INHERITANCE OF FREEZING STRESS IN SOUTH AFRICAN POTATO (*SOLANUM TUBEROSUM)* GERMPLASM

by

Carien Venter

Submitted in fulfilment of the requirements for the degree

Magister Scientae Agriculturae

Faculty of Natural and Agricultural Sciences
Department of Plant Science: Plant Breeding
University of the Free State
BLOEMFONTEIN

October 2006

Supervisor : Prof. C.S. van Deventer

ACKNOLEDGEMENTS

To my heavenly Father who gave me the privilege to do research and to discover the wonderful work of His almighty, remembering that He will always look after me, as He does to nature.

I would also like to express my sincere gratitude to the following persons and organisations for their contribution towards the success of this dissertation:

- My promoter, Prof. S.C. van Deventer for his kindness, guidance and advice in my study.
- Agric Risk Specialist (ARS) providing the necessary funds to complete this study.
- GWK Douglas and LNR Roodeplaat, Pretoria for their advice and providence of planting material.
- PW Pelser of OMNIA for his advice and help with the nutrition applications for each trail
- Elizma Koen for her help and patience in the laboratory
- The Department of Plant Breeding and it's personnel for the use of their research facilities and their kindness.
- My friends and family for their encouragement, love, motivation and understanding while completing this study.

I wish to dedicate this work to my grandma, Ria Venter in whom I share my great love for plants and flowers.

TABLE OF CONTENTS

List of abbreviations

ABA	abscisic acid
ANOVA	analysis of variance
ARC	Agricultural Research Council, South Africa
ARS	Agricultural Risk Specialists, Bloemfontein, South Africa
B.C.	before Christ
°C	degrees Celsius
C1	clone one generation
C.V.	coefficient of variation
CHI	cycloheximide
CIP	cold induced polypeptides
cm	centimeter
df	degrees of freedom
EDTA	ethylenediaminetetra acetic acid
g	gram
G x E	genotype by environment interaction
Gs	growth stage
h	hour(s)
h^2	heritability
ha	hectares
kDa	kilo Dalton
ℓ	liter
LSD	least significant difference
M	molar
mg	milligram

min	minute
ml	milliliter
mM	millimolar
MS	mean squares
pH	acidity
rpm	revolutions per minute
s	seconds
SDS	sodium dodecyl sulfate
Sp.	species (singular)
Spp.	Species (plural)
Temp	temperature
Tris	tris(hydroxymethyl) aminomethane
USA	United States of America
µg	microgram
µl	microlitre

List of tables

List of figures

CHAPTER 1

Introduction

Potatoes can be regarded as the vegetable that made remarkable history more than once. Its history is intimately linked with globalization (Wallin, 2006). Ancient potato growers of the Peruvian Bolivian Andes in South America discovered many types of small, bitter, wild potatoes 200 B.C., from where it spread until today to almost 50 countries. In 1995 the potato was the first vegetable grown in outer space to feed astronauts on their long missions (Anonymous, 2005c).

During the centuries the potatoes gained popularity and is now known as one of the major food crops grown worldwide. It is the world's fifth largest food crop (Alleman *et al*., 2004) and is the most widely distributed crop in the world (Beukema and Van der Zaag, 1990). Approximately 300 million tons of potatoes are annually produced world wide (Anonymous, 2005a). With the exception of milk products, potatoes are the most consumed food (Anonymous, 2005c). China (21%), the Russian Federation (12%), Ukraine (6%) and the USA (7%) are the major potato producing countries of the world (Anonymous, 2005a). Li (1985) reported that potato production exceeds that of all other food crops in some developing countries.

Potatoes are an important food crop in South Africa. It produces approximately 0.5% of the world's total production and is ranked 29th among the largest potato producing countries (Anonymous, 2005a). Within the South African context, the gross value of potato production accounts for about 43% of all vegetables and four percent of the total agricultural production (Anonymous, 2005b). South African potato producers plant approximately 46 000 hectares with a total production of 1.44 million tons annually. The income value is approximately two billion Rands (Theron, 2003).

In South Africa potatoes are grown under a wide range of climatic conditions. With the exception of the cold winter months, potatoes are planted almost throughout the year. The most important production regions are indicated in Figure 1.1 (Potatoes, S.A. 2003).

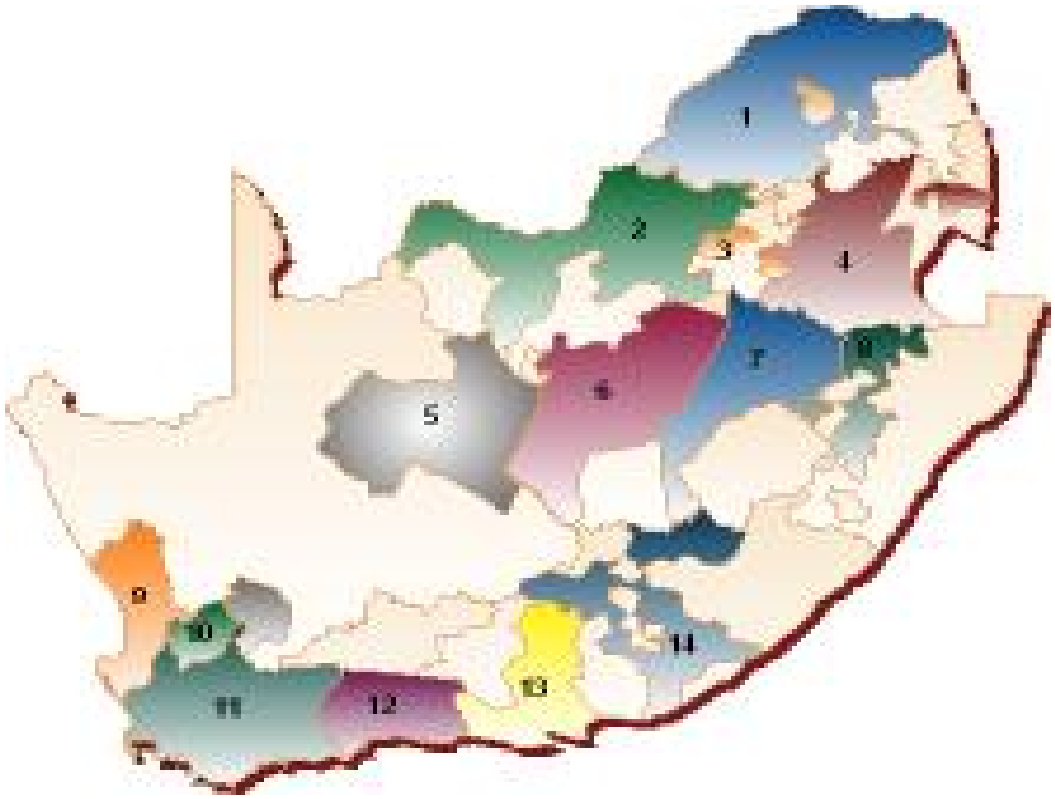


Fig. 1.1 Potato producing regions in South Africa (Potatoes S.A., 2003).

Production regions : 1 Limpopo, 2 North West, 3 Gauteng, 4 Mpumalanga, 5 Northern Cape, 6 Western Free State, 7 Eastern Free State, 8 KwaZulu-Natal, 9 Sandveld, 10 Ceres, 11 South Western Cape, 12 South Cape, 13 Eastern Cape, 14 North Eastern Cape.

Some of these production areas are subject to adverse weather conditions, which cause stress during the growth period of the plant. Freezing injury due to frost damage can be detrimental to potato plants during the early fall and late summer.

According to ARS (2005), 5.5 million hectares with a value of more than R300 million were insured against frost damage during the past eight years. The total loss of potato production due to frost damage during this period exceeded R33 million or 10 percent of the production. Yield loss due to frost damage depends mainly on the growth stages of the potato plant. The potato plant is particularly sensitive to any stress during the flowering stage. If frost damage does occur during this stage, severe yield losses can be expected.

A strategy, which potato producers can follow, is to plant cultivars which have some tolerance to freezing stress. In order to develop some tolerant cultivars, it is necessary to study the genetic variability for freezing stress in South African potato germplasm.

The main objectives of this study were to:

- Determine the genetic variability due to freezing stress in the different growth stages of two potato cultivars.

- Study alterations in protein profile expressions when potatoes are subjected to freezing stress.

- Study the inheritance of yield in C1 (Caren x Bravo) potato breeding population under conditions of freezing stress.

REFERENCES

Alleman, J., Laurie, S.M., Thiart, S. and Vorster, H.J. 2004. Sustainable production of root and tuber crops (potato, sweet potato, indigenous potato, cassava) in southern Africa. *South African Journal of Botany* 70 (1): 60-66.

Anonymous, 2005a. US Potato Statistics. United States Department of Agriculture. http://www/ers/usda/gov/Briefing/Potatoes/worldprod.htm

Anonymous, 2005b. Potato profile. Department of Agriculture Resource Centre, Directorate Agricultural Informaion Services, Pretoria, South Africa.

http://www.nda.agric.za/docs/Potatobrochure/potato.htm

Anonymous, 2005c. http://www.medicalecology.org/food.f_potatoes.htm.

ARS, 2005. Agricultural Risk Specialist, Bloemfontein, South Africa.

Beukema, H.P. and Van der Zaag, D.E. 1990. Introduction to potato production, Pudoc Wageningen, Den Haag.

Li, P.H. 1985. Potato Physiology. Academic Press Inc., London.

Potatoes S.A. 2003. Situation analysis of the South African potato industry. Final report to the Business and Institutional Development Directorate, National Department of Agriculture, Potatoes South Africa, Pretoria.

Theron, D.J. 2003. The South African potato industry in perspective. *In*: Niederwieser, J.G. (ed). Guide to potato production in South Africa. ARC-Roodeplaat, Pretoria, South Africa.

Wallin, N-B. 2006. From famine to fries. The potato has come a long way. YaleGlobal Online. The Potato. http://yaleglobal.yale.edu/about/potato.jsp.

CHAPTER 2

Literature review

2.1 Origin and genetics of potatoes

2.1.1 Wild potato species

The potato belongs to the *Solanaceae* family and was first cultivated as a staple diet in Peru almost 8 000 years ago (Wallin, 2005). During the 16th century the potato was introduced to Europe as a curiosity from the South American mountains where the potato originated (Beukema and Van der Zaag, 1990). The popularity of this vegetatively grown crop contributed to its production in the 17th century. In the 19th century, potatoes were already an important food crop and introduced to several tropical and subtropical countries by colonists from Europe (Beukema and Van der Zaag, 1990).

Some wild potato species and the regions where they commonly occur are listed in Table 2.1. The fact that these species are adapted to an extraordinary wide range of habitats, explains the way in which they have become tolerant to stress environments and their development of resistance to a wide range of pests and diseases. There are 199 wild potato species (Spooner and Hijmans, 2001) that may have traits, which can be useful for cultivated crop improvement.

Solanum tuberosum L., the most widely cultivated potato, has evolved under a very limited range of environmental conditions and is unable to resist such a wide range of environmental stresses in its current habitat (Bradshaw and Mackay, 1994).

Table 2.1 Natural growth habitat of some wild potato species (Bradshaw and Mackay, 1994).

REGION	CONDITION OF GROWTH HABITAT	WILD POTATO SPECIES
Andean mountains	frost common	*S.acaule, S.megistacrolobum*
	dry semi desert	*S.berthaultii, S.tarijense, S.neocardenasii*
	cool temperate rain forests	*S.violaceimarmoratum, S.colombianum*
Argentina	coastal plains	*S.commersonii, S.chacoense*
Mexico and USA	cactus deserts	*S.stoloniferum, S.jamesii*
	cool temperate pine and Abies forests	*S.brachycarpum, S.demissum, S.verrucosum*
South America	Woodlands	*S.vernei, S.microdontum*

A major problem with cultivated varieties is their sensitivity to low temperatures (Li and Palta, 1978). They possess very little or no frost tolerance, while some wild species like *S. acaule*, S. *chomatophila* and *S. commersonii* are considered to be frost tolerant (Li, 1977; Vega and Bamberg, 1995). These wild species withstand slight night frost in their original habitat (Hawkes, 1958; Hawkes and Hjerting, 1969).

Hijmans *et al*. (2003) analyzed the extent to which taxonomic, geographic and ecological factors can explain the presence of frost tolerance in wild potatoes. They found a greater chance of finding wild potatoes with high levels of frost resistance in regions where they originated with an annual mean temperature below 3°C than in warmer areas (such as the central and South Peruvian Andes and lowlands of Argentina).

Although the origin of the common potato, *Solanum tuberosum* subsp. *tuberosum* is obscure, it is not known from where and when the potato was first introduced into Europe (Hawkes, 1978a). What is known, is that it is adapted to cool temperate climates (Hawkes, 1978b).

Wild potato species, growing naturally from near sea level to above 400m, show a wide range of genotypic adaptation to heat, non-freezing and cold stresses (Hetherington *et al*., 1983).

Evolution in the tuber-bearing *Solanums* has taken place in such a way that the use of wild potato species in breeding programs are of great interest. To the plant breeder, potatoes with genetic characters such as resistance to pathogens, viruses, nematodes, Colorado beetle and frost (Hawkes, 1958) are available, some more readily than others.

2.1.2 Genetics

The potato evolved at the diploid level (2n=2x=24) (Beukema and Van der Zaag, 1990). During the late 1930's geneticists recognized *S. tuberosum* (the principal cultivated potato species) to be in fact tetraploid, and that it displays tetrasomic inheritance (Cadman, 1942). Most commercial potato cultivars are tetraploids (2n=2x=48), derived from *Solanum tuberosum var. andigenum*. Some diploid potatoes are also cultivated (Beukema and Van der Zaag, 1990).

Potatoes at the tetraploid level seem to have the highest yield potential (Beukema and Van der Zaag, 1990) while diploid cultivars appear to be self-incompatible.

The cross-pollinated crop has far more complex genetics than self pollinating diploids (Watanabe *et al*., 1997). Because of its small and relatively numerous chromosomes, the cultivated potato (*Solanum tuberosum* L.) is generally regarded as cytologically difficult species to study (Bradshaw and Mackay, 1994). The potato has short chromosomes and a low chiasma frequency (1.0-1.68 in diploid species), resulting in a block gene transfer with the consequence that deleterious genes may sometimes need a number of backcrossing generations to be removed, especially if they are situated near useful genes which breeders wish to retain (Hawkes, 1958). In tetrasomic inheritance, segregation is far more complicated than with disomic inheritance, while selection can be laborious and less cost effective compared with diploid selection (Watanabe, 2002).

Diploid, tetraploid and hexaploid potato species are primarily sexually fertile while odd-numbered polyploids are sterile (tri-and pentaploids), since their sterility is genetically determined (Beukema and Van der Zaag, 1990). The knowledge of the existing chromosome number of different potato species simplify interspecific crosses to the potato breeder.

Tetraploid *Solanum tuberosum* contain by far the greatest degree of variability, including tuber shape, colour, texture and biochemical composition but with a small range of disease and frost resistance (Hawkes, 1958). Some wild potato species on the other hand, show increased levels of resistance to a wide range of characters such as disease and frost tolerance, a higher vitamin C and protein content, all of which can be usefully introduced in *S. tuberosum*.

Wild tuber bearing *Solanum* species are therefore of considerable interest to potato breeders because of their resistance to pests and pathogens as well as their adaption to climatic extremes. Native types also show resistance against some physiological properties such as tuber dormancy, time of maturity, chemical composition sources and photoperiodic responses and therefor valuable sources of allelic diversity are necessary to improve the narrow genetic base of the cultivated potato. The qualities of these wild potato traits are of direct advantage to potato breeders.

2.2 Effect of low temperature stress on potato plants

Potato plants are poikilotherms, assuming the temperature of their immediate environment and therefore low temperature resistance must be due to tolerance. Plants can thus not avoid low temperatures, but some may be able to adapt hereto.

To understand the effect of low temperature stress on plants, it is necessary to know that exposure to low temperatures can induce two different types of injury to plants, namely chilling injury and freezing injury (Levitt, 1972).

As early as 1897, Molisch suggested that low temperature levels in the absence of freezing, should be called 'chilling injury'. Chilling temperatures can be defined as any temperature that is low enough to produce injury to the plant, but not low enough to freeze the plant (temperatures above the freezing point of water). Freezing injury on the other hand, occurs only if the environmental temperature is below 0°C (freezing point of water) and may be defined as the freezing potential of the environment to induce injury to plants (Levitt, 1972).

Plants from tropical or subtropical climates such as rice (when in flower) and sugar cane, may suffer chilling injury at 15°C (Adir, 1968; Tsunoda *et al*., 1968). South Africa on the other hand, has a semi-arid climate where both extremely high and low temperatures occur. Frost killing of the haulm of the potato plant, affects the foliage and limits the growing season (Bradshaw and Mackay, 1994). Such freezing-induced cellular dehydration is the most wide spread cause of damage in potatoes when environmental temperatures drop below 0°C.

2.2.1 The freezing process

Cultivated potato species survive temperatures down to -3°C. At night, when environmental temperatures drop below -3°C (below the freezing point of water 0°C), a rapid spread of a low intensity thermal signal occurs in the plant tissue, resulting in the formation of ice crystals in extra cellular spaces and the freezing of leaf water (Bradshaw and Mackay, 1994; Pearce, 2001).

According to Levitt (1972) ice crystals first form in large vessels of leaves from where freezing proceeds along the vessels from a few nucleation points. Once ice had been formed in the vessels, it spreads through internal cellular spaces to

the living cells of the plant. Crystals form at the expense of water vapour in the air and of the amount of surface of water on the cell walls (Levitt, 1972).

Ice formation within the vessels results in intercellular ice formation, causing the vapour pressure in intercellular spaces to drop sharply (Levitt, 1972). As the tissue temperature drops below the freezing point of the cell contents, the vapour pressure will be higher than that of internal cellular ice, causing water to diffuse to the intercellular ice through the plasma membrane. Ice crystals on external surfaces of the cell wall grow, while the cell itself contracts due to water loss (Levitt, 1972; Li, 1985). Since the water potential of ice is lower than that of liquid water, extra cellular ice crystals grow by drawing water from surrounding cells (cytoplasm) until the water potential of the ice and cell is equal. This results in dehydrated cell content where the water potential of the ice falls as the environmental temperature falls (Gusta *et al*., 1975).

As the temperature drops at a constant rate, diffusion of cell water to external ice loci will continue, resulting in a steady increase in the cell sap concentration. A major part of the plant tissue will now consist of intracellular spaces filled with ice (Levitt, 1972).

According to Franks (1985) recrystallization may occur, which is the growth of larger ice crystals at the expense of smaller ice crystals which lead to the formation of large ice masses. This process is favored by prolonged exposure to moderate and high freezing temperatures as readily happens in nature. Ice masses, creating damage, can separate cell layers and create cavities such as the separation of the epidermis from underlying tissues (Levitt, 1980).

The cell membrane keeps extracellular ice out of the cell, leaving the cell in a supercooled state (not frozen) (Pearce, 2001). Kitaura (1967) assumed that the appearance of hoar frost on leaves may inoculate the internal tissues rather than to cause spontaneous ice formation in the vessels. If ice forms at a sufficient low

temperature due to rapid cooling, Mazur (1963) calculated that ice crystals may be small enough to penetrate the plasma membrane and therefore induce intracellular freezing.

Cold induced dehydration may cause leaf tissue to exhibit irreversible damage, even if ice crystals do not form (Sukumaran and Weiser, 1972). Nevertheless, under normal frost conditions, ice melts before damage to the cell can take place, but if the temperature falls further and frost is severe, dehydration of the cell occurs and cells may freeze, causing death to the plant (Pearce, 2001).

2.2.2 Frost injury and physical changes

Plants that are subjected to chilling injury (as well as some that are not) are usually killed by the first occurrence of frost (Molisch, 1897). Scholander *et al.* (1953) on the other hand, noted that some plants that are native to cold climates may be frozen solid at low temperatures without any signs of injury occurring. Nevertheless, freezing temperatures result in damage to plants of all gradations, in some cases reducing photosynthetic areas (leaves), delaying maturity or even killing the entire plant.

After a night of severe frost, potato plants are characterized by a water-soaked, dark green appearance due to infiltration of the intercellular spaces with liquid (frost water/ice crystals) and the lost of turgor due to cellular dehydration (Figure 2.1).

Levitt (1972) defines freezing injury in two distinct classes: indirect injury occurs when extracellular freezing take place (a), (since there is no direct contact between the ice crystals and the protoplasm) and direct injury (b) due to the presence of ice in the protoplasm (intracellular freezing). It can thus be said that extracellular ice formation (freezing) leads to intracellular freezing that results in cell dehydration.

Fig. 2.1 Appearance of a frost damaged potato plant (left) and a control plant (right). The leaves of frost damaged plants appear dark green, water soaked and stiff due to the lost of turgor.

Ice crystals are formed intracellular, damaging the protoplasmic structure and membranes (Palta and Li, 1980). The dehydration exerts mechanical stress on the cell membranes (Iljin, 1933) due to the contraction of the cells resulting in damage to the plasma membrane caused by non-uniform stretching or compressing. In severe cases, plant cells die while the plasma membrane becomes freely permeable (Levitt, 1972).

Cell walls are more elastic and can return rapidly to their original position, putting pressure on the plasma membrane at sites of cell wall-membrane attachments (Li, 1985).

Besides cell membrane and wall changes, a number of other changes have been observed in cells due to freezing temperatures. A damaged membrane structure may cause electrolytes and other solutes leakage (Stout *et al*., 1980). Palta and

Li (1980) noticed a swelling of the protoplasm, mitochondria and chloroplasts in *S. tuberosum* and *S. acaule* in plant cells at freezing temperatures lower than the initiation of damage. The membrane lipid composition also altered (Steponkus *et al*., 1988) while some solutes such as sugars, proline, betaines (Xin and Brows, 2000) and proteins, also called dehydrins, (Close, 1996) may accumulate.

Frost damaged plants may die days or even hours after exposure to freezing temperatures that caused injury. Frost injury may sometimes be reversible according to the severity. If tissue is uninjured, the cell reabsorbs the intercellular water and regains turgor while air enters the spaces so that the water-soaked appearance is quickly lost. Injured cells are unable to reabsorb water (Wiegand, 1906).

