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THE EXPRESSION OF COLD RESISTANCE GENES IN WHEAT CULTIVARS

by

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Chapter 1
INTRODUCTION

The scatter of plant life over the globe from warm equatorial regions to frigid polar regions during the course of evolutionary time has resulted in an array of developmental, biochemical and physiological adaptations to cope with the adversities of decreasingly lower atmospheric and soil temperatures (Grace, 1987; Levitt, 1956, 1972, 1980; Sakai and Larcher, 1987). This has become increasingly apparent during the last ten millennia of agricultural development, because of people's desire and expanding need to grow crop plants well beyond their native ranges (Guy, 1990). What is not generally understood is the multitude of low temperature constraints that must be balanced with overall competitiveness or fitness (Guy, 1990). Thus, a vast diversity of adaptive and avoidance strategies have evolved to cope with the myriad variation of low-temperature-stress (Sakai and Lacher, 1987). Many higher plants can withstand the freezing of tissue water that few other higher eukaryotes can tolerate (Guy, 1990). Molecular approaches to the study of the responses of plants to low temperature will, in all likelihood, reveal higher orders of complexity not yet apparent. When utilized within a biochemical or physiological approach, molecular genetics offers great potential for understanding how plants adjust metabolism to functions at low non-freezing temperatures and how they tolerate freezing (Guy, 1990).

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

More than 40 years ago, accumulation of soluble protein in cold acclimated cortical bark cells of black locust trees was first correlated with freezing tolerance (Simovitch and Briggs, 1953). Study of the seasonal variation in protein content and hardiness of the bark cells demonstrated the accumulation of soluble proteins in fall, which closely paralleled the induction of freezing tolerance (Siminovitch and Briggs, 1953). Throughout the winter the protein concentration remained high but declined rapidly during the breaking of dormancy and the resumption of growth in the spring. The decline of soluble protein closely matched the loss of freezing tolerance. The pioneering studies by Siminovitch and Briggs prompted numerous studies of the protein content of plants during cold acclimation. Out of this cumulative effort it was established that the accumulation of soluble proteins during cold acclimation was a general response (Chen and Li, 1980; Levitt, 1972), although not universal (Guy and Haskell, 1987). Therefore, it is not surprising that freezing tolerance may not be explained as a simple function of the soluble protein concentration (Guy, 1990). There are many reasons why some plants might accumulate soluble proteins during cold acclimation; the exception of a protoplasmic augmentation hypothesis no

clear mechanistic rationale for conferring greater freezing tolerance has been proposed for this hardening response (Guy, 1990).

This knowledge could be very beneficial in cold resistance studies on South African wheat cultivars. Nearly 544 394 hectares of wheat were insured against frost damage over the last ten years in South Africa, of which 47 062 hectares were damaged by frost. This led to an average loss of income close to 6.7 million Rands annually (Willemse, 1999). This high level of frost damage indicates that wheat cultivars with a high level of tolerance to freezing are needed.

This study was done to determine if high molecular weight proteins could be used in order to screen for freezing tolerance in South African wheat, and to determine the inheritance patterns of these proteins from the parents to the hybrid F1 and F2 progeny.

Chapter 2
LITERATURE REVIEW

Temperature stress in wheat

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

According to Aitken (1965) cold requirements in wheat is directly involved with early and late cultivation, and thus influence the adaptability of a cultivar in a specific cultivating area. In South Africa, cultivars with an obliged or qualitative cold requirement are suited for areas like the Free State, which is characteristicly very cold in the winter. Cultivars with a lower degree of cold requirements are more appropiate under mildly cold conditions in the winter, like in the Western Cape (Stander and Laubscher, 1974).

Temperature stress inhibits the growth, development and thus, the yield of wheat in at least three ways. Firstly the development from emergence through tillering, stem elongation, flowering and grain fill is driven by growing degree-days or accumulated heat units. Secondly, wheat requires a certain minimum time within a favourable temperature range to go from seed to seed. The ideal temperatures for the growth and development of wheat are between 10 and 24°C. Providing no other limiting factors such as too much or too little water or light, influence the normal plant development, the accumulation of growing degree-days within this temperature range leads to large, well tillered plants with wide leaves and big heads. Thirdly, wheat plants are sensitive to temperature extremes during critical stages of development. The results of these extremes include frost injury to the internodes and florets, winterkill, frozen leaves or roots and heat damage (Cook and Veseth, 1991).

Wienhues (1960) maintained that low temperatures facilitate the initiation of culms in spring wheat, and that large differences exist between the temperature requirements of different cultivars.

The minimum temperature for growth is 3 to 4°C, the optimum temperature is 25°C, and the maximum is 30 to 32°C (Briggle, 1980).

Cold acclimation temperatures and frost tolerance

The primary winter stress is low temperature (Fowler *et al.,* 1981). The main limitation of field survival trials in the determination of the cold-hardiness potential of varieties is that results are often inconclusive, due either to complete winter kill, or a lack of it (Limin and Fowler, 1993). Furthermore, variation in factors such as soil moisture, soil fertility, disease, ice encasement, and smothering can have an indirect effect on suvival by limiting the level of cold hardiness that a plant can attain (Limin and Fowler, 1993). To prevent the above limitations it is necessary to carry out repeated experiments in diverse locations. They are, however, rather expensive and the experimental errors made on such occasions are usually large (Fowler and Charles, 1979). The limitations mentioned above have led to a search for rapid and efficient laboratory methods for evaluating cold resistance.

Although occasionally frost-resistant varieties may manifest this property already in the non hardened state (Chen *et al.,* 1983), cold acclimation determines a correct frost resistance estimation which demonstrates all the discrepancies between varieties (Chen *et al.*, 1983; Bridger *et al.*, 1994).

Cold hardiness of crop plants has not been markedly improved as a result of conventional plant breeding programmes since the beginning of the century, partly due to the lack of objective, physiological markers of frost resistance (Blum, 1985).

Cold hardiness in *Triticeae*

The lack of improvement in cold hardiness of winter wheat varieties through the use of traditional plant breeding methods may be because most of the genetic variability for this character in the common wheat gene-pool has been exploited (Limin and Fowler, 1993). This has led to an investigation of cold hardiness within related species, which vary in their relationship to wheat from sharing one or more genomes and being highly crossable with wheat, to distant relatives which are very difficult to cross with wheat.

Common winter wheat is a hexaploid composed of three basic genomes: A, B and D. The progenitor species supplying the A and D genomes are *T. monococcum* and *T. tauschii*, respectively. The B genome is thought to be related to a group of species carrying the genome designated S (Sears, 1981). A large number of accessions of *T. monococcum* (AA) and *T. tauschii* (DD) have been screened for cold hardiness (Limin and Fowler, 1985). Both species showed variability for cold hardiness, although *T. tauschii* was found to contain the most cold-hardy accessions. However, none of the *T. tauschii* accessions were as cold hardy as winter wheat varieties currently available. Within the group of species formerly designated as members

of the genus *Aegilops*, and now included in *Triticum* (Sears, 1981), most accessions had only poor to moderate cold hardiness relative to hardy winter-wheat varieties (Limin and Fowler, 1985). One *A. cylindrica* (CD genome) accession, which had a cold-hardiness level equal to the hardy wheat variety Norstar, was an exception to this generalisation. Among the close common wheat relatives, the most hardy lines have been found in the *T. turgidum* var. durum group (AB genome; Fowler *et al.*, 1977). However, once again none of these lines equalled the cold hardiness of the most hardy *T. aestivum* varieties.

The most cold-hardy genotypes of the genus *Triticum* have been found in the aestivum group (ABD genome)(Limin and Fowler, 1993). Common hexaploid (AABBDD) wheat is believed to have resulted from natural hybridisation of cultivated tetraploid (AABB) wheat with the weedy *T. tauschii* (DD) species. The addition of the D genome of *T. tauschii* to the AB genome of *T. turgidum* may have achieved greater cold hardiness. This natural hybridisation was duplicated in the laboratory using selected lines of known cold hardiness from both parental species (Limin and Fowler, 1982). Crosses were made among these selected lines and the resulting hybrid plants were chromosome-doubled with colchicine to produce synthetic hexaploid (AABBDD) wheat. In some crosses, the most cold-hardy accessions or lines of each group were selected as parents. None of the synthetic hexaploids exceeded the most hardy *T. turgidum* (AB genome) parents in cold hardiness, suggesting that the addition of the D genome to the AB genome of tetraploid wheat would not have given the original hexaploid wheats a significant initial cold-hardiness advantage. Based on these observations, it appears that the high level of cold hardiness present in hexaploid wheats evolved through mutation and/or recombination followed by selection at the hexaploid level.

