factors such as plant composition, especially cryoprotectants produced during hardening play an important role in protecting critical tissue against freezing injury in hardy cereals with relatively high moisture content. Olien (1967) and Shearman et al. (1975) found that kinetic inhibitors of polysaccharide origin of hardy wheat and rye interfere with freezing processes and affect freezing hardiness of rye and wheat. Levitt (1980) reviewed other metabolic substances which were implicated in freezing hardiness.

Since moisture is critical in determining freezing

hardiness, " What could be the morphological and anatomical characteristics of plants that will determine moisture levels?". In plant system, the water conducting system or xylem vessels contain large amounts of very diluted water. Large xylem size with almost pure water would allow freezing to start and progress freely in xylem vessels. Indeed, in severely damaged crowns, the vascular system was observed to be destroyed. Olien (1980) suggested that moisture content may relate to xylem size. However, in this study, observations made on free hand sections indicated that there was no significant difference with regard to size of xylem vessels of hardy and non-hardy genotypes of the two species (Table 4). An observation on the parenchymatous layers of the root cross sections of five accessions (Fig. 2) indicated that cell sizes seemed to be different for the two species with the hexaploid having larger cells than the diploid. There may not be, however any difference in cell size within species. Furthermore, TA1651 of T. tauschii apparently has smaller parenchymatous cells than the T. aestivum cv. Winoka, yet TA1651 has higher leaf moisture and is more tender than Winoka (Fig. 2A and 2D). Although it is rational to believe that with small cell size, during freezing, water diffuses at a faster rate to the intercellular space, thus, intracellular freezing is avoided. However, it could also be argued that diffusion of water depends on membrane permeability as well. Furthermore, reducing water content also takes place during hardening and intracellular freezing is

TABLE 4. Numbers and size of roots, tillers, crown, stele and xylem of five selected hardy and non-hardy accessions of *T. aestivum* and *T. tauschii*.

	Number		Diameter (mm)				
Accession	Root	Tiller	Root	Tiller	Crown	Stele	Xylem
TA1651- (T)	9.75a(A)	6.25a(A)	.89a(A)	1.76bc(A)	3.66a(A)	.3486ab(A)	.0330a(A)
TK91-455-2++(T)	5.00b	5.00a	.42d	1.40c	2.26c	.2677b	.0287a
RL5257- (T)	6.25b	4.50a	.52c	1.58c	2.64b	.3007ab	.0339a
Winoka++ (C)	8.50ab	5.75a	.66b	2.08ab	3.62a	.3724a	.0351a
Genesee+ (C)	10.25a	5.75a	.85a	2.33a	3.98a	.3765a	.0362a

-: non-hardy accessions

+: moderately hardy

++: very hardy accessions

(A): Treatments in the same column with one or more of the same letters are not significantly different at the .05 level, using DNMRT.

(C): *T. aestivum*

(T): *T. tauschii*

FIGURE 2. Root cross sections of 5 genotypes of the hexaploid wheat T. aestivum and diploid T. tauschii with different degrees of freezing hardiness (mag. 83X)

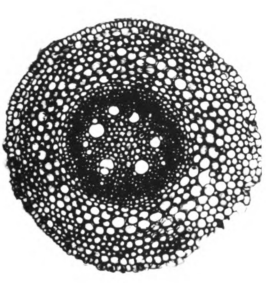
A. T. aestivum c. v. Winoka, a very hardy cultivar

B. T. aestivum c. v. Genesee, a moderately hardy cultivar

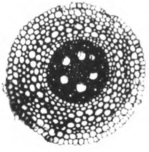
C. T. tauschii TK93-471, a very hardy accession

D. T. tauschii TA1651, a non-hardy accession

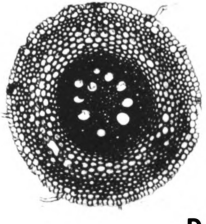
E. T. tauschii RL5257, a non-hardy accession



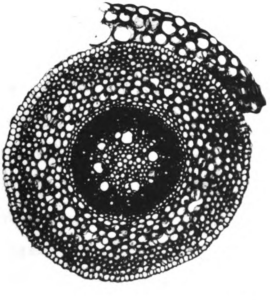
A



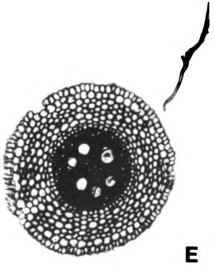
C



D



B



E

not a concern for hardened wheat. The cell water could not remain in a supercooled state to freeze intracellularly due to abundant ice nuclei in the soil. In HIF, it is the second stage of freezing where many water molecules freeze simultaneously which cause an explosion of ice crystal growth that is lethal to the tissues. Therefore, the ability of plant tissues to resist this stress is to have less water, which depends on the ability to take in water during mid-winter thaw. Roots which are very susceptible to freezing are the first to die. Water uptake at very low temperatures depends on the crowns to absorb water, which in turn, depends on the hydrophilic properties of the crown tissues. This may explain why conflicting results with regard to cell size have been encountered frequently in the literature (Levitt, 1956). Wiegand (1906) reported that more ice was found in the scale than in the young shoot of buds and twigs of 27 plant species in winter months. Wiegand attributed this to cell size differences. However, his data also indicated that Hicora alba, one of the species in which ice was not present at -18 C also had a large cell size. The cell size was even larger than that of some species where abundant amounts of ice were found in bud scales and leaves (Weigand, 1906). Recently, in many species, it has been shown that the bud scale act as a sink for water from bud primordia to migrate to (Quamme, 1975, George and Burke, 1977). This allowed the bud primordia with low moisture to remain supercooled and avoid freezing stress. Weigand's data

showed that moisture content of species that had no ice in the buds was lower than that of those with ice crystals in the buds (Weigand, 1906). Factors that are responsible for high moisture in plants depend on the ability of the plant components to bind with water or the hydrophilic properties of the substances that plants contain at a certain specific stage of their life cycle. This in turn depends on the plant response to its environment. In other words, environment and the internal genetic regulation allow certain substances to be produced as plants proceed through their life cycle. At various stages, i.e. during hardening, the plant has low moisture whereas during dehardening at higher temperatures, plant water content is high (Gusta and Fowler, 1976).

With regard to root diameter, the hardy T. aestivum c. v. Winoka had a significantly smaller root diameter than the cultivar Genesee of the same species. These two cultivars had a comparable crown diameter despite their difference in hardiness (Table 4). For the T. tauschii accessions tested, TK91-455-2 was the most hardy, its crown and root diameters were significantly different and smaller than the two other non-hardy lines. However, this relationship between hardiness and root and crown diameters did not extend to RL5257 and TA1651. These two accessions were not different in terms of hardiness in spite of the fact that RL5257 had smaller crown and root diameters than TA1651 (Table 4). Crown and stele diameters, root and tiller numbers were not related to hardiness in two species tested (Table 4).

The findings that anatomical and morphological measure ments, i.e. cell, xylem and stele sizes, root , crown and tiller diameters, were not related to freezing hardiness of 5 genotypes of the diploid T. tauschii and hexaploid T. aestivum indicated that hardiness of these lines is more of a physiological nature. The negative correlation of hardiness and leaf moisture is self-evident for the last statement. As pointed out by Levitt (1980), "when a relationship between hardiness and morphological or anatomical characteristics occur, this is indirect, due to the accompanying physiological factors". There was no such relationship on the morphological indicators measured on the 5 genotypes in this study. The physiological aspects of hardiness which is not only species but also genotypically specific may be of primary causes.

III. INTERSPECIFIC HYBRIDIZATION OF T. TAUSCHII AND T. TURGIDUM

Crossability and embryo culture

There was more suscessful fertilization with T. tauschii as the female. Failures were encountered when T. turgidum accessions were used as females. Therefore reciprocal crosses were abandoned. There were only 3 hybrids with T. durum as the female parents and 30 hybrids with T. tauschii as the female parent (Table 5). The cause of failures of fertilization of T. tauschii pollen in the durum wheat ovaries was not known.

Table 5. Number of interspecific hybrids of T. tauschii and T. turgidum var durum made in the spring and summer of 1982.

