

CHAPTER 1 GENERAL INTRODUCTION

Triticale (X *Triticosecale* Wittmack) is a man made cereal crop that was developed from crosses between wheat (*Triticum* sp. L.) and rye (*Secale cereal* L.) (Oettler 2005). Since its development, triticale has spread to every part of the world with most of the producing countries concentrated in Europe. The crop is mainly used as a feed grain in the livestock industries (Bird *et al.* 1999; Salmon *et al.* 2004) besides being the best alternative crop in the marginal areas or poor soil conditions (Jessop 1996). Only a small percentage of the crop is used as human food (Mergoum *et al.* 2004; Oettler 2005).

Triticale is a minor winter cereal crop both in terms of area and production in Australia (Table 1.1). Its distinguishing characters such as tolerance to drought, acid soils and waterlogged conditions, which are typical of Australian agriculture, have led to an increased adoption of triticale (Cooper *et al.* 2004). However, it is still a minor crop with GRDC (2008) reporting that triticale along with durum wheat and oats contribute 5% to the gross value of grain production. Most of the triticale is produced in NSW, Victoria and SA while smaller quantities are produced in other states such as WA, Tasmania and Queensland (Table 1.2). Since it is a newer crop and only grown over the last few decades, it is yet to overcome the competitive advantages of already established crop such as wheat and barley. However, in the global context Australia contributes a significant proportion to the total triticale production. As in other countries, it is mainly used as a stock feed in Australia (van Barneveld 2002).

Table 1.1 Summary of statistics for Australian wheat, barley and triticale (grain). Area is in '000 ha and production in '000 t (source: ABARE 2009).

	Wheat		Barley		Triticale	
	Area	Production	Area	Production	Area	Production
2002-03	11 170	10 132	3 864	3 865	408	327
2003-04	13 067	26 132	4 477	10 382	445	826
2004-05	13 399	21 905	4 645	7 740	389	611
2005-06	12 443	25 150	4 406	9 482	347	676
2006-07	11 798	10 822	4 182	4 257	369	199
2007-08	12 578	13 569	4 902	7 159	360	450
2008-09	13 151	20 938	4 790	7 669	355	503

Table 1.2 Production ('000 t) of triticale in different states (source: ABARE 2009).

	NSW	Victoria	Queensland	WA	SA	Tasmania	Total
2002-03	121	105	0	23	68	10	327
2003-04	294	295	2	73	153	8	826
2004-05	284	200	1	47	73	6	611
2005-06	266	237	1	42	123	8	676
2006-07	78	70	0	12	36	2	199
2007-08	177	158	1	28	82	5	450
2008-09	198	176	1	31	91	6	503

The winter cereals, including triticale, in Australia are characterized by a spring habit which develops to flowering and maturity in spring or early summer. Unfortunately, low temperatures caused by radiation frosts during this period are of particular importance in all the grain growing regions. Radiation frosts occur when the night temperatures fall below zero under conditions of clear skies, calm cold air, dry atmospheric conditions and temperature inversions (Frederiks *et al.* 2008). More importantly, the timing of the spring radiation frosts and the flowering stage of crops may coincide incidentally thereby threatening crops yields. Though crops are susceptible to frost throughout their growing stages, flowering is the most susceptible stage owing to little or no additional protection by the leaf sheath (Single 1964; Paulsen and Heyne 1983; Marcellos and Single 1984; Cromey *et al.* 1998) and the formation of external ice nucleation in the floral parts at the time of frost (Single and Marcellos 1974). As a result of these circumstances, complete or serious yield losses can be produced when frost occurs between the booting and grain ripening stages (Rebbeck *et al.* 2007b). While exact revenue losses from frost are difficult to define due to its direct and indirect effects, it is estimated that the frost costs Australian grain industry more than AU\$ 100 million annually (GRDC 2009). Therefore, identifying the winter cereals lines/varieties with putative reproductive frost tolerance continues to be a main aim of frost research in Australia.

Some data on the varietal reaction of winter cereals such as wheat and barley to frost have been documented from SA (Reinheimer *et al.* 2004) and Queensland (Frederiks *et al.* 2004, 2005, 2008). An investment of AU\$ 10 million has been made in frost research in the last decade, though the progress in genetic improvement has been slow (GRDC

2009). Significant research has been also conducted on the mechanisms of frost damage mainly on wheat by authors (e.g. Single 1961, 1964, Single and Marcellos 1974; Marcellos 1977; Marcellos and Single 1984; Single 1988) in 1970's and 1980's. However, to the writer's knowledge, there exists no data on triticale frost tolerance in Australia. Nonetheless, a recent study by Roake *et al.* (2009) reported that growers cited frost susceptibility as one of the main constraints in triticale production and expansion. Therefore, this research was executed with the following questions.

- i. Is there any difference in frost tolerance in triticale lines/varieties and how does triticale compare with wheat and barley during the flowering stage?
- ii. Could cold hardening at the reproductive stage favorably affecting the frost tolerance in triticale and
- iii. Can increased potassium nutrient levels contribute to increased frost tolerance in triticale?
- iv. Can glasshouse raised pot plants transferred to the field for frost events, be used in a suitable protocol for screening for relative frost tolerance in cereals?

In order to achieve these objectives, a series of experiments were conducted in the 2009 and 2010 seasons as follows.

- Experiment 1: This experiment was conducted in the 2009 season to compare the frost tolerance between eight different triticale lines/varieties. Simultaneously, three wheats and one barley were also evaluated to compare tolerances between the species.
- Experiment 2: Four triticale varieties/lines that showed varying degrees of tolerance in 2009 were further examined in the 2010 season to confirm the previous season results. In addition, two new wheat varieties from the frost study program in WA were also evaluated to assess their comparative frost tolerance.

- Experiment 3: Cold hardening has been reported to enhance frost tolerance in winter cereals. While adequate information on cold hardening in the vegetative stage is available, data is limited that examines cold hardening at the reproductive stage. Therefore, the triticale variety H426 was used in 2010 to understand the role of cold hardening at the reproductive stage in offering frost tolerance.
- Experiment 4: The favorable role of potassium in abiotic stress tolerance has been reported. It is possible that crops suffering from potassium deficiency will be more susceptible to frost. Thus, this experiment aimed to understand the role of potassium nutrition in frost tolerance. The triticale variety H426 was used to assess whether potassium fertilizer could reduce frost susceptibility on a low potassium soil.

This introduction has covered the rationale and main objectives of the research. The literature review in Chapter 2 consisted of brief overview of the triticale crop, the occurrence and significance of spring radiation frosts and various agronomic practices and plant mechanisms to resist frost damage. The general materials and methods employed throughout the experiments are discussed in Chapter 3. In Chapter 4, the varietal evaluation of different triticales and other winter cereals for two seasons of 2009 and 2010 were collated and presented as one Chapter. Chapter 5 presented the role of cold hardening followed by potassium nutrition in Chapter 6. Finally, a general discussion and conclusions are provided in Chapter 7.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

This chapter will provide a brief overview of the triticale crop followed by an assessment of the different aspects of frost problems in winter cereals. As triticale was bred by crossing wheat and rye, and there exists no or limited data on frosting in triticale, the literature review will centre on the frost tolerance information on winter cereals such as wheat, barley and rye.

2.2 Overview of triticale crop

Triticale (X *Triticosecale* Wittmack) is a hybrid cereal crop that was developed from crosses between wheat (*Triticum* sp.L.) and rye (*Secale cereal* L.). During crossing, wheat was generally used as the female parent while rye was used as the male parent (Oettler 2005). The history of its breeding dates back to the 19th century when a number of workers had artificially bred the crop which led to the production of sterile plants (Wilson 1875 cited in Sapra *et al.* 1971) and fertile plants (Rimpau 1891 cited in Franke 1991). Since then, considerable progress has been made in triticale breeding particularly with the usage of *in vitro* culture techniques to make the embryo fertile and colchicine to double the plant chromosomes (Ammar *et al.* 2004).

There are two types of triticale, primary and secondary, depending on the ploidy levels or generations of crosses. Primary triticales are the immediate product of chromosome doubling of the wheat-rye cross, while the secondary triticales are the product from the crosses of the primary triticales (Thiemt and Oettler 2008). The main aim of the primary triticale breeding was to create a new species that had the desirable characters from both parents. The female parent (wheat) was expected to furnish features such as high yield, bread making quality and short height while the male parent (rye) was to provide characters such as winter hardiness and disease resistance. However, the desired characteristics could not be established in the primary triticales and hence secondary triticales had to be developed either through crosses of primary triticales, crosses of

secondary triticales with either of the parents or two or three way crosses of primaries with secondary triticales (Wolski 1990 cited in Oettler 2005).

2.3 Importance of triticale in the world

Triticale has moved forward at a fast pace from being a scientific curiosity to a viable and well established crop (Franke 1991; Oettler 2005). It is now widely accepted around the globe that triticale is a potential crop for human consumption, a high value feed grain and an alternative crop in the marginal areas or poor soil conditions. It has been estimated that in 2003, the area under triticale was approximately 3 million ha worldwide (FAO 2003 cited in Mergoum *et al.* 2004). Its contribution to global cereal production has been estimated at more than 6 million tonnes per year (Varughese 1996). In 2004, there were more than 200 varieties available in more than 35 countries (Mergoum *et al.* 2004). The most notable countries in its production are Poland, Germany and France in Europe while Australia also contributes significantly to the global production (Table 2.1).

Table 2.1 Major triticale growing countries in the world in 2008 (source: FAOSTAT).

Country	Area ('000 ha)	Production ('000 t)
Poland	1333	4460
Germany	399	2382
France	343	1821
Belarus	458	1819
Hungary	132	509
Australia	376	503
Lithuania	98	311
Sweden	50	263
Czech Republic	58	256
Austria	46	251
China	246	215
Denmark	40	185
Brazil	76	185

2.3.1 Triticale as a human food

Since its first development, triticale has not been widely accepted as human food as was originally expected. Many workers (e.g. Pena 1996; Tohver *et al.* 2005) researched its feasibility as human food and concluded that it could be valuable for making bread and other food products although there was genetic variation in bread making quality.

Cooper (1985 cited in Cooper *et al.* 2004) suggested that triticale could be a food source that reduced obesity and diabetes problems. Organizations such as the International Centre of Wheat and Maize Improvement Centre also worked on improving the nutritional quality and breeding of triticale as a grain crop (Ammar *et al.* 2004). However, triticale as a main cereal for bread making is constrained by variations in bread making quality, low and inferior gluten content and lower flour yield (Oettler 2005). Further, wheat and rye have already been established as the traditional bread cereals and hence consumers' preference to triticale may take some time to promote.

2.3.2 Triticale as an animal feed

The existence and expansion of this crop is globally attributed to its high value as feed for animals (Oettler 2005). Salmon *et al.* (2004) remarked that most triticales are used as animal feed and fodder and a small percentage goes for human consumption. It can be used with all types of animals with any formulations or only as a cereal component. The presence of higher starch digestibility (Bird *et al.* 1999) and low gluten and beta glucans (Salmon *et al.* 2004) best fit triticale as ruminant feed compared to other cereals such as wheat, barley and oats. Apart from its usage as grain for feeding, the forage of triticale can also be used for grazing and animal feeding.

2.3.3 Triticale in abiotic stresses conditions

Besides its nutritional qualitative trait, triticale adoption can also be attributed to its tolerance to unfavourable growing conditions and nutrient uptake efficiencies. The crop is highly tolerant to soil with low pH, and high concentrations of aluminum and salinity (Qualset and Pinto 1996). It is resistant to many diseases, tolerant to drought and both major and minor element deficiencies (Jessop 1996; Varughese 1996). The crop has also higher nutrient uptake efficiencies particularly nitrogen and phosphorus (Mergoum *et al.* 2004). Further, triticale is a cold tolerant crop and uses water more efficiently than oats and rye while Cooper (1996) considered triticale as an excellent break crop for wheat. Triticale was found to be the best alternative crop and advantageous in marginal and stressed environments for either grain, grazing or straw production (Mergoum *et al.* 2004).

2.4 What is frost?

The definition of frost varies from one author to another. For example, Cunha, 1982 (cited in Snyder and de Melo-Abreu 2005) defined frost as,

“The formation of ice crystals on surfaces, either by freezing of dew or a phase change from vapour to ice”.

On the other hand, some authors define frost as,

“A condition in which temperatures fall below 0°C” (Sakai and Larcher 1987) and

“Occurrence of an air temperature of 0°C or lower, measured at a height of between 1.25 and 2.0 m above soil level, inside an appropriate weather shelter” (Snyder and de Melo-Abreu 2005).

Whatever the definition may be, frost is generally regarded to have occurred when air temperature fall sub zero.

2.5 Types of frost

There are two major frost types, namely radiation and advection frost. The environmental factors that contribute to the occurrences of these frosts vary from one type to another. For example, radiation frost occurs when there is clear, calm, temperature inversions and the temperature is greater than 0°C during daylight hours (Snyder and de Melo-Abreu 2005). On the other hand, advection frost is caused by large scale incursions of cold air coupled with windy atmospheric conditions and temperatures can be less than 0°C even during daylight hours. Advective frost is more typical of higher latitudes (Ivory and Whiteman 1978).

Amongst them, radiation frost is the most common occurrence and the most destructive type both in Australia and other parts of the world (Rebbeck *et al.* 2007a). It not only affects cereal crops but even horticultural crops and pasture plants. Given its greater significance both globally and within Australia and practicability for the present research,

only radiation frost will be covered further in this paper. The word 'frost' therefore will be exclusively implied to mean 'radiation frost' hereafter unless specified. More specifically, it will be the spring radiation frost that will be discussed throughout this paper as it is a common significant event.

2.6 How do frosts occur?

Radiation frost occurs when there is calm cold air coupled with clear skies and dry atmospheric conditions (Frederiks *et al.* 2008). There is usually a temperature inversion and air temperatures that are above 0°C during the day but which fall to subzero during the night (Snyder and de Melo-Abreu 2005). Under such conditions, a major flux of energy is lost to the night sky by long wave radiation from the plant structures in the upper layers of the crop at a rate proportional to the temperature difference between the plant surfaces and any cooler surrounding body (Fuller and Legrice 1998). All this energy via long wave radiation is absorbed by the sky which acts as an absorbant black body (Jones 1983 cited in Fuller and Legrice 1998). Thus, a negative temperature profile is created through the canopy as the loss of heat through the upper boundary of the crop is greater than that supplied to it from below by the soil heat flux (Marcellos and Single 1975). As a result the plants develop a lower temperature than the surrounding air and dew forms if the air relative humidity is sufficiently high. The formation of ground frost then becomes inevitable when the temperature falls below 0°C and continued dehumidification of the air allows ice crystal growth (Fuller and Legrice 1998).

Radiation frost can be further sub classified as hoar frost and black frost. A hoar frost occurs when the dew on the ground freezes to give a white coating of ice on exposed surfaces (Snyder and de Melo-Abreu 2005; Rebbeck *et al.* 2007a). Hence it is also called 'white frost'. On the other hand, 'black frost' occurs when the temperature drops subzero but without any white ice on the surfaces. Since frost formation is partly driven by humidity, black frost is more common in dry or drought years where moisture levels are low.

2.7 Importance of frost

Spring radiation frost causes significant yield losses to winter crops such as wheat, barley and triticale globally particularly when it occurs at the time of flowering or anthesis. In the temperate regions, frost risk is not a major issue as winter crops flower in summer when the temperature is generally not lethal. In contrast, the winter crops in Mediterranean regions and subtropical areas attain the reproductive stage in late winter or early spring when the days are short and frost risk is more likely (Single 1985, 1988). Ironically, optimum yield is obtained when the crops flower in late winter due to more water availability (Woodruff and Tonks 1983) and crops escape frost damage. The grain growing regions in other parts of the world also experience frost as one of the major yield constraints (Shroyer *et al.* 1995).

In Australia, spring frost at flowering time causes a significant economic loss to individual growers, and the country's grains industry, through direct and indirect yield losses. The problem is more severe in the eastern grain growing regions, such as NSW and Queensland (Kelleher 1988), where the heat and drought in summer restrict the growing season when the day time temperatures are ideal for growth but night temperatures fall to sub zero levels (Fuller *et al.* 2007). Moreover, the mild winter in these regions prevent the development of cold hardiness leading to softer plants and rapid crop development. On the other hand, the grain growing regions in southern Australian temperate zones experience winters that are uniformly cold leading to slower crop development, build up of cold hardiness and later ear emergence where the frost risk is considerably lower (Kelleher 1988). However, frost is still seen as a major constraint in grains production in the south (Reinheimer *et al.* 2004). Apart from cereal grains, radiation frost is also considered as one of the principal limiting abiotic factors for pulse crop production in Australia (Maqbool *et al.* 2010).

The existing data supports the difference in the relative amount of damage between the northern and other parts of Australia. For instance, in the eastern Australia, the yield loss due to direct frost damage in wheat and barley is estimated at AU\$ 50-100 million per year (Frederiks *et al.* 2008). In NSW alone, a heavy frost in the 1965 season wiped

out 50% of the state wheat crop (Fitzsimmons 1966 cited in Boer *et al.* 1993). However, the annual monetary loss due to frost in southern states such as Victoria and SA is comparatively less than the north eastern states with AU\$ 9.2 million from direct yield losses and AU\$ 22.5 million from indirect yield losses (GRDC undated). Frost is also a significant problem in the wheat growing regions of WA and a direct revenue loss of more than AU\$ 100 million has been reported from a late September frost in 2008 (GRDC 2009). On a national level, the loss from frost is estimated at more than AU\$ 100 million per year (GRDC 2009). Losses from frosts before anthesis are likely to be much less, as frost damage to crops prior to heading is not a common phenomenon and even if it occurs, yield recovery to a certain extent is assured from the later formed tillers (Single 1988).

In addition to direct yield loss, frost also causes economic losses by downgrading crop quality through lower organic matter digestibility and metabolizable energy (Richardson *et al.* 2001). Frost also causes reductions in grain size, decreases in flour extraction, a decrease in dough strength and baking quality, and an increase in flour ash and α -amylase activity (Allen *et al.* 2001). It is estimated that frost causes a monetary loss of AU\$1.9 million from quality downgrading in Victoria and SA (GRDC undated). Richardson *et al.* (2001) reported that severe frost in October 1998 across southern NSW and Victoria compelled grain growers to dispose of their frosted grains as feed grain at low prices as they could not meet the standard set for bread wheats.

2.8 Crops susceptibility to frost damage

Much of the literature concerning frost tolerance in winter cereals is confusing and inconsistent. For example, Fowler and Carles (1979 cited in Fowler 2008) reported that among cereals, rye was the most freezing tolerant while oats was the least freezing tolerant. They nominated relative resistance to freezing of cereals as in the order of (from most resistant): rye > common wheat > triticale > durum wheat > barley > oats. Webb *et al.* (1994) and Snyder and de Melo-Abreu (2005) also made similar observations and the latter authors cited the relative resistance to freezing of cereals as in the order of (from most resistant): rye > bread wheat > triticale > barley > oats and

durum wheat. These findings, however, contradict Rebbeck *et al.* (2007b) who reported triticale as the most susceptible cereal crop followed by wheat, barley, rye and oats. GRDC (undated) also noted oats and barley to be more frost tolerant than wheat by 4°C and 2°C respectively. While species difference in frost tolerance may exist, frost damage is also determined by other factors such as crop growth stage and other environmental conditions.

2.9 Temperatures causing frost damage

The severity of frost is generally measured by the temperature below zero and this has the crucial role in determining the amount of damage to cereals. The literature substantiates that frost occurs at sub zero temperatures, though Hammer and Rosenthal, 1978 (cited in Stone *et al.* 1996) commented that the relationship between minimum temperature and frost occurrence is not exact. GRDC (2009) suggested that a temperature recording of 2.2°C in a weather station is likely to result in frost damage and this can be lethal for flowering crops. The general baseline temperature nominated for frost damage is a fall of night air temperature to below 2°C as measured in a Stevenson screen (Rebbeck *et al.* 2007b).

Woodruff (1988) reported that a critical ear temperature that caused severe yield loss in cereals was in the range of $-3.8 \pm 0.6^{\circ}\text{C}$. This is in agreement with that reported by Marcellos and Single (1984) who also found disruption of membranes of wheat ears at temperatures down to -4°C but warmer than the -5°C value identified by Fuller *et al.* (2007). Single and Marcellos (1974) reported that a temperature of -3.9°C is a severe stress at flowering time and a cultivar that could tolerate this temperature would hardly suffer yield loss. Single (1988) nominated the damaging temperature range as -3°C to -5°C for cereal crops. It was however argued by Shroyer *et al.* (1995) that temperatures that are slightly below freezing can cause severe damage during the reproductive stages and hence significantly reduces the yield (Table 2.2).

Table 2.2 Damaging temperatures that cause spring freeze injury to wheat and yield effects (data from Shroyer *et al.* 1995).

Growth stage	Approximate injurious temp. (2 hours)	Yield effect
Tillering	–11 ⁰ C	Slight to moderate
Jointing	–4 ⁰ C	Moderate to severe
Boot	–2 ⁰ C	Moderate to severe
Heading	–1 ⁰ C	Severe
Flowering	–1 ⁰ C	Severe
Milk	–2 ⁰ C	Moderate to severe
Dough	–2 ⁰ C	Slight to moderate

2.10 Factors influencing the frost damage to crops

Frost susceptibility and injury is a complex interaction. While factors such as the effects of crop stages and species differences have been discussed, this section will cover some of the other important factors that influence the level of frost injury in winter cereals. They are freezing temperatures, length of exposure to the freezing temperatures, soil moisture, soil fertility, topography and rate of thawing (Livingston and Swinbank 1950; Rebbeck *et al.* 2007c).

2.10.1 Freezing temperature effects

The amount of injury produced is influenced by degree of low temperature experienced (Livingston and Swinbank 1950; Cromey *et al.* 1998; Shroyer *et al.* 1995). It is commonly believed that there will be more frost damage with decreasing temperature. Livingston and Swinbank (1950) in their freezing chamber study demonstrated that at 0°C and above, there was no damage to wheat heads even if they were exposed for 14 hours. In contrast, significant injury was done when the same plants were exposed to –4°C for just 2 hours.

The types of freezing injury are also affected by the different temperatures. The main form of freezing injury was expansion induced lysis in cereals such as non acclimated rye protoplasts, when the temperature range was –2.5 to –5°C (Dowgert and Steponkus 1984) or the effects of mechanical and adhesive forces of growing ice crystal at –10°C

(Gusta *et al.* 2005). However, the predominant form of injury was freeze-induced dehydration when the temperature declines below -6°C (Dowgert and Steponkus 1984) or -10°C (Gusta *et al.* 2005). Temperature also affects the spread of ice within the freezing plant with slower spread at -1.5°C and -2°C than at and below -2.5°C (Pearce and Fuller 2001).

2.10.2 Length of exposure to freezing temperatures

One of the key factors that determine the amount of injury is timing of the exposure to low temperature (Livingston and Swinbank 1950; Shroyer *et al.* 1995; Cromeey *et al.* 1998; Stoddard *et al.* 2006). Livingston and Swinbank (1950) noted no injury when the wheat heads were treated at -2°C for 2 hours but the injury increased with the length of exposure. Further, the severity of damage caused at -4°C was much greater with 4 hour exposure than with the 2 hours exposure. Under such exposure (-4°C for 4 hours and more), the heads exhibited approximately 50% floret sterility even if the wheat heads were protected by the boot section of the leaf sheath. Ivory and Whiteman (1978) also documented that when freezing temperature (-3.5°C) duration was increased from 0.5 to 4 hours, the foliar damage increased by 45 and 36% in *Setaria anceps* and *Panicum maximum* respectively. However, it was argued by Marcellos and Single (1975) that frost damage is related more to the degree of cold than other factors such as duration of sub zero temperatures. Levitt (1980) also stated that frost injury is independent of the duration the plant tissue is below a particular temperature for short periods (2 to 24 hours).

2.10.3 Plant moisture content

While maintenance of adequate soil moisture content was found to be favourable for minimizing frost damage due to more heat storage and transfer, plant moisture content was reported to have an inverse relationship with frost damage. Livingston and Swinbank (1950) reported that wheat heads given light rain (hence wetted) and followed by frost were injured more than dry heads. Dry plants were found to supercool to a lower temperature than wet plants (Wisniewski *et al.* 2009). Ivory and Whiteman (1978) also reported a significant decrease in foliar damage of a grass species (*Panicum maximum*)

when there was low water content before frosting. The detrimental effect of moisture on freezing was considered to be due to the acceleration of nucleation of ice (Pearce and Fuller 2001) brought about by bacteria such as *Pseudomonas syringae* and *Erwinia herbicola* (Pearce 2001). In addition, moisture status (plant water potential) also governs the lowest temperature it can reach during freezing (Rebbeck *et al.* 2007a). There was a drop by about 1°C of freezing point for each 12 bar drop in water potential in the range of 0 to 10°C (Maylands and Cary 1970). Moreover, there is a wealth of literature where moist conditions have been simulated by sprinkling water in freezing chamber studies to initiate freezing (e.g. Single 1966; Single and Marcellos 1974; Fuller *et al.* 2007).

2.10.4 Soil fertility

The level of soil fertility has been cited as one of the factors affecting the level of frost damage. Livingston and Swinbank (1950) found that the heads of plants grown on a highly fertile soil sustained the greatest injury compared to plants grown on a low fertility soil. It is assumed that such plants grow luxuriously with high moisture content thereby predisposing the crop to freezing injury (Shroyer *et al.* 1995).

Plants cultivated on highly organic soils that are fairly dry were also reported to be susceptible to more frost damage due to reduced thermal conductivity and heat storage capacity (Snyder and de Melo-Abreu 2005) thereby causing lower temperatures. Further, moisture content does not affect the heat transfer and storage capacities in such soils. The increase in minimum soil temperature of 1°C to 3°C was noticed when the organic soil was mixed with mineral soil (Valmari 1966, cited in Snyder and de Melo-Abreu 2005) clearly indicating the negative effect of fertile soil on frost damage. Therefore, in high frost prone areas, planting crops in organic soils should be avoided.

2.10.5 Rate of thawing

The rate of thawing after a frost event is also believed to contribute to some amount of damage. Slow thawing is less injurious than fast thawing (Reaney and Gusta 1999). Slowing the thawing rate will increase the rehydration of cells in plants that were exposed to extracellular freezing and hence reduce the damage due to fast thawing

(Mazur 1969). Slowing thawing rate also avoids the intracellular ice formation and hence reduce any damage (Guy 2003). In fact, there are reports from areas such as California where citrus farmers operate wind machines in the morning after a frosty night to slow the thawing rate.

2.11 Frost damage to cereal crops

Radiation frost can cause significant damage to cereal crops through nucleation and growth of ice crystals thereby injuring the cells. Westwood (1978) remarked that the actual injuries caused to plants during frost are the formation of ice, not the cold temperature. The plasma membrane has been found to be the most vulnerable organ to freezing injury resulting from the osmotic stresses and cellular dehydration that occurs during freezing (Steponkus 1984; Pearce and Ashworth 1992). There are two types of freezing, namely 'intracellular freezing' and 'extracellular freezing', which are mediated by intrinsic nucleators and extrinsic/heterogenous nucleators respectively. The moisture which is inevitably required by heterogenous nucleators to function is provided by condensation onto plant surfaces during radiation frost conditions. The ice nucleating active bacteria such as *Pseudomonas syringae* and *Erwinia herbicola* were reported to produce protein that can nucleate freezing at temperatures as high as -2°C (Pearce 2001).

Intracellular freezing causes direct damage where the water crystallizes inside the cell through internal nucleation (Pearce 2001). This process was found to be lethal as it mechanically damages the protoplasm structures (Levitt 1980; Guy 1990). However, the degree of damage in this type of freezing is governed by the rate of drop in temperature and cooling before freezing, with faster cooling leading to more intracellular freezing (Maqbool *et al.* 2010). Plants grown in a glasshouse and then transferred to the field were found to supercool to a much greater degree than the field grown plants due to absence of extrinsic ice nucleators (fungi and bacteria) in the glasshouse plants.

Extracellular freezing on the other hand is the presence of ice exclusively in the regions of the tissues outside the cell (Guy 1990) leading to cellular dehydration and injury.

Thus, extracellular freezing is an indirect but more damaging event than the intracellular freezing (Snyder and de Melo-Abreu 2005). Levitt (1980) too stated that the main form of freezing injury in nature was extracellular freezing and there was no evidence of intracellular freezing. During external freezing, ice is formed on the extracellular surface resulting in a sudden drop in water potential outside the cell (Xin and Browse 2000). This water potential gradient results in movement of water from the cell/cytoplasm by osmosis leading to cellular dehydration (Yu and Griffith 2001). As a result, frosted grains possess blisters on their surfaces indicating that the outer tissues have been killed by frost (Cromeey *et al.* 1998). Therefore, as freezing injury is caused by cellular dehydration, other unfavourable environmental conditions such as drought stress and salt stress also share many features and genes in common (Xin and Browse 2000) and a frost problem can be often confused with drought effects (Cromeey *et al.* 1998; Snyder and de Melo-Abreu 2005). However, moisture stress such as in drought conditions can cause spikelet sterility at the tip and base of the head (Edwards and Roberts 2008).

It is important to understand the mechanism of ice spread within the plant to determine the susceptibility of various plant parts. Stomata are known as the gateway for the exchange of gases. In the same manner, it has been also considered that stomata act as the main entry for the ice to spread into the extracellular spaces (Snyder and de Melo-Abreu 2005). Wisniewski *et al.* (1997) showed that freezing occurs first on the leaf surface and ice then enters leaves through stomata. Additionally, hydathodes which are located at the tip of leaves also serve as an important channel through which ice spreads into the plants (Pearce and Fuller 2001). In field grown plants, fissures caused by wind or pests may also act as possible routes for ice to spread into the leaf (Pearce and Ashworth 1992). Pearce and Fuller (2001), using infrared video thermography studies, found that freezing first occurred in leaves in contact with ice. Ice then spread to the next older leaves followed by the younger leaves and then finally to secondary tillers. Further, the spread of ice in the younger leaves happened twice as quickly as in older leaves. They reported an overall average of 2.6 cm s^{-1} rate of longitudinal spread of freezing in barley leaf blades which is higher than the 2 cm s^{-1} noted by Single and Marcellos (1981) in non acclimated wheat.

There is some disagreement concerning the mechanism of frost damage in acclimated and non acclimated plants as significant changes in the plants occur during cold acclimation. It is, however, commonly agreed that cellular dehydration leads to a phase change in the fraction of the membrane lipids from a bilayer to a non layered structure (Pearce 2001). Thus, the alterations in the membrane lipid composition are seen as one of the important factors in frost tolerance (Hughes and Dunn 1996). One of these changes can be the utilization of sugars that were synthesized during cold acclimation or from fructan hydrolysis to prevent plasmolysis by increasing osmotic pressure within cells (Livingston III *et al.* 2009).

2.12 Stages of crop that are susceptible to frost damage

Every stage of a crop is frost susceptible throughout its growing period (from vegetative through to the reproductive stage) and can be damaged by frost. However, flowering has been found to be the most sensitive stage (Figure 1) (Single 1964; Single and Marcellos 1974; Shroyer *et al.* 1995). Rebbeck *et al.* (2007b) also noted crop flowering as the most critical stage and the greatest losses in grain yield and quality were produced when a frost event occurred between the booting and grain ripening stages. Minor variations in temperature during flowering cause significant injury and ultimately yield loss.

