GENETIC VARIABILITY OF TOLERANCE TO FREEZING IN SOUTH AFRICAN WHEAT CULTIVARS

by

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List of abbreviations

ABA	abscisic acid
ANOVA	analysis of variance
APS	ammonium persulphate
Bis	NN'-methylenebisacrylamide
CB10	number of protein bands in coleoptiles after 10 days (hardened)
CB20	number of protein bands in coleoptiles after 20 days (hardened)
CB3/10	difference in number of protein bands between unhardened and hardened coleoptiles
CB3	number of protein bands in coleoptiles after 3 days (unhardened)
CB30	number of protein bands in coleoptiles after 30 days (hardened)
CB4	number of protein bands in coleoptiles after 4 days (unhardened)
CB4/20	difference in number of protein bands between unhardened and hardened coleoptiles
CB5	number of protein bands in coleoptiles after 5 days (unhardened)
CB5/30	difference in number of protein bands between unhardened and hardened coleoptiles
cm	centimetre(s)
cont.	continue
CV	coefficient of variance
DB	double band
DF	degrees of freedom
dH_2O	distilled water

EDTA	ethylenedinitrilotra-acetic acid disodium salt
et al.	*Et alii*
etc.	*Et cetra*
Fig.	figure
g	gram(s)
GCA	general combining ability
h	hour(s)
h^2	heritability
H_2O	water
ha	hectare(s)
HCL	hydrochloric acid
HMW	high molecular weight
kDa	kilo Daltons
LL-12°C	leaf length at -12°C
LL-6°C	leaf length at -6°C
LSD	least significant difference
LT50	lethal temperature
m	metre(s)
mA	milli Ampere
mg	milligram(s)
ml	millilitre(s)
mm	millimetre(s)
mM	milli Molar
Mpa	milli Pascal

NS	not significant
PMSF	phenylmethylsulfonyl fluoride
r	correlation
R	Rand
RB10	number of protein bands in roots after 10 days (hardened)
RB20	number of protein bands in roots after 20 days (hardened)
RB3	number of protein bands in roots after 3 days (unhardened)
RB3/10	difference in number of protein bands between unhardened and hardened roots
RB30	number of protein bands in roots after 30 days (hardened)
RB4/20	difference in number of protein bands between unhardened and hardened roots
RB4	number of protein bands in roots after 4 days (unhardened)
RB5	number of protein bands in roots after 5 days (unhardened)
RB5/30	difference in number of protein bands between unhardened and hardened roots
RL-6°C	root length at -6°C
RL-12°C	root length at -12°C
rpm	revolutions per minute
S-12°C	percentage survival at -12°C
S-6°C	percentage survival at -6°C
SCA	specific combining ability
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate poly acrylamide gel electrophoresis

TEMED	N,N,N',N'-tetrametylenediamine
Tris	tris[hydroxymethyl]aminomethane
t/ha	ton per hectare
°C	degrees Celsius
$°Ch^{-1}$	degrees Celsius per hour
µl	microlitre(s)
°N	degrees north
°S	degrees south
>	larger than
<	smaller than
%	percentage
%LLR	percentage leaf length reduction
%RLR	percentage root length reduction
%SR	percentage survival reduction

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Chapter 1

Introduction.

On 5 November 1922 two archeologists, Howard Carter and Lord Carnavon, discovered the tomb of king Tutankhamen in Egypt. As predicted they found amazing treasures and artworks in the tomb. However they also found bags of wheat, an indication that wheat was already a primary food source almost 3000 years ago (Fensham, 1979). Even today in the twentieth century wheat is still an important food source.

Wheat is cultivated in all nine provinces of South Africa, with the Free State being the largest wheat producing province, contributing around 1.08 million metric tons of the total production in 1997/98 (Table 1.1). During 1997/98 nearly 2.2 million metric tons of wheat were produced In South Africa. This wheat was produced on 1.38 million hectares with an average yield of 1.5 t/ha.

Up to 1945, South Africa could supply adequate wheat for domestic wheat consumption. After 1945, the demand for wheat products showed an increasing trend, which has partly been associated with population and economic growth in South Africa (Marasas, Anandajayasekeram, Tolmay, Martella, Purchase and Prinsloo, 1997). Domestic consumption has increased by approximately four percent over the past two decades, and amounted to 2.5 million metric tons in 1997/98. Fig.1.1 shows the wheat production in South Africa compared to the domestic consumption (Willemse, 1999).

Human consumption accounts for almost all of the domestic requirements and is determined by the demand for end products, like bread and flour. A total of 1.126 million metric tons of flour and meal were used by bakeries and other processors during 1992/1993. Wheat for animal feed is not consciously produced in South Africa,

Table 1.1 Wheat production and production areas

Province	1990/91		1991/92		1992/93		1993/94		1994/95		1995/96		1996/97		1997/98	
	Ha*	Ton	Ha	Ton	Ha	Ton	Ha	Ton	Ha	Ton	Ha	Ton	Ha	Ton	Ha	Ton
Western Cape	334576	489354	327563	496479	334932	627251	417997	742796	397795	738144	400800	819175	403000	808000	400000	550000
Northern Cape	53011	227342	53632	227953	55031	276393	52465	252262	55268	384796	56000	278242	68000	345000	65000	284000
Free State	1030630	713403	946454	1107414	301321	249946	529319	788955	515482	451371	819000	638828	702000	1217000	790000	1080000
Eastern Cape	5838	17387	7029	11200	5942	17885	15209	24808	16110	37753	16550	29817	17000	18000	13000	22000
Kwazulu-Natal	6377	24145	5378	20391	4530	19940	4304	15021	3635	22023	3500	12412	5000	24000	5800	21000
Mpumalanga	20875	67810	17097	67430	7285	44761	7824	37788	12095	72149	10500	36708	17000	76000	22000	88000
Northern Province	3620	12276	3258	16921	203	8577	10327	39034	10827	46700	11400	13503	20000	65000	17000	68000
Gauteng	2992	8217	2130	8676	324	9138	2235	7597	2861	9057	2800	9347	1800	8000	3000	16500
North West	92713	142437	71215	176521	33932	62182	25118	67063	25418	90248	42600	130480	60000	13900	66500	154000
Total	1550632	1702371	1433756	2132985	743500	1316073	1064798	1975324	1039491	1852241	1363150	1968512	1293800	2574900	1382300	2283500

* Hectares

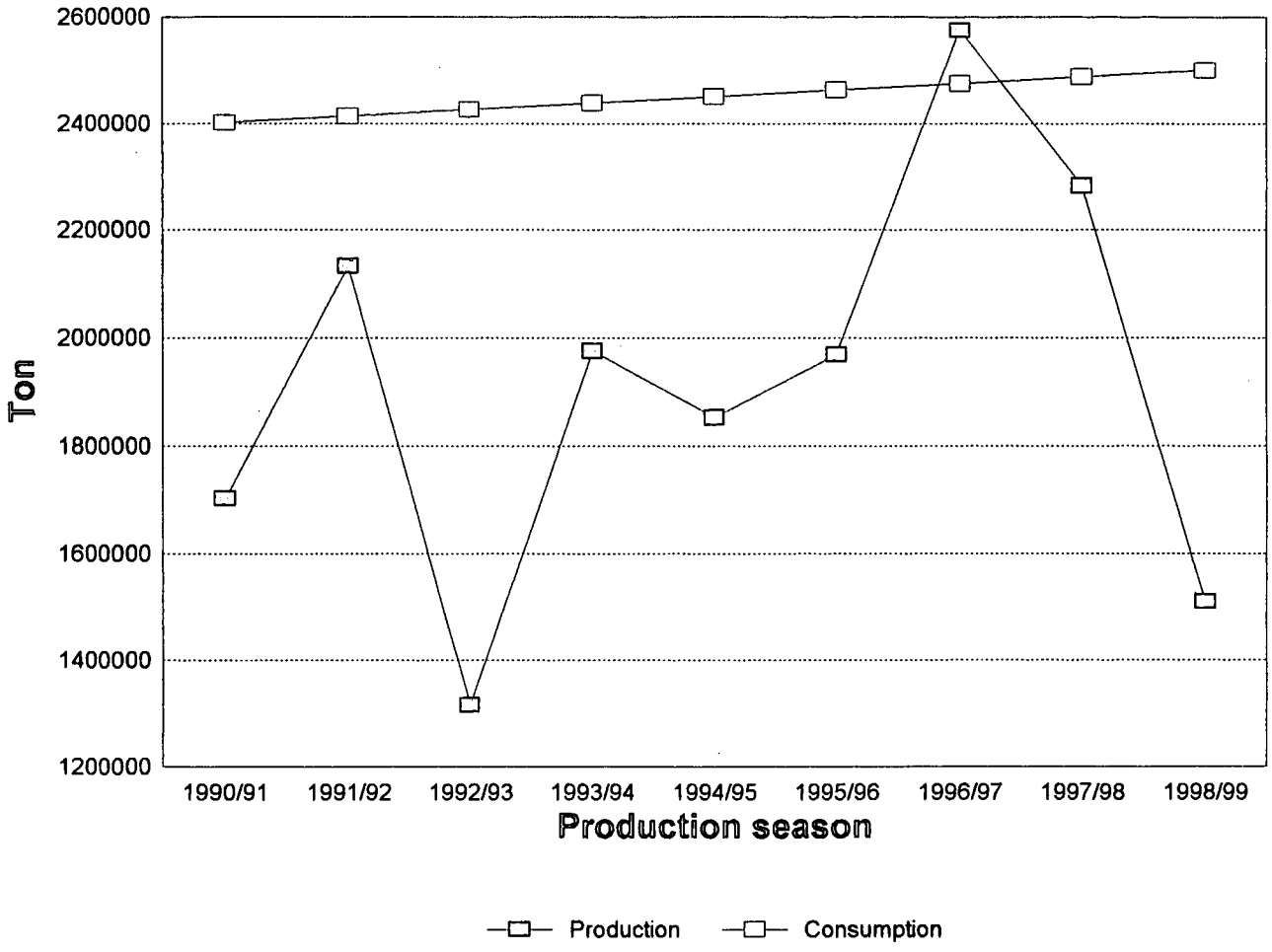

Fig. 1.1 South African wheat production and consumption.

and only becomes available from local production, depending on the quality as influenced by climatic and cultivating conditions. The availability of wheat for animal feed therefore, varies each year. Wheat bran is an important feed source and is available as a byproduct from the milling process. Local yellow maize is usually used as feed grain (Marasas *et al.*, 1997).

Apart from domestic production, South Africa is both an importer and exporter of wheat. This is necessary because of the erratic climate in the major wheat producing areas. This forces the local industry to deal with temporary shortages or surpluses occasionally, while still meeting domestic requirements, ensuring sufficient carry-over stock, and maintaining traditional export markets in Africa. The consumption of wheat in 1995/1996 exceeded production, necessitating an estimated 665 000 metric tons of wheat to be imported to meet the domestic shortfall and maintain traditional export markets. A stable export market for South African wheat has been established in certain African countries, which is maintained even if surplus wheat is not available in the local market. Exports of wheat to African countries are estimated at 150 000 metric tons for the year 1995/1996 (Wheat Board Annual Report, 1995/1996).

Nearly 544 394 hectares of wheat were insured against frost damage over the last ten years of which 47 062 hectares were damaged by frost. This led to an average loss of income close to 6.7 million Rands annually (Pienaar, 1999; Vosloo, 1999). This high level of frost damage indicates that wheat cultivars with a high level of tolerance to freezing are needed.

The level of tolerance to freezing, genetic variability, combining ability and inheritance of tolerance to freezing in South African wheat cultivars has never been studied. To cultivate wheat and run a breeding programme successfully this

information is essential.

The objectives of this study were:

- to screen South African wheat cultivars for tolerance to freezing at -6°C and -12°C
- to study the genetic variability for tolerance to freezing of South African wheat cultivars at -6°C and -12°C
- to study the use of high molecular weight proteins to screen for tolerance to freezing.

Chapter 2

Literature review

2.1 Wheat

2.1.1 General. Wheat (*Triticum aestivum* L.) is grown all around the world. More land is devoted to the production of wheat than to any other commercial crop. Wheat is also the main food grain consumed directly by humans (Briggle and Curtis, 1987; Cook and Veseth, 1991).

Wheat is a cool season crop, but it flourishes in many agronomic and climatic zones. Production is concentrated between latitudes 30 to 60°N and between 27 to 40°S (Percival, 1921; Nuttonson, 1955). However, it is known that wheat is also grown outside these areas, for example in the Northern hemisphere wheat is cultivated from within the Arctic circle to the equator, provided that it is cultivated at locations of sufficiently high elevation (Briggle and Curtis, 1987).

The minimum temperature for growth is 3 to 4°C, the optimum temperature is 25°C and the maximum is 30 to 32°C (Briggle, 1980). Wheat grows well in well-drained soils from sea level to 3000m above sea level. However, in some tropical countries wheat is grown from 2000 to 3200m above sea level (Briggle and Curtis, 1987). In Tibet wheat is cultivated at 4270 to 4570m above sea level (Percival, 1921). Wheat can be cultivated in most areas with an annual rainfall ranging from 250 to 1750mm (Briggle and Curtis, 1987). Most of the wheat growing areas, however have a rainfall of 375 to 875mm annually (Leonard and Martin, 1963).

A crop of wheat is harvested somewhere in the world during every month of the year (Percival, 1921; Briggle, 1980). In the temperate zone of the northern hemisphere most of the harvest occurs between April and September, while in the southern

hemisphere the harvest occurs from October to January (Briggle and Curtis, 1987).

Bread has been a basic food for man throughout recorded history, and will probably be for a much longer period. It remains the principal food product made from wheat (Briggle and Curtis, 1987; Cook and Veseth, 1991). A significant amount of wheat is also used as animal feed. The actual quantity depends entirely on the price of wheat in relation to maize (*Zea maize* L.) and other feed grains (Briggle and Curtis, 1987). Small amounts of wheat and wheat flour are also used by various industries. Wheat starch is used in the industry for laundering, paper laminating and corrugating, adhesives, textiles, wallpaper and paper additives (Miller, 1974). Wheat starch is derived from low grade or damaged wheat, or from the least desirable flour fractions after milling (Briggle and Curtis, 1987).

2.1.2 Classification. All wheats belong to the genus *Triticum* (Table 2.1). This genus along with *Hordeum*, *Secale*, *Aegilops*, *Bromus* and *Agropyron* make up the most important groups in the grass family *Gramineae* (*Poaceae*)(Cook and Veseth, 1991). In the grass family one or more flowered spikelets are sessile and alternate on opposite sides of a rachis, forming a true spike (Knott, 1987). In 1753 Linnaeus proposed the first classification of wheats based on morphological and physiological differences (Bozzini, 1988). In 1918, Sakamura showed that the wheats fall into three categories (Knott, 1987; Bozzini, 1988; Cook and Veseth, 1991).

The basic number of chromosomes in wheat is seven. Diploid wheats have 14 chromosomes (two sets of seven chromosomes, one set from each parent). Tetraploid wheats have 28 chromosomes (four sets of seven chromosomes), and hexaploid wheats have 42 chromosomes (six sets of seven chromosomes) (Cook and Veseth,

Table 2.1. Botanical classification of wheat (Cook and Veseth, 1991)

Kingdom	Plant
Class	Angiospermae
Subclass	Monocotyledoneae
Family	Gramineae
Sub group	Hordeae
Genus	*Triticum*

1991, Feldman, 1976)

2.1.3 Origin and evolution. The origin and early domestication of wheat were traced through their chromosomal makeup. Table 2.2 lists all the wild, primitive and modern cultivated wheats (Feldman, 1976).

The chromosome makeup of wheat must be understood before its evolution can be interpreted. Wheat falls into three categories (Table 2.3). One group has the usual two sets of chromosomes (diploid), the second group has four sets of chromosomes (tetraploid), and the third group has six sets of chromosomes (hexaploid) (Sears, 1981; Cook and Veseth, 1991).

Wheats are subdivided into species according to similarities of their basic chromosome sets, referred to as their respective genomes. Genomes are identified as A, B, C and D and the corresponding diploids are AA, BB, CC, and DD respectively (Cook and Veseth, 1991).

Evidence indicates that diploid wheats such as einkorn were among the first wheats to be harvested and cultivated. Einkorn (AA), was developed from a type of wild grass native to the arid regions of Asia (Orth and Shellenberger, 1988; Cook and Veseth, 1991). These grasses were adapted to the steppes or semi-arid areas, characterised by winter rains and dry summers, and developed with available autumn-winter moisture to reach maturity in late spring or summer (Bozzini, 1988). Wild einkorn wheats can still be found in Turkey, Iraq and Iran (Cook and Veseth, 1991).

Tetraploid wheats are thought to have arisen in prehistoric times through a natural cross between two diploid species. This process, called amphidiploidy, was made possible when the pollen from one diploid species contributed both sets of its

Table 2.2. Wild, primitive and modern wheats (Feldman, 1976)

Wild wheats	
Triticum monococcum var. *boeoticum*	diploid (AA)
Triticum tauschii	diploid (DD)
Triticum turgidum var. *dicoccoides*	tetraploid (AABB)
Triticum timopheevii	tetraploid (AADD)
Triticum aestivum	hexaploid (AABBDD)
Primitive cultivated wheats (6000-8000 B.C.)	
Triticum monococcum var. *monococcum*	einkorn, diploid (AA)
Triticum turgidum var. *dicoccum*	emmer, tetraploid (AABB)
Triticum turgidum var. *durum*	tetraploid (AABB)
Triticum aestivum var. *spelta*	hexaploid (AABBDD)
Triticum aestivum var. *compactum*	hexaploid (AABBDD)
Triticum aestivum var. *aestivum*	hexaploid (AABBDD)
Modern cultivated wheats	
Triticum turgidum var. *durum*	durum, tetraploid (AABB)
Triticum aestivum var. *spelta*	spelt, hexaploid (AABBDD)
Triticum aestivum var. *compactum*	club wheat, hexaploid (AABBDD)
Triticum aestivum var. *aestivum*	bread wheat, hexaploid (AABBDD)

Table 2.3. The classification of wheat according to ploidy levels (Cook and Veseth, 1991)

Diploids (2n=14, AA) - einkorn wheat

Triticum boeoticum Boiss. - wild einkorn wheat

Triticum monococcum L. - einkorn wheat

Tetraploids (2n=28, AABB) - durum and emmer wheat

Triticum durum Desf. - durum wheat

Triticum dicoccum Schrank - emmer wheat

Triticum dicoccoides Korn. - wild emmer wheat

Triticum turgidum L. - poulard, rivet or cone wheat

Triticum polonicum L. - polish wheat

Triticum carthilicum Nevski. - persian wheat

Triticum persicum Vav. - persian wheat

Triticum timopheevii

Hexaploids (2n=42, AABBDD) - bread wheat

Triticum aestivum L. - bread wheat

Triticum compactum Host. - club wheat

Triticum spelta L. - spelt wheat

Triticum macha Dek. & Men. - spelt wheat

Triticum sphaerococcum Perc. - shot wheat

Triticum vavilovii Jakubz.

Triticum zhukovskyi Men. & Er.

seven chromosomes(AA), rather than just the one set (A) typically donated in the usual hybridisation, and these combined through the usual process of fertilization with both sets of chromosomes of another diploid species (BB). This match produced a new fertile species, known as wild emmer, with four sets of chromosomes (AABB), representing both the AA and BB genomes (Cook and Veseth, 1991).

It was concluded that hexaploid bread wheat originated by the process of amphidiploidy. The cross may have occurred between a wild diploid wheat like species with the genome DD and domesticated emmer (AABB). The result was a new fertile species with six sets of chromosomes (AABBDD) Fig. 2.1 shows the proposed origins and relationships of wheat (Knott, 1987; Orth and Shellenberger, 1988; Cook and Veseth, 1991).

2.2 Physiology of cold stress

2.2.1 Temperature. Temperature stress inhibits the growth, development and thus, the yield of wheat in at least three ways. Firstly the development from emergence through tillering, stem elongation, flowering and grain fill is driven by growing degree-days or accumulated heat units. Secondly, wheat requires a certain minimum time within a favourable temperature range to go from seed to seed. The ideal temperatures for the growth and development of wheat are between 10 and 24°C. Providing no other limiting factors such as too much or too little water or light, influence the normal plant development, the accumulation of growing degree-days within this temperature range leads to large, well tillered plants with wide leaves and big heads. Thirdly, wheat plants are sensitive to temperature extremes during critical stages of development. The results of these extremes include frost injury to the internodes and florets, winterkill,

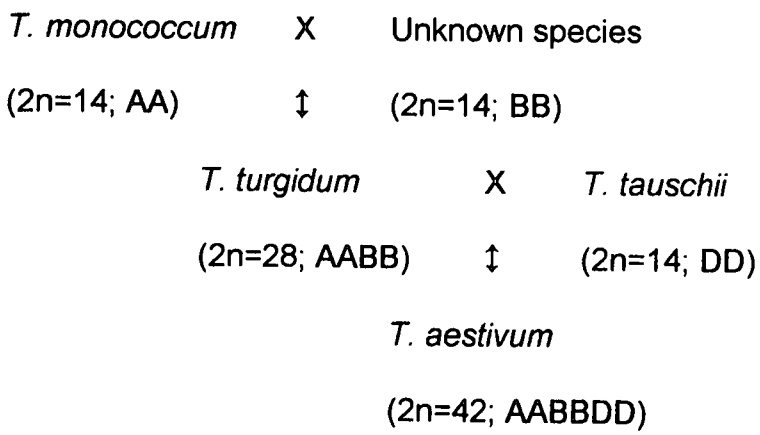

Fig. 2.1. Proposed origins and relationships of wheat (Cook and Veseth, 1991)

frozen leaves or roots and heat damage (Cook and Veseth, 1991). Figure 2.2 illustrates the favourable, unfavourable and lethal effects of various temperatures on wheat plants.

2.2.2 The freezing of plants. At sub zero temperatures, ice forms in the intracellular spaces where water is the purest. In nature, changes in air temperature are slow (1 to 10°Ch^{-1}) while changes in soil temperature are even slower (1 to 5°Ch^{-1}). With such slow cooling rates, water can readily migrate to areas of lower vapour pressure created by the ice. Ice does not grow uniformly through the tissue, but rather at preferred sites, which can accommodate the growing crystall. Ice may first form in the veins or between the epidermis and mesophyll of leaves (Idle, 1966; Olien, Marchetti and Chomyn, 1968). In the crowns, ice may form in the vascular systems where large amounts of free water are available (Olien, 1981). This may disrupt the vascular connections between the upper and lower part of the plant.

Ice is partitioned from the protoplasm by the cell wall and plasma membrane. Since no ice forms inside the protoplast, the cell fluids remain in a liquid state. Not all the water in a cell freezes at once due to dissolved cell solutes and its interaction with cellular components (Glasstone, 1964).

Prior to freezing the freezing point depression of cells is generally in the range of -1 to -1.5°C (0.5 - 0.7 osmolal). More than 60% of the crown tissue water content is frozen at -4 °C , which results in an increase in the cellular fluids to approximately 5 osmolal. This increased solute concentration prevents the interior of the cell from freezing. At -10 °C, over 90% of the total freezable water is frozen and the cell becomes severely dehydrated. At -20 °C, nearly all of the freezable water is frozen,

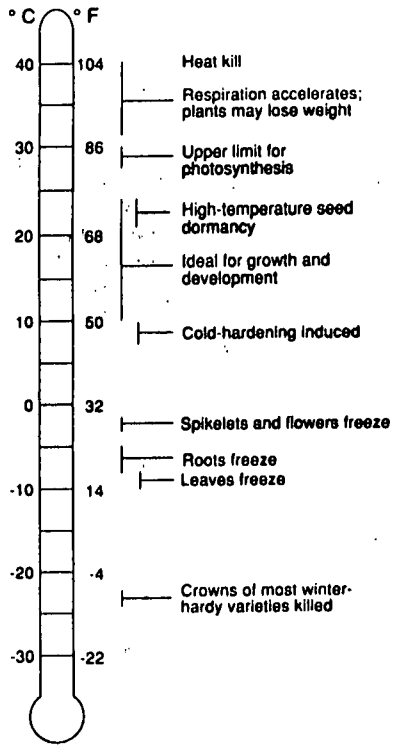

Fig. 2.2. Favourable and unfavourable temperatures for wheat (Cook and Veseth, 1991).

except for a small fraction that is held tightly by the cellular compounds (it is not available for freezing even at -40 °C). The quantity of water that migrates from the cell to the growing ice crystal is therefore a function of the temperature (Gusta, Burke and Kapoor, 1975).

During freezing-induced dehydration, the plasma membrane remains attached to the cell wall, causing the cell to collapse (Levitt, 1956; Salcheva and Samygin, 1963; Siminovitch and Scarth, 1938). If the extracellular spaces are too small to accommodate the growing ice crystall, the cells are crushed, ruptured, or separated by the splitting of the cell walls along the middle lamella (Levitt, 1956). As the cell wall collapses, the protoplasm is pushed against the outer cell wall in the form of a ring and the plasmatic strains break (Alden and Herman, 1971). Upon thawing, the cells become rehydrated and expand back to their original volume. If the cell has been killed by the freeze-thaw cycle, the protoplast may break away from the cell wall and shrink in size. This phenomenon has been termed pseudo or frost plasmolysis. If the cells have been injured by freezing, their membranes leak and the cells are unable to regain full turgor (Gusta and Chen, 1987).

The reduced semi-permeability properties of cell membranes because of freeze injuries has led many researchers to suggest that the membranes are the primary site of injury. Cellular compounds start to leak from the tissue immediately upon thawing, with no measurable lag period. Depending on how homogenous the tissue is, there is little or no ion leakage prior to exposure to the killing temperature. Results based on microscopic observations, fluorescent changes in cells under frozen conditions, and nuclear magnetic resonance studies suggest that freezing injury occurs during the freezing process upon cooling below the frost-killing temperature (Salcheva and

Samygin, 1963; Rajashekar, Gusta and Burke 1980).

2.2.3 Sensitivity of tissue to freezing. Wheat leaves can tolerate -7 to -9°C when not hardened and -12 to -18°C when hardened, depending on the cultivar and the age of the leaves. Mature leaves are more sensitive to cold than young and developing leaves. Roots are killed at temperatures below -3 to -5°C, but such freezing happens rarely, since they are generally protected from freezing by their location in the soil. Reproductive tissues may be injured at temperatures as mild as -2 to -3°C (Cook and Veseth, 1991).

2.2.4 Frost resistance. Harrington (1936) cautioned that attempts to introduce disease resistance or other traits into wheat may reduce its frost resistance. Marcellos and Burke (1979) demonstrated that leaves of several unhardened spring wheat cultivars are able to tolerate temperatures as low as -7 to -9°C. Leaves of the spring wheat cultivars Kite, Manitou and Oxley in the hardened state tolerated -9 to -10 °C before injury become apparent. The leaves of Norstar and Cheyenne winter wheats can tolerate -18 and -12 °C respectively, when cold hardened (Marcellos and Burke, 1979; Chen, Gusta and Fowler, 1983; Gusta and Chen, 1987;). Gusta and Chen (1987) observed that leaves of field grown Columbus and Neepawa wheat collected from mid to late July survived temperatures as low as -8 °C. Temperatures at the time of collection were between 25 and 30 °C, indicating that the leaves possess considerable frost tolerance in the absence of hardening conditions (Gusta and Chen, 1987). Although the leaves of wheat plants may possess a considerable degree of frost tolerance, the reproductive tissues of the developing ear are considerably less resistant

to freezing and may be injured at -1.8°C (Single and Marcellos, 1974).

Frost damage to young, developing ears is usually not recognised until after heading is complete, but it can occur anytime after the onset of stem elongation. If the growing point is killed before heading, the main stem or tiller will die (Fig. 2.3). When this happens, new shoots develope from the base of the plant. Heads damaged by frost are empty and bleached white. Frost damage to heads during the boot or early heading stages may affect the entire head, the tip only (Fig. 2.4), the base only (Fig. 2.5), both the tip and the base, or occasionally the middle section only.

Frost damage is sometimes confused with drought damage which can also result in empty, bleached white tips on the heads (Cook and Veseth, 1991). The floral parts within the flag leaf sheath may avoid freezing by super cooling, even though the rest of the plant is frozen. This is due to the inability of the ice front to travel across the node of the stem or rachis to the developing ear. Once the ear has emerged, however it may be nucleated by atmospheric ice or infected with ice-nucleating bacteria. Depending on how glaucous the lemma, palea and awns are, the exposed floral and reproductive tissues may super cool even when exposed to frost (Single and Marcellos, 1974). Apparently the waxy surface prevents contact between atmospheric freezing nuclei and internal tissue moisture (Gusta and Chen, 1987).

Super cooling provides one mechanism by which frost sensitive plants or tissues have developed to avoid freeze damage. Lindlow, Arny and Upper (1982) suggest that plants do not contain intrinsic ice nuclei active above -8 to -11°C. Marcellos and Single (1976) demonstrate that dry wheat leaves will super cool to temperatures as low as -14°C, with the majority of leaves super cooling to -10°C. Gusta and Chen (1987) determined the degree of super cooling of various plant organs

A B C D

Fig. 2.3. Cold damage to the growing points of wheat plants before heading (A.B,C = damaged, D = undamaged).

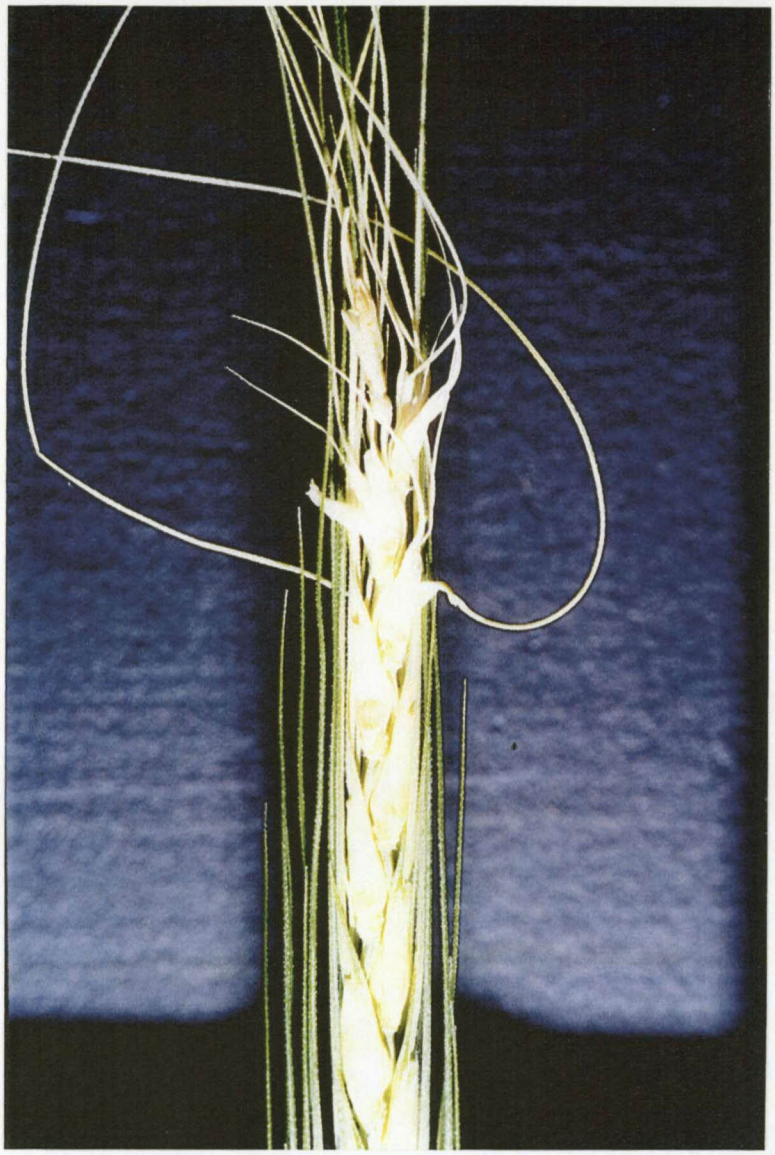

Fig. 2.4. Cold damage to the ear tip of a wheat plant.