T. tauschii X T. turgidum var durum	DTE[a]	DCM[b]	#PTL[c]	CT[d]	F/S[e]	#S[f]	TS[g]
Hardy X hardy							
1.TK91-4552XPI293918	12	18	5	WK	2/7	4	4
Hardy X moderately hardy							
2.TK93-471XPI295422	12	41	3	WK	6/3	22	22
Moderately hardy X hardy							
3.KU2133XPI293918	14	19	1	WK	4/14	49	49
4.KU2119XPI344544	14	19	1	WK,TK	1/0,1/3	14,8	22
Hardy X nonhardy							
5.TK92-467XPI191380	19	20	1	WK	2/1	16	16
6.TK93-471XPI191380	12	14	3	WK	8/9	78	78
7.TK93-471XCI15296	19	19	1	WK	4/13	18	18
8.TK91-455-2XPI191380	15	24	5	WK	5/11	22	22
9.KU2119XCI15296	10	22,43	2	CS,G	0/1,1/0	0,4	4
Non hardy X hardy							
10.TK57-324XPI293918	9	13	1	CS,G	1/0,1/1	14,1	15
11.TK57-324XPI344544	11,19	13,30,20	5	CS,G,WK,TK	0/2,1/2,3/9,4/15	0,5,4,35	44
12.TA1651XPI326214	17	55	1	WK	1/1	7	7
13.AE1XPI344544	12	13	1	G,WK,TK	1/1,1/0,1/0	20,1,3	24
14.RL5257XPI293918	11,12	13,19	3	CS,G,WK	1/0,1/0,1/6	3,4,51	58
15.RL5263XPI404584	11	22	1	CS,G	0/1,1/1	0,11	11
16.RL5263XPI404583	died		1				
17.RL5263XM1CH	26	31	1	CS,G	0/1,2/1	25,7	32
18.TK57-324XPI372430	11	15	5	CS,G,WK	4/4,3/7,2/4	19,31,5	55
19.TA1644XM1CH	15	19	1	CS,G	0/2,2/1	0,16	16
20.TA1645XPI344544	14	12	2	CS,G,WK,TK	1/4,0/4,1/14,1/6	2,0,26,9	37
21.TK57-322XM1CH	11	18	1	WK,TK	4/4,3/5	9,19	8
22.TA1651XPI293918	died		1				
Non hardy X non hardy							
23.RL5257XCI15296	16	31	2	CS,G,WK,TK	1/0,2/0,6/4,1/0	1,4,40,13	58
24.TK57-324XCI15296	12	15	1	WK	1/1	6	6
25.KU2829XCI11246	14	23,33	2	CS,G,WK,TK	3/0,1/1,8/4,4/0	27,3,60,4	94
26.Ae1XPI383552	11	22	1	CS,WK	1/0,1/1	6,21	27
27.Ae1xCI15296	13	23	1	WK,TK	0/3,0/1	0	0
28.KU2071XPI372430	15	21	1	WK	3/11	36	36
29.RL5257XPI191380	14	28	1	WK	0/1	0	0
T. turgidum var durum X T. tauschii							
Hardy X non hardy							
30.PI326314xTA1651	19	15	1	WK	6/0	11	11
31.PI293918xTA1651	27	9	2	WK,TK	1/8,1/4	4,3	7
32.PI293918xTK57-324	14	74	1	WK	died	0	0
33.PI345703XTK57-324	14	60	1	WK	died	0	0

a: DTE: Days from date of pollination to date of embryo culture
b: DCM: Days in culture media
c: #PLT: number of plants from embryo culture
d: CT: colchicine techniques used: CS: Cauderon and Saigne's method (1961), G: Gill's method, WK: Winkle and Kimber's methods (1976), TK: Thiebaut and Kesha's method (1978), see materials and methods
e: F/S: Numbers of fertile clones/ Numbers of sterile clones. The ratio coresponds to the colchicine technique used in d, separated by a coma (,)
f: #S: numbers of seeds collected from each of the colchicine technique indicated in d separated by a coma (,)
g: TS: total seeds collected from clones that set seeds.

The age of the embryo for a successful culture also agreed with that reported by others for interspecific and intergeneric crosses of species within the tribe Triticinae which fall in the range of 10-19 days (Winkle and Kimber, 1978; Chueca et al, 1977, Thiebault and Kasha, 1979). In two instances, 26 to 27 days after pollination were required before the embryos were rescued (Table 5, RL5263xMICH and PI293918xTA1651). The embryo should have a well-defined scutellum and be well-advanced in differentiation for a successful development into plants in artificial culture. The embryo should be allowed to develop as much as possible on the plant and rescued as soon as there was a sign of embryo abortion. At this stage, the color of the seed coat turns from green to yellow. However, if the seed coat has turned completely yellow, chances were that the embryo had already deteriorated and would not germinate. Abnormal development of the embryo was also observed if the scutellum was not in contact with the media (Chueca et al, 1977). The majority of the plants germinated in the laboratory after 3 to 7 days in the media. In general, after 13-31 days in an artificial media, plants were ready to be transplanted into pots and kept in the greenhouse. In several cases, embryos were kept in culture for 43 to 74 days (Table 5). These embryos were recultured at least 3 times in fresh media before they finally developed into plants.

Colchicine Treatment

Since colchicine is a health hazard and expensive, it

Colchicine Treatment

Since colchicine is a health hazard and expensive, it was best if less colchicine was used with satisfactory results. Cauderon and Saigne (1961) and Gill's (personal communication) methods of colchicine treatment gave the highest survival rate but a large amount of colchicine was needed. The number of seeds collected from these two techniques was not very impressive. Winkle and Kimber's technique was superior to that of Thiebaut and Kasha in that it did not kill many clones. This technique was selected for subsequent treatments of clones with colchicine. Based on observations on the ratio of fertile/sterile clones and number of seed set, the colchicine treatment seemed to be more successful for certain hybrids than others. For example, the colchicine treated TA1651xPI293918 did not set seeds after the colchicine treatment, the original clones were transplanted several times and treated again with colchicine but they failed to produce seeds and died. Spontaneous doubling of chromosomes is not a rare phenomenon and is genetically determined (Gill, personal communication, 1982). Kihara (1944 cited Kihara, 1982) obtained the first interspecific hexaploid hybrid by spontaneous doubling of chromosomes. This was not the case in this study as clones that were not treated with colchicine remained sterile.

For accessions used in the interspecific crosses, there was no indication that hybrid lethality took place. This was different from that observed by Nishikawa (1960) where

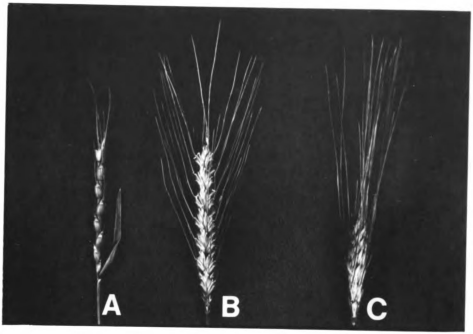
crosses involving the two species resulted in a range of hybrids from fully vital to lethal at various stages of development. Death of the hybrids in this study was due to colchicine treatment and/or to aging with a sterile flower.

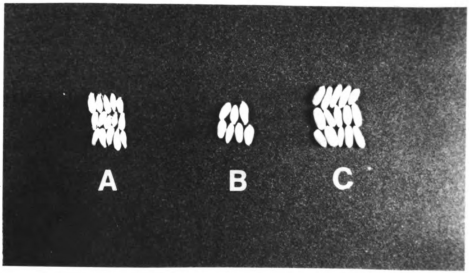
Morphological characteristics of the interspecific hybrids

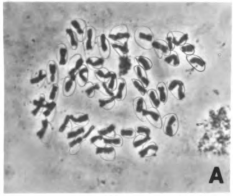
Phenotypically, the hybrids obtained matched the description of McFadden and Sears (1946). They were true hybrids. They had both the characteristics of the T. tauschii and T. turgidum parents. All hybrids were awned (Fig. 3) and had tall culms as the durum. From a distance, the young hybrids at the vegetative stage could be distinguished from their tetraploid parents by their dark green stem while the tetraploids had a lighter stem color. The surface of the durum culm was covered with a layer of gray wax which the hybrids did not have. Although the heads of the hybrids and their male parents were very similar (Fig. 3), the glume shape could be used to distinguished the two plant types. The hybrids had square glumes and the durum had pointed tip glumes. Hybrid plants had very broad leaves. Hybrid seeds were as large as that of the durum parents (Fig. 3). The hybrid TK57-324xPI372430 had the brown awn color similar to that of its male parent. The above described characteristics were apparently inherited from the tetraploid parents. On the other hand, the morphological characteristics of the diploid parents were also expressed in the hybrids i.e. higher tiller numbers, tight and square glume. In Ae1x PI344544, the shape of the seeds was as long and slender as

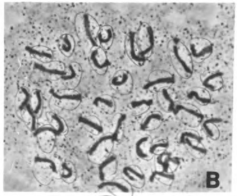
Fig. 3. Spikes and seeds of an interspecific hybrids and its parents A: T. tauschii, B: hybrid and C: T. turgidum var durum

Fig. 4. Somatic chromosomes of the interspecific hybrids of a random cell of A: RL5257XC115296 (56 chromosomes) and B: TK57-324XPI293918 (42 chromosomes)









Recount 1. 56?

42 ok

that of the diploid parent (Fig. 3). Several hybrids had very distinct characteristics of the diploid parents in that the color of their heads turn black when they mature. These were the hybrids of RL5257xCI15296, RL5257xPI293918 KU2119x CI15296, TK91-455-2xPI293918 and KU2133xPI293918. Two hybrids TK57-324xCI15296 and TK93-471xCI15296 had hulled seeds as that of the T. tauschii parents. In both cases, CI15296 served as the male parent. While the hybrid of RL5257xPI293918 had a black head, the hybrid of TK57-324 with the same male partner had a white head at the maturing stage.