The synthetic hexaploid wheats produced by Limin and Fowler (1982) are basically raw amphiploids combining the genomes of two separately evolved species. Therefore, it was theorised that they may provide a source of unexploited genes for cold hardiness that were not expressed because of a lack of integration or synchrony between the separately evolved genetic systems combined in the raw amphiploid. To investigate this possibility, the synthetic hexaploids derived from the most cold-hardy parents were crossed to the hardy winter wheat variety Norstar, thereby providing the opportunity for integration of these possibly cryptic cold-hardiness genes into the genetic structure of modern wheat (Limin and Fowler, 1989). Several F2-derived F3 lines with cold hardiness potentials similar to Norstar were identified in the progeny of these crosses. The most cold-hardy genotypes within these populations were backcrossed to Norstar, or crossed to other equally cold-hardy common wheat varieties. Since all parental lines in the study were approximately equal in cold hardiness, a single-temperature freeze-test utilising the killing temperature of Norstar was employed to identify the most hardy plants in the subsequent F2

populations of these crosses. Plants surviving this cold stress were grown out to produce F3 lines that were then screened to determine if any *T. turgidum* or *T. taushii* genes had resulted in improved cold hardiness compared to the parental wheat varieties. None of the F2-derived F3 lines were found to exceed the cold hardiness of the modern hardy common wheat parents. These observations have been interpreted as evidence that the modern *T. eastivum* varieties probably contain the same or superior genes to those found in their progenitor species. This suggests that the search for new genes for exploitation will have to be extended to the more distant relatives of wheat.

Potential gene donors should have outstanding cold hardiness, since integration of distantly related genetic material into a wheat background will require considerable effort (Limin and Fowler, 1993). Excellent cold hardiness and good crossability makes rye (*Secale cereale*) a potential source of genes for the improvement of cold hardiness in wheat. However, the addition of the entire rye genome to wheat, to form triticale, has not produced the expected new crop plant combining the cold hardiness of rye with wheat. Dvorak and Fowler (1978) found that seven octaploid triticales (AABBDDRR) achieved only the hardiness level of their wheat parents. In triticale, the wheat genomes appear to suppress the expression of cold-hardiness genes known to be present in rye. Similar results were found in hexaploid (AABBRR) triticale (Limin and Fowler, 1985), indicating that the D genome of wheat could not be solely, if at all, responsible for the suppression of rye cold-hardiness genes in octaploid triticale.

Agropyron (wheatgrass) species are also a potential source of genes for cold hardiness. Perennial wheats or wheat/Agropyron hybrids have often been reported to have greater cold hardiness than the most frost-resistant winter wheats, or even hardy winter rye (Fedorov, 1970). In spite of these reports, no commercial varieties have been produced utilising this germplasm to improve cold hardiness in wheat. In all cases, there was a loss of alien gene expression, including cold hardiness, following breeding efforts to recover other desirable characters of common wheat.

Cold acclimation of wheat: amino acids involved

Cold acclimation of wheat leads to a significant rise in soluble protein concentrations, especially in the leaves of winter wheat (Charest and Phan, 1990). The content and nature of proteins seem to play an important role in the cold hardening process (Cloutier, 1983).

Proline accumulation was found to be very important in winter varieties like Kharkov, specifically in the crown of the plant (Charest and Phan, 1990). Other studies have also documented proline synthesis or the presence of proline precursors in leaves and roots (Machackova *et al.*, 1989).

Two metabolic pathways, glutamite and ornithine metabolism, are very important in proline formation.

It is well known that proline accumulates in plants during adaptation to various environmental stresses such as drought, salinity and high and low temperatures (Lalk and Dröffling, 1985; Machackova *et al.*, 1989). It also accumulates in other ectotherms, such as bacteria, when they are stressed. Because proline levels increase in stress-resistant organisms, it may be used as an index of resistance.

Proline has specific properties (e.g. osmotic efficiency) allowing it to play many important metabolic roles in stressed plants. Proline can serve as a nitrogen and carbon source which can be used during recovery from stress (Jager and Meyer, 1977). It is also involved in cell osmoregulation and in protection of proteins during dehydration. Finally, proline can act as an enzymatic regulator.

Resistance to freezing in plants is determined by their genetic constitution and by the environment. In winter resistant plants, such as winter wheat, a broad variance in genetically fixed freezing resistance exists. The expression of freezing resistance is affected by environmental factors, especially by low temperature (Levitt, 1980; Sakai and Larcher, 1987). The cold hardening process involves several physiological, biochemical and biophysical changes, among them an increase in dry weight, in sugar and free amino acid (especially proline) concentration (Benko, 1986; Chu *et al.,* 1978; Kaldy and Freyman, 1984; Kushad and Yelenosky, 1987), changes in the physical state and chemical composition of membranes (Uemura and Yoshida, 1984; Huner *et al.*, 1989), in protein composition (Dörffling and Askman, 1989; Perras and Sarhan, 1989), and in the levels or hormones, especially abscisic acid (ABA) (Wightman, 1979; Lalk and Dörffling, 1985; Taylor *et al.*, 1988). It has been suggested that ABA may trigger some of the processes which are responsible for freezing resistance, for example formation of freezing tolerance proteins (Dörffling and Askman, 1989; Perras and Sarhan, 1989).

In a study conducted by Dörffling *et al.* (1990), changes in dry weight, osmotic potential, abscisic acid and free proline contents were measured during cold hardening of nine winter wheat varieties differing in freezing resistance. During the first weeks of cold hardening dry weight and proline levels increased and the osmotic potential decreased parallel to the development of freezing resistance. Dry weight reached a broad maximum between the seventh and tenth week of hardening. ABA levels had a sharp maximum around the seventh week of hardening, when the dry weight increase began to cease. Maximal levels of proline were observed seven to 10 weeks after the start of cold hardening. The mean and final dry weights, the mean, maximal and

final proline contents, as well as the maximal ABA contents of the nine varieties correlated significantly with freezing resistance measured at the end of the hardening period.

The conclusion that a casual relationship exists between proline levels and freezing resistance leads to the idea that breeding for higher freezing resistance could be achieved by selection and breeding for high proline levels (Dörffling *et al.*, 1990).

Variety-specific discrepancies in ABA which correlate positively with freezing resistance correlate positively with freezing resistance have also been observed, but increased levels of ABA during cold hardening were transient. Although freezing resistance was measured after the ABA peak had appeared, a positive correlation between freezing resistance and the ABA maximum was observed. This indicates that ABA may serve as a signal triggering processes responsible for the increase of freezing resistance, among which may be the formation of proline (Bornmann and Jansson, 1980) and an alteration in the membrane composition (Uemura and Yoshida, 1984; Farkas *et al.*, 1985).

As with dry weight and proline, ABA levels may serve as a criterion of freezing resistance in early diagnostic tests. Machackova *et al.* (1989) concluded from studies with only three wheat varieties and four putative markers that ABA levels were the best marker for frost hardiness, followed by proline. In the study conducted by Dörffling *et al* (1990), the most significant correlation was found between maximum ABA levels and freezing resistance. This finding supports the conclusion of Mahcackova *et al.* (1988). However, ABA determinations are time-consuming and laborious, even when immunoassays are used, so that, from practical point of view, preference should be given to proline as a metabolic marker for freezing resistance.

Chilling injury

Many plants of tropical origin are susceptible to chilling injury below 10°C and their survival is limited by low temperature in temperate climates. This phenomenon has ecological as well as economic ramifications as many cereals, vegetables and fruit are chilling sensitive. Symptoms of chilling injury vary with plant species, organ, severity of stress and pre-stress treatment.