Frost does not cause economic damage to cereals when it occurs during the vegetative stage as plants have the ability to induce high levels of frost tolerance (Kosova *et al.* 2008). Fuller *et al.* (2007) and Frederiks *et al.* (2005) reported freezing tolerance by wheat cultivars to -20°C during the vegetative stage. The growing parts and reproductive organs are still inside the plant sheath and protected by the warmer soil conditions (Rebbeck *et al.* 2007b). However, as growth progresses, these organs are exposed from the boot and become susceptible to harsh environmental factors such as frost (Zhong *et al.* 2008). The crop tolerance to low temperature is totally lost when it reaches the reproductive stage and severe damage occurs even at moderate temperatures of -5°C to -7°C (Fuller *et al.* 2007; Frederiks *et al.* 2008) due to little or no additional protection by the leaf sheath (Single 1964; Paulsen and Heyne 1983;

Marcellos and Single 1984; Cromeey *et al.* 1998) and the formation of external ice nucleation in the floral parts at the time of frost (Single and Marcellos 1974). The tolerance to radiation frost and plant age is thus inversely related and frost tolerance at the vegetative stage is not related to reproductive frost tolerance (Maqbool *et al.* 2010).

The location of the leaves in a crop induces differences in their sensitivity to frost. Generally, the lower leaves and the base of the plant are less susceptible to frost damage as warmer temperatures are encountered in the lower canopy (Marcellos 1977; Pearce and Fuller 2001). Soil also furnishes considerable heat which is trapped by the lower leaves acting as a blanketing effect (Single 1988). The flag and second leaves are the most vulnerable to frost damage and experience the coldest temperatures during frost (Marcellos and Single 1975). Conversely, they are also the main suppliers of photosynthates to the ear. The crown also plays a significant role in winter cereals ability to adapt to low temperatures as it synthesizes antifreeze proteins (Perras and Sarhan 1989) and polysaccharides that provide osmotic protection and anti freezing property (Livingston III and Henson 1998) during cold hardening. The crown was also reported to interrupt the ice travel to other plant parts (Pearce and Fuller 2001). However, Maqbool *et al.* (2010) reported a contrasting scenario in legumes where the lowest areas of the canopy were seriously damaged by frost due to settlement of dense chilled air in a differently shaped canopy.

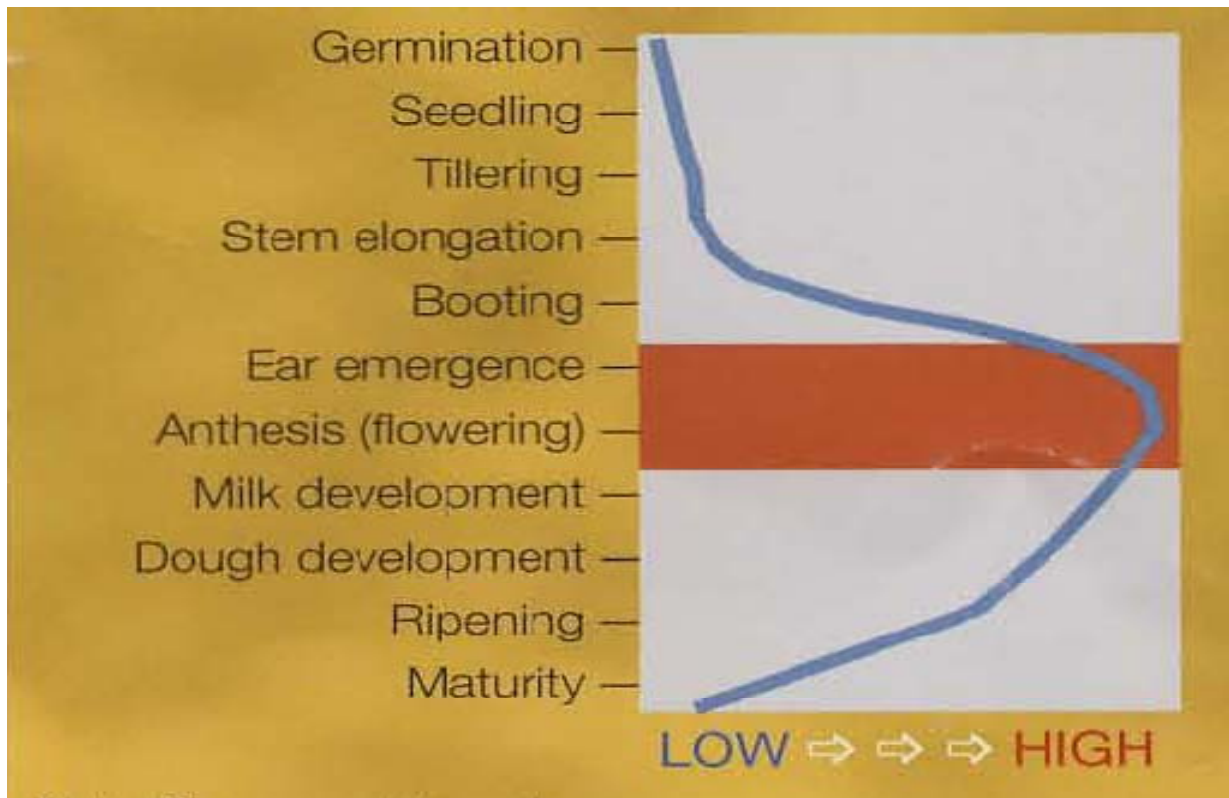


Figure 1. Cereal susceptibility to frost damage at various developmental stages. (Figure is not to scale and taken from GRDC 2009). The dark area in the middle of the diagram is the most critical stage for frost damage.

2.13 Symptoms of frost damage

After a frost event, crops exhibit different symptoms according to the site or organ that has been affected. The symptoms differ from one stage to another depending on the susceptibility of the crop parts and the intensity of the frost. It is also important to know the differences between the normal and frosted plants to readily observe the changes or differences between the treatments.

2.13.1 Symptoms at vegetative (tillering) stage

The occurrence of frost at this stage is not as destructive as at the other growth stages. However, it does affect some of the plant parts (mainly leaves and tillers) as the crop is in the vegetative phase. The injured foliage appears brown and scorched (Rebbeck *et*

al. 2007b), twisted and yellow (Shroyer *et al.* 1995) and with necrosis at the tip of the leaves (Marcellos 1977; Paulsen and Heyne 1984; Shroyer *et al.* 1995). The damaged tillers prevent the growth of their stems thereby leading to emergence of secondary tillers. Thus, there appears a mixture of normal and late tillers resulting in uneven maturity and a slight reduction in yield (Shroyer *et al.* 1995). Further growth of affected plants is restricted or arrested depending on the severity of frost but in general the subsequent growth of the plants is resumed when there are warmer temperatures. Marcellos (1977) observed that the mildly frosted/stressed plants resume their photosynthetic activities at normal rates by the following day while severely affected plants could not recover over this period.

2.13.2 Symptoms at vegetative (booting) stage

When the stem is frosted it has a pale green to white ring around it which is sunken, rough and easy to squeeze (Rebbeck *et al.* 2007b). The stem can also be distorted, cracked and shriveled often leading to lodging (Single 1961; Paulsen and Heyne 1984). As a result, the water and nutrient transporting mechanism of the plant is affected. Nonetheless, no association between stem frosting and grain production was observed (Banath and Single 1976) as long as lodging did not occur (Single 1975 cited in Banath and Single 1976).

The heads within the boots may be either trapped by the frost or, in some instances, emerge like a normal head. However, the alteration in colour from normal green to yellow or white after emergence will indicate frost damage. Such heads fail to produce fertile florets.

2.13.3 Symptoms at flowering and post flowering stage

Flowering is the most sensitive stage and hence maximum damage is done when frost occurs at this stage. Its sensitivity can be attributed to the complete exposure of florets to the outside environment where low temperatures during frost bring the greatest detrimental effect. The male reproductive organs (anthers) are more sensitive compared to the female parts to low temperatures (Shroyer *et al.* 1995). Normal anthers are bright

yellow in colour but frost affected anthers are dry, pale yellow or white, and banana shaped (Rebbeck *et al.* 2007b) and shriveled (Maes *et al.* 2001). In the self pollinated crops such as wheat and triticale, the damage to male reproductive organs can result in complete or partial sterility (Shroyer *et al.* 1995).

2.13.4 Symptoms at grain development and maturity

The severity of frost will largely determine the level of partial or complete sterility. When the frost is severe, it leads to complete sterility while mild frost leads to partial sterility. Frost can cause sterility in some florets, whole spikelets or whole ears depending on the severity of frost (Single and Marcellos 1974; Maes *et al.* 2001). Further, any differences in flowering time affect the damage as flowering starts from the centre of the head and then proceeds to the top spikelet one day later and to the basal spikelet three days later (Cromey *et al.* 1998). Thus, damage as aborted spikelets can be seen anywhere in the head (Edwards and Roberts 2008) as flowering does not occur at the same time in all the florets during freezing (Shroyer *et al.* 1995). It is suspected that the meiosis stage of flowering was more susceptible and spikelets at that particular stage were more damaged by frost (Whaley *et al.* 2004). Similar symptoms of random frost damage in wheat heads were also documented by other authors (Single and Marcellos 1974; Paulsen and Heyne 1984; Maes *et al.* 2001; Whaley *et al.* 2004).

Under a mild frost event, some kernels continue to develop but they eventually turn brown from a white colouration and exude a clear to straw colored liquid when squeezed (Rebbeck *et al.* 2007b). In contrast, the normal developing grain is light to dark green and exudes white milky dough when squeezed. Frost also leads to deformation of the grain shape to a shriveled and wrinkled appearance while the normal grains are plump in shape (Shroyer *et al.* 1995). Cromey *et al.* (1998) observed that the frosted grains were clearly shriveled in the upper spikelets with surfaces that were buckled and wrinkled as compared to smooth surfaces of the unfrosted grains. As expected, the frosted kernels were of inferior quality and light in weight (Livingston and Swinbank 1950; Preston *et al.* 1991).

2.14 Frost management strategies: frost tolerance strategies

Winter cereals such as wheat, barley and triticale may survive frost through one of the frost resistance strategies, namely, frost tolerance or frost avoidance. Frost tolerance is the ability to tolerate ice formation in the tissues without injury (Maqbool *et al.* 2010) which is usually determined as LT₅₀ values, the lethal temperature when 50% of samples die (Kosova *et al.* 2008). Frost avoidance on the other hand is the ability of the plant to avoid low temperatures or to avoid potentially damaging ice formation in tissues by lowering the freezing point or super cooling (Frederiks *et al.* 2004), through physical barriers (Maqbool *et al.* 2010) or through various agronomic practices (Rebbeck *et al.* 2007c, 2007d).

2.14.1 Delay in sowing

Manipulation of flowering time through appropriate selection of varieties and planting date is one of the important components in frost risk management in cereals and other annual crops. It is the tradition of the growers to carry out early sowing in areas with low frost risk and delay planting in frost prone paddocks. Postponing planting and use of longer season varieties delays head emergence and allows the crop to escape frost during flowering which is the most critical stage (Gomez-Macpherson and Richards 1995; Shackley and Anderson 1995). Frosting research in WA has demonstrated that sowing of long season varieties significantly avoids frost damage.

Planting longer maturing varieties can lead to a time conflict between two rotation crops (Zhong *et al.* 2008). While a delay in sowing minimizes the frost risk, it increases the risk of drought due to rising temperatures and declining soil water levels that usually occur at the end of the season during the grain filling period and hence reduces the yield potential (Frederiks *et al.* 2004). The average decrease in grain yield from delayed sowing was 20 kg/ha/day (McDonald *et al.* 1983; Woodruff and Tonks 1983; Shackley and Anderson 1995) which is approximately a 5% yield loss (Hayman *et al.* 2007). This value was, however, lower than the data obtained by Keer *et al.* (1992) who found yield decline of 35 kg/ha/day. On a weekly basis the yield decline for each week at anthesis is delayed past the optimum date was 15% (Kerr *et al.* 1992) and 16% (McDonald *et al.*

1983). Trials in SA and WA have also confirmed the loss of grain yield from late planting (Table 2.3). In monetary values, the loss by delayed sowing in WA alone was about AU\$ 18.4 million (Hayman *et al.* 2007).

Similar to late sowing, early planting of winter cereals before the optimum period was detrimental. This is because warmer temperatures accelerate the development of these winter cereals that require little or no vernalization, thereby not allowing the plants to accumulate sufficient dry matter to produce a large number of kernels and the optimum grain yield (Anderson and Smith 1990). However, research has demonstrated that earlier sowing could enhance yield potential by approximately 1% for each day flowering is advanced (Woodruff and Tonks 1983) provided frost problems do not occur.

The other detrimental effects of delayed sowing are a general reduction in dry matter production, grain size and number of heads and kernels per head, which resulted in fewer kernels per m² (Woodruff and Tonks 1983; Kerr *et al.* 1992). However, there was an increase in grain protein as a consequence of a decrease in grain size due to late planting (Shackley and Anderson 1995).

Table 2.3 Estimated cost of delayed sowing of wheat to minimize the impact of frost (data from Hayman *et al.* 2007).

Site	Days delay	Loss (kg/ha)	Loss (\$/ha)	Average loss (\$/ha)
Merredin, WA	18	360	79	16
Narrogin, WA	10	200	44	14
Geranium, SA	26	520	114	24
Booleroo, SA	21	420	92	16

2.14.2 Growing hay on frost prone areas

One of the main aims of farm managers is to optimize economic returns which require the utilization of his land holdings with minimum risk. Thus, in areas where the risk of severe frost is inevitable, growing hay may be a viable option to reduce the farm

business exposure to frost (Rebbeck *et al.* 2007c). Hay production is a good strategy as crops avoid the frost sensitive period when mowing is done soon after flowering. Among the cereal varieties, awnless varieties are best suited for the hay enterprise.

2.14.3 Cultivating tolerant varieties

The cultivation of tolerant varieties can be seen as one of the most economical strategies in combating the frost problem. It has been recognized that development of winter cereals with increased adaptation to frost will substantially elevate cereal production in Australia (Frederiks *et al.* 2008). However, identifying reliably tolerant varieties within the commercially available varieties has been a challenge as it involved routine screening of the cultivars, demanding considerable human and financial resources. In addition, unpredictable natural frost events and differences in crops phenology such as flowering have constrained the evaluation program (Collins 2009). Single and Marcellos (1974) in their wheat breeding program revealed that variation in the tolerance of wheat cultivars to frost damage does exist. They found the commercial cultivars Florence and Kite were the most resistant varieties. Kite had been incorporated in the present study to confirm its tolerance.

Frost research in SA has identified three barley varieties (cv. Franklin sib, Amagi Nijo and Haruna Nijo) as frost tolerant (Reinheimer *et al.* 2004). However, the field evaluation of these barley varieties in the Queensland environment did not support the findings of the SA work. Indeed, the control variety Gilbert, which is susceptible, performed better than or equal to these tested lines under four frost events (Frederiks *et al.* 2005). Shroyer *et al.* (1995), in fact, cautioned that there is little scope to increase the freezing resistance in winter cereals such as wheat varieties as there is no difference in susceptibility to frost at the same growth stage. The varietal differences reported elsewhere are likely to have been caused by differences in the plant growth stages when freezing occurred.

2.14.4 Soil heat bank

The management of the soil heat bank is crucial to store the heat during the day time and release it into the crop canopy during the night (Rebbeck *et al.* 2007c, 2007d). In soil heat management, soil moisture is an important factor as it governs the thermal conductivity and heat content of soils. Heavier textured soils such as clays hold more moisture (and therefore heat available for release during the night) than light textured soils such as sandy soils.

Braunak and Mayer (2002, cited in Rebbeck *et al.* 2007c) noted more frost damage to crops grown on lighter soil types due to lower soil moisture and the more reflective nature of these soils. However, it was argued by Veisz and Tischner (1995) that more plants are damaged by frost in wet, waterlogged areas than the drier areas. They found less frost damage to winter wheat at lower soil moisture levels and the damage increased considerably with increasing water content. Greater moisture content leads to non equilibrium freezing which is more damaging than the equilibrium freezing (Olien 1967). Non equilibrium freezing is, “the sudden loss of liquid water from the intercellular spaces through the conversion to ice, resulting in rapid loss of water from within the cells, as well as physical distortion and mechanical shearing forces from the ice crystal growth” (Olien 1967). On the other hand equilibrium freezing is, “a process in which a three way vapour pressure equilibrium exists among liquid water outside of the protoplasts, liquid water inside the protoplasts, and ice crystals and liquid water between the cells” (Olien 1964 cited in Skinner and Mackey 2009). For the best protection, it has been recommended that the upper layer of soil should be just moist but not saturated (Snyder and de Melo-Abreu 2005). More specifically, a water level of 25 mm for light sandy soils and 50 mm for heavy clay soils (Snyder and de Melo-Abreu 2005) to a depth of 30 cm were found to be adequate as the variation of diurnal temperature below 30 cm is insignificant (Snyder *et al.* 1987 cited in Snyder and de Melo-Abreu 2005).

2.14.5 Delving

Delving or the rolling of the soil which eventually break up clods and compacts the topsoil was found to improve the heat storage and transfer due to a decrease in soil porosity and increase in thermal conductivity and heat capacity (Snyder and de Melo-Abreu 2005). Sandy soils are known to have poor heat storage and thermal conductivity due to more and larger air spaces. Hence in frost prone areas with sandy soil types, delving and clay spreading are suggested as some of the alternative options in conjunction with other practices. In delving, specialized cultivation equipment with wide bladed tynes of 150 mm width, 480-720 mm depth and about 1.5m apart (Rebbeck *et al.* 2007c, 2007d) are used to bring up the deep clay subsoil to mix with the sandy topsoil. Such operations bring about 60-75 tonnes of clay per hectare to the top soil. The potential effects of delving operations are reduced albedo, increased water holding capacity and, in non wetting sand, increased hydraulic conductivity (Rebbeck *et al.* 2007d) resulting in enhanced heat storage and release during the frosty night that may attenuate frost damage.

Research in one of the most frost prone areas in SA by Rebbeck *et al.* (2007c, 2007d) found delving operation to be an effective management practice in reducing frost damage. They estimated the reduction of frost risk up to 80% due to the delving operation. As a consequence of the reduced frost damage, the wheat crop in the delved soil yielded significantly higher when compared to the untreated control soil. Further, the average difference in canopy height minimum temperature between delved and control soils were 0.3-0.4°C. This small difference in temperature was responsible for the higher yield in the delved treatment as small changes in the minimum temperature at the ear level will have a significant impact on the grain set in winter cereals such as wheat (Marcellos and Single 1984; Shroyer *et al.* 1995; Maes *et al.* 2001). Increased yield was also attributed to higher temperatures, improved availability of nutrients and water, reduction of soil mechanical impediment and change in soil colour in the delved soil.

2.14.6 Stubble retention

Minimizing the retention of stubble in the field, either through cutting, burning or slashing, was presumed to be desirable in reducing frost damage. This was because stubble materials such as straw and mulches act as an insulator thereby increasing the temperature below the stubble and preventing heat from escaping. Research in SA and WA reported colder air temperatures at head height between 0.38°C to 1°C (Rebbeck *et al.* 2007c) with stubble retention. Despite the differences in temperature, the findings were not consistent as Woodruff (1988) and Rebbeck *et al.* (2007c) concluded that the presence of stubbles did not appear to increase or decrease the frost damage irrespective of the method of stubble retention.

2.14.7 Manipulating Airflow through canopy

It has been suggested that use of blended varieties (mixing of two or more different varieties with different agronomic characteristics such as height and maturity) in the same frost prone paddocks will minimize frost damage. The difference in crop heights is expected to facilitate the airflow through the crop canopy and allow heat to rise from the soil to crop heads during frosty conditions. In a blending trial, Rebbeck *et al.* (2007c) noted a higher temperature of 0.13°C in the plot of the wheat variety Buckley, with a more open canopy, erect leaves and narrow flag leaf as compared to Tamaroi durum wheat which possessed a broader floppy flag leaf. However, researches in SA and WA have demonstrated that blended varieties may not be a useful strategy at a paddock scale but it may prove worthwhile at the whole farm level in reducing yield losses. Further, the wise strategy would be to sow different varieties in different paddocks with different flowering dates so that a single severe frost does not wipe out the entire crop.

2.14.8 Row Spacing

The other agronomic practice thought to contribute to minimizing frost damage is the adoption of wide row spacing and hence the low plant population densities. Wide row spacing may facilitate more airflow through crops thereby allowing the soil heat to rise up to the crop head height during a frost event. In addition, crops at low population density develop slowly as compared to high population density thereby minimizing the risk of exposure of reproductive apices to frost (Kirby and Faris 1970). Woodruff (1988) reported a reduction in the incidence of light frost at ear height with wider spacing due to more air turbulence and better drainage of cold air away from the crop. However, subsequent trials in SA and WA found no significant effect on reducing the frost damage by wide spacing despite receiving more heat during the day and early morning (Rebbeck *et al.* 2007c). In the United Kingdom, Whalley *et al.* (2004) also reported no role of plant population densities in frost damage. Instead it was determined more by sowing time with more frost damage with a low plant population when sown on 23 September and more damage with a high plant population when sown on 25 October.

2.15 Role of cold acclimation in frost tolerance

Cold acclimation is the exposure of plants for a certain period to low non-freezing temperatures before freezing and this was found to enhance frost tolerance (Levitt, 1980). It is also termed cold hardening. Cold acclimation of plants can be achieved by exposing the plants to temperatures in the range of 2-8°C (Hughes and Dunn 1996; Xin and Browse 2000), below 10°C (Gusta *et al.* 2005) or below 12°C (Guy 2003). However, induction of cold acclimation varies within and among species and some genotypes could develop cold tolerance even after exposure to warm temperatures of 15 to 17°C for 2 days (Fowler 2008). The acclimation period for rye is longer than wheat as rye initiates acclimation at much warmer temperatures (Săulescu and Braun, 2001). Marcellos (1977) reported that plants raised in the field are at least 2⁰ hardier than their glasshouse counterparts.

The increase in frost tolerance is brought about by the induction of genetic, physiological and morphological changes when exposed to low, non-freezing temperatures (Guy

1990; Thomashow 1999). The most prominent changes are the accumulation of cold regulated proteins such as *wcs120* (Sarhan *et al.* 1997) and different cryoprotectants such as sugars, sorbitol, polyol, proline, polyamines, glycinebetaine and quaternary ammonium compounds (Guy 1990; Close 1996; Vagujfalvi *et al.* 1999). The decrease in concentration of ice nucleation active bacteria, or an increase in solute content of the plant tissue, or both, were also related to cold acclimation and frost tolerance (Snyder and de Melo-Abreu 2005).

The length of exposure/hardening needed for maximum tolerance, and the tolerance level produced, varies between plant species, season and time of treatment. For example, tolerance is at the minimum level in spring and summer seasons while maximum tolerance is reached in winter (Guy 1990). Antikainen and Griffith (1997) reported that winter rye and wheat survived temperatures of -27°C and -20°C respectively after cold acclimation for 7 weeks at temperatures of $5/2^{\circ}\text{C}$ (day/night). A similar trend was also reported by Perras and Sarhan (1989) who found LT_{50} temperature of -19 to -16°C for hardened wheat plants (acclimated for 40 days at $6/2^{\circ}\text{C}$) and -6 to -3°C for unhardened control plants. Thomashow (1999) found that non acclimated rye was damaged at -5°C while the same rye could survive -30°C after cold acclimation for a certain period of time. This difference in tolerance was reasoned to be due to non-functioning of the protective proteins at warm temperatures/unhardened conditions (Rebbeck *et al.* 2007a). Further, exposure of plants to environmental conditions such as water stress, wind, and ultra violet rays also contributes to a small increase in freezing tolerance (Gusta *et al.* 2004).

However, cold acclimation is a highly complex process involving many biochemical and physiological changes (Hughes and Dunn 1996; Xin and Browse 2000). It is similar to the complex trait of freezing tolerance involving multigenetic inheritance (Thomashow 1999).

2.16 Mechanism of frost resistance/tolerance

2.16.1 Resistance due to glaucousness and pubescence

The presence of hydrophobic wax covering (glaucousness) in certain wheat varieties was found to induce less freezing injury than the uncovered varieties (Single and Marcellos 1974). Its positive role in yield contribution under drought conditions though efficient water use has already been demonstrated (Richards *et al.* 1986). The reason for this minimal freezing damage is due to the protective function of the hydrophobic surfaces of the lemma, palea and awn to prevent contact between atmospheric freezing nuclei and internal tissue moisture thereby avoiding external ice nucleation. However, later but limited experiments with glaucous and non glaucous varieties by Marcellos and Single (1984) showed little evidence of the role of glaucousness in frost tolerance. Further, the role of the awn in providing frost resistance has also been argued by the studies of Single (1988) where he found no protective barriers to ice formation between the awn, glume and ovum. Also, any differences in temperature between the awned and awnless wheat was not conclusive (Panozzo *et al.* 1999).

Trethowan *et al.* (1998), (cited in Maes *et al.* 2001), pointed out that wheat varieties with pubescent (covered with fine hairs) glumes were more tolerant to frost than the non pubescent ones. Research by Maes *et al.* (2001) also suggested that glume pubescence is one of the possible factors that will influence the damaging effects of frost as the temperature inside the florets of all the pubescent plants was higher than the non pubescent plants with a simultaneous delay in the time to reach damaging temperatures. The floret temperature of pubescent plants varied between 0.25 to 0.47°C when the floret temperature of non pubescent plants reached 0°C. Further, pubescent plants reached the freezing temperature of –3°C by 2.5 to 3.5 minutes later than their non pubescent counterparts. However, the role of glume pubescence as a physical barrier to ice formation was ruled out as super cooling was not observed in the varieties with pubescence during the experiment.

2.16.2 Resistance due to rachis and rachilla nodes

The rachis and rachilla nodes are thought to have a role in providing frost tolerance. It is suggested that they arrest the spread of ice crystals to the freezing boundary from the leaves and peduncle upwards to the reproductive organs (Single 1964; Single and Marcellos 1974). Subsequent research by Pearce and Fuller (2001) and Fuller *et al.* (2007) made similar observations. Pearce and Fuller (2001) noted an interruption of ice travel in the crown region of barley plants while Fuller *et al.* (2007) observed a limited number of damaged florets in the ears after a frost event, suggesting the rachilla acted as an effective barrier in preventing ice movement. The location of this barrier is thought to be in the tracheal terminal of the node (Single and Marcellos 1981).

The effectiveness of these barriers, however, depends on the freezing temperature level. Pearce and Fuller (2001) demonstrated that the delay of ice spread due to barriers was only for a few seconds when the temperature was below -2°C . On the other hand, the delay was prolonged or indefinite when the temperature was above -2°C . Therefore, it was suggested that barriers will be only effective in the mildest frost and will not necessarily reduce ice spread throughout the plant.

2.16.3 Resistance due to vernalization and vernalization genes

The another factor put forward in frost tolerance in winter cereals is the phenomenon of vernalization, that is the need of a sufficiently long cold period before the transition to reproductive growth (Kosova *et al.* 2008). Sutka (2001) utilised molecular studies to establish a close genetic linkage between vernalization and frost tolerance. Snape *et al.* (2001) reported that the genes responsible for determining vernalization are *Vrn* and in barley the three major genes responsible for vernalization requirement are located on chromosomes 4, 7 and 5 (Takahashi and Yasuda 1970 cited in Hughes and Dunn 1996) and on group 5 chromosomes in wheat (Fletcher 1988). Thus, late maturing varieties with higher vernalization requirements were found to possess more resistance to frost damage than the early maturing varieties and vice versa (Prasil *et al.* 2005).

The varieties that have vernalization requirement such as winter wheat possess greater frost tolerance than their counterparts such as spring wheat that do not need vernalization (Prasil *et al.* 2004). This results from the expression of the vernalizing genes for a longer time and at a higher level during the longer vegetative phase (Prasil *et al.* 2004). Wheat in autumn is exposed to low temperatures and hence frost tolerance is automatically induced whereas the rising temperature in spring leads to resumption of growth and development, and loss of frost tolerance. Mahfoozi *et al.* (2001a) also reported a decline of frost tolerance in wheat plants after the vegetative/reproductive transition. The vernalization requirement is temporary and once a wheat crop has satisfied its requirement, it not only losses its frost tolerance gradually but also its ability to re-establish a high level of frost tolerance. Photoperiod, which is also a major controlling mechanism for transition from a vegetative to reproductive stage, did not affect the vernalization requirement (Mahfoozi *et al.* 2001a). Vernalization effects on frost tolerance are effective only in the vegetative stage and not in the reproductive phase (Limin and Fowler 2006). It was also demonstrated by Fuller *et al.* (2007) that cereals such as wheat were unable to express the gene for acclimation after head initiation.

2.16.4 Resistance due to cryoprotectants

The accumulation of different compounds during cold acclimation with cryoprotectant properties has been suggested to increase frost tolerance (Guy 1990). These cryoprotectants provide freezing resistance by either preventing adhesions to the plasma membrane, by moderating ice growth during the freezing process (Reaney and Gusta 1999), or by increasing membrane stability during desiccation (Snyder and de Melo-Abreu 2005). The most commonly identified cryoprotectants are disaccharide and trisaccharide sugars, sorbitol, polyol, proline, polyamines, glycinebetaine and quaternary ammonium compounds (Guy 1990; Close 1996; Vagujfalvi *et al.* 1999).

Proline is one of the most notable metabolites that offers considerable frost tolerance. Its concentration was found to increase during cold hardening and was positively related with increased freezing tolerance in winter wheat and winter barley (Dorffling *et al.*

1997). Later investigations by Tantau *et al.* (2004) also found increased accumulations of proline during cold acclimation and a positive relationship between the amount of proline and frost tolerance by barley plants.

Different sugars such as glucose, sucrose and raffinose were also observed in the freezing tolerant plants and their contents were temperature dependent. Larsson *et al.* (1992) demonstrated that when wheat plants were cold acclimated at 2°C for 30 days, the level of these sugars almost doubled from 78 to 143 mg g⁻¹ dw with raffinose exhibiting a prominent increase. These sugars, however, declined rapidly on deacclimation at 20°C. Sasaki *et al.* (2001) also reported a decrease freezing tolerance in cabbage when there was low sucrose content in leaves.

While cryoprotectants such as sugars may contribute to some degree of frost tolerance, Zhong *et al.* (2008) argued that the wheat cultivar Jing 411 had a higher soluble sugar level but was less frost resistant. Thus, frost resistance is not simply related to soluble sugar levels. Moreover, the role of sugars as cryoprotectants is uncertain as most of the studies to date have sampled sugars in whole plant or plant organs such as leaves, roots or crowns (Livingston III and Tallury 2009). The death of the plants from freezing, however, can be a consequence of the death of specific regions or cells (Livingston III *et al.* 2006). Plants deploy multiple mechanisms to ensure freezing tolerance and soluble sugars alone are not sufficient for complete freezing tolerance (Xin and Browse 2000).