Table 5 summarizes the number of plants obtained from the embryo culture, number of hybrids that survived the colchicine treatment, number of fertile and sterile clones and number of seeds collected from the fertile clones.

Cytological characteristics of the interspecific hybrids

Cytological studies indicated that there was tremendous variation in chromosome numbers of the colchicine treated interspecific hybrids derived from a single embryo as compared to that of the control lines T. aestivum cv. Genesee and the T. tauschii accessions TK57-324 and KU2133 (Table 6 and App. 9). There was a constant chromosome number for T. tauschii while a difference of only 3-4 chromosomes was found for T. aestivum cv. Genesee and for a single random root from the interspecific hybrid RL5257xCI15296. If this 3-4 chromosome number difference is established as the counting error in this study, then the variation in the

Table 6. Somatic chromosme counts of the colchicine treated interspecific hybrids of T. tauschii (2n=14) and T. turgidum var durum (2n=28)

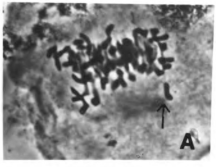
Hybrid Combination	Chromosome counts		
	#cells	min.	max.
1. TK93-471XPI191380	12	42	54
2. TK57-324XPI293918	15	33	43
3. TA1651XPI326314	15	32	51
4. RL5257XCI15296 (A)	11	42	53
4. RL5257XCI15296 (B)	10	31	56
5. KU2119XPI344544	11	41	55
6. TK92-467-1XPI191380 (c)	10	42	56
7. Ae1XPI344544	16	34	44
8. RL5263XPI404584	10	28	42
9. RL5257XPI293918 (c)	11	42	55
10.TK93-471XCI15296	10	42	52
15.RL5263XMICHURINKA	10	32	42
16.TK93-471XPI293422	11	35	45
17.KU2133XPI293918	11	39	54
18. TK91-455-2XPI191380(A)	11	29	54
18. TK91-455-2XPI191380(B)	13	35	55
TK57-324XPI344544 (d)	10	20	28
Ae1XPI344544 (d)	10	18	25
TK93-471XPI191380 (d)	10	12	27
KU2119XCI15296 (d)	10	16	27
RL5263XPI404583 (d)	10	17	21
TK57-324 (b)	10	14	14
KU2133 (b)	10	14	14
CI15296 (b)	16	26	41
GENESEE (b)	16	39	42
Rl5257XCI15296(e) Root 1	4	53	56
Root 2	10	51	55

(A) and (B): Plant from another embryo but of same hybrid combination
(b): Control genotypes
(c): Meiotic observations were made on these hybrid combination
(d): Plants that were not treated with colchicine
(e): Chromosome counts from a single root of the genotype.

number of chromosomes of interspecific hybrids was too large a number to fall in a counting error range. There was almost one genome increase for the colchicine treated hybrids with 56 chromosomes (Fig. 4A, Table 6). This erratic variation was explained by observations of meiotic chromosomes. Univalent, trivalents during metaphase I (Fig. 5 A and B) of hybrid combinations 4 (RL5257xCI15296) and 6 (TK92-467-1xPI191380) were observed. Lagards were also observed in anaphase I and II (Fig. 5 C and D). This explained the presence of micronulei in dyad and tetrad stages (Fig. 5 E and F). Chromosome elimination would certainly take place which could result in microspores with fewer chromosomes than other. An abnormal tetrad in which four microspores were formed but only three microspores contained chromosomes was found. The fourth microspore had only a trace of chromosomes (Fig. 5 G). For this cell, cytokinesis took place without complete karyokinesis which resulted in a higher number of chromosomes than that of the other two microspores.

Chromosome numbers of clones that were not treated with colchicine were also counted (Table 6). For RL5263xPI404583, the difference was negligible. For other hybrid combinations, variation in chromosome numbers ranged from 7 to 15 chromosomes (Table 6). The durum accession CI15296 had chromosome numbers ranging from 26-41 (Table 6). This was unexpected as the chromosome number of durum wheat had been determined as 28 (Feldman, 1976). Zohary et al. (1969)

Fig.5. Meiotic chromosomes: Univalents (open arrows) and trivalents (solid arrows) in Metaphase I of A: TK92-467x PI191380 and B: RL5257xPI293918. Lagging chromosomes in C: Anaphase I and D: Anaphase II of TK92-467-1xPI191380 (arrows). Micro nuclei formation in E: dyad and F: tetrad of RL5257xPI293918. G: Incomplete karyokinesis for a tetrad of TK92-467-1xPI191380: pollen grain on the bottom right has only a trace of chromosomes while that on the top right has more chromatin and a micronucleus.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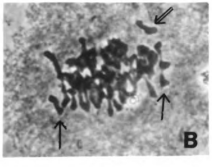
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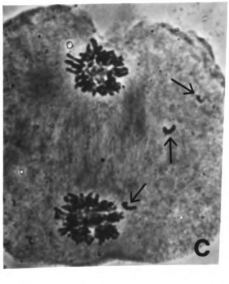
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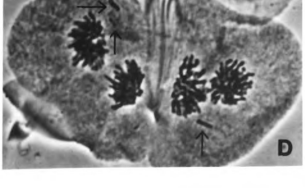
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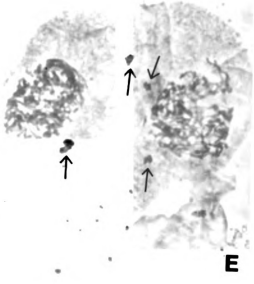
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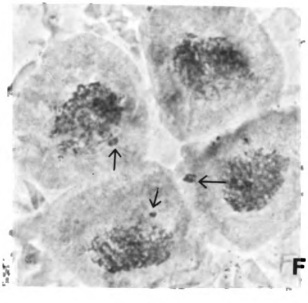
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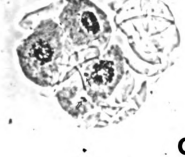
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observed that introgression of diploid to tetraploid species occurred very frequently in nature. It is not known if this type of introgresion occurred for the CI15296 grown in the greenhouse.

Variation in somatic chromosome counts of the interspecific hybrids agreed with that reported previously. The synthesized wheats from the two species AB and D genomes developed by Kihara were more or less unstable cytologically (Ono and Tanaka, 1967). Even for progenies derived from a hybrid plant between T. persicum and Aegilops squarosa strangulata with 21 bivalents showed variation in chromosome numbers from 38 to 44 (Tabushi, 1957). Aneuploid plants were observed even at generation 9 of an F6 plant with 42 chromosomes (Tabushi, 1964). Ono and Tanaka (1967) found that for some combinations, after 18 or 19 years, chromosome pairing remained unstable while variation in chromosome numbers was narrow. One strain even had more univalents after 18 or 19 years of synthesis than it had 6 to 8 years after synthesis. These strains synthesized by the Japanese did not go through colchicine treatment but were formed by unreduced gametes. This indicated that in the absence of colchicine, chromosome aberration was not an exception for synthesized hexaploid wheat. In this study, colchicine was used to double the chromosome number in order to restore fertility for the hybrids. This treatment might contribute to variation in chromosome number of the plants originating from a single embryo. Abnormality of the spindle fiber and germ pore was

induced by colchicine (Dover, 1972). Chromosome groupings were also induced by colchicine treatment (Levan and Lotty, 1949). Two groups of chromosomes were observed frequently. Berger et al. (1952) and Franzke and Ross (1952) and Davison et al (1983) reported that unequal distribution of chromosomes was prevalently observed between two groups. Davison et al. (1983) observed the chromosomes of two groups underwent restitution and binucleate interphase cells were formed. The distribution of chromosomes into two groups was functionally equivalent to chromosome nondisjunction. Aneuploid nuclei arose when the two groups contained different chromosome numbers. This may help in explaining the occasional failure to obtain the chromosome doubling by the use of colchicine as a result of unequal grouping of chromosomes after the treatment. To start with an aneuploid plant, one would expect that chromosome instability of the progenies would be a consequence. Colchicine treatment effect as well as the interspecific nature of the hybrids could account for the variation in chromosomes. Partial or complete chromosome elimination of a genome in interspecific and intergeneric embryos grown in culture is a well-known phenomenon. The genomes A, B, D that are contained in cultivated hexaploid wheats had more than 8,000 years to evolve together. In the interspecific hybrids which were created in this study, genomes AB and D came from accessions with a diverse range and tney may have evolved in different paths. Their genomes may be well-differentiated to the extent that genetic

harmony in the colchicine treated interspecific hybrids may not be attained. This might result in unsynchronization of chromosome segregation at meiosis. Multipolar spindles were not observed in this study, however, this phenomenon was observed frequently in interspecific and intergeneric hybrids which also gave rise to aneuploids.