Fig. 2.5. Cold damage to the ear base of a wheat plant.

collected from field-grown wheat plants. Leaves super cooled the least, followed by the stem and nodes. The awns, lemma, and palea super cooled 2 to 4°C more than the leaves.

Marcellos and Single (1976) concluded that under conditions of a radiation frost neither airborne particles nor ice crystals induced frost formation. In a review on ice nucleation in plants, Lindlow (1983) identified three species of bacteria commonly found as epiphytes on leaf surfaces which are extremely effective ice nucleators at warm sub zero temperatures. The most common ice nucleation active bacteria were *Pseudomonas syringae* (Van Hall), followed by *Erwinia herbicola* (Dye) and *Pseudomona fluorescence* (Mugila). Isolates of these bacteria are effective ice nucleators at -2°C (Lindlow, Arny, and Upper, 1982; Lindlow, Arny, Barchet and Upper, 1978). Wheat plants growing in controlled environment chambers have little or no ice nucleating bacteria on their leaves and do not freeze when exposed to temperatures as low as -8 °C for up to 6 hours. However, if these plants are sprayed with ice nucleating bacteria, freezing occurs within minutes at approximately -3 °C (Gusta and Chen, 1987).

Under field conditions, low populations of ice nucleating bacteria were found on wheat leaves in the spring in comparison to the higher populations found in late summer, when plants start to mature. The increase in ice nucleating bacteria results in a decrease in super cooling of the plant. It appears that the ice nucleating bacteria may play a more significant role in causing frost damage in the fall than in the spring (Gusta and Chen, 1987).

2.2.5 Cold hardiness. The critical process for the survival of winter wheat during periods of extreme cold is hardening. Hardening must be completed in time and it must not be lost too soon in the spring (Cook and Veseth, 1991). Cold hardening is under genetic control and is induced by temperatures below 10°C (Paulsen, 1968; Svec and Hodges, 1972; Fowler and Gusta, 1977). The energy to drive the metabolic process is obtained either through photosynthesis or from energy reserves in the seed (Andrews, 1960; Olien, 1967). Cold hardiness is not a static condition, but changes with time, temperature, day length, maturity, soil moisture, plant moisture, nutrition and physiological age (Cook and Veseth, 1991; Gusta and Chen, 1987).

Significant correlations have also been reported between ability to survive the winter and growth habit of wheat (Hayes and Aamodt, 1927; Quisenberry, 1931). In general, spring wheats tended to be less hardy than winter wheats, but this relationship was not absolute (Brule-Babel and Fowler, 1988). In contrast Cahalan and Law (1979) found no evidence of genetic linkage between cold hardiness and vernalization requirement, confirming that a winter growth habit enhances the ability to survive sub zero temperatures.

2.2.6 Winter survival. Winter wheat plants are killed outright by low temperatures when their crowns are killed. Low temperatures may kill the leaves, but as long as the crowns are not killed, recovery is still possible in the spring. Crowns of cultivars developed for areas subject to winterkill harden enough to withstand temperatures down to -24°C. Even a few hours at these temperatures can be lethal to other wheat cultivars. A snow cover can provide the isolation necessary to prevent lethal temperatures in the crown zone, even during periods when air temperatures decrease to -34°C or lower.

On the other hand, leaves of plants are highly vulnerable to winterkill if it snows too soon in the fall, before the hardening process is complete (Cook and Veseth, 1991).

The outright freezing of wheat however, is not the only reason for the failure of wheat to survive some winters. Plants may die from smothering under ice, or desiccation when exposed to cold, dry winds while the water is in the solid form rather than the liquid form. Winter wheat is also subject to snow mold and root diseases caused by low temperature fungi. Much so-called winterkill involves fungi that parasitise roots and crowns, limiting the ability of the plants to survive the winter (Cook and Veseth, 1991).

2.2.7 Factors affecting winter survival. Depending on the location and crop species, reasons for winterkill vary from year to year. The primary causes are heaving, smothering, physiological drought and freezing of the plant tissues (Salmon, 1933). Ice encasement is a major cause of death in areas of high rainfall (Andrews, Pomeroy and De la Roche, 1974). Flooding has also been shown to reduce winter hardiness (Olien, 1967). In Western Canada, the primary cause of winter injury appears to be related to low soil temperatures between November and February. It is essential during this period to have an insulating layer of snow to prevent the soil temperature from going below the critical temperature of the winter cereal. For example the crowns of Norstar can survive exposure to -23°C for 12h without being killed, but a brief exposure to -24°C may completely kill the crown tissue (Gusta and Chen, 1987).

In areas with prolonged periods of sub zero temperatures, snow (10-15cm) is essential for survival of winter wheat. Snow is required as an insulator to trap residual soil heat and buffer wheat plants against air temperature extremes. If a uniform snow

cover is not maintained it can lead to winterkill. It was concluded that only 8-10cm of snow in standing stubble was adequate for the survival of hardy winter wheat. Under these conditions the soil temperature at crown depth would rarely be colder than -11°C even if air temperatures were below -22 to -35 °C (Aase and Siddoway, 1979; Fowler, 1983).

In addition to an insulating effect, snow also protects wheat plants against the dehydration effects of sub zero temperatures. Freezing induces severe dehydration of tissue. At -5°C the water potential is reduced to -60MPa and at -15 °C it is reduced to -185MPa. The leaves of winter wheat are much more sensitive than crowns to the desiccating effects of sub zero temperatures (Fowler, 1983).

Cold injury increases with the length of exposure to low temperatures (Cook and Veseth, 1991; Gusta, Fowler and Tyler, 1982). Fully cold hardened winter wheat crowns can tolerate -15°C for 5 to 6 days, but can only tolerate -18°C for 24 hours and -23°C for 12 hours. Crowns of hardy cultivars could be held at -12°C for a period of 15 days with little or no damage. Plants held at -3°C under a snow cover for 5 months lose almost all of their cold hardiness or succumb to the continuous frost.

Although a cultivar may achieve considerable hardiness by early winter, this hardiness may be lost due to various factors. Continuous freezing and thawing results in increased injury from ice crystal growth with each freezing cycle (Gusta and Fowler, 1977; Olien, 1969). A midwinter thaw results in flooding of the crown, which increases tissue water content. Gullord, Olien and Everson (1975) duplicated these conditions in a controlled freeze test and found that the temperature at which flooded crowns were killed was 2 to 7°C warmer than for non-flooded crowns.

2.2.8 Morphological changes associated with tolerance to freezing. Different parts of winter cereals possess different levels of cold hardiness. In comparison to the crown and herbage, the roots have only a limited capacity to cold harden. Chen, Gusta and Fowler, (1983) reported that the roots of Norstar and Puma (rye) cold hardened to only -6 to -7°C, whereas the crowns cold hardened to -20 and -30°C, respectively. Gusta and Chen (1987) found that after four months storage at -3 to -4°C, the adventious roots on hardy cultivars were dead while the crowns were still alive.

Tillers on the same plant did not possess the same degree of tolerance to freezing. The young and intermediate tillers survived the winter better than the older tillers (Legge, Fowler and Gusta, 1983).

Legge (1979) observed that following a freeze, tillers regenerated from adventious buds rather than from the intercalary meristem. Olien (1961) indicated that it may be due to injury of the xylem vessels and the cells of the central and lower region of the crown. This region has a high moisture content due to the xylem vessels and the presence of large vacuolated cells. Thus, large ice crystals could form, resulting in mechanical damage to the tissue. This would reduce the vascular connection between shoots and the roots, or result in injury to the tissue which normally gives rise to adventitious roots (Pauli, 1961; Beard and Olien, 1963). Auxiliary buds may escape injury due to their small undifferentiated cells and their less rigid tissue, compared to the stem region of the parent tiller (Gusta and Chen, 1987).

An inverse relationship exists between cold hardiness and the number of tillers, leaves, crown root numbers and crown root length (Fowler and Gusta, 1977). Crown and leaf water content, plant erectness, crown phosphorus content, and total crown sugar content were highly correlated with field survival. Leaf water content, combined

with plant erectness, was as good an indicator of winter field survival as a controlled freeze test (Fowler, Gusta and Tyler, 1981).

It was suggested that cultivars which have their crowns deep in the soil survive the winter better than cultivars with a shallow crown, because the crown and the coleoptile tiller are more insulated from temperature extremes (Ferguson and Boatwrigt, 1968; Levitt, 1956). Crown depth is influenced by genotype, depth of seeding, soil temperature, and light. However, Fowler and Gusta (1977) found no correlation between crown depth and winter survival of four hardy winter wheats, and no difference in soil temperature could be detected by thermocouples placed next to the crown or next to the coleoptile tiller.

Unhardened cells have a large central vacuole and a thin peripheral cytoplasm, whereas cold-hardened cells have a dense cytoplasm with many vacuoles and a large central nucleus. During hardening there is an increase in the quantity of membranes, particularly the plasma membrane (Pomeroy and Siminovitch, 1971).

Steponkus, Dowgert, Evans and Gordon-Kammin (1982) studied the freezing behaviour of rye protoplasts under a microscope and observed that non-acclimated protoplasts were unable to expand back to their original volume during a freeze-thaw cycle and consequently lysed. They attributed lysing to the loss of plasma membrane material during freeze-induced contraction. Upon re-hydration the plasma membrane material lost could not be added back fast enough to the plane of the membrane before expansion-induced lysis occurred. In the case of acclimated protoplasts, large surface contractions were reversible and injury was not due to the events that occurred during contraction.

Fresh weight, dry weight, water content and total soluble carbohydrate were

determined for ten winter wheat and 18 winter triticale lines. Correlation between crown dry weight , water content and survival suggest that those traits might be used as predictors of tolerance to freezing (McIntyre, Chen and Mederick, 1988).

2.2.9 Metabolic changes associated with tolerance to freezing. Siminovitch, Gffeller and Rheaume (1967) demonstrated that cold hardening results in changes in the cellular constituents. Cells labelled with radioactive leucine tested at 21 and 2°C absorb the radio label at nearly the same rate. The rate of turnover of label tested at 21°C is several times higher than in cells tested at 2°C. These results suggest that for cells to cold harden there must be a slow down in growth accompanied by an increase in cell metabolites (Gusta and Chen, 1987).

Many studies have been conducted on the degree of lipid unsaturation and fluidity of the membranes, since they are the primary site of injury. Marked growth temperature dependant alterations in the fatty acid composition and unsaturation of the mitochondrial phospholipids were observed during hardening of four wheat cultivars (Miller, De la Roche and Pomeroy, 1974). Structural transitions occur at lower temperatures in cold grown material and were quantitatively greater in winter hardy cultivars. Farkas, Deri-Hadlaczky and Belea (1975) also concluded that the degree of lipid unsaturation was correlated with cultivar hardiness. De la Roche, Pomeroy and Andrews (1975) found that the change in lipid unsaturation of the membranes was the same in four wheat cultivars differing in cold hardiness. The increased unsaturation of lipids at hardening temperatures may be more related to vernalisation than to cold hardening (De Silva, Weinberger, Kates and De la Roche, 1975).

Uemura and Yoshida (1984) analysed a plasma membrane enriched fraction

from cold hardened winter rye seedlings and found that the degree of fatty acid unsaturation and proportion of phospholipid classes changed only slightly during hardening. These results suggest that fatty acid changes may not be as dramatic as once thought for cold hardening of winter cereals.

A high positive correlation exists between crown water content and cold hardiness in cereal species. However, between species this correlation does not hold. For example, when fully hardened, Norstar winter wheat and Puma rye have the same moisture content, but differ by 10°C in cold hardiness. The decrease in water content with cold hardening is due to an increase in dry matter accumulation at low temperatures and not to a water deficit.

In an examination of 34 characters Fowler *et al.* (1981) found that crown and leaf water content were the best predictors of survival ability. The exposing of wheat cultivars to acclimation temperatures resulted in a decrease of tissue water content (Brule-Babel and Fowler, 1989). This observation is in agreement with results reported by other researchers (Metcalf, Cress, Olien and Everson, 1970; Fowler and Charles, 1979; Tyler, Gusta and Fowler, 1981; McKersie and Hunt, 1987). However, based on observations by Brule-Babel and Fowler (1989), it was concluded that, to be effective as a screening method for cold hardiness, measurements of tissue water content should be made on fully acclimated plants for which the acclimation conditions have been rigorously controlled. A short dehydration period at room temperature will cold harden winter cereals. The increased cold hardiness persists after the plants have been rehydrated (Cloutier and Siminovitch, 1983).

In wheat it has been observed that following cold-hardening treatment, the levels of endogenous abscisic acid (ABA) increase to a greater extent in the frost tolerant

varieties than in frost-sensitive ones (Dorfling, Schulenburg, Lesselich and Dorfling, 1990 ; Machakova, Hanisova and Krekule, 1989). Cell suspension cultures of Norstar winter wheat growing at 21°C can tolerate -30°C after four days treatment with ABA (Chen and Gusta, 1983). Thus, it appears that the low temperature requirement for hardening can be bypassed in special circumstances. Caliba, Tuberosa, Kocsy and Sutka (1993) studied the relationship between frost tolerance and ABA accumulation in callus of three wheat cultivars differing in the level of frost tolerance. Following cold hardening, the increase in ABA level in the calli of the two frost tolerant cultivars was significantly higher than in those of the frost susceptible cultivar. They concluded that ABA accumulation might be a trait of interest to select for, in the order to achieve higher levels of tolerance to freezing.

The electric conductivity method, also known as the ion leakage method, was introduced into frost hardiness studies by Dexter, Tottingham and Graber (1930; 1932). This method is based on the assumption that during frost damage, cell membranes lose their semi-permeability and ions are leaked from the cells to the effusate. Accordingly, the greater the damage to the plant, the higher the conductivity value of the effusate (Hommo, 1994).

Palfi, Gulyas, Rajki and Csuez (1988) studied the correlation between frost tolerance and the proline levels in shoots and roots of wheat and rye. They found that the cold induced proline concentration varied significantly between the two varieties. The difference was 35.5% for wheat and 40.7% for rye. With these values it is possible to characterize the cold and frost tolerance of different varieties. Peruanskiy and Stacenko (1981) established significant differences between wheat and rye for frost tolerance. According to Peruanskiy and Stacenko (1981) the increase of proline

content is directly proportional to the grade of frost tolerance and they also demonstrated significant differences between frozen young shoots of different varieties. Paquin and Pelletier (1981) showed that the proline level in the leaves and roots of wheat varieties increase with their frost tolerance, but only till the falling of the leaves. The higher the frost tolerance the greater is the proline accumulation.

2.2.10 High molecular weight proteins and tolerance to freezing. The appearance of high molecular weight proteins in response to cold hardening has been reported in studies with different plant species including wheat. The expression of these proteins was positively correlated with the variety and organ specific degree of frost tolerance. In spite of the reports on formation of proteins related to cold hardening, the exact function of none of them has been identified. Some of the proteins may not be responsible for frost tolerance (Guy, 1990; Abromeit, Askman, Sarnighausen and Dorfling, 1992).

A study of the seasonal variation in protein content and hardiness of cells demonstrate the accumulation of soluble proteins in fall, which closely correlates with the induction of freezing tolerance (Siminovitch and Briggs, 1953). Perras and Sarhan (1989), Danyluk and Sarhan (1990) and Abromeit *et al.* (1992) found proteins in the range of 200kDa with a similar isoelectric point (6.8) in winter wheat cultivars after cold hardening.

Freezing tolerant winter wheat synthesized two hydrophyllic high molecular weight proteins (240 and 115 kDa) at 3°C, while freezing sensitive wheat was unable to produce similar proteins (Rochat and Therrien, 1975).

Sarhan and Perras (1987) analysed the electrophoretic patterns of soluble

protein fractions from cold tolerant winter wheats, Frederick and Norstar, and cold sensitive spring wheat, Glenlea. One and two dimensional gel electrophoresis analysis revealed that the cold hardening conditions induced changes in the soluble protein patterns. The most important is the accumulation of a high molecular weight in the range of 200kDa. This protein accumulated at a higher concentration in cold tolerant cultivars compared to the cold sensitive cultivar, suggesting a correlation between the degree of freezing tolerance and the accumulation of this specific protein. In addition, the intensity of three protein bands(48, 47 and 42 kDa) increased while that of five others (93, 89, 80, 67 and 63 kDa) decreased during hardening. These changes occurring in the three cultivars suggest that these proteins are part of the metabolic adjustments in response to low temperatures rather than a specific change associated with the development of tolerance to freezing.

Abromeit, *et al.* (1992) studied changes in two dimensional gel electrophoretic patterns of soluble proteins from two winter wheat varieties, Roughrider and Capelle. These two varieties differ in frost tolerance. The soluble proteins were obtained from the shoots of dark grown unhardened seedlings and dark grown cold hardened seedlings. The cold hardening at 2°C increased the frost tolerance in both varieties. Two dimensional gel electrophoretic studies revealed that cold hardening caused the appearance of a group of up to seven high molecular weight proteins (150-176 kDa), which were not present on gels of unhardened seedlings. Their induction by cold hardening was more pronounced in the cultivar Roughrider (frost tolerant) than in the cultivar Capelle (less frost tolerant). Kinetic studies revealed that in Roughrider the high molecular weight protein pattern was completely expressed 48 hours after the beginning of cold hardening.

Zhou, Arakawa, Fujikawa and Yoshida (1994) identified proteins that were induced by cold acclimation in wheat. Two cultivars with different genetic ability to cold-acclimate, namely Chinese Spring (spring wheat) and Norstar (winter wheat) were used. Cold acclimation induced remarkable changes in the electrophoretic patterns of plasma membrane proteins. Levels of polypeptides with molecular masses from 22-31kDa decreased in both the root and shoot plasma membranes for both cultivars. By contrast, levels of polypeptides of 89, 83, 52, 23, 18, and 17 kDa increased specifically in the shoots of winter wheat. The increases in the levels of the 23, 18 and 17 kDa polypeptides were proportional to the development of freezing tolerance.

2.3 Breeding for tolerance to freezing

The Crimean wheat cultivars introduced from the USSR at the turn of the century provided the basic germplasm for the production of extremely freezing tolerant red winter wheats in North America (Quisenberry and Reitz, 1974). Almost all the hard red wheats grown on the Great Plains were developed from hybrids involving the Crimean cultivars. Kharkov M22, a selection from the USSR wheats, was for long regarded as the most cold hardened cultivar, until it was replaced by Norstar in 1977. Two USSR cultivars Alabaskaja and Ulianovkia, are significantly more cold hardy than Kharkov and slightly more cold hardy than Norstar. Stushnoff, Fowler and Brule-Babel (1983) concluded that the genetic variability for winter hardiness has been largely exhausted. Fowler, Limin and Gusta (1983) found that the only examples of transgressive segregation for greater winter hardiness were from crosses between parents of moderate or poor hardiness, and selections from these were not as hardy as existing genotypes. In crosses involving extremely hardy parents, transgressive segregation for

increased cold hardiness has not been reported (Orlyuk, 1976; Shelepov, Kul'chitsbaya and Shelepova, 1980). This may be due to the fact that all the hardy genotypes identified today are from Crimean stock and possess common genes for cold hardiness. Thomas and Gaudet (1983) suggest that, since all the hardy wheats are derived from Russian varieties, perhaps some gain could be made from Chinese introductions. The Chinese wheat E85 has considerable winter hardiness and it crosses readily with rye. It may possess genes for winter hardiness different from the Russian selections (Everson, Olien, Peare, Worral and Webster, 1976; Thomas and Gaudet, 1983).

Attempts to transfer the cold hardiness from rye to winter wheat resulted in the production of triticale that is only about as cold hardy as the wheat parent (Larter, 1973; Dvorak and Fowler, 1978). The cold hardiness of the rye is apparently suppressed in triticale. Grafius (1981) backcrossed wheat to a *Triticum aestivum* X *Agropyron trichophorum* hybrid and selected wheat like plants whose cold hardiness were similar to rye. Fedorov (1970) reported that wheat-Agropyron amphyploids (2n=56) were as cold hardy as hardy ryes.

Limin and Fowler (1982) produced a range of amphyploids from inter specific crosses between *Triticum durum* and *T. aestivum*, *T. dicoccum*, *T. araraticum*, T. *ventricosum*, and *T. tauchii*. None of the amphyploids exceeded the most hardy *T. durum* parent in cold hardiness.

Brule-Babel (1985) working with reciprocal crosses of hardy by less hardy wheat parents, could not find any evidence of a cytoplasmic effect on cold hardiness or on the nuclear expression of cold hardiness.

2.4 Genetic control of tolerance to freezing

There are two primary methods for evaluating the cold tolerance potential of wheat. These are survival under field conditions and the use of different morphological and metabolic characters. Differences are correlated with field survival (Fowler *et al.*, 1981). Field survival is considered to be the ultimate test for a cultivar's cold tolerance. However, field survival trials are often inconclusive due either to complete winterkill or a lack of it (Fowler and Gusta, 1979). Variation in stress levels within field trials also makes it difficult to identify small but important differences among cultivars even when differential winterkill does occur (Fowler, 1979). Because of these inherent limitations in field trials there has been a continuing search for rapid and efficient methods to predict tolerance to freezing.

Just about every biochemical, physiological and morphological character changes in the plant during cold acclimation. Based on these changes, a large number of prediction tests are possible (Fowler *et al.*, 1981). Due to this attribute, controlled freeze tests have been utilized for many years (Hill and Salmon, 1927; Weibel and Quisenberry, 194; Roberts and Grant, 1968; Fowler, Siminovitch and Pomeroy, 1973; Thomas, Schaalje and Roberts, 1988). Compared to field tests, they have the advantage of speed, greater control over stress levels and the opportunity for replications over time. Results from controlled freeze tests employing a single minimum temperature were found to be highly correlated with those obtained from field trials when cultivars having a wide range of cold tolerance were studied (Metcalf *et al.*, 1970; Fowler *et al.*, 1973; Bridger, Falk, McKersie and Smith, 1994;).

Cell suspension cultures and embryo cultures provide the opportunity to determine whether there is somaclonal variation for cold hardiness within winter wheat.

Cell suspension cultures of winter wheat, exposed to cold hardening conditions, will cold harden within a week (Chen and Gusta, 1982; 1983). Abscisic acid induced freezing resistance in Norstar cell cultures as low as -30°C within four days at 21°C (Chen and Gusta, 1983). Regenerated wheat callus, derived from immature embryos, can be stored at liquid nitrogen temperatures if cryoprotectants are added. Plants can be regenerated from these cultures and provide the breeder with a larger number of plants from a single cross. Since wheat cultures can be cold hardened in vitro, studies of the genetics of hardiness from a single cross are possible (Gusta and Chen, 1987).

The genetics of cold tolerance was studied in detail in winter wheat with a method using complete diallel crosses (Gullord, 1974; Puchkov and Zhirov, 1978; Parodi, Nyquist, Patterson and Hodges, 1983; Sutka, 1994). Their data showed that cold tolerance is controlled by an additive-dominance system. Sutka (1981, 1984, 1994) revealed a preponderance of additive genetic variance. Sutka (1994) also indicated that non-additive genetic variation is present as dominance only. The dominant genes acted in the direction of lower cold tolerance and the recessive genes in the direction of a higher level of cold tolerance.

2.5 Genetic variability of tolerance to freezing

2.5.1 Survival. Damania and Tahir (1993) screened 46 lines selected from accessions of wheat and its wild and primitive forms originating in West Asia and North Africa. These regions represent diverse ecological areas. The check cultivar TAM 105 was the most cold tolerant, with 75% of the plants surviving the screening test. The most tolerant group was *T. urartu*, whereas for *T. boeoticum* and *Ae. squarrossa* the reaction to cold ranged from zero tolerance to moderate tolerant. The other groups were mostly

susceptible, including *T. urartu* collected from Armenia, a region known for its harsh environment.

Brule-Babel and Fowler (1989) studied the survival of nine cultivars. Analysis of variance indicated that there were significant differences in their survival ability. A Duncan's multiple range test of means indicated that they fell into five distinct cold hardiness groups. Manitou and Capelle Desprez remained in classes by themselves, while Nugaines and Besostaya 1 formed another group. Cheyenne, Minter and Winalta formed the fourth group and Kharkov 22MC and Norstar formed the hardiest group. McKersie and Hunt (1987) studied the winter survival of 34 winter wheats in Ontario, USA, analyses of variance of freezing tolerance showed that the genotype effects were significant.

Tagmaj'yan and Kolbasina (1972) compared the frost resistance of 156 cultivars. When the reaction of the cultivars to -14°C was compared with that of the Soviet standards, 15 proved especially hardy and five moderately so. Fowler and Limin (1987) screened extensively diverse hexaploid wheat types collected from Afganistan. No lines were found to surpass the cold hardiness potential of the hardiest commercial wheat cultivars presently produced in North America. Hommo (1994) studied the field survival of 23 winter wheat, 13 rye, 5 triticale and 11 winter barley cultivars in order to estimate their winter survival potential under Finnish conditions. A wide range of winter hardiness levels were observed. Ten winter wheat and 18 winter triticale lines were obtained from the provincial cereal breeding programme at Lacombe, they were screened for cold tolerance using a LT50 (temperature at which 50% of test plants die) method. Three wheat cultivars (Norstar, Ulianovka, Winalta) were provided as checks. The LT50 of Norstar was -16.3°C, whereas Ulianovka exhibited a LT50 of -13.9°C.

McIntyre *et al.* (1988) found that Ulianovka had survival rates higher than Norstar in three out of five years of field trails. The test line (Kharkov/Ulianovka) exhibited a higher LT50 value than Norstar.

2.5.2 Leaf length. Marcellos and Burke (1979) demonstrated that leaves of several unhardened spring wheat cultivars are able to tolerate temperatures as low as -7 to -9°C. Leaves of spring wheat Kite, Manitou and Oxley in the hardened state tolerated -9 to -10°C before injury become apparent. The leaves of Norstar and Cheyenne winter wheats can tolerate -18 and -12°C respectively, when cold hardened (Gusta and Chen, 1987; Chen *et al*, 1983; Marcellos and Burke, 1979). Gusta and Chen (1987) observed that leaves of field grown Columbus and Neepawa wheat collected from mid to late July survived temperatures as low as -8°C. Temperatures at the time of collection were between 25 and 30°C, indicating that the leaves possess considerable frost tolerance in the absence of hardening conditions. Although the leaves of wheat plants may possess a considerable degree of frost tolerance, the reproductive tissues of the developing ear are considerably less resistant to freezing and may be injured at -1.8°C (Single and Marcellos, 1974).

Veisz and Sutka (1993) used leaf regrowth to measure cold tolerance of some Chinese Spring ditelosomics. After freezing the leaves were cut off with scissors a few centimetres above the soil, so that regrowth could be evaluated. After 16 days, the plants were rated on a 0 to 5 scale. Those who had died were scored 0, while the well developed tillering plants scored 5 points.

2.5.3 Root length. Gullord *et al.* (1975) evaluated freezing hardiness for 14 winter wheat cultivars and eight selections under controlled freezing conditions. Freezing was obtained by decreasing the temperature slowly in hardened wheat crowns containing high and low amounts of water respectively. Freezing hardiness was assessed in terms of lower peripheral crown meristem (root) regrowth on a 0 (dead) to 5 (undamaged) scale. The results show that the described freezing procedures easily discriminate between genotypes when freezing hardiness is evaluated. Several of the selections and cultivars have freezing hardiness-genes in common.

Le, Reicosky, Olien and Cress (1986) studied the freezing hardiness of 51 accessions of *T. tauchii* and 35 accessions of *T. turgidum* using a root regrowth scale. Freezing resistance for the *T. tauchii* accessions ranged from non-hardy to as hardy as the check (Winoka). Although the *T. turgidum* accessions were less hardy than Winoka, some accessions of this species approached the hardiness levels of Winoka in a low intensity test.

2.6 Combining ability of tolerance to freezing

2.6.1 Survival. Reports of cold hardiness levels in the F1 generation of winter wheat crosses have been widely variable. F1 hybrids in some spring x winter crosses have been reported to resemble the less hardy parent, hardy parent or to be intermediate in cold hardiness. Brule-Babel and Fowler (1988) reported that all winter wheat by Manitou spring wheat crosses resulted in spring habit F1 hybrids that were significantly more hardy than the parental midpoint.

2.6.2 Leaf length. No literature is available on leaf growth under freezing conditions.

2.6.3 Root length. No literature is available on root growth under freezing conditions.

2.7 General combining ability : specific combining ability ratio for tolerance to freezing(GCA:SCA ratio)

2.7.1 Survival. Sutka (1994) found a high GCA:SCA ratio for the percentage survival of winter wheat in a controlled freezing test. The ratio of 14:6 revealed a preponderance of additive genetic variance. No significant average maternal differences or other reciprocal differences were found between the reciprocal crosses. Sutka (1981; 1984) has reported a high GCA:SCA ratio indicating mainly additive genetic variance. Similarly Gullord (1974) concluded that cold hardiness was controlled by partial dominant genes which were mainly additive in their effects.

2.7.2 Leaf length. No literature is available on the GCA:SCA ratio of leaf growth under freezing conditions.

2.7.3 Root length. No literature is available on the GCA:SCA ratio of root growth under freezing conditions.

2.8 Inheritance of tolerance to freezing

Studies on the genetic nature of cold hardiness in wheat most frequently report that this character is genetically complex and quantatively inherited (Worzella, 1947; Quisenberry and Clark, 1929). Gullord *et al.* (1975) indicated that cold tolerance may

not be a single trait, but a complex of tolerances to different types of freezing stresses. Sutka (1994) indicated that cold tolerance is controlled by an additive dominance system. Results of the diallel analyses indicated both additive and non-additive gene action. The variance and covariance for percentage survival, averaged over reciprocal crosses were calculated. The regression coefficient was significantly different from zero but not significantly different from unity. This indicated that non-additive genetic variation is present as dominance only. The dominant genes acted in the direction of lower cold tolerance and the recessive genes in the direction of a higher level of cold tolerance.

Parodi *et al.* (1983) reported that cold hardiness of F1 hybrids was determined mainly by specific combining ability (SCA) or specific heterosis or additive by additive gene action.

Limin and Fowler (1993) concluded from crosses of amphyploids with Norstar that there was partial dominance for cold hardiness. Many of the F2-derived F3 lines were equal to Norstar in hardiness, suggesting that only a few genes are involved in hardiness.