2.2.3 Cold acclimation and hardening

Cold acclimation is the ability of a plant to become cold tolerant upon exposure to low non-freezing temperatures (Levitt, 1972; Levitt, 1980) by alteration of their tissue and cellular freezing tolerance. Cold acclimation is therefore the outcome of biochemical and physiological processes associated with the increase in cold tolerance (Guy, 1990).

Hardening of a plant is normally accompanied by an acclimation of one or more substances synthesized by the plant. Plants can be hardened by exposing them for a couple of weeks to temperatures a few degrees above the freezing point (Levitt, 1972). Chen and Li (1976) mentioned that cold acclimation of potato plants can be achieved within three weeks by stepwise lowering of temperatures. Direct exposure of plants to constant day-night low temperatures (Chen *et al*., 1979) can cause cold acclimation within two weeks.

Tuber bearing *Solanum* species can be classified according to Chen and Li (1980a) into five groups on the basis of their ability to acclimate cold and on the basis of leaf frost hardiness (Table 2.2).

1. Frost resistant potato species - able to cold-acclimate
2. Frost resistant potato species - unable to cold-acclimation
3. Frost sensitive potato species - able to cold-acclimation
4. Frost sensitive potato species - unable to cold-acclimation
5. Chilling sensitive potato species

According to Li (1977) the major difference between tender (cultivated) and hardy (wild) potato species is their ability to tolerate more frozen water at frost killing temperatures.

Low temperature exposure is not the only way known to induce freezing tolerance in plants. The treatment of stem and cell cultures and seedlings with Abscisic acid (ABA) at non-acclimating temperatures can change their freezing tolerance (Chen *et al*., 1979; Chen and Gusta, 1983). Limited desiccation can also increase the freezing tolerance of plants (Levitt, 1951; Chen *et al*., 1975).

The cold-acclimation process in any plant include major changes in plant functions such as the adjustment of the metabolism and basic cellular functions to the biophysical constraints imposed by low temperatures leading to the development of freezing tolerance (Guy, 1990).

An increase in frost hardiness in potatoes initiates after three days of cold acclimation (Chen and Li, 1982). When cold-acclimated plants are exposed to freezing temperatures, it shows a negative effect on the developed cold-acclimation.

Table 2.2 The classification of tuber-bearing *Solanum* species on their ability to cold-acclimate and on the basis of frost hardiness of the leaves (Chen and Li, 1980a).

Categories and species	Killing temperature (°C)	
	Before acclimation	After acclimation
Group 1: **FROST RESISTANT**		
ABLE TO COLD-ACCLIMATE		
S.acaule	-6	-9
S. commersonii	-4.5	-11.5
S. multidissectum	-4	-8.5
S. chomatophilum	-5	-8.5
Group 2: **FROST RESISTANT**		
UNABLE TO COLD-ACCLIMATE		
S. bolviense	-4.5	-4.5
S. megistacrolobum	-5	-5
S. sanchae-rosae	-5.5	-5.5
Group 3: **FROST SENSITIVE**		
ABLE TO COLD-ACCLIMATE		
S. oplocense	-3	-8
S. polytrichon	-3	-6
Group 4: **FROST SENSITIVE**		
UNABLE TO COLD-ACCLIMATE		
S. brachistotricum	-3	-3
S. cardiophyllum	-3	-3
S. fendleri	-3	-3
S. jamesii	-3	-3
S. kurtzianum	-3	-3
S. microdontum	-3	-3
S. pinnatisectum	-3	-3
S. stenotomum	-3	-3
S. stoloniferum	-3	-3
S. sucrense	-3	-3
S. tuberosum	-3	-3
S. venturii	-3	-3
S. vernei	-3	-3
S. verrucosum	-3	-3
Group 5: **CHILLING SENSITIVE**		
S. trifidum	-3	dead

De-acclimation (the loss of freezing tolerance) in some species appear to be a more rapid process than cold-acclimation. The rate of de-acclimation varies with the degree of temperature rise, the exposure period to warm temperatures and the genetic composition of the plant. Frost hardiness will decline in one day to the pre-acclimating level of the plant when cold-acclimated potato plants are exposed to a warm temperature regime (20°C day/night) (Chen and Li, 1980a). According to these editors de-acclimation is initiated at about 2-3h after exposure to warm temperatures. Other plants such as winter cereals and apple twigs require several days for complete de-acclimation (Howell and Weiser, 1970; Gusta and Fowler, 1976).

2.3 Effect of freezing temperatures on the growth, development and yield of potato plants

The potato is a member of the *Solanaceae* family, including crops such as tomato, pepper, tobacco and eggplant as well as some weeds and alkaloid drug plants. Some ornamental plants like *Petunia* and *Schizantus* also belong to this family of plants. Most of the plants belonging to this plant family are produced from true seed. The potato however, differs from the rest and grows vegetatively from tuber 'seed' pieces.

Potato plants grow from tubers and develop adventitious roots at the nodes of the underground stems and stolons. The plant generally roots shallowly. According to Rowe (1993) the development and growth cycle of the potato can be divided into five distinct life stages (Figure 2.2).

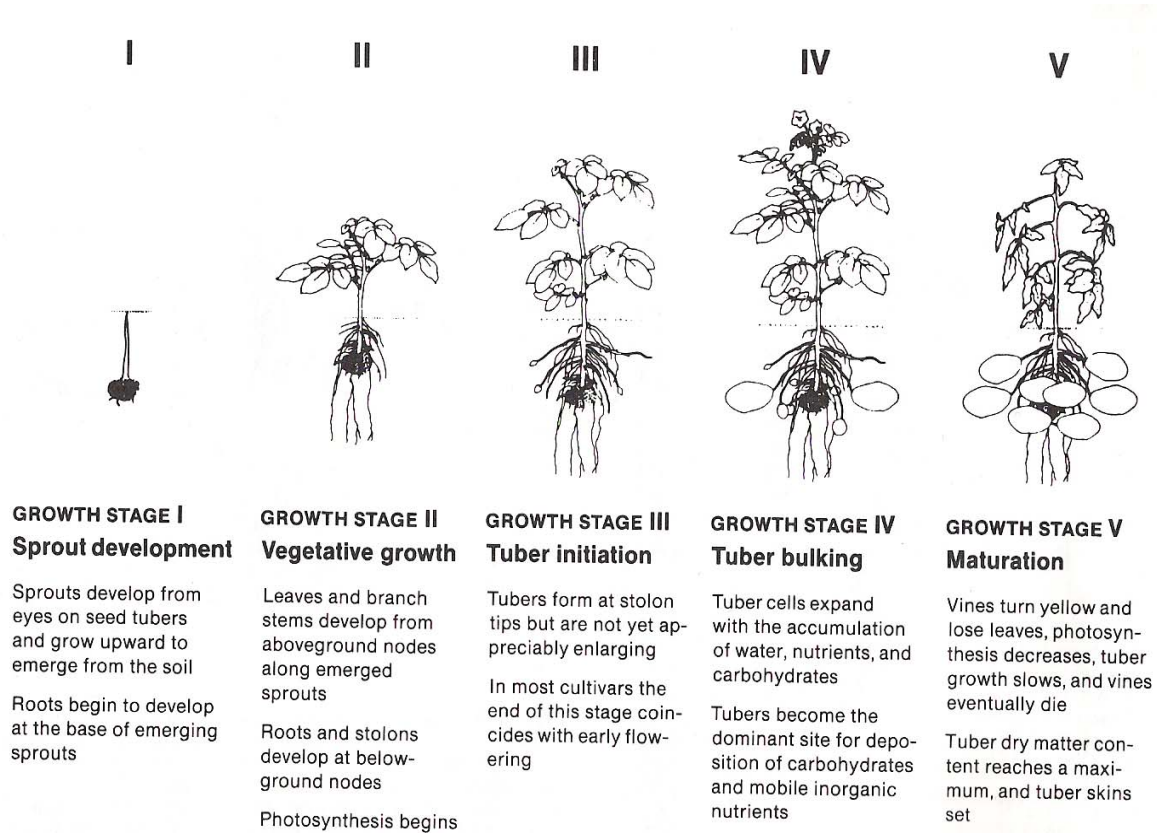


Fig. 2.2 Growth stages of the potato (Rowe, 1993).

Although the cultivated potato *S. tuberosum* is known to grow well in cool temperate environments, with a optimum temperature range of 20 – 25°C for normal growth (Smith, 1977; Beukema and Van der Zaag, 1990), it is frost sensitive (Li, 1977).

Frost or freezing temperatures may occur during any growth stage of a potato crop and as a result induce non-lethal or lethal injury to the stems and leaves of the plants. In contrast with most crops, potatoes respond differently to frost damage since tubers are carried below ground and are not affected directly. Especially early in the season, during spring and late autumn, frost can be detrimental to this crop.

Visually, frost damaged potato plants show a wilted appearance, with dark green, water-soaked leaves and stems (Figure 2.3). Damaged plant material may turn yellow (chlorosis) after some days and finally to brown (necrotic).

Fig. 2.3 An example of frost damage during the early reproductive growth stage of potatoes. Some plants show greater tolerance (plants on the right) to freezing temperatures than others (on the left).

2.3.1 Growth Stage 1 - Sprout development

Soil temperature has been shown to have a great affect on the emergence rate of potato plants. If soil temperatures are below 10°C, growth will be very slow if at all, while pathogens may attack the seed piece (Dean, 1994). Sprouts develop from the eyes on the seed tubers, growing upwards to emerge from the soil. Tiny roots develop at the base of the emerging sprouts. Because the plant at this stage is unable to photosynthesize, the seed piece serves as an energy source for further growth.

Water management prior to crop emergence is important. Post plant irrigation, before sprouts emerge, is usually not advisable since saturated soils favor seed piece decay (*Pythium)*.

Simulated defoliation on emerging potato plants has been studied to detect its relationship to yield loss (Shields and Wyman, 1984). Young potato plants showed a decreased sensitivity according to yield loss compared to plants defoliated at later growth stages. These authors suggested that defoliation may disrupt the process of tuber initiation but plants recover quickly by regrowth from auxillary buds that remain undamaged on stems.

2.3.2 Growth Stage 2 - Vegetative growth

Branch stems and leaves develop from above ground nodes along emerging sprouts. Stolons (under ground stems) and roots develop at below ground nodes. As the plant grows and leaves develop, photosynthesis produce carbohydrates as a source of energy for further plant growth and development. This stage, where all vegetative parts of the plant form, starts at plant emergence and lasts until stolon tips start to swell.

During vegetative growth, roots start to develop, absorbing nutrients from the soil. For the potato plant, 75%-85% available soil water is preferable during vegetative growth (Rowe, 1993). Adequate hilling before row closure is an important cultivation practice during this growth stage. Hilling ensure that tubers initiated during stage three be sufficiently covered with soil to avoid damage (greening and sunscald).

The sensitivity of potato plants to defoliation during vegetative growth may differ, since short, medium and long growth cultivars may require different length of periods to complete tuber formation. A transition in the utilization of resources between sites in the plant occur during the vegetative and reproductive growth

phase. The majority of sources swift toward flowering and tuber growth (Sparks and Woodbury, 1967).

2.3.3 Growth Stage 3 - Tuber initiation

Stolons are underground stems, developing firstly at the basal nodes of the stem (closest to the seed piece) and then developing progressively upward. Lateral buds can potentially develop either in a shoot or as a stolon to produce a tuber. Exposure to light may cause stolons (aerial tubers) to become green and to convert to leaf axils (shoots) (Figure 2.4). High temperatures during development may also cause stolons to migrate to the soil surface and become shoots. Normal stolon growth results in tuber formation.

The number of eyes in a tuber varies depending on the size of the tuber and growth conditions (Beukema and van der Zaag, 1990). Tubers form at stolon tips and enlarge during growth stage three (Figure 2.5). This growth stage is the shortest and lasts for almost two weeks, while most cultivars end this period to coincide with early flowering. Tuber initiation starts when stolon tips enlarge into tubers (Figure 2.5). Tubers (underground stems) are adapted to store food and to serve as a source of vegetative reproduction.

Fig. 2.4 When stolons migrate upwards and became exposed to light they convert to leafy shoots.

High temperatures delay and may even prevent tuberization, explaining why potato production regions are concentrated in relatively cool growing areas (Dean, 1994). According to Winkler (1971) the tubers furthest from the foliage (deepest in the ground) usually obtain the largest size.

This short, tuber initiation growth stage requires proper water management, to ensure the health of the developing crop. Eighty to ninety percent of available soil water must be maintained at growth stages three and four to favor rapid plant growth. Nitrogen is often applied with irrigation water, but application during tuber bulking is most effective (Rowe, 1993).

Defoliation during full bloom results in the greatest yield loss (Shields and Wyman, 1984). Sensitivity to defoliation in this growth stage indicates a continued resource shift while most resources are being directed underground, toward tuber growth.

Partial or complete freezing damage to potato plants annually results in extensive reductions in potato yield. The reduction in potato yield is thus the greatest if frost occurs just after flowering or during tuber initiation when the plant is most sensitive to damage (Beresford, 1967; Harris, 1978; Shields and Wyman, 1984; Beukema and Van der Zaag, 1990). Damage is cultivar specific since different cultivars show sensitivity at different growth stages (Takatori *et al*., 1952; Snyder and Michelson, 1959; Murphy and Goven, 1962).

2.3.4 Growth Stage 4 - Tuber bulking

Tuber cells expand due to accumulation of water, nutrients and carbohydrates. During tuber bulking the developed tubers become the dominant site for deposition of carbohydrates and mobile inorganic nutrients within the plant (Rowe, 1993).

Fig. 2.5 During tuber initiation, stolon tips start to swell.

Moisture stress during the tuber bulking period may increase sensitivity, causing the potato plant to die early. Over night irrigation must be avoided to prevent excessive movement of nitrates below the root zone as well as the erodation of planting hills that cause low-set tubers to be exposed causing greening and sunscald. The incidence of leaf diseases may be reduced by management of the plant canopy to reduce the duration of leaf wetness. Insect and disease management should be at peak activity during this growth stage.

The optimum range for tuber growth is between 15°C - 20°C. As temperatures rise to 27°C, tuber dry mass and tuber number decrease (Yamaguchi *et al*., 1964). High temperatures increase aging (senescence).

Moisture stress reduces plant and tuber growth, resulting in a decrease in yield. If moisture stress occurs during tuber bulking, growth conditions of tubers may result in abnormally-shaped tubers (Robins and Domingo, 1956).

In this growth stage, defoliation results a reduced in sensitivity to the plant according to yield loss (Shields and Wyman, 1984). A possible reason for this decrease in sensitivity may be that potato plants in their full-grown phase show diminished foliage growth (Sparks and Woodbury, 1967).

2.3.5 Growth Stage 5 - Maturation

During this growth period photosynthesis gradually decreases as vines turn yellow and lose leaves. The tuber growth rate slows and the vines eventually reach a maximum dry matter content while tuber skins thicken ('set'). The amount of water and nitrogen applied to the crop should be reduced. Excessive moisture during the later stages of maturation of the potato plant may result in sprouting of mature tubers.

Adequate water should be available throughout the growth and development of a potato crop. Uniform soil moisture minimizes plant stresses that lead to reduced tuber yield and quality. Irrigated potato fields should never be allowed to dry below 60%-65% of field capacity (Rowe, 1993).

Compared to earlier growth stages, simulated defoliation of mature potato plants results in reduced sensitivity to cold stress as indicated by a reduction in yield loss (Shields and Wyman, 1984).

2.4 Metabolic changes and alterations in protein expression in potatoes caused by exposure to freezing temperatures

Metabolic and biochemical responses of plants at low temperatures have been correlated but no understanding of how cold-acclimation leads to an increased frost tolerance could be reached (Steponkus, 1984). Studies on cold-acclimation focused on some rapid physiological and molecular responses when plants are exposed to low temperatures. These studies provide new insight to the cold-acclimation process.

2.4.1 Sugars

According to Levitt (1956) the sugar content of tissue is commonly proportional to its freezing tolerance. Sugars normally increase in the fall as plants harden and decrease in the spring as they deharden. An increase in sugar content of

potatoes during cold-acclimation can also be associated with increased frost hardiness (Levitt, 1980). Chen and Li (1980b) identified a starch and sugar increase occurring simultaneously during cold-acclimation whereas the increase in sugars was greater in cold-acclimated potato species such as *S. commersonii* than in non-acclimating potato species such as *S. tuberosum*.

The importance of sugar accumulation in the development of freezing tolerance at exposure to low temperatures has been demonstrated by the fact that tolerance is lost if sugar accumulation is blocked (Guy *et al*., 1980).

Li and Palta (1978) mentioned that the leaf water content of *Solanum* species decreases during an increase in cold-acclimation. However, there is no relationship with increased frost hardiness. This may be partly due to displacement of water in the cell when sugars accumulate. Water content therefore is frequently inversely related to cold hardiness (Levitt, 1956).

2.4.2 Abscisic Acid

Li *et al*. (1989) suggested that ABA is a signal transducer, which transmits environmental signals such as low temperatures into biochemical responses of plants. Chen *et al*. (1983) observed that ABA could induce frost hardiness in potato plants while the ABA content increases when plants are subjected to acclimating temperatures. The elevation of the osmotic concentration triggers the endogenous ABA to increase. During cold-acclimation an increase in ABA only occurs in *S. commersonii* (able to cold-acclimate) but not in *S. tuberosum* (unable to cold-acclimate) (Chen *et al*., 1983). When exogenous ABA is applied to plants, frost hardiness has been shown to increase (Chen *et al.*, 1979; Tseng and Li, 1991) not only in potato plants, but also in other crops such as winter wheat, rye and bromegrass (Chen and Gusta, 1983).

Exogenous application of ABA can substitute for low temperature exposure (Chen *et al*., 1975; Lee *et al*., 1992) whereas desiccation is similar to extracellular freezing (i.e. the removal of water from the cell) of plants.

2.4.3 Membranes

The biochemical and physical restructuring of cell membranes is a well known feature of plants when cold acclimating take place. According to Steponkus (1984) the plasma membrane is considered to be the primary site of freezing injury and cold acclimation. Reconstruction within the lipid components of the protoplast membranes of cold hardened rye was shown to change the cryostability of the plants (Steponkus and Lanphear, 1968).

2.4.4 Lipids

Levitt (1941) noted a correlation between lipid content and hardiness. Cold-acclimated *S. acaule* showed a total lipid increase during cold-acclimation, while non-acclimated *S. tuberosum* did not (Chen and Li, 1980b). The increase in phospholipids suggest that the development of frost hardiness in the wild potato species, *S. acaule* could be associated with changes in membrane properties.

2.4.5 Cryoprotectants

Exposing plants to low temperatures may cause the accumulation of low molecular weight compounds with a cryoprotectant activity (Guy *et al*., 1980; Chen and Li, 1980b) such as disaccharide and trisaccharide sugars, polyol, sorbitol, quaternary ammonium compounds, glycinebitaine, praline and polyamines. They may function as cryoprotectants by sustaining the ordered vicinal water around proteins (Yancey *et al*., 1982) or to stabilize membranes (Anchordoguy *et al*., 1987).

2.4.6 Proteins

Much research has been done on the hardening and cold acclimation of potato plants and plantlets (Tseng and Li, 1990; 1991; Lee *et al*., 1992; Ryu and Li, 1994). Low temperatures stimulate the production of free abscisic acid (ABA) in potato plants (Chen *et al*., 1983). The increased ABA concentration leads to changes in gene expression (Tseng and Li, 1990; 1991), while inducing the synthesis of certain protein species (Gayler and Glasziou, 1969). According to Tseng and Li (1990) the cold acclimation process in potato plants require the *de novo* synthesis of proteins.

Chen *et al*. (1983) used cycloheximide (CHI), a cytoplasmic protein synthesis inhibitor, to indicate that low temperatures trigger a change in endogenous ABA which induces the synthesis of proteins resulting in the development or frost hardiness. This inhibitor blocks protein synthesis, resulting in no increase in ABA levels of the plant, causing cold hardiness not to develop (Ryu and Li, 1994).

At low temperatures cold-acclimated tissue can synthesize proteins faster than non-acclimated tissue (Guy *et al*., 1985). Some studies indicated that new proteins can appear within one day and sometimes an hour after exposure to low temperatures (Guy and Haskell, 1987). Tseng and Li (1990) identified at least 23 cold induced polypeptides (CIPs) that were newly synthesized during 14 days of acclimation of potatoes. The appearance of CIPs may probably be the result of *de novo* protein synthesis.

Chen and Li (1980b) observed a linearly related increase in total soluble proteins during cold-acclimation in cold acclimated potato species (*S. acaule and S. commersonii*) with a net increase in frost hardiness. This relationship was not observed in non-acclimated *S. tuberosum* after the same procedure of cold treatment was used. *S. acaule* and *S. commersonii*, the wild potato species, which are able to cold acclimate showed an increase in soluble protein (Figure 2.7) parallel to the increase in cold hardiness. The hypothesis that soluble

protein(s) are responsible for the increase in hardiness may exist. In contrast, *S. tuberosum*, the cultivated potato which is unable to synthesise proteins at low temperatures (Figure. 2.6), explains why it can not be hardened. Chen and Li (1980b) concluded that their results support the hypothesis that nucleic acid and protein metabolism are involved in the process of plant hardening of *Solanum* potatoes.

At hardening temperatures, both soluble proteins and 4S RNA may accumulate in potato foliage, although no true tolerance is developed (Li and Weiser, 1969). Cox and Levitt (1976) suggested that only those plant species that have the ability to conduct active protein synthesis at low temperatures have the capability to cold-acclimate.

Numerous studies using electrophoretic separation of proteins have shown qualitative and quantitative differences between non-acclimated and cold acclimated plants (McCown *et al*., 1968). New protein species were present in cold acclimated and freezing tolerant plants, while being absent in non-acclimated plants (Kacperska-Palacz *et al*., 1977).

Some enzyme variation has been observed in studies with plants subjected to low temperatures, compared to plants that were maintained at warmer temperatures. The enzyme ribulose bisphosphate carboxylase/oxygenase purified from freezing sensitive and tolerant potato species demonstrated structural differences during tolerance (Huner *et al*., 1981).

Rorat *et al*. (1997) reported that many different genes are induced in a cold tolerant potato *Solanum sogarandinum* during cold acclimations. These genes are suspected to be involved in different cellular functions during cold resistance, some protecting the chloroplast and cell functions under cold condition, while others may be involved in metabolic adjustments to cold.