One theory of chilling injury proposes that it is dominated by a phase change of the lipids in the mitochondria membrane. The temperature at which chilling-sensitive plants fail to grow in the short term, and die in the long term, is called the "critical" temperature. This temperature corresponds with the temperature at which the membrane lipids undergo a two-dimensional phase transition from a disordered state to a more ordered state as the temperature drops, and

this will affect the conformation of enzymically active proteins within the membrane and therefore alter the kinetics of reactions catalysed by membrane-associated enzymes.

Cold hardiness

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

Significant correlations have also been reported between ability to survive the winter and growth habit of wheat (Hayes and Aamodt, 1927; Quisenberry, 1931). In general, spring wheat tended to be less hardy than winter wheat, but this relationship was not absolute (Brule-Babel and Fowler, 1988).

Winter survival

Winter wheat plants are killed outright by low temperatures when their crowns are killed. Low temperatures may kill the leaves, but as long as the crowns are not killed, recovery is still possible in the spring. Crowns of varieties developed for areas subject to winter kill harden enough to withstand temperatures down to –24°C. Even a few hours at these temperatures can be lethal to other wheat varieties. A snow cover can provide the insulation necessary to prevent lethal temperatures in the crown zone, even during periods when air temperatures decrease to –34°C or lower. On the other hand, leaves of plants are highly vulnerable to winter kill if it snows too soon in the autumn, before the hardening process is complete (Cook and Veseth, 1991).

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

Wheat protein composition

A protein is a primary product of a structural gene and therefore it serves as a marker for that particular gene. Genes are coupled into genetic systems and because of this, proteins may also serve as markers for such systems, including chromosomes and the genome as a whole. Because of this, the totality of protein markers gives considerable insight into genome or genotype structure and could be used to resolve genetic and breeding problems. The protein band pattern of electropherograms shows only genotypic variations, while environmental factors can be excluded to a large extent.

Protein Structure

It is necessary to study the endosperm (morphology), polypeptide subunits, amino acids, as well as total nitrogen content in order to understand the protein structure of wheat. Criteria of these proteins include cellular function and location, processing value, chemical characteristics and amino acid content. Genetic aspects have been examined at most of these levels. It is important to build up an integrated picture of the genetics of protein composition at all of these levels, because of interactions occurring between them. The study of the genetics of these components complement the direct study of this character which itself is inherited in a complex manner. Genetic studies of all of these fractions as dissociated polypeptides must obviously be related to studies of the native proteins for the results to have proper significance for variety evaluation.

Proteins are a complex group of natural polymers of which each protein is unique and performs a specific function in the plant from which it is derived. All proteins consist of more or less 20 different L-alpha amino acids that differ from one another in the side chain attached to the tetrahedral alpha-carbon, along with a hydrogen atom and the amino and carboxyl groups. The numbers of each amino acid incorporated into the polymer chain give origin to the uniqueness of each protein.

The primary stucture of the protein is the number of each species of amino acid and the sequence of its incorporation through formation of peptide bonds. This unique sequence of amino acid residues determines the three-dimensional structure of the protein through defining the possibilities for interaction of any residue with other residues in the chain. Much of the complexity of gluten proteins is due to the extensive duplication and diversification of structural genes needed for their synthesis.

The use of proteins to determine sensitivity to freezing

High temperatures induce protein denaturation (Bernstam, 1978), so that many temperature-related injuries are due to thermolabile enzyme inactivation (Langridge, 1963). Low temperatures

cause the breakdown of protoplast structure as a result of water crystal formation (Langridge, 1963); they also induce a reduction of available energy due mainly to a decrease in the ATP content of the tissues. On the other hand, temperatures higher and lower than that considered optimal can modify membrane permeability (Ono and Murata, 1981). Membranes seem to be the primary site damaged by temperature stress (Levitt, 1980). Temperatures ranging from –12 to 60°C modifies some of the parameters of senescence. Chlorophyll content remains unchanged at all these temperatures. The sol AA content shows a small increase at 40°C. Permeability is the parameter showing the most important changes when temperatures are higher or lower than 20°C. However the permeability increase is more important at higher (40 and 60°C) than at lower (-12 and 0°C) temperatues. Generally, the permeability enhancement bears a close relation to the hydroperoxide content, except 60°C when the hydroperoxide content is lower than the initial value.

The process of cold hardening in hardy plants is controlled by a genetic system regulated directly or indirectly by environmental conditions, particularly low temperature. Previous work indicates that low temperatures required in the hardening process induces genetic and metabolic responses which enable the plants to develop a significant cold tolerance and withstand freezing stress during winter. It has been suggested that specific proteins may be newly synthesized or accumulated during hardening in order to increase the resistance of cell and organelle membranes to freezing. These altered patterns of protein synthesis may be the result of low temperature-induced regulation of gene expression at the transcriptional level as indicated by the increase in DNA-dependent RNA polymerases I and II and soluble Rnases activities observed in wheat during hardening. However, there is no conclusive evidence of the presence of specific cold tolerance proteins.

According to the study conducted by Sarhan and Perras in 1987, is it apparent from the comparison of hardened and unhardened plants that hardening conditions induce a marked accumulation of a large polypeptide with a molecular weight of 200 kD. The intensity of this band was higher in the more hardy varieties Norstar and Frederick compared to the less hardy variety Glenlea. It appears that during hardening the 200 kD protein increased substantially in both hardy varieties Norstar and Frederick. This increase was more pronounced in Norstar, which is more cold tolerant. On the other hand, the increase was relatively small in Glenlea, the cold sensitive variety. This observation suggests a correlation between the degree of freezing tolerance and the accumulation of the specific protein. The early appearance of this protein during hardening and its presence at the end of the hardening process suggests a possible involvement in the acquisition, the development and the maintenance of the increased freezing

tolerance. The relation between the accumulation of the protein and the degree of freezing tolerance could result from differential gene expression during hardening in the three varieties.

The comparison of protein patterns of hardened and control unhardened plants shows the content of at least eight proteins was modified upon hardening. Among these polypeptides some were accumulated in cold treated seedlings (molecular weight 48, 47 and 42 kD), while others (molecular weight 93, 89, 80, 67 and 63 kD) tended to decrease in intensity. Most of these modifications occur in the three varieties and appear to be part of the metabolic adjustments in response to low temperature treatment rather than a specific change associated with the cold hardening capacity.

Various plants have been found to accumulate or synthesize high storage proteins (HSP) in response to high temperature treatment. The synthesis of these HSPs was found to be correlated with high temperature tolerance, which suggests that they may be involved in protecting plant cells against lethal high temperature. Cold hardy plants may have a similar mechanism which enables them to synthesize cold-tolerance proteins. The high molecular weight polypeptide detected during hardening could be part of this specific group of proteins. The presence of this large protein in the soluble fraction may suggest a possible regulatory role associated with the metabolic changes known to occur during the onset of cold hardening and particularly at the enzyme level. It is also possible that this cold-tolerance protein is involved in the processes leading to the plasma membrane alterations observed during cold hardening (Sarhan and Perras, 1987).

Protein synthesis was studied again by Sarhan and Perras (1989) in leaves, coleoptiles and roots during cold hardening of freezing tolerant winter wheat and freezing sensitive spring wheat. The results showed that cold hardening induces important changes in the soluble protein patterns depending upon the tissue and variety freezing tolerance. Among the proteins induced or increased in hardened tissues, some were expressed at a higher level in the freezing tolerant varieties than in the sensitive one, indicating a correlation between the synthesis and accumulation of these proteins and the degree of freezing tolerance. These proteins, suggested to be freezing tolerance proteins, may have an important role in the cellular adaptation to freezing.