Results of this study indicated that the phenomenon of chromosome doubling after the colchicine treatment cannot be taken for granted. It is always necessary to check on the chromosome constitution of the colchicine treated interspecific hybrids to determine their chromosome number and stability. In this study, since freezing resistance existed in both species, it may be wise to select for hybrid plants with at least 42 chromosomes to be used in the breeding program. There is evidence that amphiploid plants existed within the colchicine treated interspecific hybrids with 2n=42 (Fig. 4B). Furthermore, crossing with the current cultivars should be carried out as soon as the hybrids were formed to prevent the loss of some chromosomes which may carry freezing resistance. This practice will not only allow maximum genetic exchange of the cultivated wheat with all the freezing resistant genes in the two progenitor species but also assure stability for the population derived from crosses of the cultivated species and the colchicine treated interspecific hybrids.

IV. FREEZING RESISTANCE OF THE INTERSPECIFIC HYBRIDS OF *T. TAUSCHII* AND *T. TURGIDUM*

Within species, results in Table 7 and 8 agreed with that recorded previously. The known hardy accessions of both species in the freezing evaluation section also ranked high in HIF and LIF in these tests.

In general, the derived hybrids did not outperform the hardy parental accessions in either the HIF or LIF. They were either comparable to the hardiest parent or inferior to it (Table 7 and 8). Freezing resistant genes from both species were expressed in the recombined hybrid in HIF. Regardless of the species of the uncommon parent, hybrid performance depended upon the hardiness levels of the uncommon parent. For example, the cross of RL5257 with the hardy *T. turgidum* (D) PI293918 hybrid combination 4 (HC 4) resulted in a hardier hybrid than that with the non-hardy D CI15296 (HC 10) (Table 7). The cross of PI293918 with the hardier *T. tauschii* (T) TK57-324 resulted in a hardier hybrid (HC 11) than one with the tender T RL5257 (HC 4)(Table 7). Similarly, in HC 5 and 7 where the common hardy D PI344544 crossed with hardier T KU2119 resulted in a hardier hybrid than that with the more tender T Ae1 (Table 7). In addition, when the uncommon parents were comparable in freezing resistance, their crosses with a common parent gave rise to hybrids with comparable levels of hardiness. This was the case for hybrids HC 3, HC 8 and HC 9. The T

Table 7. High intensity freezing resistance of the colchicine treated hybrids of T. tauschii and T. turgidum var durum

Hyrid Combination	Hardiness Rating Values									
	T.tauschii		Hybrid		T.turgidum(D)		T. aestivum Gen		T. aestivum Win	
	(M) +	++	(M) +	++	(M) +	++	(M) +		(M) +	
1. TK57-324xPI372430	.94c	CD	1.55c	D	1.00c	E	2.14b		2.90a	
2. TK93-471xPI293422	2.65ab	A	2.70ab	A	2.15b	BC	2.14b		2.90a	
3. TK92-467-1xPI191380	2.50ab	AB	2.00bc	BCD	1.75c	CD	2.14bc		2.90a	
4. RL5257xPI293918	.25d	E	1.50c	D	2.90a	A	2.14b		2.90a	
5. Ae1xPI344544	.10e	E	.90d	E	2.90a	A	2.14b		2.90a	
6. KU2133xPI293918	1.35c	C	1.85bc	CD	2.45b	AB	2.14b		2.90a	
7. KU2119xPI344544	2.05b	B	2.05b	BCD	2.55ab	AB	2.14b		2.90a	
8. TK91-455-2xPI191380	2.40b	AB	1.89bc	BCD	1.75c	CD	2.14bc		2.90a	
9. TK93-471-1xPI191380	2.55ab	AB	1.65c	CD	1.75c	CD	2.14bc		2.90a	
10.RL5257xPI15296	.45d	DE	.55d	E	1.42c	DE	2.14b		2.90a	
11.TK57-324xPI293918	.94c	CD	2.55ab	A	3.00a	A	2.14b		2.90a	
12.RL5263xPI404584	.60e	DE	2.30bc	ABC	2.70ab	AB	2.14bc		2.90a	
Mean	1.39d		1.84c		2.19b		2.14b		2.90a	

(D): T. turgidum var durum
(M): Mean of two high intensity freezing tests at -15.3oC and 12.2oC (based on a rating scale 0= dead, 1= 1 or 2 roots, 2= 2 or 3 roots, 3= more than 3 roots (non-damage))
+: Treatments in the same row with a common letter are not significantly different at the .05 level, using DNMRT
++: Treatments in the same column with a common capital letter are not significantly different at the .05 level, using DNMRT.

Table 8. Low intensity freezing resistance of the colchicine treated hybrids of T. tauschii and T. turgidum var durum

Hybrid Combination	Hardiness Rating Values							
	T.tauschii		Hybrid		T.turgidum(D)		T.aestivum Gen.	T.aestivum Win.
	(M)+	++	(M) +	++	(M) +	++	(M) +	(M) +
1. TK57-324xP1372430	.68c	BCD	2.00a	AB	1.65ab	CD	1.78ab	1.84a
2. TK93-471xP1293422	2.00a	A	2.20a	AB	2.00a	ABCD	1.78a	1.84a
3. TK92-467-1xP1191580	1.20b	ABCD	1.58ab	BC	1.80ab	BCD	1.78ab	1.84a
4. RL5257xP1293918	.47d	CD	1.10cd	CD	2.20a	ABC	1.78ab	1.84ab
5. Ae1xP1344544	.45c	CD	2.00b	AB	2.80a	A	1.78b	1.84b
6. KU2133xPI293918	1.80bc	A	1.26c	BCD	2.60a	AB	1.78bc	1.84bc
7. KU2119xP13444544	1.45c	AB	2.47a	A	2.40ab	ABC	1.78c	1.84bc
8. TK91-455-2xP1191580	1.30a	ABC	1.95a	ABC	1.30a	D	1.78a	1.84a
9. TK93-471xPI191380	1.60a	A	2.15a	AB	1.30b	D	1.78a	1.84a
10.RL5257xC115296	.45c	CD	.60c	D	1.65ab	CD	1.78a	1.84a
11.TK57-324xP1293918	1.20c	ABCD	2.11ab	AB	2.50a	ABC	1.78bc	1.84bc
12.RL5263xP1404584	.39d	D	2.45ab	A	2.60a	AB	1.78c	1.84bc
Mean	1.08c		1.82ab		2.05a		1.78b	1.84ab

(D): T. turgidum var durum
(M): Mean of two low intensity freezing tests at -14.4oC (based on a rating scale of 0= dead, 1= 1 root, 2= 2 or 3 roots, 3= more than 3 roots=non-damage)
+: Treatments in the same row with a common letter are not significantly different at the .05 level, using DNMRT
++: Treatments in the same column with a common capital letter are not significantly different at the .05 level, using DNMRT.

accessions TK93-471, TK92-467-1 and TK91-455-2 were not significantly different in terms of freezing resistance. It was reasonable that their hybrids obtained from crosses with a common durum (PI191380) were comparable. Hybrid combination 2 between a hardy T and a moderately hardy D resulted in a hybrid with the highest hardiness level (2.7) and HC 10 with both non-hardy parents had the lowest freeze rating value. The predictability of the hybrid performance based on the parents indicated that freezing resistance genes were expressed in an additive fashion in these hybrids (Table 7).

Although some degree of predictability of hardiness of the hybrids was indicated for HIF, there were some exceptions for the HIF. Hybrid combination 11 between a less hardy T parent and a hardy D parent was significantly hardier than that derived from a moderately hardy T x hardy D (HC 6). Hybrid combination 4 between a non-hardy T x hardy D and HC 10 between the same D and a moderately hardy T were comparable. Non-additive types of gene action were indicated for the above described examples.

In the LIF, the unpredictable nature of hardiness of the hybrids based on parental hardiness was most pronounced. In crosses that had one common parent and the other uncommon parents at different hardiness levels, hardiness of the resulting hybrids were not significantly different. This was the case for HC 5 and 7 and 4 and 6 (Table 8). In addition, HC 1 and 10 had comparable parental hardiness but HC 1 was

hardier than HC 10. This suggested the behavior of the genes in a non additive fashion. This result of LIF agreed with that of Limin and Fowler (1982) in their low intensity type test. One difference between Limin's and Fowler's data and data of this study was that the average hardiness rating of their study was comparable to the tender T parents while the hybrids developed in this study was hardier than T and comparable to that of D parents (Table 8).

It was also observed that for HC 4 and 10 with a non hardy T parent (RL5257), hybrid performance tended to be depressed. With a hardier T, the hybrids were comparable to the T parent (HC 2 and 9). In HC 1, 3, 5 and 7, hardiness of the hybrids was comparable to the D parent. This indicated that freezing resistance from both the T and D parents were being expressed in the hybrid in the LIF freezing (Table 8).