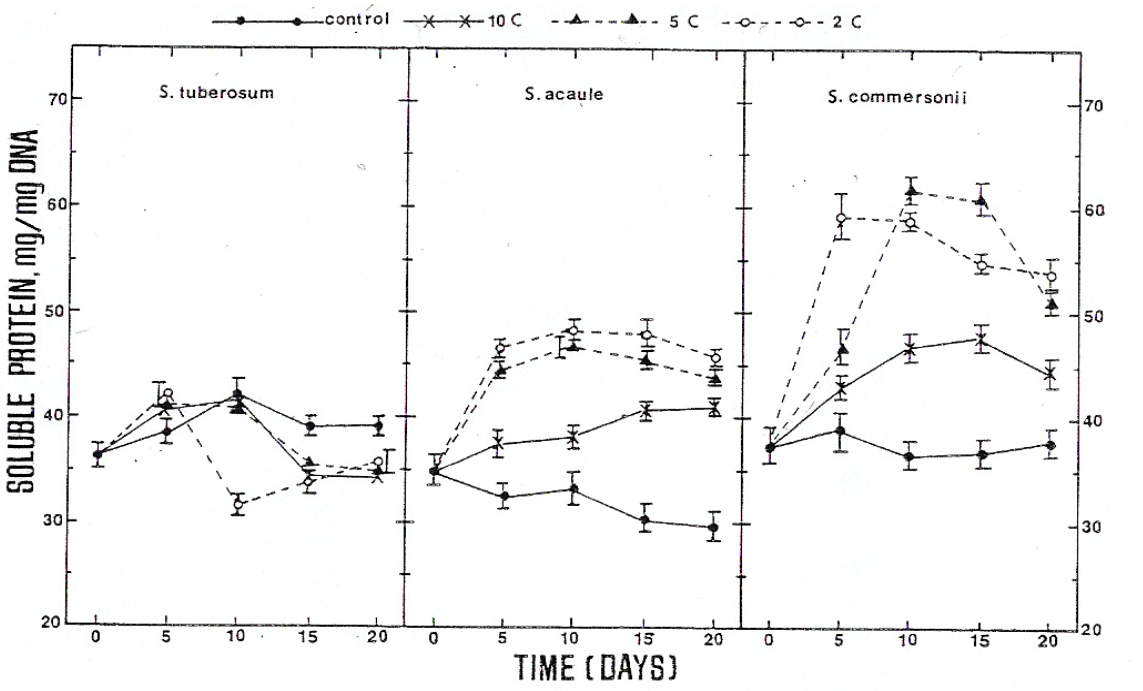


Fig. 2.6 Soluble protein changes in potato leaves exposed to low temperatures during cold acclimation (Chen and Li, 1980b).

2.4.7 Other alterations observed

According to Molisch's hypothesis, starvation may also lead to a plant's death at chilling temperatures, since the respiration rate may exceed the rate of photosynthesis (Molisch, 1896). Starvation may also occur in cases where carbohydrates are broken down more rapidly than they are synthesized when chilling sensitive plants are exposed below the compensation point at chilling temperatures (Selwyn, 1966).

Some research showed a protein breakdown (proteolysis) without an equally resynthesis at low temperatures, that may cause injury to plants (Levitt, 1972). According to Pentzer and Heinze (1954) low temperature exposure causes a disturbance in the normal balance of biochemical processes of the plant, resulting in the accumulation of a cell toxin that causes injury to plants.

2.5 Inheritance studies on yield of potato

The late blight attack during 1845 that led to the Irish Famine was the main trigger for breeding potatoes resistant to this dreaded disease. This was also probably the first occasion where breeding for resistance such as to plant diseases was initiated (Alleman *et al*., 2004). Severe disease outbreaks that threaten manhood have not only stimulated breeding activities but also the collection of new germplasm from the place of origin (Beukema and Van der Zaag, 1990).

Compared to other crops, potato breeding showed slow progress since 1960 (Glendinning, 1983) and appears to be relatively less successful over the years, primarily attributed to the genetic complexity of the potato and its narrow genetic base (Simmonds, 1969) from which parents originate.

Due to the complex tetraploid genetic composition and quantitave nature of breeding targets, it still appears to be a challenge for breeders to introduce wild potato genomes into cultivated potato species. The high degree of ploidy makes the potato genetically diverse, but also difficult to create new cultivars (Dean, 1994).

In potato breeding, the genotype remains constant from generation to generation since the potato propagates vegetatively. Any selection at the F1 stage of breeding will stay true to type while breeding targets can be gained in one generation of selection on single traits. Yet most traits are quantitatively inherited (potato leaf roll virus, tuber quality traits and yield components), making conventional breeding inefficient because of the slow progress. According to Hawkes (1945) pollen sterility is the main technical problem for potato breeders. Other hindrances are self-incompatibility of diploid species or complete sterility of others. Self-incompatibility of potatoes appears to be incomplete since some species show self-fertility and self-sterility under different conditions (Hawkes, 1945). Some potato genotypes do not flower or their buds and flowers drop

(abscission) before being fully opened. Parents to be crossed have to flower over a sufficient length of time. Ovule sterility may also hamper the process of incorporating wild potato genes into cultivated potato species.

2.5.1 Breeding for frost resistant potatoes

Wild potato species growing naturally at high latitudes show resistance to frost. Frost resistance for potatoes in the *Solanum* family was first discovered by Russian breeders. *S. acaule* is a small compact wild potato plant, with narrow leaflets and a compact growth habit (Mastenbroek, 1956). This potato can withstand temperatures of -8°C to -10°C and is known as the most resistant type of potato (Razumov, 1935).

Limited success has been achieved to improve frost resistance in potato cultivars over the years (Palta and Simon, 1993). Resistance is generally assumed to be polygenically inherited, though some indications have been found that major genes are also involved (Hawkes, 1958). Freezing tolerance is a complex polygenic trait (Stone *et al.*, 1993; Chen *et al*., 1999) with various components of hardiness that may not necessarily be controlled by the same genes (Palta and Simon, 1993; Stone *et al*,. 1993). Mastenbroek (1956) suggested that cold tolerance depends on a number of dominant genes with quantitative or cumulative effects. He concluded that one or a few major genes and some genes with modified effects might be involved in the inheritance of cold tolerance.

Freezing tolerance and a plant's ability to cold-accumulate are under independent genetic control (Stone *et al*., 1993; Vega *et al*., 2003). Thus to improve frost hardiness successfully, both characters have to be transferred to cultivated potato species (Palta and Simon, 1993). Stone *et al*. (1993) demonstrated that freezing tolerance comprises both non-acclimating freezing tolerance and cold-acclimating capacity.

According to Hawkes (1945), breeders of frost resistant potatoes made primarily use of two wild potato species; *S. acaule* and *S. demissum*. *S. demissum* is crossed easily with *S. tuberosum*, while hybrids also show qualities of blight-resistance. *S. acaule* seems to be more difficult to cross with the cultivated *S. tuberosum* but hybridized more easily with diploid species. Since *S. acaule'*s pollen fails to fertilize other species, it had to be used as the female parent in crosses. Both *S. tuberosum* and *S. acaule* are tetraploid potatoes and therefore difficult to cross because they are widely separated into two quite distinct series (Hawkes, 1945). Hybrids derived from the same series and same chromosome numbers on the other hand seems to nearly always be successful.

Part of *S. acaule'*s frost resistance can be transferred to second backcross hybrids while the best progeny showed a resistance of -5.5°C (Mastenbroek, 1956). Studies showed that cold tolerance of *S. acaule* can not be linked to, or is not closely correlated with other characters (Mastenbroek, 1956).

REFERENCES

Adir, C.R. 1968. Testing rice seedlings for cold water tolerance. *Crop Science* 8: 264-265.

Alleman, J., Laurie, S.M., Thiart, S. and Vorster, H.J. 2004. Sustainable production of root and tuber crops (potato, sweet potato, indigenous potato, cassava) in Southern Africa. *South African Journal of Botany* 70 (1): 60-66.

Anchordoguy, T.J., Rudolph, A.S., Carpenter, J.F., Crowe, J.H. 1987. Modes if interaction of cryoprotectants with membrane phospholipids during freeing. *Cryobiology* 24: 324-331.

Beresford, B.C. 1967. Effect of simulated hail damage on yield and quality of potatoes. *American Potato Journal* 44: 347-354.

Beukema, H.P. and Van der Zaag, D.E. 1990. Introduction to potato production, Pudoc Wageningen, Den Haag.

Bradshaw, J.E. and Mackay, G.R. 1994. Potato Genetics. University Press Cambridge, United Kingdom.

Cadman, C.H. 1942. Autotetraploid inheritance in the potato: some new evidence. *Journal of Genetics* 44: 33-52.

Chen, P., Li, P.H. and Weiser, C.J. 1975. Induction of frost hardiness in red osier dogwood stems by water stress. *Horticultural Science* 10: 372-374.

Chen, P.M. and Li, P.H. 1976. Effect of photoperiod, temperature and certain growth regulators on frost hardiness of *Solanum* species. *Botanical Gazette* 137: 105-109.

Chen, H.H., Gavinlertvatana, P. and Li, P.H. 1979. Cold acclimation of stem-cultured plants of leaf callus of *Solanum* species. *Botanical Gazette* 140: 142-147.

Chen, H.H. and Li, P.H. 1980a. Characteristics of cold acclimation and deacclimation in tuber-bearing *Solanum* species. *Plant Physiol*ogy 65: 1146-1148.

Chen, H.H. and Li, P.H. 1980b. Biochemical changes in tuber bearing *Solanum* species in relation to frost hardiness during cold acclimation. *Plant Physiology* 66: 414-421.

Chen, H.H. and Li, P.H. 1982. Potato cold acclimation. *In*: Li, P.H. and Sakai, A (eds.). Plant Cold Hardiness and Freezing stress. Volume 2. Academic press New York. pp. 5-22.

Chen, H.H. and Gusta, L.V. 1983. Abscisic acid-induced freezing resistance in cultured plant cells. *Plant Physiology* 73: 71-75.

Chen, L.L., Li, P.H. and Brenner, M.L. 1983. Involvement of abscisic acid in potato cold acclimation. *Plant Physiol*ogy 71: 362-365.

Chen, Y-KH., Bamberg, J.B. and Palta, J.P. 1999. Expression of freezing tolerance in the interspecific F1 and somatic hybrids of potatoes. *Theoretical and Applied Genetics* 98: 995-1004.

Close, T.J. 1996. Dehydrins: Emergence of a biochemical role of a family of plant dehydration proteins. *Plant Physiology* 97: 289-290.

Cox, W. and Levitt, J. 1976. Interrelations between environmental factors and freezing resistance of cabbage leaves. *Plant Physiology* 57: 553-555.

Dean, B.B. 1994. Managing the potato production system. Food Products Press, New York.

Franks, F. 1985. Biophysics and biochemistry at low temperatures. Cambridge. Cambridge University Press.

Gayler, K.R. and Glasziou, K.T. 1969. Plant enzyme synthesis: hormonal regulation of invertase and peroxidase synthesis in sugar cane. *Planta* 84: 185-194.

Glendinning, D.R. 1983. Potato introductions and breeding up to the early 20th century. *New Phytologist* 94: 479-505.

Gusta, L.V., Burke, M.J. and Kapoor, A. 1975. Determination of unfrozen water in winter cereals at subfreezing temperatures. *Plant Physiology* 56: 707-709.

Gusta, L.V. and Fowler, D.B. 1976. Effect of low temperature on dehardening and rehardening of winter cereals. *Canadian Journal of Plant Science* 56: 673-678.

Guy, C.L. 1990. Cold acclimation and freezing stress tolerance: role of protein metabolism. *Annual Reviews of Plant Physiology and Plant Molecular Biolology* 41: 187-223.

Guy, C.L. and Haskell, D. 1987. Induction of freezing tolerance in spinach is associated with the synthesis of cold acclimation induced proteins. *Plant Physiol*ogy 84: 872-878.

Guy, C.L., Niemi, K.J. and Brambl, R. 1985. Altered gene expression during cold acclimation of spinach. *Proceedings. National Academy of Science. United States of America* 81: 3673-3677.

Guy, C.L., Yelenosky, G. and Sweet, H.C. 1980. Light exposure and soluble sugars in citrus frost hardiness. *Florida Scientist* 43: 268-273.

Harris, P.M. 1978. The Potato Crop. Chapman and Hall, London.

Hawkes, J.G. 1945. The indigenous American potato and their value in plant-breeding. *Empire Journal of Experimental Agriculture* 13: 11-40.

Hawkes, J.G. 1958. Significance of wild species and primitive forms for potato breeding. *Euphytica* 7: 257-270.

Hawkes, J.G. 1978a. History of the potato. *In*: Harris, P.M. (ed). The potato crop. Chapman and Hall, London. pp. 1-14.

Hawkes, J.G. 1978b. Biosystematics of the potato. *In*: Harris, P.M. (ed). The potato crop. Chapman and Hall, London. pp. 15-67.

Hawkes, J.G. and Hjerting, J.P. 1969. The potatoes of Argentina, Brazil, Paraguay and Uruguay. Oxford University Press, London.

Hetherington, S.E., Smillie, R.M., Malagamba, P. and Huaman, Z. 1983. Heat tolerance and cold tolerance of cultivated potatoes measured by the chlorophyll fluorescence method. *Planta* 159: 119-124.

Hijmans, R.J., Jacobs, M., Bamberg, J.B. and Spooner, D.M. 2003. Frost tolerance in wild potato species: Assessing the predictivity of taxonomic, geographic and ecological factors. *Euphytica* 130: 47-59.

Howell, G.S. and Weiser, C.J. 1970. Fluctuations in the cold resistance of apple twigs during spring dehardening. *Journal of American Society for Horticultural Science* 95: 190-192.

Huner, N.P.A., Palta, J.P., Li, P.H. and Carter, J.V. 1981. Comparison of the structure and function of ribulose bisphosphate carboxylase-oxygenase from a cold-hardy and nonhardy potato species. *Canadian Journal of Biochemistry* 59: 280-289.

Iljin, W.S. 1933. "Uber den kaelrerod de pflanzen und sein ursachen". *Protoplasma* 20: 105-124, as sighted by Li, P.H. 1985.

Kacperska-Palacz, A., Dlugokecka, E., Breitenwald, J. and Wcislinska, B. 1977. Physiological mechanisms of frost tolerance: possible role of protein in plant adaption to cold. *Biologia Plantarum* 19: 10-17.

Kitaura, K. 1967. Supercooling and ice formation in mulberry trees. *In*: Asahina, E. (ed.). Cellular injury and resistance in freezing organisms. (Institution of Low Temperature Science), Hokkaido, pp.143-156.

Lee, S.P., Zhu, B., Chen, T.H.H. and Li, P.H. 1992. Induction of freezing tolerance in potato (*Solanum commersonii*) suspension cultured cells. *Physiologia Plantarum* 84: 41-48.

Levitt, J. 1941. Frost killing and hardiness of plants. Burgess, Minneapolis, Minnesota.

Levitt, J. 1951. Frost drought and heat resistance. *Annual Review of Plant Physiology* 2: 245-268.

Levitt, J. 1956. The hardiness of plants. Academic Press, New York, p. 278.

Levitt, J. 1972. Physiological Ecology. A Series of Monographs, Texts and Treatises. Responses of plants to environmental stress. Academic Press, New York.

Levitt, J. 1980. Responses of Plants to Environmental Stresses: Volume 1, Chilling, freezing and high temperature stresses. Academic Press, New York 2nd Edition.

Li, P.H. 1977. Frost killing temperatures of 60 tuber-bearing *Solanum* species. *American Potato Journal* 54: 452-456.

Li, P.H. 1985. Potato Physiology. Academic Press Inc, London.

Li, P.H. and Weiser, C.J. 1969. Influence of photoperiod and temperature on potato foliage protein and 4S RNA. *Plant and Cell Physiology* 10: 929-934.

Li, P.H., Ryu, S.B., Tseng, M.J. and Chen, T.H.H. 1989. Induction of plant cold hardiness. *In*: Randall, D.D. and Blevins, D.G. (eds). Current topics in plant biochemistry and physiology. Volume 8, University of Missouri – Columbia, MO. pp. 21-40.

Li, P.H. and Palta, J.P. 1978. Frost hardening and freezing stress in tuber bearing *Solanum* species. *In*: Li, P.H. and Sakai, A. (eds). Recent Advances in Plant Cold Hardiness and Freezing Stress: Mechanisms and Crop Implications. Academic Press, New York. pp. 49-71.

Mastenbroek, C. 1956. Some experiences in breeding frost-tolerant potatoes. *Euphytica* 5: 289-297.

Mazur, P. 1963. Kinetics of water ions from cells at subzero temperatures and the likelihood of intracellular freezing. *Journal of General Physiology* 47: 347-369.

McCown, B.H., Beck, G.E. and Hall, T.C. 1968. Plant leaf and stem proteins. I. Extraction and electrophoretic separation of basic, water-soluble fraction. *Plant Physiology* 43: 578-582.

Molisch, H. 1897. "Untersuchugen uber das erfrieren der pflanzen". *Fischer Jena* pp.1-73 as cited by Levitt, J. 1972.

Molisch, H. 1896. "Das erfrieren von pflanzen bei temeraturen uben dem eispunkt". *Sitzber. Kaiserlichen. Akad. Wiss. Wies. Math. Naturwiss. Kl* 105: 1-14. as cited by Levitt, J. 1972.

Murphy, J.J. and Goven, M.J. 1962. The effect of simulated hail damage on yield and quality of potatoes in Maine. *Maine. Agricultural Station. Bulletin* 607.

Palta, J.P. and Li, P.H. 1980. Alterations in membrane transport properties by freezing injury in herbaceous plants: evidence against rupture theory. *Physiologia Plantarum* 50: 169-175.

Palta, J.P. and Simon, G. 1993. Breeding potential for improvement of freezing stress resistance: genetic separation of freezing tolerance, freezing avoidance and capacity to cold acclimate. *In*: Li, P.H. and Christensson, L. (eds). Advances in plant cold hardiness. CRC Press, Boca Raton. pp. 299-310.

Pearce, R.S. 2001. Plant freezing and Damage. *Annals of Botany* 87: 417-424.

Pentzer, W.T. and Heinze, P.H. 1954. Post-harvest physiology of fruits and vegetables. *Annual Review of Plant Physiology* 5: 205-224.

Razumov, V. 1935. Frost resistance of some potato species 1935. Bulletin of Applied Botany, Lenin grad. Ser 3 (6) 221-226.

Robins, J.S. and Domingo, C.E. 1956. Potato yield and tuber shape as affected by severe soil moisture deficits and plant spacing. *Agronomy Journal* 48: 488-492.

Rorat, T., Grygorowicz, W.J., Berbezy, P. and Irykowski, W. 1997. Isolation and expression of cold specific genes in potato (*Solanum sogarandium*). *Plant Science* 133: 57-67.

Rowe, R.C. 1993. Potato health management. APS Press, USA.

Ryu, S.B. and Li, P.H. 1994. Potato cold hardiness development and abscisic acid. II. De novo synthesis of proteins is required for the increase in free abscisic acid during potato (*Solanum commersonii*) cold acclimation. *Physiologia Plantarum* 90: 21-26.

Scholander, P.F., Flagg, W., Hoch, R.J. and Irving, L. 1953. Studies on the physiology of frozen plants and animals in the Arctic*. Journal of Cellular and Comparative Physiology* 42: 1-56.

Selwyn, M.J. 1966. Temperature and photosynthesis II. A mechanism for the effects of temperature on carbon dioxide fixation. *Biochimica et Biophysica Acta* 126: 214-224.

Shields, E.J. and Wyman, J.A. 1984. Effect of defoliation at specific growth stages on potato yields. *Journal of Economic Entomology* 77: 1194-1199.

Simmonds, N.W. 1969. Prospects for potato improvement. Scottish Plant Breeding Station Forty-Eighth Annual Report 1968 -1969. pp.18-38.

Smith, O. 1977. Potatoes: Production, Storing, Processing. 2nd Ed – AVI Publishing Co., Westport, C.T. pp. 342-404.

Snyder, G.B. and Michelson, L.F. 1959. The effect of simulated hail damage on potatoes. *Massachusetts Agricultural Experiment Station Bulletin* 506a.

Sparks, W.C. and Woodbury, G. W. 1967. Stages of potato plant growth. *Idaho Agricultural Experiment Station Bulletin 309.*

Spooner, D.M. and Hijmans, R.J. 2001. Potato systematics and germplasm collecting 1989-2000. *American Journal of Potato Research* 78: 237-268, 395.

Steponkus, P.L. and Lanphear, F.O. 1968. The relationship of carbohydrates to cold acclimation of *Hedera helix* L. cv. Thorndale. *Physiologia Plantarum* 21: 777-791.

Steponkus, D.L. 1984. Role of plasma membrane in freezing injury and cold acclimation. *Annual Review of Plant Physiology* 35: 543-548.

Steponkus, P.L., Uemura, M., Balsamo, R.A., Arvinte, T. and Lynch, D.V. 1988. Transformation of the cryobehaviour of rye protoplasts by modifications of the plasma membrane lipid composition. *Proceedings of the National Academy of Sciences, USA* 85: 9026-9030.

Stone, J.M., Palta, J.P., Bamberg, J.B., Weiss, L.S. and Harbage, J.F. 1993. Inheritance of freezing resistance in tuber-bearing Solanum species. Evidence for independent genetic control of non-acclimated freezing tolerance and cold acclimation capacity. *Proceedings of the National Academy of Sciences* 90: 7869-7873.

Stout, D.G., Majak, W. and Reaney, M. 1980. *In vivo* detection of membrane injury in freezing temperatures. *Plant Physiology* 66: 74-77.

Sukumaran, M.P. and Weiser, C.J. 1972. Freezing injury in potato leaves. *Plant physiology* 50: 564-567.

Takatori, F.H., Sparks, W.C. and Woodbury, G.W. 1952. A study of simulated hail injury on potatoes. *Idaho Agricultural Experimental Station Bulletin* 22.

Tseng, M.J. and Li, P.H. 1990. Alterations of gene expression in potato (*Solanum commersonii*) during cold-acclimation. *Physiologia Plantarum* 78: 538-547.

Tseng, M.J. and Li, P.H. 1991. Changes in protein synthesis and translatable messenger RNA populations associated with ABA-induced cold hardiness in potato (*Solanum commersonii*). *Physiologia Plantarum* 81: 349-358.