The inheritance of cold hardiness in wheat was studied in 20 crosses among five parents ranging from spring wheat to hardy winter wheat. Analysis of F1 and F2 populations indicated that genetic control of cold hardiness in spring x winter crosses was partially dominant. The F2 derived F3 lines confirmed this conclusion since all distributions were skewed to the hardier end of the population ranges. In contrast, the F1 and F2 populations of winter x winter crosses did not differ significantly in hardiness from their parental midpoints. Thus, no dominance was exhibited in these crosses and genetic control was most likely additive. Distributions of F2 derived F3 lines agreed

with the premise that genetic control of cold hardiness was additive in winter x winter crosses. Consequently, the choice of parents would determine whether cold hardiness acted in a dominant or additive fashion. Since cytoplasmic effects were not implicated, crosses in either direction could be used (Brule-Babel and Fowler, 1989).

Synthetic hexaploid wheat produced by combining tetraploid wheat (AB genome) with *T. tauchii* (D genome), was crossed to modern hexaploid wheat (ABD genome) in an attempt to introduce new cold hardiness genes into the common hexaploid wheat gene pool. The cold hardiness levels of F1 hybrids ranged from similar to parental means to equal to the hardy parent, indicating that cold hardiness was controlled by both additive and dominant genes. Heritability estimates for cold hardiness ranged from 63 - 70% indicating that selection for cold hardiness should be effective in populations arising from crosses between common and synthetic hexaploid wheat (Limin and Fowler, 1993). Sutka (1994) calculated values of 81.1 and 97.55% for narrow and broad heritability respectively. This indicated a high heritability for cold tolerance. High heritability estimates for cold hardiness were also reported in wheat by Brule-Babel and Fowler (1988) and Sutka (1984; 1981). These estimates indicated cold hardiness was a heritable character and, provided genetic variability is present, selection for cold hardiness should be effective.

Monosomic and substitution analyses have made it possible to locate genes determining cold resistance on the chromosomes (Jenkins, 1971; Cahalan and Law, 1979; Veisz and Sutka, 1993;). Various authors reported that at least 10 of the 21 pairs of chromosomes are involved in the control of cold resistance (Sutka, 1981; Poysa, 1984; Sutka and Kovacs, 1985; Roberts, 1986; Sutka, Kovacs and Veisz, 1986; Sutka and Snape, 1989). On the basis of freeze testing experiments using substitution

material it was established that chromosomes 2B, 4B, 4D, 5A, 5B, 5D and 7A increased the level of cold tolerance, whereas chromosomes 1B, 3A, 3D, 6A and 7D (Poysa, 1984; Veisz and Sutka, 1989; Sutka, 1994;) showed a decreased cold resistance levels. Chromosomes 5A and 5D have been implicated most frequently and they appear to carry major genes. Snape, Law, Parker and Worland (1985) developed single chromosome recombinant lines, from the cross between the substitution line Hobbit (*Triticum spelta* 5A) and Hobbit. These lines were tested for cold hardiness and they could be classified into two distinct classes according to sensitivity to freezing, with *T. spelta* 5A lines exhibiting sensitivity to freezing (survival rates 5-25%) and the Hobbit 5A lines exhibiting resistance (survival rates 60-80%). The contribution of chromosomes, 5A and 5D, in the control of frost resistance and winter hardiness is very significant (Sutka, 1981; Roberts, 1986; Sutka *et al.*, 1986; Galiba and Sutka, 1989).

The transmission of these genes involved in tolerance to freezing is possible using conventional breeder's tools like crossing and selection, but the narrow gene pool available for these programmes is a limiting factor (Limin and Fowler, 1983). The frost tolerance of the frost sensitive spring wheat variety Saratovskaya 29 was improved to such an extent, by substituting the 5A chromosome with that of the extremely frost resistant varieties Albidum 11, Ulyanovka or Lutecens 230, that it became capable of surviving freezing at -14°C (Sutka, 1994). Brule-Babel and Fowler (1988) reported that all winter wheat by Manitou spring wheat crosses resulted in spring habit F1 hybrids that were significantly more hardy than the parental midpoint, indicating some dominance for cold hardiness.

Chapter 3

Materials and Methods

3.1 Tolerance to freezing of South African wheat cultivars

3.1.1 Materials

Three wheat lines from the USA, one from Germany and 24 South African wheat cultivars were screened for tolerance to freezing. Norstar was included as the freezing tolerant check (Fowler *et al.*, 1981). The genotypes represented three different growth habits: spring, facultative and winter. Table 3.1 shows the origin and growth habit of the 28 wheat genotypes screened for tolerance to freezing.

3.1.2 Methods

The artificial freezing test used was a modification of the procedure used by O'Connor, Reaney and Gusta (1993). The experimental unit consisted of two sets of 32 seeds, planted in four replicates of eight seeds. Each replicate was fully randomized. The seed was planted at a depth of 2cm in polyethylene seed trays filled with vermiculite. One hour after the first watering hardening was started in the dark at 2 to 4°C. The trays were covered with black plastic bags to reduce evaporative losses.

On day 21 the seed trays were rewatered and placed in a growth chamber at a temperature of 15 to 22°C and a day length of 14h. The seedlings were watered with 20ml water three times a week and received nutrient supplement once a week.

On day 49 the seedlings were at growth stage 13 to 15 (Zadoks, Chang and Konzak, 1974) and ready for the artificial freezing test. Screening was done at two different temperatures, -6 and -12°C. The control treatment of -6°C was used because it is the average minimum temperature during cold spells recorded in the summer

Table 3.1. Cultivars screened for tolerance to freezing

Entry	Cultivar	Origin	Growth habit
1	Snack	South Africa	Spring
2	SST 66	South Africa	Spring
3	Adam Tas	South Africa	Spring
4	SST 822	South Africa	Spring
5	SST 825	South Africa	Spring
6	Palmiet	South Africa	Spring
7	Kariega	South Africa	Spring
8	Scheepers 69	South Africa	Facultative
9	SST 363	South Africa	Facultative
10	PAN 3349	South Africa	Facultative
11	PAN 3211	South Africa	Facultative
12	Karee	South Africa	Facultative
13	PAN 3364	South Africa	Facultative
14	Limpopo	South Africa	Facultative
15	Gariep	South Africa	Facultative
16	Belinda	South Africa	Winter
17	SST 966	South Africa	Winter
18	SST 936	South Africa	Winter
19	Tugela DN	South Africa	Winter
20	PAN 3235	South Africa	Winter
21	PAN 3232	South Africa	Winter
22	Caledon	South Africa	Winter
23	Betta	South Africa	Winter
24	Molen	South Africa	Winter
25	Carolus	Germany	Winter
26	Jager	USA	Winter
27	Kavkaz	USA	Winter
28	Norstar	USA	Winter

rainfall wheat producing areas.

One set of seed trays were frozen at -6°C (control) and the other at -12°C in the dark. After one hour the seedlings were allowed to thaw at a rate of 2°C an hour. The roots and leaves of the seedlings were cut to a length of 40mm below and above the crown respectively.

The seedlings were transplanted in 1 litre pots containing a sandy soil. These pots were placed in a greenhouse with natural day light, a temperature of 15 to 21°C, and a day length of 14 hours. The pots were grouped to maintain the same randomization used during the freezing procedure. The plants were watered daily and received a nutrient supplement twice a week.

On day 70 the plants were removed from the pots and the seedlings were washed to remove the soil. The tolerance to freezing of the seedlings was obtained by calculating the average percentage survival, leaf length and root length.

3.1.2.1 Survival. Plants which showed root and leaf growth were counted as surviving plants. The surviving plants in each replication were counted and the percentage survival at -6 (control) and -12°C were calculated for each genotype. The reduction in survival with the reduction in temperature from -6 to -12°C was calculated as percentage survival reduction.

3.1.2.2 Leaf length. The average leaf length after exposure to -6 (control) and -12°C was measured for each genotype. The percentage reduction in leaf length with the reduction in temperature was calculated for each genotype.

3.1.2.3 Root length. The average root length after exposure to -6 (control) and -12°C was measured for each genotype. The percentage reduction in root length with the reduction in temperature was calculated for each genotype.

3.1.3 Statistical analysis

3.1.3.1 Analysis of variance. An analysis of variance (ANOVA) for each of the freezing tolerance parameters as well as a combined analysis was done. Agrobase version 1.4 was used to do the analysis. The t-test was used to calculate the least significant difference (LSD) for each set of data, at a 95 or 99% confidence level, to determine significant differences between cultivars.

3.2 Genetic variability, combining ability and inheritance of tolerance to freezing

3.2.1 Materials

Six wheat cultivars were identified according to their growth habit to be used as parents in a diallel cross (Table 3.2). Norstar and Tugela DN were included as the winter types, PAN 3349 and Karee as facultative types and SST 66 and Snack as the spring types. Norstar was the freezing tolerant check. The parents were crossed in a full diallel, including all crosses in both directions. In order to generate enough F1 seed the maximum number of crosses were made for every combination. The experimental material consisted of the six parents and 30 F1-hybrids. The 30 F1-hybrids consisted of 15 crosses and 15 reciprocal crosses. The parents and crossing combinations are listed in Table 3.2.

Table 3.2. Parents and crossing combinations for the 6x6 full diallel cross

Entry	Cross	Genotype
1	Norstar	Norstar
2	Tugela DN	Tugela DN
3	PAN 3349	PAN 3349
4	Karee	Karee
5	SST 66	SST 66
6	Snack	Snack
7	1x2	Norstar/Tugela DN
8	1x3	Norstar/PAN 3349
9	1x4	Norstar/Karee
10	1x5	Norstar/SST 66
11	1x6	Norstar/Snack
12	2x1	Tugela DN/Norstar
13	2x3	Tugela DN/PAN 3349
14	2x4	Tugela DN/Karee
15	2x5	Tugela DN/SST 66
16	2x6	Tugela DN/Snack
17	3x1	PAN 3349/Norstar
18	3x2	PAN 3349/Tugela DN
19	3x4	PAN 3349/Karee
20	3x5	PAN 3349/SST 66
21	3x6	PAN 3349/Snack
22	4x1	Karee/Norstar
23	4x2	Karee/Tugela DN
24	4x3	Karee/PAN 3349
25	4x5	Karee/SST 66
26	4x6	Karee/Snack
27	5x1	SST 66/Norstar

Table 3.2. (Cont.) Parents and crossing combinations for the 6x6 full diallel cross

Entry	Cross	Genotype
28	5x2	SST 66/Tugela DN
29	5x3	SST 66/PAN 3349
30	5x4	SST 66/Karee
31	5x6	SST 66/Snack
32	6x1	Snack/Norstar
33	6x2	Snack/Tugela DN
34	6x3	Snack/PAN 3349
35	6x4	Snack/Karee
36	6x5	Snack/SST 66

3.2.2 Methods

Exactly the same method as described in Chapter 3.1.2 was used to evaluate the genotypes in the diallel cross. The experimental unit consisted of two sets of 32 seeds, planted in four replicates of eight seeds for each cultivar. The seed was planted at a depth of 2cm in polyethylene seed trays filled with vermiculite. One hour after the first watering hardening was begun in the dark at 2 to 4°C.

On day 21 the seed trays were rewatered and were placed in a growth chamber with a temperature of 15 to 21°C. On day 49 the seedlings were at growth stage 13 to 15 and ready for the controlled freezing test. The roots and leaves of the seedlings were cut to a length of 40mm below and above the crown respectivily. One set of seed trays were frozen in the dark at -6°C (control), and the other at -12°C. After one hour the seedlings were allowed to thaw at a rate of 2°C an hour.

For the recovery period the seedlings were transplanted in one litre pots containing a sandy soil. On day 70 the plants were removed and the freezing tolerance of the seedlings was then obtained by calculating the percentage survival, leaf length and root length.

3.2.2.1 Survival. Plants which showed root and leaf growth were counted as surviving plants. The surviving plants in each replication were counted and the percentage survival at -6 and -12°C was calculated for each cultivar. The reduction in survival with the reduction in temperature from -6 to -12°C was calculated as percentage survival reduction.

3.2.2.2 Leaf length. The average leaf length after exposure to -6 and -12°C was measured for each genotype. The percentage reduction in leaf growth with the reduction in temperature was calculated for each genotype.

3.2.2.3 Root length. The average root length after exposure to -6 and -12°C was measured for each genotype. The percentage reduction in root length with the reduction in temperature was calculated for each genotype.

3.2.3 Statistical analysis

3.2.3.1 Variability. Analysis of variance (ANOVA), mean squares, coefficients of variation (CV) and standard errors were calculated for the patents and the F1's with Agrobase version 1.4. The least significant differences (LSD) for each set of data were calculated at a 95 or 99% confidence level.

3.2.3.2. Combining ability. The aim of the diallel cross was to determine the combining ability of the parent lines for tolerance to freezing. The general combining value of a genotype determines its crossing value. The crossing value of the cultivar is a function of its additive gene action, while the additive gene action is responsible for the similarity between the parents and offspring. The specific combining ability is a result of loci which exhibit dominance and interaction.

Griffing (1956a) distinguished four methods and two models for the analysis of diallel data. For this study Model 1, Method 1 was used. Model 1 implies that the experimental material is to be regarded as the population about which inferences are to be made. Method 1 implies a full diallel with the parents, one set of F1's and

reciprocal F1's. The diallel cross was analysed with Agrobase version 1.4 based on the analyses of Griffing (1956a, 1956b).

3.2.3.3 GCA : SCA ratio. The GCA of each tolerance to freezing parameter was divided by its SCA to produce a GCA:SCA ratio for each parameter. A high ratio indicated additive gene action, while a low GCA:SCA ratio indicated specific gene action.

3.2.3.4 Heritability. The regression of offspring on the parents was used to determine heritability based on the computational formulas of Becker (1984).

3.3 Use of high molecular weight proteins to screen for tolerance to freezing

3.3.1 Materials

The high molecular weight proteins (HMW-proteins) of the six wheat genotypes used as parents in the diallel cross (Chapter 3.2) were used to screen for tolerance to freezing. The genotypes included were Norstar, Tugela DN, PAN 3349, Karee, SST 66 and Snack. Norstar was included as the freezing tolerant check.

3.3.2 Methods

3.3.2.1 Screening for tolerance to freezing. Exactly the same method as described in Chapter 3.1.2 was used to screen the tolerance to freezing of the six wheat genotypes. The experimental unit consisted of two sets of 32 seeds, for each cultivar. The seed was planted 2 cm deep in polyethylene seed trays filled with vermiculite.

After the first watering, hardening was begun in the dark at 2 to 4°C. On day 21 the seed trays were placed in a growth chamber at a temperature of 15 to 21°C. On day 49 the seedlings were ready for the controlled freezing test. The roots and leaves of the seedlings were cut to a length of 40mm. One set of seed trays was frozen in the dark at -6°C (control), and the other set were frozen at -12°C in the dark for one hour. The seedlings were transplanted in 1 litre pots containing a sandy soil. On day 70 the plants were removed and their tolerance to freezing was obtained by calculating the percentage survival, leaf length and root length.

3.3.2.2 Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE). This method was a modified version of the methods used by Sarhan and Perras (1987) and Singh, Shepherd and Cornish (1991).

Electrophoresis involved five basic steps:

1. Gel preparation (choosing the type and concentration of polymeric network to separate the proteins)
2. Sample preparation (extraction of proteins to obtain a solution containing either all the proteins or only a particular class of protein)
3. Sample separation (fractionation is provided by different rates of movement of the proteins in a gel matrix caused by differences in charge and size)
4. Gel staining (protein stains are used to colour the protein bands, yielding a banding pattern or fingerprint)
5. Gel interpretation (the comparison of banding patterns between treated and untreated samples separated under the same conditions)

1. Gel preparation

A discontinuous mini gel system was used, which required the formation of two gel layers, the separating gel in which separation took place and the stacking gel on which samples were applied and in which the protein zones were concentrated to give very thin starting zones.

Stock solutions

Separating buffer

Tris[hydroxymethyl]aminomethane (Tris)	45.41g
dH_2O	460ml
Titrate to pH 8.8 with Hydrochloricacid (HCl)	
Soduim dodecyl sulfate (SDS)	1.0g
Make up to 500ml with dH_2O	
Store at 4°C	

Separating acrylamide (30% Ac/1% crosslinker)

Acrylamide	75g
NN'-Methylenebisacrylamide (Bis)	0.75g
dH_2O	181ml
Make up to 250ml with dH_2O	
Store at 4°C	

Stacking buffer

Tris	6.06g
dH_2O	190ml

Titrate to pH 6.8 with HCl

SDS	0.4g

Make up to 200ml with dH_2O

Store at 4°C

Stacking acrylamide (35% Ac/1.5% crosslinker)

Acrylamide	87.5g
Bis	1.32g
dH_2O	181ml

Make up to 250ml with dH_2O

Store at 4°C

Electrode buffer

Cathode buffer

Tris	30.28g
Glysine	144g
SDS	10g

Make up to 1000ml with dH_2O

Anode buffer

Tris	30.28g
dH_2O	800ml

Titrate to pH 8.4 with HCl

Make up to 1000ml with dH_2O

Separating gel. (10% uniform) Mix 14ml separating buffer, 1.3ml separating acrylamide and 4.7ml H_2O. Add 55µl N,N,N',N'-Tetramethylenediamine (Temed) and 65µl 10% freshly made Ammonium persulphate (APS). Pour gel.

Stacking gel. Mix 5ml stacking buffer, 1.3ml stacking acrylamide and 3.7ml H_2O. Add 20µl Temed and 50µl freshly made APS. Pour the stacking gel on top of the separating gel and insert sample-loading positions.

2. Sample preparation

Germination and growing. Thirty seeds of each cultivar was surface sterilized with 3% NaOCl for three minutes and imbibed at 22°C for 12h in sterile water. The seed was spread on moist filter paper and allowed to germinate in the dark for two days. Seedlings were then either kept at 22°C for three more days (control, unhardened) or transferred to 4°C for 30 days (cold hardened). Based on their dry weight, control seedlings of 3, 4 and 5 days old correspond in terms of physiological age to seedlings hardened respectively for 10, 20 and 30 days.

Protein extraction

Stock solutions

Extraction buffer

12.5mM Tris	0.3g
2mM Ethylenedinitrilotraacetic acid disoduim salt (EDTA)	0.15g
10mM Mercapto-ethanol	140µl
2mM Phenylmethylsulfonyl fluoride (PMSF)	3.5mg

Titrate to pH 6.8 with HCl

Make up to 200ml with dH_2O

Sample buffer

12.5 mM Tris	0.1g
10% Gliserol	5ml
2.3% SDS	1.15g
Bromofenolblue	0.2g

Titrate to pH 6.8 with HCl

Make up to 50ml with dH_2O

The coleoptiles and roots of the hardened and unhardened seedlings were separated. 0.5-1.0g of the coleoptile or root was ground in a mortar and homogenized in 6ml extraction buffer. The homogenate was centrifuged at 12 000rpm for 10 minutes, placed in a tube with an excess cold asetone (-20°C) for 12h. The homogenate was centrifuged again at 12 000rpm for ten minutes. The asetone was removed and the proteins were dried in a vacuum dryer. Sample buffer (60µl) was added and the

proteins were left to dissolve. The protein solvent was then heated for one minute in a water bath at 70°C to assist complete denaturation.

3. Sample separation

Twenty microlitre protein and 20µl sample buffer were loaded into the sample wells. The gels were run at 65mA and 120 to 140 volts. The running time was approximately 30 minutes. The temperature was kept at 15°C. The current were switched off once the sample buffer reached the bottom of the gel.

4. Gel staining

Fixing solution

Methanol	400ml
Glacial acetic acid	100ml
H_2O	500ml

Staining solution

Trichloroacetic acid	30g
dH_2O	170ml
Coomassie Blue	0.1g
Methanol	9ml

The gel was removed from the glass plates and immersed in the fixing solution for one hour and overnight in the staining solution. The efficiency of shaking is very important to get uniform results and should therefore be optimized so that the fluid

circulates without breaking the gel during staining and destaining. The stained gels were rinsed in distilled water for a few hours before interpretation and photography (Wrigley, 1992).

5. Gel interpretation

A Rainbow™ [^{14}C]methylated protein molecular weight marker (Amersham International, England) was used as a standard from which test results were derived. The HMW-protein bands for hardened and unhardened coleoptile and root samples were compared. The number of new protein bands produced by each cultivar was observed. Any change in protein band intensity was also noted.

3.3.2.3 Phenotypic correlations. Phenotypic correlations were calculated for all the cold tolerance characteristics. These characteristics included: percentage survival, leaf length, root length, the number of protein bands produced in hardened and unhardened coleoptiles and the number of protein bands produced in hardened and unhardened roots. Agrobase version 1.4 was used for the analysis. A confidence level of 94% was used to determine significant correlations between characteristics.

Chapter 4

Results and Discussion

4.1 Tolerance to freezing of South African wheat cultivars

South African wheat cultivars were screened for tolerance to freezing. One set of seedlings was frozen at -6° (control), while the other was frozen at -12°C. The cultivars represented the following growth habits: winter, facultative and spring. Norstar was included as the freezing tolerant check.

4.1.1 Survival. An ANOVA indicated a relatively low but significant level of variance for percentage survival between cultivars at -6°C(control) (Fig. 4.1). The percentage survival at -6°C ranged from 100% (Norstar) to 71.9% (PAN 3211).

At -12°C, significant differences were observed between genotypes. Norstar (93.75%), Scheepers 69 (87.50%) and Kavkaz (84.38%) had significantly higher levels of percentage survival. Genotypes such as SST 822(43.75%), Caledon (43.75%), SST 363 (40.63%), SST936 (40.63%), Betta (40.63%), Gariep (40.63%), Limpopo (37.50%) and Snack (34.38%) had a low percentage survival at -12°C (Fig. 4.2).

The percentage reduction in survival varied from 64.29% (Snack) to 6.25% (Norstar). The low percentage reduction in survival of Norstar (6.25%), Scheepers 69 (6.70%), Belinda (7.14%), Kavkaz (9.82), Molen (12.50%) and Carolus (17.56%) indicated a high level of tolerance to freezing. Genotypes such as Snack (64.29%), Betta (57.59%) and Gariep (56.70%) exhibited a low level of tolerance to freezing (Fig. 4.3).

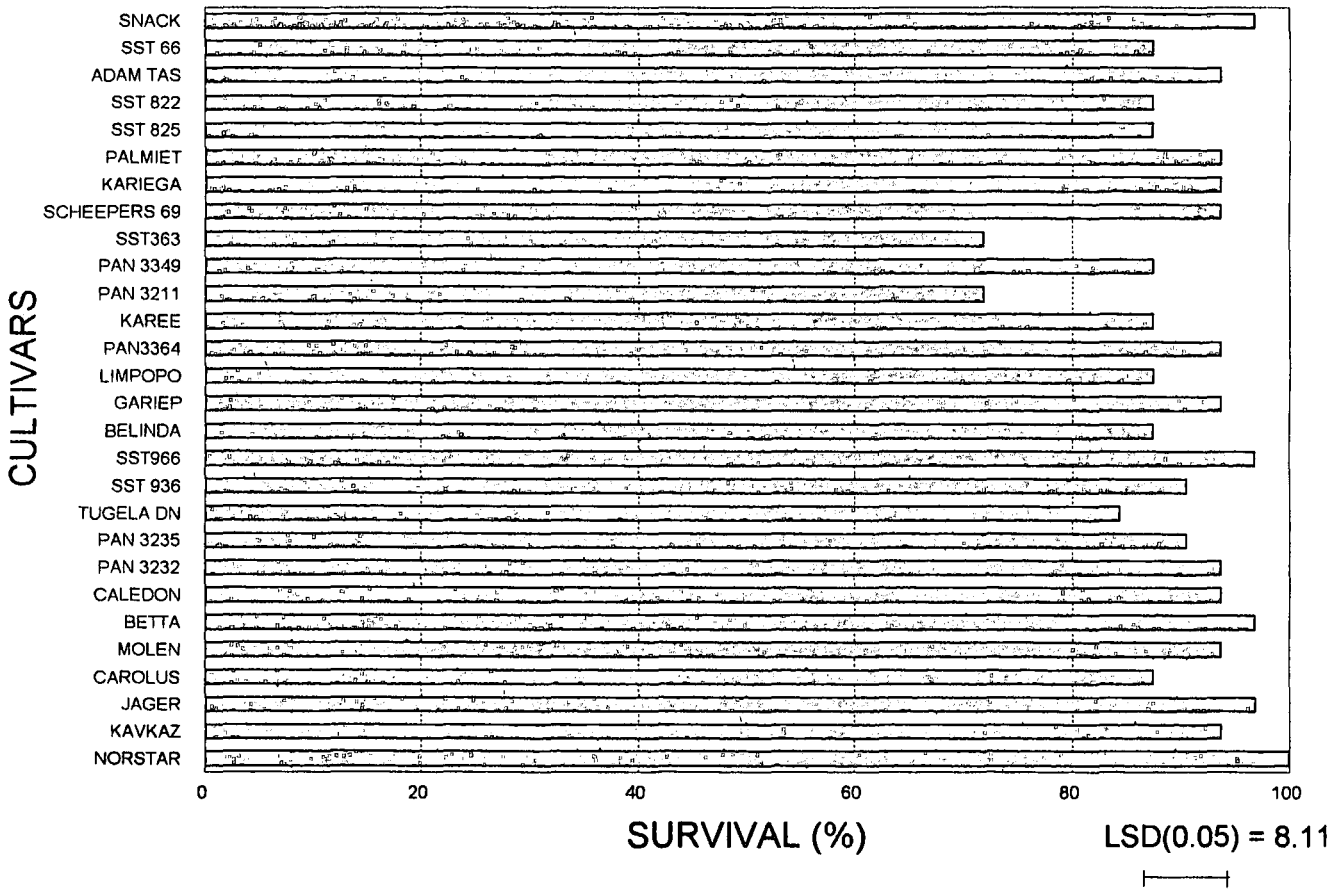

Fig. 4.1. Percentage survival of South African wheat cultivars frozen at -6°C.

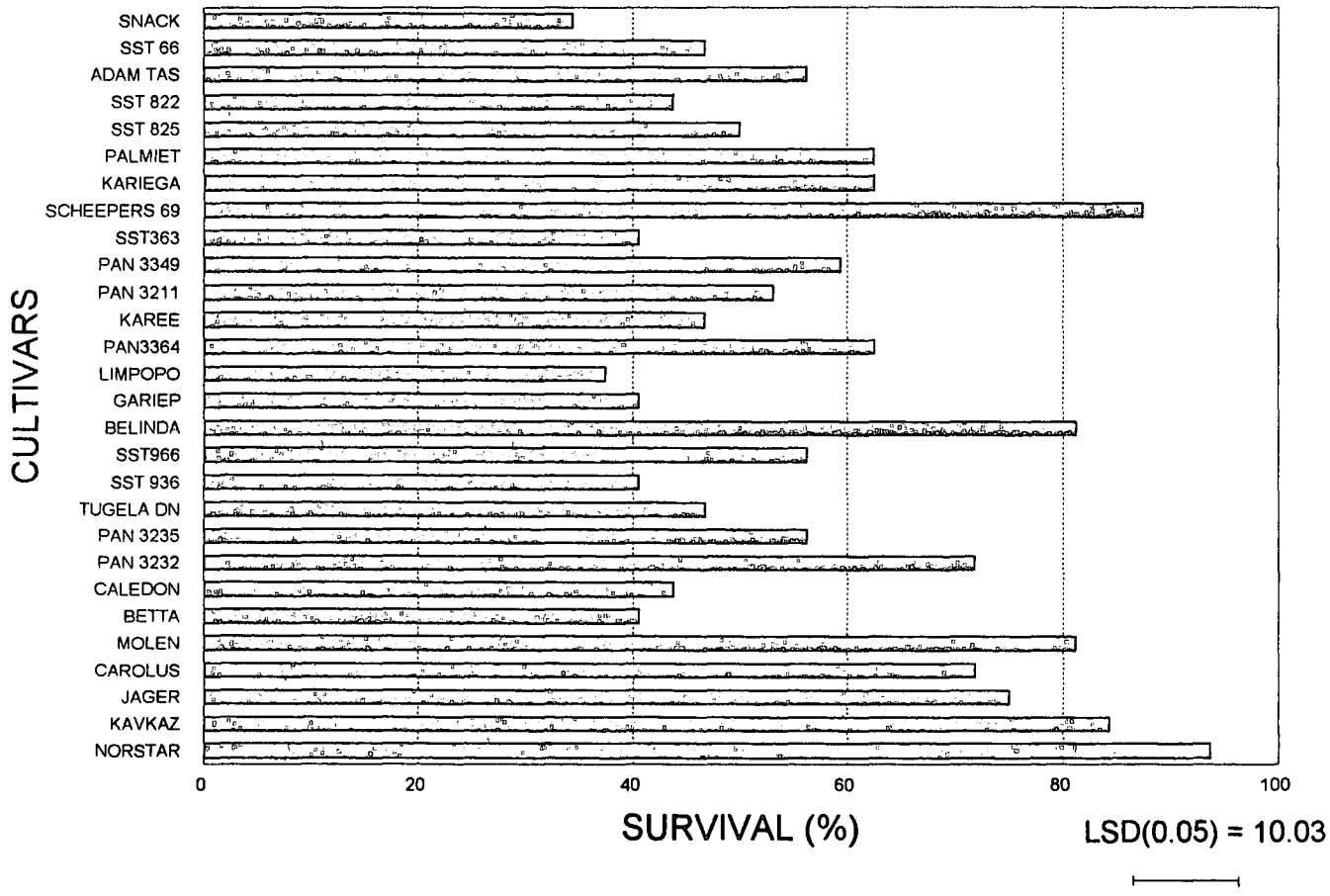

Fig. 4.2. Percentage survival of South African wheat cultivars frozen at -12°C.