Analysis of hardiness of the hybrids formed from crosses with a common parent and the other parents with different hardiness levels indicated that both additive and non additive types of gene action for HIF and non additive type of gene action for LIF were at work when allopolyploids were formed from T. tauschii and T. turgidum. The above results were in agreement with that reported previously for freezing hardiness in a variety of crop species. For cereals, using diallele analysis for barley (Eunnus et al, 1960), wheat (Gullord, 1974), both additive and non-additive effects of freezing resistance were reported.

The synthesized interspecific hybrids of HC 2 and 11

were as hardy in HIF as the most hardy hexaploid wheat Winoka in this study. This study of the HIF freezing resistance of T. tauschii, T. turgidum and T. aestivum, data study indicated that the maximum HIF freezing resistance of the accessions of the three species appears to be comparable.

In the HIF, eight HC were comparable to Genesee whereas 4 HC were less hardy. In LIF, the result for Winoka was lower than expected while that of T. aestivum c.v. Genesee check was in agreement with previous data. Difficulties in controlling the crown moisture might bring about the rather unusual results that are encountered from time to time in LIF. This has occurred in a previous experiment where Winoka was comparable to Genesee (App. 7). Comparisons, therefore, were made with Genesee for LIF. Table 8 indicated that two HC (7 and 12) were hardier and two HC (4 and 10) were less hardy than Genesee and the remainder was comparable to it.

Relatively, hardy lines in HIF were also hardy in LIF. A correlation coefficient of $r=.664$ ($P<.05$) was obtained for the means of hybrids in HIF and LIF tests whereas a correlation coefficient of $r=.891$ ($P<0.001$) was obtained for T and $r=.817$ ($P<.04$) was obtained for D. Freezing resistance of HC 2, 11 and 12 remained relatively stable in both test levels, although there was a slight change in the order of ranking. These three hybrids were among the most hardy as far as freezing resistance was concerned in both HIF and LIF tests.

Except for the low correlation coefficient ($r = .53$) and

low significant value ($P<.1$) for LIF of hybrid plants, results of data collected in fall 83 for HIF and LIF tests of 15 HC (with 9 HC used in this experiment) which were infected with Fusarium (App. 10 and 11) also supported that reported here. Correlation analysis of means of 9 HC that appeared in both fall and winter and spring tests showed a high correlation coefficients of .896 ($P<.0015$) for T, .947 ($P<.0003$) for hybrid plant and .845 ($P<.004$) for D in HIF. For LIF tests of 83 and 84, a correlation coefficient of .787 ($P<.01$) was obtained for T and .891 ($P<.001$) for D. Disease infection in this case did not appear to affect hardiness results of the test plants. Diseases have been known to reduce survival rate of winter barley after the stress of freezing (Smith and Olien, 1977, 1978).

The hardiest interspecific hybrids were only comparable to Winoka and they were not hardier than the hardiest parents. However, these hybrids may contain different genes or different levels of expression for hardiness from the ones that are now in current cultivars. Further study on genetic recombination of the interspecific hybrids and current cultivars will elucidate this point.

SUMMARY AND CONCLUSIONS

SUMMARY

Freezing resistance of 51 accessions of T. tauschii and 35 accessions of T. turgidum were evaluated under high and low intensity freezing conditions. Leaf moisture studies were conducted for selected hardy and non-hardy accessions of both species. Size of crown, tiller, root, stele and xylem diameters and number of roots and tillers were taken from 5 genotypes of hardy and non-hardy types of T. aestivum and T. tauschii. Interspecific hybrids were made from selected accessions of the progenitor species of wheat. Morphological and cytological observations and freezing resistance of the interspecific hybrids at both high and low intensity freezing levels were noted. Results indicated that:

1/ There was variation in freezing hardiness within both progenitor species of cultivated hexaploid wheat T. aestivum. The highest level of freezing resistance of both species only approached that of the hardy T. aestivum c. v. Winoka under either high and/or low intensity freezing.

2/ Within species, leaf moisture was related to freezing hardiness in the T. tauschii and T. turgidum accessions tested.

3/ There was no relationship between hardiness and number of tillers and roots or size of crown, tiller, root, stele and xylem of the 5 hardy and non-hardy genotypes measured. Freezing hardiness of the genotypes investigated appeared to be of a physiological nature rather than associated with morphological or anatomical factors.

4/ The interspecific hybrids were morphologically intermediate between the two parental species. They were true hybrids.

5/ The F3 generation of the colchicine treated hybrids was a mixture of euploids and aneuploids. Chromosome stability of the hybrids is in doubt.

6/ The interspecific hybrids were not hardier than the hardiest parents at either the high or low intensity freezing tests.

7/ Freezing hardiness of both species was expressed in the hybrids.

8/ In high intensity freezing, both additive and non-additive types of gene action were at work while non-additive types of gene action were more pronounced in low intensity freezing.

9/ Two interspecific hybrids had comparable hardiness to T. aestivum c. v. Winoka in the high intensity freezing.

CONCLUSIONS:

Data indicated that the highest level of freezing hardiness of the D and AB accessions evaluated was only comparable to that of T. aestivum c. v. Winoka. This may be ex-

plained by the fact that Tsunewaki's observations were only on growth habit (1969). Growth habit and freezing hardiness are two different genetic components of winter hardiness (Cahalan and Law, 1979; Gratius, 1980). The possession of the strong growth habit on the D genome does not mean that the genome D has high freezing hardiness. Law and Jenkins (1970) found two chromosomes containing genes for cold hardiness in the D genome and they agreed with Tsunewaki (1969) that the D genome has allowed the hexaploid wheat to be more adapted to continental conditions than the tetraploids. These ideas were also shared by Feldman (1976). However, a critical look at the study of Law and Jenkins indicates that the variety Capelle Deprez used is a more tender line than Genesee (Fowler and Gusta, 1979). It is not surprising that genes for cold hardiness were found on only 3 chromosomes with 2 on the D genome and 1 on the A genome for this variety. It is also arguable that with 2 chromosomes on the D genome out of the total 3 chromosomes that control cold hardiness in a tender accession such as Capelle Deprez should not be considered as high concentration. It is more meaningful if the chromosome substitution study is done on hardier lines, i.e., Kharkov, Winoka, North Star etc. It would not be surprising if more than 3 chromosomes on all three genomes which contain hardiness genes could be found since data of this study indicated that the AB genome contains accessions which were as hardy as that of the D and ABD genomes. This indicated that either genome A or B in the AB accessions

also contained hardiness genes. Natural populations of the species of the A genome were found in massive stands in cold areas at elevation as high as 2000 meters in southeastern Turkey and Iran (Harlan and Zohary, 1966). In addition, the A genome has been found to contain genes for hardiness (Law and Jenkins, 1970; Cahalan and Law, 1979). It is desirable to investigate not only the D but also the A and B genomes for freezing hardiness genes.

The data also indicated that the addition of the D genome into the AB genome or vice versa increased the hardiness of the hybrids (ABD genome) if the D or AB accessions were hardy. Furthermore, the maximum level of freezing hardiness of the accessions of AB, D and ABD genomes was comparable. The range of variation for the above was also comparable. This suggested that 1/ there was no complementary genetic system for freezing resistance that was brought together from both species and 2/ the genetic system for freezing hardiness of the progenitor species and their derivatives was similar.

Data of this study indicated that variation in the diploid, tetraploid and hexaploid with regard to freezing resistance was more or less of the same magnitude and freezing resistance of the hardiest accessions of the three species was comparable. This indicated that evolution of freezing hardiness genes of the three species may be similar.

It is not known if freezing hardiness genes of the two

progenitor species are similar to those which exist in the cultivated gene pool. Further studies on genetic analysis of crosses between the current cultivars and the interspecific hybrids will elucidate this point. Recessive genes for freezing resistance could be uncovered from these studies as well. These studies should further evaluate the breeding value of these hybrids. Chromosome instability is another factor that has to be considered in ultilizing these interspecific hybrids for freezing resistance improvement. Care should be taken in preventing loss of freezing genes on chromosomes that may be eliminated. It is necessary to obtain cytologically stable and hardy hybrids. Chromosome number of each individual plant should be checked before it is used in a crossing program.

Finally, it is necessary to recover the phenotype of the current cultivars by backcross breeding. Even if there are different freezing resistant genes with different levels of expression in an adapted background, by introducing freezing hardiness from wild species to cultivated species, many wild traits which are undesirable will be introduced as well. To get rid of these wild traits, one has to backcross to cultivated varieties, the genes for hardiness with low heritability may be lost altogether during backcrossing. Strategies have to be devised to recover the phenotype of the current cultivars and not lose the freezing hardy genes. This task is certainly not as easy as the transferring of traits controlled by major genes such as insect and disease

resistance from the close relatives to cultivated T. aestivum. Regardless of all of the optimism expressed by Grafius (1980), Feldman and Sears (1981), Johnson (1972) and Johnson and Waines (1977) with regard to using genetic variation from the wild relatives, especially the D genome, the prospect of improving a polygenic trait such as freezing resistance by genetic transfers from the close relatives will certainly not be one that will give a quick return.