Tsunoda, K., Fujimura, K., Nakahari, T. and Oyamado, Z. 1968. Studies on the testing method for cooling tolerance in rice plants. I. An improved method by means of short turn treatment with cool and deep water. *Japanese Journal of Breeding* 18: 33-40.

Vega, S.E. and Bamberg, J.B. 1995. Screening the U.S. potato collection for frost hardiness. *American Potato Journal* 72: 13-21.

Vega, S.E., De Rio, A.H., Jung, G., Bamberg, J.B. and Palta, J.P. 2003. Marker-assisted genetics analysis of non-acclimated freezing tolerance and cold acclimation capacity in a backcross *Solanum* population. *American Journal of Potato Research* 80: 359-369.

Wallin, N-B. 2005. From Famine to Fries: The potato has come a long way. YaleGlobal Online. The Potato. http://yaleglobal.yale.edu/about/potato.jsp

Watanabe, K., Golmirzaie, A.M. and Gregory, P. 1997. Chapter 10. Use of biotechnology tools in potato genetics resources management and breeding. *In*: Watanabe, K. and Pehu, E. (eds). Plant biotechnology and plant genetics

resources for sustainability and productivity. Landes, R.G. Co., Georgetown, Texas, USA. pp. 145-154.

Watanabe, K.N. 2002. Challenges in biotechnology for abiotic stress tolerance in roots and tubers. JIRCAS Working report. *Japan*. pp.75-83.

Wiegand, K.M. 1906. The occurrence of ice in plant tissue. *Plant World* 9: 25-39.

Winkler, E. 1971. "Kartoffelbau in Tirol. II. Photosynthesis – vermagen und respiration von verschiedenen kartoffel sorten. *Potato Research* 13: 1-18, as cited by Dean, 1994.

Xin, Z. and Brows, J. 2000. Cold comfort farm: The acclimation of plants to freezing temperatures. *Plant Cell and Environment* 23: 893-902.

Yamaguchi, M., Yimm, H. and Spurr, A.K. 1964. Effects of soil temperature on growth and nutrition of potato plants and tuberization, composition and periderm structure of tubers. *Proceedings of the American Society for Horticultural Science* 84: 412-423.

Yancey, P.H., Clark, M.E., Hand, S.C., Bowlus, R.D. and Somero, G.N. 1982. Living with water stress: evolution of osmolyte systems. *Science* 217: 1214-1222.

CHAPTER 3

Effect of freezing temperatures on the growth, development and yield of potato plants

3.1 Introduction

Solanum tuberosum L. ssp. *tuberosum* is known to grow well in cool temperate environments. It is generally agreed that optimum potato growth (Beukema and Van der Zaag, 1990) and maximum netto photosynthetic rates (Dwelle *et al*., 1981) occur between 20 - 25°C. Despite this relatively low temperatures suitable for potato production, cultivated potatoes are sensitive to extremely low temperatures (Li, 1977).

Compared to wild potato species such as *S.acaule*, *S.commersonii* and *S. chomatophilum* (which are considered to have high levels of frost tolerance), the cultivated potato, *S.tuberosum* possesses very little or no frost tolerance (Li, 1977).

Worldwide, potato producers suffer high economic losses annually due to freezing temperatures. Frost injuries occur frequently where potatoes are cultivated when the temperature drops below -2°C, resulting in limited growth. The South African climate can be described as harsh for potato production, especially due to extremely high or low temperatures that can occur during tuber growth. Frost mainly occurs during early spring or late autumn, resulting in major yield reduction in the potato producing regions of South Africa (ARS, 2005). Up to date, no cultivar could be developed which can tolerate the extreme frost conditions that prevail in South Africa from time to time.

When the potato plant experiences its first freezing temperatures of the growing season, severe damage may occur. Although frost occurs sporadically and cannot be prevented, hardening may help to tolerate the plant (Chen *et al*., 1979).

Yield reduction mostly depends on the severity of the damage and the plant's growth stage when frost occurs. Research on yield loss as result of defoliation of potato plants have been done in hail studies (Takatori *et al*., 1952; Snyder and Michelson, 1959; Murphy and Goven, 1962; Beresford, 1967) and insect defoliation studies (Cranshaw and Radcliffe, 1980, Hare, 1980; Wellik *et al.*, 1981; Ferro *et al*., 1983). Potato plants have a high capacity to recover from defoliation depending primarily on the growth stage and the extent of defoliation. The intensity of frost injury can be observed in a range of symptoms such as damaged foliage, injured stems or the entire killing of plants. These symptoms cause a reduction in photosynthetic area of the plant, resulting in a lower yield or crop failure (Esterada, 1978). Zehnder *et al*. (1995) calculated thresholds for plant leaf losses at plant emerge, flowering and the late reproductive growth stage that would result in yield loss. Entomologists have calculated an economic injury level as a result of insect defoliators feeding on potato plants, which provides the relationship between leaf area reduction and yield loss (Stone and Pedigo, 1972; Berry and Shields, 1980).

Apart from genetic differences, other factors such as plant age, stage of development (Hudson, 1961; Richardson and Weiser, 1972) and cultivar type (Takatori *et al*., 1952; Snyder and Michelson, 1959; Murphy and Goven, 1962) may also affect resistance. It has been found that potato plants display the highest sensitivity to damage at flowering, resulting in greater yield reduction than with damage occurring at any other time earlier or after flowering (Beresford, 1967; Harris, 1978; Shields and Wyman, 1984; Beukema and Van der Zaag, 1990). But, freezing temperatures during any time of the growing season of this crop can affect the final yield.

The objective of this study was to investigate the sensitivity of two potato cultivars to freezing stress during various growth stages and to determine its effect on yield.

3.2 Materials and methods

3.2.1 Plant material and growth conditions

Two South African potato cultivars, Darius and BP1 were used. Darius was planted during February 2005 while BP1 was planted during November 2005. These two cultivars were planted at different times, because of a lack of greenhouse space. Darius and BP1 were used because they are widely grown in potato-cultivated areas in South Africa, where frost commonly occurs. One hundred and fifty six potato plants of each cultivar were grown. Tubers of each separate cultivar were planted on the same day. The plants were grown from single tubers (one per pot) under controlled glasshouse conditions at a temperature regime of 15/26°C (night/day). Tubers were planted in sandy loam soil in pots (42 cm diameter and 30 cm high – 41.6 ℓ). Each pot contained 35 kg soil. Thirty gram of 2:3:2 (N:P:K) per pot was given to each pot at planting, while 6.7 g KNO_3 was given seven and 13 weeks after planting. Plants were watered each second to third day during growth.

3.2.2 Freezing injury

The potato plants were subjected to freezing temperatures at three different growth stages:

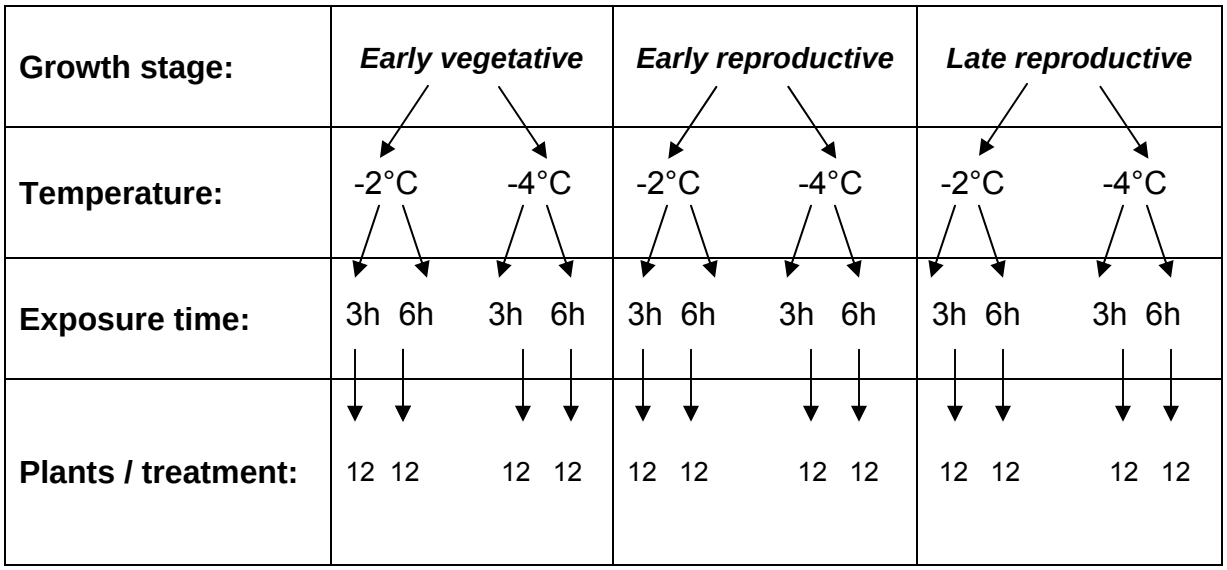
(i) late vegetative - approximately three weeks after planting

(ii) early reproductive - approximately five weeks after planting - flowering

(iii) late reproductive stage - approximately 10 weeks after planting.

When the potato plants reached the respective growth stages, 48 potted plants of each cultivar were placed in a low temperature (4°C) walk-in chamber. Twelve

control plants per cultivar remained untreated in the glasshouse. The plants remained at 4°C for a four hour period to ensure that all the plants were at the same temperature (Lindstrom *et al*., 1992). The pots were then placed in the freezing chambers on a 4 cm thick foam sheet for isolation and to prevent soil water from freezing. The 48 pots were then divided into two groups of 24 each, which were then exposed to -2°C and -4°C respectively. Twelve pots of each group of 24 pots were removed after three hours and the other 12 pots after six hours of exposure. A schematic illustration of the various treatments is given in Figure 3.1.



Control plants: 12 per cultivar

Fig. 3.1 Schematic illustration of the potato trial layout and different freezing treatments.

After exposure to -2°C and -4°C for three and six hours, the potato plants were taken from the cold chambers and put at room temperature (± 20°C) for 24 hours. The treated pots were finally moved back to the glasshouse where they remained until harvest.

3.2.3 Characteristics measured

The following characteristics were measured on the control and treated plants:

i) Percentage stem damage - was calculated by dividing the number of dead auxiliary buds by the total number of buds for each stem for each plant.

ii) Percentage leaf damage - was assessed by calculating the percentage damaged leaf area on the remaining auxiliary buds for each plant.

iii) Yield - total mass (in g) of tubers produced by each plant.

iv) Average tuber diameter - the sum of each tuber's length (mm) and width (mm) divided by two and then divided by the total number of tubers per plant.

v) Average tuber mass - yield per plant divided by the total number of tubers produced by each plant.

vi) Number of tubers - the total number of tubers produced per plant.

5.2.4 Statistical analysis

a) Due to a lack of greenhouse space, Darius and BP1 were planted at different times. Therefore analysis of the two cultivars were done separately. The data pertaining to the six characteristics measured were subjected to factorial analysis, using stem damage as factor 1, leaf damage as factor 2, yield as factor 3, average tuber diameter as factor 4, average tuber mass as factor 5 and number of tubers per plant as factor 6. Data obtained were calculated as a relative percentage according to the mean of untreated plants. The genotypic means for all traits measured were used to compare the performances of the two cultivars. Means were separated by using least significant differences (LSD). The SAS 9.1 computer program was utilized to perform the factorial analysis.

b) Correlation coefficients were obtained by using the software program, AGROBASE (2000) to examine the degree of association between characters.

3.3 Results and discussion

3.3.1 Analysis of variance for stem and leaf damage

Separate factorial analysis was done on stem and leaf damage data of Darius and BP1, since the two cultivars were planted at two different planting dates. Data on each characteristic were expressed as percentage damage according to the method described above.

a) Darius

Results of the analysis of variance obtained for stem and leaf damage for Darius are shown in Table 3.1.

Stem and leaf damage

Highly significant differences ($P<0.01$) were observed between growth stages for both stem and leaf damage. A significant ($P<0.05$) interaction was observed between temperature and time of exposure for stem damage per plant. This indicated that different levels of stem damage can occur with different times of exposure. Highly significant ($P<0.01$) differences occurred between the growth stage x temperature and growth stage x temperature x time interactions for leaf damage. These interactions complicate the screening of potatoes for freezing damage since it indicates different levels of leaf damage for different temperature and time intervals.

b) BP1

Results of the factorial analysis obtained for stem and leaf damage for BP1 are shown in Table 3.2.

Stem and leaf damage

All variables and interactions differed highly significantly ($P<0.01$) from each other for leaf damage. Highly significant ($P<0.01$) differences occurred between the different growth stages. The growth stage x temperature interaction was highly significant ($P<0.01$) for leaf damage per plant, while the growth stage x time interaction only differed significantly ($P<0.05$) for the amount of leaf damage per plant. Significant differences were observed for the growth stage x temperature x time interaction. The significant interactions for stem and leaf damage caused by the different temperatures and time variables will weaken the relationship between plant damage and yield loss. The coefficients of variation for stem and leaf damage were above expectations which will further complicate the relationship between freezing damage and yield loss.

3.3.2 Analysis of variance for measured yield traits

Factorial analysis was done on the yield and yield characteristic data pertaining to each of the six yield factors of Darius and BP1, using average tuber diameter as factor 1, average tuber mass as factor 2, number of tubers as factor 3 and yield as factor 4. Data of each characteristic were calculated as a relative percentage according to the average of untreated plants.

a) Darius

Results of the analysis of variance obtained from measured traits of Darius are shown in Table 3.3. Growth stage was highly significant ($P<0.01$) for tuber diameter, tuber mass and yield per plant. Temperature showed significant differences ($P<0.05$) for yield per plant. A significant ($P<0.05$) interaction occurred between growth stage and temperature for yield per plant. The growth stage x temperature interaction indicates that the various freezing levels had affected the three growth stages differently. The coefficients of variation (CV%) for this trial were relatively low.

Table 3.1 Analysis of variance for stem and leaf damage per plant of Darius.

Source	Mean Squares	
	Stem damage	**Leaf damage**
Gs	43845.19762**	13067.31786**
Temp	31313.19601**	1162.94120
Time	460.03086	1690.96853
Gs x temp	77.46526	19467.92166**
Gs x time	1481.52293	92.37703
Temp x time	3627.05700*	89.46358
Gs x temp x time	506.18718	8751.17945**
Error	641.4548	651.9274
CV (%)	59.34190	75.12107

*,** = significant at P=0.05 and 0.01 probability levels respectively; Gs=growth stage.

Table 3.2 Analysis of variance for stem and leaf damage per plant of BP1.

Source	Mean Squares	
	Stem damage	**Leaf damage**
Gs	78351.7404**	17389.74066**
Temp	13235.4001**	36.16051
Time	3908.1731**	135.18250
Gs x temp	6371.6648**	3917.75865**
Gs x time	1593.0163**	2480.80961*
Temp x time	3491.1713**	2009.58949
Gs x temp x time	3232.7645**	2607.94956*
Error	233.1406	599.7844
CV (%)	21.57595	118.7706

*,** = significant at P= 0.05 and 0.01 probability levels respectively; Gs=growth stage.

Tuber diameter, tuber mass and yield per plant

Yield and yield components (tuber diameter and tuber mass) showed highly significant ($P<0.01$) differences for the various growth stages and are shown in Figure 3.2. Tuber diameter, tuber mass and yield followed the same pattern during growth as a result of the freezing treatment. Tuber diameter, tuber mass and yield were the highest for potato plants treated during the late vegetative growth stage. Plants damaged during vegetative growth, had a greater ability to recover from damage, resulting in new plant growth and recovery to take place early in the growing season. Plants treated during early reproductive growth caused an interruption in tuber formation, resulting in tubers with the lowest tuber diameter, tuber mass and yield. When potato plants were treated during late reproductive growth, second best values for tuber diameter, tuber mass and yield per plant were obtained. Potato plants damaged during late reproductive growth delayed tuber growth and maturity, causing in tubers to maintain almost the same shape and size until harvest.

Growth stage x temperature interaction

A highly significant ($P<0.01$) interaction between the different growth stages and temperatures occurred for yield in Darius. Relative percentages of the means for the interaction are shown in Table 3.4. Yield of the potato plants treated during late vegetative growth showed a decrease when temperatures droped lower. Plants treated during early reproductive growth showed a significant decrease in yield when temperatures decreased. This result may explain why potato plants in their early reproductive growth stage show a high sensitivity to lower temperatures, resulting in a significant reduction in yield. Yield of plants treated during their late reproductive growth showed an increase as temperatures decreased but did not differ significantly from each other.

Table 3.3 Analysis of variance for yield characteristics of Darius.

Source	df	Mean Squares			
		Relative average tuber diameter	Relative average tuber mass	Relative average number of tubers	Relative average yield
Gs	2	2762.77**	12426.32**	1665.97	8526.42**
Temp	1	433.66	691.53	1306.14	2645.53*
Time	2	34.99	32.03	0.02	10.28
Gs x temp	1	184.09	1250.66	207.36	1718.12*
Gs x time	2	19.23	94.43	1517.55	984.73
Temp x time	1	0.07	71.62	110.08	258.85
Gs x temp x time	1	61.20	445.56	2677.88	659.42
Error		126.24	458.70	911.56	476.47
CV (%)		12.79	31.29	28.03	28.71

*,** = significant at P= 0.05 and 0.01 probability levels respectively; Gs=growth stage.

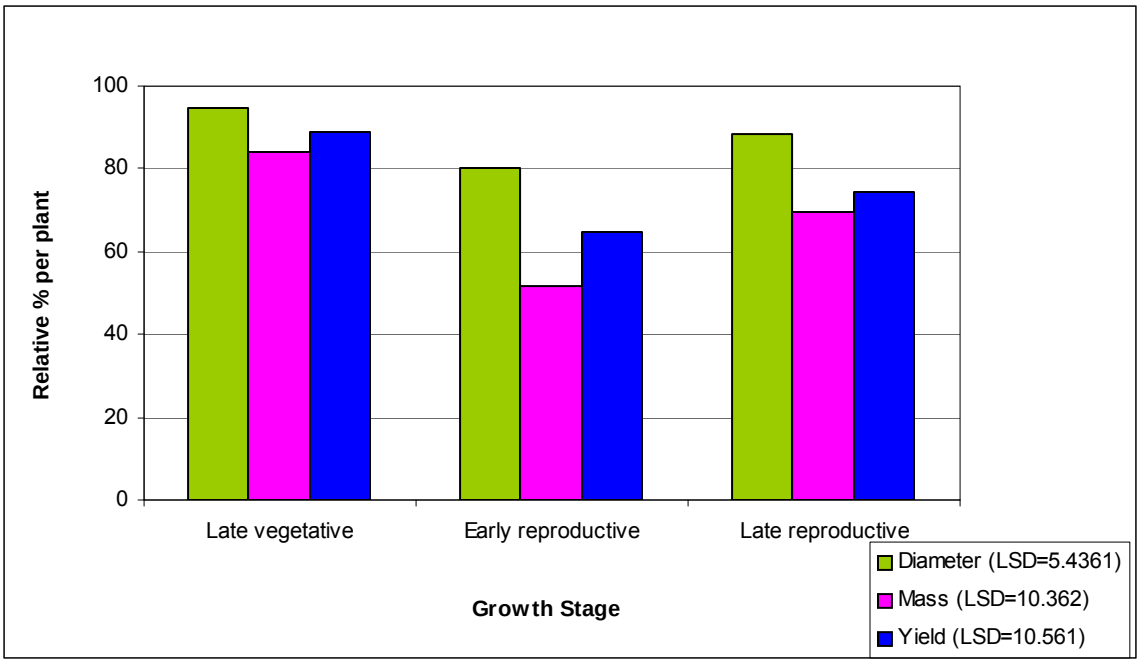


Fig 3.2 Relative percentages of tuber diameter, tuber mass and yield per plant for Darius at different growth stages.

Table 3.4 Means for growth stage x temperature interaction for yield per plant of Darius.

Growth Stage	Temperature		
		- 2°C	-4°C
	Late vegetative	96.24 a	81.89 ab
	Early reproductive	69.45 b	49.83 c
	Late reproductive	72.33 b	76.60 b

* Means followed by the same letter in the same row did not differ significantly at P=0.01.

b) BP1

Results of the analysis of variance for measured traits of BP1 are shown in Table 3.5. Growth stages differed highly significantly ($P<0.01$) for yield and yield components (tuber diameter, tuber mass, number of tubers and yield per plant). Yield showed highly significant ($P<0.01$) differences to temperature. Tuber weight and yield were significantly ($P<0.05$) influenced by the growth stage x temperature interaction. Number of tubers and yield per plant were highly significantly ($P<0.01$) influenced by the temperature x time interaction. The coefficient of variation (CV%) for this trial was extremely high compared to Darius. This may be explained in terms of different environmental factors that occurred during the growth of this cultivar in the glasshouse such as extreme high temperatures and mildew.

Tuber diameter, tuber mass, number of tubers and yield per plant

Figure 3.3 represents the yield and yield components (tuber diameter, tuber mass and number of tubers per plant) that showed highly significant ($P<0.01$) differences at the various growth stages. All four variables followed the same pattern during growth as a result of the freezing treatment. Tuber diameter, tuber mass, number of tubers and yield were the highest for potato plants treated during the late vegetative growth stage. Plants damaged during vegetative growth, had a stronger ability to recover, resulting in new plant growth and recovery to take place early in the growing season. Plants treated during early reproductive growth caused an interruption in tuber formation, resulting in tubers with the lowest tuber diameter, tuber mass, number of tubers and yield. When potato plants were treated during late reproductive growth, second best values for each of the four variables were obtained. Potato plants damaged during late reproductive growth delayed tuber growth and maturity, resulting in tubers to maintain almost the same shape and size till harvest.

Growth stage x temperature interaction

Tuber mass was significantly influenced by the growth stage x temperature interaction. Results of the relative mean percentages of tuber mass at different growth stages and temperatures are shown in Table 3.6. Tuber mass decreased significantly ($P<0.01$) with treatment during late vegetative growth and as temperatures decreased. A significant reduction in tuber mass was observed with treatment during the early reproductive stage of development, while no differences were observed between the -2°C and -4°C treatments. Tuber mass increased slightly when temperature treatment decreased to -4°C during late reproductive growth. These results indicate that different temperature treatments affected tuber mass differently.