The underlying physiological and molecular mechanisms of freezing tolerance of plants are poorly understood. It has been suggested that the development of cold resistance may depend on altered gene expression and the synthesis of new proteins. Perras and Sarhan (1989) reported on changes in the soluble protein patterns of cold hardened etiolated wheat seedlings. Similar to

their study in 1987, previously mentioned, a high molecular weight protein in the range of 200kD appeared in hardened plants and its accumulation was correlated with freezing tolerance. Guy and Haskell (1987) have also found three high molecular weight induced proteins (160, 117 and 85 kD) in spinach during cold acclimation. On the other hand Mohapatra *et al.* (1987) reported the appearance of several small polypeptides ranging from 11 to 38 kDa in cold acclimated alfalfa seedlings. Using cell suspension cultures, Joghson-Flanagan and Singh (1987) working with winter rape and Robertson *et al.* (1988) with bromegrass, found low molecular weight peptides ranging from 20 to 48 kD during the induction of freezing tolerance. These discrepancies could be due to species discrepancies or to the different experimental approaches. However, it is difficult to determine whether these changes are associated with growth at low temperature or with the development of freezing tolerance.

Cold hardening in higher plants is a complex process involving many genes. The nature of the adaptation process and the intensity of the response varies among plant species and among the different varieties of wheat. The comparison of the gene expression in different genotypes, varying in their degree of freezing tolerance, appears to be a valid way to identify the modifications specifically related to the development of freezing resistance from those related to the low temperature stress.

Using this approach, Perras and Sarhan (1989) were able to associate the accumulation of a high molecular weight polypeptide of 200 kD with the cold hardening of etiolated wheat seedlings. The analysis of the steady state protein patterns of shoots showed that cold hardening induced modifications in the expression of at least 12 peptides. The most noticeable changes were the induction of a high molecular weight polypeptide of 200 kD and the repression of a 157 kD polypeptide in hardened seedlings. These changes were more pronounced in the two cold tolerant cutivars Fredrick and Norstar, indicating a correlation between the synthesis of these peptides and the degree of freezing tolerance of the different genotypes. The induction of the 200 kD protein in the early stages of cold hardening and its accumulation throughout the whole hardening process suggests that this low temperature induced protein may be required for acquisition, the development and/or the maintenance of the increased freezing resistance in wheat. The analysis of the *in vivo* protein synthesis revealed that a number of proteins were newly expressed or repressed, while many others were differently regulated by the cold hardening conditions. At least eight new polypeptides appeared in hardened tissues.

The appearance of these new peptides in the protein synthesis patterns of hardened what tissues suggested that cold acclimation induced modifications at the gene expression level. Among the polypeptides induced by the hardening conditions, some were synthesized at a higher rate in the

two cold tolerant cutivars than in the cold sensitive one, indicating a correlation between the degree of expression of these genes and the degree of tolerance reached by the different genotypes. These proteins ([200 kD] in the three tissues, [36 kD] in leaves, and [64 kD and 52 kD] in roots) were therefore considered as part of a potential family of FTPs, *e.g.* proteins strongly associated with the development and the maintenance of the increased freezing resistance. The induction of a 200 kD protein as well as the increased synthesis of a 75 kD peptide concomitantly in all tissues suggested that part of the genetic regulation associated with the increased freezing tolerance took place at the whole plant level. On the other hand, the induction of specific proteins in the leaf, crown and root tissues indicated that the regulation of some freezing tolerance genes was tissue specific. This finding was supported by the fact that the synthesis rate of some proteins, detected in more than one tissue, was differently regulated in different tissues during cold hardening. The most significant change detected in the protein syntheses patterns during cold hardening was the induction of the 200 kD freezing-tolerance protein in the leaf, crown and root tissues.

Substitution analysis of frost resistance in wheat callus culture

The inheritance of the frost resistance and winter hardiness of wheat seedlings under artificial and natural conditions has been investigated by several researchers using diallel analysis, F2 monosomics and chromosome substitution lines. Most of the evidence indicates that frost resistance and winter hardiness in wheat are quantitative characters resulting from the action of genes at several loci.

Monosomic and substitution analyses have made it possible to locate genes determining cold resistance on the chromosomes (Jenkins, 1971). Various authors reported that at least 10 of the 21 pairs of chromosomes are involved in the control of cold resistance (Sutka, 1981; Poysa 1984; Sutka and Kovacs, 1985; Roberts, 1986; Sutka and Snape, 1989). On the basis of freeze testing experiments using substitution material it was established that chromosomes 2B, 4B, 4D, 5A, 5B, 5D and 7A increased the level of cold tolerance, whereas chromosomes 1B, 3A, 3D, 6A and 7D (Poysa, 1984; Sutka, 1994) showed decreased cold resistance levels. Chromosomes 5A and 5D have been implicated most frequently and they appear to carry major genes. Snape *et al.* (1985) developed single chromosome recombinant lines, from the cross between the substitution line Hobbit (*Triticum spelta* 5A) and Hobbit. These lines were tested for cold hardiness and they could be classified into two distinct classes according to sensitivity to freezing, with *T. spelta* 5A lines exhibiting sensitivity to freezing (survival rates 5-25%) and the Hobbit 5A lines exhibiting resistance (survival rates 60-80%).

The most effective genes in the control of frost resistance in seedlings are carried on chromosomes 5A and 5D, which have a more pronounced effect at a lower freezing temperature (Sutka, 1981; Sutka and Veisz, 1988). Chromosomes of the 5th homoelogous group also play a role in the genetic control of winter hardiness (Sutka *et al.*, 1986).

If wheat calli are hardened in the dark and then frozen at –12 °C, certain genes responsible for frost resistance could be detected on chromosomes 5A, 5D and 7A, corresponding to the results obtained when freezing the seedlings. The effect of chromosomes 6A and 6D can only be detected when freezing calli, but not when freezing seedlings. The biochemical process of cold hardening in calli kept in the dark is probably not identical with that taking place in seedlings growing in the light. After hardening seedlings in the dark at 0.8 °C for seven weeks, followed by –5 °C for eight weeks Roberts (1986) found that chromosome 6A was involved in cold hardiness. This chromosome is also involved in the control of winter hardiness (Sutka *et al.*, 1986), so it is doubtful whether its effect can be explained purely on the basis of hardening in the dark

Chromosome 2B is also responsible for frost resistance in seedlings, as the calli of the 2B substitution line did not show frost resistance using TTC staining. In this case the question of the role of illumination again arises. There must be some relation between frost resistance and the response to day length. Illumination, and consequently day length, promotes the accumulation of sugars leading to the hardening of plants, which in turn increases the frost resistance. According to data publishes by Welsh (1973) and Law *et al.* (1978) on alien substitutions, the response to day length is associated with chromosomes 2B and 2D. It can be concluded that both in calli and in seedlings, under both artificial and natural conditions, the genetic control of frost resistance is determined mainly by low temperature.

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

Evaluating cold hardiness

In a recent review of cold stress, Steponkus (1984) noted the failure of "innumerable correlative studies of biochemical changes that occur during the period of cold acclimation" to lead us to an understanding of how cold acclimation offers the tolerance of cells, tissues and plants to freezing and thawing stresses. This failure has renewed attempts to comprehend the mechanisms of injury at the probable primary site of freeze thaw injury; the plasmalemma or plasma membrane (Steponkus, 1984). While mechanistic approaches to the cryobehavior of the plasmalemma showed great promise (Gordon-Kamm and Steponkus, 1984), a new approach provides insight about which biochemical and physiological changes are mainly responsible for altered freezing tolerance. This approach is based on an early idea that variation in hardiness in the plant kingdom and seasonal variation within the tissues of a single species have a genetic or molecular basis (Weiser, 1970).

A significant component of winter hardiness in cold hardy plants is the capacity to undergo cold acclimation. Virtually all temperate perennial, and many annual and biennial plants native to regions of the world subject to subzero temperatures can alter their tissue and cellular freezing tolerance upon exposure to low, nonfreezing temperature (Levitt, 1972, 1980; Sakai and Larcher, 1987). The term cold acclimation is most often used to describe the outcome of the myriad biochemical and physiological processes associated with the increase in cold tolerance.