APPENDIX

APPENDIX A

Homogeneity of variance and Lilliefors test for normality.

Homogeneity of variance

Example of setting aside several accessions with means less than .5 to obtain homogeneity of variance.

In test # 8, when all accessions were included in the analysis of variance, the Bartlett test indicated that the variance was not homogenous with the Chi-square value of 2074 for 33 df. When accession numbers 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 71, 72, 75, 80, 83 and 1 with means<.5 were set aside, the Bartlett test indicated that there was homogenous of variance with Chi-square value of 9.41542 for 13 df (Tables 9 and 10).

Test for normality of residuals (or experimental errors)

The Lilliefors test for normality was conducted as follows:

a/ Taking means freeze rating values of group of 5 plants for the remaining 14 accessions which showed homogeneity of variance.

b/ Calculating individual residual of each of the mean values generated from a by: 1/ Substracting the respective mean rating of each accession from the means of 5 plants each. and 2/ Dividing this value to the square root of the error mean square (obtained from the ANOVA based on means of 5 plants each of each accession) to obtain the residuals.

Values of residuals calculated were:

-3.11, -1.39, -1.12, -1.12, -.975, -.93, -.87, -.68, -.60, -.56, -.56, -.35, -.35, -.33, -.27, -.27, -.25, -.145,

-.145, -.1, -.06, .12, .14, .27, .37, .41, .41, .41, .415, .415, .50, .643, .643, .684, .726, .788, .91, .975, 1.09, 1.1, 1.14, 1.16, 1.24, 1.24, 1.35, 1.35, 1.47.

c/ Plotting these residuals values on the 95% Lilliefors Bound for Normal Samples. There were a total of 47 residuals. The critical curve used for checking normality of the sample was the one with n=50.

d/ Fig. 6 indicated that there was only very small deviation from normality at the intermediate range (not at the critical tail of the curve) and this permitted a regular analysis of variance for the remaining 14 accessions of freeze test #8.

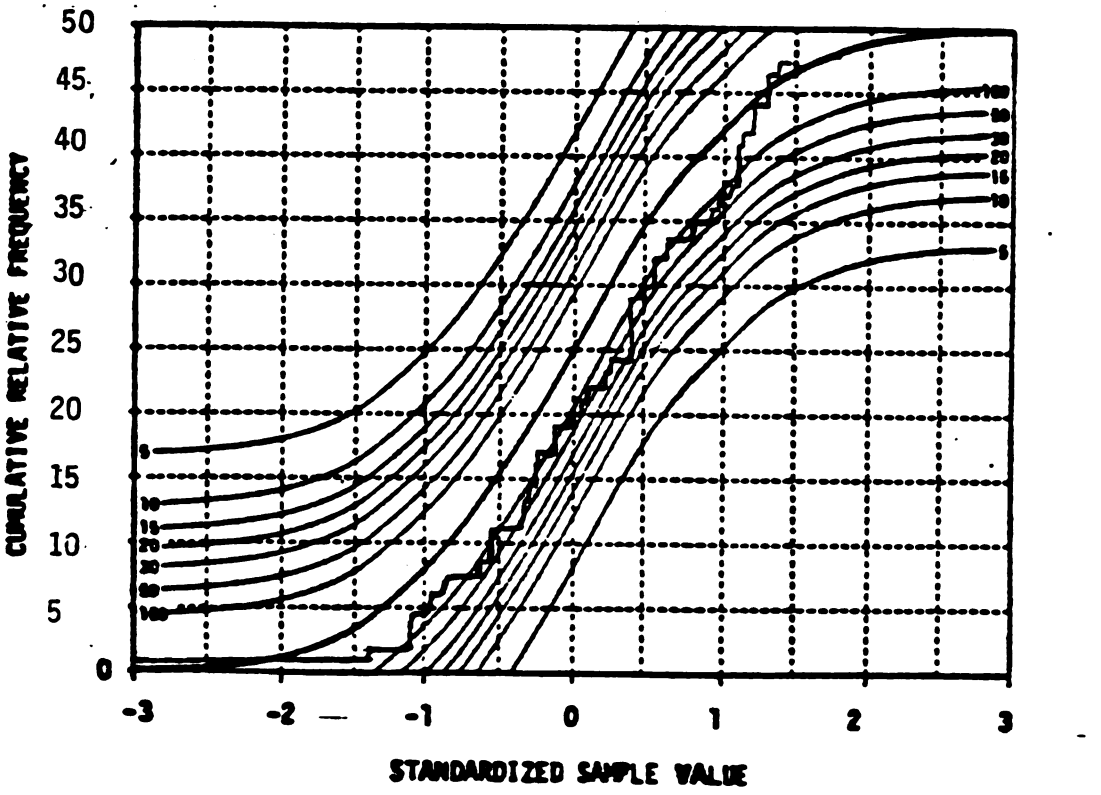

Fig. 6. Test for normality of the residuals of the freeze rating values of accessions used in test #8

Table 9. Analysis of variance of the low intensity freezing test #8 with all tested accessions included in the analysis.

One way ANOVA grouped over variable 0

with values from 1 to 83

Variable 1

	DF	SS	MS	F	PX
Total	605	810			
Between	33	460	13.94	22.78	0
Within	572	350	0.61		

Var. 0	VARIABLE Number	Sum	Average	SD	SE
1	31	12	0.39	0.84	0.14
2	42	84	2.00	1.04	0.12
52	19	47	2.47	0.70	0.18
53	16	36	2.25	0.93	0.20
54	13	13	1.00	1.15	0.22
55	16	32	2.00	1.15	0.20
56	15	0	0.00	0.00	0.20
57	16	0	0.00	0.00	0.20
58	18	5	0.28	0.57	0.18
59	19	0	0.00	0.00	0.18
60	19	0	0.00	0.00	0.18
61	17	0	0.00	0.00	0.19
62	21	0	0.00	0.00	0.17
63	15	0	0.00	0.00	0.20
64	19	0	0.00	0.00	0.18
65	18	0	0.00	0.00	0.18
66	18	2	0.11	0.47	0.18
67	17	1	0.06	0.24	0.19
68	17	4	0.24	0.75	0.19
69	17	26	1.53	1.37	0.19
70	19	27	1.42	1.17	0.18
71	21	6	0.29	0.78	0.17
72	10	1	0.10	0.32	0.25
73	14	32	2.29	1.14	0.21
74	13	25	1.92	1.12	0.22
75	18	2	0.11	0.47	0.18
76	14	27	1.93	1.00	0.21
77	17	33	1.94	1.03	0.19
78	13	0	0.00	0.00	0.22
79	15	10	0.67	1.18	0.20
80	18	8	0.44	0.92	0.18
81	17	28	1.65	1.11	0.19
82	18	10	0.56	0.98	0.18
83	16	2	0.13	0.50	0.20
TOTAL	606	473	0.78	1.16	0.05
Within				0.78	

BARTLETT'S TEST
CHI-SQUARE= 2074.967
Number of DF= 33
ONE OR MORE FACTOR LEVELS HAVE A VARIANCE OF ZERO.
THIS WILL CAUSE A LARGE CHI SQUARE.

Table 10. Analysis of variance of the low intensity freezing test # 8 with only selected accessions with mean >.5 included in the analysis.

ONE WAY ANOVA GROUPED OVER VARIABLE 2
STRAIN
WITH VALUES FROM 1 TO 83

VARIABLE 8
RATING 0=0 1=1 2=2 3=3 TO 5

	DF	SS	MS	F	PX
TOTAL	249	350			
BETWEEN	13	76	5.85	5.04	0
WITHIN	236	274	1.16		

VAR. 2	VARIABLE NUMBER	SUM	AVERAGE	SD	SE
2	42	84	2.00	1.04	.17
52	19	47	2.47	.70	.25
53	16	36	2.25	.93	.27
54	13	13	1.00	1.15	.30
55	16	32	2.00	1.15	.27
69	17	26	1.53	1.37	.26
70	19	27	1.42	1.17	.23
73	14	32	2.29	1.14	.24
74	13	25	1.92	1.12	.30
76	14	27	1.93	1.00	.29
77	17	33	1.94	1.03	.24
79	15	10	.67	1.18	.28
81	17	28	1.65	1.11	.24
82	18	10	.56	.98	.25
TOTAL	250	430	1.72	1.19	.07
WITHIN				1.08	

BARTLETT'S TEST
CHI-SQUARE= 9.41942
NUMBER OF df= 13

Appendix B

LS media for embryo culture.