2.16.5 Resistance due to Cold Regulated Proteins

The ability of crops to develop frost tolerance is also related to the accumulation of the cold regulated (cor) proteins. Crosatti *et al.* (1996) observed the accumulation of two cor proteins namely cor14a and cor14b in winter and spring cultivars and hence a varying degree of cold tolerance between these two cultivars. Specifically, cor14b is one of the best characterized cold regulated genes in cereals (Crosatti *et al.* 2003) and is located in the stomata component of the chloroplast (Crosatti *et al.* 1999). Its expression is strictly regulated by cold (Cattivelli and Bartels 1990). Giorni *et al.* (1999) in barley studies also

noted higher amounts of cor14 in the winter cultivar Onice than in the spring variety Gitane with associated greater frost tolerance by the winter variety.

2.16.6 Resistance due to Antifreezing Proteins (AFPs)

The accumulation of AFPs by winter crops was also linked to the development of frost resistance. Hoshino *et al.* (1999) reported that plants that could overwinter produce two types of proteins, namely AFPs and cryoprotective proteins, and these two were closely related to frost resistance. Griffith *et al.* (1992, 2005) found these types of proteins in the apoplast of winter rye leaves and crowns. AFPs offer frost tolerance by binding to the surface of the ice crystals and thereby modifying ice crystal growth (Griffith *et al.* 1992; Hon *et al.* 1995), inhibiting intracellular ice growth (Hoshino *et al.* 1999) and controlling the site of ice formation (Griffith and Antikainen 1996). AFPs do not prevent freezing and have no specific antifreeze activity (Griffith *et al.* 2005) but merely lower the temperature at which ice is stable by 0.3°C (Griffith and Antikainen 1996). Its antifreeze activity, however, was observed only in the frost tolerant monocotyledons and was not directly related to the amount of proteins accumulated in the apoplast/leaves (Antikainen and Griffith 1997). These AFPs were found to increase their activities during cold acclimation while the weak activity of AFPs was noted in the non-acclimation stage (Griffith *et al.* 1992).

The accumulations of AFPs were also positively linked to other factors. Yu and Griffith (2001) reported induction of these proteins in the rye crop when plants were exposed to drought. In a similar way, the treatment of rye leaves with ethylene led to the accumulation of AFPs which invariably possessed antifreezing activity (Yu *et al.* 2001). Further, AFPs that were produced by cereals were similar to the pathogenesis related proteins (Hon *et al.* 1995; Pearce 2001; Yu and Griffith 2001).

Some reports suggest the engineering transfer of fish AFPs into different crops may assist in frost tolerance. Such inclusion has resulted in lowering the freezing temperature by 1.8°C (Cutler *et al.* 1989 cited in Pearce 2001). However, the results were contradictory as the incorporation of fish AFPs failed to improve the survival

attributable to antifreeze effects in transgenic plants such as tobacco (Kenward *et al.* 1999) and potato (Wallis *et al.* 1997). Literature on the transferal effects of such fish AFPs into cereals to date is lacking.

2.16.7 Resistance due to gases

The exposure of crop plants to specific atmospheric gases is also linked to the development of frost hardiness and susceptibility. Carbon dioxide was found to play a positive role in frost resistance. Veisz and Tischner (1995) reported that exposing cereals such as wheat and triticale to higher atmospheric carbon dioxide content had a positive effect on the hardening process and hence there was less frost damage to these plants than the controls. The carbon dioxide contents used in the experiment were $350 \mu\text{mol mol}^{-1}$ for the control treatment and $750 \mu\text{mol mol}^{-1}$ for the higher carbon dioxide content. As expected, the increase in carbon dioxide concentration led to an increase in leaf area. However, the elevated carbon dioxide had a deleterious effect during a frosting event with some tree species such as *Pinus sylvestris* (Repo *et al.* 1996), *Eucalyptus pauciflora* (Lutze *et al.* 1998) and *Ginkgo biloba* (Terry *et al.* 2000). It was established that higher carbon dioxide levels led to a decrease in stomatal conductance (Drake *et al.* 1997) which reduced transpiration and resulted in increased leaf temperatures due to a decrease in evaporative cooling (Siebke *et al.* 2002). Higher leaf temperatures under elevated carbon dioxide was noted to interfere with the cold acclimation process and predisposed the plant to more frost damage (Barker *et al.* 2005; Loveys *et al.* 2006). However, the results concerning the relationship between elevated carbon dioxide and freezing tolerance were inconsistent; this may have been influenced by many circumstances such as species-specific responses in terms of higher carbon dioxide, the mechanisms evolved by species to develop freezing tolerance and individual experimental conditions (Loveys *et al.* 2006).

Plants treated with ozone were found to be more susceptible to frost damage (Barnes and Davison 1988; Wolfenden and Wellburn 1991; Skarby *et al.* 1998). Ozone is a strong oxidant and membrane lipids, along with contents of chloroplasts are sensitive to oxidative damage (Silfver *et al.* 2008). Thus, Paakkonen *et al.* (1996) reported that

ozone decreased the chlorophyll components and photosynthetic rates in most of the tree species studied. The gas is also thought to react with proteins and fatty acid components of cell membranes, and enzymes that are responsible for solute transportation and osmoregulation (Heath 1980). Different experimental circumstances, including the use of gases that disrupt the physiological, anatomical and biochemical properties of cell membranes may be detrimental in relation to cold tolerance, as membrane dysfunction is a key determinant of low temperature injury (Levitt 1980).

2.16.8 Resistance due to ABA

The naturally occurring plant hormone ABA has been found to offer frost tolerance in plants such as wheat and rye (Chen and Gusta 1983) and potato (Chen *et al.* 1983). Four kinds of evidence were put forward to support the role of ABA in freezing tolerance. Firstly, there was an increase in the endogenous level of ABA when plants were exposed to low temperature (Daie and Campbell 1981; Chen *et al.* 1983). Secondly, ABA deficient plants failed to possess freezing tolerance which however was restored by adding ABA (Heino *et al.* 1990; Gilmour and Thomashow 1991). Thirdly, there was an increase in cold hardiness and freezing tolerance when plants were exogenously applied with ABA (Rikin *et al.* 1976). Applying ABA to the roots of rye, at non acclimating temperatures, increased freezing tolerance from -3 to -9°C after three days of exposure (Gusta *et al.* 2005). Fourthly, plants treated with ABA induce activity of all the cold regulated genes (Gilmour and Thomashow 1991) and the role of cold regulated genes in frost tolerance have been demonstrated (Giorni *et al.* 1999).

Chen and Gusta (1983) reported that in wheat and rye, ABA induces freezing tolerance extremely rapidly over a period of two to four days and the optimum ABA concentration needed in plants to induce freezing tolerance was in the range of 7.5×10^{-5} molar. Under such concentration conditions the LT_{50} values of wheat and rye were found to be -33°C and -31°C respectively. The concentration of ABA was found to be highest in soils which are acidic and with low moisture content, and lowest in moist, neutral and moderately alkaline soils (Hartung *et al.* 1996). Further, ABA concentration was found to increase during the vegetative period in maize fields (Hartung *et al.* 1996) and by cold

acclimation (Chen *et al.* 1983; Săulescu and Braun 2001). The greatest accumulation was found to be in the root tips (Hartung *et al.* 2002).

However, later studies in winter rye by Yu and Griffith (2001) and Griffith *et al.* (2005) found no significant role of ABA in providing crops with antifreezing activity. This was because ABA induced the accumulation of the apoplastic proteins that are immunologically similar to AFPs but without any anti-freezing property (Yu and Griffith 2001). In addition, ABA concentration may be another limiting factor for the development of freezing tolerance as a concentration of 7.5×10^{-5} molar is needed for effective hardiness (Chen and Gusta 1983).

2.17 Fructan in frost tolerance

Olien and Clark (1993) reported that variation in water soluble carbohydrates is also implicated in frost tolerance. They found that snow covered plants of barley, wheat and rye had a higher percentage of water soluble carbohydrates such as fructan while on the other hand, exposed plants (with no snow covered) had higher percentage of water soluble carbohydrates such as sucrose and fructose. Thus, it was believed that during freezing stress the proportion of fructan converted to sugar increases thereby resulting in fructan depletion. Vagujfalvi *et al.* (1999) also reported that wheat genotypes with higher contents of total water soluble and fructan contents had greater frost tolerance and these carbohydrates increased with the cold acclimation.

Fructan is found in almost every part of plant such as the stems, leaves, inflorescence and seeds. Five different types were described (Vijn and Smeekens 1999) of which wheat was reported to possess a branched (graminin) type (Bancal *et al.* 1992). Tognetti *et al.* (1990) found that when wheat plants were grown at 4°C for 17 days, both the levels of sucrose and fructose increased dramatically and these sugars were dominant in more cold tolerant cultivars. Suzuki and Nass (1988) also reported a similar trend of fructan accumulation with low temperatures, though they indicated that a low DP (less than 7) has less correlation with freezing resistance than higher DP (7 or higher) fructan in wheat, triticale and several rye varieties. DP is the process in which small molecules

called monomers combine each other to form a macromolecules called as polymers (Oadian 2004).

The presence of fructan may increase solute concentrations and hence freezing tolerance of plants as a high solute concentration depresses the freezing point of cell contents (Burke *et al.* 1976; Snyder and de Melo-Abreu 2005). Further, fructan may decrease the adhesive energy developed from competition for interfacial liquid between growing extracellular ice and hydrated plant surfaces (Olien 1974). It also stabilizes membranes during freezing and dessication stress (Hinch *et al.* 2000) by inserting part of the polysaccharide into the lipid head group region of the membrane (Livingston III and Tallury 2009). In addition, fructan is believed to act as a source of hexose sugars which could reduce the freezing point of liquids during freezing (Pontis 1989, cited in Valluru and Van den Ende 2008).

However, it was argued by Pollock (1984) and Cairns and Pollock (1988) that fructan accumulation with cold acclimation, and its role in freezing tolerance, are debatable as both the fructan synthesis and accumulation are governed by sucrose level, and not by low temperature. Thus, the association between fructan and abiotic stress tolerance is a highly complex phenomenon (Livingston III and Tallury 2009) and may depend on differences between the size and structure of fructan molecules, along with its localization in the whole plant (Livingston *et al.* 2006). Generally, fructan and sucrose, along with other sugars or carbohydrates vanish gradually with the onset of spring conditions and rising temperatures, leading to the loss of freezing tolerance in winter cereals (Stupnikova *et al.* 2002).

2.18 Role of nitrogen in frost tolerance

Inorganic fertilizers are inevitably required to furnish the essential nutrients for the optimum growth and development of the crop plants. The elements considered as major ones are nitrogen, phosphorus and potassium. While a deficiency of these nutrients has always been detrimental, their excess application, particularly nitrogen, has been found to be disadvantageous from the frost damage perspective.

Nitrogen is commonly applied to increase yield, but is increasingly being applied in later growth stages to increase grain protein levels (Rebbeck *et al.* 2007c). However, these practices of higher rates and later nitrogen application have been found to be detrimental as they predispose crops to more frost damage by increasing the freezing point of the plant tissue (Rebbeck *et al.* 2007c), increasing the crop biomass and diluting the concentration of the water soluble carbohydrates and other materials that otherwise act as an anti-freeze agent in the crops (Karow 1999 cited in Rebbeck *et al.* 2007c) and accelerating luxurious growth with high moisture content (Shroyer *et al.* 1995). Late applications of nitrogen also reduce the hardening of plants and encouraged new growth of plant parts (Gusta *et al.* 1982) with fewer solutes (Snyder and Melo-Abreu 2005). Ivory and Whiteman (1978) reported higher incidence of frost damage in two tropical grasses following higher nitrogen application.

A trial in WA with varying nitrogen fertilizers rates (nil: 32, low: 42, medium: 52 and high: 62 kg N/ha) and split applications (basal dose to all treatments and top dressings at Z31 and Z39 stages to low, medium and high treatments) showed significant frost damage with more distorted grains in the high and later applied treatments (Rebbeck *et al.* 2007c). The better grain quality in the low nitrogen treatments also resulted in higher profits as this grain was graded as general purpose, compared to feed in the higher nitrogen treatments. However, risk management has also to be assessed as there will be lower grain yield and protein in absence of a frost event. Thus, this strategy along with others may be used in areas where the frost risk is inevitable as lower nitrogen rates reduce the cost of production even if the frost occurs.

On the other hand, high concentrations of nitrogen (14-16 mg/g) was found to improve the frost hardiness in *Calluna vulgaris* (Caporn *et al.* 1994) and Scotspine seedlings (Rikala and Repo 1997). This was reasoned to be the result of stronger supercooling (i.e. delayed ice crystallization), more concentrated extracellular sap and hence higher osmotic pressure in the highest fertilizer treatment. Additionally, increased nitrogen supply could elevate the tissue concentration of compounds which are supposed to act as cryoprotectants of cellular membranes (Caporn *et al.* 1994) and reduce the rates of

photosynthesis and transpiration (L'Hirondelle *et al.* 1992). The decrease in photosynthesis and transpiration is suggested to trigger the plants into early cellular preparations for winter. However, this experiment was carried in the nursery stage (2 years old) which may not be representative of the whole plant lifecycle.

2.19 Role of potassium in frost tolerance

Potassium is one of the major elements that is required by a crop for its growth and development. It is acknowledged that potassium improves crop quality and protects the crops from biotic and abiotic stresses. It is also known to play a vital role in water regulation and photosynthesis in plants (Snyder and de Melo-Abreu 2005). Therefore, it is expected that an increase in potassium fertilization may lead to better photosynthesis and acclimatization as frost damage is the consequence of the dehydration of the protoplasm.

It is reported that increased application of potassium enhanced plant growth, photosynthetic rate, yield and increased drought resistance in crop such as Hibiscus under water stress conditions (Egilla *et al.* 2001). Therefore, the higher demand for potassium under water stressed conditions is related to the protective role of potassium against stress induced photo-oxidative damage (Kant and Kafkafi 2002). Simultaneously, plants that were fertilized with potassium possess higher leaf water potential, turgor potential and water content and lower osmotic potential than the unfertilized crops of wheat (Pier and Berkowitz 1987; Sen Gupta *et al.* 1989) and maize (Premachandra *et al.* 1991).

Apart from the aforementioned roles, potassium has been also reported to reduce the frost damage in tuberous crops such as potato (Grewal and Singh 1980) and to reduce spikelet sterility induced by low temperatures in rice (Haque 1988). It is possible that the effect of potassium in frost tolerance is related to an increase in phospholipids, membrane permeability and an improvement in biophysical and biochemical properties of cells (Kakerlerker *et al.* 1997 cited in Kant and Kafkafi 2002). Further, it is suggested that potassium regulates the osmotic and water potential of the cell sap and reduces the

electrolyte leakage caused by the freezing temperature (Singer *et al.* 1996, cited in Kant and Kafkafi 2002) and provides protection against oxidative damage caused by frost (Cakmak 2005). The increase in potassium supply also restricts nitrogen uptake leading to maintenance of a high K:N ratio which presumably reduces spikelet sterility in rice caused by low temperatures (Haque 1988). However, Ivory and Whiteman (1978) found no role of potassium in reducing the frost damage.

2.20 Evaluation of frost resistance/tolerance studies

In order to assess the differences in frost resistance/ tolerance that exist between cereal cultivars, different experimental methods such as field, field-laboratory and laboratory methods have been used (Prasil *et al.* 2007). Workers (e.g. Fuller and Legrice 1998) reported the use of an artificial frosting system where the frost is simulated. Zhong *et al.* (2008) regarded employment of such artificial chamber as the most reliable evaluation method as varieties with different lengths of growing season can be synchronized and treated at the same time. The temperature control under such laboratory conditions can also be precisely monitored and hence provide scope for investigating a range of temperatures (Pearce and Ashworth 1992). However, this method is constrained by being able to screen only a few varieties due to the large amount of work involved. Further, the freezing chambers were found to be unsuitable for screening for in-head frost resistance (Frederiks *et al.* 2008) and the method of cooling may set up a gradient in water potential between the cooling surface and the specimen, which may lead to dehydration of the specimen (Pearce and Ashworth 1992). In addition, the true effects of wind, topography, moisture on both the soil and the plant, the duration of freezing and other environmental conditions which occur in natural freezing conditions cannot be precisely simulated in controlled chambers (Paulsen and Heyne 1984).

On the other hand, the field evaluation under natural frost conditions can be a challenge due to the unpredictable nature of frost events, both in terms of timing and severity (Stoddard *et al.* 2006; Fuller *et al.* 2007). Screening varieties using natural frost has been also of limited success due to differences between varieties in their stage of development when the frost event occurs (Zhong *et al.* 2008). To obtain reliable

information on relative frost resistance/tolerance, crops have to be treated at the same developmental phase and same temperature (Zhong *et al.* 2008). Thus, it is of paramount importance to have an efficient, simple and rapid evaluation procedure in place for frosting trials. Equally important is the inclusion of field experiments to confirm the results developed in laboratory experiments (Pearce and Fuller 2001). For instance, Single (1988) found that one of the derivatives of *vulgare* type showed excellent freezing tolerance in the glasshouse but the behavior was variable when treated in the field. In a similar way, Reinheimer *et al.* (2004) reported three barley varieties (Amagi Nijo, Haruna Nijo and Franklin sib) as frost tolerant varieties in SA while Frederiks *et al.* (2005) found no tolerance when tested under Queensland conditions. The likely reasons attributed to these contrasting findings were the genotype by environment interactions, differences in nature of frost events in SA and Queensland and variations in the experimental methods.

2.21 Summary

This review has covered the importance of triticale and the effects of spring radiation frosting in winter cereals and the nature of frost effects. Additionally, the mechanisms of damage, various agronomic practices to mitigate the problem and plants' ability to avoid or minimize the damage have also been discussed.

It was obvious that the effect of radiation frost is of considerable importance both in Australia and overseas due to its direct and indirect effect on grain yields. In addition, all the stages of crop development are susceptible to frost with flowering being the most sensitive stage with significant yield penalties from booting onwards. Given this particular problem, the evaluation and selection of frost resistance in winter cereals has been the main priority of frost research in Australia.

Work has been commenced and/or in progress for the selection of frost resistant varieties of wheat and barley. However, little or no research has been done in evaluation of triticale varieties against frost. Thus, the following experiments reports the findings of

frost tolerance in triticale varieties compared with other winter cereals and other frost associated issues of two years, 2009 and 2010.

CHAPTER 3 GENERAL MATERIALS AND METHODS

3.1 Introduction

This chapter details about the procedures used throughout the experiments and will be similar to those reported in Chapters 4-6 unless specified. All the experiments were conducted at UNE, Armidale (30.51°S, 151.66°E; 1000 m a.s.l) in 2009 and 2010.

3.2 Plant materials

In 2009, 8 triticales (X *Triticosecale* Wittmack cv. Bogong, Tahara, H20, H151, H418, H426, JRCT 74 and JRCT 400), 2 bread wheats (*Triticum aestivum* L. cv. Kite and Ventura), 1 durum wheat (cv. Bellaroi) and 1 barley (*Hordeum vulgare* L. cv. Kaputar) were tested. The cereal lines/varieties were sourced from Agronomy and Soil Science, UNE or supplied by Jeremy Roake, University of Sydney.

In 2010, four triticales (Tahara, H20, H426 and JRCT 400) and three wheats (Kite, Young and Wyalkatchem) were evaluated. The triticales that showed some tolerance in 2009 experiment were selected for their further confirmation. In addition, two wheats from WA were included to observe the performance of their varieties used in their frost research program. Seeds of Young and Wyalkatchem were supplied by Ben Biddulph, Department of Agriculture and Food, WA.

In both the years Tahara was used as a standard line since it is still used as the variety for varietal comparisons and is still used as a commercial line in some locations. The brief characteristics of all the varieties/lines tested are given in Table 3.1.

Table 3.1 Characteristics of the different tested cereal lines/varieties.

Crop	Line/Variety	Characteristics	Reference
Triticale	Bogong (H127)	Grain variety, early to mid season flowering, fully awned, stiff strawed, good resistance to all rusts. Released by UNE, Armidale in 2008. CIMMYT line (102160) <i>Pedigree: ARDI_1/TOPO 1419//ERIZO_9/3/LIRON_1-1/4/FAHAD_4/FARAS_1</i>	Wheeler and Saunders (2009)
	Tahara	Mid season, fully awned, resistance to CCN and RLN & hence preferred variety in cereal rotations. CIMMYT line (unknown pedigree)	Wheeler and Saunders (2009)
	H20	High yielding line which performed very well near Spring Ridge and Liverpool Plains. CIMMYT line (33 ITSN-No.45).	
	H151	Early maturing, taller line with large heads. CIMMYT line (102160). <i>Pedigree: POLLMER_1.2//150.83/2*WALRUS_1/3/FAHAD_1</i>	
	Canobolas (H418)	Mid season awned variety. Tolerant to acid soil and resistant to stem and leaf rust. <i>Pedigree: DAHBI_6/3/ARDI_1/TOPO 1419//ERIZO_9</i>	McRae <i>et al.</i> (2009)
	H426	High yielding and early maturing line. CIMMYT line (102128). <i>Pedigree: POLLMER_1.1/3/FARAS_2//SIKA 26/HARE_337</i>	
	Berkshire (JRCT 74)	Main season and a high yielding variety. Moderately resistant to stem and stripe rust and resistant to leaf rust. <i>Pedigree: YOGUI_1/TAPIR//2*FARAS_1/3/ERIZO_11/YOGUI_3/5/ASAD*2/JUN//ANOAS_5/3/SONNI_6/4/ASAD/ELK 54//ERIZO_10</i>	McRae <i>et al.</i> (2009)
	JRCT 400	Possessing high starch but low fibre content. Has a high energy level for pigs. <i>Pedigree ARDI_1/TOPO 1419//ERIZO_9/4/GAUR_1/3/MUSX/LYNX//STIER_12-3/5/LASKO/IBEX//ERIZO_9/6/150.83/2*WALRUS_1:</i>	Roake <i>et al.</i> (2009)
Wheat	Ventura	Hard quality, high yielding, main season semi-dwarf spring wheat. Resistant to stem rust, leaf rust and moderately susceptible to stripe rust. Tolerant to acid soils.	McRae <i>et al.</i> (2009)

	Kite	Older hard quality frost resistant wheat (No longer recommended by NSW DPI).	
	Young	Hard quality and early to mid season variety. Tolerant to acid soils. Moderately resistant to stem and leaf rust and CCN.	McRae <i>et al.</i> (2009)
	Wyalkatchem	Large grain size variety. Possess acceptable dough properties and higher water absorption.	Cornish <i>et al.</i> (2002)
Durum wheat	Bellaroi	Variety with exceptional pasta making quality. Resistant to stem and leaf rust, stripe rust and resistant to yellow leaf spot. Resistant to common root rot but highly susceptible to crown rot.	McRae <i>et al.</i> (2009)
Barley	Kaputar	2-row white aleurone variety, short stiff straw and awns heavily anthocyanin pigmented. Resistant to leaf rust, powdery mildew, and covered smut.	Doyle and Johnston (1994)

3.3 Plant growth

Three seeds of each variety to be tested were sown into pots 7.5 x 7.5cm x 20 cm deep containing a grey vertosol. The characteristics of soil used are given in Table 3.2. A week after emergence, plants were thinned to one healthy plant per pot. Twelve pots of each variety were sown weekly over a five week period to generate a wide spread of flowering times. This facilitated the selection of varieties to be frosted at the most susceptible stage (flowering time) and to assess them at exactly the same developmental stage. Nitrogen was top dressed at the rate of 50 mg/kg of soil (50 kg N/ha) after a month of sowing. Other crop requirements such as irrigation and plant protection measures were provided as required for optimum plant growth. More importantly, plants were well irrigated prior to frosting as frost damage is worse in dry soils (GRDC 2009). Plants were placed randomly in the glasshouse compartment both before and after the frost treatments.

Plants were raised in the naturally lit glasshouse at the UNE green house complex with temperatures of 20/10⁰C (day/night). Tillers were tagged when the emergence of the top

spikelet on each head was observed, and the number of days to anthesis was monitored for each variety. Anthesis generally occurred 14 days after apical spikelet emergence for triticale, 10 days for wheat, and at emergence for barley. To ensure that barley was within ± 5 days, a few spare plants were monitored after the awn emergence before the actual trial. The flowering and apical spikelet emergence occurred after 5-7 days of awn emergence.

Table 3.2 Soil test results of Laureldale soil.

Nutrient	Measurement
Total C	2.35%
N	0.18%
pH	5.20
NO ₃ ⁻ - N	35.00 mg/kg
NH ₄ ⁺ - N	3.66 mg/kg
Colwell P	85.20 mg/kg

3.4 Frosting treatments

3.4.1 2009 Experiment

Since the main objective of this research was to evaluate the relative frost tolerance in different triticale varieties/lines and other winter cereals at flowering time, frost treatments were imposed when plants were at the flowering stage. Though winter cereals such as wheat usually complete flowering in about 4 days (Edwards and Roberts 2008), plants that were ± 5 days of anthesis were selected to get sufficient numbers of plants. Maes *et al.* (2001) also reported of selecting wheat plants at either anthesis or 5 days post anthesis in their freezing studies. The likelihood of frost occurrence in the locality was monitored through the Bureau of Meteorology website and frost treatments were decided when temperatures were forecast to fall below 0°C overnight.

Selected plants were taken to an open paddock in the evening at around 5 pm and spaced approximately 40cm apart to allow free air flow and avoid the influence of a thick crop canopy. The pots were buried to prevent freezing of the roots, and plants with weak

stems loosely tied to bamboo stakes to prevent lodging. Four Thermocron iButton DS1922L temperature loggers were tied to bamboo stakes and placed at 60 and 110cm above the ground that recorded temperatures at 10 minute intervals. Such placement of loggers at two heights was felt necessary as some varieties (mainly wheats and barley) were short statured while others (mainly triticales) were tall in height. For determining the floret exposure to frost, the minimum temperature at 110cm for the night was averaged. After overnight exposure, plants were returned to the glasshouse at around 9am and grown to maturity.

3.4.2 2010 Experiment

The frosting treatment in 2010 season was modified from the lessons learnt in the 2009 trial. Since the experiment was dealing with natural frost, it is unsurprising that the intensity of frost complicated the experimental results. In addition, the frost forecast from the Bureau of Meteorology did not match the actual frost occurrence. As a result of these unavoidable circumstances, many plants were completely killed in the 2009 season due to severe frost despite the forecast of desirable temperatures. Further, as the research aimed to evaluate frost tolerance at the flowering stage, plants had to be treated when they were at the flowering window (± 5 days). Therefore, the susceptible plants were exposed even during the severe frost and rescued once the desired temperature has attained. In this way, the heavy damage was avoided and frost temperature was maintained only for short time.

Plants were selected and placed in the open space in a similar way to 2009 season. To prevent freezing of roots and soil, pots were placed inside a bigger pot and surface was covered with potting mix as similar to Single (1966). As plant height can have a confounding effect, plant height was equalised by elevating the short statured plants (mostly wheats) so that the heads of all plants were at same height. Four Thermocron iButton DS1922L temperature loggers were placed at head height (110 cm) that recorded the temperatures at one minute intervals. The frost temperatures were monitored frequently by reading two data loggers alternatively. After the attainment of desired temperature, plants were transferred to a cool room with the temperature of

4.5⁰C to avoid fast thawing. Plants were finally transferred to the glasshouse the following day at 9 am and grown to maturity. Similar methods of maintaining momentary freezing temperatures were also reported in previous frosting research (Banath and Single 1976; Ivory and Whiteman 1978).

3.5 Data collection and statistical analysis

Different parameters such as plant height, date of first spike emergence and location of tillers were recorded once the crop attained maturity. The plants that were outside the flowering window were omitted from analysis for consistency. Frost damage was assessed in terms of ear fertility viz. number of grains set per spikelet, similar to measurements by other workers (Marcellos and Single 1984; Maes *et al.* 2001).

Data was analyzed by using data analysis software R (R Development Core Team 2010) using a generalized linear model with quasibinomial distribution. Quasibinomial distribution is useful where the variances of population are not equal and deviances are large (Crawley 2007). In smaller deviances and equal variances, binomial distribution was used. Main effects were considered to be temperature and genotypes and each individual plant was considered as a random replicate. Significant difference was calculated at the $P < 0.05$ probability level. The differences between means were separated by 95% confidence interval. It is a conservative comparisons method but provides information on both location and precision that can be used to make significance decision (Payton *et al.* 2003).

CHAPTER 4 EVALUATION OF TRITICALE VARIETIES AND OTHER WINTER CEREALS (WHEAT AND BARLEY)

“Frost tolerance at flowering in winter cereals is a complex trait and is far from being completely understood”- (Maes et al. 2001).

4.1 Introduction

Spring radiation frost is of significant importance in all the grain growing regions of Australia causing important yield and revenue losses to the national economy. The susceptibility of cereal crops to frost increases with the progression in its development and becomes most vulnerable during reproductive stage. The coincidence of spring frost occurrence and the crops' flowering stage has been unfortunate given the susceptibility of the crop at this stage. Further, frost also causes severe damage when it occurs between the booting to grain formation stage.

Given the economic loss caused by frost during flowering, the screening and nomination of in-head frost tolerant varieties in winter cereals has been the main focus of frost research in Australia. This expectation, however, is yet to be fully realized as evaluation of frost tolerance particularly at reproductive stage is complex (GRDC 2009). Notwithstanding with this constraint, research is continuing in WA, SA and Queensland to determine the frost tolerance of winter cereals during flowering (GRDC 2009).

While research on the relative frost tolerance in winter cereals such as wheat and barley have been documented, there is no information on frost tolerance in triticale. Additionally, a recent study (Roake *et al.* 2009) reported that 41% of the surveyed triticale producers mentioned unavailability of frost tolerant triticale varieties as the most serious problem in production and further expansion. As there has been no study on triticale frosting, farmers are unable to choose triticales tolerant to frosts at the flowering stage. Therefore, this study was aimed to assess the relative frost tolerance in different triticale cultivars and how triticale compared with other winter cereals, wheat and barley.

4.2 Materials and methods

4.2.1 Plant materials

Plants were grown and maintained as described in Chapter 3 (section 3.2 and 3.3).

4.2.2 Frosting treatments

Frosting treatments were imposed as explained in Chapter 3 (section 3.4.1 and 3.4.2).

4.2.3 Fructan and nitrogen analysis

In order to determine the varietal differences in fructan content, the mainstems (including leaves and head) were sampled during flowering for fructan analysis. Further, the effect of frost on protein content was studied by analyzing the grain protein with and without frost treatment.

The plant samples for fructan were collected by harvesting shoots above the soil. For grain protein analysis, the grains were collected from both frosted and control plants. The samples were dried in the oven at 40°C for 72 hrs, followed by grinding to 0.5 mm screen size. Fructan and nitrogen content were determined by NIR (Inframatic 8260) analysis by Futari Grain Technology services in Narrabri. The nitrogen analysis was calibrated at 2180, 2100 and 1680 nm wave lengths, while fructans were measured at 2310, 2100, 1818 and 1778 nm wave lengths. To obtain protein percentage, the nitrogen percent was multiplied by 6.25.

4.2.4 Effect of frost during grain filling

In 2010, there were some triticales and wheats which could not be treated during flowering due to absence of frost. However, frost occurred 2 weeks after flowering when the crop was at grain filling stage. In order to know the effect of frost during such post flowering stage, plants were exposed as described in Chapter 3 (section 3.4.1).

4.3 2009 RESULTS

4.3.1 Frost events

Plants were exposed to one of six frost events between June and August at the University of New England, Armidale, at an altitude of 1000m. Temperatures were measured at -6.6 , -4.2 , -3.5 , -1.9 , and -1.7°C , while the minimum temperature measured in the glasshouse for control treatment was 8°C (Appendix 1). The -6.6°C frost killed all plant shoots for both triticales and wheats, leading to complete sterility (data not presented).