Growth stage x temperature interaction for yield

Yield was significantly influenced by the growth stage x temperature interaction (Table 3.5). Relative percentages for yield with treatment at different growth stages and the relevant temperatures are shown in Table 3.7. Yield decreased significantly with treatment during late vegetative growth and as freezing temperatures increased. Yield slightly decreased with treatment during early reproductive growth and as temperatures decreased. Treatment of plants in their late reproductive growth resulted in an insignificant increase in yield. This indicates that low temperatures occurring at late plant growth may stimulate tuber growth with a consequent increase in yield.

Temperature x time interaction for number of tubers

The number of tubers per plant was highly significantly ($P<0.01$) influenced by the temperature x time interaction of the treatments. Results on the relative percentages of number of tubers per plant are given in Table 3.8. The number of tubers per plant showed an increase with the -2°C treatment and when exposure was extended to six hours. Potato plants exposed to -2°C for a shorter time (3h) showed a lower tuber number per plant in comparison with the six hour treatment. The results indicate that short periods of slight freezing temperatures

may inhibit tuber formation. The plants might become more tolerant to freezing temperatures after six hours of exposure to -2°C. The number of tubers per plant decreased significantly when exposed to -4°C for six hours. When plants were exposed to -4°C for six hours, severe damage to the tuber formation process resulted, with a consequent reduction in number of tubers per plant.

Temperature x time interaction for yield

Yield per plant was highly significantly ($P<0.01$) influenced by the temperature x time interaction. Results on relative percentages of yield per plant are shown in Table 3.9. When potato plants were exposed to -2°C, yield increased slightly when time of exposure was extended to six hours. Potato plants subjected to low temperatures for a short time may acclimate to lower temperatures. This may explain the increase in yield when exposure time increased. Plants exposed to -4°C showed a lower yield when the exposure time was increased to six hours. These results indicate that time of exposure to different freezing temperatures will not necessarily affect yield in exactly the same way.

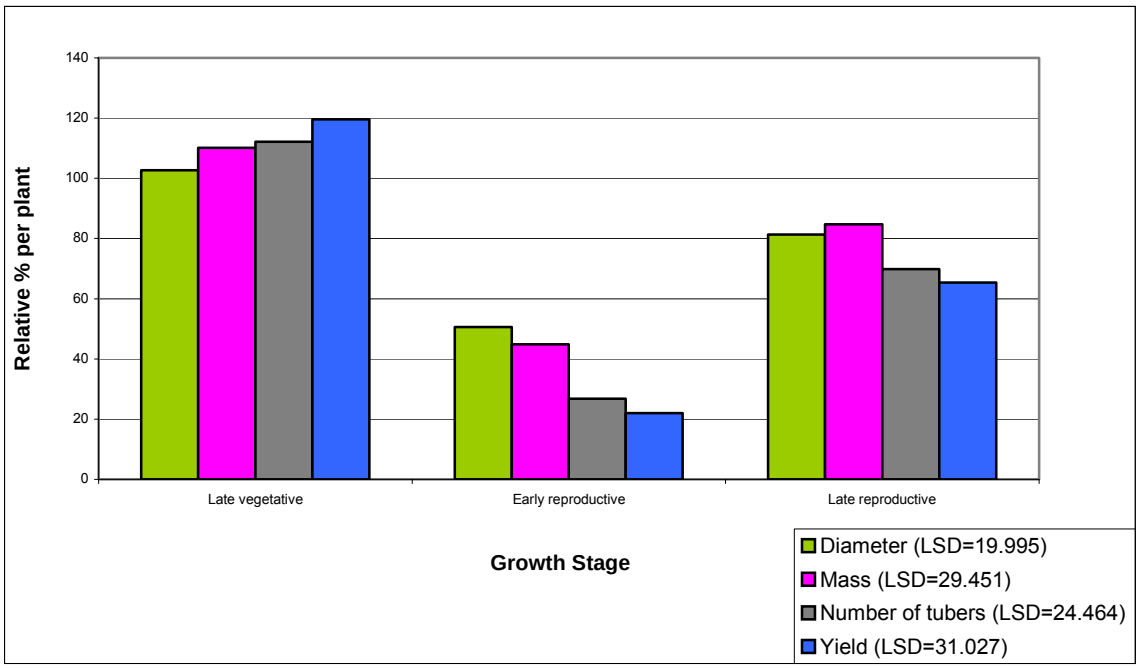


Fig. 3.3 Relative percentage tuber diameter, tuber mass, number of tubers and yield per plant for BP1 at different growth stages.

Table 3.5 Analysis of variance for yield characteristics per plant for BP1.

Source	df	Mean Squares			
		Relative average tuber diameter	Relative average tuber mass	Relative average number of tubers	Relative average yield
Gs	2	31919.57**	57346.71**	96734.88**	135610.76**
Temp	1	4.82	7854.28	6726.76	32486.12**
Time	2	741.88	157.49	563.22	881.14
Gs x temp	1	4997.04	13366.69*	6352.35	13162.18*
Gs x time	2	98.96	2862.72	1206.69	2865.07
Temp x time	1	524.22	4014.66	23642.72**	37913.84**
Gs x temp x time	1	2725.00	27.66	1232.65	0.55
Error		1707.83	3705.18	2556.56	4112.25
CV (%)		52.84	76.17	72.69	92.94

*, ** = significant at P=0.05 and 0.01 probability levels respectively; Gs=growth stage.

Table 3.6 Means of growth stage x temperature interaction for tuber mass per plant on BP1.

Growth Stage	Temperature	
	- 2°C	-4°C
Late vegetative	125.541a	94.728b
Early reproductive	54.145bc	17.015c
Late reproductive	74.431b	95.055ab

* Means followed by the same letter in the same row did not differ significantly at P=0.01.

Table 3.7 Means of growth stage x temperature interaction for yield per plant on BP1.

Growth Stage	Temperature	
	- 2°C	-4°C
Late vegetative	152.094a	87.019b
Early reproductive	25.827cd	10.683d
Late reproductive	63.922bc	66.879bc

* Means followed by the same letter in the same row did not differ significantly at P=0.01.

Table 3.8 Means of temperature x time interaction for number of tubers per plant for BP1.

Time	Temperature	
	- 2°C	-4°C
3h	62.036	71.528
6h	75.000	68.182

Table 3.9 Means of temperature x time interaction for yield per plant for BP1.

Time	Temperature	
	- 2°C	-4°C
3h	66.047	67.907
6h	77.843	56.424

3.3.3 Comparison of yield loss between Darius and BP1

Results on average yield losses for Darius and BP1 cultivars resulting from freezing temperatures during various growth stages are shown in Figures 3.4 and 3.5 respectively. The yield response of both cultivars followed the same reaction to freezing damage at the various growth stages. Potato plants damaged during the late vegetative growth stage showed the lowest yield loss compared to damage during the other two growth stages tested. Plants in the early reproductive growth stage showed the highest sensitivity to freezing temperatures, resulting in the greatest yield loss. The yield loss due to freezing temperatures was slightly higher with exposure during the late reproductive growth stage than with treatment in the early vegetative stage. Of the two

cultivars tested, BP1 showed greater yield loss with treatment during the most sensitive growth stage (early reproductive) and when damaged by freezing temperatures (Figure 3.4). This result may indicate that Darius is more tolerant to freezing stress during this critical growth period. Freeze treated plants of BP1 during late vegetative growth preformed better, resulting in a yield increase of almost 20%. Low temperatures may stimulate this cultivar during the early vegetative growth to enhance yield (Figure 3.5).

3.3.4 Correlation between measured traits

The correlation coefficients between yield and yield components were calculated on the combined data obtained from Darius and BP1. Percentage leaf and stem damage as measured for the various treatments were also included in the correlation matrix. Correlation coefficients between the different characteristics measured are shown in Table 3.10. Yield correlated significantly with tuber diameter (r=0.61), tuber mass (r=0.71) and number of tubers (r=0.75). Stem and leaf damage were significantly negatively correlated with yield. Stem damage (r=-0.38) had the most pronounced negative effect on potato yield. Stem damage caused the largest reduction in number of tubers per plant (r=0.43) while leaf damage mainly reduced the tuber mass (r=0.25). Tuber diameter was significantly correlated with tuber mass (r=0.81).

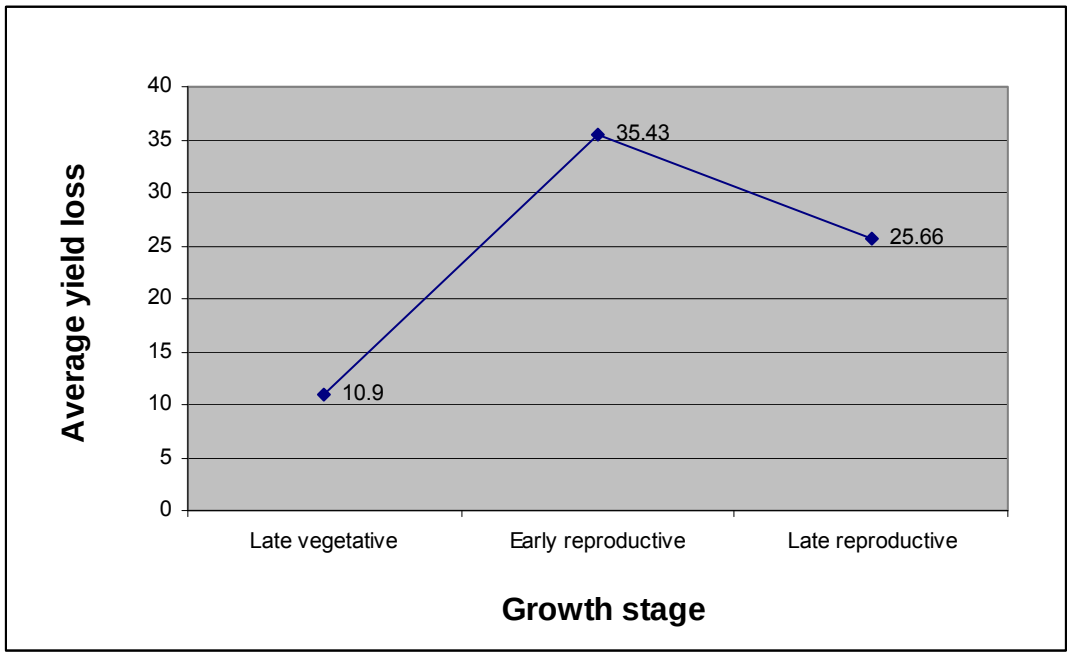


Fig. 3.4 Average yield loss resulting from exposure of Darius to freezing temperatures.

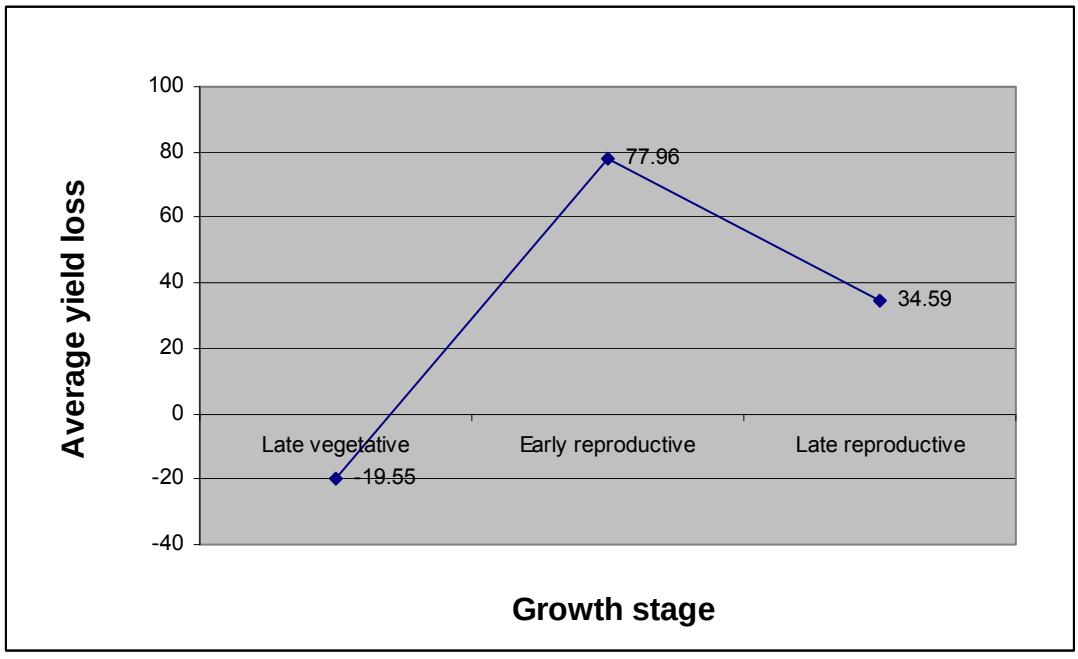


Fig. 3.5 Average yield loss resulting from exposure of BP1 to freezing temperatures.

Table 3.10 Correlation matrix for measured yield traits per plant of combined data on Darius and BP1.

	Stem damage	Leaf damage	Relative tuber diameter	Relative tuber mass	Relative number of tubers
Leaf damage	0.0643				
Relative tuber diameter	-0.2511**	-0.1987**			
Relative tuber mass	-0.1848**	-0.2596**	0.8176**		
Relative number of tubers	-0.4310**	-0.0954	0.4911**	0.2901**	
Yield	-0.3839**	-0.2531**	0.6179**	0.7114**	0.7503**

*,** = significant at P=0.05 and 0.01 probability levels respectively.

3.4 Conclusions

In both cultivars tested, tuber diameter, tuber mass and yield per plant were similarly influenced by freezing temperatures. Young plants damaged by freezing may die completely, but may recover quickly by regrowth from auxillary buds remaining undamaged. Damage to the potato plant at this growth stage, may delay the process of tuber initiation but yield loss would not necessarily occur (Shields and Wyman, 1984).

When potato plants in the late vegetative growth stage are exposed to freezing temperatures, plant growth could sometimes be stimulated to result in better yields. It may be possible for potato plants to become more tolerant to freezing temperatures the longer they are exposed to it.

Potato plants damaged during early reproductive growth (flowering stage) showed the greatest yield loss. Potato plants in this growth stage undergo a transmission phase where the majority of resources are being shifted toward flower production and tuber growth (Sparks and Woodbury, 1967). Recovery following defoliation in this phase would be more difficult, resulting in a greater reduction in yield.

Potato plants in the late reproductive growth stage showed a reduced sensitivity to yield loss compared to plants in the early reproductive growth stage. This result corresponded with the findings of Shields and Wyman (1984). Leaf growth during this late growth stage is almost reduced to zero (Sparks and Woodbury, 1967). Plants thus showed a lower sensitivity to leaf and stem damage as indicated by the final yield loss.

Although no association between tuber traits and freezing tolerance could be found in previous studies (Ross and Rowe, 1965; Esterada, 1978) in this study relative yield was shown to be positively correlated with tuber diameter, tuber

mass and number of tubers per plant for treated plants. Any factor that might affect one of these tuber properties during growth would largely affect the final yield. In a previous study no significant correlation could be found between freezing tolerance and tuber traits (Chen *et al*.,1999) indicating that freezing tolerance is independently genetically controlled.

This study showed significant interactions between growth stage x temperature and growth stage x time of exposure for stem and leaf damage in most situations. This indicates that the various freezing levels affected the three growth stages differently. The temperature x time interaction was also significant for yield in BP1. This indicated cold-acclimated that the various freezing temperatures and times of exposure will not necessarily affect potato yield similarly. The only way to deal with significant interactions is the use of regression analysis to predict yield loss for each growth stage according to stem and leaf damage measured. Of the two cultivars tested, Darius showed the highest tolerance to frost damage.

REFERENCES

AGROBASE, 2000. Agrobase user's guide and reference manual. Agronomix Software Inc., Canada.

ARS, 2005. Agricultural Risk Specialist, Bloemfontein, South Africa.

Beresford, B.C. 1967. Effect of simulated hail damage on yield and quality of potatoes. *American Potato Journal* 44: 347-354.

Berry, R.E. and Shields, E.J. 1980. Variegated cutworm: leaf consumption and economic loss in peppermint. *Journal of Economic Entomology* 73: 607-608.

Beukema, H.P. and Van der Zaag, D.E. 1990. Introduction to potato production, Pudoc Wageningen, Den Haag.

Chen, H.H., Gavinlertvanta, P. and Li, P.H. 1979. Cold acclimation of stem cultured plants and leaf callus of *Solanum* species. *Botanical Gazette* 140: 177-147.

Chen, Y-K.H., Palta, J.P. and Bamberg, J.B. 1999. Freezing tolerance and tuber production in selfed and backcross progenies derived from somatic hybrids between *Solanum tuberosum* L. and *S. commersonii* Dun. *Theoretical and Applied Genetics* 99: 100-107.

Cranshaw, W.S. and Radcliffe, E.B. 1980. Effect of defoliation on yield of potatoes. *International Bibliography, Information and Documentation* 73: 131-134.

Dwelle, R.B., Kleinkopf, G.E. and Pavek, J.J. 1981. Stomatal conductance and gross photosynthesis of potato (*Solanum tuberosum* L.) as influenced by irradiance, temperature and growth stage. *Potato Research* 24: 49-59.

Esterada, R.N. 1978. Plant Cold Hardiness and Freezing Stress. Breeding frost resistant potatoes for the tropical highlands. Academic press, Inc.

Ferro, D.N., Morzuch, B.J. and Margolies, D. 1983. Crop loss assessment of the Colorado potato beetle (Coleoptera*: chrysomelidae*) on potato in western Massachusetts. *Journal of Economic Entomology* 76: 349-356.

Hare, J.D. 1980. Impact of defoliation by Colorado potato beetle on potato yields. *International Bibliography, Information and Documentation* 73: 369-373.

Harris, P.M. 1978. The Potato Crop. Chapman and Hall, London.

Hudson, M.A. 1961. The limitations of a leaf test for assessing the frost resistance of the tuber-bearing *Solanums*. *Euphytica* 10: 169-179.

Li, P.H. 1977. Frost killing temperatures of 60 tuber-bearing *Solanum* species. *American Potato Journal* 54: 452-456.

Lindstrom, O.M.; Olson, D.J. and Carter, J.V. 1992. Degree of undercooling and injury of whole potato plants following exposure to -2°C for 6 or 12 hours. *Horticultural Science* 27(3): 244-246.

Murphy, J.J. and Goven, M.J. 1962. The effect of simulated hail damage on yield and quality of potatoes in Maine. *Maine Agricultural Station, Bulletin* 607.

Richardson, D.G. and Weiser, C.J. 1972. Foliage frost resistance in tuber-bearing Solanums. *Horticultural Science* 7(1): 19-22.

Ross, R.W. and Rowe, P.R. 1965. Frost resistance among the *Solanum* species in the IR-1 potato collection. *American Potato Journal* 42: 177-185.

Shields, E.J. and Wyman, J.A. 1984. Effect of defoliation at specific growth stages on potato yields. *Journal of Economic Entomology* 77: 1194-1199.

Snyder, G.B. and Michelson, L.F. 1959. The effect of simulated hail damage on potatoes. *Massachusetts Agricultural Experiment Station, Bulletin* 506a.

Sparks, W.C. and Woodbury, G.W. 1967. Stages of potato plant growth. *Idaho Agricultural Experiment Station, Bulletin* 309.

Stone, J.D. and Pedigo, L.P. 1972. Development and economic injury level of the green clover worm on soybean in Iowa. *Journal of Economic Entomology* 65: 197-201.

Takatori, F.H., Sparks, W.C. and Woodbury, G.W. 1952. A study of simulated hail injury on potatoes. *Idaho Agricultural Experimental Station, Bull*etin 22.

Wellik, M.J., Slosser, J.E. and Kirby, R.D. 1981. Effects of simulated insect defoliation on potatoes. *American Potato Journal* 58: 627-632.

Zehnder G., Vencil, A.M. and Speese, J. 1995. Action thresholds based on plant defoliation for management of Colorado Potato Beetle (Coleoptera: *Chrysomelidae*) in potato. *Journal of Economic Entomology* 88: 155-161.

CHAPTER 4

Alterations in protein expression in potatoes exposed to freezing temperatures

4.1 Introduction

Cold acclimation is defined as the need for a plant to acquire freezing tolerance during exposure to low non-freezing temperatures (Levitt, 1980). Nevertheless, severe damage can occur in potato plants when exposed to freezing temperatures caused by early frost, without previous hardening of the plant.

When plants are exposed to environmental stresses, metabolic changes take place that lead the plant to adapt (Guy, 1990). Weiser (1970) was the first to propose that the development of cold hardiness in plants require alterations in gene transcriptions, as well as the *de novo* synthesis of unique proteins that normally do not occur under non acclimating conditions.

Numerous studies using electrophoretic separation of proteins have shown qualitative and quantitative differences between non-acclimated and cold acclimated plants (McCown *et al*., 1968). New protein bands were present in cold acclimated and freezing tolerant plants, while being absent in non-acclimated plants (Kacperska-Palacz *et al*., 1977).

Various scientists conducted research on the hardening and cold acclimation of potato plants and plantlets (Tseng and Li, 1990; 1991; Lee *et al*., 1992; Ryu and Li, 1994). Low temperatures stimulate the production of free abscisic acid (ABA) in potato plants (Chen *et al*., 1983). The increased ABA concentration leads to changes in gene expression (Tseng and Li, 1990; 1991). The ABA increase is regulated by *de novo* synthesized proteins in response to low temperatures (Ryu and Li, 1994). Cold hardiness develops finally by metabolic adjustments or

structural modifications (Li *et al*., 1989). Mechanisms which lead to the understanding of how cold acclimation changes the tolerance and biochemical responses of cells and tissues are still unclear (Guy, 1990). It has been confirmed that some biochemical alterations enable the potato plant to acclimate to low temperatures (Levitt, 1980). The netto increase of soluble proteins show a positive correlation with the development of frost hardiness in potato plants during cold acclimation (Chen and Li, 1980b). When protein synthesis in potato plants is inhibited by cycloheximide (CHI), (a cytoplasmic protein synthesis inhibitor), the production of free ABA is blocked (Ryu and Li, 1994). Protein synthesis thus plays a central role in the development of tolerance to low temperatures in potato plants.