LS major	g/liter medium
NH_4NO_3	1.65
KNO_3	1.90
$CaCl_2.2H_2O$	.44
$MgSO_4.7H_2O$	.37
KH_2PO_4	.17
LS minor	**mg/liter medium**
H_3PO_4	6.2
$MnSO_4.H_2O$	.0168
$ZnSO_4.7H_2O$	.0106
KI	.83
$Na_2MoO_4.2H_2O$	.25
$CuSO_4.5H_2O$	.025
$CoCl_2.6H_2O$	.025
	g/liter medium
Na_2EDTA	.0373
$FeSO_4.7H_2O$	.0278
	mg/liter medium
Vitamin	
Thiamin	.2
Inositol	100
Amino acid	
Tyrosine	10
Arginine	10
Glycine	10
	g/liter medium
Sucrose	30
Agar	8

Adjust to pH 5.7 with KOH .01N, the medium autoclaved for 15 min. under 15psi. Let it cool down and pour to Falcon Petri plates (5.5 cm dia.) under aseptic condition. Media can be stored in the cold room for a month.

Linsmaier E. M. and F. Skoog. 1965. Organic factor requirements of tobacco tissue cultures. Physiol. Plant. 18: 100-127

Appendix C

1.Preparation of Aceto- carmine

1. Heat 100ml of 45% acetic acid (55ml H20 + 45 ml of glacial acetic acid).
2. Add .5g of carmine.
3. Stir thoroughly.
4. Add drop by drop of saturated ferric hydroxide to the hot solution until it turns dark but does not precipitate. A bead of citrate ferric can be added to the hot solution as a substitute if Fe2(OH)3 is not available.
5. Cool the solution, filter and store in the refrigerator.

2. Preparation of Feulgen Staining

1. Weigh out 1g basic fuschin (Certified for Feulgen nuclear reaction)
2. Weigh out 3g. sodium metabisulfite.
3. Boil 200ml of distilled water to 100 °C.
4. Cool to 80 °C.
5. Add basic fuschin to H20 and stir.
6. Cool to 50 °C.
7. Add 30ml of 1N HCl to disolve stain.
8. Pour into brown stock bottle.
9. Add sodium metabisulfide into solution in brown bottle.
10. Shake well and let the bottle stand in the dark (cover brown bottle with aluminum foil).
11. Add .5 to .75 g neutral bone charcoal to decolorize the solution.
12. Shake well and store in the refrigerator.
13. The stain is ready for use in 24 hours. Stain should be clear; if not, it will not work.
14. A small amount of the stock solution should be filtered into a small brown dropping bottle for use. Both filtered stain and stock stain must be kept in the refrigerator.

Appendix D

Procedure for seed treatment with Vitavax

1. Take 10ml of Vitavax (emulsion form)
2. Add 70ml of water
3. Stir thoroughly
4. Add the seeds to be treated to the Vitavax solution
5. Stir the seeds in vitavax solution for 1 min.
6. Decan the seeds from the solution, using a small strainer.
7. Leave the treated seeds to be dried on layers of paper towell or old newspapers.
8. The solution can be re-used again for other batches of seeds to be treated.
9. Make sure that the solution does not contain the seeds from the previous treatment to prevent seed contamination.

Appendix E

Screening of T. tauschii for high intensity freezing resistance.

Temperature: Accession	Test#1 -11.7°C Rating(M)	Test#3 -12.2°C Rating(M)	Temperature Accessions	Test#5 -14°C Rating(M)	Test#11 -12.2°C Rating(M)
TK92-467-1	2.45	2.89	TK91-455-1	2.70	2.65
TK91-455-2	2.37	2.30	KU2133	2.33	(N)
TK91-454	1.89	2.20	P1428563	2.05	(N)
TK75-405	1.80	2.10	GENESEE	1.58	.54
TK93-471	1.76	2.53	HUDSON (B)	1.10	.11
TK75-400	1.67	2.40	RL5263	.95	1.42
TK64-354	1.40	1.20	KU2829A	.55	1.24
TK57-317	1.19	1.55	RL5257	.50	.28
TK57-318	.82	.74	Ae3	.50	1.00
KU2119	.79	2.22	KU2122	.42	1.41
KU2017	.75	1.79	P1220641	.37	.12
KU2071	.70	.63	P1317392	.30	.25
GENESEE (C)	.70	1.53	P1317394 (S)	.25	.54
TK57-324	.60	1.05	TA1642	.10	.06
TK61-324	.60	.47	TA1644	.10	.00
HUDSON (B)	.58	1.13	P1220642 (S)	.10	.06
H80-105-3	.53	1.42	P1431599	.10	.82
KU2113	.47	1.16	P1431600	.10	.50
AE1	.16	.28	KU2021	.05	.06
9435	.16	.60	TA1645	.00	(N)
TA1647	.15	.21	KU20-9	.00	(N)
WIS 2086 (S)	.05	(N)	KU2010	.00	.11
TA1651	.05	.00	KU2085 (S)	.00	(N)
P1276985	.00	.32	KU2110	.00	.11
KU2066	.00	.31	KU2832	(N)	1.25
			9364	.00	.67
			9436	.00	.26
			P1220326	.00	.11
			P1431598	.00	.33
			P1431601	.00	.93
			Winoka (C)	(N)	1.50
			Frankemuth(C)	(N)	.67
			Augusta (C)	(N)	.94
			B6310 (C)	(N)	.44
			P1326314 (D)	(N)	.59
			P1345703 (D)	(N)	.31
			P1293918 (D)	(N)	1.73
			C115296 (D)	(N)	.29
Error M.S.	.26	.29		.30	.29
LSD min.	.54	.69		.55	.68
max.	.72	.75		.85	.99

(B) Barley, (C) Common wheat, (D) Durum accessions.
(S) Spring habit.
(N) not available.
(M) Means of 3 to 4 replications, each rep. consists of 5 plants.

Appendix F

Screening of T. turgidum var durum for high intensity freezing resistance.

Temperature	Test#7 -11°C	Test#9 -14.4°C	Test#13 -14°C
Accession	Rating(M)	Rating(M)	Rating(M)
CI15296	2.35	.35	1.24
GENESEE (C)	2.29	1.65	.89
PI293918	2.24	2.95	1.94
HUDSON (B)	1.97	.25	.31
PI326314	1.94	1.84	1.41
PI428688	1.94	2.47	1.55
PI404583	1.78	1.35	1.00
PI404584	1.78	2.05	1.56
PI345703	1.67	2.75	1.60
PI428689	1.50	2.06	.64
CI11246	1.50	.18	.60
CI15304	1.22	.05	.59
PI352440	.44	.11	.07
PI344544	1.15	1.75	1.78
PI372432	.94	1.75	1.11
PI352456	.89	.00	.81
PI352380	.69	.05	.00
CI11245 (S)	.67	.00	(N)
PI352402 (S)	.47	.00	(N)
PI372430	1.21	.06	.46
PI383357	.41	.00	.52
PI428690	.35	.00	.18
CI15386 (S)	.20	.00	(N)
PI352454	.18	.00	.18
PI418199	.18	.00	.17
PI352457	.16	.00	.00
PI352452	.11	.00	(N)
PI352453	.10	.00	(N)
PI352455 (S)	.05	.00	.38
PI352371 (S)	.00	.00	.21
PI352372 (S)	.00	.00	.00
PI352450	.00	.00	.00
PI352451 (S)	.00	.15	(N)
PI383359	.00	.00	.85
PI293422	(N)	(N)	1.83
PI372431	(N)	(N)	1.80
PI191380	(N)	(N)	.90
Augusta (C)	(N)	(N)	1.84
B6310 (C)	(N)	(N)	1.20
Frankemuth (C)	(N)	(N)	.85
Error M. S.	.15	.26	.24
LSD min.	.40	.62	.51
max.	.71	.85	.89

(M) Means of 3 to 4 replications, each rep. consists of 5 plants
(B) Barley, (C) Common wheat accessions
(S) Spring habit, (N) not available.

Appendix G

Screening of T. tauschii for low intensity freezing resistance.

Temperature Accession	Test#2 -14.3°C Rating(M)	Test#4 -16°C Rating(M)	Temperature Accessions	Test#6 -13.3°C Rating(M)	Test#14 -15°C Rating(M)
			TK91-455-1	(N)	2.72
TK93-471	2.45	1.50	GENESEE	2.34	.17
TK92-467-1	2.33	1.25	Ae3	2.25	1.12
TK91-455-2	2.71	(N)	P1431599	1.85	.28
TK57-317	2.45	1.17	RL5263	1.50	.82
KU2119	2.45	.86	P1431600	1.45	.00
TK91-454	2.52	.75	KU2829A	1.45	.29
KU2071	2.10	.68	KU2110	1.40	.00
TK75-405	2.48	.60	P1317394 (S)	1.28	.00
TK57-318	2.38	.40	KU2010	1.40	.06
TK64-354	2.67	.35	P1431598	1.15	.47
9435	(N)	.32	KU2021	1.11	.20
TK57-322	2.88	.26	TA1644	1.05	.37
KU2113	(N)	.22	P1317392	.94	.00
GENESEE	2.85	.16	9436	.90	.00
TK61-324	2.58	.15	KU20-9	.85	(N)
KU2017	1.85	.15	RL5257	.80	.33
TK57-324	2.71	.10	TA1642	.79	.06
TA1647	(N)	.05	TA1645	.75	.06
WIS2086	1.10	(N)	HUDSON (B)	.69	.00
TA1651	(N)	.00	KU2085 (S)	.68	(N)
Ae1	1.67	.00	KU2832	.63	.00
P1276985	1.00	.00	P1220642	.42	.00
KU2066	(N)	.00	KU2122	(N)	1.05
HUDSON (B)	.54	.00	P1431601	(N)	.69
			P1220641	(N)	.00
			P1220326	(N)	.06
			Winoka (C)	(N)	.89
			Augusta (C)	(N)	.50
			Frankemuth(C)	(N)	.26
			B6310 (C)	(N)	.28
			P1293918 (D)	(N)	.40
			P1326314 (D)	(N)	.86
			P1345703 (D)	(N)	.70
			C115296 (D)	(N)	.17
Error M.S.	.21	.28		.33	.31
LSD min.	.61	.62		.66	.71
max.	.65	.76		.81	.95

(M) Means of 3 to 4 replications, each rep. consists of 5 plants.
(B) Barley, (C) Common wheat, (D) Durum accessions.
(S) Spring habit, (N) not available.