4.3.2 Frost tolerance of triticales lines/varieties

Frost of -4.2°C significantly reduced grains set compared to the control and there was a significant effect between the varieties ($P < 0.05$). Variety H20 had the highest floret survival while Tahara, Bogong and H151 had the poorest survival rate (Figure 2). H418 suffered the most with no florets surviving.

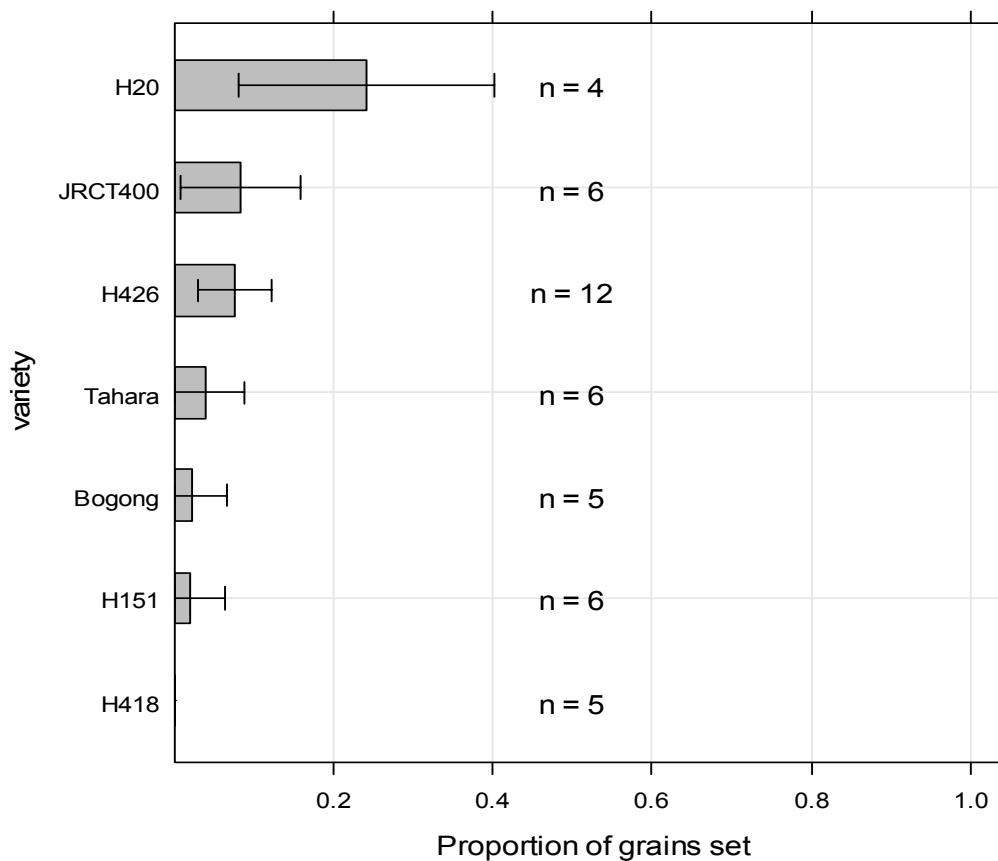


Figure 2. Proportion of grains set in different triticale varieties at -4.2°C (2009). Bars are the lower and upper 95% confidence interval. n is the number of heads frosted.

The -3.5°C frost also had a significant effect ($P < 0.05$) as compared to control on the grains set but there was no difference between the triticale genotypes ($P > 0.05$) (Figure 3). Unfortunately, Bogong and H151 could not be evaluated in the -3.5°C frost treatment as there were no plants at anthesis during that frost.

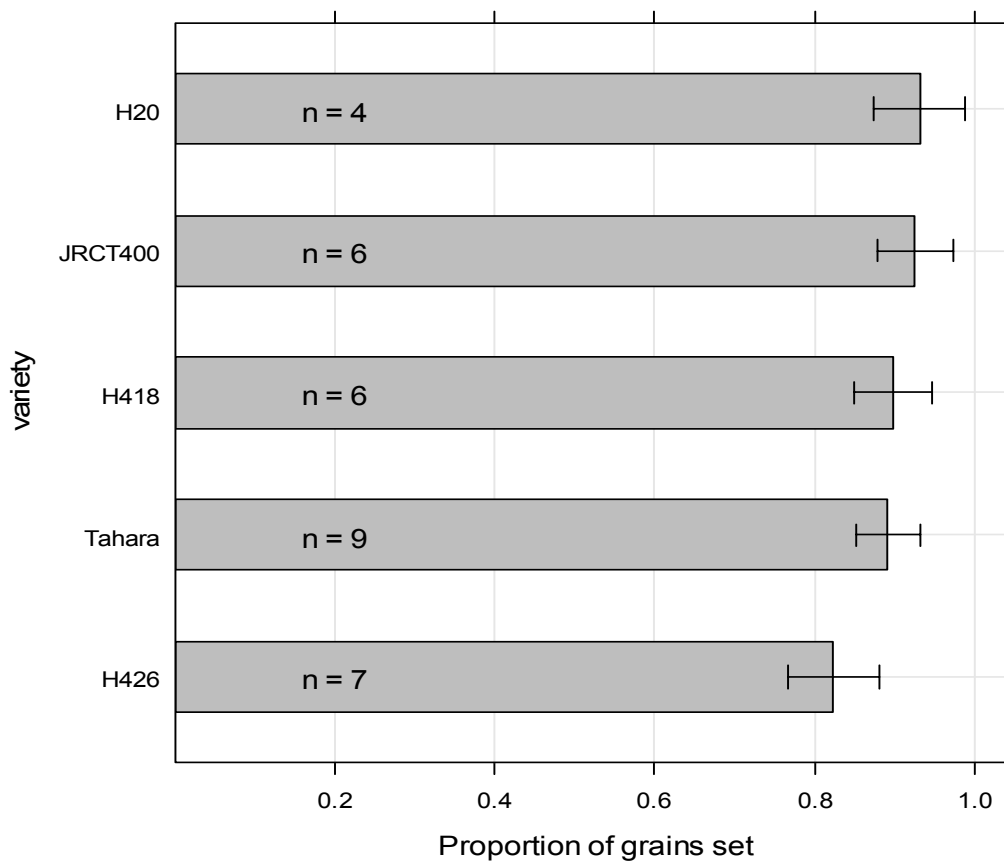


Figure 3. Proportion of grains set in different triticale varieties at -3.5°C (2009). Bars are the lower and upper 95% confidence interval. n is the number of heads frosted.

There was no significant effect ($P > 0.05$) of the -1.9°C (Figure 4) and -1.7°C (Figure 5) frosts on the floret survival. However, the varieties differed statistically ($P < 0.05$) at both temperatures. This significance may have been largely caused by the low ear fertility of JRCT 74 which seems inherently to have this character. Practically, this has less implication as the difference in grains set is small despite the statistical effect. Moreover, statistical significant effects do not guarantee practical significance as the magnitude of the effect is not provided by the P value (Utes and Heckard 2007).

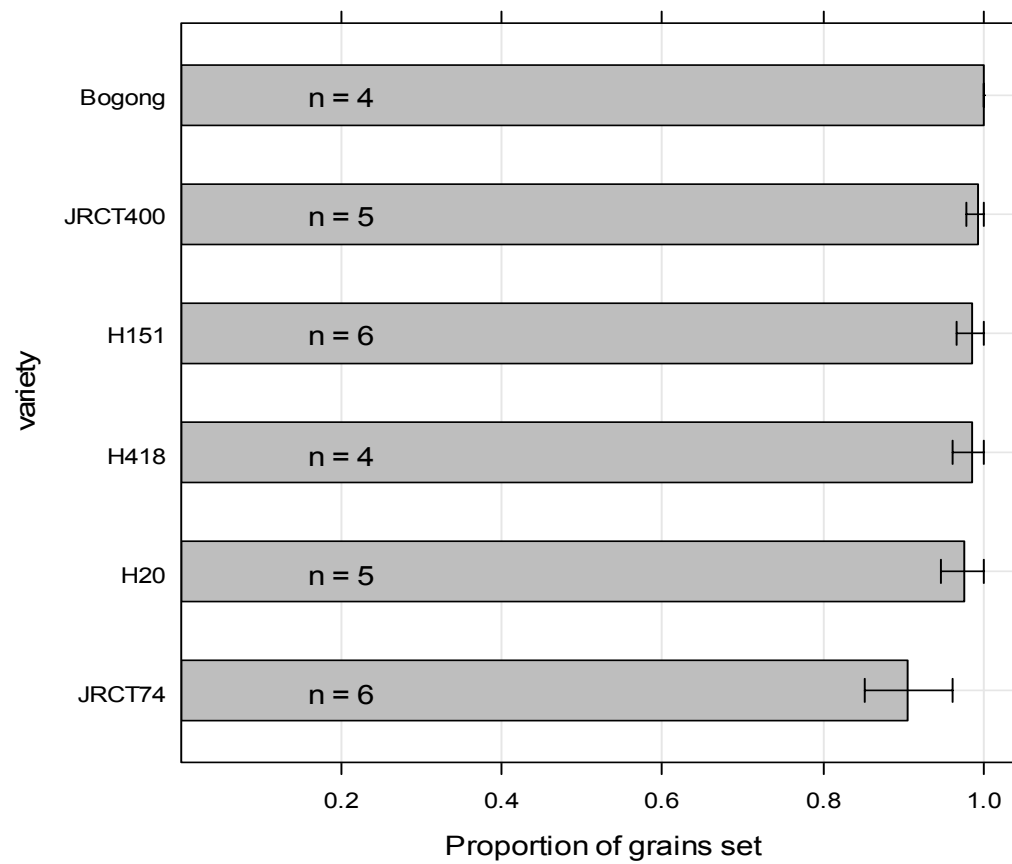


Figure 4. Proportion of grains set in different triticale varieties at -1.9°C (2009). Bars are the lower and upper 95% confidence interval. There is no bar in Bogong as the grains set was 100%. n is the number of heads frosted.

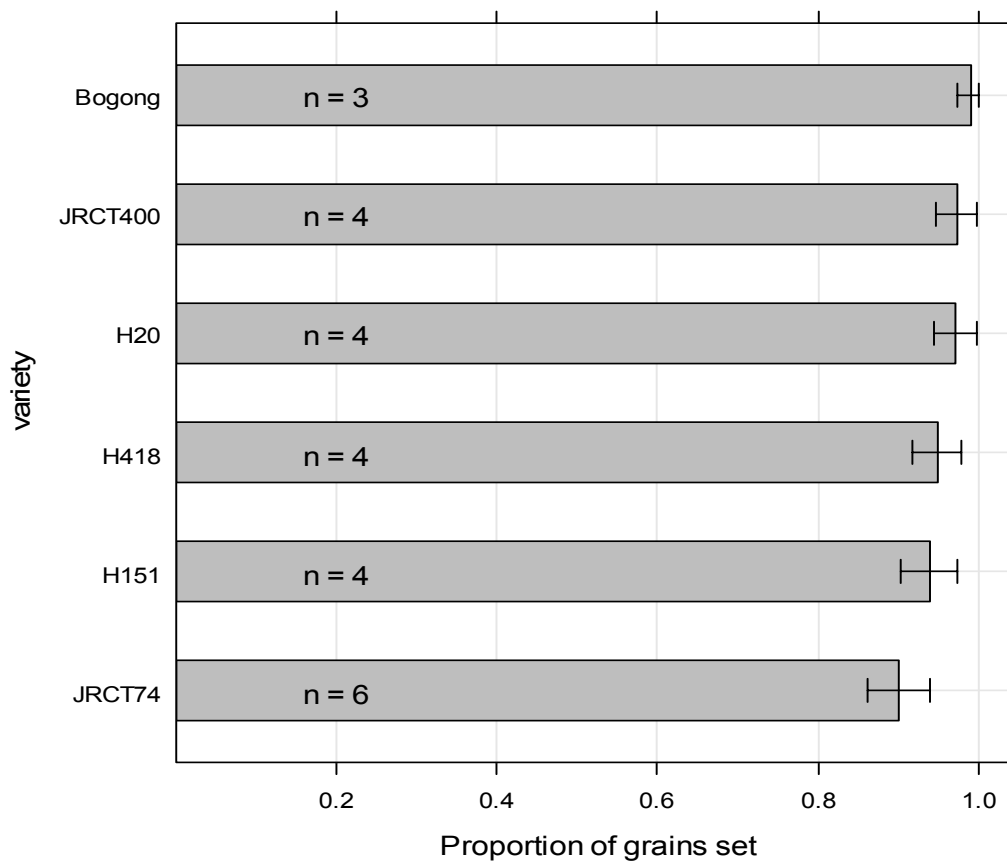


Figure 5. Proportion of grains set in different triticale varieties at -1.7°C (2009). Bars are the lower and upper 95% confidence interval. n is the number of heads frosted.

On analyzing the floret survival of control plants (8°C), the statistics showed significant effect between the varieties ($P < 0.05$). This must have been caused due to varying capacity in grains set among different varieties (Figure 6). Practically, the difference will have no major field impact as the grains set in all the varieties were $> 95\%$.

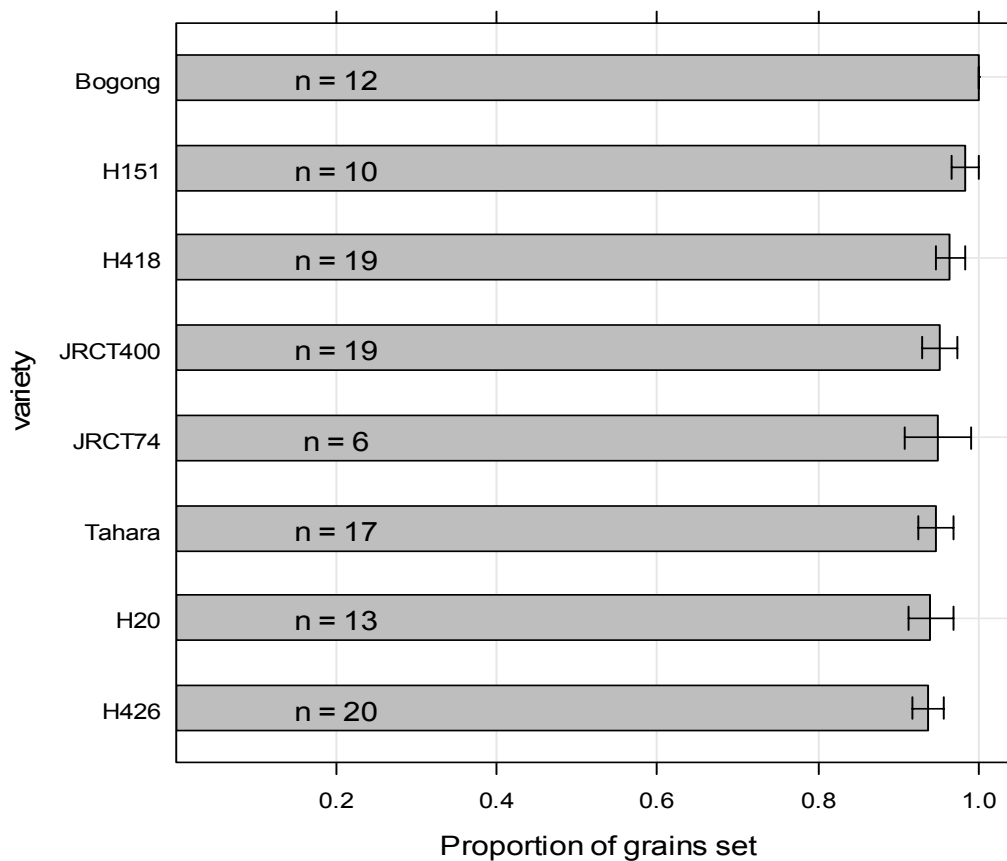


Figure 6. Proportion of grains set in different triticale varieties at 8°C (2009). Bars are the lower and upper 95% confidence interval. There is no bar in Bogong as the grains set was 100%. n is the number of heads frosted.

4.3.3 Frost tolerance of wheat

When compared to triticales, wheats suffered more at the –4.2°C frosting treatment. All the heads were completely dead with 100% sterility (data not analyzed and presented).

The frost of –3.5°C affected the grains set significantly ($P < 0.05$). Also both variety and height had a significant ($P < 0.05$) effect on grains set in the wheats, with better grains

set occurring in taller plants. At any height, Bellaroi had better grains set than Kite or Ventura (Figure 7). No significant effect of height was seen in the triticale varieties at any frost event.

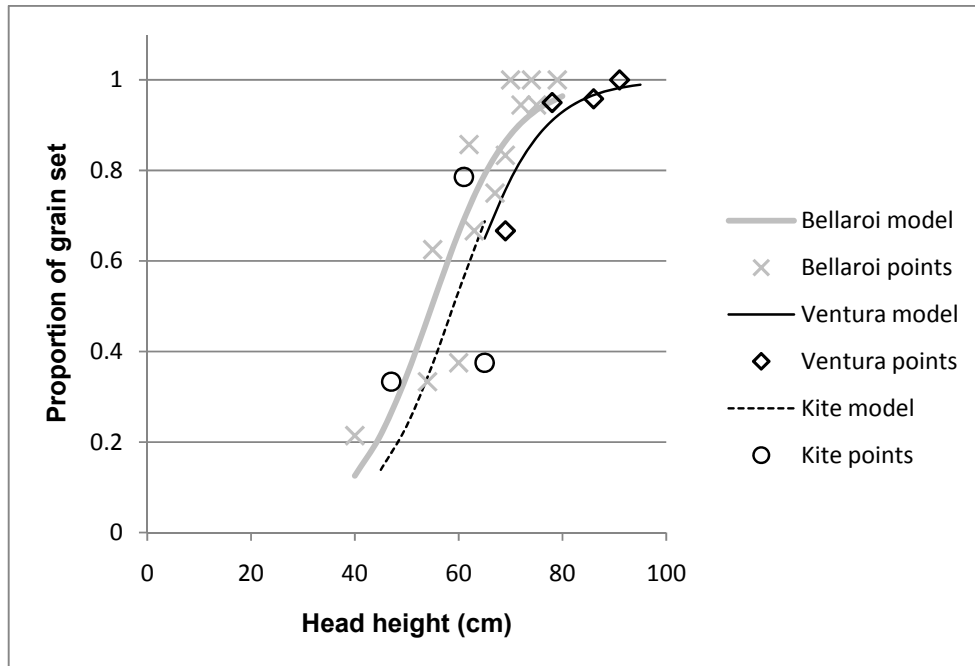


Figure 7. Actual and modelled grains set, and height in different wheat varieties at -3.5°C (2009).

Statistics showed no significant ($P > 0.05$) difference among the wheat varieties in grains set in control plants (8°C) (Figure 8). There was a tendency of lower grains set in some of the varieties such as Kite but the difference was very small.

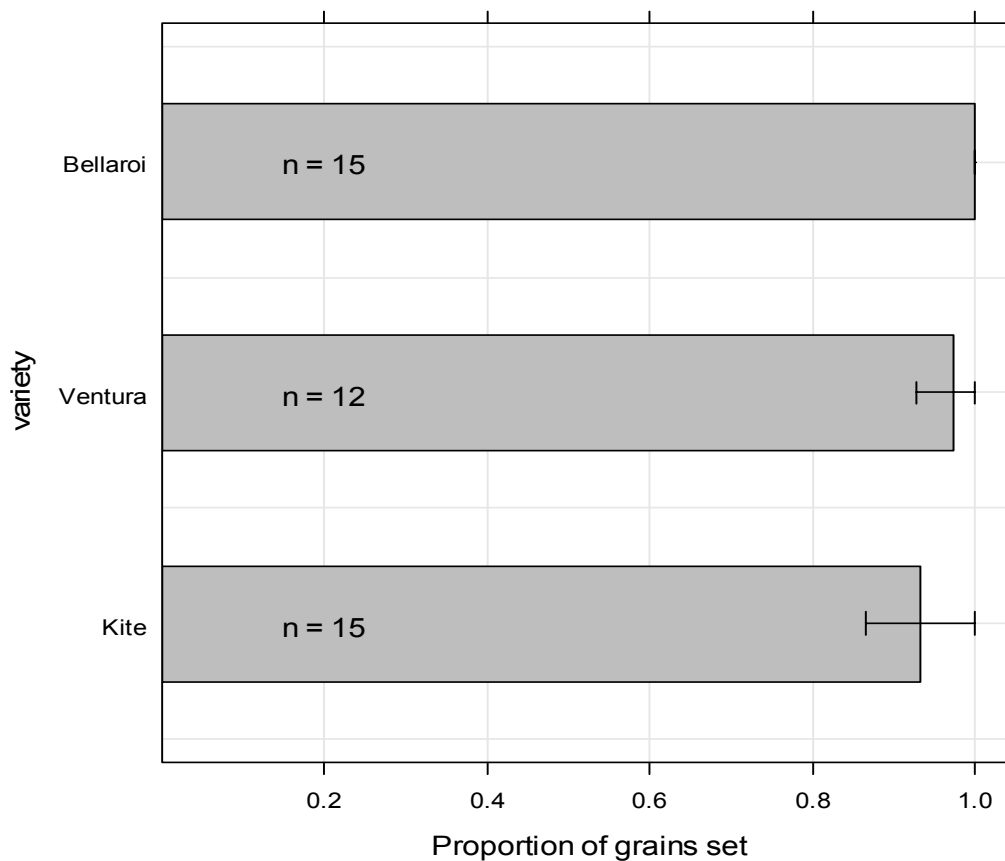


Figure 8. Proportion of grains set in different wheat varieties at 8°C (2009). Bars are the lower and upper 95% confidence interval. There is no bar in Bellaroi as the grains set was 100%. n is the number of heads frosted.

4.3.4 Frost tolerance of species

Statistical analysis found significant effects of both temperatures (−4.2°C and −3.5°C) and crop species ($P < 0.05$). At −4.2°C, there was little effect on barley floret survival while other two species, triticale and wheat, were severely affected (Figure 9). None of the wheat survived at this temperature. The −3.5°C frost also had an impact on the level of grains set but the damage was much less compared to the severe frost of −4.2°C (Figure 10).

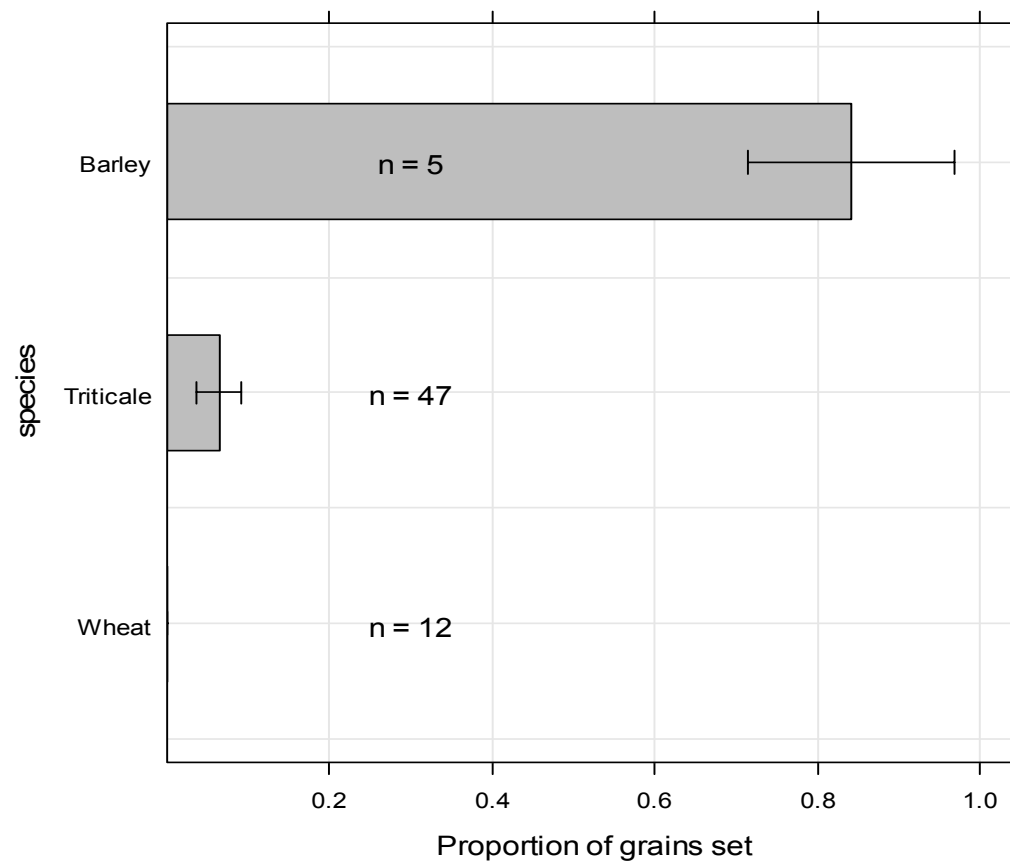


Figure 9. Proportion of grains set in different species at -4.2°C (2009). Bars are the lower and upper 95% confidence interval. n is the number of heads frosted.

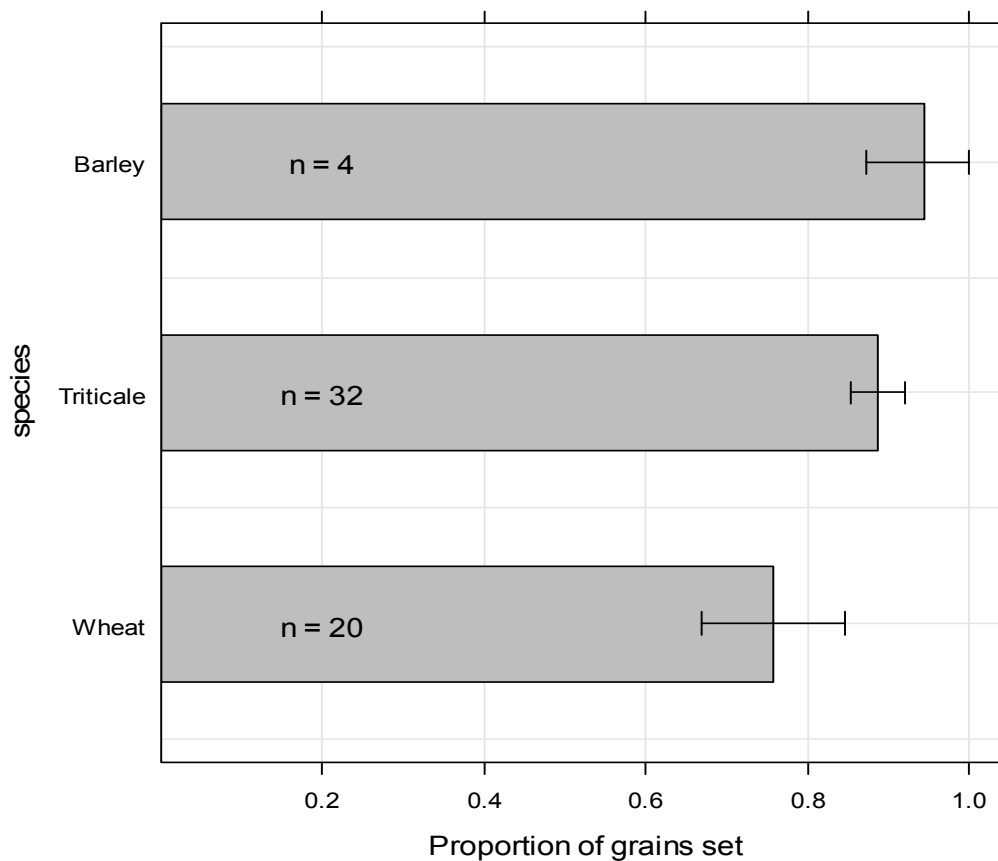


Figure 10. Proportion of grains set in different species at -3.5°C (2009). Bars are the lower and upper 95% confidence interval. n is the number of heads frosted.

4.4 2010 RESULTS

4.4.1 Frost events

Plants were treated to one of six frost events of varying intensities between mid June to late July. The frost temperatures measured were -6.5 , -4.5 , -3.9 , -3.5 , -2.8 and -2°C , while the minimum glasshouse temperature measured was 8°C (Appendix 1). As in 2009, the -6.5°C frost killed all the plant shoots overnight and hence resulted in 100% infertility (data not presented). Therefore, to avoid such fatality and to obtain discriminating temperatures, the technique was modified where plants were brought inside once the desired frost temperature had been attained. These expectations,

however, were not fully achieved as there were three nights (-3.5 , -2.8 and -2°C treatments) where temperatures did not go to lower temperatures despite monitoring for the whole night. Therefore, the application of modified method can be considered only for two treatments (-4.5 and -3.9°C).

4.4.2 Frost tolerance of triticale varieties

The analysis of variance at -4.5°C showed a significant effect of both temperature and varieties ($P < 0.05$). Frost greatly reduced the grains set for all the varieties to less than 50% (Figure 11). H426 had the highest grains set while Tahara yielded the lowest level of grain setting. H20 and JRCT 400 appeared intermediate in their frost tolerance.

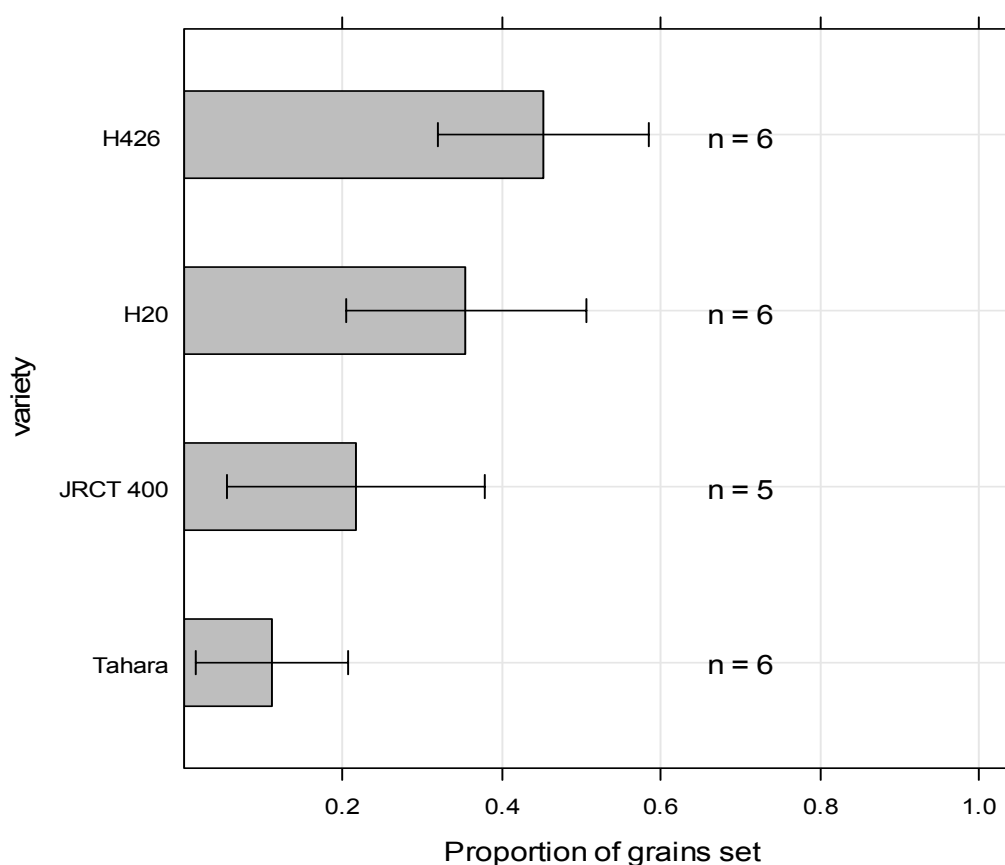


Figure 11. Proportion of grains set in different triticale varieties at -4.5°C (2010). Bars are the lower and upper 95% confidence interval. n is the number of heads frosted.