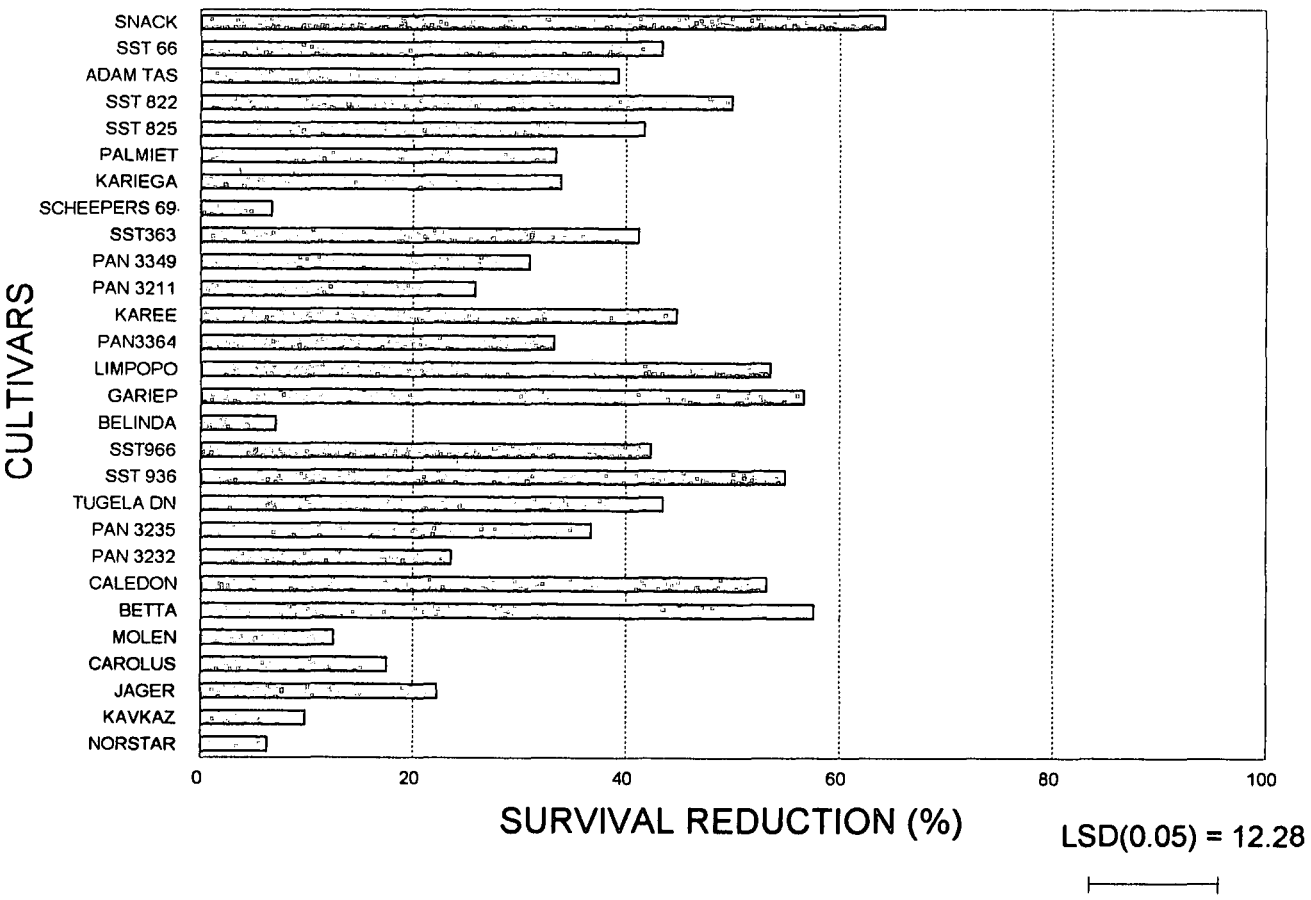

Fig. 4.3. Percentage reduction in survival of two sets of South African wheat cultivars frozen at -6°C and -12°C respectively.

4.1.2 Leaf length. The leaf length at -6°C varied from 332.03mm (Scheepers 69) to 161.43mm (Carolus)(Fig. 4.4).

Significant differences were observed for leaf length at -12°C. The leaves of Tugela DN (245.25mm) were significantly longer than the rest of the genotypes. Snack (76.20mm) and Carolus (77.73mm) showed the weakest leaf regrowth at -12 °C(Fig. 4.5).

Cultivars were significantly different from each other for percentage leaf length reduction. Snack (69.82%) and Kariega (61.95%) had significantly higher values for percentage leaf length reduction, which indicated a low level of tolerance to freezing. Caledon (7.78%), PAN 3232 (8.31%), SST 966 (10.18%), SST 936 (10.25%) and Norstar (10.40%) exhibited a low percentage leaf length reduction, which indicated a high level off tolerance to freezing (Fig. 4.6).

4.1.3 Root length. The roots of Tugela DN (247.88mm) and Scheepers 69 (224.13mm) were significantly longer than the roots of the other genotypes at -6°C. The roots of PAN 3235 (76.13mm) and SST 363 (90.25mm) were significantly shorter than the roots of the other genotypes (Fig. 4.7).

At -12°C the root length of the cultivars ranged from 147mm (Norstar) to 40.45mm (Palmiet). Norstar (147.05mm), Belinda (146.33mm), Jager (136.33mm), Kavkaz (136.28mm) and Carolus (129.13mm) exhibited the longest roots at -12°C. Palmiet (40.45mm), Kariega (41.93mm), Snack (49.68mm), SST825 (50.45mm) and Adam Tas (51.50mm) showed the weakest root growth at -12°C(Fig.4.8).

Significant differences were observed for percentage root length reduction, this indicated different levels of tolerance to freezing. Kavkaz (2.46%) and Norstar (4.05%) exhibited the lowest percentage root length reduction, indicating a high level of

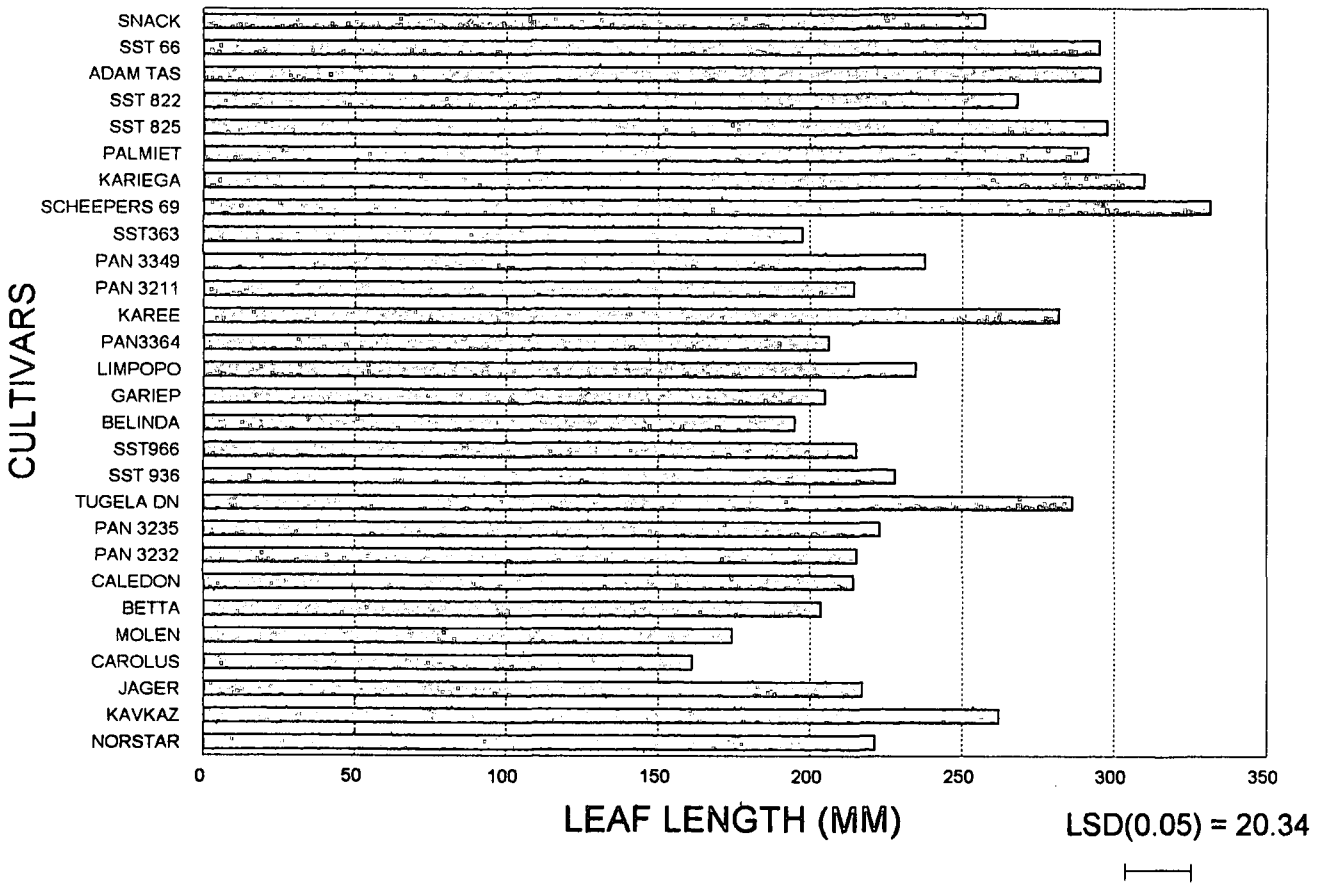

Fig. 4.4. Leaf length of South African wheat cultivars frozen at -6°C.

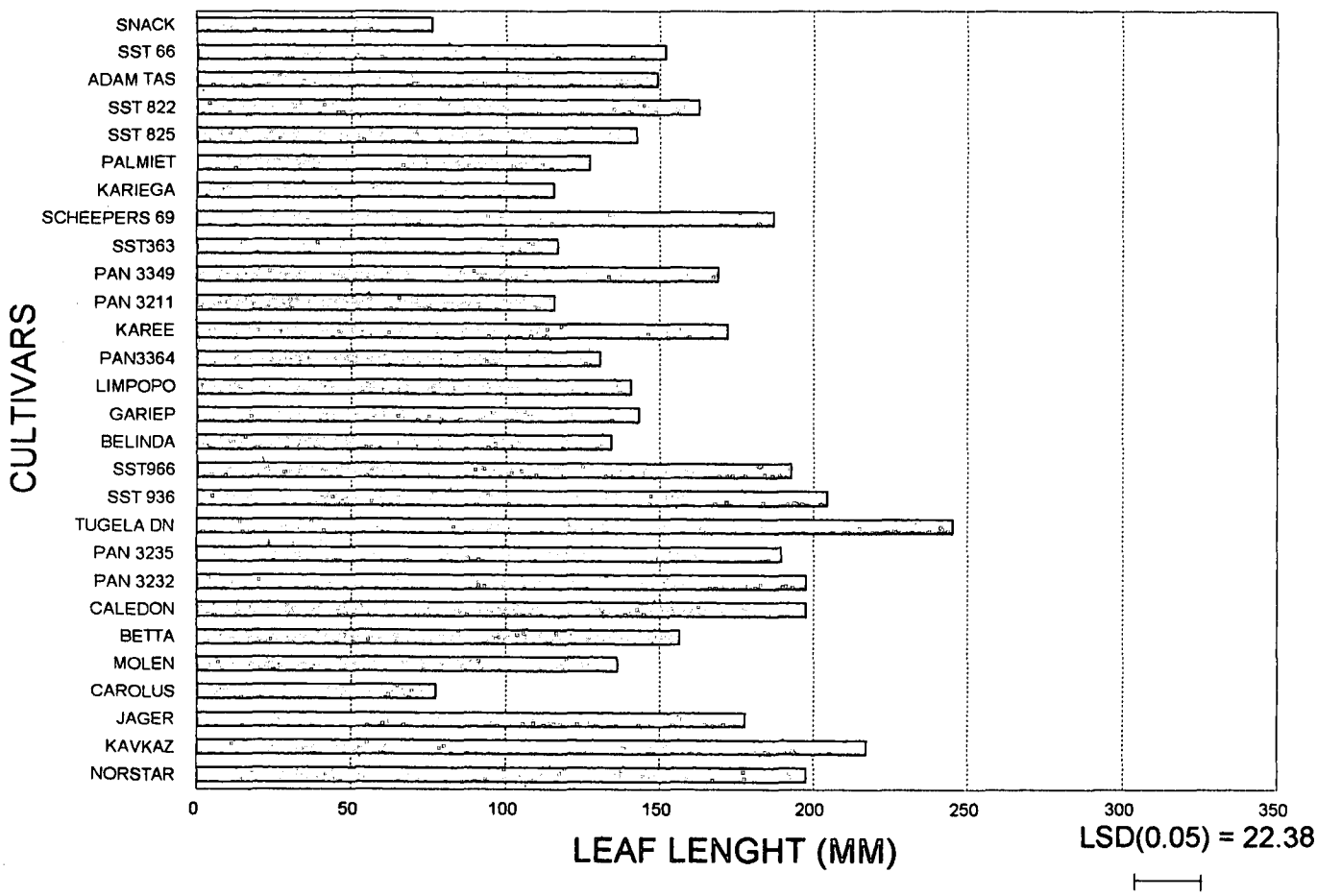

Fig. 4.5. Leaf length of South African wheat cultivars frozen at -12°C.

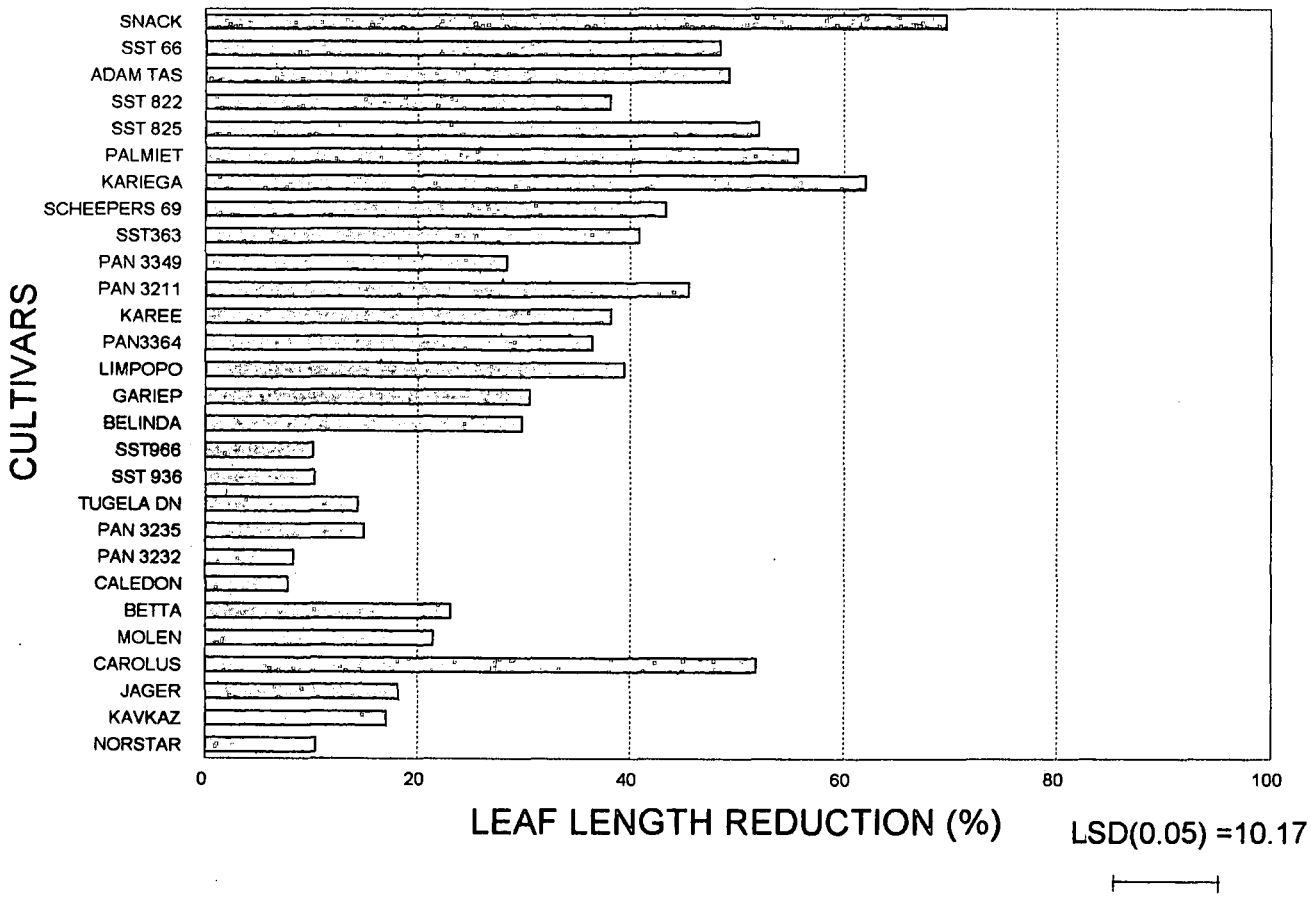

Fig. 4.6. Percentage reduction in leaf length of two sets of South African wheat cultivars frozen at -6°C and -12°C respectively.

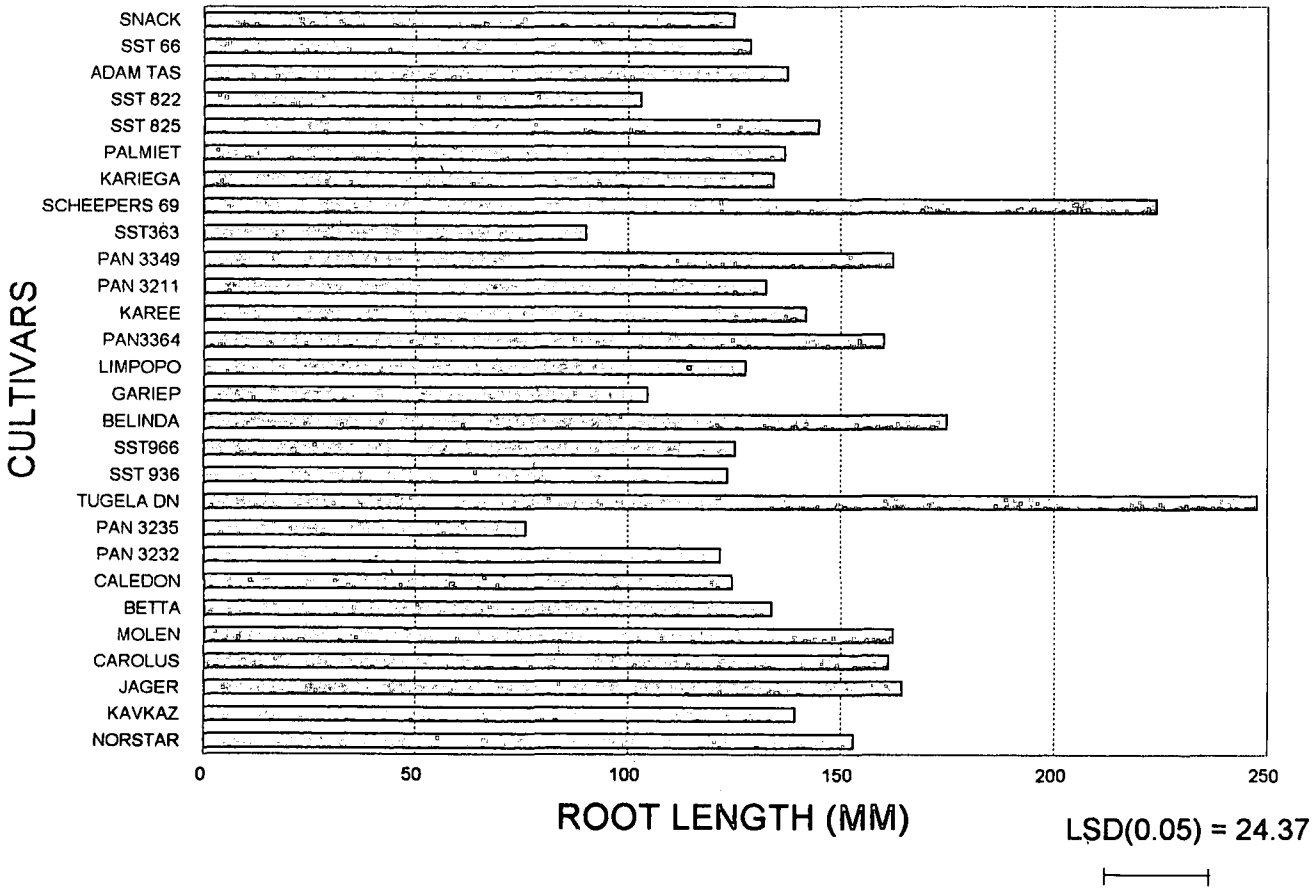

Fig. 4.7. Root length of South African wheat cultivars frozen at -6°C.

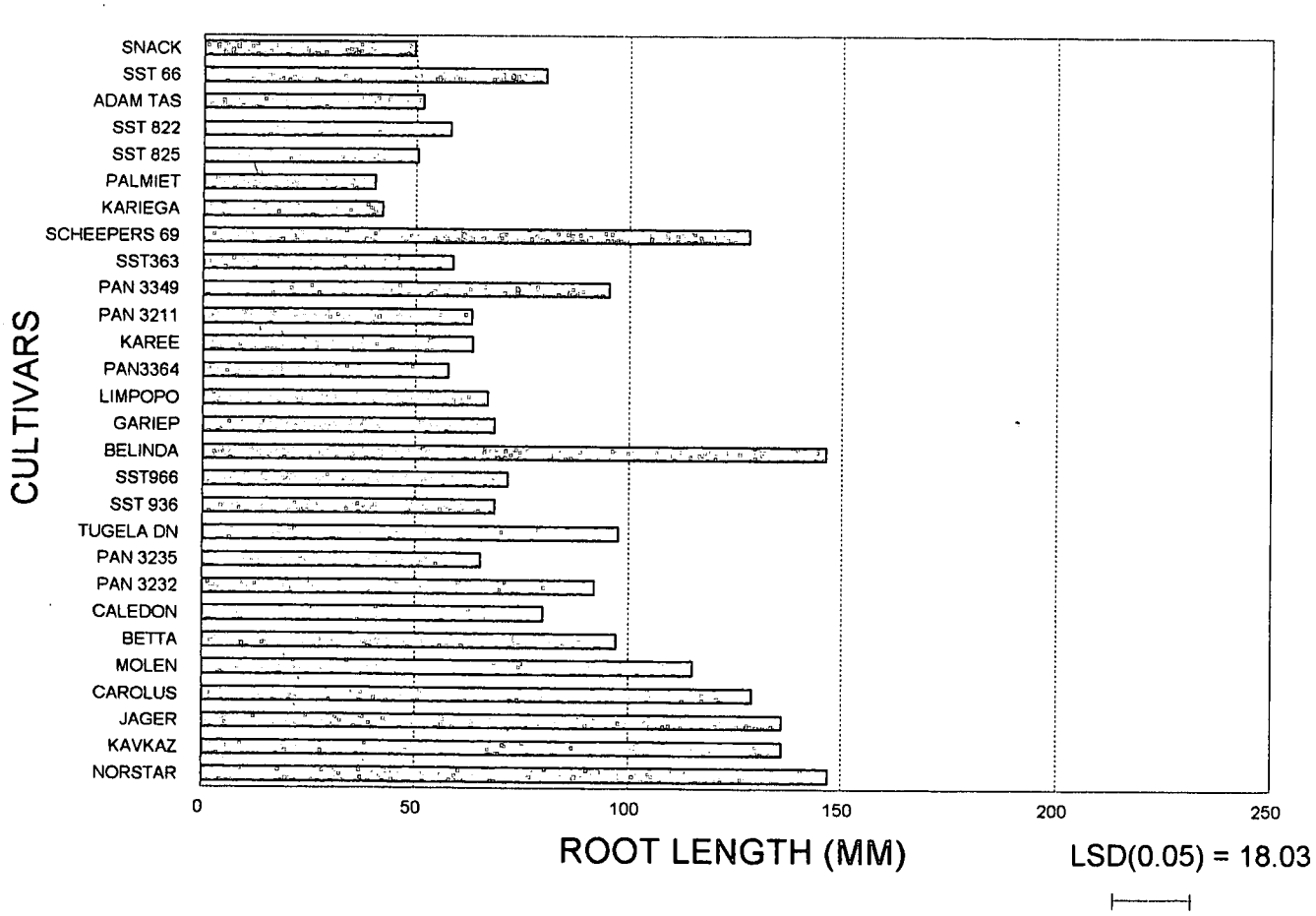

Fig. 4.8. Root length of South African wheat cultivars frozen at -12°C.

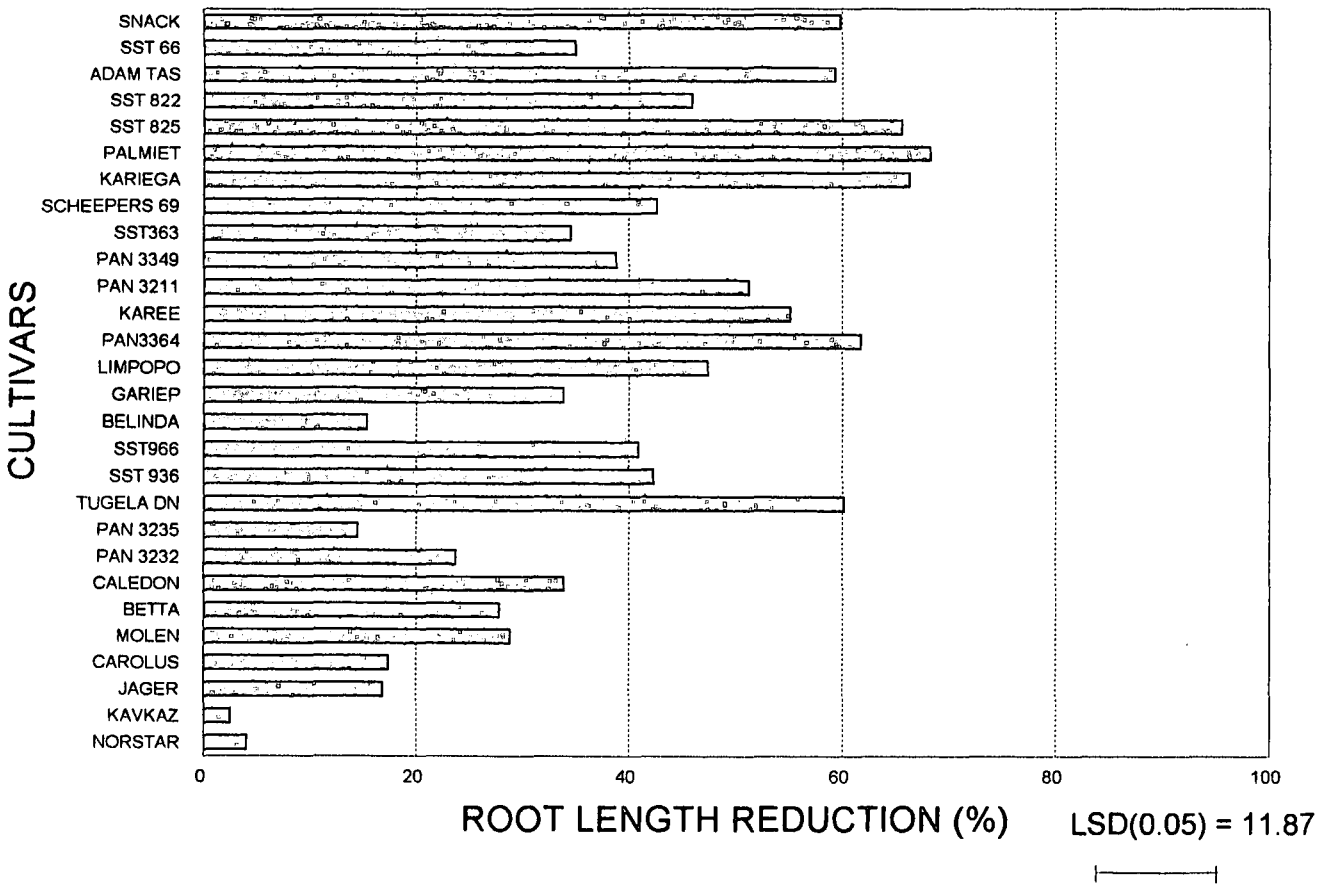

Fig. 4.9. Percentage reduction in root length of two sets of South African wheat cultivars frozen at -6°C and -12°C respectively.

tolerance to freezing. Genotypes with a high percentage root length reduction included Palmiet (68.31%), Kariega (66.31%) and SST 825 (65.65%). These values indicated a poor level of tolerance to freezing (Fig. 4.9).

4.2 Genetic variability, combining ability and inheritance of tolerance to freezing

4.2.1 Genetic variability

The ANOVA (Table 4.1) showed that the different temperature treatments were significantly different ($p<0.01$) from each other for the different freezing tolerance parameters, percentage survival, leaf length and root length. Percentage survival showed significant variance for temperature/replication interaction. Highly significant differences also existed between genotypes. Significant differences in percentage survival, leaf length and root length for the temperature/genotype interaction were also observed. This demonstrate the important influence of temperature on the genotypic x environment (GXE) interaction.

4.2.1.1 Survival. The percentage survival of Norstar at -6°C was significantly higher than the survival rate of Tugela DN, PAN 3349, Karee, SST 66 and Snack. The percentage survival of PAN 3349 and Snack was significantly higher than the survival rate of Tugela DN, Karee and SST 66.

The following F1-hybrids showed no significant difference for percentage survival at -6°C, Karee/PAN 3349, Karee/SST 66, Norstar/PAN 3349, SST 66/PAN 3349, PAN 3349/ Karee, Norstar/Karee, PAN 3349/SST 66, Karee/Norstar, SST 66/Norstar, Karee/Tugela DN, SST 66/Tugela DN, Snack/SST 66, PAN 3349/Norstar, Norstar/Tugela DN, Tugela DN/PAN 3349, Tugela DN/SST 66, SST 66/Karee, PAN

Table 4.1. Mean squares derived from the diallel ANOVA for cold tolerance characteristics

Source	D.F.	Survival	Leaf length	Root length
Temperature	1	95247.94**	919707.03**	619662.78**
Replications*Temperature	6	242.15**	1608.99NS	266.99NS
Genotypes	35	5196.97**	52800.82**	23765.23**
Temperature*Genotypes	35	507.98**	6800.67**	7986.14**
Error	210	79.95	1126.54	866.46

* $p \leq 0.05$
** $p \leq 0.01$
NS not significant
D.F. degrees of freedom

3349/Tugela DN, Tugela DN/Karee, SST 66/Snack and Tugela DN/Norstar. The following F1-hybrids exhibited significantly lower survival rates than the other F1-hybrids at -6°C, Tugela DN/Snack, Snack/Tugela DN, Snack/PAN 3349, PAN 3349/Snack, Snack/Norstar and Norstar/Snack. This lower survival rate might be the result of a dominant gene for cold sensitivity inherited form Snack (Fig. 4.10).

At -12°C, Norstar, had a significantly higher survival rate than Tugela DN, PAN 3349, Karee, SST 66 and Snack, while the percentage survival of PAN 3349 was significantly higher than Tugela DN, Karee, SST 66 and Snack. The percentage survival of Tugela DN, Karee and SST 66 at -12°C were significantly higher that the percentage survival of Snack. These results confirmed the tolerance to freezing of Norstar and the sensitivity of Snack to low temperatures.

The following F1-hybrids exhibited significantly higher survival rates at -12°C, Karee/Norstar, SST 66/PAN 3349, Karee/PAN 3349, SST 66/Norstar and Tugela DN/Norstar. PAN 3349/Snack, Tugela DN/Snack, Snack/PAN 3349, Norstar/Snack, Snack/Norstar and Snack/Tugela DN were unable to survive exposure to -12°C. This poor level of survival ability at -12°C was also an indication of the dominant gene for cold sensitivity inherited form Snack (Fig. 4.11).

Norstar showed a significantly lower percentage reduction in survival from -6 to -12°C than PAN 3349, Tugela DN, Karee, SST 66 and Snack. That was an indication of a high level of tolerance to freezing in Norstar. Snack had a significantly higher percentage survival reduction than Norstar, Tugela DN, PAN 3349, Karee, SST 66 and Snack, indicating a sensitivity to low temperatures. SST 66, Karee and Tugela DN had a higher reduction in survival than PAN 3349, but significantly lower than Snack.