Tseng and Li (1990) identified at least 23 cold induced polypeptides (CIPs) that were newly synthesized during 14 days of acclimation of potatoes. The appearance of CIPs may probably be the result of *de novo* protein synthesis.

Rorat *et al*. (1997) reported that many different genes are induced in a cold tolerant potato, *Solanum sogarandinum,* during cold acclimation. These genes are expected to be involved in different cellular functions during cold treatments, some protecting the chloroplast and cell functions, while others may be involved in the metabolic adjustments to cold temperatures.

Chen and Li (1980b) compared *Solanum acaule* (which is frost tolerant and able to acclimate to cold) and *Solanum tuberosum* (which is frost sensitive and incapable of cold acclimation). They found that sugar, starch and water content differ after stepwise acclimating conditions. RNA and soluble protein content also showed levels of changes when compared to untreated controls.

Previous research used stem cultured plantlets as plant material for evaluation of the cold hardiness of potato plants by using the conductivity test (Chen *et al*., 1983; Tseng and Li, 1990, 1991; Ryu and Li, 1994; Rorat *et al*., 1997; Rorat *et*

al., 1998). Lee *et al*. (1992) used leaf calli to form cultures that were treated with ABA to induce hardening. These cultures were used to determine the freezing tolerance of cells by the TTC reduction assay and fluorescein diacetate vital staining. The use of whole plant systems for cold hardiness studies were in most cases limited (Lee *et al*., 1992).

Chen and Li (1980a; b), Sukumaran and Weiser (1972) and Lindstrom *et al*. (1992) made use of whole potted plants propagated from seed tubers. According to Lindstrom *et al.* (1992) it is important to examine whole plants since they may respond differently to sub-zero temperatures than small leaf sections or disks.

Proteins are the direct evidence of gene expressions under certain circumstances. The objective of this research was to study the protein alterations when cultivated potato plants were subjected to freezing temperatures during the late vegetative, early reproductive and late reproductive growth stages, without previous hardening.

4.2 Materials and methods

4.2.1 Plant material

One hundred and fifty six potato plants of Darius, a South African potato cultivar, were planted during February 2006. The plants were grown from single tubers (one per pot) under controlled glasshouse conditions at a temperature regime of 15/26°C (night/day). Tubers were planted in sandy loam soil in pots (42 cm diameter and 30 cm high – 41.6 ℓ). Each pot contained 35 kg soil. Thirty grams of 2:3:2 (N:P:K) nutrients were given to each pot with plant, while 6.7 g KNO_3 were given seven and 13 weeks after planting. Plants were watered each second to third day during growth.

4.2.2 Freezing injury

The potato plants were subjected to freezing temperatures at three different growth stages:

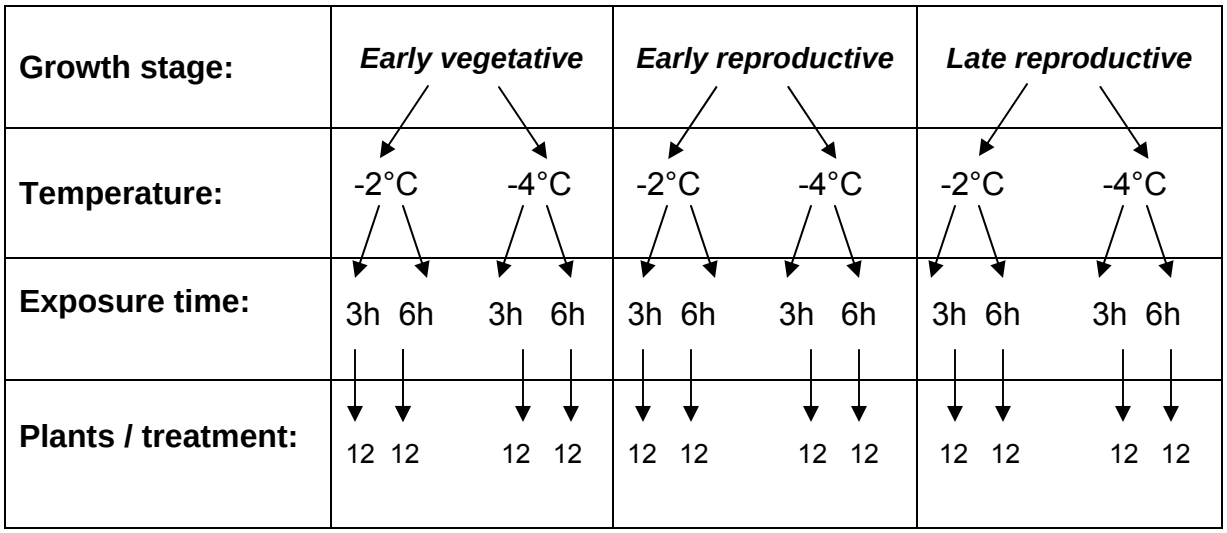
(i) late vegetative - approximately three weeks after planting

(ii) early reproductive - approximately five weeks after planting - flowering

(iii) late reproductive stage - approximately 10 weeks after planting.

When the potato plants reached the respective growth stages, 48 potted plants were placed in a low temperature (4°C) walk-in chamber. Twelve control plants remained untreated in the glasshouse. The plants remained at 4°C for a four hour period to ensure that all the plants were at the same temperature (Lindstrom *et al*., 1992). The pots were then placed in the freezing chambers on a 4 cm thick foam sheet, for isolation and to prevent soil water from freezing. The 48 pots were then divided into two groups of 24 each, which were then exposed to -2°C and -4°C respectively. Twelve pots were removed after three hours and the other 12 pots after six hours of exposure. A schematic illustration of the various treatments is given in Figure 4.1.

After exposure to -2°C and -4°C for three and six hours, the potato plants were taken from the cold chambers and put at room temperature (± 20°C) for 24 hours. The pots were finally moved back to the glasshouse where they remained until harvest.



Control plants: 12 per cultivar

Fig. 4.1 Schematic illustration of the potato trial layout and different freezing treatments.

4.2.3 Sample material

Three to four of the leaves near the growth tip of each potato plant were collected after treatment during the three different growth stages, to determine protein alterations as a result of freezing temperatures. Leaf samples collected from the plants that remained in the glasshouse were used as controls. Leaves were also collected five days after cold treatment, as well as from control plants, to compare protein profile alterations. Sample leaves were put in labeled plastic bags, immersed in liquid nitrogen and stored at -80°C until analysis.

4.2.3 Protein extraction and analysis

Leaves were ground to a very fine powder in liquid nitrogen with a mortar and pestle. Approximately 0.5 g powder was sampled and transferred to a cold cortex tube. Extraction buffer (50mM Tris pH 7.5, 20mM EDTA) was added to the powdered leaf tissue in an approximate ratio of 1:3 (mass of tissue: extraction buffer). The tubes were vortexed for 10 seconds and centrifuged for 15 min at

10 000 rpm. The supernatant was recovered and stored in Eppendorfs at -20°C until further use.

4.2.4 Determination of protein concentration

To determine the soluble protein concentration of each sample, 10µl residue of above supernatant was extracted with 150µl distilled water and 40µl BioRad. The protein concentration of each extract was determined according to the Bradford (1976) method.

4.2.5 Gel preparation

Equal amounts of total proteins of each sample were resuspended with ice-cold acetone for two hours at -20°C and centrifuged at 10 000 rpm for 10 min. The supernatant was discarded and the remaining pellet was air-dried. The pellet was resuspended in 25µl sample buffer (Tris pH6.8; 6 µl BioRad XT Sample Buffer 4x). The protein solvent was heated for one minute at 70°C in a water bath. Protein samples were separated by electrophoresis on a 10% (uniform) SDS poly acrylamide gel. Equal amounts of proteins (50 µg) of each sample were loaded into the sample wells. Electrophoresis was performed at a constant power of 15 Watt for approximately 70 minutes.

4.2.6 Gel staining

Gels were immersed in the fixing solution (40% methanol, 10% glacial acetic acid, 250ml distilled water) for approximately five hours and left in the staining solution (15% trichloroacetic acid, 1liter distilled water, 0.05% Coomassie Blue, 5% methanol) overnight. The gels were finally destained and rinsed in distilled water before interpretation and photography.

4.2.7 Gel interpretation

A Precision Plus Protein™ Standard (BioRad; CA 94547) molecular weight marker was used as a standard from which test results were derived. Protein bands of the freeze treated leaves were compared with the bands obtained from the untreated leaves. The number of polymorphisms produced at different growth stages and freezing temperatures was recorded. Changes in the intensity of protein bands were also noted.

4.3 Results and discussion

4.3.1 The effect of -2°C exposure for three hours on protein expression

Late vegetative growth stage. A new band of 37 kDa was observed with the late vegetative growth stage after a three hour treatment at -2°C, which was not present in control plants. The 23 kDa and 250 kDa bands showed a decreased band intensity. The intensity of the 250 kDa band decreased after five days, while the intensity of the 50 kDa band increased after five days.

Early reproductive growth stage. A new band of 33 kDa was observed with the early reproductive growth stage. The 23 kDa band which was present in the control plants, disappeared after the treatment and remained absent for five days after the freezing treatment. A new band of 29 kDa developed five days after the treatment.

Late reproductive growth stage. Three new bands of 18 kDa, 20 kDa and 29 kDa were observed with the late reproductive growth stage. The intensity of the 150 kDa band decreased while the intensity of the 33 kDa band increased. A new band of 150 kDa appeared after five days while the 29 kDa band showed a decreased intensity and the 33 kDa band an increased intensity.

4.3.2 The effect of -2°C exposure for six hours on protein expression

Late vegetative growth stage. The 165 kDa band was absent in the late vegetative growth stage after a six hour treatment at -2°C. The 165 kDa band appeared again five days after the treatment. Two new bands of 25 kDa and 48 kDa developed in the treated plants after five days. The 38 kDa and 56 kDa bands showed increased protein intensity.

Early reproductive growth stage. Only one new band of 30 kDa was formed in the early reproductive growth stage. Thirty-six kDa and 38 kDa bands showed an increased intensity in protein concentration. A new band of 165 kDa appeared five days after the freezing treatment. The newly formed 30 kDa band remained present in the potato plants for five days after the treatment, while the intensity of the 38 kDa band decreased.

Late reproductive growth stage. Two new bands of 40 kDa and 47 kDa were formed in the late reproductive growth stage. The 21 kDa and 92 kDa bands showed an increased intensity. The 35 kDa band showed increased protein intensity for five days after the freezing treatment, while the intensity of the 25 kDa and 31 kDa bands decreased. Two bands of 47 kDa and 92 kDa disappeared five days after the freezing treatment.

4.3.3 The effect of -4°C exposure for three hours on protein expression

Late vegetative growth stage. Three new bands of 35 kDa, 40 kDa and 80 kDa (Fig. 4.2a) were formed in the late vegetative growth stage after the plants were subjected to -4°C for three hours. The intensity of the 20 kDa and 73 kDa bands increased after the freezing treatment. The intensity of the 250 kDa band decreased after exposure to -4°C while still remained present five days. The 73 kDa band disappeared five days after treatment. The 50 kDa band showed a decreased intensity while the 73 kDa band disappeared five days after treatment (Figure 4.2a).

Early reproductive growth stage. Three new bands of 40 kDa, 75 kDa and 165 kDa were formed in the early reproductive growth stage (Fig. 4.2a). The intensity of the 35 kDa and 49 kDa bands increased with freezing treatment. After five days, plants showed a new band of 23 kDa, while the 88 kDa band's intensity decreased (Figure 4.2a).

Late reproductive growth stage. Only one new band of 70 kDa developed in the late reproductive growth stage (Figure 4.2b). The 35 kDa band's intensity increased while the 30 kDa, 60 kDa and 150 kDa bands' intensity decreased. All plants were dead five days after treatment (Figure 4.2b).

4.3.4 The effect of -4°C exposure for six hours on protein expression

Late vegetative growth stage. The 70 kDa band's intensity increased while the 150 kDa band decreased in the early reproductive growth stage after the six hour exposure at -4°C. A new band of 45 kDa developed five days after freezing treatment. The 70 kDa and 150 kDa bands' intensity decreased while the 55 kDa band's intensity increased after five days.

Early reproductive growth stage. A new band of 25 kDa developed in the early reproductive growth stage. The intensity of the 90 kDa band increased during treatment while still showing an increased intensity five days after freezing treatment.

Late reproductive growth stage. Three new bands of 49 kDa, 70 kDa and 85 kDa developed in the late reproductive growth stage. The intensity of the 150 kDa band decreased. Plants were already dead five days after treatment.

Protein profiles can help to identify the maturity of the potato plant. Mature plants showed increased band intensity at 35 and 40 kDa (Figure 4.2b). These data confirm previous findings by Li (1985), who observed decreased subunits of 80 kDa with increasing tuber size (tubers reaching maturity) (Li, 1985). Patatin, the predominant soluble glycoprotein of potato plants can be noticed on gels at approximately 45 kDa (Racusen and Foote, 1980) on gels (Figure 4.2b).

Protein profiles of potato plants that survived and those that died because of frost were compared. The presence of 25, 32, 55 and 175 kDa bands could be observed in plants which survived frost in their late vegetative growth stage. These bands were absent in the dead plants. These results indicate that potato plants which are able to accumulate these proteins, enhance cold tolerance. A 75 kDa protein accumulated at a higher concentration in frost tolerant plants (Figure 4.2b). Potato plants surviving freezing temperatures during late reproductive growth showed a decreased band intensity at 39, 65 and 85 kDa. These results indicate that freezing stress caused a significant change in the protein profiles of the potato plants.

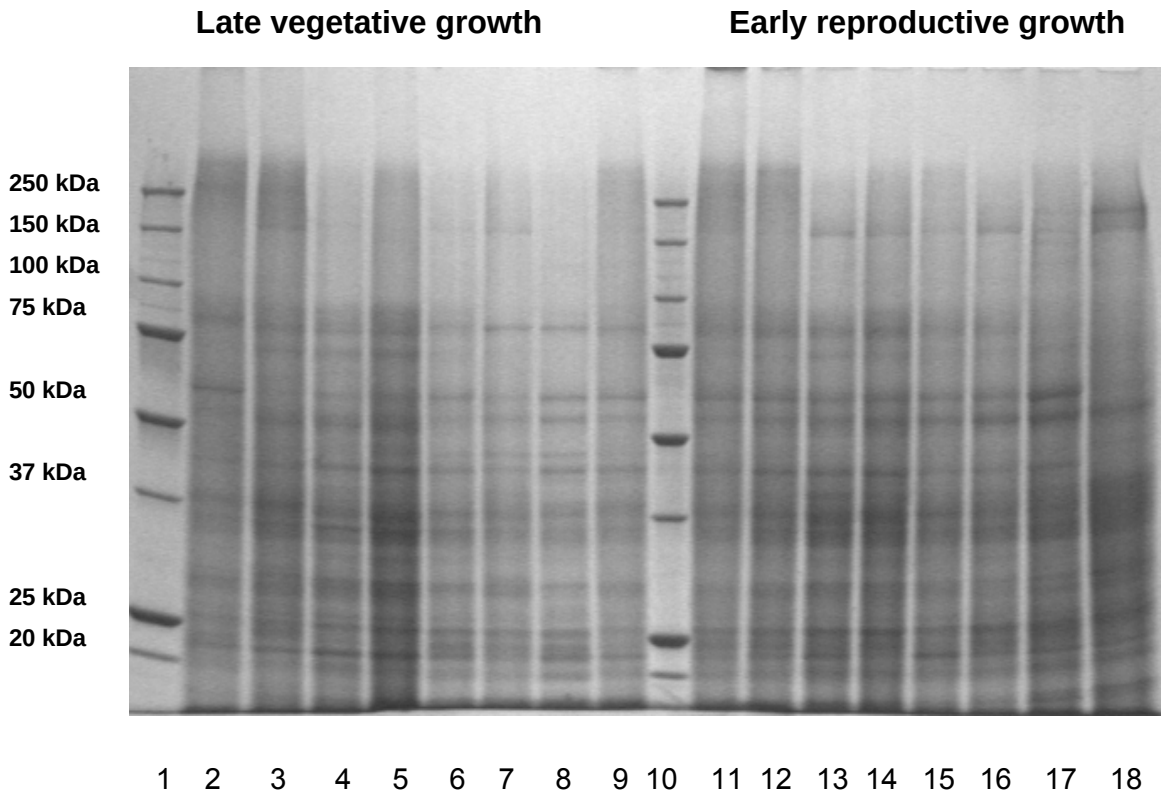


Fig. 4.2a Potato leaf proteins of Darius, separated on SDS PAGE gel. Molecular marker (lane 1), controls (lanes 2&3), plants exposed to -4°C for three hours (lanes 4&5), controls after five days (lanes 6&7), plants exposed to -4°C for three hours after five days (lanes 8&9). Molecular marker (lane 10), controls (lanes 11&12), plants exposed to -4°C for three hours (lanes 13&14), controls after five days (lanes 15&16), plants exposed to -4°C for three hours after five days (lanes 17&18).

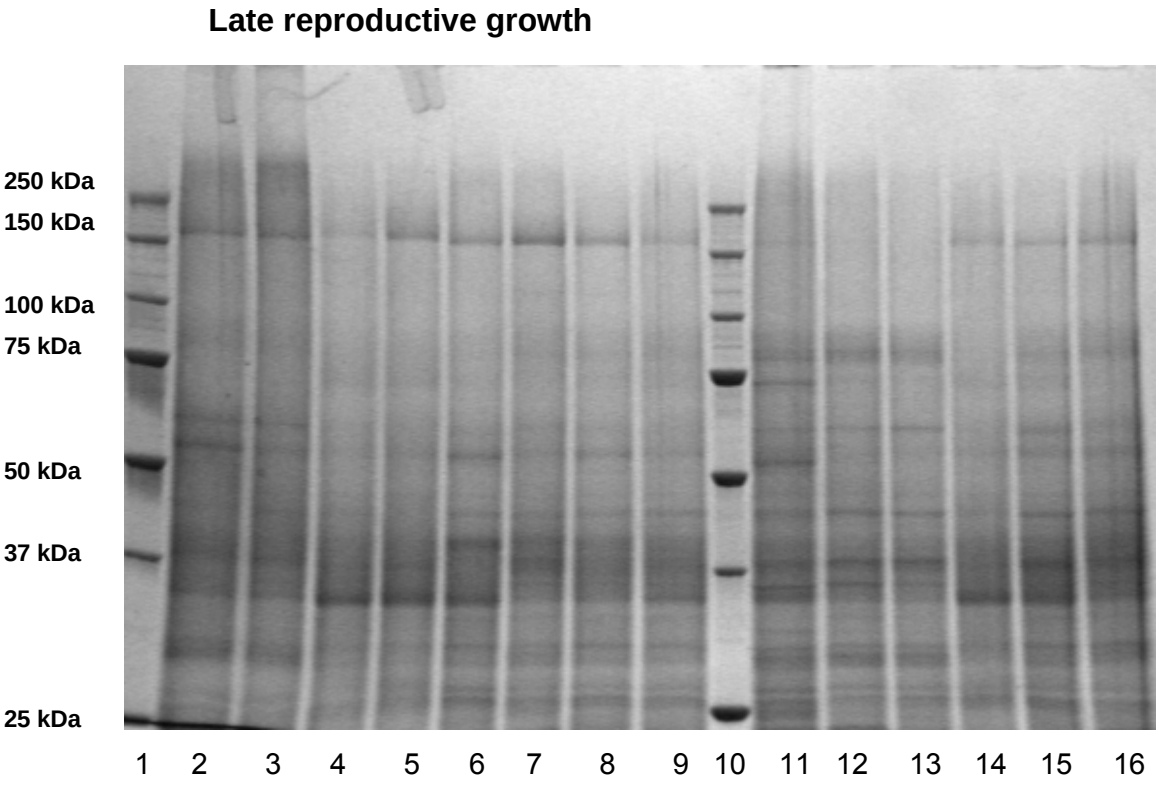


Fig. 4.2b Potato leaf proteins of Darius, separated on SDS PAGE gel. Molecular marker (lane 1), controls (lanes 2&3), plants exposed to -4°C for three hours (lanes 4&5), controls after five days (lanes 6&7), plants exposed to -4°C for three hours after five days (lanes 8&9). Molecular marker (lane 10), Plants in their late vegetative growth that survived freezing temperatures (lane 11) and those who died after exposure (lanes 12&13). Plants in their late reproductive growth that survived freezing temperatures (lane 14) and those who died (lanes 15&16).

4.4 Conclusions

Electrophoretic analysis showed that freezing temperatures for three and six hours at different growth stages resulted in alterations in the protein profile. Despite the fact that plants were exposed to freezing temperatures, quantitative protein changes were found during tuber development in a previous study (Li, 1985). Protein profile alterations should therefore be compared within different growth stages (Coccucci *et a*l., 1972).

Potato plants exposed to freezing temperatures during the late vegetative growth stage developed a new band in the region of 35/37 kDa after three hours. This band was absent in control plants. This band may play an important role in short term protection of potato plants against low temperatures. The 250 kDa band showed a decrease in protein intensity when exposed to freezing temperatures. Plants containing this protein may be sensitive to freezing stress.

When potato plants in their early reproductive growth stage were subjected to freezing temperatures for six hours, a new band in the 25/30 kDa region developed. Potato plants containing this band may be tolerant to freezing stress during the early reproductive stage.

A new protein band in the 47/49 kDa region was formed in the late reproductive stage with the six hour freezing treatment. The intensity of the 33/35 kDa band increased while the intensity of the 150 kDa band decreased after three hours. These results indicate a stimulation of protein production due to freezing stress while the production of other proteins were suppressed by freezing stress.

In a previous study, Tseng and Li (1990) acclimated *S. commersonii*, (the wild, frost tolerant potato). The presence of seven new CIPs could be observed at 21, 22, 25, 33, 46, 50 and 70 kDa after just one day of acclimating conditions at 5°C. The presence of some of these protein bands observed may be used to identify frost tolerant potato plants.

The appearance of cold induced polypeptides (CIPs) depends naturally on relative rates of individual polypeptide synthesis, but also on their stabilities for short periods (Tseng and Li, 1990). In their study they found that plantlets acclimated for up to 14 days produced more CIP's, some disappeared while others stayed stable and prominent throughout the period. This may explain why some high molecular weight proteins that formed after three hours of exposure in this study disappeared after six hours. Some unique proteins (25, 30, 47, 49 and 70 kDa bands), which were not present after the three hour treatment, were observed after six hours of freezing exposure. These alterations in protein synthesis profiles over time indicate that changes in protein synthesis are time dependent (Tseng and Li, 1990).