The frost treatment of -3.9°C had a significant effect of temperature ($P < 0.05$) compared to control, but there were no differences between varieties in floret survival ($P > 0.05$) (Figure 12). The non significant effect could be most likely due to small sample size and the large amount of variation with data.

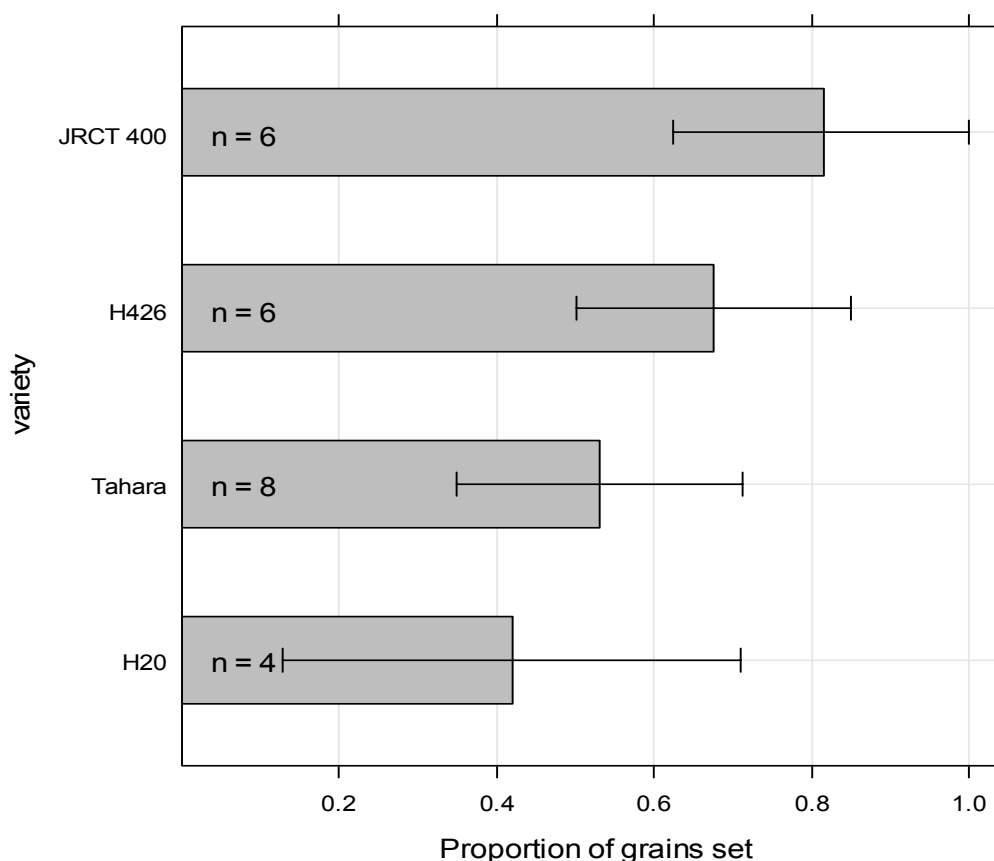


Figure 12. Proportion of grains set in different triticale varieties at -3.9°C (2010). Bars are the lower and upper 95% confidence interval. n is the number of heads frosted.

The statistical analysis found significant effects of -3.5°C temperature on the floret fertility ($P < 0.05$) compared to the control. However, different triticale genotypes did not differ statistically ($P > 0.05$) at this -3.5°C frost. All the varieties had more than 85%

floret survival at this temperature (Figure 13) indicating that no substantial yield losses would occur at this frost.

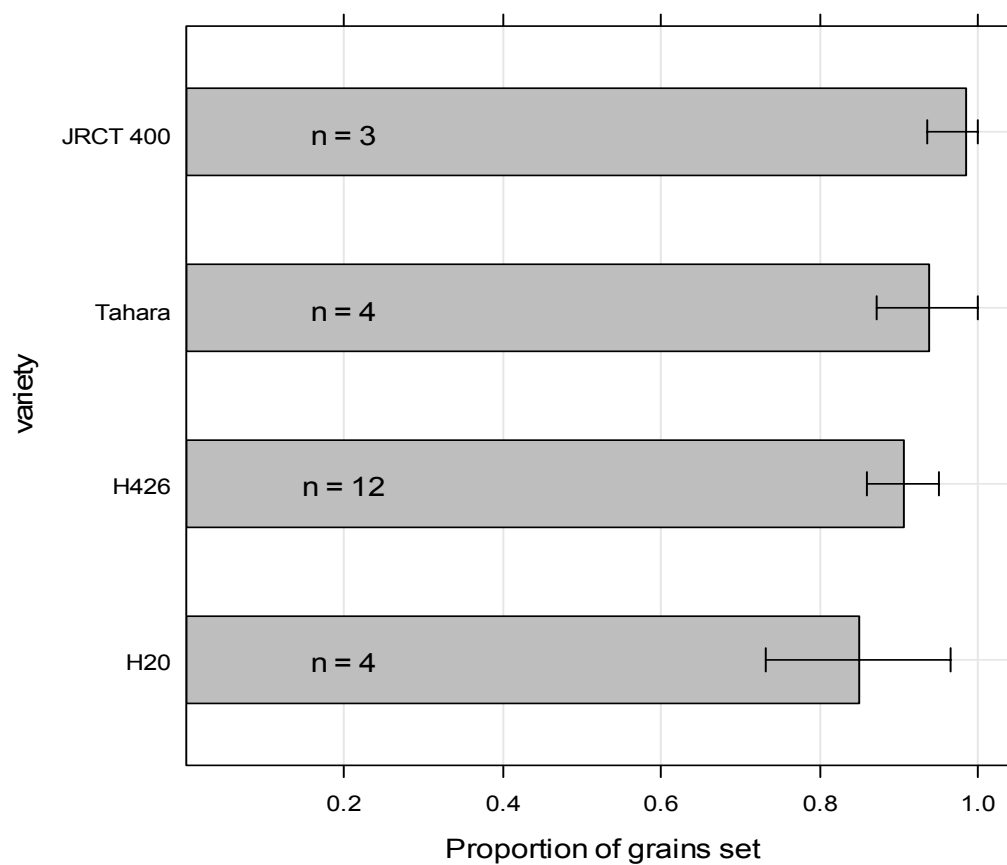


Figure 13. Proportion of grains set in different triticale varieties at -3.5°C (2010). Bars are the lower and upper 95% confidence interval. n is the number of heads frosted.

There was no significant difference ($P > 0.05$) between the unfrosted (8°C) triticale varieties. As seen in Figure 14, the grains set was above 95% in all varieties.

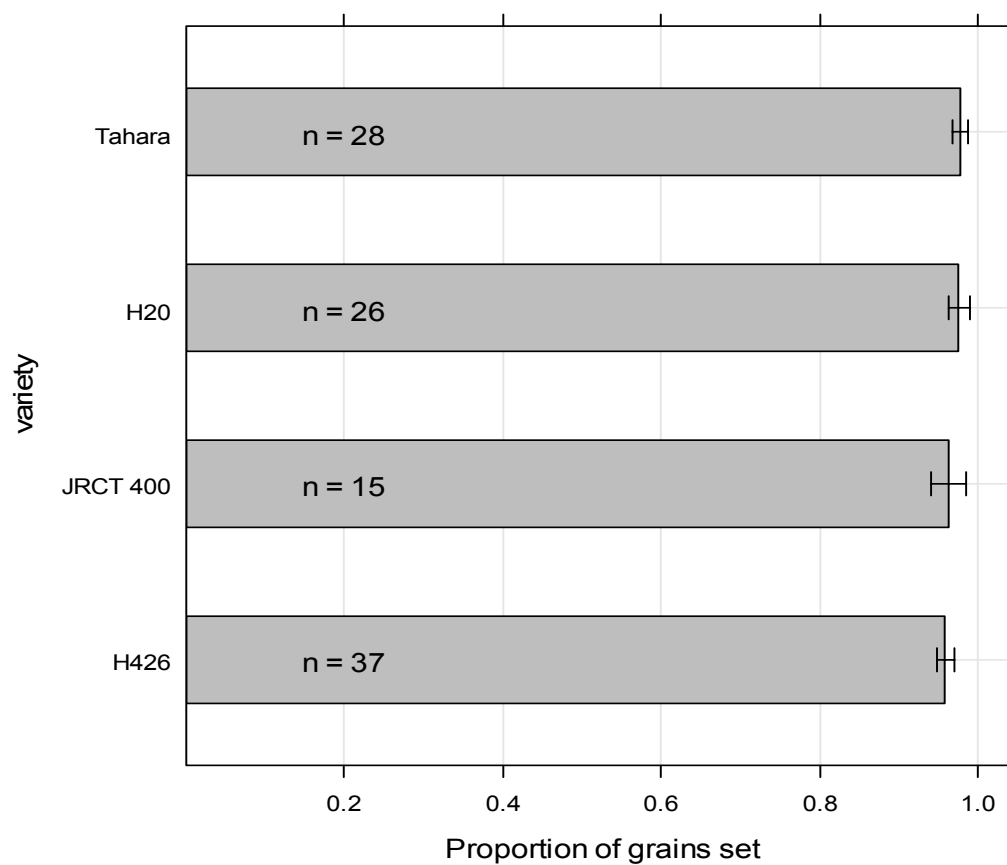


Figure 14. Proportion of grains set in different triticale varieties at 8°C (2010). Bars are the lower and upper 95% confidence interval. n is the number of heads.

4.4.3 Frost tolerance of different wheat varieties

The frost of -4.5°C caused significant damage to the floret fertility ($P < 0.05$) (Figure 15) compared to control. However, no significant differences in grain setting between the wheat varieties were observed at this temperature ($P > 0.05$), again associated with large error bars.

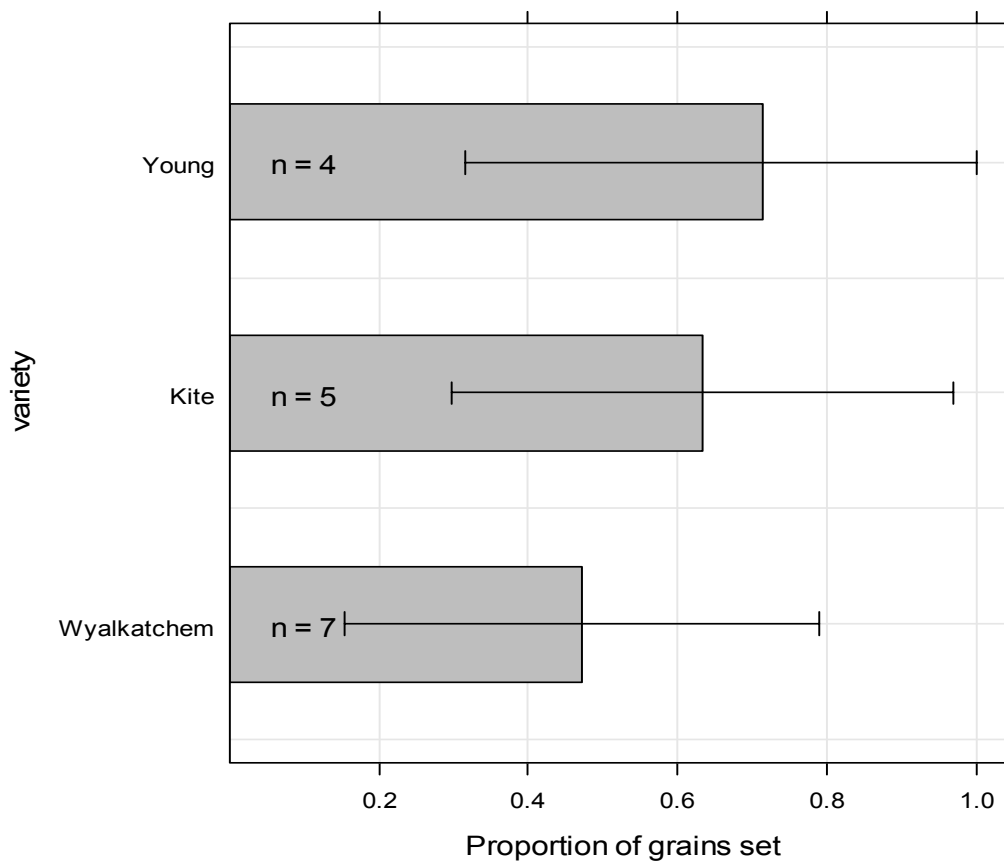


Figure 15. Proportion of grains set in different wheat varieties at -4.5°C (2010). Bars are the lower and upper 95% confidence interval. n is the number of heads frosted.

As in triticales, the frost temperature of -3.9°C had a significant effect on grains set ($P < 0.05$) but no significant difference among the wheat varieties ($P > 0.05$) (Figure 16). It is likely that the small sample size of Young as compared to other two varieties at this frost event could have affected the statistics.

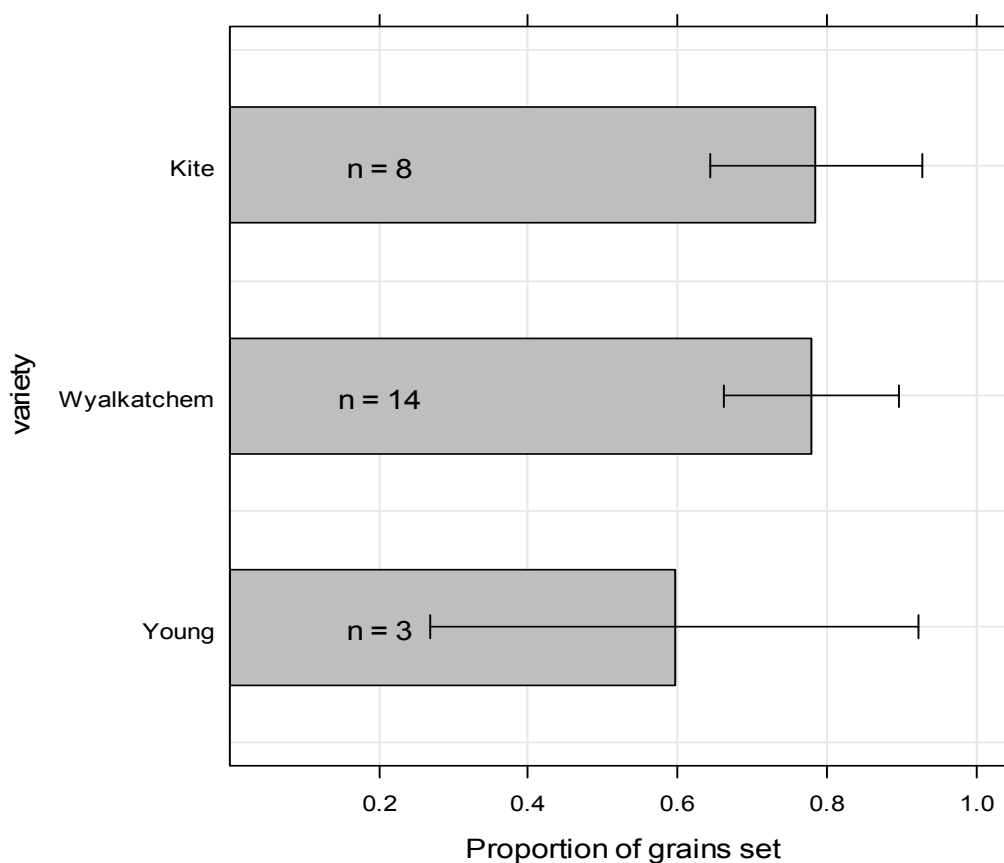


Figure 16. Proportion of grains set in different wheat varieties at -3.9°C (2010). Bars are the lower and upper 95% confidence interval. n is the number of heads frosted.

The mild sub zero temperature -2°C had no significant effects on the ear fertility ($P > 0.05$) in wheat (Figure 17).

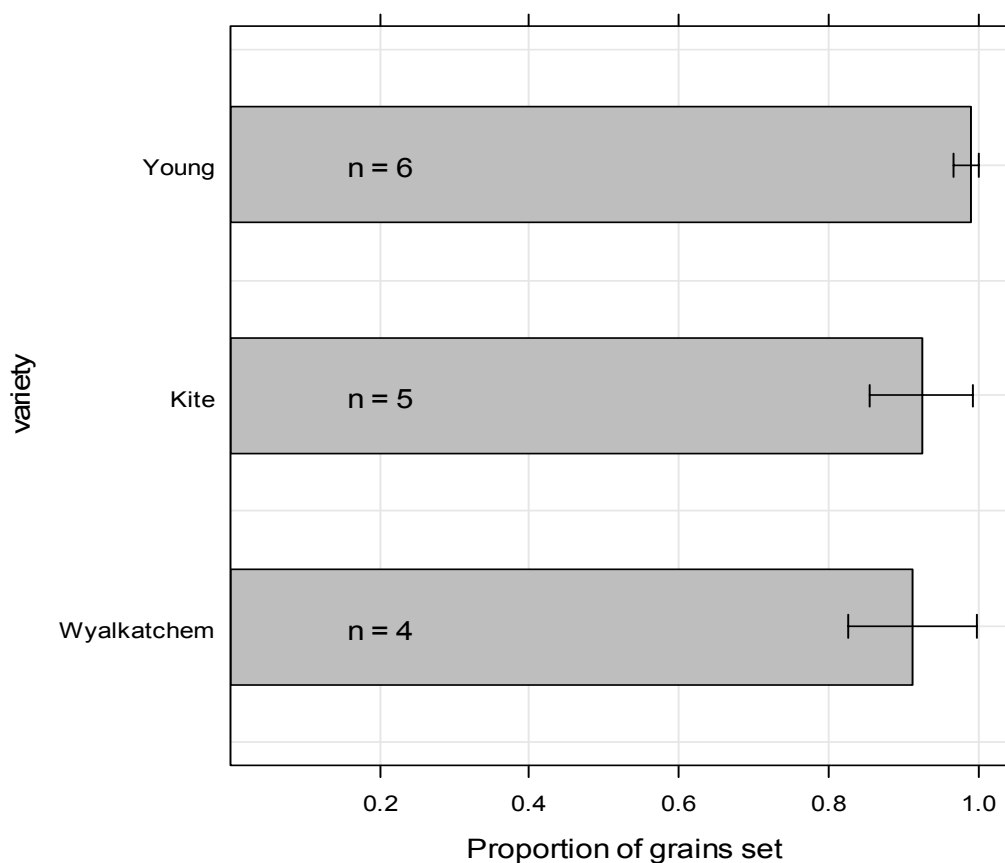


Figure 17. Proportion of grains set in different wheat varieties at -2°C (2010). Bars are the lower and upper 95% confidence interval. n is the number of heads frosted.

The analysis of floret survival found significant difference ($P < 0.05$) between the wheat varieties in control (8°C) treatment. The floret fertility of 100% in Young as compared to non 100% fertility in other two varieties must have influenced the statistics. However, as seen in the Figure 18, the differences are small, with all varieties having more than 95% grains set.

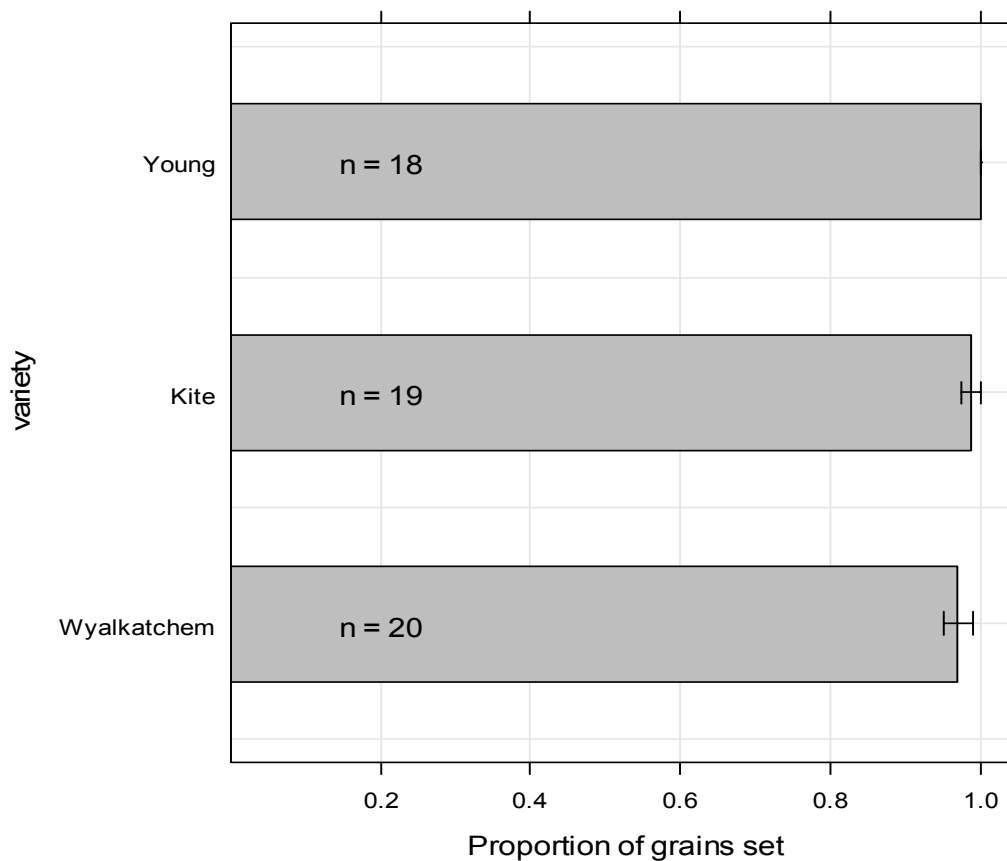


Figure 18. Proportion of grains set in different wheat varieties at 8°C (2010). Bars are the lower and upper 95% confidence interval. There is no bar in Young as the grains set was 100%. n is the number of heads frosted.

4.4.4 Species tolerance

The statistics found a significant impact of both -4.5°C frost temperature and species ($P < 0.05$) on floret survival. Triticales were more damaged than wheat, with the grains set in wheat being almost twice that of triticales at this frost (Figure 19).

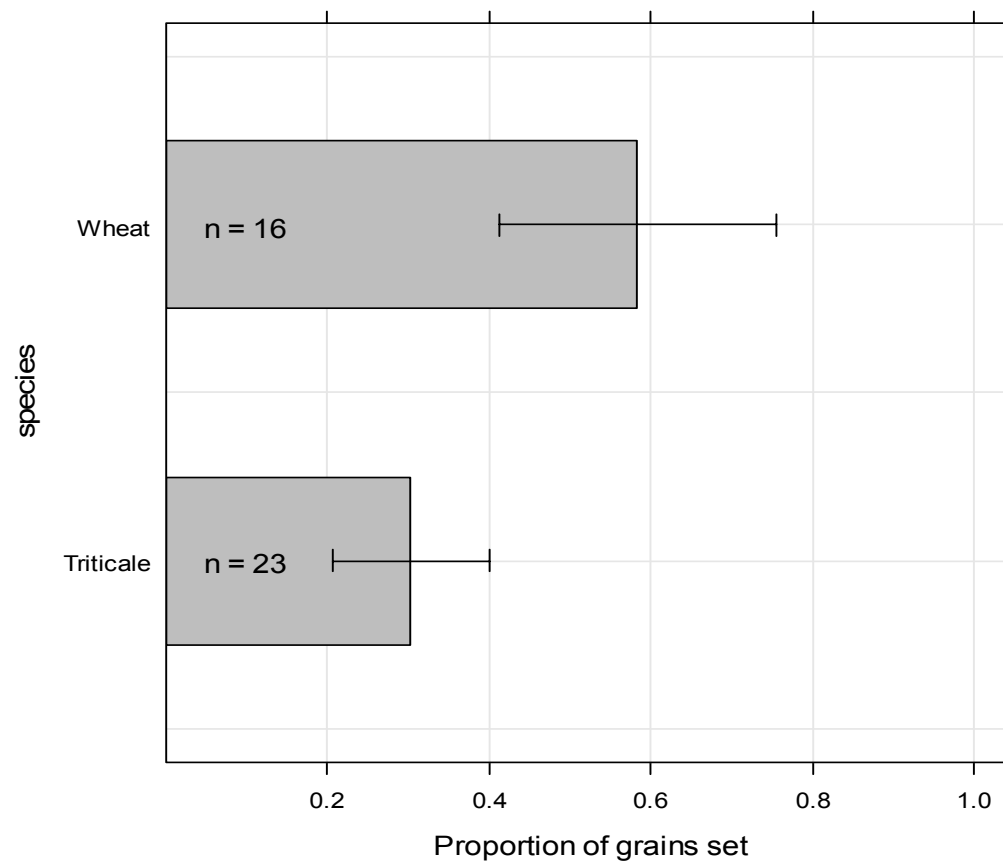


Figure 19. Proportion of grains set in triticale and wheat at -4.5°C (2010). Bars are the lower and upper 95% confidence interval. n is the number of heads frosted.

Grain setting in the two species was found to be significantly reduced by a frost temperature of -3.9°C ($P < 0.05$). However, the two species did not differ statistically ($P > 0.05$) (Figure 20). There was a suggestion that wheat continued to set more grain than triticale. The grains set in both the species at -3.9°C frost improved compared to the frost of -4.5°C .

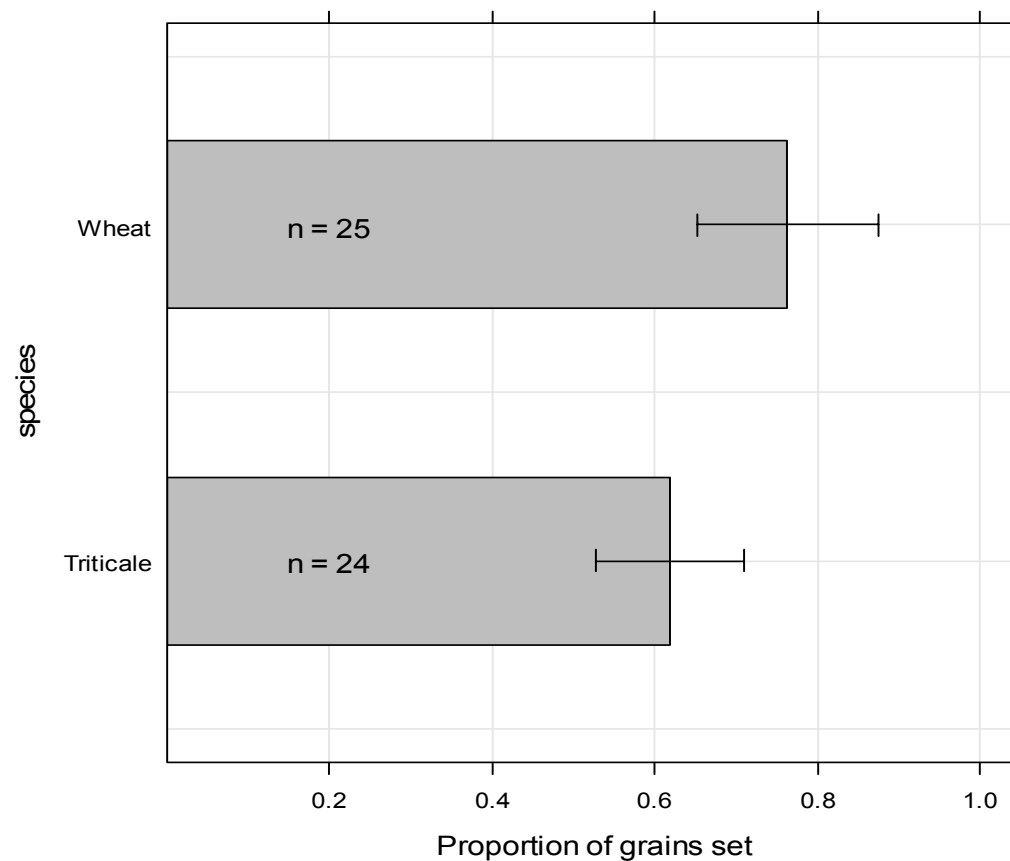


Figure 20. Proportion of grains set in triticale and wheat at -3.9°C (2010). Bars are the lower and upper 95% confidence interval. n is the number of heads frosted.

The milder sub zero temperatures of -2.8°C and -2°C had no significant effect on the floret survival ($P > 0.05$). Species also showed no significant difference ($P > 0.05$) at -2 (Figure 21) and -2.8°C (Figure 22) frost events.

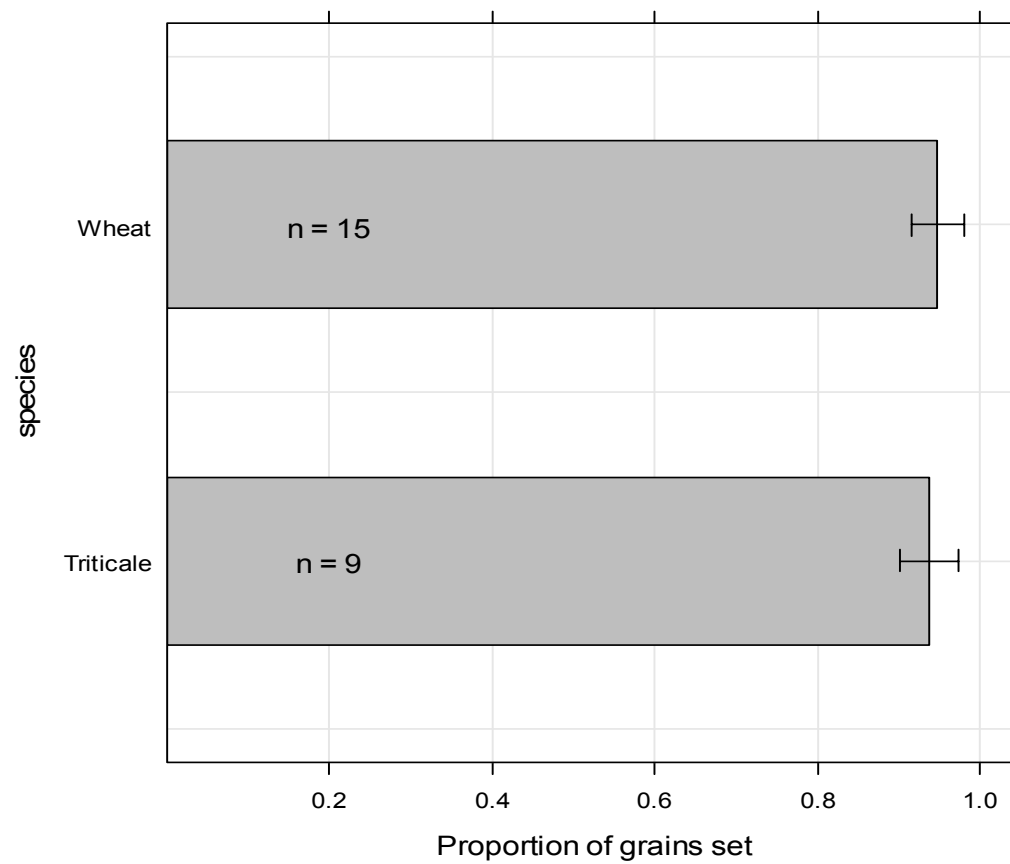


Figure 21. Proportion of grains set in triticale and wheat at -2°C (2010). Bars are the lower and upper 95% confidence interval. n is the number of heads frosted.