Two aspects of cold acclimation that have been addressed by genetic analyses are the inheritance of the minimum survivable temperature and the timing of the photoperiodic induction of freezing tolerance. The few reports on the genetics of minimum survivable temperature associated with cold acclimation have supported a dominant/recessive pattern of inheritance of the winter hardiness character (Gullord *et al*, 1975) which appears to be coupled in an additive-dominance system (Sutka, 1981; Sutka and Veisz, 1988). Chromosomal substitution with wheat has indicated additive gene effects were greater than the dominance effects (Sutka and Veisz, 1988). As freezing stress became more severe, dominant/recessive character became reversed. This is consistent with the observation that under one set of conditions genes for winter hardiness appeared to act in a dominant manner, while at other times they acted recessively (Worzella, 1932). More specifically, at mild stress levels tolerance appeared to be a dominant trait, while under more extreme freezing stress conditions tolerance exhibited a recessive character (Sutka and Veisz, 1988). This supports the hypothesis that different genes affect tolerance at different levels of stress (Gullord *et al*, 1975). Limin and Fowler (1988) examined the inheritance of minimum survivable temperature in interspecific and intergenic hybrids of *Triticum* and related genera. The freezing tolerance of F1 hybrids that maintained the parental chromosome number

was near the midpoint between the parental expression, indicating a quantitative character controlled by a number of additive genes.

Limitations have led to a continuing search for rapid and efficient laboratory methods for evaluating cold tolerance. Care must be taken in interpreting data from genetic studies that have employed methods which do not identify the killing temperature of each cold-hardened phenotype. For example, where discrepancies in cold hardiness between two parents is controlled by additive gene action, and the single minimum temperature used in the freeze test or field trial falls between the killing temperatures of the less-hardy parent and the F1 hybrid (with additive gene action the F1 should have a cold-hardiness level intermediate to its parents), then most of the F1 and all of the hardy parent population should survive. This could lead to a mistaken interpretation of the data, and the conclusion that there was dominance for cold hardiness. Depending upon the single minimum temperature used, cold hardiness may appear to be expressed as a dominant character under low-stress or a recessive character under high-stress environments. A series of test temperatures that identify the LT_{50} (minimum temperature at which 50% of the plants will survive) levels of cold-hardened plants reduces the likelihood of this type of error. Controlled freeze tests that estimate LT_{50} levels of cold-hardened plants are destructive. However, LT_{50} estimates have the highest precision and heritability (Fowler *et al.*, 1981; 1983) of all cold-hardiness-prediction tests. Unfortunately, there is not a reliable non-destructive test that can be used to effectively measure cold hardiness on a single-plant basis at this time (Limin and Fowler, 1993).

Genetics of cold hardiness in wheat

Studies into the genetic nature of cold hardiness in wheat most frequently report this character as genetically complex and quantitatively inherited (Quisenberry and Clarke, 1929; Worzella, 1942). Almost every morphological, physiological and biochemical character has been observed to change during cold acclimation in plants (Levitt, 1980). However, many of these observed changes may not be primarily responsible for cold hardiness. The evaluation of cold hardiness of varieties and segregating populations can also be complicated by high experimental errors and deficiencies in testing methodology.

The genetic control of winter hardiness is complex partly determined by cold resistance genes on group 5 chromosomes and chromosomes 4B, 4D and 7A (Law and Jenkins, 1970; Jenkins, 1971; Puchow and Zhirov, 1978; Suthka, 1984). The group 5 chromosomes and 7A are also known to control vernalisation requirements. By studying the behavior of group 5 aneuploids and intervarietal chromosome substitutions, it was demonstrated that there was no correlation between vernalisation requirements and freezing resistance. This indicates that winter-hardiness

is determined by two separate genetic systems on group 5 chromosomes, one providing frost resistance, the other frost avoidance (Cahalan and Law, 1979).

It was also concluded from crosses of amphiploids with Norstar (winter wheat) that there is partial dominance for cold hardiness (Limin and Fowler, 1983). Many of the F2-derived F3-lines were equal to Norstar in hardiness, suggesting that only a few genes are involved in hardiness.

No evidence could be found of a cytoplasmic effect on cold hardiness, or on the nuclear expression of cold hardiness on reciprocal crosses of very hardy by less hardy wheat parents (Brule-Babel, 1985).

Even though the actual structural relationships of chromosomal proteins and DNA have not been directly determined in wheat, the chromosomal proteins themselves have been studied.

An important set of chromosomal proteins that have been studied in wheat are the RNA-polymerases. The availability of wheat embryos on a large scale has allowed the insulation of three protein complexes, RNA-polymerase I, RNA-polymerase II, and RNA-polymerase III. These proteins show numerous low-molecular-weight units, as well as the major sub unit structures with molecular weights of 100 to 200 kd, analogous to those of other organisms.

Gullord *et al.* (1975) indicated that cold tolerance may not be a single trait, but a complex of tolerances to different types of freezing stresses. Sutka (1994) indicated that cold tolerance is controlled by an additive dominance system. Results of the diallel analyses indicated both additive and non-additive gene action. The variance and covariance for percentage survival, averaged over reciprocal crosses were calculated. The regression coefficient was significantly different from zero but not significantly different from unity. This indicated that non-additive genetic variation is present as dominance only. The dominant genes acted in the direction of lower cold tolerance and the recessive genes in the direction of a higher level of cold tolerance.

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

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

The inheritance of cold hardiness in wheat was studied by Brule-Babel and Fowler (1989) in 20 crosses among five parents ranging from spring wheat to hardy winter wheat. Analysis of F1 and F2 populations indicated that genetic control of cold hardiness in spring x winter crosses was partially dominant. The F2 derived F3 lines confirmed this conclusion since all distributions were skewed to the hardier end of the population ranges. In contrast, the F1 and F2 populations of winter x winter crosses did not differ significantly in hardiness from their parental midpoints. Thus, no dominance was exhibited in these crosses and genetic control was most likely additive. Distributions of F2 derived F3 lines agreed with the premise that genetic control of cold hardiness was additive in winter x winter crosses. Consequently, the choice of parents would determine whether cold hardiness acted in a dominant or additive fashion. Since cytoplasmic effects were not implicated, crosses in either direction could be used (Brule-Babel and Fowler, 1989).

Synthetic hexaploid wheat produced by combining tetraploid wheat (AB genome) with *T. tauchii* (D genome), was crossed to modern hexaploid wheat (ABD genome) in an attempt to introduce new cold hardiness genes into the common hexaploid wheat gene pool. The cold hardiness levels of F1 hybrids ranged from similar to parental means to equal to the hardy parent, indicating that cold hardiness was controlled by both additive and dominant genes. Heritability estimates for cold hardiness ranged from 63 to 70% indicating that selection for cold hardiness should be effective in populations arising from crosses between common and synthetic hexaploid wheat (Limin and Fowler, 1993). Sutka (1994) calculated values of 81,1 and 97.55% for narrow and broad heritability respectively. This indicated a high heritability for cold tolerance. High heritability estimates for cold hardiness were also reported in wheat by Brule-Babel and Fowler (1988) and Sutka (1984; 1981). These estimates indicated that cold hardiness was a heritable character and, provided genetic variability is present, selection for cold hardiness should be effective.

Reports of cold-hardiness levels in the F1 generation of winter-wheat crosses have been widely variable. F1 hybrids in some spring x winter crosses have been reported to resemble the less hardy parent (Hayes and Aamodt, 1927), or the hardy parent, or to be intermediate in cold hardiness (Worzella, 1935). Brule-Babel and Fowler (1988) used the LT_{50} method to evaluate the cold hardiness of common wheat crosses that spanned a wide range of cold-hardiness levels. In almost all instances, crosses between winter-habit parents produces F1 and F2 populations with mean cold-hardiness levels that were not significantly different from the parental midpoint. These observations would be expected if additive gene action controlled the expression of cold hardiness in wheat. Genetic control of cold hardiness in barley (Rohde and Pulham, 1960), rye (Brule-Babel and Fowler, 1989), and wheat (Sutka, 1981) was also found to be mainly additive. Brule-Babel and Fowler (1988) reported that all winter wheat by "Manitou" spring-wheat crosses

resulted in spring-habit F1 hybrids that were significantly more hardy than the parental midpoint, indicating some dominance for cold hardiness. Further, based on the distribution of F2-derived F3 lines, there appeared to be a genetic linkage between the Vrn_1 gene for growth habit, which is located on chromosome 5A (Law *et al.*, 1978), and a gene or genes controlling cold hardiness. Chromosome 5A has also been identified in other studies (Cahalan and Law, 1979; Sutka and Kovacs, 1985) as having a major influence on cold hardiness. The possibility that the Vrn_1 gene could be acting pleiotropically, affecting both growth habit and cold hardiness could not be excluded (Limin and Fowler, 1993).