The following F1-hybrids exhibited significantly higher percentages of reduction

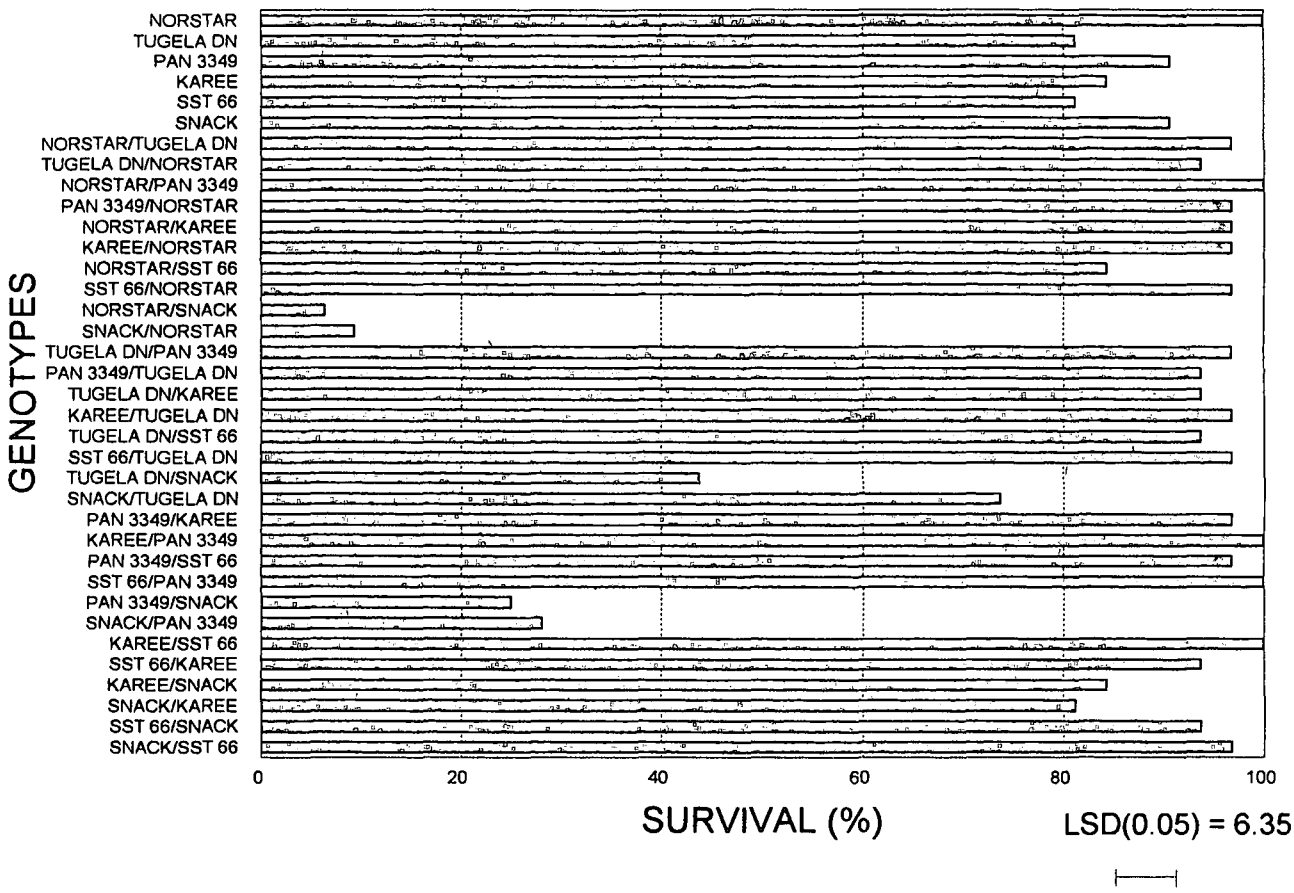

Fig. 4.10. Percentage survival of genotypes frozen at -6°C.

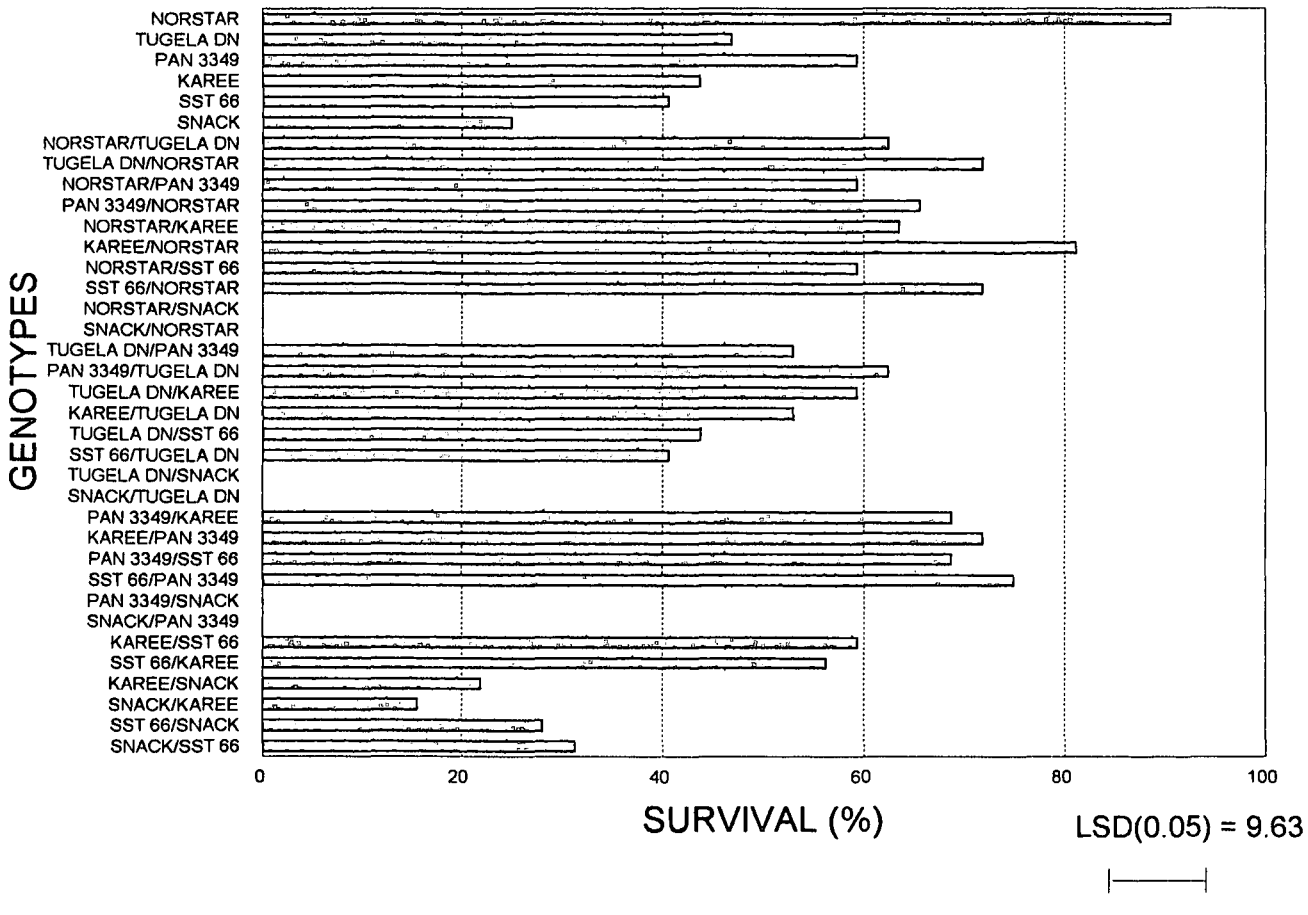

Fig. 4.11. Percentage survival of genotypes frozen at -12°C.

in survival, PAN 3349/Snack, Snack/PAN 3349, Norstar/Snack, Tugela DN/Snack, Snack/Tugela DN and Snack/Norstar, which indicated sensitivity to low temperatures. SST 66/Norstar, SST 66/PAN 3349, Tugela DN/Norstar and Karee/Norstar had significantly lower percentages of survival reduction. The low percentage reduction in survival indicated a tolerance to freezing, which might be additive gene action as well as genes for tolerance to freezing inherited from Norstar (Fig. 4.12).

4.2.1.2 Leaf length. The leaf length of Norstar, PAN 3349 and Snack were not significantly different from one another but were significantly shorter than the leaves of Tugela DN, SST 66 and Karee at -6°C. The results at -6°C might be influenced by the interaction between temperature and growth habit of the parent cultivars.

SST 66/Karee, Karee/Snack, Karee/SST 66 and Snack/Karee had significantly longer leaves at -6°C than the other F1-hybrids. The following F1-hybrids Snack/Tugela DN, Snack/PAN 3349, PAN 3349/Snack, Tugela DN/Snack, Snack/Norstar and Norstar/Snack displayed a significantly shorter leaf length at -6°C than the other F1-hybrids. The results indicated that the inheritance of a cold sensitivity gene from Snack, which induced shorter leaves at -6°C. A freezing tolerance gene inherited from Karee as well as the growth habit of SST 66, Karee and Snack, might have induced longer leaves on the different F1-hybrids (Fig. 4.13).

At 12°C the leaves of Tugela DN and Norstar were significantly longer than the leaves of PAN 3349, Karee, SST 66 and Snack. Although significantly shorter than the leaves of Norstar, the leaves of PAN 3349, Karee and SST 66 were still longer than those of Snack. The leaves of Snack were significantly shorter than the leaves of the other cultivars, confirming its sensitivity to low temperatures.

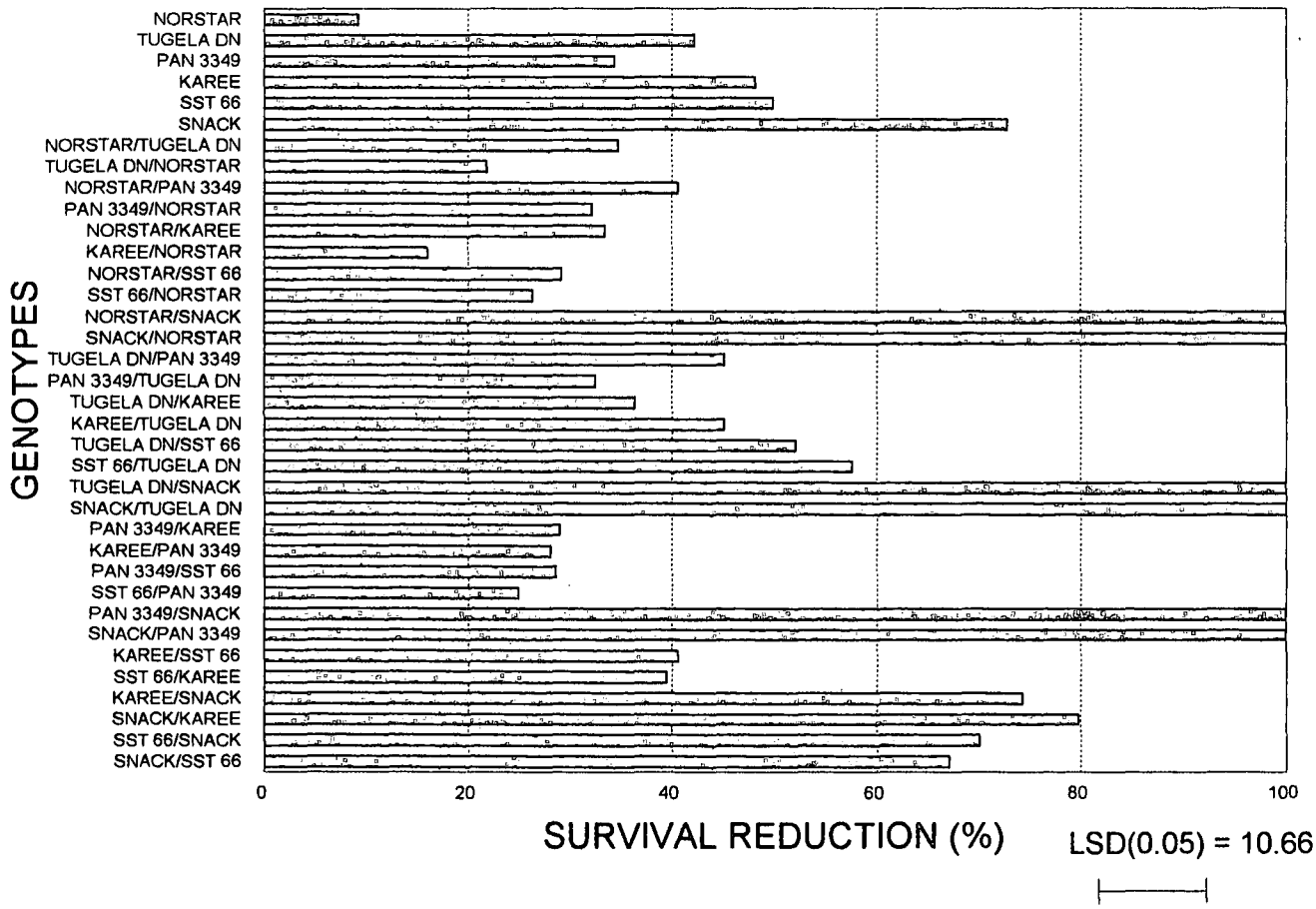

Fig. 4.12. Percentage reduction in survival of two sets of genotypes frozen at -6°C and -12°C respectively.

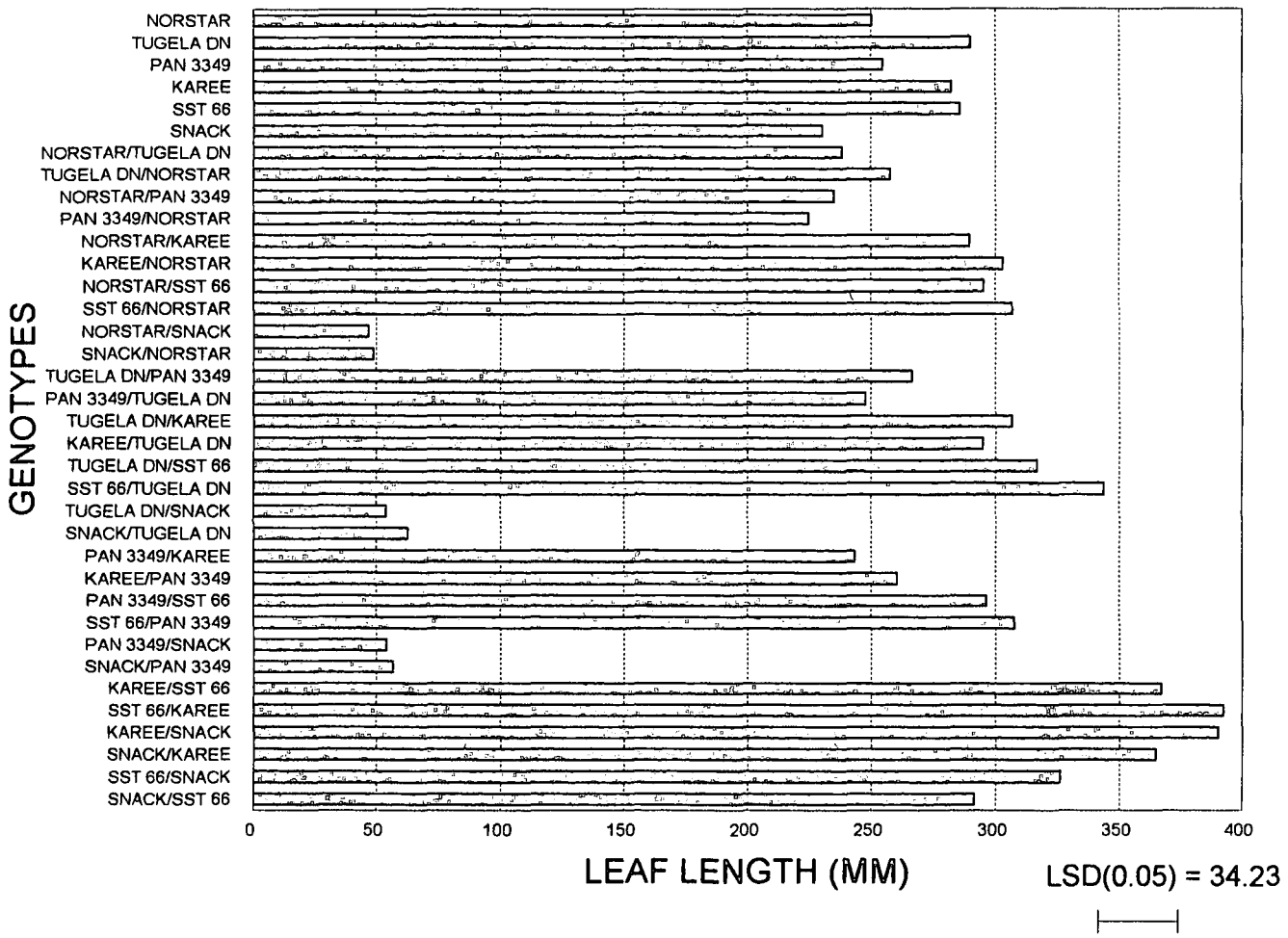

Fig. 4.13. Leaf length of genotypes frozen at -6°C.

At -12°C significant variation for leaf length existed among the F1-hybrids. These Fi-hybrids included Tugela DN/Norstar, Tugela DN/Karee, Karee/Tugela DN, SST 66/Norstar, Karee/Norstar and Norstar/Tugela DN. Some of the genotypes were unable to survive the -12°C treatment, their leaf length was scored as zero. These genotypes included PAN 3349/Snack, Tugela DN/Snack, Snack/PAN 3349, Norstar/Snack, Snack/Norstar and Snack/Tugela DN. Crosses with Snack were highly sensitive to low temperatures (Fig. 4.14).

The percentage reduction in leaf length of Norstar and Tugela DN was significantly lower than the reduction in leaf length in PAN 3349, Karee, SST 66 and Snack. The percentage reduction in leaf length of PAN 3349 was significantly higher than that for Tugela DN and Norstar, but still significantly lower than the reduction in leaf length of Karee, SST 66 and Snack. The percentage reduction in leaf length of Snack was significantly higher than the other cultivars. This high reduction in leaf length indicated a sensitivity to grow at extremely low temperatures.

The following F1-hybrids had a 100% reduction in leaf length because they were killed by the -12°C treatment, PAN 3349/Snack, Snack/PAN 3349, Norstar/Snack, Tugela DN/Snack, Snack/Tugela DN and Snack/Norstar. This result indicated once again that a cold sensitive gene was inherited from Snack. Norstar/Tugela DN, Tugela DN/Norstar and Karee/PAN 3349 had a significantly lower percentage of leaf length reduction than the other genotypes. Although these genotypes exhibited a high level of tolerance to freezing, they were not more freezing tolerant than the tolerant check, Norstar (Fig. 4.15).

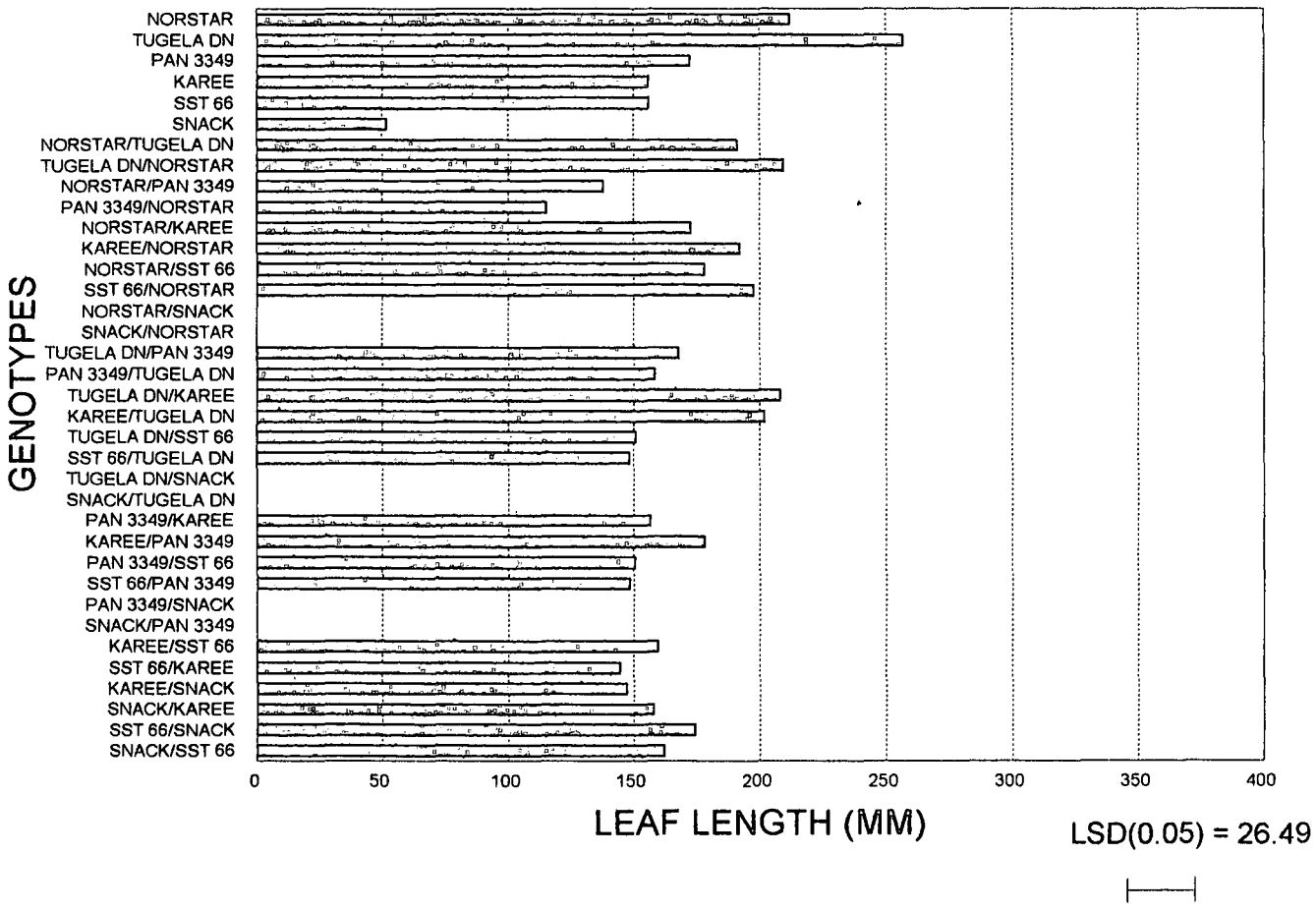

Fig. 4.14. Leaf length of genotypes frozen at -12°C.

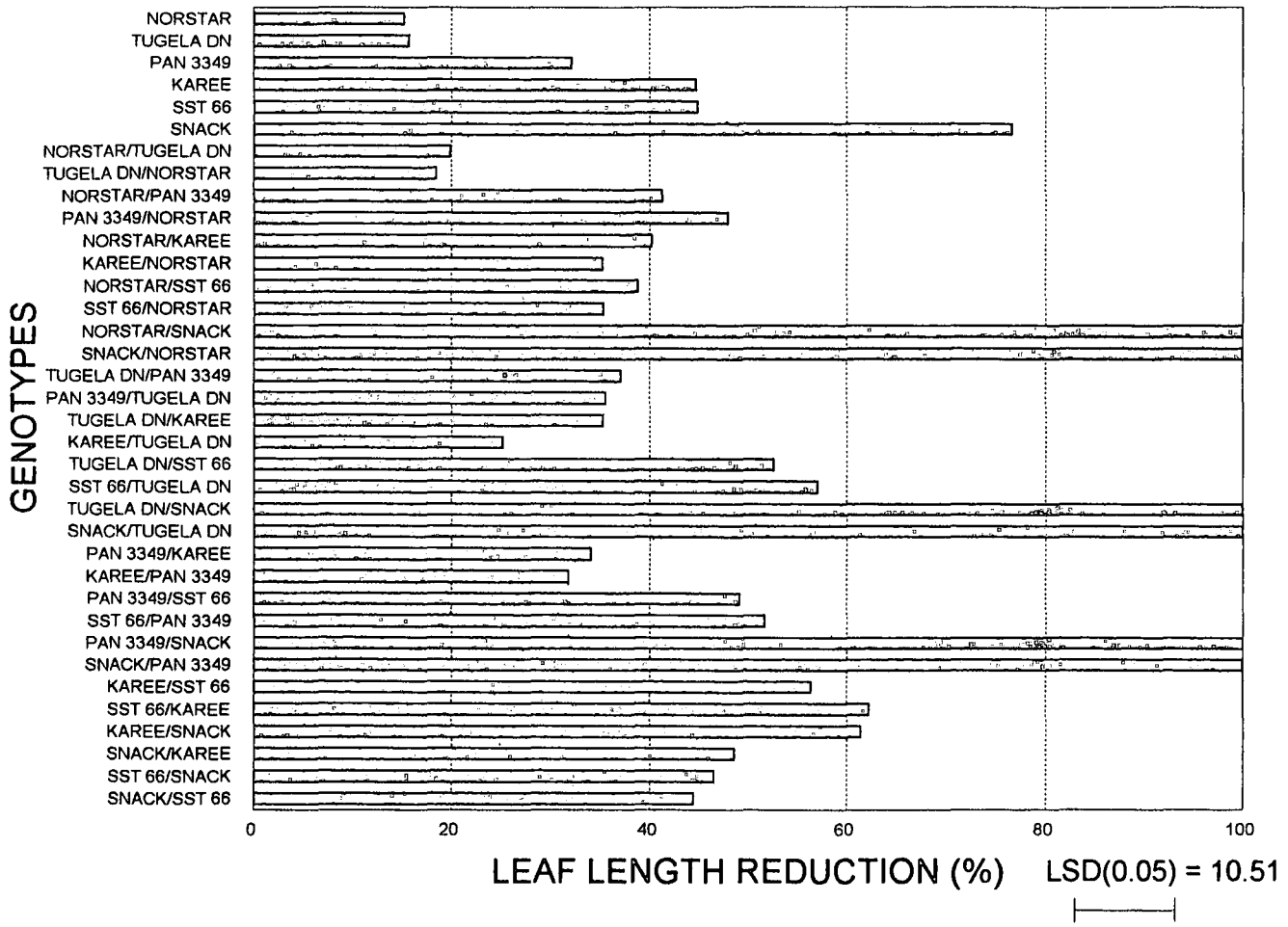

Fig. 4.15. Percentage reduction in leaf length of two sets of genotypes frozen at -6°C and -12°C respectively.

4.2.1.3 Root length. At -6°C the roots of Tugela DN were significantly longer than the roots of Norstar, PAN 3349, Karee, SST 66 and Snack. The root lengths of Norstar, PAN 3349 and SST 66 were not significantly different from each other. The roots of Karee and Snack were significantly shorter than the roots of the other parent cultivars at -6°C.

The roots of three F1-hybrids, Karee/Norstar, SST 66/Norstar and Norstar/SST 66 were significantly longer at -6°C. The roots of the following F1-hybrids were significantly shorter at -6°C, Norstar/Snack, Snack/Norstar, Snack/PAN 3349, PAN 3349/Snack, Tugela DN/Snack and Snack/Tugela DN (Fig. 4.16).

The additive effect of genes for tolerance to freezing inherited from Norstar, SST 66 and Karee made these F1-hybrids more freezing tolerant than Norstar. The inheritance of the freezing sensitive gene or lack of additive gene action resulted in Snack crosses with poor tolerance to freezing.

At -12°C the root length of Norstar was significantly longer than the root length of Tugela DN, PAN 3349, Karee, SST 66 and Snack, an indication of the ability of Norstar to tolerate low temperatures. The root lengths of Tugela DN and PAN 3349 were not significantly different from each other but were significantly longer than the roots of Karee, Snack and SST 66. The roots of Snack were significantly shorter than the roots of the other parental cultivars.

The root length of the F1-hybrids, Norstar/Tugela DN and Tugela DN/Norstar, were significantly longer than the roots of the other F1-hybrids at -12°C. The root length of the rest of the F1-hybrids were not significantly different from one another, except for the F1-hybrids which were scored zero because they did not survive the -12°C treatment. These F1-hybrids were PAN 3349/Snack, Tugela DN/Snack,

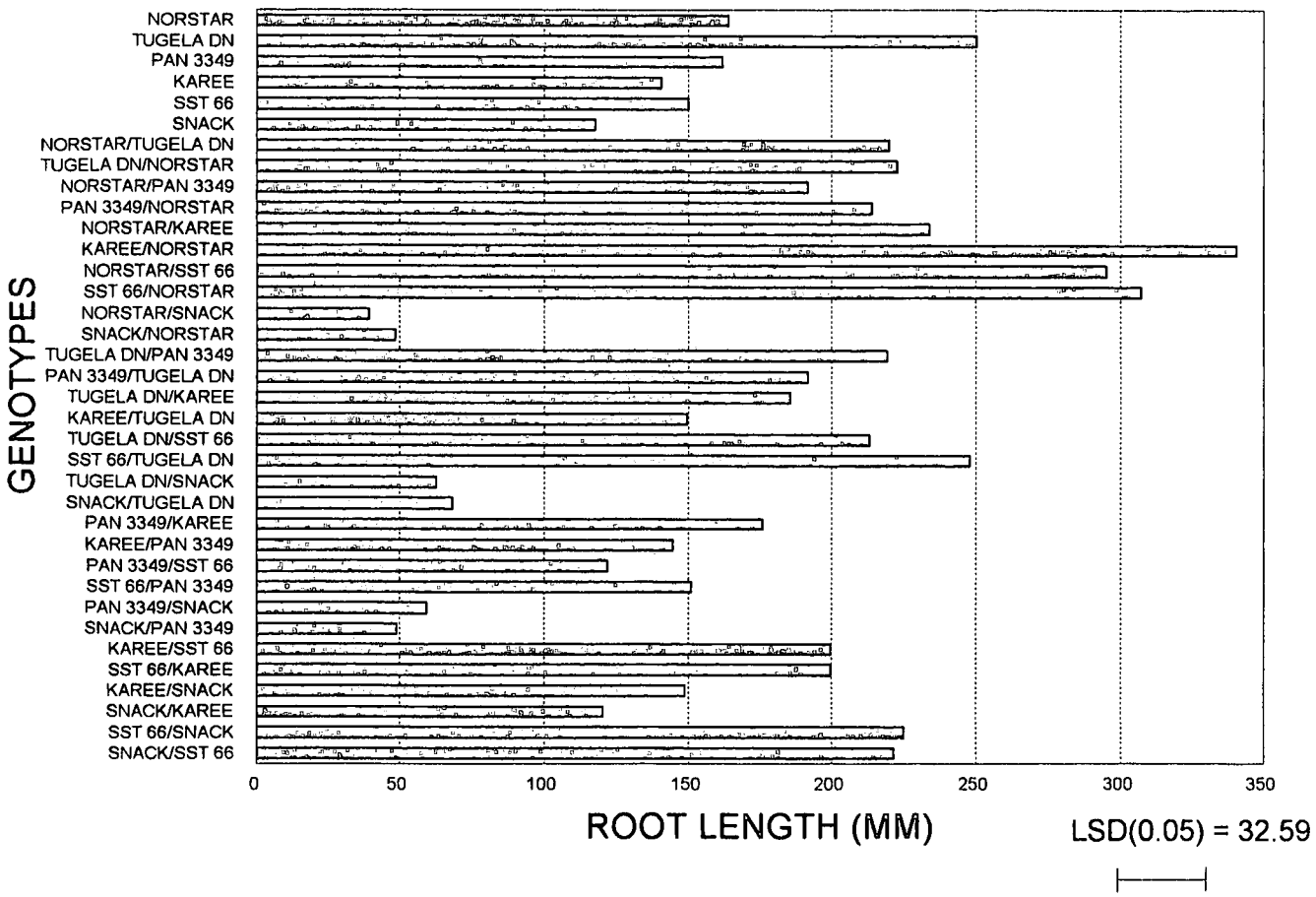

Fig. 4.16. Root length of genotypes frozen at -6°C.