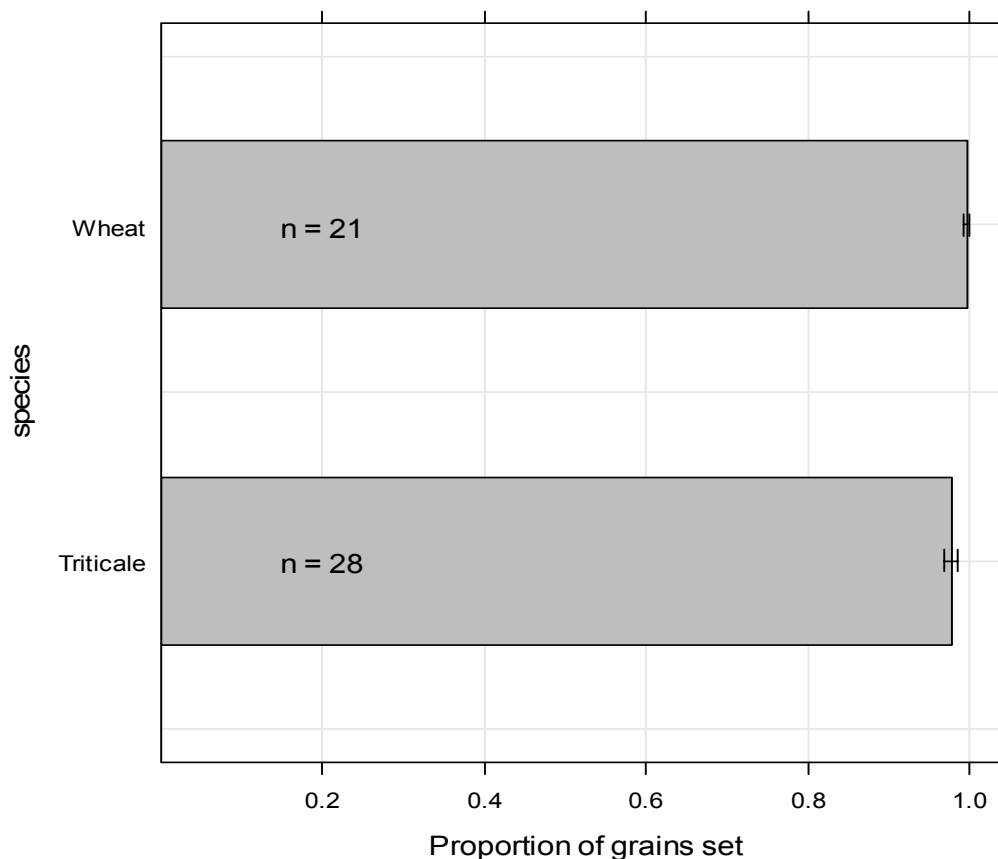


Figure 22. Proportion of grains set in triticale and wheat at -2.8°C (2010). Bars are the lower and upper 95% confidence interval. n is the number of heads frosted.

4.4.5 Frost damage symptoms

The lethal temperatures that caused the most obvious symptoms in the crop heads were -4.2°C and -4.5°C in 2009 and 2010 respectively. The patterns of damage caused by these two frost events were irregular or random in all the varieties. In the case of fertile spikelets, they were located individually or in groups at different positions along the rachis (Plates 1-10).

It was also observed that a heavy frost of -5.8°C 2 weeks after flowering (during grain filling) could seriously affect the filling of the grains making it unsuitable for commercial purposes. The frosted grains were shriveled and pinched, while the unfrosted grains

were normal in shape (Plates 11-12). As expected, the kernel weight of the frosted grains was reduced compared to unfrosted grains (Table 4.1).



Plate 1. H426 at -4.2°C (2009).



Plate 2. H20 at -4.2°C (2009).



Plate 3. JRCT 400 at -4.2°C (2009).



Plate 4. Tahara at -4.2°C (2009).



Plate 5. H426 at -4.5°C (2010).



Plate 6. H20 at -4.5°C (2010).

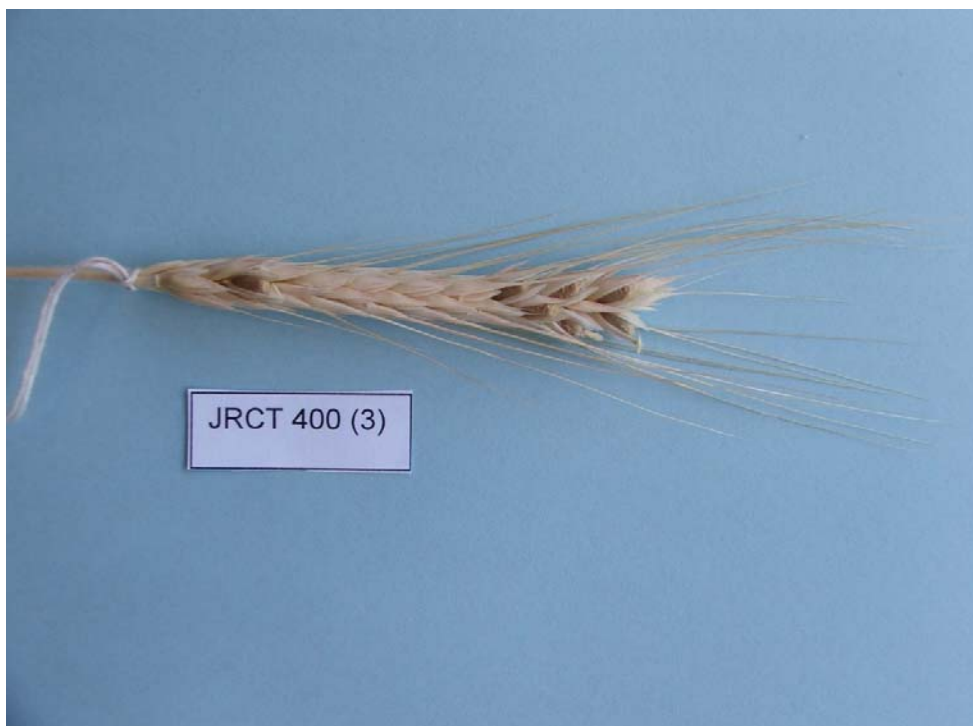


Plate 7. JRCT 400 at -4.5°C (2010).



Plate 8. Tahara at -3.9°C (2010).



Plate 9. Wyalkatchem at -4.5°C (2010).



Plate 10. Young at -3.9°C (2010).



Plate 11. Grain shape of unfrosted and frosted H20 (triticale) at -5.8°C (2010).



Plate 12. Grain shape of unfrosted and frosted Young (wheat) at -5.8°C (2010).

4.4.6 Fructan and protein analysis

There was no difference in fructan content between the varieties, or between the hardening treatments (Table 4.1). In all the varieties/treatment, the fructan content was negative, suggesting that the actual amount of fructan in the plants was very low and below the range the NIR analysis is calibrated for.

On the other hand, there was a suggestion of higher protein levels in frosted samples than unfrosted grains. JRCT 400 and Tahara were not frosted at grain filling stage due to unavailability of plants.

Table 4.1 Fructan, protein content and 100 seed weights in different varieties/treatment.

Variety/Treatment	Fructan (%)	Protein (%)		100 seed weight (g)	
		Unfrosted	Frosted	Unfrosted	Frosted
H20	−7.8	10.0	14.8	6.2	1.3
Kite	−7.0	11.5	13.0	5.5	1.6
H426	−5.5	8.9	12.7	5.8	1.5
Wyalkatchem	−5.0	11.8	10.2	4.7	2.7
JRCT 400	−4.7	12.0	-	-	-
Tahara	−4.4	9.7	-	-	-
Young	−2.0	11.2	13.0	4.0	2.0
H426 Hardened	−5.4				
H426 Non-hardened	−4.3				
<i>P</i> value	> 0.05				

Data for fructan is average of 3 plants, and protein content and 100 seed weight from a single plant of each variety.

4.5 DISCUSSION

4.5.1 Tolerance of triticales varieties

The identification of winter cereals/varieties with putative reproductive frost tolerance has been the main priority of Australian frost research, and will remain a much desired outcome in the near future (GRDC 2009). This is because frosts at reproductive stages of growth cause the most extensive damage by nucleating the ice on floral parts, thereby affecting the grain development (Single and Marcellos 1974; Cromey *et al.* 1998). Thus, winter cereals with a greater frost tolerance would have a great impact on

cereal production in Australia (Frederiks *et al.* 2008). The work presented here suggests that there may be some varietal differences in frost tolerance as the tested varieties differ in ear fertility, particularly at frost temperatures below -3.9°C .

There were changes in the relative position of frost tolerance of triticales except Tahara between the years at frost temperatures especially below -4°C . The main contributing factors to these alterations are not completely understood but difference in experimental procedures, especially the frost treatments, could be one of the effects. In 2009, the plants were kept in the field overnight, while for 2010 treatment the minimum temperature was only achieved momentarily, and plants were rescued thereafter. The duration of frosting has adverse effects with more damage from prolonged treatment (Livingston and Swinbank 1950; Crome *et al.* 1998) which could have happened in 2009 through overnight exposure. The data from -4.2°C frost in 2009 and -4.5°C in 2010 support this possibility, with more damage occurring in 2009 even though the temperature was warmer. Another factor could be photo bleaching, which is reported to injure plants in the morning after a natural frost episode (Gusta *et al.* 2009). This may have contributed in 2009 as plants were returned to the glasshouse at 9 am in the morning, although bleaching of plants was not observed. Collectively, the data suggests that there may be some variations in the frost tolerance in commercial triticales cultivars given the variation in grains set in 2009. This observation was confirmed in 2010 indicating that Tahara may be not the best option in frost prone areas that experience frost temperatures of less than -4°C as it suffered the most in both years. The relative frost tolerance of the remaining triticales could not be determined as they varied markedly between the two years.

4.5.2 Tolerance of wheat varieties

The existence of differing levels of frost tolerance in wheats has been reported (Snyder and Melo-Abreu 2005). However, the present work mostly found no differences between varieties as all the wheats were completely damaged at -4.2°C in the 2009 season. Bellaroi was found to be more frost tolerant at the -3.5°C frost in 2009, but head height was also found to be significant in this event, which could confound the results. While

Bellaroi had plants covering a range of heights, the small number of plants of Kite and Ventura frosted were limited in their range of heights.

Plant heights are considered to be one of the confounding factors in frost research given the differences in temperature profiles at varying heights during the radiation frost (Reinheimer *et al.* 2004). Wheat plants experienced cooler temperatures by 0.1 to 0.4°C compared to triticales in these two frost events in 2009 due to their shorter heights. Such changes may seem minimal but keeping the temperature even 0.1°C above the critical point can have a great impact on avoiding frost damage (GRDC undated). This problem was avoided in 2010 by ensuring all heads were at a similar height. The observation of cooler temperatures at lower heights in the present study is contrary to what is experienced practically in the field, as lowest temperatures are encountered at crop head height during the radiative frost event (Single 1988; Panozzo *et al.* 1999) due to the insulating effects of the crop canopy. Marcellos and Single (1975) in a field study reported that surface of the crop was colder by as much as 2°C than in the middle region of the canopy. As the effect of thick canopy was intentionally avoided by spacing the pots widely, the cooler temperatures at lower heights would have been inevitable in present study.

It would be not appropriate to compare the relative frost tolerance of wheat varieties for both years as the tested varieties were different in each year except for Kite. Varieties in 2009 were included to assess the differences between bread and durum wheats, while the varieties used in 2010 were the standards of the WA frost research program. However, the overall performance of wheats including Kite were remarkable in 2010 given that all the varieties had more than 50% grains set even at severe frost temperatures of -4.5°C (Figure 15). It is highly possible that the modified technique in 2010 would have contributed to this difference. The exposure of plants for short duration may have been not enough to cause substantial damage as frost damage is also related to duration of exposure (Livingston and Swinbank 1950).

4.5.3 Species tolerance

The nomination of the most frost tolerant winter cereal species has been a subject of confusion and debate given the difference in experimental procedures used by different workers, and overlap among species. However, the present study found barley is the most tolerant crop among the winter cereal species and varieties studied. This concurs with the findings of other publications (Rebbeck *et al.* 2007b: GRDC undated) whose nomination for the most frost tolerant species was barley compared to wheat and triticale. The result is, however, contradictory to that of Synder and de Melo-Abreu (2005) who suggested that triticale was more tolerant than barley.

It is highly likely that the flowering habit of barley crop must be one of the contributing factors in possessing greater frost tolerance. The crop was flowering when it was still in the booting stage, unlike triticales and wheat. GRDC (2009) also reasoned that barley's tolerance to frost is because of its distinguishing characteristic of flowering in boot, which provides some additional protection. Such protective measures would be desirable as small alterations in minimum temperature at ear level affect the grains set considerably in winter cereals (Maes *et al.* 2001).

The susceptibility of triticale to frost was considered as one of the limiting factors in the expansion of triticale in Australia (Roake *et al.* 2009). Thus, knowing the relative tolerance particularly between wheats and triticales would have been a great outcome from this study. However, the 2009 data was confounded by height differences and can not be used to compare the species. In contrast, wheats performed better than triticales in the 2010 season when the heights were equalized. The 2010 finding suggests that wheat is more tolerant than triticale though data is not confirmative over years. Therefore, more data and trials would be needed to confirm this. Limin and Fowler (1991) also reported that triticales are no better than wheats in cold hardiness as its expression of cold-hardiness genes transferred from rye is suppressed by wheat genomes.

4.5.4 Frost damage symptoms

The symptoms of frost damage depend strongly on the crop developmental stages and frost intensity (Single and Marcellos 1974; Shroyer *et al.* 1995; Rebbeck *et al.* 2007b). The damage in present study was found to be in a random/irregular fashion, similar to that documented by other workers (Single and Marcellos 1974; Marcellos and Single 1984; Whalley *et al.* 2004). This randomness must have been influenced by the timing of frost and stage of flowering as winter cereals start flowering from the middle of the spike and progress towards the top and bottom of the spikes (Edwards and Roberts 2008). Cromeey *et al.* (1998) reported that frost at early flowering resulted in sterility at the centre of the spike, while frost at later flowering led to sterility at the top and bottom of the spikes. Further, the position of ears within the crop inducing differential exposure to cold temperatures, and varying levels of insulation on different ear sides during freezing events, have also been suggested as having an influence in the field (Whalley *et al.* 2004). Alternatively, there may have been some mechanism such as barriers within the rachis, which prevented ice nucleation in these regions.

4.5.5 LT₅₀ values/damaging temperatures

There is a common belief that winter cereals experiencing even a minor sub-zero temperature during flowering will be always detrimentally affected (Shroyer *et al.* 1995). However, the present study documented no such significant damage even if the crops were at the most sensitive flowering stage. The ear fertility of different species that experienced -2°C and warmer frost temperatures had more than 95% of the florets survive (Figure 5), which is comparable to the non frosted plants. This minimal damage may have been due to warmer temperatures influencing the rate of ice propagation within the plant. For instance, Pearce and Fuller (2001) reported that ice travel in barley was much slower in warmer frost temperatures (-1.5 to -2°C) than in severe frosts (below -2.9°C). Similar observations of higher rates of ice propagation at lower temperatures were also noted in alpine cushion plants (Hacker *et al.* 2010). Further, Marcellos (1977) demonstrated that plants suffering from mild frost events had the capacity to resume photosynthesis normally by the following day, while severely stressed plants failed to recover.

The nomination of a critical temperature either at species or variety level has been a challenge given the inconsistency results from both controlled and field frost experiments. Thus, a temperature range of -3 to -5°C has been considered generally as the lethal temperature for winter cereals, especially at the time of flowering (Single and Marcellos 1974; Single 1988; Woodruff 1988). However, the degree of injury is highly sensitive to temperature and there can be severe damage to an extent of complete ear sterility when the temperature drops even 1°C below its threshold (Marcellos and Single 1984). The current study noted that the temperatures below -3.9°C could be lethal for winter cereals, particularly wheat and triticale, as there was substantial reduction in the grain set. Single and Marcellos (1974) also documented -3.9°C as the damaging frost for winter cereals at flowering stage. Some damage could also be expected from other frost temperatures such as -3.5°C , but the yield loss would not be as severe. The damage from temperatures of -3.9°C and below could be due to ice nucleation, with Hacker *et al.* (2010) reporting initiation of ice nucleation around -4°C during the frost.

Fuller *et al.* (2007) reported that winter cereals such as unacclimated wheat possessed frost resistance inherently down to -5°C at reproductive stage. However, present data showed no such resistance in wheat, given that all the varieties succumbed to complete infertility in 2009, and the best performing variety had only 70% of its florets surviving in 2010 at -4.5°C frost (Figure 15). While there may be multiple factors influencing the results, one could be the growth environment with Marcellos (1977) reporting field plants to be a least 2° harder than their glasshouse counterparts.

Several morphological traits such as glaucousness and glume pubescence have been implicated in offering frost tolerance (Single and Marcellos 1974; Maes *et al.* 2001). In a similar way, the involvement of carbohydrates such as fructans has been suggested (Vaguifalvi *et al.* 1999; Hinch *et al.* 2000; Livingston III *et al.* 2009). In the present study, no distinguishing morphological traits such as glaucousness or glume pubescence were observed in any of the tested varieties. Further, the fructan analysis showed no significant differences between varieties and in fact, the fructan content of all varieties were negative. Negative numbers (Table 4.1) are likely to be because the

values were beyond the calibrated range of the standard curve, but suggest that fructan levels were very low. Low values may be the result of environmental conditions (adequate nitrogen and water, and reduced light in glasshouse) which resulted in good plant growth and no fructan accumulation. Similar negative values were seen in field grown plants in spring 2010, where high nitrogen levels and frequent rain led to rapid growth (Brenda Rose pers. comm. 2010). Reduced accumulation of fructan due to adequate irrigation (Goggin and Setter 2004), and adequate nitrogen (Wang and Tillberg 1996) is well documented in winter cereals. This result suggests difference in fructan levels had no influence on frost tolerance in this trial, but does not exclude it having a role in the field. In the future work, it would be highly desirable to measure fructan in multiple NIRs to cross check the results, and if possible deploy other chemical tests such as High Performance Liquid Chromatography method of Huynh *et al.* (2008), which is claimed to be a reliable method.

Frost at grain filling stage also has the implication through reduction of grain weight and affecting the quality characteristics. In the current study too, there was a weight reduction and suggestion of increased protein production in frosted grains. Similar observation of higher protein content in frosted grains was also reported by Richardson *et al.* (2001), though Preston *et al.* (1991) found decreased protein content when frost occurs at this stage. Such significant frost damage may downgrade the grains to feed grade as they may not be suitable for milling.

CHAPTER 5 ROLE OF COLD HARDENING IN FROST TOLERANCE

“Cold acclimation is very complex, involving many biochemical and physiological changes” (Xin and Browse 2000).

5.1 Introduction

The exposure of plants for a certain period to low non-freezing temperatures has been reported to increase frost tolerance (Hughes and Dunn 1996; Guy 2003). During this exposure, many biochemical and physiological changes occur in plants which may positively affect frost tolerance (Xin and Browse 2000). Some of these changes include alterations in gene function, proteins, carbohydrates and ABA (Guy 1990; Sarhan *et al.* 1997; Vagujfalvi *et al.* 1999; Gusta *et al.* 2005).

There is considerable literature that has documented the role of cold acclimation and frost tolerance in winter cereals at the vegetative stage (e.g. Cloutier 1983; Gray *et al.* 1997; Limin and Fowler 2006). However, vegetative frost tolerance is not related to reproductive frost tolerance, and the general trend is for a decline in tolerance to frost as plants transit from the vegetative to the reproductive phase (Kosova *et al.* 2008). However, there are limited studies that have examined the relationship between cold acclimation and frost tolerance at the reproductive stage.

Therefore this study was aimed to assess the frost tolerance of a triticale in response to cold hardening.

5.2 Materials and Methods

5.2.1 Plant growth

A triticale variety, H426 was tested for this experiment. The plants were grown and maintained as per the method described in Chapter 3. At head emergence, plants of the same maturity were randomly allocated to either the hardening or non-hardening treatment.

5.2.2 Cold hardening

The cold hardening treatment began 2-3 days after emergence of the apical spikelet. Selected plants were taken to the cool room with a temperature of 4.5°C and no lighting at 8 pm, and transferred back to the glasshouse at 9 am the following day. This practice was continued till plants attained the flowering stage and was allocated to a frosting treatment. Plants received between 11 to 16 nights of hardening. The daily duration of cold hardening and dehardening constituted 13 and 11 hrs respectively. Every precaution was taken to avoid physical damage to plants during the transfer, although damage was not likely as the distance between glasshouse and cold room was limited. This hardening system is peculiar to the current study as the plants were only cold hardened during the night. From the practical perspective the procedure is justifiable as plants in the field can experience similar fluctuating conditions, leading to dehardening and rehardening.

5.2.3 Frosting treatments

Half of the hardened plants, and half of non-hardened plants, were selected for the frosting treatment, while the remainder plants were retained as control plants. Frosting treatments were imposed as described in Chapter 3 (Section 3.4.2).

5.3 Results

The analysis of floret survival found a significant difference both due to the -4.5°C frost and the hardening treatment ($P < 0.05$) (Figure 23). Grains set in the non hardened plants was almost double that of the hardened plants in the frosted treatment.

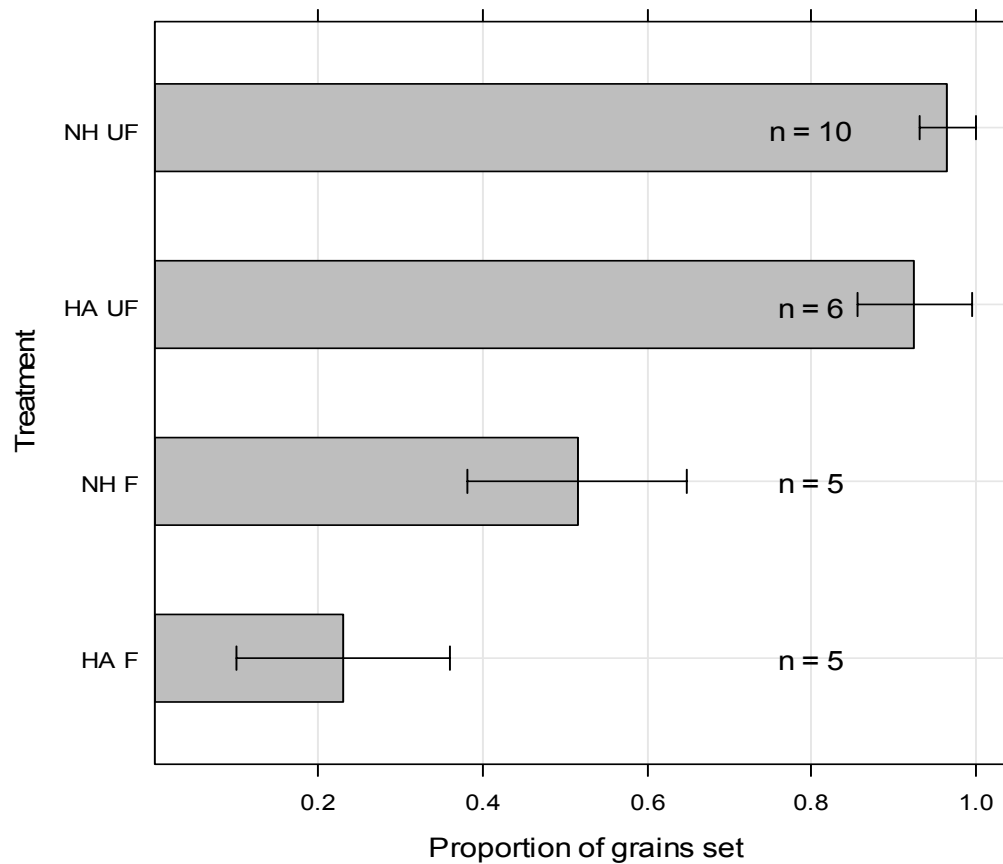


Figure 23. Proportion of grains set in variety H426 at -4.5°C (2010). Bars are the lower and upper 95% confidence interval. HA, Hardened; NH, Non-hardened; F, Frosted; UF, Unfrosted. n is the number of heads frosted or unfrosted.

There was a significant effect ($P < 0.05$) on grains set in both the -3.9°C and -3.65°C frosts. However, the hardening treatment didn't significantly influence ($P > 0.05$) grains set in either of frost (Figures 24 and 25). In both the treatments, hardened plants were more damaged than the non hardened plants.

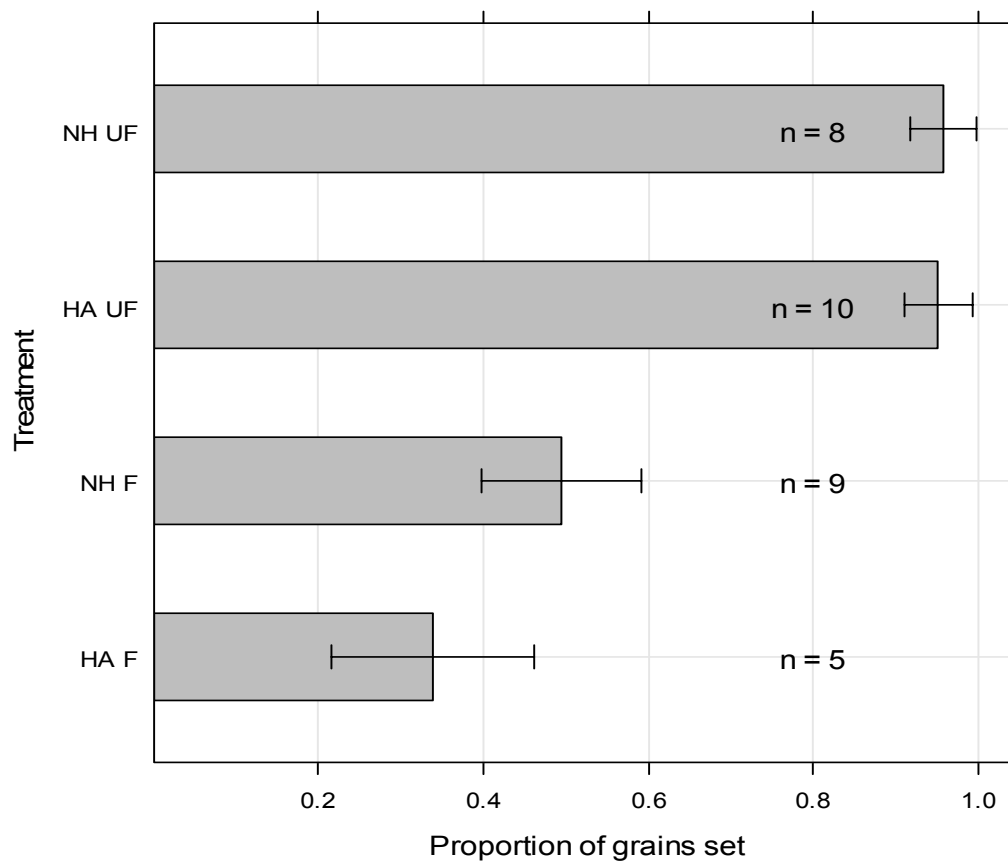


Figure 24. Proportion of grains set in variety H426 at -3.9°C (2010). Bars are the lower and upper 95% confidence interval. HA, Hardened; NH, Non-hardened; F, Frosted; UF, Unfrosted. n is the number of heads frosted or unfrosted.

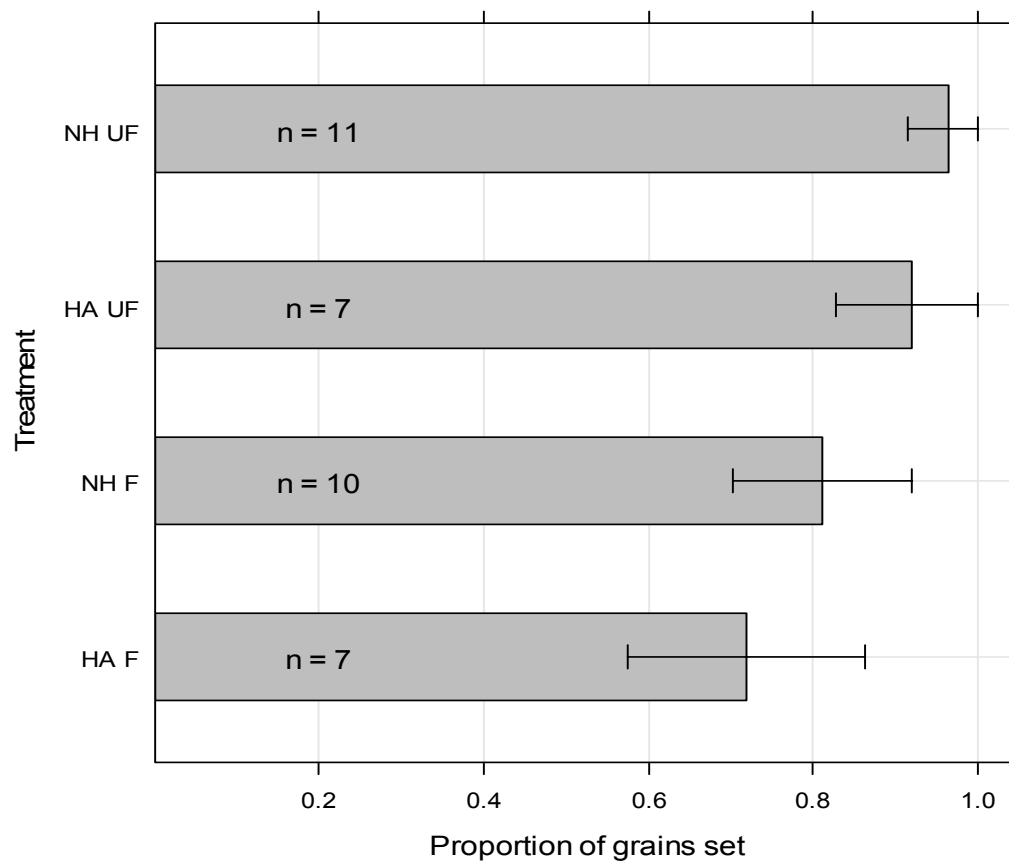


Figure 25. Proportion of grains set in variety H426 at -3.65°C (2010). Bars are the lower and upper 95% confidence interval. HA, Hardened; NH, Non-hardened; F, Frosted; UF, Unfrosted. n is the number of heads frosted or unfrosted.

There was a significant effect of -3.5°C frost on the floret survival ($P < 0.05$) but no influence of hardening ($P > 0.05$) (Figure 26). However, the field significance of the frost treatment will have less implication practically than colder frosts, given that both the treatments had considerable grain set. The trend for hardened plants to suffer more damage continued but the difference between treatments was small.