Progress in breeding for increased cold hardiness

In 1929, Quisenberry and Clarke stated "The possibility of developing hardier varieties through breeding has been recognised for many years...(the development of) Minhardi and Minturki varieties in Minnesota demonstrated the possibilities of success". There is little grounds for this optimum today, as progress in the intervening years has been minimal. Old varieties, such as Minhardi released in 1902, and Kharkov 22MC (a Canadian selection from the Russian variety Kharkov) released in 1912, are still among the most cold-hardy varieties present today in western Canada. Efforts to produce more cold-hardy lines by recombining the genetic variability for cold hardiness available in the varieties listed in Table 1 have met with little success, and the Russian introductions Albaskaja and Ulianovkia remain marginally more cold hardy than other available lines (Fowler *et al.*, 1983). Recovery of cold hardiness does not appear to be difficult if at least one parent is cold hardy, but as a general rule segregates surpassing the parents are almost never found in crosses involving parents with a high degree of cold hardiness (Oryuk, 1976; Brule-Bable and Fowler, 1988), indicating a lack of transgressive segregation for the trait.

Objectives in breeding for winter hardiness in wheat

The varieties of wheat are commonly divided on the basis of their growth habit into spring or winter types, although there is an intergrading from the spring into the winter growth habit. The spring type includes the hard red spring, the durum, and a few of the white varieties. The hard and soft red winter varieties and most of the varieties of white wheat are winter types. Some spring type varieties of the white wheats are autumn-planted where the winters are mild. Winter varieties differ from spring varieties a) the development of a more or less prostrate type of seedling growth until subjected to cold weather, (b) the capacity for hardening when exposed to cold, and (c) the ability to withstand freezing temperatures after hardening.

Within the winter wheats there are wide discrepancies in the inherent hardiness of varieties. The varieties range from non-hardy, some of which can scarcely be distinguished from spring varieties, to the most winter resistant. The introduction of the Turkey hard wheat from Russia,

with its inherent resistance to winter injury, made possible the safe production of winter wheat in the central Plains states. In South Africa the term "summer wheat" is popularly used for wheat sown in spring or early summer, depending on the eccurrence of the first summer rains (Nel and Small, 1969). With the development of more hardy varieties, winter wheat production is gradually pushing into areas where only spring-seeded wheats could be grown before.

The most common causes of winter injury in wheat are (a) freezing as a result of low temperatures and/or insufficient soil moisture and (b) heaving. Freezing is in many respects a desiccation process and wheats resistant to winter injury are often resistant also to drought. Heaving results from the alternate freezing and thawing of heavy soils and results in the wheat plant being lifted and its roots sheared off or torn loose from the soil. Many environmental factors may affect the amount of winter injury. These include (a) moisture content of the soil, (b) snow cover, (c) hardening of the plant, (d) physical condition and fertility of the soil, (e) time and rate of planting, and (f) disease and insect injury.

Winter injury as a result of low temperature and/or insufficient soil moisture is more common in the dry land areas than in humid areas where wheats are better protected by snow. The main cause of winter damage is usually insufficient soil moisture combined with low temperatures. Heaving is most severe where soil moisture is abundant and is the more common method of winter injury in humid areas. In general, the more hardy varieties of soft wheat are fairly resistant to winter injury by heaving. But when soft wheats were carried westward by early settlers in the States, they did not survive the combined effects of low temperature and inadequate soil moisture. The Turkey hard wheats survive better than the soft wheats under dry, cold conditions, but they may be more severely injured than the soft wheats if exposed to heaving. This distinction is important in the adaptation of hard and soft winter wheats to the areas where each is now produced. The geographic areas in which winter injury by freezing and by heaving predominate are not sharply defined. Killing by both cold and heaving is common. Also, killing in early autumn, before wheat has become properly hardened, or in late spring, after it has lost its dormancy, is common.

The comparative hardiness of wheat varieties can be measured by growing them over a large geographic area, where they will be subjected to winter injury in a wide range of climatic conditions. Another method of testing the hardiness of wheat varieties is to grow them in pots or flats in a greenhouse and, after suitable hardening procedures, subject them to low temperatures in specially designed freezing chambers. The latter technique measures only the resistance to cold. Measurements of injury by freezing tests have been found in many cases to correlate closely with field tests.

The complex inheritance of winter hardiness is again indicated by the results of a cross made at the Indiana Agricultural Experiment Station between Poole and Minhardi varieties of wheat. The Poole parent variety averaged 24.1 and 23.3 percent survival in an artificial freezing test, and the Minhardi variety averaged 53.0 and 51.5 per cent survival. The survival of the F1 plants was 36.5 per cent, midway between the parents. The survival of the F2 plants, estimated from the survival of the F2 families, varied from 7.5 to 52.5 per cent with an average of 34.6 per cent. Non winter-hardy transgressive segregates were recorded as well as lines as hardy as Minhardi. In this cross, no lines more hardy than the hardy parent were obtained. The results indicate that the inheritance of cold resistance is determined by many genes, which is characteristic of the inheritance of quantitative characters.

Factors that interact to affect cold hardiness

The contradictory evidence found in the literature concerning the relationship of cell size and cold tolerance, and the cold tolerance of diploids versus polyploids, can also be found in the Triticeae, according to Limin and Fowler (1985). Within the triticeae the most cold-hardy *Triticum* and *Aegilops* species were found to be polyploids (Limin and Fowler, 1985), that do in fact have large cells compared to the diploids. The diploid *Secale* variety "Puma" is, however, much more cold hardy than these polyploids. Results obtained in previous experiments (Limin and Fowler, 1988; 1989) suggest some possible explanations for these apparently contradictory findings. There appear to be three important factors interacting to determine cold hardiness in plants: (1) within and between various species there exist genes conferring different degrees of cold hardiness; (2) there appear to be gene dosage effects; and (3) superimposed upon both the quantity and quality of cold-hardiness-conferring genes is the effect of cell size.

The small cell size of diploids appears to give them a potential cold-hardiness advantage over large-celled polyploids. This small size advantage can reach its greatest potential when very effective cold-hardiness-conferring genes are evolved and incorporated, such as found in *S. cereale*. Where superior cold-hardiness genes do not exist, a plant group may expand its cold-hardiness-limiting range by "loading up" on existing genes by means of polyploidy. This dosage effect may then be further enhanced by selection pressure for smaller cell size within the polyploid nucleotype. A system such as this has been proposed to explain, in part, the evolution of cold hardiness in hexaploid wheat (Limin and Fowler, 1989).

Future improvement of cold-hardiness in wheat

According to Limin and Fowler (1991) it is apparent that significant improvement of cold hardiness in wheat will only be accomplished through the use of nontraditional plant-breeding methods.

Species such as *S. cereale* and *Ag. cristatum* are potential gene donors that appear to be much more cold hardy than any of the ancestors from which common wheat has evolved and, therefore, must possess superior cold hardiness genes. These diploid species are also easier to manipulate in a cytogenetic programme because of their smaller number of chromosomes compared with potential polyploid cold-hardiness-genes donors (Limin and Fowler, 1993). In addition, their genomes have already been added to wheat in at least the amphiploid form.

Cold-hardiness genes from diploid species are poorly expressed in the amphiploid form of wheat. Their poor expression seems to be due to the very large cell size of the amphiploid, and also due to the ratio of inferior wheat genes to those of the hardy donor species at each particular locus affecting cold hardiness (Limin and Fowler, 1993). Expression of superior alien cold-hardiness genes may be made possible by the addition of homologous pairs (disomic alien additions) of the alien chromosomes to wheat. These genes may be expressed in addition lines because the cell size should be much smaller in these 44 chromosome plants (42 wheat + 2 alien chromosomes) as opposed to the 56 chromosome amphiploids (42 wheat + 14 alien chromosomes). Cold-hardy derivatives of wheat/*Agropyron* crosses have been reported to have increased levels of cold hardiness (Grafius, 1981). Production of a series of these lines representing each separate pair of the donor species chromosomes in combination with wheat could allow for the identification of alien chromosomes carrying major cold-hardiness genes. This assumes that there is no dominance (homoeoallelic dominance) of the related wheat genes.