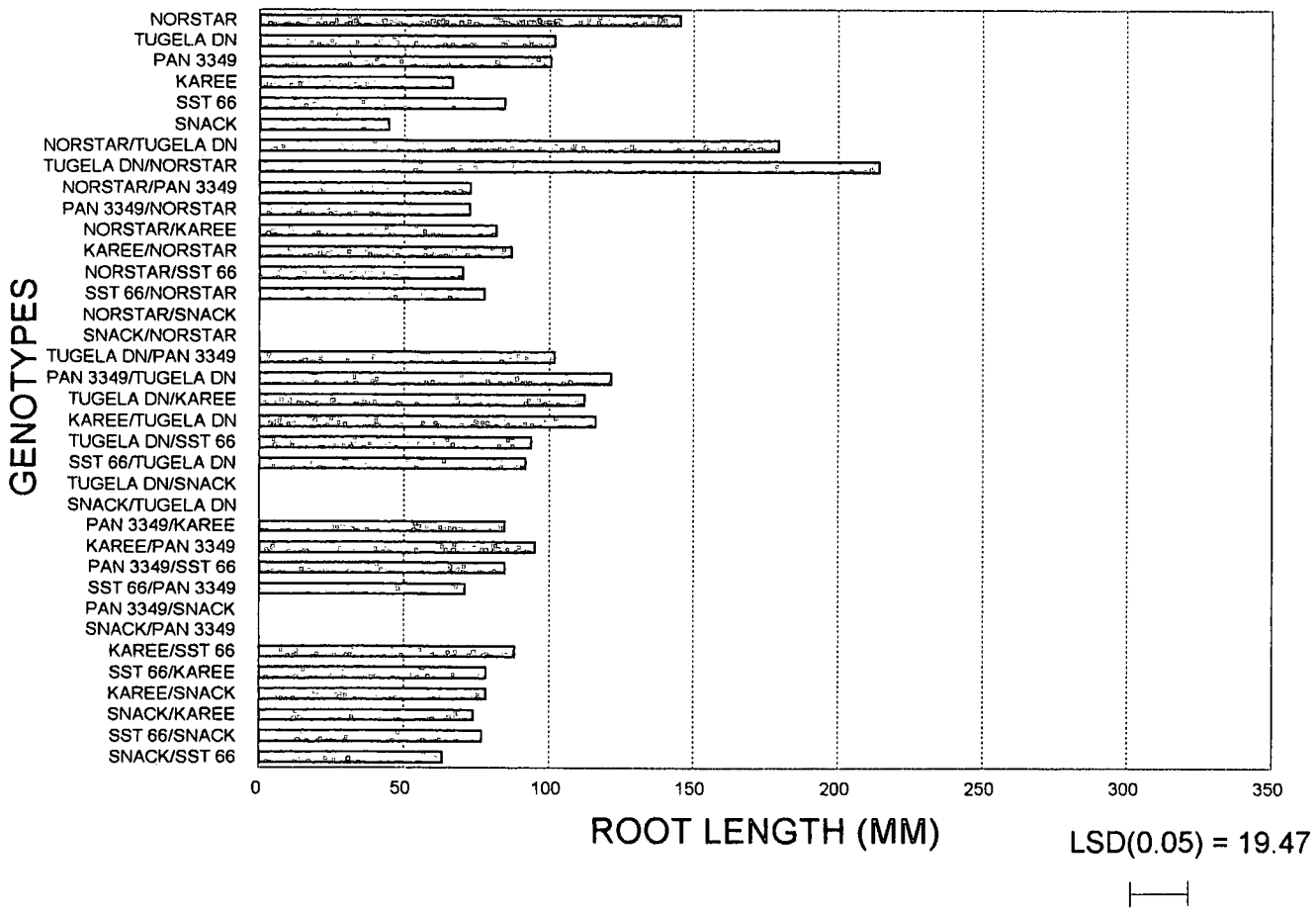

Fig. 4.17. Root length of genotypes frozen at -12°C.

Snack/PAN 3349, Norstar/Snack, Snack/Norstar and Snack/Tugela DN. Once again a cross with Norstar resulted in a F1-hybrid with a higher level of freezing tolerance than the check. This might be the result of additive gene action (Fig. 4.17).

The roots of Norstar exhibited a low percentage root length reduction which was significantly lower than the rest of the parent cultivars. The percentage root length reduction of SST 66 and PAN 3349 was significantly less than the reduction of Karee, Tugela DN and Snack. The percentage root length reduction of Snack and Tugela DN was significantly higher than the other parent cultivars which indicated a sensitivity to freezing.

The percentage root length reduction of the F1-hybrids, Norstar/Tugela DN and Tugela DN/Norstar were significantly lower than the other F1-hybrids and the freezing tolerant check, Norstar. This may be the result of heteroses and confirmed the additive gene action of freezing tolerance. The percentage root length reduction of PAN 3349/Snack, Snack/PAN 3349, Norstar/Snack, Tugela DN/Snack, Snack/Norstar and Norstar/SST 66 was 100% because they did not survive the -12°C treatment (Fig. 4.18).

4.2.2 Combining ability

Survival. Significant differences in the general combining ability (GCA) and specific combining ability (SCA) were observed for percentage survival at -6°C. The reciprocal crosses were not significantly different from each other at -6°C. The GCA and SCA for percentage survival at -12°C were significantly different, but the reciprocal crosses were not significantly different from each other. A similar observation was made for the GCA and SCA of percentage survival reduction. The reciprocal crosses were also not significantly different (Table 4.2).

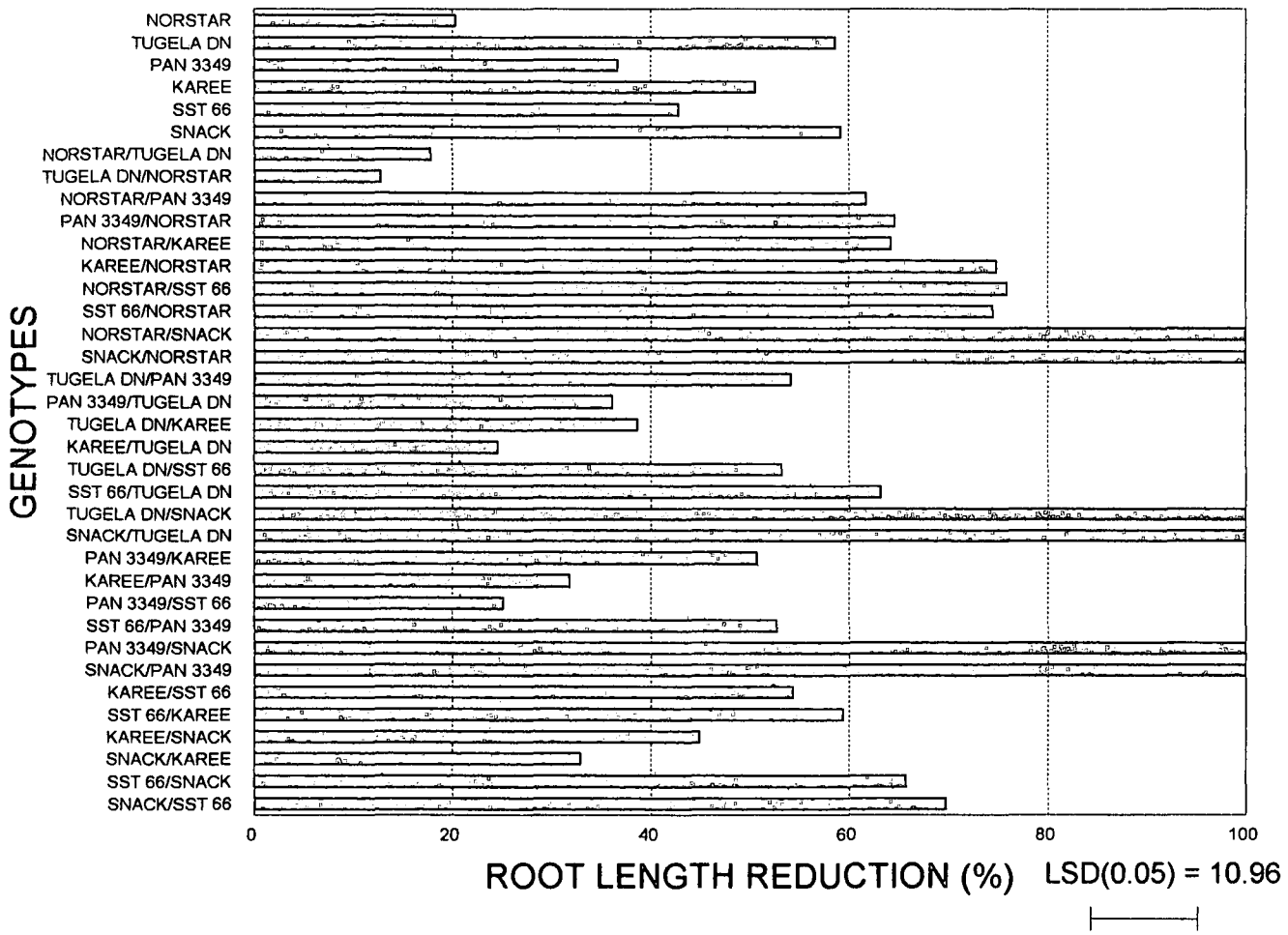

Fig. 4.18. Percentage reduction in root length of two sets of genotypes frozen at -6°C and -12°C respectively.

Table 4.2. Mean squares derived from an ANOVA for combining ability in a 6x6 diallel

		Survival			Leaf length			Root length		
Source	D.F.	-6°C	-12°C	%reduction	-6°C	-12°C	%reduction	-6°C	-12°C	%reduction
GCA	5	1984.38**	3539.61**	3784.03**	35732.71**	19385.15**	2361.49**	16677.49**	8245.57**	1297.74**
SCA	15	1004.23**	446.52**	460.20**	10923.26**	5213.89**	709.45**	6381.64**	3127.67**	980.83**
Reciprocals	15	10.09NS	25.72NS	28.74NS	168.32NS	89.45NS	10.94NS	666.51*	38.12NS	69.32NS
Error	105	12.11	27.86	34.14	352.30	210.97	33.23	319.30	113.93	36.14

* $p \leq 0.05$
** $p \leq 0.01$
NS not significant
D.F. degrees of freedom

Leaf length. At -6°C the GCA and SCA for leaf length were significantly different, while the reciprocal crosses were not. Significant differences for leaf length were also observed for the GCA and SCA at -12°C. The reciprocal crosses were not significantly different at -12°C. The GCA and SCA for percentage leaf length reduction were significantly different. The reciprocal crosses were not significantly different (Table 4.2).

Root length. The GCA and SCA for root length at -6 and -12°C were significantly different. The reciprocal crosses at -6°C were not significantly different, but the reciprocal crosses at -12°C were significantly different. The GCA and SCA for percentage root length reduction were significantly different. No significant differences for the GCA and SCA of the reciprocal crosses for root length reduction were observed (Table 4.2).

No significant differences were observed between reciprocal crosses, except for root length at -6°C. This indicated that cytoplasmic effects did not play any roll in tolerance to freezing (Table 4.2).

4.2.2.1 General combining ability.

Survival. The general combining abilities for percentage survival of Karee (10.156) and SST 66 (10.677) were significantly higher than those of the other parent cultivars for percentage survival at -6°C. The GCA for percentage survival of PAN 3349 (2.344), Tugela DN (2.083) and of Norstar (-0.781) were significantly higher than the GCA of the spring wheat cultivar, Snack (-24.479).

The GCA for percentage survival of Norstar (13.976) was significantly higher than for the rest of the cultivars at -12°C. The cultivars PAN 3349 (7.726), Karee

(7.465) and Snack (5.382) had a significantly higher GCA for percentage survival than Snack (-33.681) and Tugela DN (-0.868) at -12°C. Tugela DN had a significantly higher GCA for percentage survival than Snack.

The GCA of Norstar (-13.442) for percentage survival reduction was significantly lower than the other cultivars. The GCA of Karee (-7.940), PAN 3349 (-7.035) and SST 66 (-6.490) for percentage survival reduction was significantly lower than Tugela DN (-0.351) and Snack (35.239) (Table 4.3).

Leaf length. At -6°C the GCA for leaf length of Karee (62.757) and SST 66 (65.892) was significantly higher than the other cultivars. The GCA of Tugela DN (-4.629) was significantly higher than Norstar, PAN 3349 and Snack. The GCA for leaf length at -6°C for Norstar (-23.181) and PAN 3349 (-28.451) was significantly higher than Snack (-72.386).

AT -12°C the GCA for leaf length of Karee (29-976), Tugela DN (23.174) and SST 66 (21.163) was significantly higher than the other cultivars. The GCA for leaf length of Norstar (12.319) was significantly higher than PAN 3349 and Snack. The GCA of PAN 3349 (-9.483) was significantly higher than Snack (77.149).

The GCA for percentage leaf length reduction of Norstar (-8.919), Tugela DN (-7.441) and Karee (-6.995) was significantly lower than the other cultivars. PAN 3349 (-2.010) and SST 66 (-2.720) both had a GCA for percentage leaf length reduction significantly lower than Snack (28.084) (Table 4.3).

Root length. At -6°C Norstar (27.559) and SST 66 (34.319) had significantly higher GCA's than the other cultivars. The GCA's of Tugela DN (14.122) and Karee (9.090)

Table 4.3. GCA effects for tolerance to freezing characteristics

	Survival			Leaf length			Root length		
Genotypes	-6°C	-12°C	%reduction*	-6°C	-12°C	%reduction*	-6°C	-12°C	%reduction*
Norstar	-0.781	13.976	-13.422	-23.181	12.319	-8.919	27.559	19.215	-0.318
Tugela DN	2.083	-0.868	-0.351	-4.629	23.174	-7.441	14.122	26.455	-6.065
PAN 3349	2.344	7.726	-7.035	-28.451	-9.483	-2.010	-18.993	-4.306	-3.331
Karee	10.156	7.465	-7.940	62.757	29.976	-6.995	9.090	6.017	-9.419
SST 66	10.677	5.382	-6.490	65.892	21.163	-2.720	34.319	0.6944	-0.946
Snack	-24.479	-33.681	35.239	-72.389	-77.149	28.084	-66.097	-48.076	20.078
$LSD_{(0.05)}$	2.834	4.299	4.759	15.287	11.830	4.695	14.554	8.693	4.896

LSD least significant difference
* a low value indicates a high cold tolerance and *vica verca*

was significantly higher than those of PAN 3349 and Snack. The GCA for root length at -6°C of PAN 3349 (-18.993) was significantly higher than for Snack (-66.097).

At -12°C, the GCA for root length of both Tugela DN (26.445) and Norstar (19.215) was significantly higher than those of the other cultivars. The GCA of Karee (6.017), SST 66 (0.694) and PAN 3349 (-4.306) was significantly higher than Snack (-48.097).

The GCA for percentage root length reduction of Karee (-9.419) and Tugela DN (-6.065) was significantly lower than the other cultivars. The GCA of SST 66 (-0.946), Norstar (-0.318) and PAN 3349 (-3.331) was significantly lower than Snack (20.078) (Table 4.3).

Norstar and Karee had the best overall GGCA, followed by Tugela DN and SST 66. PAN 3349 was better than Snack (Table 4.3).

4.2.2.2 Specific combining ability.

Survival. At -6°C Norstar/PAN 3349 (14.58) and Norstar/Tugela DN (11.72) had a significantly higher specific combining ability (SCA) for percentage survival than the rest of the Norstar crosses. Norstar/Karee (5.21) had a significantly higher SCA for percentage survival than Norstar/SST 66 and Norstar/Snack. The SCA for percentage survival of Norstar/SST 66 (-1.56) was significantly higher than the SCA of Norstar/Snack (-49.22). Tugela DN/PAN 3349 (8.59) had a significantly higher SCA for percentage survival than the other Tugela DN crosses at -6°C. The SCA for percentage survival of Tugela DN/Karee (0.78) and Tugela DN/SST 66 (0.26) was significantly higher than Tugela DN/Snack (-16.15). The SCA for percentage survival of PAN 3349/Karee (3.65) and PAN 3349/SST 66 (3.13) was significantly higher than

PAN 3349/Snack (-33.59), at -6°C. Karee/Snack (14.84) had a significantly higher SCA for percentage survival than Karee/SST 66 (-6.25) at -6°C. At -6°C SST 66/ Snack (26.82), had a significantly higher SCA for percentage survival than any other cross. Norstar/Snack (-49.22) had the lowest SCA for percentage survival at -6°C.

At -12°C, Norstar/Tugela DN (8.16) and Norstar/Karee (6.08) had significantly higher SCA values for percentage survival than the other Norstar crosses. Norstar/SST 66 (0.35) and Norstar/PAN 3349 (-5.12) both had a significantly higher SCA for percentage survival than Norstar/Snack (26.22). The SCA for percentage survival at -12°C of Tugela DN/PAN 3349 (5.03) and Tugela DN/Karee (3.73) was significantly higher than Tugela DN/SST 66 (-8.25) and Tugela/Snack (-11.37). At -12°C the SCA for percentage survival of PAN 3349/SST 66 (12.85) and PAN 3349/Karee (9.20) was significantly higher than PAN 3349/Snack (-19.97). The SCA for percentage survival of Karee/SST 66 (0.95) and Karee/Snack (0.95) was not significantly different at -12°C. SST 66/Snack had a SCA for survival at -12°C of 12.07. SST 66/Snack and PAN 3349/SST 66 had the highest SCA for percentage survival at -12°C.

The SCA values for percentage survival reduction of Norstar/Tugela DN (-9.07), Norstar/Karee (-5.05) and Norstar/SST 66 (-3.53) was significantly lower than the other Norstar crosses. Norstar/PAN 3349 had a significantly lower SCA for percentage survival reduction than Norstar/Snack (26.99). Tugela DN/PAN 3349 (-4.97) and Tugela DN/Karee (-2.13) had significantly lower SCA values for percentage survival reduction than Tugela DN/SST 66 (10.48) and Tugela DN/Snack (13.92). PAN 3349/Karee (-7.65) had a significantly lower SCA for percentage survival reduction than the other PAN 3349 crosses. The SCA for percentage survival reduction of PAN 3349/SST 66 (10.88) was significantly lower than PAN 3349/Snack (20.60). The SCA

of the Karee crosses, Karee/SST 66 (3.34) and Karee/Snack (-1.4), was not significantly different. The SST 66/Snack had a SCA for percentage survival of -11.19. SST 66/Snack had the lowest SCA for percentage survival reduction (Table 4.4).

Leaf length. At -6°C Norstar/PAN 3349 (29.40) had a significantly higher SCA for leaf length than Norstar/Karee, Norstar/SST 66 and Norstar/Snack. The SCA of Norstar/Tugela DN (23.80), Norstar/Karee (4.60) and Norstar/SST 66 (6.50) for leaf length at -6°C was significantly higher than Norstar/Snack (-108.90). Tugela DN/PAN 3349 (38.00) had a significantly higher SCA for leaf length than Tugela DN/Karee and Tugela DN/Snack. At -6°C Tugela DN/SST 66 (17.00) and Tugela DN/Karee (-9.40) both had a significantly higher SCA value for leaf length than Tugela DN/Snack (-116.80). PAN 3349/SST 66 (12.20) had a significantly higher SCA for leaf length than the other PAN 3349 crosses at -6°C. The SCA for leaf length of PAN 3349/Karee were significantly higher than PAN 3349/Snack (-95.90). At -6°C the SCA's for leaf length of Karee/Snack (135.10) was significantly higher than the other Karee cross, Karee/SST 66 (-0.60). The SST 66/Snack cross had a SCA for leaf length at -6°C of 62.90. Karee/Snack had the highest SCA for leaf length at -6°C.

The SCA for leaf length at -12°C of the Norstar crosses was not significantly different, except for Norstar/Snack (-74.47), which was significantly lower. At -12°C the SCA for leaf length of the following Tugela DN crosses, Tugela DN/PAN 3349 (10.08) and Tugela DN/Karee (12.68) was significantly higher than Tugela DN/Snack (-85.32). The SCA for leaf length of the PAN 3349 crosses were not significantly different at -12°C. Karee/Snack (60.38) had a significantly higher SCA for leaf length than Karee/SST 66 (-38.43). The SST 66/Snack cross had a SCA for leaf length at -12°C

Table 4.4. SCA effects for tolerance to freezing characteristics

	Survival			Leaf length			Root length		
Genotypes	-6°C	-12°C	%reduction*	-6°C	-12°C	%reduction*	-6°C	-12°C	%reduction*
Norstar/Tugela DN	11.72	8.16	-9.07	23.80	25.59	-14.83	-13.15	91.63	-35.92
Norstar/PAN 3349	14.58	-5.12	5.65	29.40	-15.51	4.15	22.09	-21.74	9.24
Norstar/Karee	5.21	6.08	-5.05	4.60	1.10	2.30	77.88	-20.43	21.67
Norstar/SST 66	-1.56	0.35	-3.53	6.50	14.97	-2.70	66.96	-25.74	18.87
Norstar/Snack	-49.22	-26.22	26.99	-108.90	-74.47	29.39	-90.18	-51.09	22.68
Tugela DN/PAN 3349	8.59	5.03	-4.97	38.00	10.08	-5.62	37.77	9.71	-2.97
Tugela DN/Karee	0.78	3.73	-2.13	-9.40	12.68	-0.27	-28.18	1.89	-10.42
Tugela DN/SST 66	0.26	-8.25	10.48	17.00	-34.13	13.58	9.40	-14.04	7.63
Tugela DN/Snack	-16.15	-11.37	13.92	-116.80	-85.32	27.91	-55.25	-58.33	28.43
PAN 3349/Karee	3.65	9.20	-7.65	-34.40	7.77	-9.50	-2.44	8.46	-3.49
PAN 3349/SST 66	3.13	12.85	10.88	12.20	-1.60	3.72	-51.30	1.72	-14.33
PAN 3349/Snack	-33.59	-19.97	20.60	-95.90	-52.66	22.48	-33.38	-27.57	25.69
Karee/SST 66	-6.25	-0.95	3.34	-0.60	-38.43	17.62	-16.38	-3.16	9.61
Karee/Snack	14.84	-0.95	-1.14	135.10	60.38	-17.52	19.16	38.48	-29.26
SST 66/Snack	26.82	12.07	-11.19	62.90	84.82	-31.28	82.31	37.56	-9.02
$LSD_{(0.05)}$	4.172	6.328	7.005	22.502	62.304	6.911	21.422	12.796	7.207

LSD least significant difference

* a low value indicates a high cold tolerance and *vica verca*

of 84.82. SST 66/Snack and Karee/Snack had the highest SCA for leaf length at -12°C, which indicated specific gene action.

Norstar/Tugela DN (-14.83) had a SCA for percentage leaf length reduction significantly lower than the other Norstar crosses. The SCA for percentage leaf length reduction of Norstar/PAN 3349 (4.15), Norstar/Karee (2.30) and Norstar/SST 66 (-2.70) were significantly lower than Norstar/Snack (29.39). Tugela DN/PAN 3349 (-5.62) and Tugela DN/Karee (-0.27) had a SCA value for percentage leaf length reduction significantly lower than Tugela DN/SST 66 and Tugela DN/Snack. The SCA for percentage leaf length reduction of Tugela DN/SST 66 (13.58) was significantly lower than Tugela DN/Snack (27.91). PAN 349/Karee (-9.50) had a SCA for percentage leaf length reduction significantly lower than the other PAN 3349 crosses. PAN 3349/SST 66 (3.72) had a significantly lower SCA for percentage leaf length reduction than PAN 3349/Snack (22.48). The SCA for percentage leaf length reduction of Karee/Snack (-17.52) was significantly lower than Karee/SST 66 (17.62). The SST 66/Snack cross had a SCA for percentage leaf length reduction of 84.82. The high negative value of SST 66/Snack (-31.28) indicated a high level of specific combining ability for percentage leaf length reduction. (Table 4.4).

Root length. At -6°C Norstar/Karee (66.96) and Norstar/SST 66 (66.96) had significantly higher SCA values for root length than the other Norstar crosses. Norstar/PAN 3349 (22.09) had a significantly higher SCA for root length at -6°C than Norstar/Tugela DN and Norstar/Snack. The SCA for root length at -6°C of Norstar/Tugela DN (-13.15) was significantly higher than Norstar/Snack (-90.18). At -6°C the SCA for root length of Tugela DN/PAN 3349 (37.77) was significantly higher

than the other Tugela DN crosses. Tugela DN/SST 66 (9.40) had a significantly higher SCA for root length at -6°C than Tugela DN/Karee and Tugela DN/Snack. The SCA for root length of Tugela DN/Karee (-28.18) was significantly higher than Tugela DN/Snack (-55.25). At -6°C the SCA for root length of PAN 3349 was significantly higher than for PAN 3349/Snack (-33.38) and PAN 3349/SST 66 (-51.30). Karee/Snack (19.16) had a significantly higher SCA for root length than Karee/SST 66 (-16.38). The SST 66/Snack cross had a SCA for root length at -6°C of 82.31. SST 66/Snack had the highest SCA for root length at -6°C.

At -12°C Norstar/Tugela DN (91.63) had a significantly higher SCA for root length than the other Norstar crosses. Norstar/PAN 3349 (21.74), Norstar/Karee (20.43) and Norstar/SST 66 (25.74) had significantly higher SCA value for root length than Norstar/Snack (51.09). The SCA value for root length at -12°C for Tugela DN/PAN 3349 (9.71) and Tugela DN/Karee (1.89) was significantly higher than Tugela DN/SST 66 and Tugela DN/Snack. At -12°C, Tugela DN/SST 66 (-14.04) had a significantly higher SCA for root length than Tugela DN/Snack (-58.33). PAN 3349/Karee (8.46) and PAN 3349/SST 66 (1.72) had significantly higher SCA values for root length than PAN 3349/Snack (-27.57). The SCA for root length at -12°C of Karee/Snack (38.48) were significantly higher than Karee/SST 66 (-3.16). The SST 66/Snack cross had a SCA for root length of 37.56. Karee/Snack and SST 66/Snack had the highest SCA for root length at -12°C.

The SCA for percentage root length reduction of Norstar/Tugela DN was significantly lower than for the other Norstar crosses. Norstar/PAN 3349 (9.24) had a significantly lower SCA for percentage root length reduction than Norstar/Karee (21.67), Norstar/SST 66 (18.87) and Norstar/Snack (22.68). The SCA for percentage root

length reduction of Tugela DN/Karee (-10.42) was significantly lower than the other Tugela DN crosses. Tugela DN/PAN 3349 had a significantly lower SCA for percentage root length reduction than Tugela DN/SST 66 and Tugela DN/Snack. The SCA for percentage root length reduction of Tugela DN/SST 66 (7.63) was significantly lower than for Tugela DN/Snack (28.43). PAN 3349/SST 66 (-14.33) had a significantly lower SCA for percentage root length reduction than the other PAN 3349 crosses. PAN 3349/Karee (-3.49) had a significantly lower SCA for percentage root length reduction than PAN 3349/Snack (25.69). Karee/Snack (-29.26) had a significantly lower SCA for percentage root length reduction than Karee/SST 66 (9.61). The SST 66/Snack cross had a SCA for percentage root length reduction of -9.02. Norstar/Tugela DN had the lowest SCA for percentage root length reduction (Table 4.4).

SST 66/Snack had the best overall specific combining ability, followed by Norstar/Tugela DN, Karee/Snack, PAN 3349/Karee and Norstar/Karee (Table 4.4).

4.2.3 GCA:SCA ratio

The nature of genetic variance, whether additive or specific was studied using the GCA:SCA ratio (Table 4.5). A high ratio would indicate the greater prevalence of additive genes and a low ratio would indicate specific gene action.

Survival. The GCA:SCA ratio for percentage survival at -6°C was 1.98, which indicated additive and specific gene action. For survival at -12°C (7.93) and for percentage survival reduction (8.22) the high GCA:SCA ratios indicated additive gene action (Table 4.5).

Table 4.5. GCA : SCA ratio for tolerance to freezing characteristics

	Survival			Leaf length			Root length		
	-6°C	-12°C	%reduction	-6°C	-12°C	%reduction	-6°C	-12°C	%reduction
GCA	1984.38**	3539.61**	3784.03**	35732.71**	19385.15**	2361.49**	16677.49**	8245.57**	1297.74**
SCA	1004.23**	446.52**	460.20**	10923.26**	5213.89**	709.45**	6381.64**	3127.67**	980.83**
GCA:SCA ratio	1.98	7.93	8.22	3.27	3.72	3.33	2.61	2.64	1.32

GCA General combining ability
SCA Specific combining ability
* $p \leq 0.01$
** $p \leq 0.01$

Leaf length. The GCA:SCA ratios for leaf length at -6°C (3.27), -12°C (3.33) and percentage leaf length reduction (2.61) indicated additive as well as specific gene action (Table 4.5).

Root length. The GCA:SCA ratios for root length at -6°C (2.61) and -12°C (2.64) indicated additive as well as specific gene action. The GCA:SCA ratio of 1.32 for percentage root length reduction indicated specific gene action (Table 4.5).

The percentage survival at -12°C and percentage survival reduction exhibited the strongest additive gene action, for the quickest genetic improvement selections must be made from a population using one of these two characteristics.

4.2.4 Heritability

Survival. The heritability for percentage survival was 66.13% at -6°C, 88.18% at -12°C and 88.45% for percentage survival reduction (Table 4.6).

Leaf length. The heritability for leaf length was 76.01% at -6°C, 78.13% at -12°C and 76.07% for percentage leaf length reduction (Table 4.6).

Root length. The heritability for root length was 71.34% at -6°C, 71.78% at -12°C and 56.07% for percentage root length reduction (Table 4.6).

The relatively high heritability for percentage survival at -12°C (88.18%)and the percentage survival reduction (88.45%) indicated that selection for these two characters will improve the freezing tolerance of wheat.

Table 4.6. Heritability of tolerance to freezing characteristics

	Survival			Leaf length			Root length		
	-6°C	-12°C	%reduction	-6°C	-12°C	%reduction	-6°C	-12°C	%reduction
h^2 (%)	66.13	88.18	88.45	76.01	78.13	76.07	71.34	71.78	56.07
SD±	3.48	5.28	5.84	18.77	14.52	5.76	17.86	10.67	6.01

h^2 (%) heritability
SD± standard deviation

4.3 Use of high molecular weight proteins to screen for tolerance to freezing

The six parental wheat cultivars, used in the diallel study, were used to study the use of high molecular weight protein (HMW-protein) bands to screen for freezing tolerance. The cultivars included were Norstar, Tugela DN, PAN 3349, Karee, SST 66 and Snack. Norstar was included as the freezing tolerant check. The SDS-PAGE method was a modified version of the methods used by Singh *et al* (1991) and Sarhan and Perras (1987). Electrophoresis involved five basic steps of gel preparation, sample preparation, sample separation, gel staining and gel interpretation. Based on their dry weight, control seedlings of 3, 4 and 5 days corresponded in terms of physiological age to seedlings hardened respectively for 10, 20 and 30 days.