Appendix H

Screening of *T. turgidum* var *durum* for low intensity freezing resistance.

Temp. Accession	Test#8 -14°C Rating(M)	Test#10 -16°C Rating(M)	Test#12 -17°C Rating(M)
PI293918	2.47	2.20	1.68
PI404583	2.29	1.71	1.88
PI326314	2.25	1.69	.94
GENESEE (C)	2.00	1.02	1.12
PI345703	2.00	2.37	2.05
PI428688	1.93	2.38	2.28
PI428689	1.94	1.50	1.92
PI404584	1.92	2.20	2.00
CI15296	1.65	.37	.38
PI372430	1.53	1.00	.58
PI372432	1.42	1.56	1.94
PI344544	1.00	1.85	1.94
CI11245 (S)	.65	.17	(N)
CI15304	.56	.89	.78
CI11246	.44	.92	.32
HUDSON (B)	.39	.05	.00
PI383357	.29	.11	.50
PI352402 (S)	.28	.19	(N)
PI352440	.24	.78	.20
CI15386 (S)	.13	.00	(N)
PI418199	.11	.00	.00
PI352457 (S)	.11	.15	.33
PI383359	.10	.00	.00
PI352380	.06	.05	.16
PI352371 (S)	.00	.00	.11
PI352372 (S)	.00	.00	.13
PI352450	.00	.00	.00
PI352451 (S)	.00	.00	(N)
PI352452	.00	.00	(N)
PI352453	.00	.05	(N)
PI342454	.00	.11	.18
PI352455 (S)	.00	.00	.33
PI352456	.00	.24	.19
PI428690	.00	.31	.28
PI293422	(N)	(N)	2.14
PI372431	(N)	(N)	1.89
PI191380	(N)	(N)	.50
Frankemuth(C)	(N)	(N)	.58
B6310 (C)	(N)	(N)	.89
Error M. S.	.23	.28	.22
LSD min.	.59	.65	.56
max.	.80	.97	.85

(M) Means of 3 to 4 replications, each rep. consists of 5 plants.
(B) Barley, (C) Common wheat accessions, (S) Spring habit,
(N) not available.

Appendix I

Somatic chromosme counts of the colchicine treated interspecific hybrids of T. tauschii (2n=14) and T. durum (2n=28)

Hybrid Combination	Chromosome counts														
	Cell Number														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. TK93-471XPI191380	42	42	42	42	42	44	45	47	50	51	53	54			
2. TK57-324XPI293918	33	34	35	36	36	38	39	39	40	40	42	42	42	43	43
3. TA1651XPI326314	32	33	38	38	39	39	40	42	42	42	42	42	42	42	51
4. RL5257XCI15296(A)	42	42	42	44	45	45	47	48	48	49	53				
4. RL5257XCI15296(B)	31	48	51	51	53	54	55	55	56	56					
5. KU2119XPI344544	41	42	42	44	44	44	46	49	53	53	55				
6. TK92-467-1XPI191380(c)	42	42	43	43	44	46	47	50	50	56					
7. Ae1XPI344544	34	34	35	35	36	37	37	39	39	39	40	40	41	41	43	44
8. RL5263XPI404584	28	29	33	34	36	40	40	41	41	42					
9. RL5257XPI293918(c)	42	46	46	48	49	51	51	52	53	55	55				
10.TK93-471XCI15296	42	44	46	47	48	48	49	50	51	52					
15.RL5263XMICHURINKA	32	34	34	40	40	40	40	42	42	42					
16.TK93-471XPI293422	35	37	39	40	40	41	42	42	42	42	45				
17.KU2133XPI293918	39	42	44	48	48	49	49	50	51	53	54				
18.TK91-455-2XPI191380(A)	29	30	36	45	45	46	47	49	51	54	54				
18.TK91-455-2XPI191380(B)	35	42	44	44	45	46	46	47	47	49	49	53	55		
TK57-324XPI344544(d)	20	21	21	21	21	21	23	23	25	28					
Ae1XPI344544(d)	18	18	19	19	20	21	21	21	22	25					
TK93-471XPI191380(d)	12	16	18	19	21	22	22	23	25	27					
KU2119XCI15296(d)	16	18	19	19	20	20	20	21	22	27					
RL5263XPI404583(d)	17	18	18	19	19	20	20	20	21	21					
TK57-324(b)	14	14	14	14	14	14	14	14	14	14					
KU2133(b)	14	14	14	14	14	14	14	14	14	14					
CI15296(b)	26	26	27	27	28	30	33	35	35	35	36	36	36	38	39	41
GENESEE(b)	39	40	40	41	41	42	42	42	42	42	42	42	42	42	42	42
RI5257XCI15296(e) Root 1	53	53	56	56											
Root 2	51	52	52	52	52	53	54	55	55	55					

(A) and (B): Plant from another embryo but of the same hybrid combination
(b)Control genotypes
(c): Meiotic observations were made on these hybrid combination
(d): Clones that were not treated with colchicine
(e): Chromosome counts from a single root of the genotype

Appendix J

High intensity freezing resistance of the Fusarium infected colchicine treated interspecific hybrids of T. tauschii and T. turgidum var durum tested in the fall of 1983.

Hybrid combination	Hardiness Rating Values		
	T. tauschii	Hybrid	T. turgidum(D)
1. TK57-324XPI372430	1.40	1.55	1.10
2. TK93-471XPI293422	(N)	(N)	(N)
3. TK92-467-1XPI191380	2.15	2.75	2.25
4. RL5257X293918	.37	1.40	2.20
5. AE1XPI344544	.45	1.15	2.40
6. KU2133XPI293918	(N)	(N)	(N)
7. KU2119XPI344544	1.50	2.00	2.70
8. TK91-455-2XPI191380	(N)	(N)	(N)
9. TK93-471-1XPI191380	1.55	1.65	1.70
10.RL5257XCI15296	.42	.40	1.74
11.TK57-324XPI293918	1.30	2.80	2.50
12.RL5263XPI404584	.65	2.50	2.75
13.TA1651XPI326314	.15	1.60	1.56
14.PI326314XTA1651	.20	.60	1.84
15.TK57-324XCI15296	1.15	1.35	1.30
16.TK57-324XPI344544	1.80	2.00	2.60
17.RL5263XMICH	.70	1.60	2.35
18.TK93-471XCI15296	1.75	1.75	1.40
GENESEE	1.15		
WINOKA	2.03		

(D): T. turgidum var durum
(N): Not available

Appendix K

Low intensity freezing resistance of the *Fusarium* infected colchicine treated interspecific hybrids of *T. tauschii* and *T. turgidum* var *durum* tested in the fall of 1983.

Hybrid combination	Hardiness Rating Values		
	T. tauschii	Hybrid	*T. turgidum*(D)
1. TK57-324XPI372430	.35	1.10	1.10
2. TK93-471XPI293422	(N)	(N)	(N)
3. TK92-467-1XPI191380	1.42	1.50	1.60
4. RL5257X293918	.55	.95	2.20
5. AE1XPI344544	.70	.50	2.05
6. KU2133XPI293918	(N)	(N)	(N)
7. KU2119XPI344544	1.65	.65	2.05
8. TK91-455-2XPI191380	(N)	(N)	(N)
9. TK93-471-1XPI191380	1.15	1.55	.75
10. RL5257XCI15296	.65	.15	.65
11. TK57-324XPI293918	.79	1.85	2.16
12. RL5263XPI404584	.63	1.95	2.60
13. TA1651XPI326314	.20	.80	1.35
14. PI326314XTA1651	.00	.95	1.37
15. TK57-324XCI15296	.65	.60	.75
16. TK57-324XPI344544	.63	1.10	2.70
17. RL5263XM1CH	.50	.85	1.40
18. TK93-471XCI15296	1.80	.55	.70
GENESEE	2.01		
WINOKA	2.50		

(D): *T. turgidum* var *durum*
(N): not available

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