To conclude, the protein profiles of potato plants can be used to screen plants (cultivars) for tolerance to freezing stress and to predict the maturity of the plant. Tolerance to freezing temperatures in potato plants can be associated with the production and accumulation of several proteins. The duration of exposure of potato plants to freezing temperatures will affect the production and accumulation of different proteins.

More accurate results can be derived with the monitoring of plant protein profiles over a longer period of time after exposure to freezing temperatures. The utilization of root tips, tubers or stem materials, the use of other extraction methods and of different coloring techniques should also be tested. These results indicate that freezing temperatures do have an effect on the production and accumulation of proteins in potato leaves.

REFERENCES

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.

Chen, H.H. and Li, P.H. 1980a. Characteristics of cold acclimation and deacclimation in tuber-bearing Solanum species. *Plant Physiology* 65: 1146-1148.

Chen, H.H. and Li, P.H. 1980b. Biochemical changes in tuber-bearing *Solanum* species in relation to frost hardiness during cold acclimation. *Plant Physiology* 66: 414-421.

Chen, H.H., Li, P.H. and Brenner, M.L. 1983. Involvement of abscisic acid in potato cold acclimation. *Plant Physiology* 71: 362-365.

Cocucci, S.M., Garlashi, F.M., Bianchi, E. and Marre, E. 1972. Changes of the protein synthesis system during the maturation of potato tubers. *Physiologia Plantarum* 27: 200-225.

Guy, C.L. 1990. Cold acclimation and freezing stress tolerance: role of protein metabolism. *Annual Review of Plant Physiology and Molecular Biology* 41: 187-223.

Kacperska-Palacz, A., Dlugokecka, E., Breitenwald, J. and Wcislinska, B. 1977. Physiological mechanisms of frost tolerance: possible role of protein in plant adaption to cold. *Biologia Plantarum* 19: 10-17.

Lee, S.P., Zhu, B., Chen, T.H.H. and Li, P.H. 1992. Induction of freezing tolerance in potato (*Solanum commersonii*) suspension cultured cells. *Physiologia Plantarum* 84: 41-48.

Levitt, J. 1980. Responses of plants to environmental stresses. Vol 1. Academic Press, New York.

Li, P.H. 1985. Potato Physiology. Academic Press Inc. (London).

Li, P.H., Ryu, S.B., Tseng, M.J. and Chen, T.H.H. 1989. Induction of plant cold hardiness. *In*: Randall, D.D. and Blevins, D.G. (eds). Current topics in plant biochemistry and physiology. Volume 8, University of Missouri-Columbia, MO. pp. 21-40.

Lindstrom, O.M., Olson, D.J. and Carter, J.V. 1992. Degree of undercooling and injury of whole potato plants following exposure to -4°C for 6 or 12 hours. *Horticultural Science* 27(3): 244-246.

McCown, B.H., Beck, G.E. and Hall, T.C. 1968. Plant leaf and stem proteins. I. Extraction and electrophoretic separation of basic, water-soluble fraction. *Plant Physiology* 43: 578-582.

Racusen, D. and Foote, M. 1980. A major soluble glycoprotein of potato tubers. *Journal of Food Biochemistry* 4: 43-52.

Rorat, T., Grygorowicz, W.J., Berbezy, P. and Irykowski, W. 1998. Isolation and expression of cold specific genes in potato (*Solanum sogarandium*). *Plant Science* 133: 57-67.

Rorat, T., Irzykowski, W. and Grygorowics, W.J. 1997. Identification and expression of novel old genes in potato (*Solanum sogarandinum*). *Plant Science* 124: 69-78.

Ryu, S.B. and Li, P.H. 1994. Potato cold hardiness development and abscisic acid. II. *De novo* synthesis of proteins is required for the increase in free abscisic

acid during potato (*Solanum commersonii*) cold acclimation. *Physiologia Plantarum* 90: 21-26.

Sukumaran, N.P. and Weiser, C.J. 1972. An excised leaflet test for evaluating potato frost tolerance. *Horticultural Science* 7: 467-468.

Tseng, M.J. and Li, P.H. 1990. Alterations of gene expression in potato (*Solanum commersonii*) during cold acclimation. *Physiologia Plantarum* 78: 538-547.

Tseng, M.J. and Li, P.H. 1991. Changes in protein synthesis and translatable messenger RNA populations associated with ABA-induced cold hardiness in potato (*Solanum commersonii*). *Physiologia Plantarum* 81: 349-358.

Weiser, C.J. 1970. Cold resistance and injury in woody plants. *Science* 169 (3952): 1269-1278.

CHAPTER 5

Inheritance of yield in C1 (Caren x Bravo) potato breeding population under conditions of freezing stress

5.1 Introduction

With the exception of maize, the potato is grown in more countries in the world than any other crop (Li, 1985). Because of its importance as one of humanity's most valuable staple foods, much interest is given to improve some important agronomic traits in this crop. Recent potato breeding programs helped to develop new potato varieties which met market demands for reduced disease and pest related problems. Compared to other crops, potato breeding appeared to be less successful over the past years, mainly because of the genetic complexity of the plant (Dean, 1994).

Potato breeding programs may focus on the improvement of one specific character or a combination of characters such as pest resistance, enhancement of yield, specific agronomic characteristics, improvement of raw product quality (tuber morphology), storage and processing characteristics (Dean, 1994).

The first step of a breeding program is to select suitable parents for the crossing block. Parents are crossed to produce unique and desirable genotypes. After dormancy the F1 seeds are harvested and planted to produce numbers of seedlings (genotypes). Effective methods of screening should be used on the F1 plants to select superior plants with desirable characteristics derived from both parents.

Since the potato propagates by vegetative production, the genotype remains constant from generation to generation. Selection done at any stage will stay true to type. When selection for a character is done, the character's level of

heritability determines the effectiveness of selection. In clonal propagation, the broad sense heritability is appropriate to use when measuring the response to selection. According to Bradshaw and Mackay (1994) the estimation of the heritability of agronomically important traits are of great value in determining selection and breeding strategies in potato breeding.

The inheritance of frost resistance in potato plants is not a simple mechanism and is believed to be inherited polygenically (Hawkes, 1958). Cultivated potatoes are frost sensitive while some wild potato species possess frost resistance. These wild species produce low yield and bitter flavored tubers (Estrada, 1978). The ideal would be to breed a cultivated potato with high yield and acceptable taste containing frost resistance. Unfortunately, up to date the incorporation of wild and cultivated species was unsuccessful because of sterility, incompatibility and odd chromosome numbers (Beukema and Van der Zaag, 1990), but also because of the genetic complexity of the freezing tolerance trait (Stone *et al*., 1993) (Chen *et al*., 1999). In order to utilize the genetic variability for freezing stress within the South African potato germplasm, it is necessary to determine the heritability under such conditions.

The objective of this study was to estimate the heritability and calculate the phenotypic correlation coefficients between the yield and yield components under freezing stress conditions.

5.2 Materials and methods

5.2.1 Plant material

A cross was made between two South African potato cultivars, Caren (male) x Bravo (female). This was done by the ARC at Roodeplaat, South Africa during 2003. Caren was derived from a cross between Kimberley Choice and selection 890/20. Bravo was derived from a cross between BP1 x (Bismarck x Katahdin). Fruits that developed on the female plant (Bravo) were harvested and the seeds were planted separately in a seed tray in a glass house. When the seedlings

were strong enough they were replanted in the field to produce potato progenies. A random sample of 14 progenies were selected. The experimental material used in this study consisted of two parental cultivars Caren and Bravo and the 14 C1 progenies derived from this cross.

5.2.2 Freezing injury

Ten tubers of each parent and of each of the 14 C1 progenies were planted on the same day during August 2005 in a glasshouse at the University of the Free State, Bloemfontein. The tubers were planted one per pot (42 cm diameter and 30 cm high) under controlled glass house conditions with a 15/26 °C night/day temperature regime. Pots contained a mixture of sandy loam soil. Each pot contained 35 kg soil. Thirty grams of 2:3:2 (N:P:K) nutrients were given to each pot with plant, while 6.7 g KNO_3 were given to each pot seven and thirteen weeks after plant. Plants were watered each second to third day during its growth.

For each of the 16 genotypes, 10 plants were divided into two groups of five. Five plants of each entry served as a control population while the other five plants were subjected to a freezing treatment during the early reproductive growth stage.

The plants were placed in a low temperature (4°C) walk-in chamber for four hours to ensure that all plants were at the same temperature prior to freezing (Lindstrom *et al*., 1992). The plants of each entry were then subjected to a -4°C treatment for three hours. Pots were placed on a 4 cm thick foam isolation sheet in the freezing chambers to prevent soil water from freezing. After exposure to freezing temperatures the plants were left at room temperature ($\pm$ 20°C) for approximately 24 hours and finally returned to the glasshouse where they remained until harvest.

5.2.3 Characteristics measured

The following characteristics were measured on the control and treated plants:

i) Percentage stem damage - was calculated by dividing the number of dead auxiliary buds by the total number of buds for each stem of each plant.

ii) Percentage leaf damage - was assessed by calculating the percentage damaged lead area on the remaining auxiliary buds for each plant.

iii) Yield - total mass (in g) of tubers produced by each plant

iv) Average tuber diameter - the sum of each tuber's length (mm) and width (mm) divided by two and then divided by the total amount of tubers per plant.

v) Average tuber mass - yield per plant divided by the total number of tubers produced by each plant.

vi) Number of tubers - the total amount of tubers per plant produced.

5.2.4 Statistical analysis

a) An analysis of variance (ANOVA) was used to analyze the data of the six measured characteristics. The genotypic means for all traits measured were used to compare the performances of the two parental cultivars and 14 genotypes. Means were separated using least significant differences (LSD). The AGROBASE (2000) computer program was utilized to perform the ANOVA.

b) Correlation coefficients were obtained by using the software program, AGROBSE (2000) to determine the degree of association between characters. Separate analyses were preformed on the untreated, treated and differences between treated and untreated plants.

c) Heritability in the broad sense (h^2_B) was calculated from the components of variance as described by Becker (1967). Components of variance were estimated as follows:

Genotypes	$\mathbf{MS_g}$	=	$\sigma^2_e + r\,\sigma^2_{gt} + rt\,\sigma^2_g$
Genotype x Treatment	$\mathbf{MS_{gt}}$	=	$\sigma^2_e + r\,\sigma^2_{gt}$
Error	$\mathbf{MS_e}$	=	σ^2_e

$$\sigma^2_g = \frac{MS_g - MS_{gt}}{rt}$$

$$\sigma^2_{gt} = \frac{MS_{gt} - MS_e}{r}$$

$$\sigma^2_e = MS_e$$

Where: σ^2_g is the genotypic variance

σ^2_{gt} is the genotype x treatment variance

σ^2_e is the environmental variance

h^2_B is the broad sense heritability

t = treatments

r = replications

$$h^2_B = \sigma^2_g \,/\, \sigma^2_g + \sigma^2_{gt/t} + \sigma^2_{e/rt}$$

5.3 Results and discussion

5.3.1 Analysis of variance for stem and leaf damage

Mean squares for stem and leaf damage are shown in Table 5.1. Analysis of variance showed no significant differences between entries for stem damage. This indicates limited variability between entries for stem damage in the Bravo x Caren cross. Analysis of variance showed significant differences ($P<0.05$) between entries for leaf damage. Percentage leaf damage caused by freezing stress for the different entries is illustrated in Figure 5.1. Genotype 43 showed the highest sensitivity to freezing temperatures with the highest percentage leaf damage, followed by genotype 8 with 68.38% and genotype 39 with 63.19% leaf damage. Genotype 60 had the lowest leaf damage (8.93%) and therefore displayed the highest degree of resistance against freezing stress. Genotypes placed second and third were genotype 61 with 18.46% leaf damage and genotype 25 with 19.86% leaf damage. The leaf damage recorded for these genotypes was significantly lower than that of the parental cultivar, Caren. These results demonstrate the transgressive nature of poligenes controlling resistance to freezing stress in potatoes.

5.3.2 Genotypic means

The freezing treatment had a significant effect on yield and yield components. Mean squares for treatments were highly significant (Table 5.2) for average tuber diameter, average tuber mass, number of tubers and yield per plant. These results indicate that the yield and yield components were significantly reduced by the -4°C freezing treatment. Large differences were found between entries for yield and yield components. Mean squares for entries (Table 5.2) were highly significant ($P<0.01$) for average tuber diameter, average tuber mass, number of tubers and yield per plant. Entries within treatments showed only significant differences for number of tubers and yield per plant.

a) Average tuber diameter per plant

The average tuber diameter per plant for untreated and treated plants are illustrated in Figure 5.2. Freezing treatment caused a reduction in tuber diameter. The only exceptions were genotypes 43 and 54, where a slight increase in tuber diameter occurred. Although the diameters of the treated genotypes were slightly lower than those of the control plants, it was not significant. The only exception was genotype 56 of which average tuber diameter was significantly reduced by the freezing treatment. Caren produced tubers with the largest average tuber diameter. It was significantly larger than the diameters of genotypes 53, 60 and 66 in both the untreated and treated plants.

b) Average tuber mass per plant

The average tuber mass per plant was significantly reduced by the freezing treatment in three of the entries (Figure 5.3), namely the parental cultivar Caren and genotypes 39 and 56. The other genotypes showed only a slight reduction in tuber mass, indicating some kind of resistance against freezing at -4°C. The only exception was genotype 43 of which average tuber mass increased significantly with treatment. The parental cultivar Caren produced the highest average tuber mass among control plants. The tuber mass was significantly higher than the rest of the entries except for genotype 61. Genotype 43 showed the highest tuber mass among treated plants. It was significantly higher than most of the entries except for Caren and genotype 61. The average tuber mass of Caren exceeded those of genotypes 28, 53, 55, 56, 60 and 66 significantly for both treatments.

c) Total amount of tubers per plant

Freezing treatment caused a significant decrease in tuber number for six of the genotypes (Figure 5.4), namely genotypes 8, 25, 28, 55, 56 and 64. The number of tubers per plant increased significantly for genotype 43 in treated plants. Freezing treatment therefore stimulated the production of tubers in this

genotype. The number of tubers produced by Caren exceeded those of genotypes 53, 60, 61 and 66 significantly in both the untreated and treated plants.

Table 5.1 Analysis of variance for stem and leaf damage of 16 potato genotypes.

	Mean squares	
Source	**Stem damage**	**Leaf damage**
Reps	1492.555	1637.122
Entry	1209.021	1851.101*
Error	1202. 016	927.495
CV (%)	65.26	65.67

* = significant at P=0.05 probability level

Table 5.2 Mean square values derived from the ANOVA of four yield components of 16 potato genotypes.

	Mean squares			
Source	**Average tuber diameter per plant (mm)**	**Average tuber mass per plant (g)**	**Number of tubers per plant**	**Yield per plant (g)**
Treatments	1684.025**	1244.173**	232.565**	120813.622**
Reps in treatment	142.488	116.389	9.363	4864.343
Entry	586.849**	318.998**	35.859**	14976.289**
Entry in treatment	92.537	79.254	17.525**	9319.495**
Error	115.878	61.820	7.638	3693.671

*,** = significant at P=0.05 and 0.01 probability levels respectively

d) Yield

Freezing treatment caused a significant yield reduction in seven of the entries (Figure 5.5), namely Caren and genotypes 8, 28, 39, 55, 56 and 64. Although the freezing treatment caused a reduction in yield in most of the entries, the yield of genotype 43 increased significantly with treatment, indicating that genotype 43 was stimulated significantly by a short period of freezing. Caren produced the highest yield among the untreated entries. With the exception of genotypes 8 and 39 it outyielded the rest of the entries significantly. Genotype 43 revealed the highest yield among the treated plants. With the exception of Caren, its yield was significantly higher than the rest of the treated plants.

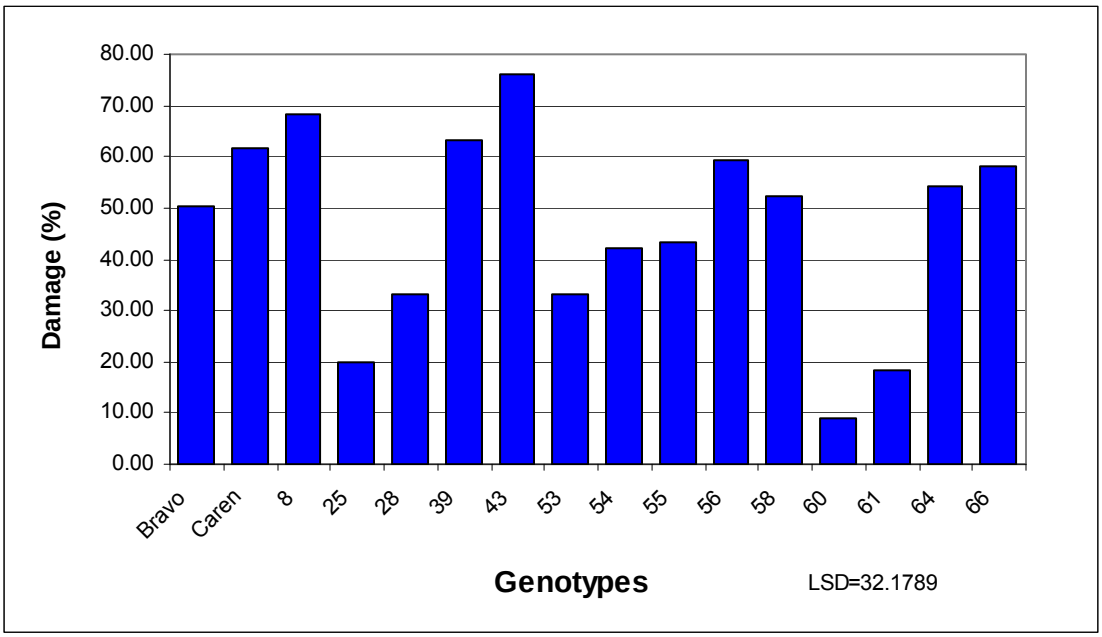


Fig. 5.1 Percentage leaf damage of 16 potato genotypes exposed to freezing temperatures.

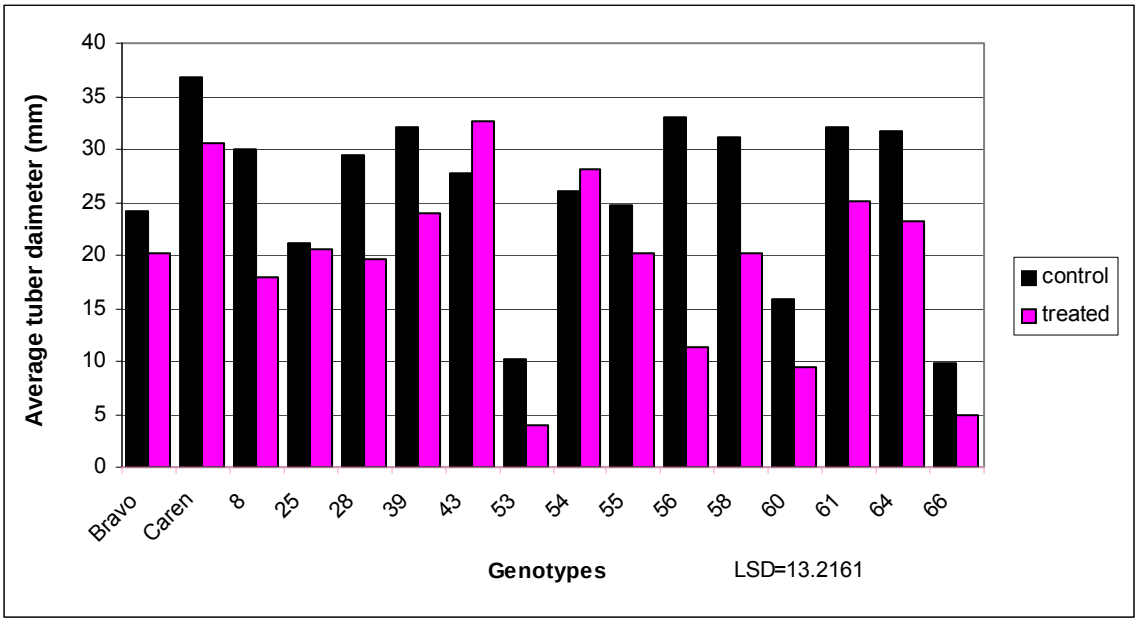


Fig. 5.2 Average tuber diameter per plant for 16 freeze treated and untreated potato genotypes.

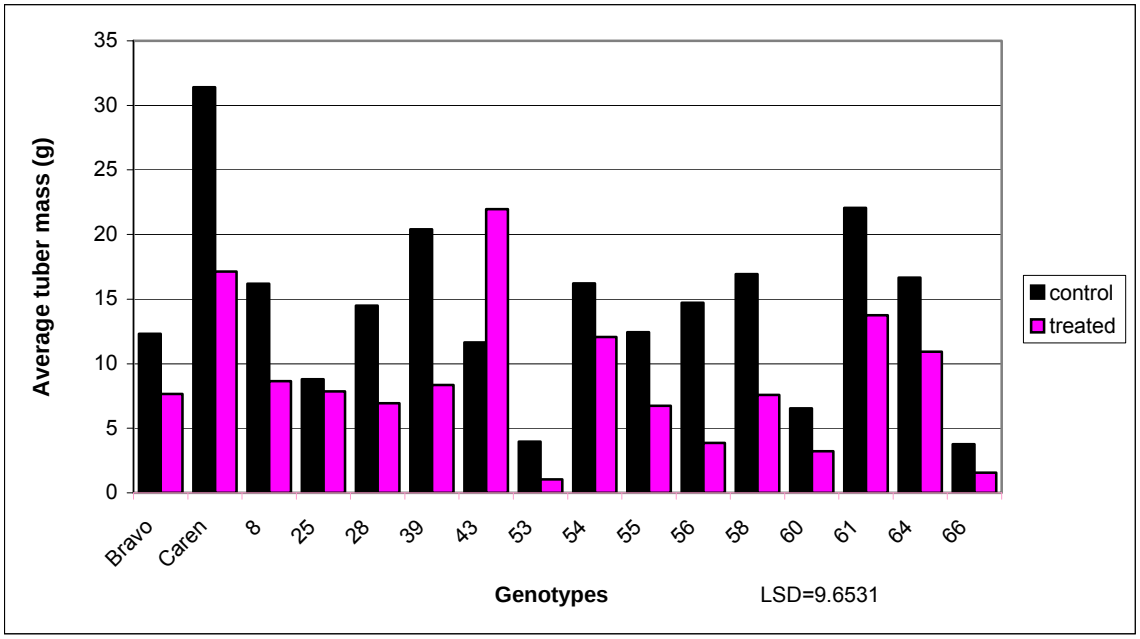


Fig. 5.3 Average tuber mass per plant for 16 freeze treated and untreated potato genotypes.