4.3.1 Screening for tolerance to freezing

The six wheat cultivars were screened for tolerance to freezing using the method described in Chapter 3.3.2.1. The tolerance to freezing of these cultivars was discussed in Chapter 4.1. The results of the artificial freezing test indicated a high level of freezing tolerance in Norstar and an intermediate level of freezing tolerance in Tugela DN, PAN 3349 and Karee. SST 66 and Snack showed a low level of tolerance to freezing with regard to percentage survival, leaf length and root length (Table 4.7).

4.3.2 Effect of cold hardening on the HMW-proteins in wheat coleoptiles

Norstar. The three day old unhardened Norstar coleoptiles exhibited 13 different HMW-protein bands, but after 10 days of cold hardening only 10 HMW-protein bands were observed (Table 4.8). After 10 days of hardening the coleoptiles lost the double band of 46kDa and a single band of 18 kDa. Intensity of the bands did not increase

Table 4.7. The tolerance to freezing of the six wheat cultivars

Cultivars	Survival (%)			Leaf length (mm)			Root length (mm)		
	-6°C	-12°C	%reduction	-6°C	-12°C	%reduction	-6°C	-12°C	%reduction
Norstar	100.00	96.62	3.38	256.50	222.25	13.35	168.25	159.75	5.05
Tugela DN	82.25	48.86	40.56	293.50	236.75	19.34	258.38	108.00	58.20
PAN 3349	93.63	58.98	37.01	244.00	165.25	32.23	170.00	106.75	37.21
Karee	83.48	45.37	45.65	280.50	164.75	41.27	147.88	69.75	52.83
SST 66	82.51	43.63	47.12	284.00	146.00	48.60	155.38	88.00	43.36
Snack	93.20	29.30	68.56	233.00	57.25	75.43	119.88	49.75	58.50

Table 4.8. HMW-proteins (kDa) in unhardened (3 days) and hardened (10 days) wheat coleoptiles

Norstar		Tugela DN		PAN 3349		Karee		SST 66		Snack	
3 days	10 days	3 days	10 days	3 days	10 days	3 days	10 days	3 days	10 days	3 days	10 days
48	48	48	0	48	48	48	48	48	48	48	48
46DB	0	46DB	0	46	0	46DB	0	46DB	0	46DB	0
0	0	0	0	0	44	0	0	0	0	0	0
40	40	40DB	40	40	40	40	40	40	40	40DB	40DB
35	35	35	35	35	35	35	35	35	35	35	35
0	0	0	0	0	0	0	0	0	0	0	0
30DB	30DB	30DB	30DB	30DB	30DB	30DB	30DB	30DB	30DB>	30DB	30DB
25	25	25	25	25	25<	25	25	25	25>	25	25
0	0	0	0	0	0	0	20	0	0	0	20
19	19	0	0	0	0	19	19	19	19	19	19
18	0	18	18	18	18	18	0	18	0	18	0
17	17	17	17	17	17	0	17	17	17	17	17
16	16	16	16	16	16	16	16	16	16	16	16
13	13	13	13	13	13	13	13	13	13	13	13
13*	10*	13*	9*	11*	11*	12*	11*	13*	10*	14*	12*

< intensity of band increased
> intensity of band decreased
DB double band
* total number of protein bands

with the 10 days hardening period.

The unhardened coleoptiles had 13 HMW-protein bands after four days. Only 10 HMW-protein bands were observed after the 20 day cold hardening period (Table 4.9). The coleoptiles lost the double band of 46 kDa, and one band of 48 kDa. The intensity of the 25 kDa band increased slightly with cold hardening.

Five day old unhardened coleoptiles had 13 HMW-protein bands. Eleven HMW-protein bands were exhibited after 30 days of cold hardening (Table 4.10). The coleoptiles lost the double band of 46 kDa. The intensity of bands with a molecular weight of 48, 40 and 25 kDa increased with the 30 days hardening period (Fig. 4.19).

Tugela DN. Unhardened Tugela DN coleoptiles produced 13 different HMW-protein bands after three days, but after 10 days of hardening the cultivar had only nine different HMW-protein bands. Tugela DN coleoptiles lost the single band of 48 kDa and the double band of 46 kDa. The intensity of the bands did not increase with the 10 day hardening period (Table 4.8).

Unhardened coleoptiles had 12 HMW-protein bands after four days, but the hardened coleoptiles had nine HMW-protein bands. The coleoptiles of Tugela DN lost bands with molecular weights of 48, 16 and 13 kDa after 20 days cold hardening. The intensity of the 30, 25 and 18 kDa bands decreased with cold hardening (Table 4.9).

Unhardened coleoptiles of Tugela DN had 12 HMW-protein bands after five days, and hardened coleoptiles had nine different HMW-protein bands after 30 days of cold hardening. The coleoptiles lost the double band of 46 kDa and the single band of 18 kDa. The intensity of the 48 and 30 kDa bands decreased, while the intensity of the 25 kDa band increased with the 30 day cold hardening period (Table 4.10).

Table 4.9. HMW-proteins (kDa) in unhardened (4 days) and hardened (20 days) wheat coleoptiles

Norstar		Tugela DN		PAN 3349		Karee		SST 66		Snack	
4 days	20 days	4 days	20 days	4 days	20 days	4 days	20 days	4 days	20 days	4 days	20 days
48	0	48	0	48	0	48	48	48	48	48	48>
46DB	0	46DB	46DB	46	0	46DB	0	46DB	0	46DB	0
0	0	0	0	0	0	0	0	0	0	0	0
40	40	40	40	40	40	40	40	40DB	40DB<	40DB	40
35	35	35	35	35	35	35	35	35	35<	35	35
0	0	0	0	0	0	0	0	0	0	0	0
30DB	30DB	30DB	30DB>	30DB	30DB>	30DB	30DB	30DB	30DB	30DB	30DB>
25	25<	25	25>	25	25<	25	25	25	25>	25	25>
0	0	0	0	0	0	0	0	0	0	0	0
19	19	0	0	0	0	19	0	19	19	19	0
18	18	18	18>	18	0	18	18	18	18<	18	0
17	17	17	17	17	17>	0	17	17	17DB	17	17
16	16	16	0	16	0	16	0	16	16DB	16	16>
13	13	13	0	13	0	13	13	13	13	13	13>
13*	10*	12*	9*	11*	6*	12*	9*	14*	14*	14*	8*

< intensity of band increased
> intensity of band decreased
DB double band
* total number of protein bands

Table 4.10. HMW-proteins (kDa) in unhardened (5 days) and hardened (30 days) wheat coleoptiles

Norstar		Tugela DN		PAN 3349		Karee		SST 66		Snack	
5 days	30 days	5 days	30 days	5 days	30 days	5 days	30 days	5 days	30 days	5 days	30 days
48	48<	48	48>	48	48	48	48	48	48	48	48
46DB	0	46DB	0	46	46>	46DB	46DB>	46DB	46DB	46DB	0
0	0	0	0	0	44	0	0	0	0	0	0
40	40<	40	40	40	40	40	40	40	40	40DB	0
35	35	35	35	35	35	35	35	35	35	35	35>
0	0	0	0	0	0	33	33	33	33	33	0
30DB	30DB	30DB	30DB>	30DB	30DB<	30DB	30DB	30DB	30DB>	30DB	30DB>
25	25<	25	25<	25	25<	25	25	25	25>	25	25>
0	0	0	0	0	0	0	0	0	0	0	0
19	19	0	0	0	0	19	0	19	19	19	0
18	18	18	0	18	18	18	18>	18	18	18	18
17	17	17	17	17	17	0	17	17	17	17	17
16	16	16	16	16	16	16	16	16	16	16	16>
13	13	13	13	13	13	13	13	13	13	13	13>
13*	11*	12*	9*	11*	12*	13*	13*	14*	14*	15*	9*

< intensity of band increased
> intensity of band decreased
DB double band
* total number of protein bands

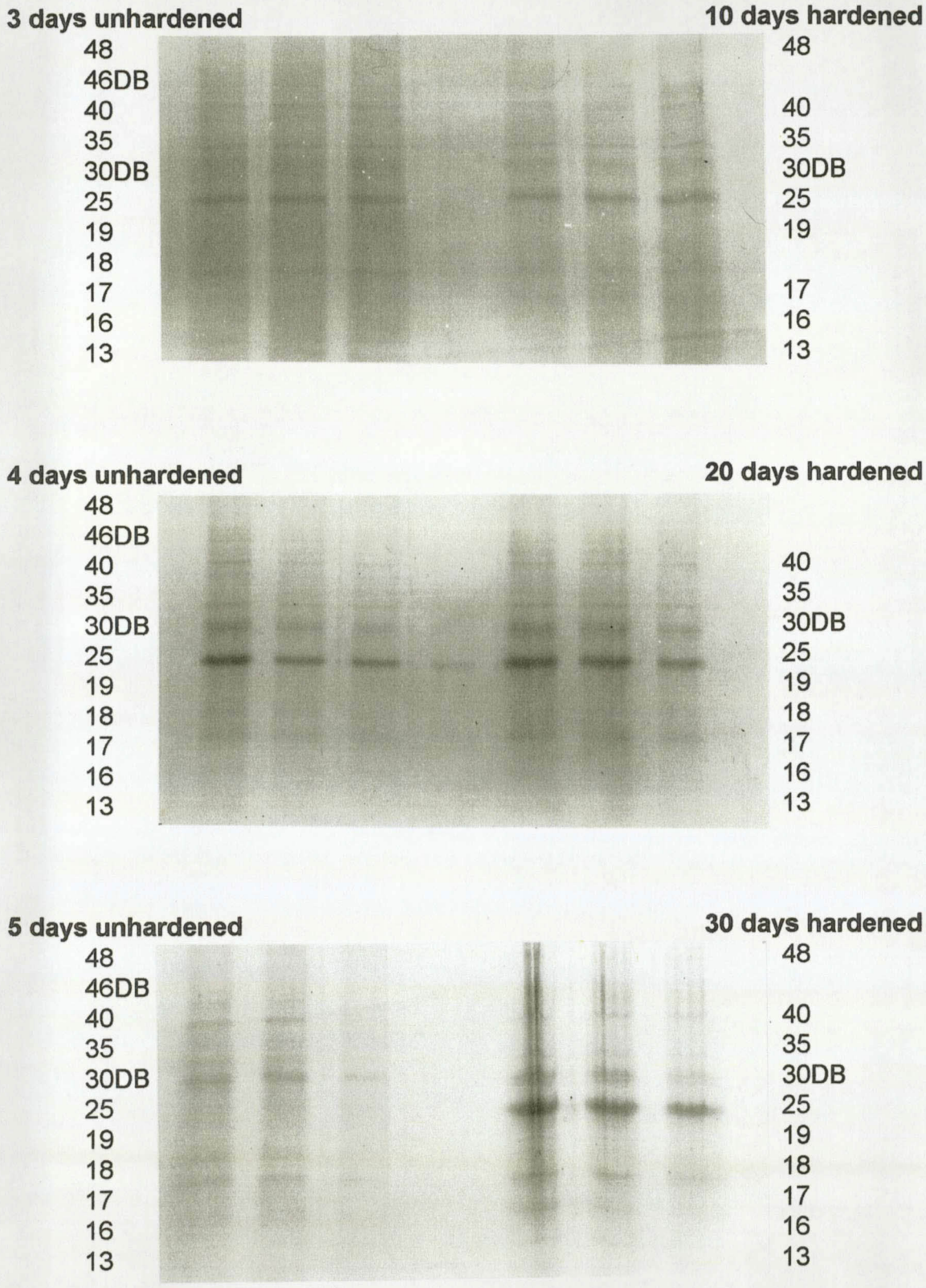

Fig. 4.19. High molecular weight protein bands produced by Norstar coleoptiles.

PAN 3349. The unhardened and hardened coleoptiles of PAN 3349 had 11 different HMW-protein bands after three and 10 days respectively. The double band of 46 kDa was lost and the intensity of the 25 kDa band increased after 10 days cold hardening. A new band of 44 kDa was produced after 10 days cold hardening (Table 4.8).

Unhardened coleoptiles had 11 HMW-protein bands after four days and hardened coleoptiles has six different HMW-protein bands after 20 days. PAN 3349 lost the 46, 48, 18, 16 and 13 kDa bands after twenty days cold hardening. The intensity of the 30 and 17 kDa bands decreased slightly while the intensity of the 25 kDa band increased (Table 4.9).

After five days the unhardened coleoptiles had 11 different HMW-protein bands, while the hardened coleoptiles had 12 HMW-protein bands after 30 days. A new band of 44 kDa was observed after 10 days cold hardening. The intensity of 30 and 25 kDa bands increased and 46 kDa decreased after 30 days cold hardening (Table 4.10).

Karee. The unhardened Karee coleoptiles exhibited 12 different HMW-protein bands after three days, while the hardened coleoptiles had 11 HMW-protein bands. After 10 days of cold hardening the coleoptiles of Karee lost single bands of 46 and 18 kDa, while it gained two bands of 17 and 20 kDa. The intensity did not increase after 10 days cold hardening (Table 4.8).

Four day old unhardened coleoptiles had 12 HMW-protein bands and the hardened coleoptiles had only nine different HMW-protein bands after 20 days. The coleoptiles of Karee lost bands of 46, 19 and 16 kDa during 20 days of cold hardening. The intensity of the bands did not increase with cold hardening (Table 4.9).

The unhardened and hardened coleoptiles had 13 HMW-protein bands after five

and 30 days respectively. A new band of 33 kDa was produced after 30 days. The coleoptiles did not lose the double band of 46 kDa, but lost a band of 19 kDa after 30 days of cold hardening. The intensity of the 18 and 46 kDa bands decreased after 30 days (Table 4.10).

SST 66. Thirteen HMW-protein bands were produced by the unhardened coleoptiles of SST 66 after three days. The hardened coleoptiles produced only 10 HMW-protein bands after 10 days. The double band of 46 kDa and a single band of 18 kDa were lost. The intensity of the 25 and 30 kDa bands decreased during the 10 days cold hardening (Table 4.8).

The unhardened and hardened coleoptiles had 14 HMW-protein bands after four and 20 days respectively. SST 66 lost the double band of 46 kDa after 20 days of cold hardening. The intensity of the 40, 35, and 18 kDa bands increased, while the intensity of the 25 kDa band decreased slightly (Table 4.9).

The unhardened and hardened coleoptiles had 14 HMW-protein bands after four and 30 days respectively. The intensity of the 30 and 25 kDa bands decreased with the 30 days hardening period (Table 4.10).

Snack. Fourteen HMW-protein bands were exhibited by unhardened coleoptiles of Snack after three days. After 10 days of cold hardening the coleoptiles of Snack had 12 HMW-protein bands. Snack lost the 46 kDa and 18 kDa band. A new 20 kDa protein band was produced after 10 days. The intensity of the bands did not increase after 10 days (Fig. 4.20).

The unhardened coleoptiles had 14 HMW-protein bands after four days, while

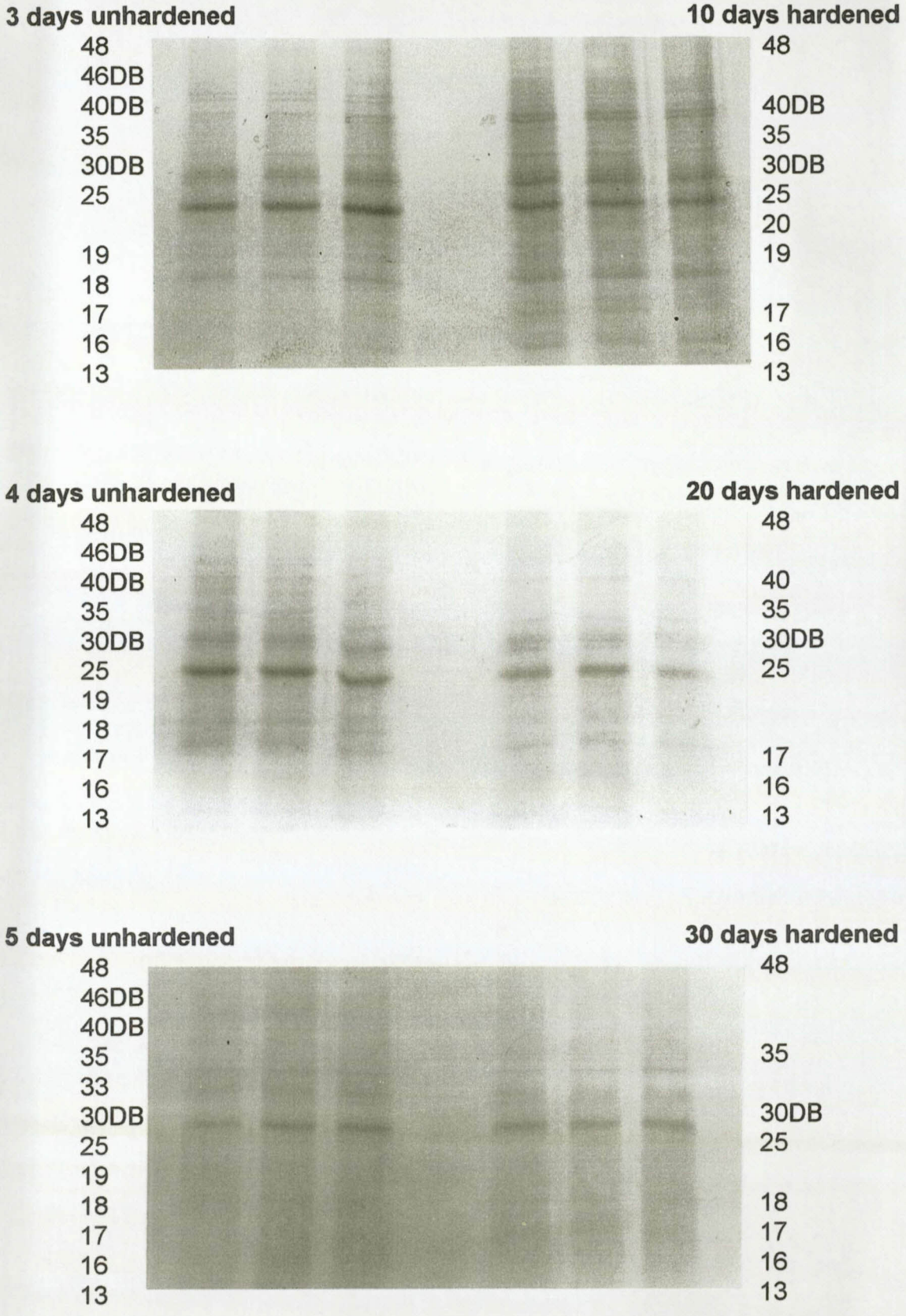

Fig 4.20. High molecular weight protein bands produced by Snack coleoptiles.

the hardened coleoptiles had only eight HMW-protein bands. Snack lost the double band of 46 kDa and three other bands of 40, 19 and 18 kDa after 20 days of cold hardening. The intensity of the 48 kDa band decreased with the 20 day cold hardening period (Table 4.9).

The unhardened coleoptiles of Snack had 15 HMW-protein bands after five days, while the hardened coleoptiles had only nine HMW-protein bands after 30 days of hardening. The coleoptiles lost bands of 46, 40, 33 and 19 kDa. The intensity of bands with a molecular weight of 35, 25,16 and 13 kDa decreased with the 30 days hardening period (Table 4.10).

4.3.3 Effect of cold hardening on the expression of HMW-proteins in wheat coleoptiles

48 kDa. Snack produced a band of low intensity after 20 days, while Norstar, PAN 3349 and Tugela DN lost it. After 30 days cold hardening Norstar produced a 48 kDa band with a very high intensity. This indicated that after 30 days the tolerant cultivar (Norstar) was able to accumulate the protein rapidly while the less tolerant cultivars were not. Accumulation of the protein in Norstar possibly started after the vernalisation requirement was satisfied. Although Snack could produce this protein, the accumulation was too slow to have an effect on its tolerance to freezing (Table 4.8-10).

46 kDa. This is a cold sensitive protein. The production of the protein was stopped during cold acclimation (Table 4.8-10).

44 kDa. No reaction to cold hardening was displayed (Table 4.8-10).

40 kDa. This band indicated a lack of tolerance to low temperatures. Cold hardening did not have an effect on the production of this protein in cultivars with intermediate and high levels of cold tolerance. In Snack, however, nothing was observed after 10 days, after 20 days one band was lost and after 30 days both bands were lost. The production of this protein was shut down with prolonged cold hardening in susceptible cultivars, indicating a sensitivity to low temperatures in susceptible cultivars (Table 4.8-10).

35 kDa. Cold hardening did not have an influence on the production of this protein. However, the intensity of the band in Snack decreased after 30 days. It might be necessary to expose the coleoptiles for a longer period to low temperatures to see if the gene is shut down with prolonged exposure to low temperatures (Table 4.8-10).

33 kDa. Karee, Snack and SST 66 produced this protein after five and 30 days. Cold hardening had no effect on the accumulation. The production might be related to the growth habit of the cultivars because Karee, Snack and SST 66 have a shorter growth period than Norstar, Tugela DN and PAN 3349 (Table 4.8-10).

30 kDa. SST 66 and Snack (cold susceptible) showed a slight decrease in intensity of this protein in reaction to cold hardening. This was an indication of susceptibility to low temperatures, since the cultivars were unable to produce the protein over proloned cold spells. If cold hardening is continued after 30 days a more definite reaction might be possible (Table 4.8-10).

25 kDa. The intensity of this protein increased with cold hardening in the more tolerant cultivars (Norstar, Tugela DN and PAN 3349), while the intensity decreased in the less tolerant cultivars (SST 66 and Snack). Cold tolerant cultivars were able to accumulate this protein rapidly when they needed protection against low temperatures (Table 4.8-10).

20 kDa. No reaction to cold hardening was displayed (Table 4.8-10).

19 kDa. No reaction to cold hardening was displayed (Table 4.8-10).

18 kDa. Norstar, Karee, Snack and SST 66 lost this band after 10 days . After 20 days Norstar, Karee and SST 66 regained the band while Snack took another 10 days to regain it. This indicated that cold tolerant cultivars were able to reproduce this protein faster than the susceptible cultivars (Table 4.8-10).

17 kDa. No reaction to cold hardening was displayed (Table 4.8-10).

16 kDa. Cultivars with intermediate and low levels of cold tolerance lost this band after 20 days cold hardening. Cold hardening had no effect on the tolerant cultivar, Norstar. This reaction indicated a sensitivity to low temperatures (Table 4.8-10).

13 kDa. Only Snack (cold susceptible) showed any reaction to cold hardening with a slight decrease in intensity, which may be an indication of susceptibility to low temperatures. If cold hardening is continued for a longer period a more definite

reaction might be possible (Table 4.8-10).

4.3.4 Effect of cold hardening on the HMW-proteins in wheat roots

Norstar. Eight different HMW-protein bands were produced by unhardened Norstar roots after three days, while seven HMW-protein bands were produced by hardened roots after 10 days. After 10 days of cold hardening a band of 68 kDa was lost (Table 4.11).

Unhardened roots had nine HMW-protein bands after four days, while 13 HMW-protein bands were observed in hardened roots after 20 days. New bands of 23 and 15 kDa were formed after 20 days cold hardening, while the intensity of several bands increased (58, 30, 25, 21, 19 and 17 kDa) (Table 4.12).

After five days, unhardened roots had eight HMW-protein bands and hardened roots had 11 HMW-protein bands after 30 days. The new band of 23 kDa was also present after 30 days cold hardening, while the intensity of the 68, 30 and 25 kDa bands increased (Table 4.13).

Tugela DN. The unhardened roots of Tugela DN exhibited 13 different HMW-protein bands after three days, while the hardened roots had 12 HMW-protein bands after 10 days. The intensity of several bands (68, 58, 30, 25, 21 and 17 kDa) increased (Table 4.11).

Thirteen HMW-protein bands were observed in unhardened roots after four days, while 17 HMW-protein bands were observed in hardened roots after 20 days. A new band of 15 kDa was produced after 20 days cold hardening and the intensity of several bands (68, 30, 25, 21, 19, 17 and 14 kDa) increased (Table 4.12).

Table 4.11. HMW-proteins (kDa) in unhardened (3 days) and hardened (10 days) wheat roots

Norstar		Tugela DN		PAN 3349		Karee		SST 66		Snack	
3 days	10 days	3 days	10 days	3 days	10 days	3 days	10 days	3 days	10 days	3 days	10 days
68	0	68DB	68DB<	68DB	68DB>	68DB	68DB<	68DB	68DB	68DB	0
58DB	58DB	58DB	58<	58DB	58DB	58DB	58DB	58DB	58DB	58DB	58DB>
0	0	46DB	46DB	46DB	46DB	0	0	0	0	46DB	0
30DB	30DB	30DB	30DB<	30DB	30DB<	30DB	30DB<	30DB	30DB	30DB	30DB<
0	0	25	25<	25	0	25	25<	25	0	25	25<
0	0	0	0	0	23	0	23DB	0	0	0	0
0	0	21	21<	21	21	21	21<	21	21DB	21	21
19	19	19	19	19DB	19DB	19	19DB	19	19	19	19<
17	17	17	17<	17	17	0	17	0	17	17DB	17DB
0	0	0	0	0	0	0	0	0	0	0	0
14	14	14	14	14	14	14	14	14	14	14	14
0	0	0	0	0	0	0	0	0	0	0	0
8*	7*	13*	12*	14*	14*	10*	14*	10*	11*	14*	10*

< intensity of band increased
> intensity of band decreased
DB double band
* total number of protein bands

Table 4.12. HMW-proteins (kDa) in unhardened (4 days) and hardened (20 days) wheat roots

Norstar		Tugela DN		PAN 3349		Karee		SST 66		Snack	
4 days	20 days	4 days	20 days	4 days	20 days	4 days	20 days	4 days	20 days	4 days	20 days
68	68DB	68DB	68DB<	68DB	68DB	68DB	68DB<	68DB	68DB	68DB	0
58DB	58DB<	58DB	58DB	58DB	58	58DB	58DB	58DB	58DB<	58DB	0
0	0	46DB	46DB	46DB	46DB	0	0	0	0	46DB	46DB
30DB	30DB<	30DB	30DB<	30DB	30DB<	30DB	30DB<	30DB	30DB>	30DB	30DB>
25	25<	25	25<	25	25<	25	25<	25	25>	25	25>
0	23DB	0	0	0	23<	0	0	0	0	0	0
21	21<	21	21DB<	21	21<	21	21<	21	21>	21	21
19	19<	19	19DB<	19DB	19DB<	19	19DB	19	19	19	19
17	17<	17	17<	17	0	0	17	0	17	17DB	17DB
0	15	0	15	0	0	0	0	0	0	0	0
0	0	14	14<	14	14	14	14	14	14	14	14
0	0	0	13	0	0	0	0	0	0	0	0
9*	13*	13*	17*	14*	13*	10*	12*	10*	11*	14	10

< intensity of band increased
> intensity of band decreased
DB double band
* total number of bands

Table 4.13. HMW-proteins (kDa) in unhardened (5 days) and hardened (30 days) wheat roots

Norstar		Tugela DN		PAN 3349		Karee		SST 66		Snack	
5 days	30 days	5 days	30 days	5 days	30 days	5 days	30 days	5 days	30 days	5 days	30 days
68	68<	68DB	68DB	68DB	68DB	68DB	68DB<	68DB	68DB<	68DB	68DB>
58DB	58DB	58DB	58DB	58DB	58	58DB	58DB<	58DB	58DB	58DB	58DB>
0	0	46DB	46DB	46DB	46DB	0	0	0	0	46DB	46DB
30DB	30DB<	30DB	30DB<	30DB	30DB<	30DB	30DB<	30DB	30DB>	30DB	0
0	25<	25	25<	25	25<	25	25<	25	25>	25	0
0	23DB	0	0	0	23<	0	0	23	0	0	0
0	0	21	21DB	21	21<	21	21	21	21	21DB	21
19	19	19	19<	19DB	19DB<	19	19DB	19	19>	19	19>
17	17	17	17	17	0	0	0	0	0	17DB	17DB>
0	0	0	0	0	0	0	0	0	0	0	0
14	14	14	14	14	14	14	14	14	0	14	14>
0	0	0	0	0	0	0	0	0	0	0	0
8*	11*	13*	14*	14*	13*	10*	11*	11*	9*	15*	11*

< intensity of band increased
> intensity of band decreased
DB double band
* total number of bands

The unhardened roots had 13 HMW-protein bands after five days, while the hardened roots had 14 HMW-protein bands after 30 days. The intensity of the 30, 25 and 19 kDa bands increased slightly after 30 days (Table 4.13).

PAN 3349. Fourteen different HMW-protein bands were displayed by unhardened and hardened roots after three and 10 days respectively. A new band of 23 kDa was produced after 10 days of cold hardening. The 25 kDa band was lost and the intensity of the 68 kDa band decreased. The intensity of the 30 kDa band increased after 10 days cold hardening (Table 4.11).

The unhardened roots had 14 HMW-protein bands after four days, while the hardened roots had 13 HMW-protein bands after 20 days cold hardening. The 17 kDa band was lost and the intensity of the 30, 25, 23, 21 and 19 kDa bands increased (Table 4.12).

The unhardened roots had 14 HMW-protein bands after five days and the hardened roots had 13 HMW-protein bands after 30 days. The 17 kDa band was lost after 30 days cold hardening, while the intensity of the 30, 25, 23, 21 and 19 kDa bands increased (Table 4.13).

Karee. Ten HMW-protein bands were produced by unhardened roots of Karee after three days, but the hardened roots produced 12 HMW-protein bands after 10 days. A new band of 23 kDa was produced after 10 days cold hardening, while the intensity of bands (68, 30, 25, and 21 kDa) increased (Table 4.11).

The unhardened roots had 10 HMW-protein bands after four days and the hardened roots had 12 HMW-protein bands after 20 days. A new band of 17 kDa was

displayed after 20 days cold hardening and the intensity of the 68, 30, 25 and 21 kDa bands increased (Table 4.12).

After five days the roots of the unhardened plants had 10 different HMW-protein bands and the hardened roots had 11 different HMW-protein bands after 30 days. The intensity of the 68, 58, 30 and 25 kDa bands increased after 30 days cold hardening (Table 4.13).

SST 66. Ten HMW-protein bands were displayed by the unhardened roots of SST 66 and the hardened roots had 11 different HMW-protein bands after 10 days. A new band of 17 kDa was produced with 10 days cold hardening. The 25 kDa band was lost after 10 days (Table 4.11).

After 20 days cold hardening the unhardened roots produced 10 different HMW-protein bands and the hardened roots produced 11 HMW-protein bands. Intensity of the 58 kDa band increased and the intensity of the 30, 25 and 21 kDa bands decreased. A new band of 17 kDa was produced after 20 days (Table 4.12).