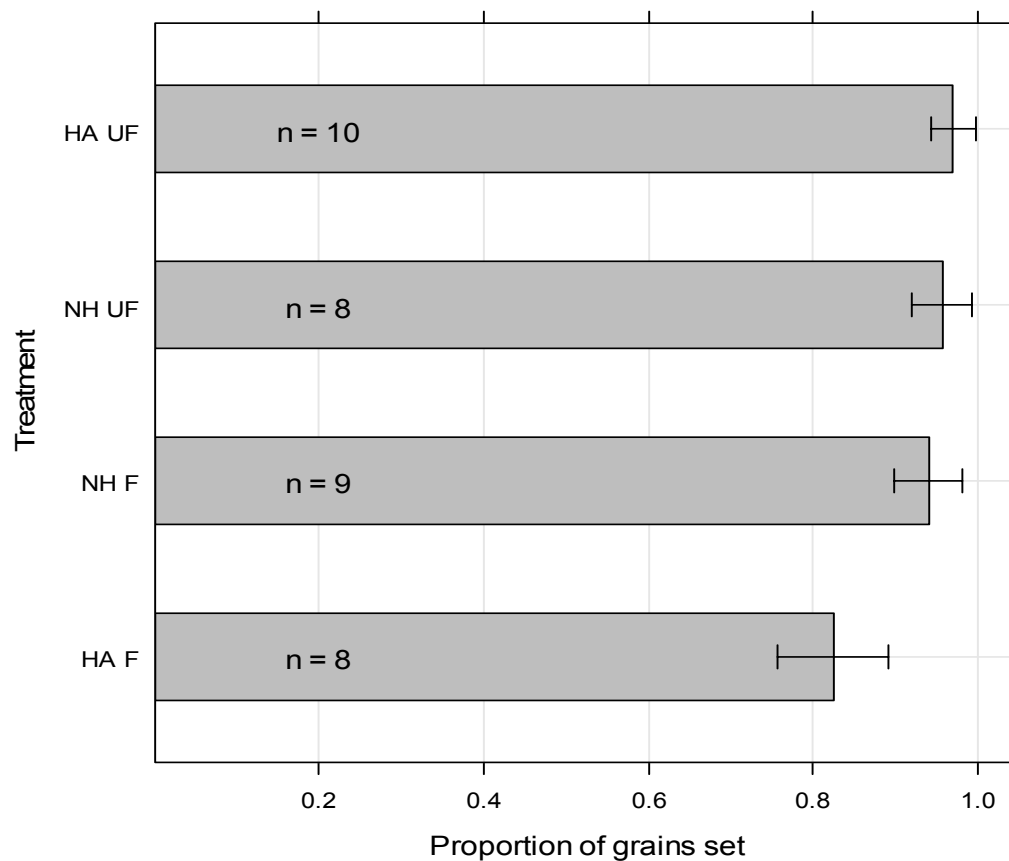


Figure 26. Proportion of grains set in variety H426 at -3.5°C (2010). Bars are the lower and upper 95% confidence interval. HA, Hardened; NH, Non-hardened; F, Frosted; UF, Unfrosted. n is the number of heads frosted or unfrosted.

At a milder frost of -2.7°C , neither the temperature nor the hardening treatment had any significant effect ($P > 0.05$) on grains set (Figure 27). Both treatments had more than 95% of their grains filled, indicating that there will be little or no loss to farmers at this temperature irrespective of the hardening treatments.

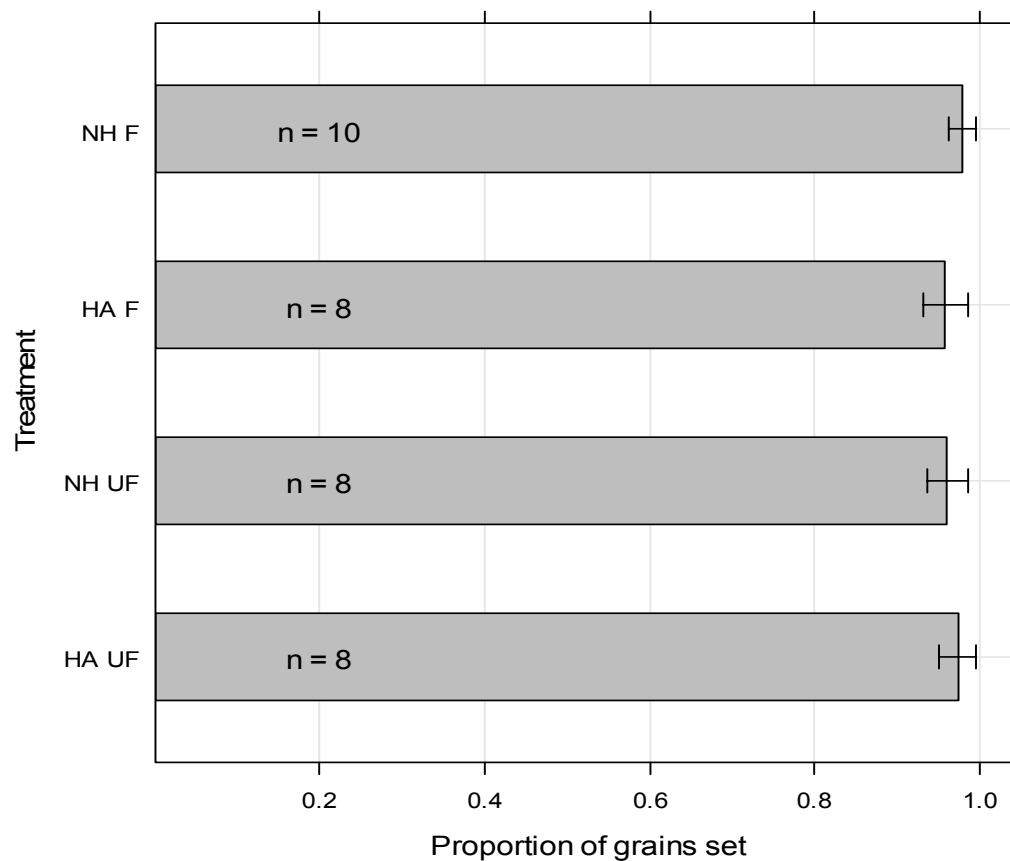


Figure 27. Proportion of grains set in variety H426 at -2.7°C (2010). Bars are the lower and upper 95% confidence interval. HA, Hardened; NH, Non-hardened; F, Frosted; UF, Unfrosted. n is the number of heads frosted or unfrosted.

The frost damage symptoms in this experiment were also similar to the varietal evaluation trials. The damage was random with surviving florets spread all over the heads (Plates 13-14), while the unfrosted plants, including the hardened plants, had normally filled heads (Plates 15-16).



Plate 13. H426 hardened plant at -3.9°C (2010).



Plate 14. H426 Non-hardened at -3.9°C (2010).



Plate 15. H426 Hardened and Unfrosted (2010).



Plate 16. H426 Non-hardened and Unfrosted (2010).

5.4 Discussion

The process of cold hardening has been suggested to increase frost tolerance in winter cereals owing to numerous physiological and biochemical alterations within the plant. However, the present study showed no positive effect of cold hardening on frost tolerance at the reproductive (flowering) stage. Similar observations of no significant effect of cold hardening at the reproductive stage have been documented by other authors in winter crops such as wheat (Fuller *et al.* 2007).

Other published reports have suggested that winter cereals lack a capacity to acclimatize once the main shoot meristem has advanced to the double ridge reproductive growth stage (Limin and Fowler 2006; Mahfoozi *et al.* 2006). This is because the loss of acclimation potential is progressive with ongoing phenological development (Limin and Fowler 2006). The stage of crop growth could be one of the factors in the non induction of frost tolerance in the present study as the crop was treated for hardening at the reproductive stage (1-2 days after apical spike emergence).

The non induction of frost tolerance in hardened plants in the present work could also be linked to the experimental procedures. Unlike other hardening trials where plants are hardened constantly with both day and night temperatures below 10°C, the plants in the current study were transferred back to the glasshouse during the day where the temperature was 20°C. This shift may have dehardened the plants, with whatever freezing tolerance compounds or genes activated during hardening disappearing when the plants are transferred back to warm temperatures during the day. The down-regulation of genes on transferring the cold hardened (2-4°C) plants to deacclimatizing temperatures (22-25°C) in winter cereals such as barley and rye has been documented (Sutton *et al.* 1992; In *et al.* 2005). Crespi *et al.* (1991) and Sasaki *et al.* (1996) also reported a reduction in carbohydrate content when hardened plants were deacclimated in wheat and cabbage respectively. In addition, the disappearance of a specific set of cold stimulated genes (*Wcs*) in deacclimated wheat has been documented (Gray *et al.* 1997). However, the role of genes or biochemical processes that are responsive to cold temperature in freezing tolerance are not clearly understood (Xin and Browse 2000).

Though *Triticeae* have been broadly categorized as winter cereals, they differ considerably especially in their need of specific growing conditions. One of these is the vernalization requirement which accordingly leads to a differentiation of two types as having winter or spring habits. This growth habit also influences cold hardening, with Mahfoozi *et al.* (2001) reporting a switching off of the expression of low temperature genes in vernalization demanding varieties once their requirement is fulfilled. Similarly, Fowler *et al.* (1996) reported that spring habit varieties had no low temperature induced genes in an up regulated condition and hence lacked low temperature tolerance even if they are cold hardened at 4°C. This was reasoned mainly due to the lack of vernalization requirement. Further, Gusta *et al.* (1997a, cited in Săulescu and Braun 2001) reported that spring cereals lack capacity to initiate acclimation at temperatures above 2°C. Additionally, it was also demonstrated that a winter wheat cultivar had higher accumulation of soluble and insoluble carbohydrates than the spring wheat after cold hardening (Hurry *et al.* 1995). Therefore, if acclimation is possible at the reproductive stage, it is unlikely that it would have been demonstrated in this trial as H426 is a spring type.

The inherent frost tolerance of the tested variety also has an influence on the degree of frost tolerance acquired through cold hardening. This is because frost resistant varieties are considered to harden faster and dehardening more slowly than the frost susceptible types (Rizza *et al.* 1997; Săulescu and Braun 2001). Thus, different varieties may be in need of different threshold temperatures for hardening, which is in contrast to the common recommendation of cold hardening in temperatures < 10°C. Therefore, it is possible that the temperature of 4.5°C used for hardening in the current trial was not appropriate, and H426 may require different threshold temperatures for optimum induction. The expression of some of the cold regulated genes is highly temperature dependent (Vagujfalvi *et al.* 2000).

Greater frost tolerance due to increased accumulation of fructan during cold hardening has been reported (Vagujfalvi *et al.* 1999). However, current study did not observe any difference in fructan content between hardened and control plants (Table 4.1). This lack

of difference may be due to the influence of irrigation and growing conditions (Stupnikova *et al.* 2002; Goggin and Setter 2004). Alternatively, the tested variety H426 may not be a tolerant cultivar as fructan was found to be accumulated higher in tolerant genotypes than susceptible ones during hardening (Vagujfalvi *et al.* 1999). Irrespective of these circumstances, the accumulation of fructan during cold hardening and its role in frost tolerance has always been debatable owing to other associated issues such as the influence of sucrose levels on fructan synthesis and accumulation (Pollock 1984; Cairns and Pollock 1988).

Contrary to expectation, the present work suggested that the hardened plants were more damaged by frost than their counterparts, although this effect was statistically significant only at one instance (-4.5°C). Low non freezing temperatures during the critical stage of meiosis have been reported to be damaging in crops such as wheat by causing male sterility (Săulescu and Braun 2001), though Demotes-Mainard *et al.* (1995) found no such effects. Male sterility due to low temperatures have been documented in crops such as sorghum (Downes and Marshall 1971; Brooking 1976), and rice (Satake and Hayes 1970). Therefore, it is possible that the frost treatment resulted in further damage of the crop physiology especially in the male reproductive systems. Male sterility is detrimental as it leads to failure of fertilization and production of fertile seed (Saini 1997). In contrast, female's fertility was unaffected (Brooking 1976) as they are less sensitive to low temperatures (Shroyer *et al.* 1995). The result suggests that cold-warm treatments are in fact a stress to the reproductive tissues, making them more susceptible to frost damage. On the other hand, stress-free such as non hardened plants are more frost tolerant and there are stresses that may mimic the effect of frost. However, it should be noted that current treatment was not a hardening, rather an imposed stress treatment.

CHAPTER 6 ROLE OF POTASSIUM NUTRITION IN FROST TOLERANCE IMPROVEMENT

“Researchers are divided about the benefits of potassium for frost protection”-
(Snyder and de Melo-Abreu 2005)

6.1 Introduction

An adequate supply of mineral nutrients is considered to be important for optimum growth and production of plants. Adequate nutrient supply is also linked to improved resistance against abiotic stress such as cold temperatures. One of the major nutrients that is implicated in minimizing frost damage is potassium (Cakmak 2005). Its favorable effect on other abiotic stresses such as drought (Andersen *et al.* 1992) and lodging (Beaton and Sekhon 1985) has been documented.

GRDC (undated) reported that crops deficient or marginal in potassium are prone to frost damage, although evidence on frost tolerance following potassium application especially in winter cereals is scanty or lacking. However, a reduction in frost damage from potassium fertilization has been reported in other crops such as potato (Grewal and Singh 1980) and rice (Haque 1988).

The main objective of this experiment was to investigate whether potassium application had any positive effect in alleviating frost damage in a selected triticale variety.

6.2 Materials and methods

6.2.1 Plant growth and soil characteristics

The triticale variety H426 was evaluated in this experiment. The soil used was a heavy vertisol (Isbell 1996) from Inverell, NSW and was known to be deficient in potassium. It was gathered from the sub-surface (10-20 cm depth). Its characteristics are presented in Table 6.1.

Table 6.1 Soil characteristics of Inverell.

Soil property	Measurement
pH (1:5 H ₂ O)	6.81
EC (dS/m) (1:5 H ₂ O)	0.64
Exchangeable K (cmol _c /kg) ^c	0.22
Exchangeable Mg (cmol _c /kg) ^c	19.01
Exchangeable Ca (cmol _c /kg) ^c	30.30
Exchangeable Na (cmol _c /kg) ^c	0.18
C (%)	1.55
N (%)	0.04
P (mg/kg)	37.0

^c extracted by 1:10 soil ammonia acetate (pH 7.0) solution

6.2.2 Potassium treatment

Muriate of potash (50% K) was used as a fertilizer source. Potassium was applied at the rate of 400 mg per kg of soil (~400 kg/ha). The required amount of fertilizer (292 mg) was dissolved in water and mixed with 730 gm of soil in a plastic bag for uniform distribution. The mixed soil was then transferred to the pot of 7.5 x 7.5cm x 20cm deep. Nitrogen fertilizer (both basal and top dressing) was provided similarly to the other experiments. The plants were kept on benches with plastic laid out to avoid the leaching of nutrients. Under no instances did the water leach during the course of experiment. Also, potassium is unlikely to move in vertisols (Carter and Singh 2004).

The control plants on the other hand were not provided with additional potassium nutrition. However, nitrogen was applied as both basal and top dressing treatments so that plant growth was not affected. Plants were kept on separate benches in order to avoid the risk of potassium leaching from the fertilized pots and being absorbed by the control pots.

6.2.3 Frosting treatment

Plants were frosted according to the procedures described in Chapter 3 (section 3.4.2).

6.2.4 Potassium analysis

In order to assess the potassium uptake, the grain and straw of both fertilized and control plants were sampled for potassium analysis. The samples were dried in the oven at 40°C for 72 hours, ground to pass 0.5 mm sieve, then digested by Sealed Chamber Digestion Method of Anderson and Henderson (1986), using a solution of 70% perchloric acid and 30% hydrogen peroxide. Sample potassium was then measured using an Inductively Coupled Plasma Atomic Absorption Spectroscopy (ICP-AAS).

6.3 Results

There was a significant reduction in grain set from the -3.9°C frost ($P < 0.05$), but no difference between the potassium treatments ($P > 0.05$) (Figure 28).

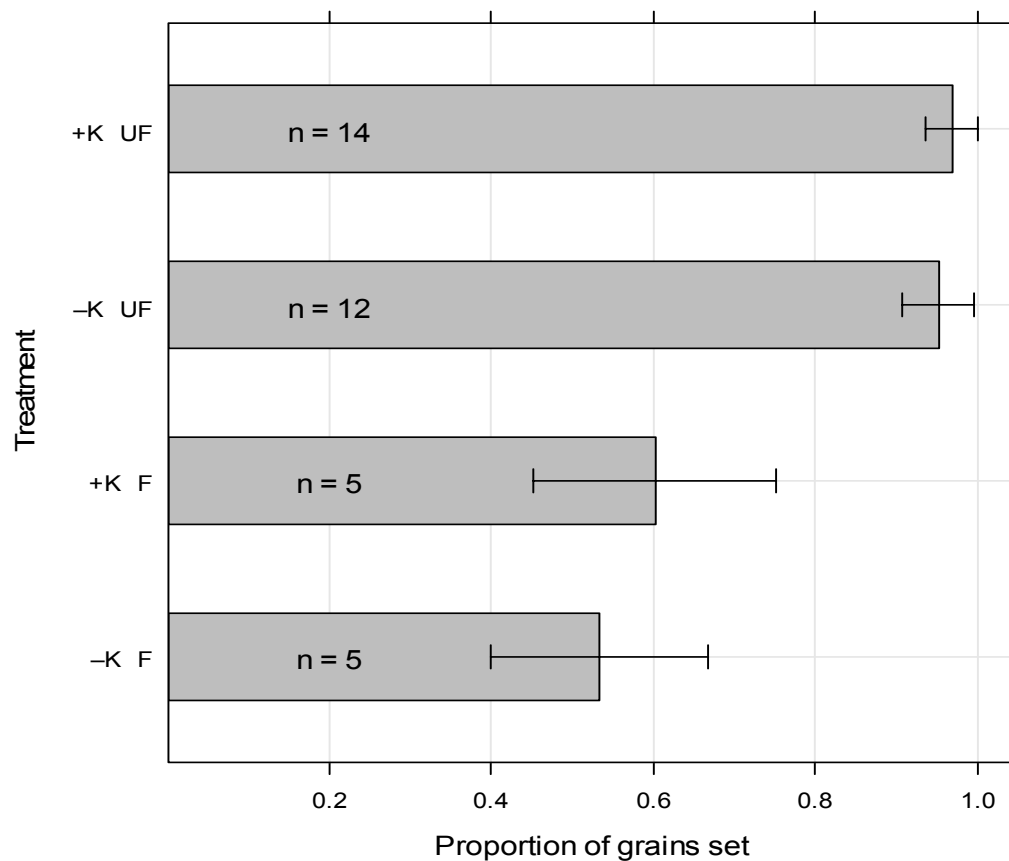


Figure 28. Proportion of grains set in variety H426 at -3.9°C (2010). Bars are the lower and upper 95% confidence interval. -K, No potassium; +K, Potassium added; F, Frosted; UF, Unfrosted. n is the number of heads frosted or unfrosted.

The statistics showed a significant effect of -3.5°C frost ($P < 0.05$) but no difference in potassium treatment on grains set ($P > 0.05$) (Figure 29). Some damage to grain yields could be expected from this frost event but it would be of reduced magnitude compared to the -3.9°C treatment.

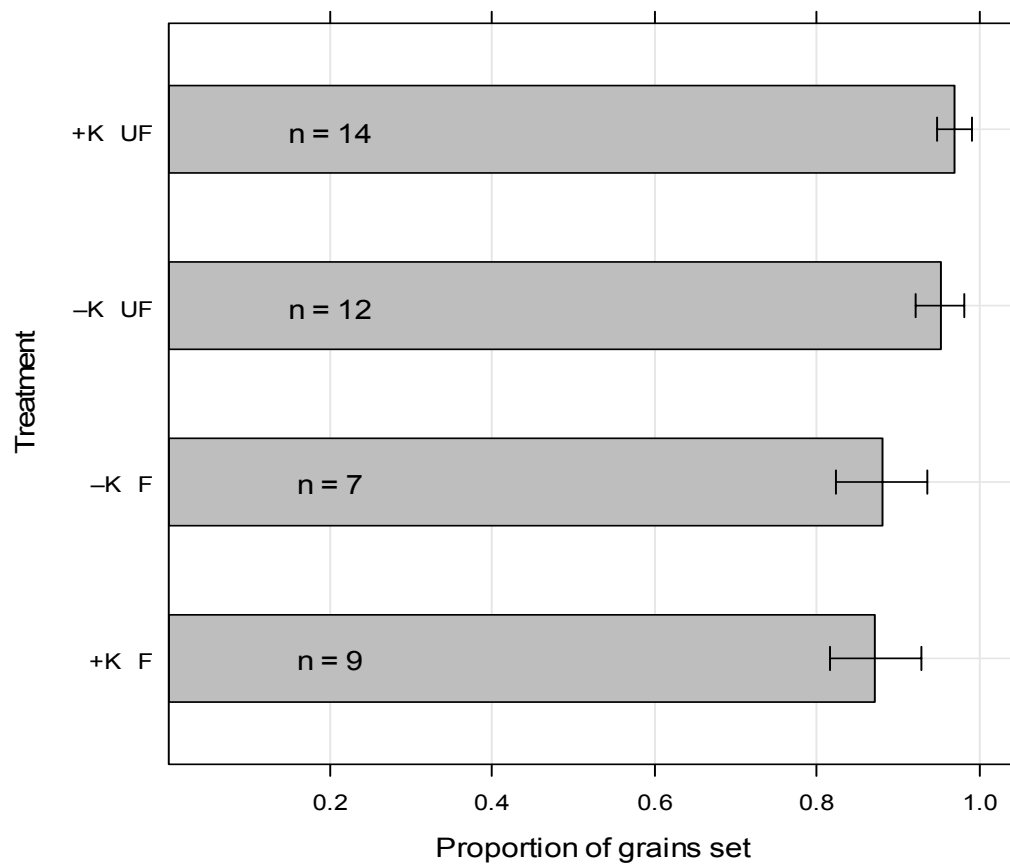


Figure 29. Proportion of grains set in variety H426 at -3.5°C (2010). Bars are the lower and upper 95% confidence interval. -K, No potassium; +K, Potassium added; F, Frosted; UF, Unfrosted. n is the number of heads frosted or unfrosted.

There was no significant effect of the -2.7°C frost or potassium treatment ($P > 0.05$) on the level of grains set (Figure 30).

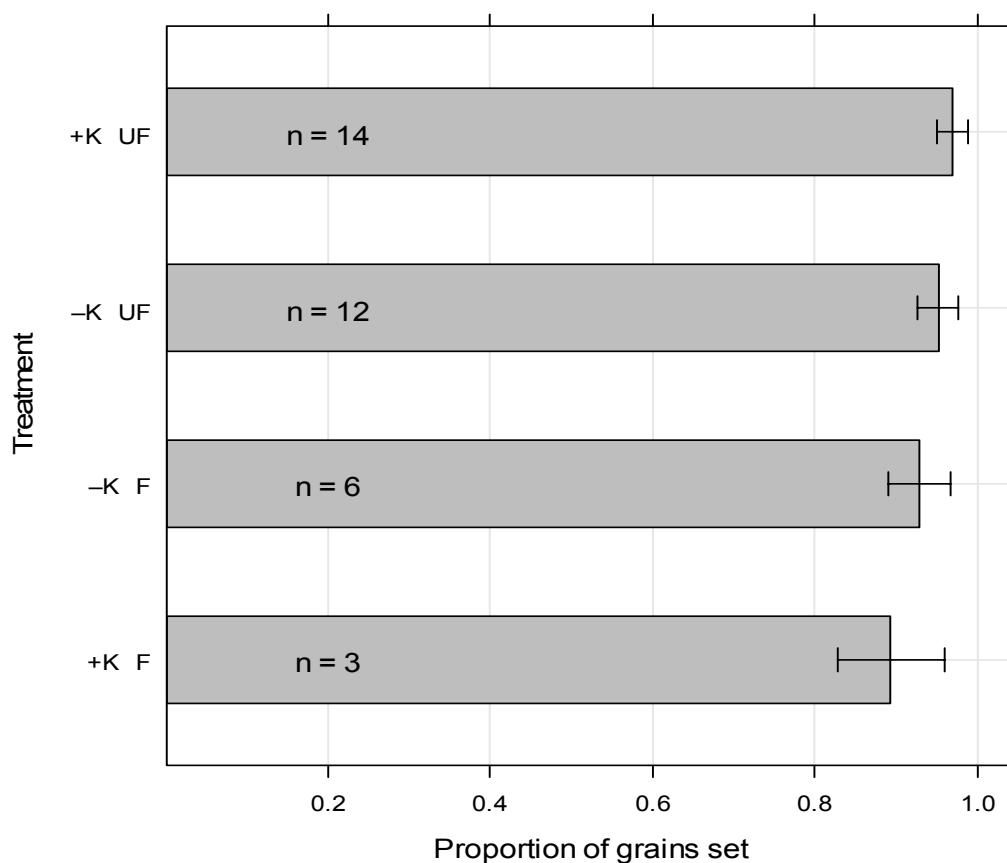


Figure 30. Proportion of grains set in variety H426 at -2.7°C (2010). Bars are the lower and upper 95% confidence interval. -K, No potassium; +K, Potassium added; F, Frosted; UF, Unfrosted. n is the number of heads frosted or unfrosted.

There was no remarkable difference in potassium concentration between the fertilized and control plants in both frosted and unfrosted treatments (Table 6.2). However, flowering was advanced by almost a week in fertilized plants compared to non-fertilized ones.

Table 6.2 Potassium concentration (data from a single sample) and days to flowering.

Plant part	Frost treatment	+ K	– K
		Potassium content (g/kg)	
Grains	Frosted	6.1	6.1
	Unfrosted	6.3	5.8
Head except grain	Frosted	9.4	8.7
	Unfrosted	10.0	9.3
Average days to flowering ($P < 0.05$)		60	66

6.4 Discussion

There is a general understanding that for plants to be able to tolerate low temperature conditions, the plants had to be adequately fertilized, particularly in relation to potassium nutrition. However, the results reported here showed no such significant effects in the tested triticale variety. Similar observations of no potassium effect on two species of grasses were also reported by Ivory and Whiteman (1978). Thus, greater frost damage is unlikely to be related to poor availability of potassium in the soil.

It has been suggested that a sufficient supply of potassium will have positive effects on morphological, physiological and biochemical aspects of plants (Pier and Berkowitz 1987; Sen Gupta *et al.* 1989; Snyder and de Melo-Abreu 2005) which in turn will enhance the plants' potential to resist frost damage (Cakmak 2005). Plants with higher potassium concentration were also considered to have lower freezing points through increased levels of carbohydrates and proteins in the cell sap (Beaton and Sekhon

1985). Further, potassium was also linked to modified gas regulation through closure and opening of stomata, which directly or indirectly, contributes to frost tolerance (Fischer 1971). However, it seems likely that these changes brought by higher potassium supply have little to offer in improved frost tolerance. At both the colder (Figure 28) and warmer (Figure 29) temperatures, the potassium fertilized plants did not exhibit significantly improved grain set compared to the untreated plants.

While potassium application has no significant effect on minimizing the frost damage, it did affect other agronomic characters especially the days to flowering. The potassium treated plants had their flowering advanced almost by a week compared to their non treated counterparts (Table 6.2). This, however, was managed in current work by multiple sowings and selecting plants that were at same developmental stage from the different sowings. While not directly relevant to the present study, it does suggest that in the field, applying potassium to a potassium deficient paddock could increase frost risk by causing earlier flowering than usual. Thus, sowing may be delayed under such condition, so that plants do not flower when the likelihood of frost is high. This, if verified under field conditions, needs to be considered in relation to the effect of a spring frost which may be more severe with early flowering.

CHAPTER 7 GENERAL DISCUSSION AND CONCLUSIONS

“We have only scratched the surface of an extremely complex problem”- Loss (1988).

Given the importance of spring radiation frosts in Australian grain industry, the present work was mainly intended to evaluate relative frost tolerance in commercial triticale varieties. Simultaneously, it was also aimed to compare frost tolerance in triticale with other winter cereals (wheat and barley), given that farmers complain triticale is the most susceptible species (Roake *et al.* 2009). Additionally, it was designed to document other associated issues in frost tolerance, such as the role of hardening at the reproductive stage and the possible effects of potassium fertilization. While these remained the prime guiding objectives of present work, it was also expected that a novel method of frost screening could be developed which would provide an alternative or supplementary method to the existing frost studies (field based and controlled temperature chambers).

The collated results of two years demonstrated that there may be a difference in tolerance level between various commercial triticale genotypes. However, the importance of the differences among the cultivars particularly, in 2009 season, needs be assessed from different angles. From a field perspective, the differences were not great and growers may continue to suffer serious losses if some frost avoidances strategies (e.g. delayed sowing) are not used. From a longer term perspective, the difference may be more useful as they suggest there may be tolerance genes which could be located and exploited through molecular and genetic studies and plant breeding programs. Confirmatory work in 2010 also showed varying grains set among the cultivars with Tahara as the most susceptible variety, though the relative tolerance order had changed slightly between the years. This is not surprising as frost researchers have experienced inconsistency in varietal rankings from year to year (Belford 2010). Thus, repeated screenings with the same protocols may be needed to confirm the true relative tolerance.

The increased frost susceptibility of wheat compared with triticale in first year (2009) was mainly due to height difference, indicating how erroneous results could be induced with different crop heights. Due to the height issue and changes in experimental procedures between the years (2009 and 2010), it is impossible to state precisely whether wheat was more tolerant than triticale or vice versa. However, barley, despite being short statured, was not affected greatly by a severe frost such as -4.2°C due to its characteristic of flowering in the boot (GRDC 2009). Thus, growing barley instead of wheat and triticale in high frost risk areas may be another frost management strategy. It is said that some farmers in WA have started replacing wheat with barley in some frost prone areas for this reason (Imma Farre pers. comm. 2010). If such a flowering habit is able to offer some degree of frost tolerance, it may be worth assessing wheat and triticale germplasm collections for material that possesses this character. However, the effects of subsequent frost events have to be considered as cereals remain susceptible until the grain maturation stage (Thakur *et al.* 2010). A severe frost, such as -5.8°C , even at 2 weeks after anthesis can be equally devastating as frosting at flowering since all the grains were shriveled and unfit for commercial purposes (Plates 12-13).

While there have been many studies on the role of hardening in vegetative frost tolerance, there has been little work done at the reproductive stage. The present study on hardening at the reproductive stage did not find a significant role of cold hardening in modifying frost tolerance in the tested triticale variety. The same observation was also made by Fuller *et al.* (2007), who reasoned that the acclimation process is not triggered when plants are at reproductive stage. Surprisingly, there was a trend of more damage in hardened plants than their counterparts, suggesting that a series of cold non-freezing temperatures during the night may have detrimental effects on grain set. While such negative effects of low non-freezing temperatures have been documented in thermophilic crops, the reasons for more damage in this winter cereal are unknown. A possible reason could be that the frost brought further destruction when reproductive organs were already stressed due to the cool nights.

The application of potassium did not bring any improvement in frost tolerance. This was possibly to be expected as the role of potassium in frost resistance has been controversial (Snyder and de Melo-Abreu 2005). While its exact contribution in frost tolerance will remain in mystery it did, however, hasten the flowering of treated plants by a week compared to the non treated ones. This suggests that it did have some influence on specific plant physiological processes in this trial, even though potassium deficiency symptoms were not observed. The lack of a potassium effect on frost tolerance could be confirmed using a severely potassium deficient soil. The change in phenological development also needs to be clarified and its implications for increasing frost risk assessed.