Once these alien chromosomes are selected, they would then have to be identified with their related (homoeologous) group of chromosomes in wheat. Unless extensive chromosomal rearrangements have occurred during the separate evolution of wheat and the donor species' chromosomes, the alien chromosome will carry genes of similar function to those of the related wheat chromosomes. Substitution of such an alien chromosome, carrying genes for superior cold hardiness, for a related (homoeologous) wheat chromosome should result in the normal wheat cell size, and a 2:1 ratio of the critical cold-hardiness-conferring genes as opposed to the 3:1 ratio for these particular genes in the amphiploid or alien addition line (Limin and Folwler, 1991).

According to Limin and Fowler (1991) some triticale lines in which particular wheat chromosomes have been removed (triticale nullisomics, 2n=54) have shown greater cold hardiness than the general amphiploid possessing complete sets of both wheat and rye chromosomes. Preliminary observations also suggest that the wheat chromosomes most affecting rye cold-hardiness expression are those unknown to carry important cold-hardiness genes. If the basic genetic system for cold hardiness has been conserved (without chromosomal translocation) between the species, the related rye chromosomes should carry corresponding (homoeoallelic) cold-hardiness

genes. The change in chromosome ratio within this group (from 3:1 to 2:1) presumably causes the increased expression of the rye cold hardiness genes, even in the large-celled amphiploid.

These observations suggest that alien substitution lines may, in fact, allow expression and exploitation of the superior cold-hardiness genes found in related species. Limin and Fowler (1991) states that further plant-breeding efforts would be required to remove linked alien genes with undesirable effects. However, this procedure could provide the opportunity of successfully breeding the impasse that has persisted and prevented any major improvement in the cold hardiness of wheat since the turn of the century.

Chapter 3

MATERIALS AND METHODS

The expression of cold resistance genes in the coleoptiles of South African wheat cultivars

3.1 Materials

Twenty six cultivars from South Africa and one from China were screened for their tolerance to freezing. The cultivars included spring, facultative and winter wheat types. Table 3.1 shows the origin and growth habit of the different wheat cultivars that were used to screen for their tolerance to freezing. The proteins were used to screen for tolerance to freezing.

Table 3.1 Cultivars screened for the expression of proteins. The origin and growht habit of the cultivars are also given, as well as the tolerance or susceptibility of the cultivars to temperatures of –6 and –12 °C

Cultivar	Growth habit	Origin	Coleoptiles*	Roots*
Adam Tas	Spring	South Africa	Susceptable	Susceptable
Betta	Facultative	Argentine	Tolerant	Tolerant
Betta DN	Winter	South Africa	N/A	N/A
Caledon	Winter	South Africa	Tolerant	Tolerant
Chinese Spring	Spring	China	N/A	N/A
Gamtoos	Spring	South Africa	N/A	N/A
Gariep	Facultative	South Africa	Tolerant	Tolerant
Hugenoot	Winter	South Africa	N/A	N/A
Letaba	Winter	South Africa	N/A	N/A
Limpopo	Facultative	South Africa	Tolerant	Susceptable
Molen	Winter	South Africa	Tolerant	Tolerant
Molopo	Winter	South Africa	N/A	N/A
Nantes	Spring	South Africa	N/A	N/A
Palmiet	Spring	South Africa	Susceptable	Susceptable
PAN 3211	Facultative	South Africa	Susceptable	Susceptable
PAN 3349	Winter	South Africa	Tolerant	Tolerant
PAN 3377	Winter	South Africa	N/A	N/A
Scheepers 69	Winter	South Africa	Susceptable	Susceptable
Snack	Spring	South Africa	Susceptable	Susceptable
SST 66	Spring	South Africa	Susceptable	Tolerant
SST 363	Facultative	South Africa	Susceptable	Tolerant

Table 3.1 continued

SST 367	Winter	South Africa	N/A	N/A
SST 966	Winter	South Africa	Tolerant	Susceptable
SST 822	Spring	South Africa	Tolerant	Susceptable
SST 57	Spring	South Africa	N/A	N/A
SST 825	Spring	South Africa	Susceptable	Susceptable
Tugela DN	Winter	South Africa	Tolerant	Susceptable

*In the last two columns the leaf and root reduction are given according to Jacobs (1999).

3.2 Methods

3.2.1 Sample preparation

Germination and growing. Thirty seeds of each cultivar were surface sterilized with 3% NaOCl for three minutes and imbibed at 22 °C for 12h in sterile water. The seed was spread on moist filter paper and allowed to germinate in the dark for two days. Seedlings were then either kept at 22 °C for three more days (control, unhardened) or transferred to 4 °C for 30 days (cold hardened). Based on their dry weight, control seedlings of 3, 4, 5, 6, 7 and 8 days old correspond in terms of physiological age to seedlings hardened respectively for 10, 20, 30, 40, 50 and 60 days.

3.2.2 Protein extraction

Stock solutions

Extraction buffer

12.5 milli Molar tris[hydroxymethyl]aminimethane (Tris)

2 mM Ethylenedinitrilotraacetic acid disodium salt (EDTA)

10 mM Meracpto-ethanol

2 mM Phenylmethylsulfonyl fluoride (PMSF)

Titrate to pH 6.8 with hydrochloricacid (HCl)

Make up to 200 ml with distilled water (dH_2O)

Sample buffer

12.5 mM Tris

10% Gliserol

2.3% Sodium dodecyl sulphate (SDS)

Bromofenolblue

Titrate to pH 6.8 with HCl

Make up to 50 ml with dH_2O

The coleoptiles and roots of the hardened and unhardened seedlings were separated. Of the coleoptile and the root, 0.5-1.0 g was ground in a mortar and homogenized in 6 ml extraction buffer. The homogenate was centrifuged at 12 000 rpm for 10 minutes and placed in a tube with an excess cold asetone (-20 °C) for 12 hours. The homogenate was centrifuged again at 12 000 rpm for ten minutes. The acetone was removed and the proteins were dried in a vacuum dryer. Sample buffer (60 µl) was added and the proteins were left to dissolve. The protein solvent was then heated for one minute in a water bath at 70 °C to assist complete denaturation.

Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE). This method was adapted from the methods used by Sarhan and Perras (1987) and Singh *et al* (1991).

3.2.3 Gel preparation

A discontinuous gel system was used, which required the formation of two gel layers. In the separating gel the separation of the proteins took place and in the stacking gel the samples were loaded to concentrate the protein zones to give very thin starting zones.

Stock solutions

Separating buffer

Tris	45.41g
dH_2O	460 ml
Titrate to pH 8.8 with HCl	
Add SDS	1.0 g
Make up to 500 ml with dH_2O	
Store at 4 °C	

Separating acrylamide (30% Ac/1% cross linker)

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

Stacking buffer

Tris	6.06 g
dH_2O	190 ml
Titrate to pH 6.8 with HCl	
Addd SDS	0.4 g

Make up to 200 ml with dH_2O
Store at 4 °C

Stacking acrylamide (35% Ac/1.5% crosslinker)

Acrylamide	87.5 g
Bis	1.32 g
dH_2O	181 ml

Make up to 250 ml with dH_2O
Store at 4 °C

Electrode buffer

Cathode buffer

Tris	30.28 g
Glysine	144 g
SDS	10 g

Make up to 1 000 ml with dH_2O

Anode buffer

Tris	30.28 g
dH_2O	800 ml

Titrate to pH 8.4 with HCl
Make up to 1 000 ml with dH_2O

Separating gel. (10% uniform) Mix 14 ml separating buffer, 1.3 ml separating acrylamide and 4.7 ml dH_2O. Add 55 µl N,N,N,N',N'-Tetramethylenediamine (Temed) and 65 µl 10% freshly made Ammonium persulphate (APS). Pour gel.