Eleven different HMW-protein bands were produced by the unhardened roots after three days and the hardened roots produced only nine HMW-protein bands after 30 days. The 14 kDa band was lost after 30 days cold hardening and the intensity of the 30, 25 and 19 kDa bands decreased. The intensity of the 68 kDa band increased with cold hardening (Table 4.13).

Snack. The unhardened roots of Snack exhibited 14 different HMW-protein bands after three days and the hardened roots had only 10 HMW-protein bands after 10 days. The 68 and 46 kDa bands were lost and the intensity of the 58 kDa band decreased after

10 days cold hardening. The intensity of the 30, 25 and 19 kDa bands increased (Table 4.11).

The unhardened roots exhibited 14 different HMW-protein bands after four days and the hardened roots had only 10 HMW-protein bands after after 20 days cold hardening. The 68 and 58 kDa bands were lost and the intensity of the 30 and 25 kDa bands decreased (Table 4.12).

Fifteen different HMW-protein bands were produced by unhardened roots after three days, while the hardened roots produced only 11 HMW-protein bands after 30 days. The intensity of the 68, 58, 19, 17 and 14 kDa bands decreased with 30 days cold hardening, while the 30 and 25 kDa bands disappeared (Table 4.13).

4.3.5 Effect of cold hardening on the expression of HMW-proteins in wheat roots

68 kDa. This protein was expressed as a single band in the tolerant cultivar, Norstar, and as a double band in the less tolerant cultivars for hardened and unhardened samples. The susceptible cultivar (Snack), lost the ability to produce this protein during prolonged periods of low temperatures, while the tolerant cultivar (Norstar) was able to accumulate it during extended cold periods. This indicated that the ability to accumulate this protein enhances cold tolerance (Table 4.11-13).

58 kDa. Only Snack (cold susceptible) showed a reaction to cold hardening with a slight decrease in intensity, an indication of susceptibility to low temperatures. If cold hardening is continued after 30 days or at a lower temperature a more definite reaction might be visible (Table 4.11-13).

46 kDa. Only Snack, PAN 3349 and Tugela DN produced this protein. Cold hardening did not have any effect on the production of this protein (Table 4.11-13).

30 kDa. This protein played an important role in tolerance to freezing. All the cultivars were able to accumulate this protein in reaction to cold hardening. This gene was activated once vernalisation was completed. Norstar, with a longer vernalisation period, only started to accumulate it after 20 days. The other cultivars with a lesser vernalisation requirement accumulated the protein after 10 days. The susceptible cultivars, Snack and SST 66, accumulated this protein for only 10 days before the intensity decreased and the band disappeared (Snack) after 30 days. The tolerant cultivars, however, accumulated the protein over long periods in reaction to low temperatures. This confirmed their tolerance to low temperatures (Table 4.11-13).

25 kDa. Like the 30 kDa protein all the cultivars were able to accumulate this protein in reaction to cold hardening. The accumulation of the protein was activated once vernalisation was completed. Cultivars with a lesser vernalisation requirement can accumulate the protein after 10 days but Norstar, with a longer vernalisation period, only started to accumulate it after 20 days. The susceptible cultivars, Snack and SST 66, accumulated this protein for only 10 days before the intensity decreased and disappeared (Snack) after 30 days. The tolerant cultivars however accumulated it over long periods, confirming their tolerance to low temperatures (Table 4.11-13).

23 kDa. The production of this protein was activated in Karee, PAN 3349 and Norstar after cold hardening. Once again the production started after 10 days in the facultative

wheats (Karee and PAN 3349) and 20 days in the winter wheat (Norstar). Indicating that vernalisation must be completed before the gene is activated. The cold tolerant cultivar can produce it for a longer period, but the less tolerant cultivars lose the ability after 20 days. The susceptible cultivar, Snack, was unable the produce this protein at any given time. The accumulation of this protein was an indication of tolerance to low temperatures (Table 4.11-13).

21 kDa. Once again this protein was accumulated in reaction to cold hardening, however accumulation ceased after 20 days. Accumulation in Norstar started after 20 days cold hardening, an indication of its longer vernalisation period. The accumulation decreased after 10 days in SST 66. Snack was unable to accumulate any extra protein in response to hardening. This protein acted as an inherent defence mechanism to low temperatures. Hardy cultivars accumulated more protein in reaction to cold, whereas susceptible cultivars were unable to the same (Table 4.11-13).

19 kDa. Accumulation of this protein was activated after 10 days in spring wheat, and 20 days in winter wheat. The production decreased after 30 days in Snack and SST 66. This protein was produced in response to low temperatures for a short time, when exposed for longer periods the accumulation stopped. This protein thus acted as an early defence mechanism against low temperatures (Table 4.11-13).

17 kDa. A double band was formed in Snack and a single band in other cultivars. This protein was sensitive to low temperatures. Norstar and Tugela DN accumulated some protein after 20 days. In Snack the production decreased after 30 days. A test period

after 30 days may reveal the total disappearance of this protein in Snack (Table 4.11-13).

15 kDa. This protein was observed only in Norstar and Tugela DN after 20 days cold hardening. It might be the reaction of a restorer gene (Table 4.11-13).

14 kDa. After 30 days production decreased in Snack. Cold hardening did not have an effect on the production of this protein (Table 4.11-13).

4.3.6 Phenotypic correlations

A correlation matrix of all characters measured is given in Table 4.14. Only values with a confidence level of 94% will be discussed. Ten different combinations exhibited a correlation value higher than 94% ($r>0.94$). Percentage survival at -12°C was significantly correlated with percentage survival reduction ($r=-0.98$) and root length at -12°C ($r=0.95$). The percentage reduction in survival was correlated with root length at -12°C ($r=-0.96$), while leaf length at -12°C was significantly correlated with percentage leaf length reduction ($r=-0.97$), and the difference between the number of protein bands produced in unhardened (4 days) and hardened (20 days) roots ($r=0.93$). Root length at -6°C also correlated with the number of protein bands produced in hardened (20 days) roots ($r=0.97$). The number of protein bands produced in unhardened (4 days) coleoptiles correlated with the number of protein bands produced in unhardened (5 days) coleoptiles ($r=0.94$). The number of protein bands produced in unhardened (3 days) roots correlated significantly with the number of protein bands produced in unhardened (4 and 5 days) roots respectively ($r=0.99$ and $r=0.98$). The

Table 4.14 Correlation matrix for tolerance to freezing characteristics

	S-6°C	S-12°C	%SR	LL-6°C	LL-12°C	%LLR	RL-6°C	RL-12°C	%RLR	CB3	CB10	CB3/10	CB4	CB20	CB4/20	CB5	CB30	CB5/30	RB3	RB10	RB3/10	RB4	RB20	RB4/20	RB5	RB30	RB5/30
S-6°C																											
S-12°C	0.62																										
%SR	-0.47	-0.98*																									
LL-6°C	-0.80	-0.05	-0.11																								
LL-12°C	-0.07	0.66	-0.77	0.59																							
%LLR	-0.12	-0.79	0.87	-0.42	-0.97*																						
RL-6°C	-0.34	0.19	-0.31	0.61	0.79	-0.70																					
RL-12°C	0.47	0.95*	-0.95*	0.10	0.79	-0.88	0.44																				
%RLR	-0.71	-0.94	0.89	0.22	-0.42	0.57	0.08	-0.85																			
CB3	0.02	-0.22	0.27	-0.30	-0.32	0.40	-0.11	-0.20	0.15																		
CB10	0.35	-0.39	0.52	-0.77	-0.88	0.76	-0.85	-0.61	0.20	0.00																	
CB3/10	0.12	-0.17	0.21	-0.42	-0.27	0.17	-0.31	-0.30	0.18	-0.76	0.60																
CB4	0.02	-0.22	0.28	-0.09	-0.51	0.55	-0.46	-0.25	0.01	0.85	0.15	-0.65															
CB20	-0.40	-0.01	-0.07	0.56	0.08	0.02	-0.03	0.07	-0.12	0.41	-0.43	-0.71	0.68														
CB4/20	-0.53	0.13	-0.28	0.77	0.40	-0.29	0.21	0.24	-0.18	0.03	-0.64	-0.53	0.26	0.89													
CB5	-0.01	-0.39	0.48	-0.18	-0.67	0.72	-0.61	-0.49	0.19	0.82	0.40	-0.44	0.94*	0.47	0.08												
CB30	-0.27	0.07	-0.13	0.29	0.02	-0.02	-0.33	0.01	-0.21	-0.50	0.00	0.22	-0.02	0.48	0.64	-0.06											
CB5/30	-0.21	0.27	-0.38	0.33	0.38	-0.41	0.08	0.27	-0.28	-0.85	-0.22	0.42	-0.53	0.12	0.47	-0.60	0.83										
RB3	-0.09	-0.61	0.65	-0.36	-0.41	0.41	0.11	-0.50	0.64	-0.07	0.34	0.44	-0.26	-0.62	-0.65	-0.16	-0.50	-0.30									
RB10	-0.58	-0.52	0.44	0.24	-0.05	0.11	0.12	-0.48	0.61	-0.67	0.14	0.71	-0.64	-0.35	-0.08	-0.47	0.30	0.50	0.47								
RB3/10	-0.50	0.05	-0.17	0.59	0.33	-0.27	0.01	-0.01	-0.01	-0.61	-0.18	0.29	-0.39	0.23	0.53	-0.32	0.78	0.80	-0.46	0.55							
RB4	-0.27	-0.51	0.57	-0.44	-0.38	0.36	0.12	-0.42	0.55	-0.05	0.33	0.42	-0.26	-0.67	-0.71	-0.18	-0.57	-0.35	0.89*	0.38	-0.54						
RB20	-0.22	0.29	-0.39	0.53	0.83	-0.77	0.97*	0.50	0.01	-0.21	-0.78	-0.19	-0.59	-0.19	0.09	-0.70	-0.37	0.08	0.09	0.14	0.05	0.12					
RB4/20	-0.19	0.60	-0.72	0.73	0.83*	-0.87	0.67	0.69	-0.39	-0.12	-0.88	-0.45	-0.26	0.34	0.59	-0.41	0.12	0.32	-0.64	-0.17	0.44	-0.63	0.69				
RB5	-0.11	-0.69	0.74	-0.39	-0.54	0.55	0.01	-0.59	0.68	0.04	0.39	0.35	-0.08	-0.50	-0.59	0.00	-0.48	-0.37	0.98*	0.40	-0.52	0.98*	-0.04	-0.73			
RB30	0.02	0.07	-0.10	0.02	0.44	-0.45	0.71	0.21	0.19	-0.33	-0.27	0.26	-0.75	-0.72	-0.49	-0.72	-0.60	-0.08	0.56	0.34	-0.19	0.60	0.79	0.18	0.40		
RB5/30	0.13	0.79	-0.88	0.42	0.89	-0.90	0.49	0.77	-0.58	-0.28	-0.60	-0.18	-0.43	0.02	0.28	-0.50	0.06	0.33	-0.63	-0.19	0.40	-0.59	0.60	0.90	-0.76	0.27	

S-6°C Survival at -6°C
S-12°C Survival at -12°C
%SR % Survival reduction
LL-6°C Leaf length at -6°C
LL-12°C Leaf length at -12°C
%LLR % Leaf length reduction
RL-6°C Root length at -6°C
RL-12°C Root length at -12°C
%RLR % Root length reduction
CB3 Number of protein bands in coleoptiles after 3 days (unhardened)
CB10 Number of protein bands in coleoptiles after 10 days (hardened)
CB3/10 Difference in number of protein bands between unhardened and hardened coleoptiles
CB4 Number of protein bands in coleoptiles after 4 days (unhardened)
CB20 Number of protein bands in coleoptiles after 20 days (hardened)
CB4/20 Difference in number of protein bands between unhardened and hardened coleoptiles
CB5 Number of protein bands in coleoptiles after 5 days (unhardened)
CB30 Number of protein bands in coleoptiles after 30 days (hardened)
CB5/30 Difference in number of protein bands between unhardened and hardened coleoptiles
RB3 Number of protein bands in roots after 3 days (unhardened)
RB10 Number of protein bands in roots after 10 days (hardened)
RB3/10 Difference in number of protein bands between unhardened and hardened roots
RB4 Number of protein bands in roots after 4 days (unhardened)
RB20 Number of protein bands in roots after 20 days (hardened)
RB4/20 Difference in number of protein bands between unhardened and hardened roots
RB5 Number of protein bands in roots after 5 days (unhardened)
RB30 Number of protein bands in roots after 30 days (hardened)
RB5/30 Difference in number of protein bands between unhardened and hardened roots
* r>0.94

number of protein bands produced in unhardened (4 days) roots also correlated with unhardened five day old roots (r=0.96).

Chapter 5

Conclusions

5.1 Tolerance to freezing of South African wheat cultivars

Tagmaj'yan and Kolbasina (1972), McKersie and Hunt (1987), Brule-Babel and Fowler (1989), Damania and Tahir (1993) studied the survival of wheat cultivars under freezing conditions. Analysis of variance indicated that there were significant differences in survival ability between genotypes. Fowler and Limin (1987) screened extensively diverse hexaploid wheat genotypes. No genotypes were found to surpass the cold hardiness potential of the most hardy commercial wheat cultivars being produced in North America at present. Hommo (1994) studied the field survival of winter wheat cultivars under Finnish conditions and a wide range of winter hardiness levels was observed. Brule-Babel and Fowler (1989) described Norstar as one of the most hardy wheat cultivars . McIntyre *et al.* (1988) found that Ulianovka had survival rates higher than Norstar, and a test line (Kharkov/Ulianovka) exhibited similar higher LT50 values than Norstar.

The South African cultivars tested can be divided into three definite classes according to their ability to survive the different test temperatures. Norstar, the cold tolerant check, displayed superior tolerance to freezing at -6 and -12°C. Scheepers 69, Belinda, Kavkaz, Molen, Carolus, Jager and PAN 3232 exhibited a high level of survival under freezing conditions. Snack however displayed a poor level of survival under freezing conditions. The rest of the cultivars showed an intermediate level of tolerance to freezing.

Marcellos and Burke (1979) found that leaves of several spring wheat cultivars were able to tolerate temperatures as low as -7 to -9 °C. The leaves of Norstar can

tolerate -18°C (Marcellos and Burke, 1979; Chen *et al*, 1983; Gusta and Chen, 1987). Gusta and Chen (1987) observed that leaves of Columbus and Neepawa wheat survived temperatures as low as -8 °C. Veisz and Sutka (1993) used leaf regrowth to measure cold tolerance of some Chinese Spring ditelosomics.

It was clear that the leaves of a number of cultivars displayed relatively high levels of tolerance to freezing. This might be ascribed to the ability of some cultivars to recover after being exposed to freezing temperatures. The leaves of Caledon, PAN 3232, SST 966, SST 936, Norstar, Tugela DN and PAN 3235 showed a high level of tolerance to freezing. The leaves of Snack and Kariega were not able to recover after exposure to the test temperatures. The rest of the cultivars had only intermediate levels of tolerance to freezing.

Gullord *et al.* (1975) and Le *et al.* (1986) evaluated the freezing tolerance of winter wheat cultivars. Tolerance to freezing was assessed in terms of root regrowth. Tolerance to freezing ranged from non-hardy to hardy.

South African cultivars can be divided into three distinct groups according to the ability of their roots to survive exposure to freezing temperatures. The roots of Kavkaz, Norstar, PAN 3235, Belinda, Jager, Carolus, PAN 3232, Betta and Molen exhibited high levels of tolerance to freezing. The roots of Palmiet, Kariega and SST 825 displayed poor levels of tolerance to freezing.

It can thus be concluded that a reasonable level of tolerance to freezing does exist in South African wheat cultivars. However, it was clear that the bulk of the cultivars possessed only an intermediate level of tolerance. It was also noted that the growth habit of cultivars had an influence on their reaction to freezing temperatures, with the winter types were definitely more freezing tolerant than the spring types.

Norstar exhibited a high level of tolerance to freezing. The fact that no South African cultivar had a higher level of tolerance to freezing than Norstar, indicated that the level of tolerance to freezing in South African wheat cultivars can be improved by the introduction of selected foreign germplasm.

5.2 Genetic variability, combining ability and inheritance of tolerance to freezing

Stushnoff *et al.* (1983) concluded that the genetic variability for winter hardiness has been largely exhausted. Fowler *et al.* (1983) found the only examples of segregation for greater winter hardiness were from crosses between parents of moderate or poor hardiness, and selections from these were not as hardy as existing genotypes. No segregation for increased cold hardiness in crosses involving extremely hardy parents, has been reported (Orlyuk, 1976; Shelepov *et al.*, 1980). Brule-Babel and Fowler (1988) reported that winter by spring wheat crosses resulted in spring habit F1-hybrids that were significantly more freezing tolerant than the parental midpoint. Other F1-hybrids in spring x winter crosses have been reported to resemble the less hardy parent, hardy parent or to be intermediate in cold hardiness.

Considerable variation in freezing tolerance was displayed by the F1-hybrids in the diallel cross. Crosses such as Snack/Norstar, PAN 3349/Snack and Snack/Tugela DN had a tolerance to freezing similar to that of Snack, the less freezing tolerant parent. Karee/Norstar, SST 66/PAN 3349, Karee/PAN 3349 and SST 66/Norstar were nearly as freezing tolerant as Norstar. It can be concluded that a high level of variability for tolerance to freezing can be obtained with crosses between freezing tolerant and less tolerant parents. This type of cross can thus result in wheat lines with

improved tolerance to freezing. The lack of significant differences between the freezing tolerance of reciprocal crosses indicated that freezing tolerances, were not affected by cytoplasmic effects.

Norstar and Karee had the highest general combining ability, which makes them suitable to be used in a breeding programme. The high level of tolerance to freezing in Norstar, combined with its general combining ability, makes the cultivar an ideal crossing parent in order to improve tolerance to freezing.

Sutka (1981; 1984; 1994) found a high GCA:SCA ratio for the percentage survival of winter wheat in a controlled freezing test. The high GCA:SCA ratio indicated additive gene action. Gullord (1974) also concluded that cold hardiness was controlled by partial dominant genes which were mainly additive in their effect.

The additive gene action was confirmed with the high GCA:SCA ratio for percentage survival at -12°C and percentage reduction in survival.

Studies on freezing tolerance in wheat concluded that it is a genetically complex characteristic and is quantitatively inherited (Quisenberry and Clark, 1929; Worzella, 1947; Gullord *et al.,* 1975; Sutka, 1994). Sutka (1994) and Brule-Babel and Fowler (1988) calculated high values for narrow and broad heritability respectively. This indicated a high heritability for tolerance to freezing. Provided genetic variability is present, selection for tolerance to freezing should be effective.

The high and accurate heritability estimates for percentage survival at -12°C and percentage survival reduction confirmed that tolerance to freezing is a highly heritable character and that selection for freezing tolerance will be effective.

5.3 Use of HMW-proteins to screen for tolerance to freezing

The reaction of high molecular weight proteins to cold hardening has been studied by numerous researchers. Sarhan and Perras (1987), Perras and Sarhan (1989), Danyluk and Sarhan (1990) and Abromeit *et al.* (1992) found proteins in the range of 200kDa in winter wheat cultivars after cold hardening. The 200kDa proteins accumulated at a higher concentration in cold tolerant cultivars. In addition, the intensity of three protein bands(48, 47 and 42 kDa) increased while that of five others (93, 89, 80, 67 and 63 kDa) decreased during hardening. Abromeit *et al.* (1992) studied changes in Roughrider and Capelle. Electrophoretic studies revealed that cold hardening caused the appearance of a group of up to seven high molecular weight proteins (150-176 kDa), which were not present on gels of unhardened seedlings. Zhou *et al.* (1994) identified proteins that were induced by cold acclimation in wheat. Two cultivars with different levels of cold tolerance, Chinese Spring and Norstar, were used. Proteins with molecular masses from 22-31kDa decreased in both the roots and shoots. By contrast, levels of polypeptides of 89, 83, 52, 23, 18, and 17 kDa increased specifically in the shoots of winter wheat. The increases in the levels of the 23, 18 and 17 kDa polypeptides were proportional to the development of freezing tolerance.

The results confirmed the different reactions of HMW-proteins in coleoptiles to cold hardening. Proteins with a molecular weight of 44, 43, 38 and 20 kDa were produced in response to cold hardening. The intensity of proteins with a molecular weight of 48, 40, 35, 25, 18, 16 and 13 kDa increased or decreased during cold hardening in a specific cultivar. The disappearance of some proteins (48, 46, 40, 33, 19, 18, 16 and 13 kDa) was also noted. These results indicated that HMW-proteins do react to cold hardening.

Accumulation of the 48 kDa protein in Norstar started after the vernalisation requirement was satisfied. Although Snack could produce this protein, the accumulation was too slow to have an effect on its tolerance to freezing. The 46 kDa protein is a cold sensitive protein, the production stopped during cold acclimation. The 40 kDa band indicated a lack of tolerance to low temperatures, cold hardening did not have an effect on the production of this protein in Norstar. In Snack, however, no accumulation was observed. Cold tolerant cultivars were able to accumulate the 25 kDa protein rapidly when they needed protection against low temperatures, an indication that the protein enhanced tolerance to freezing.

Similar reactions to cold hardening were found in the roots of the different cultivars. Two new proteins (23 and 15 kDa) were produced in response to cold hardening. The intensity of several HMW-proteins (68, 58, 30, 25, 21, 19, 17 and 14 kDa) increased or decreased in reaction to cold hardening. Several of these HMW-proteins (68, 58, 46, 30, 25, 21, 19, 17 and 14 kDa) disappeared during the process of cold hardening.

Snack lost the ability to produce the 68 kDa protein during prolonged periods of low temperatures, Norstar however was able to accumulate it during extended cold periods. This indicated that the ability to accumulate this protein enhances cold tolerance. All the cultivars were able to accumulate the 21, 25 and 30 kDa protein in reaction to cold hardening. The accumulation was activated once vernalisation was completed. Snack and SST 66 accumulated these proteins for only 10 days. The tolerant cultivars, however, accumulated them over longer periods, confirming that the accumulation of the proteins enhanced the tolerance to low temperatures. Norstar could produce the 23 kDa protein for a longer period. The susceptible cultivar, Snack

was unable the produce this protein at any given time. The accumulation of this protein indicated a high level of tolerance to low temperatures. The 19 kDa protein acted as an early defence mechanism against low temperatures, because the production decreased after 30 days in Snack and SST 66. This protein protects the plants to low temperatures for a short time. However, when exposed for longer periods the accumulation stopped.

To conclude, HMW-proteins can be used to screen for tolerance to freezing. The production and accumulation of several proteins can be associated with tolerance to freezing. Growth habit and vernalisation requirement influenced the production and accumulation of some of the proteins. Roots were more sensitive to the cold treatments. More sensitive test results can be derived with the use of longer test periods and different hardening temperatures. The intensity of the HMW-protein bands in roots was very low, other extraction and colouring methods should be tested in order to obtain more accurate results.

Some significant correlations were found between the different cold tolerance characteristics. The number of protein bands produced by unhardened roots was definitely correlated with the number of protein bands in hardened roots, but the difference between the number of protein bands of hardened and unhardened coleoptiles and roots was not correlated. This indicated that cold hardening did have an effect on the production and accumulation of proteins in wheat coleoptiles and roots.

Chapter 6

6.1 Summary

(Key words: tolerance to freezing, artificial freezing, survival, leaf length, root length, variability, combining ability, inheritance, electrophoresis, high molecular weight proteins)

In South Africa nearly 47 062 ha of wheat has been damaged by frost over the last ten years. This led to an average loss of income close to R 6.7 million annually. The objective of this study was to screen South African wheat cultivars for tolerance to freezing, to study the genetic variability for tolerance to freezing and to study the use of HMW-proteins to screen for tolerance to freezing.

An artificial freezing test was used to screen 24 South African wheat cultivars for tolerance to freezing. Tolerance to freezing was obtained by calculating the percentage survival, root length and leaf length at -6 and -12°C.

Norstar displayed superior tolerance to freezing at -6 and -12°C. Norstar, Scheepers 69, Belinda, Kavkaz, Molen, Carolus, Jager and PAN 3232 exhibited a high level of tolerance to freezing. The leaves of Caledon, PAN 3232, SST 966, SST 936, Norstar, Tugela DN and PAN 3235 showed a high level of tolerance to freezing. The roots of Kavkaz, Norstar, PAN 3235, Belinda, Jager, Carolus, PAN 3232, Betta and Molen exhibited a high level of tolerance to freezing.

A full 6X6 diallel cross was used to determine the genetic variability, combining ability and inheritance of tolerance to freezing. Norstar, Tugela DN, PAN 3349, Karee, SST 66 and Snack were used as parents. Griffing's Model 1, Method 1 was used for the analysis.

Significant differences in tolerance to freezing were displayed by the F1- hybrids. Crosses such as Snack/Norstar, PAN 3349/Snack and Snack/Tugela DN had a low level of tolerance to freezing. Karee/Norstar, SST 66/PAN 3349, Karee/PAN 3349 and SST 66/Norstar were nearly as freezing tolerant as Norstar. The lack of significant differences in the freezing tolerance between reciprocal crosses indicated that freezing tolerance was not affected by cytoplasmic effects.

Norstar and Karee had the highest general combining ability. The high GCA:SCA ratio for percentage survival at -12°C and percentage reduction in survival, confirmed the additive gene action of tolerance to freezing. The high heritability estimates for percentage survival (88.18%) and leaf length at -12°C (88.45%) confirmed that tolerance to freezing is a highly heritable character. Selection for freezing tolerance in a breeding programme will be effective.

The effect of HMW-proteins to screen for tolerance to freezing was studied using SDS-PAGE. Six wheat cultivars were included, Norstar, Tugela DN, PAN 3349, Karee, SST 66 and Snack.

The results confirmed that HMW-proteins in coleoptiles can be used to screen for tolerance to freezing. Accumulation of the 48 kDa protein in Norstar started after the vernalisation requirement was satisfied. The 46 kDa protein is a cold sensitive protein as production stopped during cold acclimation. The 40 kDa band indicated a lack of tolerance to low temperatures. Cold tolerant cultivars were able to accumulate the 25 kDa protein rapidly when they needed protection against low temperatures.

Similar reactions to cold hardening were found in the roots of the different cultivars. Snack lost the ability to produce the 68 kDa protein during prolonged periods of low temperatures. Norstar, however, was able to accumulate it during extended cold

periods. All the cultivars were able to accumulate the 21, 25 and 30 kDa protein in reaction to cold hardening. The accumulation was activated once vernalisation was completed. The 19 kDa protein acted as an early defence mechanism against low temperatures.

Production and accumulation of several proteins can be associated with increased tolerance to freezing. Growth habit and vernalisation requirement did influence the production and accumulation of certain HMW-proteins. A signifficant correlation between the leaf length and the number of HMW-protein bands produced was observed. This indicated that cold hardening did have an effect on the production and accumulation of HMW-proteins in coleoptiles and roots.

6.2 Samevatting

In Suid Afrika is daar oor die laaste tien jaar sowat 47 062 ha koring beskadig deur koue, wat gelei het tot 'n jaarlikse verlies aan inkomste van sowat R6.7 miljoen. Die doel van hierdie studie was om die vriestoleransie van Suid Afrikaanse koringcultivars te toets, om die genetiese variasie ten opsigte van vriestoleransie te bestudeer en om hoë molekulêre gewig proteïne (HMG-proteïne) te gebruik as aanduiding van vriestoleransie.

'n Kunsmatige vriestoets is gebruik om die vriestoleransie van 24 Suid Afrikaanse koringcultivars te toets. Die vriestoleransie is bepaal deur die persentasie oorlewing, blaarlengtes en wortellengtes by -6 en -12°C.

Norstar het die hoogste vlak van vriestoleransie by -6 en -12°C gehad. Scheepers 69, Belinda, Kavkaz, Molen, Carolus, Jager en PAN 3232 het ook hoë vlakke van vriestoleransie getoon. Die blare van Caledon, PAN 3232, SST 966, SST

936, Norstar, Tugela DN en PAN 3235 het die hoogste vlakke van vriestoleransie getoon. Die wortels van Kavkaz, Norstar, PAN 3235, Belinda, Jager, Carolus, PAN 3232, Betta en Molen het die hoogste vlakke van vriestoleransie getoon.

'n 6X6 dialeel is gebruik on die genetiese variasie, kombineervermoë en oorerflikheid van vriestoleransie te bepaal. Norstar, Tugela DN, PAN 3349, Karee, SST 66 en Snack is gebruik as kruisingsouers.

Betekenisvolle verskille is gevind tussen die F1-basters vir oorlewing, blaarlengte en wortellengte. Snack/Norstar, PAN 3349/Snack en Snack/Tugela DN het 'n lae vlak van vriestoleransie getoon. Karee/Norstar, SST 66/PAN 3349, Karee/PAN 3349 en SST 66/Norstar was amper net so vriestolerant soos Norstar. Geen betekenisvolle verskille tussen kruisings en hulle resiproke is waargeneem nie, dit bevestig dat daar geen sitoplasmiese invloede betrokke was by vriestoleransie nie.

Norstar en Karee het die beste algemene kombineervermoë gehad. Die hoë verhouding van algemene kombineervermoë tot spesifieke kombineervermoë bevestig die additiewe geen aksie van vriestoleransie. Die hoë oorerflikheidssyfers vir persentasie oorlewing (88.18%) en blaarlengte by -12°C (88.45) bevestig die hoë oorerflikheid van vriestoleransie.

Elektroforese van hoë molekulêre gewigproteïene is gebruik om die vriestoleransie van koring te toets. Ses koringcultivars is ingesluit in die proef, naamlik Norstar, Tugela DN, PAN 3349, Karee, SST 66 en Snack.

Die resultate het bevestig dat hoë molekulêre gewigproteïene teenwoordig in koleoptiele van koring wel gebruik kan word om te toets vir vriestoleransie. 'n 48kDa proteïen het in die koleoptiele van Norstar begin opbou nadat die vernalisasiebehoefte bevredig is. Die 46 kDa proteïen is 'n kouesensitiewe proteïn waarvan die produksie

gestaak is tydens blootstelling aan lae temperature. Die 40 kDa proteïen band was 'n aanduiding van die afwesigheid van vriestoleransie. Kouetolerante cultivars was in staat om die 25 kDa proteïen vinnig te produseer wanneer die behoefte vir beskerming teen koue onstaan het.

Soortgelyke reaksies is gevind toe die hoë molekulêre gewigproteïne in die wortels ondersoek is. Onder lae temperature was Snack nie instaat om die 68 kDa proteïen vir lang periodes te produseer nie. Norstar kon die proteien egter vir lang periodes produseer wat die cultivar bestand gemaak het teen lae temperature. Al die cultivars was in staat om die 21, 25 en 30 kDa proteïene te produseer. Die produksie van die proteïen is begin nadat die cultivars se onderskeie vernalisasiebehoeftes bevredig is. Die 19 kDa proteïen het opgetree as 'n vroeë beskermings meganisme.

Die produksie van verskeie HMG-proteïene kan geassosieer word met vriestoleransie. Die groeiperiode en vernalisasie behoefte van cultivars het 'n invloed gehad op die produksie van hierdie HMG-proteïene. 'N Betekenisvolle korrelasie tussen blaarlengte en die aantal HMG-proteïen bande geproduseer dui daarop dat koue wel 'n invloed het op die produksie van HMG-proteïene.

Chapter 7

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