The present technique of raising the plants in the glasshouse and transferring to field for natural frost event has a great potential in frost tolerance evaluation. This is because the frost damage symptoms of randomness in the crop heads in both the years were identical to those occurring under field conditions. Additionally, the method also avoids multiple frosts which are a major constraint in field based frost studies. Thus, the technique should be seen as one of the best alternatives in frost studies where the artificial and multiple frost events are to be avoided. In contrast, frost chamber studies were criticized for failing to reproduce identical symptoms to those usually observed under natural frosting episodes, especially the random head frost damage (Fuller *et al.* 2009).

One limitation of the current design is the number of plants treated in each frost event. To ensure adequate sample size over a range of frost events, weekly plantings of 12 plants per variety were conducted. Despite these efforts, a considerable number of plants could not be treated due to absence of a frost event during flowering, leading to shrinkage of sample size. The problem was further aggravated by the need to treat plants at anthesis ± 5 days. Thus, there are possibilities that in some frost events the sample size must have greatly influenced the significance of results as width of the confidence interval increases with a smaller sample size, leading to lower precision (Gardner and Altman 1986). Therefore, due attention has to be given to ensuring

sufficient (> 10) sample size in future screening programs. This could be aided by adopting some flexibility in selecting the plants outside of the flowering period as the crop remains susceptible until the grain ripening stage.

It would have been ideal to re-test all the cereals species and varieties of 2009 in 2010 season to confirm their performance. Additionally, a range of temperatures from -3.5 to -4.5°C with an interval of 0.1 to 0.2°C would have assisted in differentiating the critical damaging temperature. However, with the expectation of covering as many varieties and species as possible, some varieties of 2009 had to be excluded at the expense of new wheat varieties in 2010. Moreover, with the increase in test varieties/lines, plant maintenance and glasshouse space for their growth becomes an issue as multiple sowings have to be done. Therefore, due attention may be given in selecting the test materials such as the current commercial grown varieties, pre-released lines that have farmers' preference, lines from different breeding origins and cultivars with distinguishing morphological characters such as glaucousness, glume pubescence and triticale and wheat varieties that flower in the boot. This should be supplemented by a common protocol, especially for frost duration and intensity, though this will be a great challenge with natural frost. It was observed in the current experiments that temperatures in nature were fluctuating constantly.

It seems impossible to precisely nominate a frost tolerant variety as there are many confounding issues such as environmental factors, plant growing conditions, plant health status to name few which are influencing the tolerance level. Before a tolerant variety is made available, farmers should continue following agronomic measures such as manipulation of sowing time (mostly delayed sowing), delving (Rebbeck *et al.* 2007d) and use of blended varieties (Rebbeck *et al.* 2007c). The effectiveness of these practices in minimizing the frost damage has been acknowledged by the grains farmers and there is adequate reason for them to implement these methods in frost management.

Finally, the study found a very small difference in triticales, barley as tolerant species and no role of cold hardening and potassium fertilization in frost tolerance. Further, the study demonstrated that temperatures below -3.9°C could cause serious yield reduction in triticales and wheats at flowering. On the other hand, temperatures warmer than -3.5°C did not result in considerable damage, even with crops at the most susceptible stage, flowering. It is unfortunate that the degree of damage could not be assessed at temperature between -3.65 and -3.9°C due to the lack of suitable frosts, or susceptible plants, or both.

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APPENDIX 1 MEASURED AIR TEMPERATURES FOR EACH TREATMENT

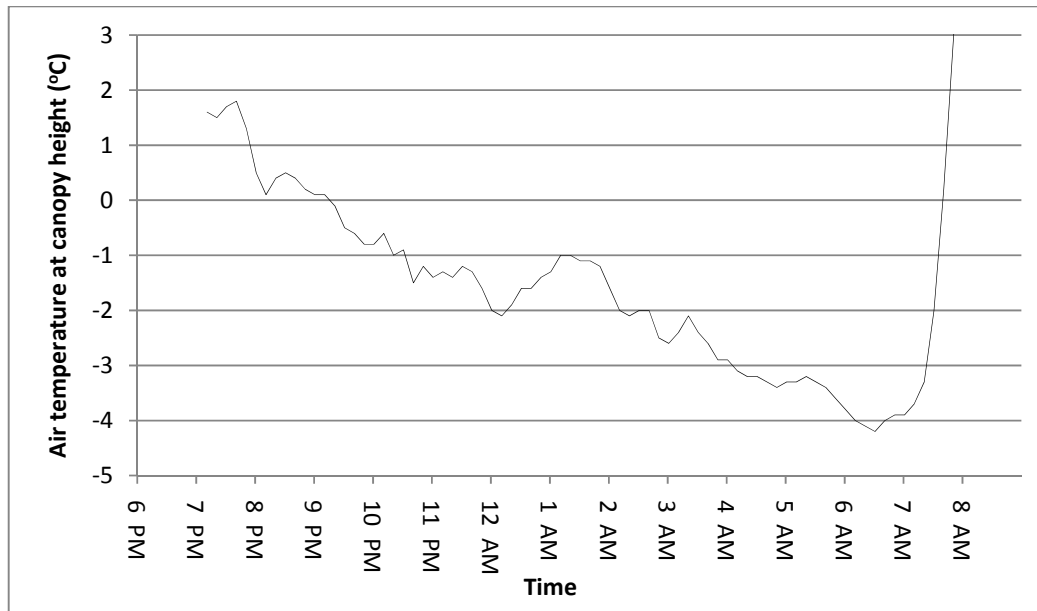


Figure 31. Measured air temperature for -4.2°C frost event on 18 July, 2009.

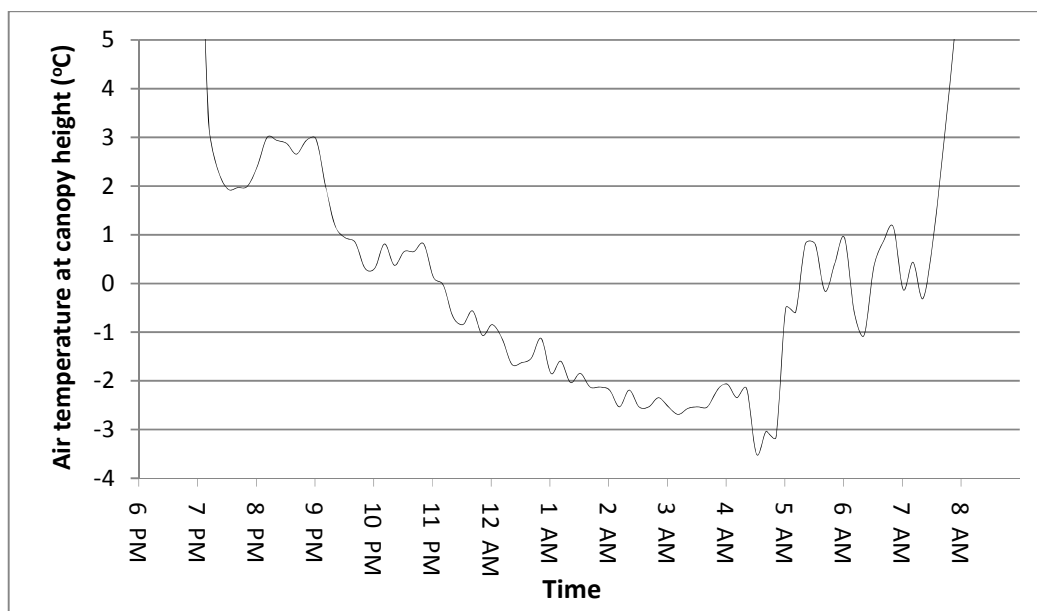


Figure 32. Measured air temperature for -3.5°C frost event on 19 July, 2009.

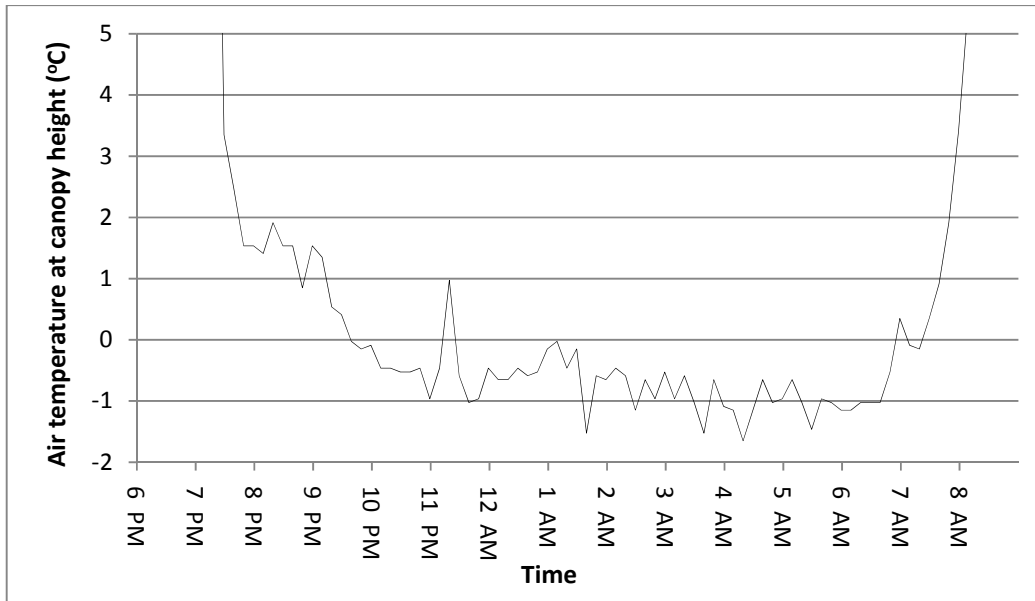


Figure 33. Measured air temperature for -1.9°C frost event on 24 June, 2009.

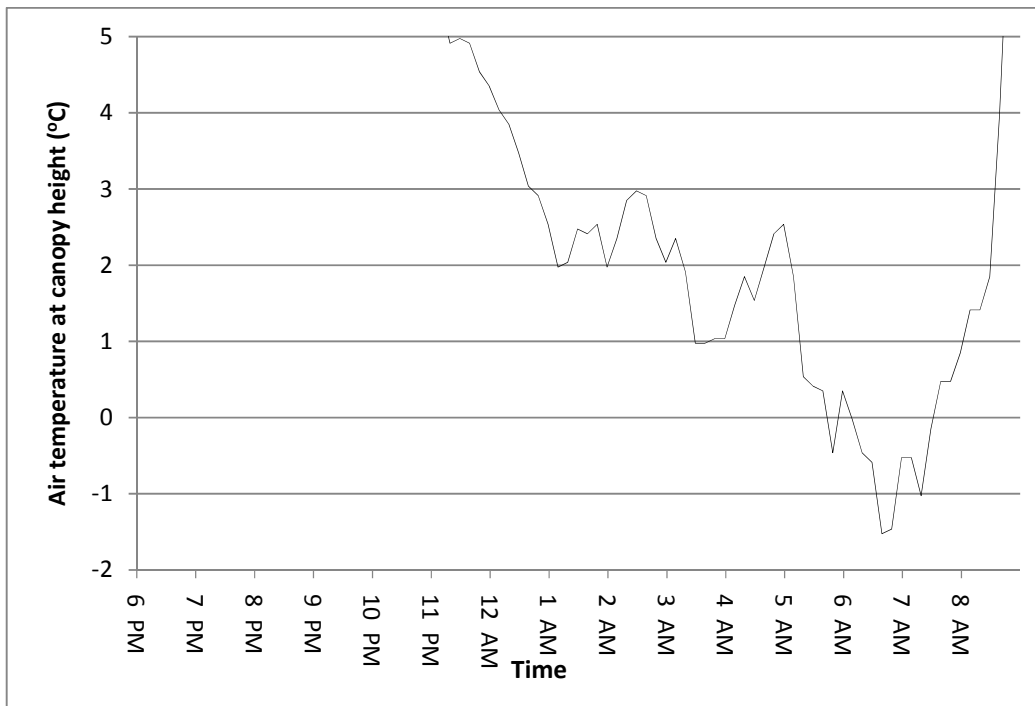


Figure 34. Measured air temperature for -1.7°C frost event on 25 June, 2009.

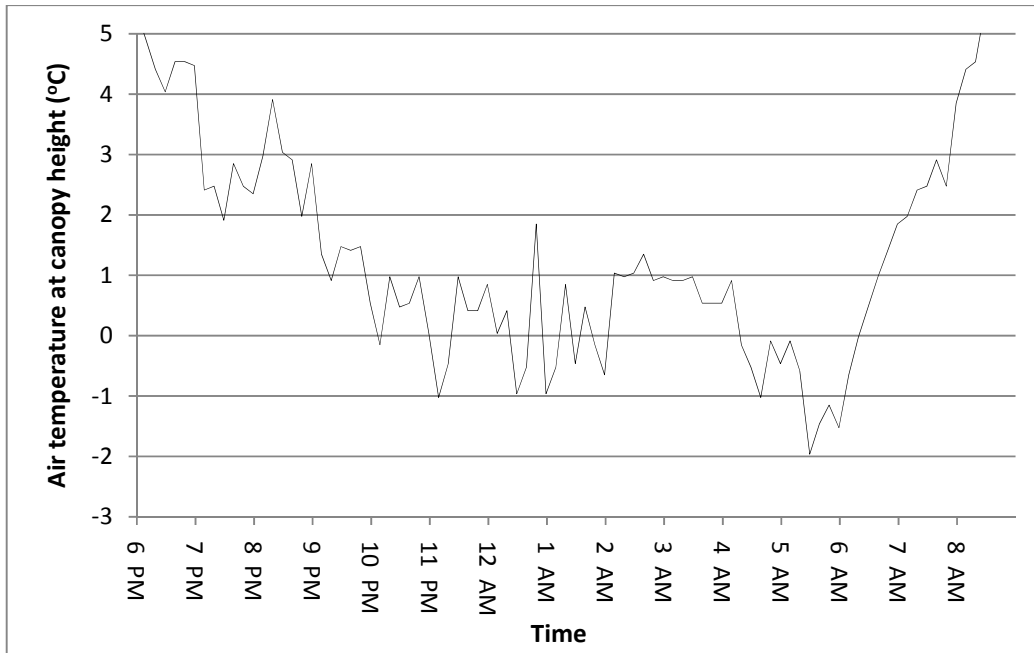


Figure 35. Measured air temperature for -2°C frost event on 19 June, 2010.

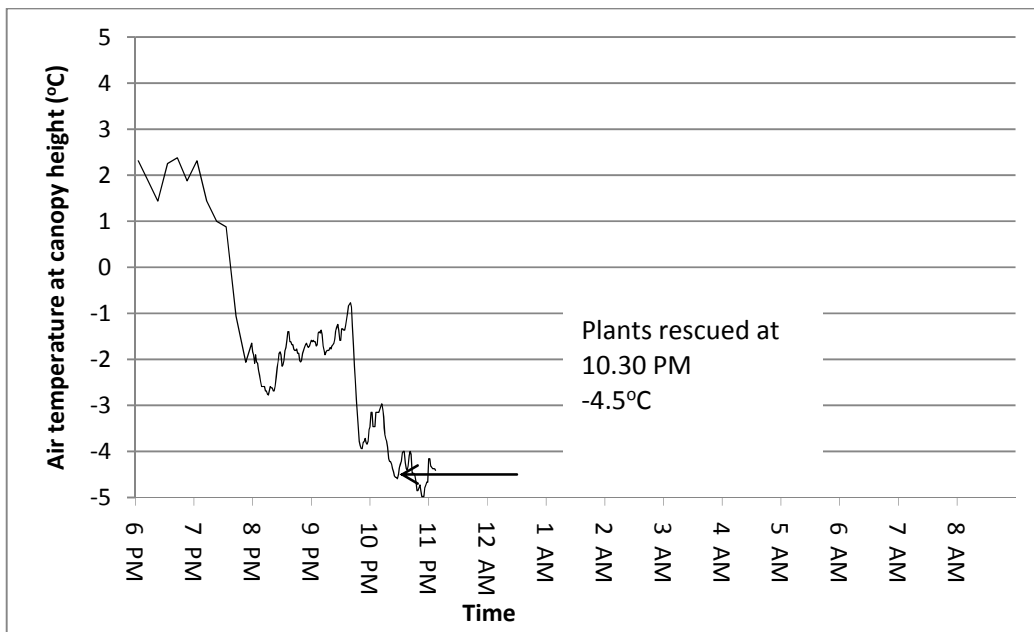


Figure 36. Measured air temperature for -4.5°C frost event on 29 June, 2010.

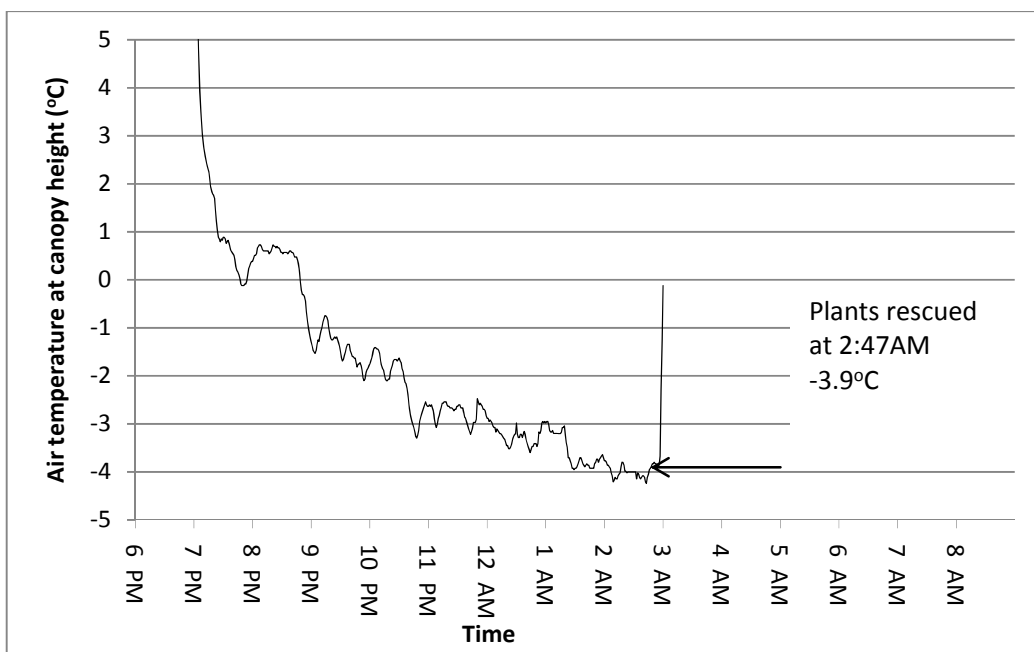


Figure 37. Measured air temperature for -3.9°C frost event on 17 July, 2010.

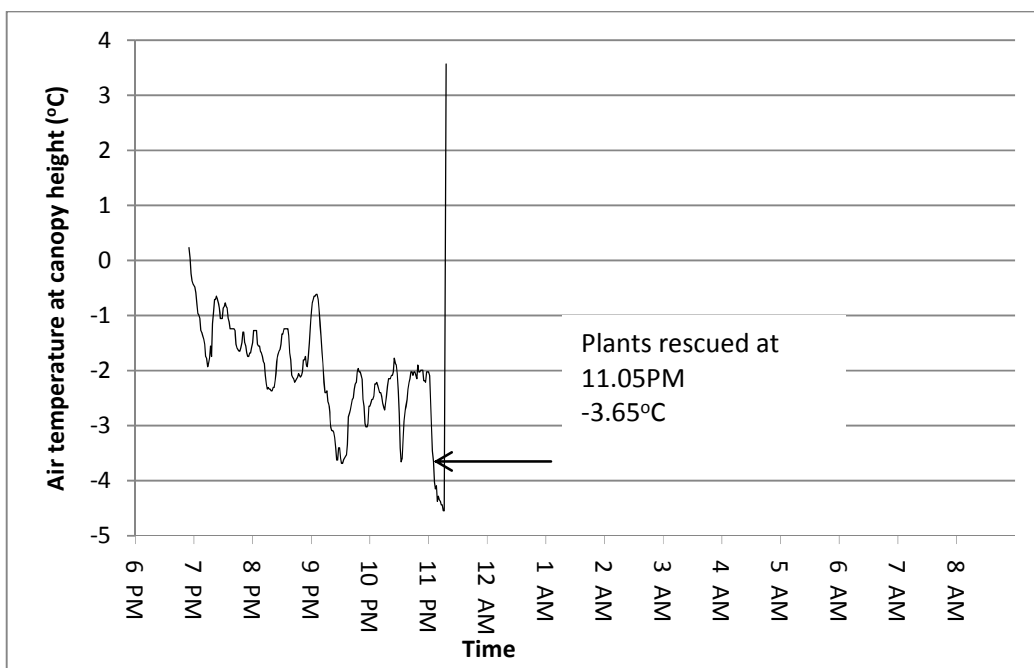


Figure 38. Measured air temperature for -3.65°C frost event on 30 June, 2010.

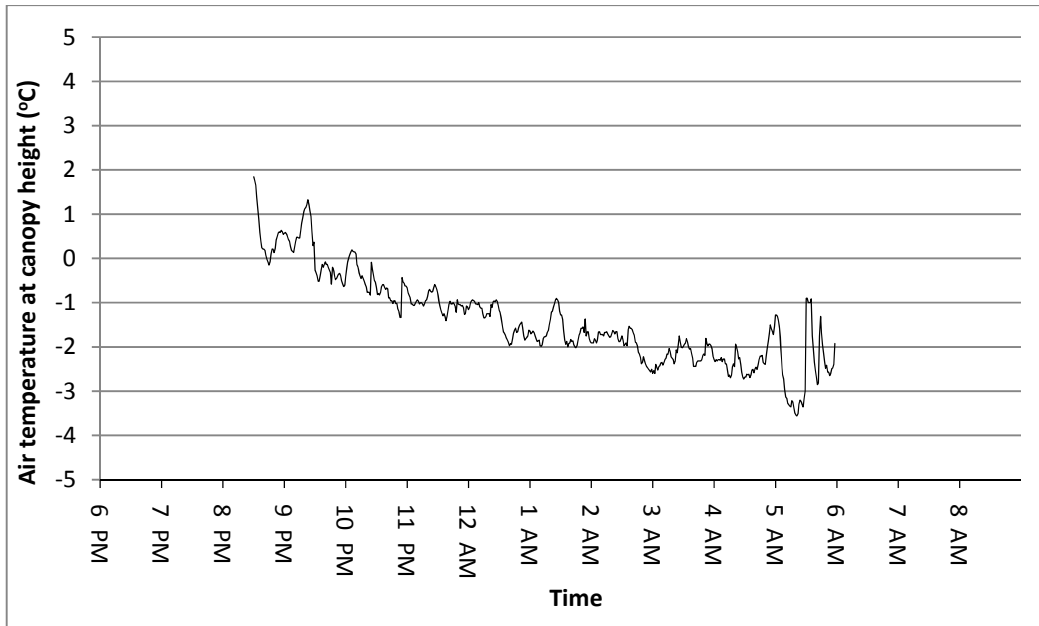


Figure 39. Measured air temperature for -3.5°C frost event on 19 July, 2010.

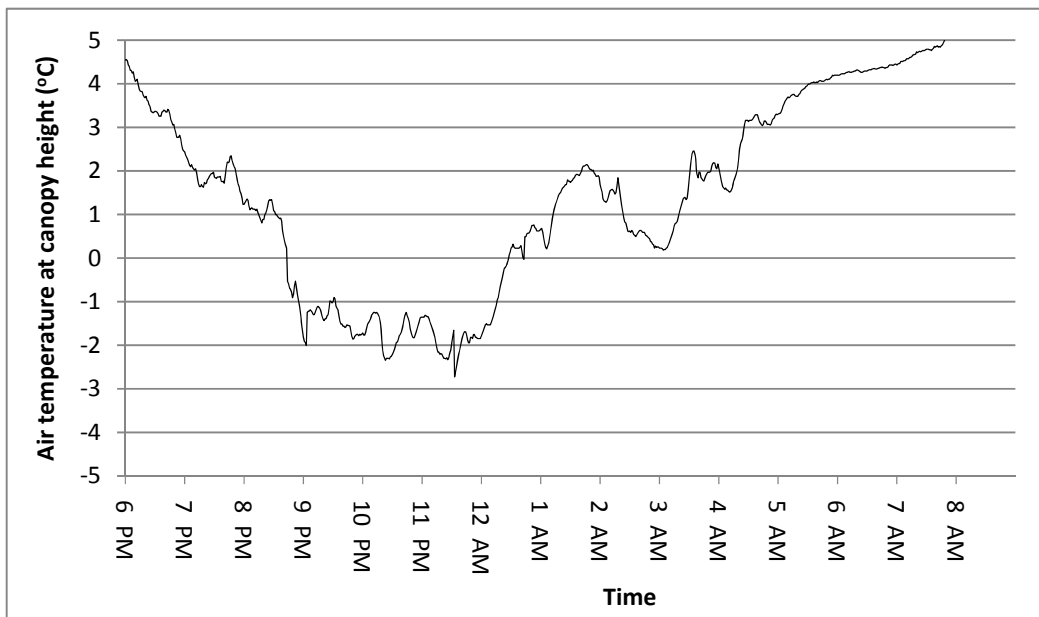


Figure 40. Measured air temperature for -2.7°C frost event on 23 July, 2010.

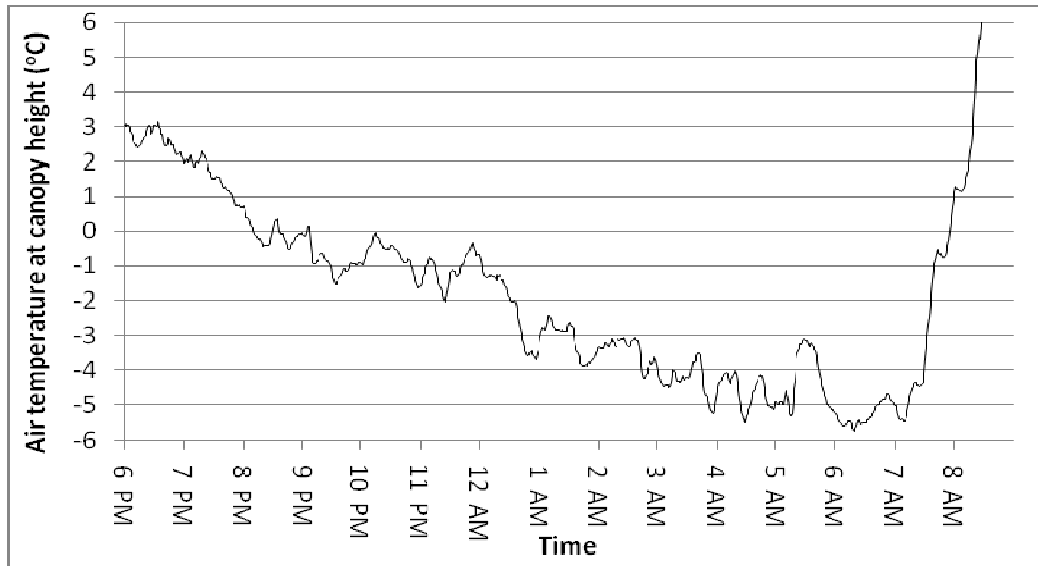


Figure 41. Measured air temperature for -5.8°C frost event on 6 August, 2010.

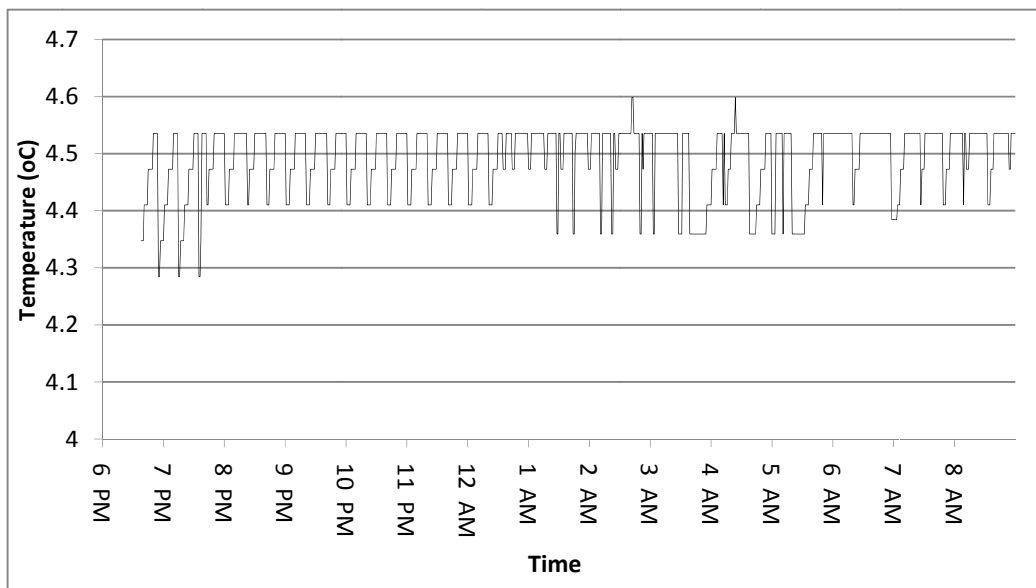


Figure 42. Measured temperature for 4.5°C in the cool room in 2010.

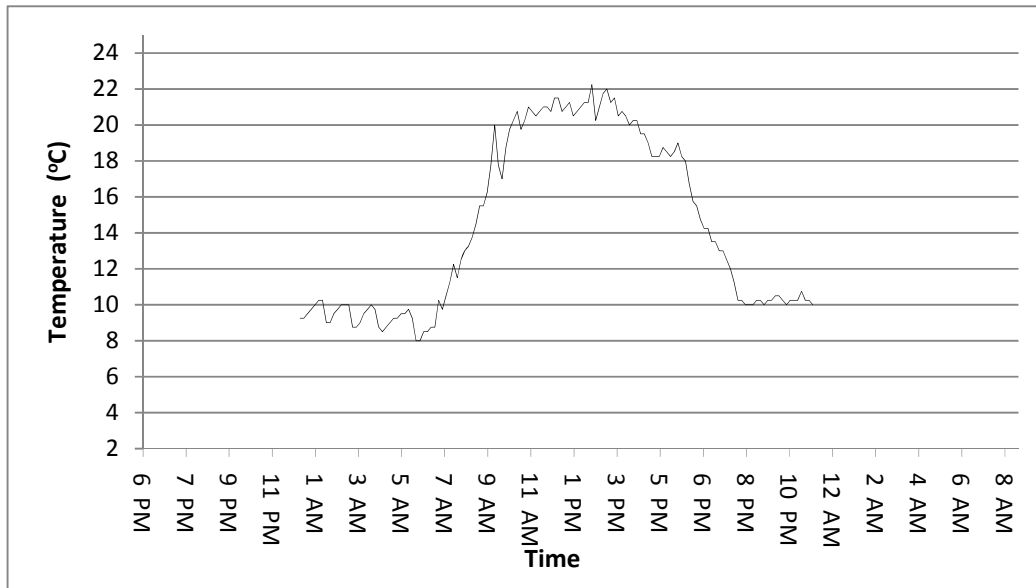


Figure 43. Typical temperatures in the glasshouse in 2009 and 2010.