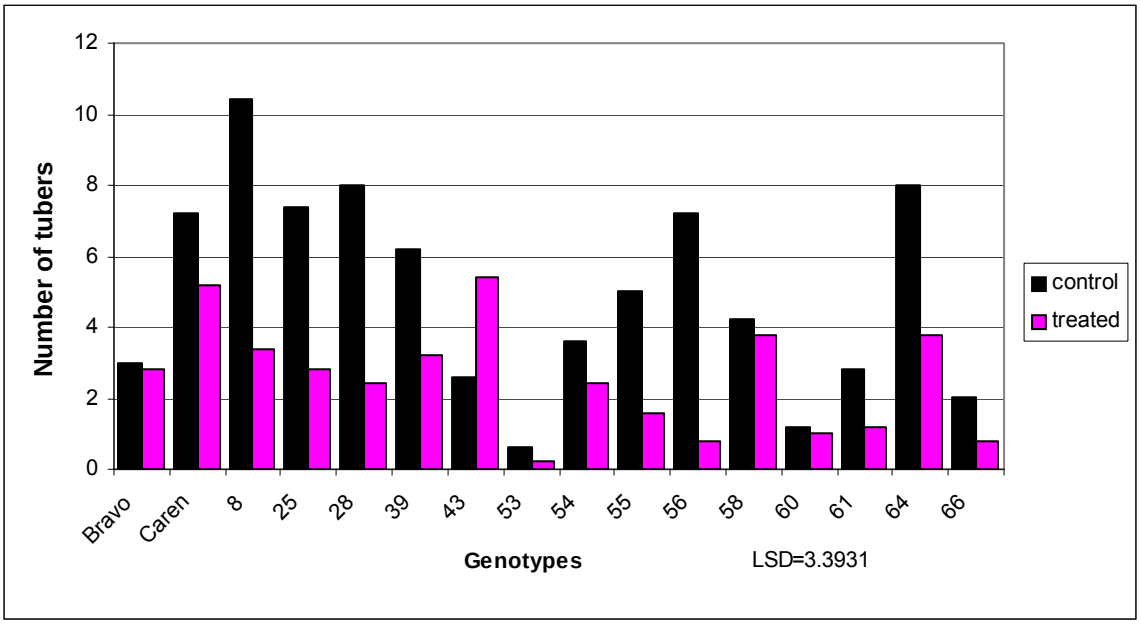


Fig. 5.4 Mean number of tubers per plant for 16 freeze treated and untreated potato genotypes.

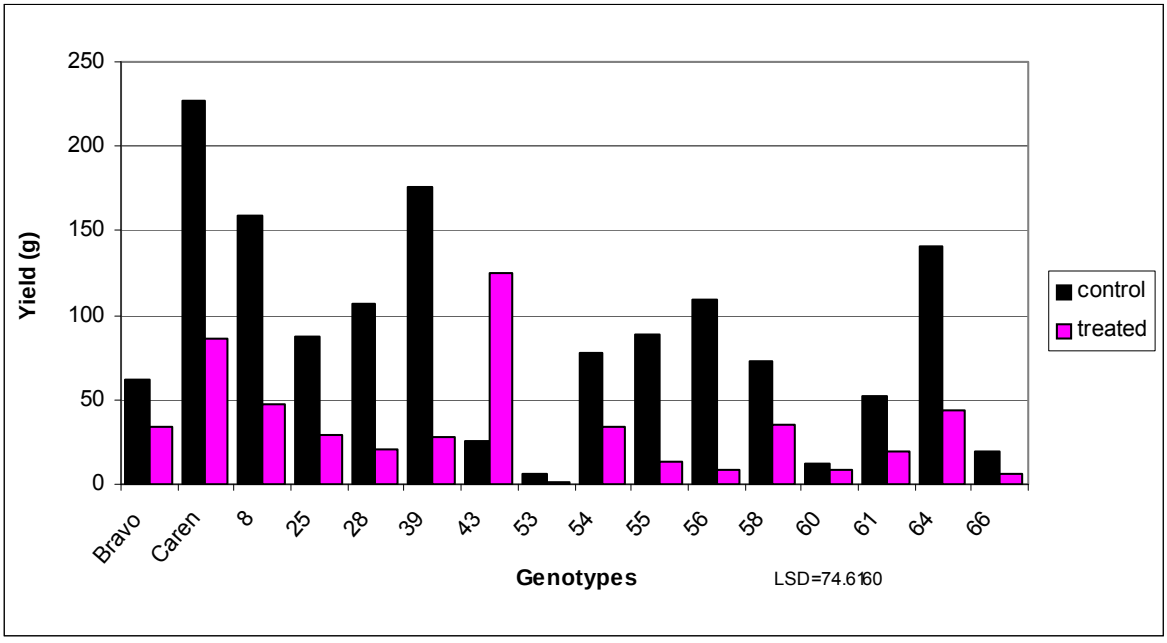


Fig. 5.5 Mean yield per plant for 16 freeze treated and untreated potato genotypes.

5.3.3 Correlations between measured traits

Correlation coefficients between the traits measured were calculated separately for untreated and treated potato plants. A third correlation analysis was done which included average stem and leaf damage as well as percentage reduction in yield and yield components due to freezing.

a) Untreated potato plants

Correlation coefficients between yield and yield components for the untreated plants are shown in Table 5.3. Highly significant ($P<0.01$) positive correlations were found between yield and tuber diameter ($r=0.61$), yield and tuber mass (0.74) and yield and tuber number ($r=0.78$) per plant. Highly significant ($P<0.01$) correlations were also observed between tuber number and number of stems ($r=0.56$), tuber number and tuber diameter ($r=0.54$) and tuber number and tuber mass ($r=0.40$) per plant. A highly significant ($P<0.01$) correlation also occurred between tuber mass and tuber diameter ($r=0.82$). The correlation coefficients between yield and number of stems were significant ($P<0.05$), but very low ($r=0.27$). The result indicates that increased tuber numbers of larger mass will result in increased yield.

b) Treated potato plants

Correlation coefficients between yield and yield components for treated potato plants are shown in Table 5.4. Highly significant ($P<0.01$) correlations were observed between yield and tuber diameter ($r=0.65$), yield and tuber mass ($r=0.79$) and yield and tuber number ($r=0.82$). Highly significant ($P<0.01$) correlations were also observed between tuber mass and tuber diameter ($r=0.88$), tuber number and tuber diameter ($r=0.64$) and tuber number and tuber mass ($r=0.56$) per plant. The correlation coefficients obtained from untreated and treated plants were similar. The only exceptions were the correlation coefficients between yield and number of stems and tuber number and number of stems

which were not correlated significantly in treated plants. This is mainly the result of stems that died due to freezing temperatures during treatment.

c) Differences between untreated and treated potato plants

A separate correlation analysis was done on percentage reduction of the various yield characteristics due to freezing stress. The results are shown in Table 5.5. A significant correlation ($P<0.05$) was observed between yield and tuber diameter ($r=0.56$). Highly significant ($P<0.01$) correlations were observed for yield and tuber mass ($r=0.86$) and yield and number of tubers ($r=0.75$) per plant. Tuber diameter was significantly ($P<0.01$) correlated with both tuber mass ($r=0.68$), and the number of tubers. Leaf and stem damage was negatively ($P<0.01$) correlated. This association is the result of the measuring method used to assess stem and leaf damage. Leaf damage was determined on the remaining part of the stem, which survived freezing temperatures. Increased stem damage therefore resulted in low leaf damage.

Table 5.3 Correlation matrix for yield and yield components of untreated potato plants.

	Number of stems	Average tuber diameter	Average tuber mass	Number of tubers
Average tuber diameter	0.0430			
Average tuber mass	-0.0842	0.8219**		
Number of tubers	0.5681**	0.5486**	0.4086**	
Yield	0.2793*	0.6163**	0.7464**	0.7865**

*,** = significant at P=0.05 and 0.01probability levels respectively

Table 5.4 Correlation matrix for yield and yield components of treated potato plants.

	Number of stems	Average tuber diameter	Average tuber mass	Number of tubers
Average tuber diameter	-0.0988			
Average tuber mass	-0.1602	0.8822**		
Number of tubers	0.1302	0.6446**	0.5669**	
Yield	-0.0554	0.6522**	0.7901**	0.8203**

** = significant at P= 0.05 probability level

Table 5.5 Correlation matrix of percentage damage between untreated and treated potato plants for six characteristics.

	Average stem damage	Average leaf damage	Average tuber diameter	Average tuber mass	Number of tubers
Average leaf damage	-0.6852**				
Average tuber diameter	0.1028	0.0927			
Average tuber mass	0.0645	0.0053	0.6874**		
Number of tubers	0.0627	0.0194	0.6447*	0.5276	
Yield	-0.0790	0.1368	0.5649*	0.8683**	0.7597**

*,** = significant at P=0.05 and 0.01probability levels respectively

5.3.4 Broad sense heritability

Variance components and heritabilities for yield and yield components are provided in Table 5.6. Variance components were relatively high for both yield and yield components. The variance components for genotype x treatment interaction were relatively low for tuber diameter and tuber mass, but proved to be important for yield and tuber number per plant. For yield, it declared more than 60% of the genotypic variance and for tuber number almost 50%. This result indicates the importance of genotype x treatment interaction for yield, which was mainly caused by the genotype x tuber interaction. Broad sense heritabilities were relatively high for yield and yield components. Broad sense heritabilies for tuber diameter (h_b=0.91) and tuber mass (h_b=0.87) were particularly high. Somewhat lower values were observed for tuber number (h_b=0.79) and yield (h_b=0.74). The lower broad sense heritabilities for tuber number and yield were mainly due to the large variance components for genotype x treatment interactions. Relatively high heritability for tuber diameter and its strong correlation with potato yield, enhances the possibility to use tuber diameter indirectly for the genetic improvement of potato yield.

In contrast with the findings of Du Plooy *et al.* (1996), broad sense heritability for average tuber diameter, mass, number of tubers and yield per plant were relatively high in this study.

Table 5.6 Variance component values and heritabilities for yield and yield components in potatoes.

	Average tuber diameter per plant	Average tuber mass per plant	Number of tubers per plant	Yield
σ^2_g	586.849	318.998	35.859	14976.289
σ^2_{gt}	92.537	79.254	17.525	9319.495
σ^2_e	115.878	61.820	7.638	3693.671
h^2_B	0.9102593	0.8744294	0.7901016	0.7486122

5.4 Conclusions

Freezing stress (-4°C) treatment in the reproductive stage of C1 (Caren x Bravo) breeding population caused significant leaf damage. The number of stems was, however, not significantly reduced by the treatment.

The study showed no definite association between leaf damage and percentage yield loss. Genotype 43 that had the highest level of leaf damage, showed a significant increase in yield under freezing stress conditions. This result emphasizes the possibility that slight freezing stress may enhance yield characteristics in genotype 43. This is probably due to the activation of proteins that help the plant to recover from freezing stress and consequently lead to improved yield. Caren yielded significantly better than Bravo under freezing stress conditions.

Genotypes 53, 60 and 66 which produced the lowest yields under freezing stress conditions, showed the lowest level of leaf damage. These genotypes should not be considered for future use in breeding programs.

Highly significant correlations were observed between yield and the three yield components (diameter, mass and number of tubers). Average tuber mass and size were not significantly associated with leaf and stem damage in this study.

The broad sense heritabilities for yield and yield components were relatively high. The high heritability for tuber diameter and its strong association with yield, emphasize the possibility to practice indirect selection in order to enhance yield.

REFERENCES

AGROBASE, 2000. Agrobase user's guide and reference manual. Agronomix Software Inc., Canada.

Becker, W.A. 1967. Manual of Quantitative genetics. Washington, Pullman.

Beukema, H.P. and Van der Zaag, D.E. 1990. Introduction to potato production. Pudoc Wageningen, Den Haag.

Bradshaw, J.E. and Mackay, G.R. 1994. Potato genetics. University Press Cambridge, Unigted Kingdom.

Chen, Y-KH., Bamberg, J.B. and Palta, J.P. 1999. Expression of freezing tolerance in the interspesific F1 and somatic hybrids of potatoes. *Theoretical and Applied Genetics* 98: 995-1004.

Dean, B.B. 1994. Managing the potato production system. Food Products Press, New York.

Du Plooy, F.I., Eisenberg, B.E., Vorster, H.J., Roux, C.Z. and Geerthsen, J.M.P. 1996. Study of the inheritance of some agronomic traits in a potato breeding population. *Journal of South African Horticultural Science* 6(1): 20-23.

Estrada, R.N. 1978. Breeding frost-resistant potatoes for the tropical highlands. Plant cold hardiness and freezing stress. Academic Press, Inc.

Hawkes, J.G. 1958. Significance of wild species and primitive forms for potato breeding. *Euphytica* 7: 257-270.

Li, P.H. 1985. Potato Physiology. Academic Press, Inc. (London).

Lindstrom, O.M., Olson, D.J. and Carter, J.V. 1992. Degree of undercooling and injury of whole potato plants following exposure to -4°C for 6 or 12 hours. *Horticultural Science* 27(3): 244-246.

Stone, J.M., Palta, J.P., Bamberg, J.B., Weiss, L.S. and Harbage, J.F. 1993. Inheritance of freezing resistance in tuber-bearing Solanum species. Evidence for independent genetic control of non-acclimated freezing tolerance and cold acclimation capacity. *Proceedings National Academy of Science, USA.* 90: 7869-7873.

CHAPTER 6

General conclusion and recommendations

Frost damage due to freezing stress causes major yield losses to potato growers in the eastern low fields of South Africa. One of the major challenges for scientists is the accurate prediction of yield losses due to stem and leaf damage for the various growth stages of the potato plant and to develop tolerant cultivars. In the past, research has only been done on defoliation of the stems and leaves and its relationship with yield loss. No information about the artificial freezing of potato plants and its effect on yield are available in literature. This research was therefore initiated to study the relationship between artificial freezing at different growth stages and yield loss of two potato cultivars.

The first problem that arose was the large variability for stem and leaf damage between plants. This increased the coefficient of variation of the experiments, which decreased the accuracy of the statistical analysis. The significant genotype x treatment interactions for stem and leaf damage as well as for yield and some of the yield components caused further difficulties, since it indicated no linear relationship between either stem or leaf damage and yield loss. This was probably due to the ability of the plants to recover. The most sensitive plants showed the best ability to recover, especially with treatment during the early growth stages.

With both cultivars Darius and BP1, the largest percentage yield loss was observed with treatment in the early reproductive growth stage. This result emphasises the sensitivity of the early reproductive growth stage to freezing stress and its importance for screening purposes. The large difference observed in yield loss between Darius and BP1 with treatment in the early reproductive growth stage, emphasizes the possibility to develop tolerant cultivars. The study indicated that potato breeders should concentrate on the ability of plants to

recover in order to develop tolerant cultivars. The study further showed a strong association between stem damage and number of tubers and leaf damage and tuber mass.

In this study large tubers were not produced, mainly due to soil compaction in the pots. In future research it should be considered to plant in a less compact growth medium such as vermiculite.

Protein analysis showed the formation of various new bands after freezing treatment at different growth stages. A connection may exist between newly formed protein bands and the ability of the plant to recover from cold stress. This needs further investigation.

The broad scense heritability for tuber diameter was significantly higher than that for yield. Due to its extremely high heritability and highly significant correlation with potato yield, it is expected that the correlated response for tuber diameter will be relatively high. It is therefore recommended that potato breeders use tuber diameter as an indirect selection criterion, rather than yield measurement to improve yield in potatoes.

CHAPTER 7

Summary

Key words: freezing stress, growth stage; heritability; proteins; *Solanum tuberosum*; yield components.

a) Frost damage caused by freezing stress is a major problem for potato growers in some parts of South Africa. In this study two South African potato cultivars, Darius and BP1, were compared for yield loss due to freezing stress during the late vegetative, early reproductive and late reproductive growth stages. The cultivars were subjected to temperatures of -2°C and -4°C for three and six hours. Significant genotype x treatment interactions were observed for stem and leaf damage in both cultivars. Genotype x treatment interactions were also significant for yield and some of the yield components. The cultivars were shown to be the most sensitive to freezing stress during the early reproductive growth stage, followed by the late reproductive growth stage. BP1 was identified to be more sensitive to freezing stress in the early and late reproductive growth stages. Stem damage was positively associated with tuber number, whereas leaf damage correlated with tuber mass.

b) Electrophoretic separations of proteins were done to study the polymorphisms as a result of freezing stress at -2°C and -4°C for three and six hours. The plants were treated during the late vegetative, early reproductive and late reproductive growth stages. The freezing treatments caused large variability in the protein profiles of Darius. Various new protein bands developed while others disappeared. Differences in the intensity of the bands were also recovered. A protein band of approximately 29/33 kDa developed consistently at -2°C treatment during the early reproductive growth stage. Plants subjected to -4°C for three and six hours developed protein bands of approximately 40/49 kDa during the three growth stages tested.

c) In this study fourteen selected C1 progenies of a Caren x Bravo cross with the two parents were included, subjected to freezing temperatures of -4°C for four hours to study the heritability of potato yield under freezing stress conditions. The plants were treated during the early reproductive growth stage (the most sensitive growth stage). Freezing stress (-4°C) treatment during the early reproductive growth stage reduced potato yield, tuber diameter, tuber mass and number of tubers significantly. Significant genetic variability was found among offspring for leaf damage, yield, tuber diameter, tuber mass and number of tubers under freezing stress conditions. Tuber diameter (r=0.56), tuber mass (r=0.86) and number of tubers (r=0.75) were significantly correlated with yield for percentage damage caused by freezing stress. The freezing treatment had no significant effect on the correlation coefficients between potato yield and yield components. Relatively high broad sence heritabilities were recorded for potato yield (h^2 =0.74) tuber diameter (h^2=0.91), tuber mass (h^2=0.87) and number of tubers (h^2=0.79).

Opsomming

a) Ryp, wat vriesskade by plante veroorsaak, hou ‘n groot bedreiging vir Suid-Afrikaanse aartappelprodusente in. Twee Suid-Afrikaanse aartappelkultivars, Darius en BP1 is in hierdie studie ten opsigte van hul opbrengsverliese wat ontstaan as gevolg van die voorkoms van vriesskade vergelyk. Hierdie eksperiment is tydens die laat vegetatiewe, vroeg reproduktiewe en laat reproduktiewe groeistadiums van die aartappelplante gedoen. Die plante van elke kultivar is blootgestel aan -2°C en -4°C vir drie en ses ure. Vir stam- en blaarskade is ‘n betekenisvolle genotipe x behandeling interaksie by beide kultivars gevind. Die genotipe x behandeling interaksies vir opbrengs en sommige van die opbrengskomponente was ook betekenisvol. Beide kultivars het die grootste sensitiwiteit teenoor vriesskade getoon vir blootstelling aan vriestemperature gedurende die vroeë reproduktiewe groeistadium, gevolg deur die laat reproduktiewe groeistadium. BP1 het groter sensitiwiteit teenoor vriesskade in die vroeë en laat reproduktiewe groeistadiums as Darius getoon. Stam- en blaarskade was positief gekorreleer met die hoeveelheid knolle per plant en knolgewig onderskeidelik.

b) Om polimorfismes as resultaat van vriesskade op aartappelplante by -2°C en -4°C blootstelling vir drie en ses ure afsonderlik te bestudeer, is aartappelblaarproteïene ektroforeties geskei. Plante is tydens die laat vegetatiewe, vroeg reproduktiewe en laat reproduktiewe groeistadiums behandel. Hierdie gesimuleerde rypbehandeling het verskeie veranderings in die kultivar se proteïenprofiel tot gevolg gehad. Verskeie nuwe proteïenbande het ontwikkel, terwyl ander verdwyn het. Verskille tussen die intensiteit van sommige bande is ook waargeneem. ‘n Proteïenband van ongeveer 29/33 kDa het herhaaldelik in die vroeë reproduktiewe groeistadium tydens die -2°C behandeling voorgekom. Aartappelplante wat by -4°C vir drie en ses ure gelaat is, het nuwe proteïenbande van ongeveer 40/49 kDa by al drie groeistadiums gevorm.

c) Veertien geselekteerde C1 nageslagte van ‘n Caren x Bravo kruising, tesame met die ouers, is blootgestel aan gesimuleerde ryptoestande van -4°C vir vier ure. Hierdie eksperiment is gedoen om die oorerflikheid van opbrengs by aartappels onder ryptoestande te bestudeer. Die plante is tydens die vroeë reproduktiewe groeistadium (die mees sensitiewe groeistadium) behandel. Rypskade (-4°C) tydens vroeë reproduktiewe groei, het die opbrengs, knoldeursnit, knolmassa en hoeveelheid knolle per plant betekenisvol verminder. Betekenisvolle genetiese variasie is tussen die nageslagte vir blaarskade, opbrengs, knoldeursnit, knolmassa en die hoeveelheid knolle per plant onder rypskade toestande gevind. Die opbrengs was betekenisvol gekorreleer met knoldeursnit (r=0.56), knolmassa (r=0.86) en die hoeveelheid knolle per plant (r=0.75) vir die persentasie skade wat ontstaan het as gevolg van die vriestemperatuurbehandeling. Hierdie lae temperatuurbehandeling het geen betekenisvolle invloed op die korrelasiekoeffisiënt vir opbrengs en opbrengskomponente gehad nie. ‘n Relatiewe hoë breësin van oorerflikheid (h^2_B) is waargeneem vir opbrengs (h^2 =0.74) knoldeursnit (h^2=0.91), knolmassa (h^2=0.87) en die hoeveelheid knolle per plant (h^2=0.79).