Stacking gel. Mix 5 ml stacking buffer, 1.3 ml stacking acrylamide and 3.7 ml dH_2O. Add 20 µl Temed and 50 µl freshly made APS. Pour the stacking gel on top of the separating gel and insert sample-loading positions.

Sample separation

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

3.2.4 Gel staining

Fixing solution

Methanol	400 ml
Glacial acetic acid	100 ml
dH_2O	500 ml

Staining solution

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

The gel was removed from the glass plates and immersed in the fixing solution for one hour and then left overnight in the staining solution. The efficiency of shaking is very important to get uniform results and should therefore be optimized so that the fluid circulates without breaking the gel during staining and destaining. The stained gels were rinsed in distilled water for a few hours before interpretation and photography (Wrigley, 1992).

3.2.5 Gel analysis

The "Molecular Analyst Fingerprinting" software of Biorad was used to analyse the gels. The gels were scanned with the Gel Doc 1000 using an UV-gel camera and VGA graphics in 256 colours as recommended. The analysing procedure consisted of three steps, namely a) the conversion of the gel, b) the normalisation of the tracks and c) the analysis of the tracks.

The normalisation settings were as follow: the resolution was set at 200 points and a smoothing factor of three was chosen (this implied that one point at either side of a data point would be averaged with the data point). To subtract the background, the rolling disk method was chosen. The principle of the rolling disk mechanism is that a disk is rolled on the inside across the curve. Every area of the curve below the imaginary trace left behind the disk will be subtracted as background. This method gives very stable and reliable background subtraction. The intensity of the background substraction was set at ten (typical settings for SDS-PAGE proteins patterns are between eight and 12). The clearest pattern was used as the standard reference pattern and all the other reference patterns were aligned to the standard reference. Normalisation of a gel is achieved by aligning the bands of all reference patterns on the gel to the corresponding ones of the standard. Non-reference tracks are interpolated gradually according to both surrounding references. At least three references were loaded on each gel for the best normalisation results.

After normalisation the gels were analysed using the main program of the "Molecular Analyst Fingerprinting" software. A densitometric curve of every replication of every cultivar was drawn and from this the migration distances were determined. The program gave the migration distance of each peak and the intensity of the band. Four classes of intensity were assigned with 1 being a very light band and 4 a very dark band. The migration distances of the peaks were compared with each other.

Gel interpretation

The protein bands for hardened and unhardened coleoptile and root samples were compared and the number of new protein bands produced by each cultivar were observed. Any change in the band intensity was also noted.

3.2.6 Determination of the molecular weights

A low range (14 to 97.4 kDa) SDS-PAGE molecular marker of Biorad was used to determine the molecular weights of the protein groups. It consisted of the following:

	Molecular weight (kDa)
Phosphorylase b	97.4
Serum albumin	66.2
Ovalbumin	45.0
Carbonic anhydrase	31.0
Trypsin inhibitor	21.5
Lysozyme	14.4

It was possible to determine the molecular weights of the protein subunits, using the Gel Doc software. The migration distance of each band was calculated as a molecular weight in kDa.

3.2.7 Correlation between the different protein bands

The correlation between the different protein bands was determined using the Spearman-rank correlation matrices of the NCSS 2000 program. The Spearman-rank calculates the correlation by applying the Pearson correlation formulas to the ranks of the data rather than to the actual data values them selves, thus reducing many of the distortions that affect the Pearson correlation. Pair-wise removal of missing values were chosen, because missing values occurring in other variables do not have an influence on the calculations.

Chapter 4

COLD RESISTANCE IN THE COLEOPTILES OF WHEAT SEEDLINGS

The protein subunits expressed during cold treatment in the coleoptiles of wheat seedlings.

The protein bands were expressed at different cold treatments and their controls. Fourteen different groups were found. Table 4.1 shows the groups and possible ranges of their different molecular weights.

Table 4.1 Protein groups with their respective migration distances and molecular weights

	Migration distances	Molecular weight – kDa
Group 1	10 – 20	76.5 – 72
Group 2	21 – 30	71 – 66.5
Group 3	31 – 40	65.5 - 61
Group 4	41 – 50	60 – 55.5
Group 5	51 – 60	54.5 – 50
Group 6	61 – 70	49 – 44.5
Group 7	71 – 80	43.5 – 39
Group 8	81 – 90	38 – 33.5
Group 9	91 – 100	32.5 – 28
Group 10	101 – 110	27 – 22.5
Group 11	111 – 120	21.5 – 17
Group 12	121 – 130	16 – 11.5
Group 13	131 – 140	10.5 – 6
Group 14	141 - 150	5 - 0

The occurrence of bands in the different groups

Table 4.2 gives a summary of the different groups, the molecular weight of the group, the occurrence of the bands in the groups at the cold treatment, and the occurrence of the bands in the groups at the controls.

Table 4.2 The occurrence of bands in the different protein groups of proteins in the wheat cultivars studied

Group	Molecular weight (kDa)	Occurrence at Cold treatment	Occurrence at Controls
Group 1	76.5 – 72	97	87
Group 2	71 – 66.5	101	100
Group 3	65.5 – 61	146	152
Group 4	60 – 55.5	125	120
Group 5	54.5 – 50	145	153
Group 6	49 – 44.5	140	155
Group 7	43.5 – 39	163	163
Group 8	38 – 33.5	171	164
Group 9	32.5 – 28	173	165
Group 10	27 – 22.5	159	149
Group 11	21.5 – 17	150	164
Group 12	16 – 11.5	152	158
Group 13	10.5 – 6	147	142
Group 14	6 - 0	106	104

The protein groups expressed most in all the different wheat cultivars were group 8 (migration distance 81 – 90) and group 9 (migration distance 91 –100) where the bands occurred 335 and 338 times, in total, respectively. The group that was expressed the least was group 1 (migration distance 10 – 20) where the bands occurred only 184 times. Group 9 (migration distance 91 – 100) occured the most in the cold treated cultivars with 173 bands, and also the most in the controls with 165 bands The protein group expressed the least in the cold treated cultivars was group 1 (migration distance 10 – 20) with 97 bands, group 2 (migration distance 21 – 30) with 101 bands and group 14 (migration distance 141 –150) with 106 bands The protein group expressed the least in the controls was group 1 with 87 bands Group 6 (migration distance 61 – 70) was expressed 140 times in the cold treated cultivars and 155 times in the controls. Group 11 was expressed 150 times in the cold treated cultivars and 164 times in the controls.

Please refer to Appendix A for all the banding patterns of the different cultivars.

Adam Tas

10 days – Adam Tas expressed 16 protein bands (Tabel 1). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by Adam Tas.

Although there were no significant differences between the total number of proteins produced, groups 1, 4 and 8 expressed an additional band under cold treatment, while groups 6, 9 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 5, 7, 8, 11 and 14 at both the control and treatment. Groups 4 and 14 expressed bands of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to four in the control.

20 days – Adam Tas expressed 11 protein bands (Table 1). The untreated sample expressed 14 protein bands indicating that treatment had a significant effect on the number of protein bands produced by Adam Tas.

After cold treatment a total number of three more protein bands were expressed than in the control. Groups 3 and 5 expressed an additional band under cold treatment, while groups 2, 6, 9, 11 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 9, 11 and 14 at the control. Groups 4, 7, 10 and 14 expressed bands of a higher intensity in the control while two bands showed a high intensity in the cold treatment. The number of bands with a high density varied from two in the treated material to six in the control.

30 days – Adam Tas expressed 11 protein bands (Table 1). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by Adam Tas.

Although there were no significant differences between the total number of proteins produced, groups 2, 6 and 12 expressed an additional band under cold treatment, while groups 1, 4 and 5 showed a reduction of one band each when treated with cold. Double bands were expressed in group 14 at both the control and treatment. Groups 6, 8, 9, 11 and 14 expressed bands of a higher intensity under treatment while six bands showed high intensity in the control population. There is no clear pattern with regard to the number of double bands between the control and the treatment in the cultivar Adam Tas. The number of bands with a high density varied from five in the treated material to six in the control.

40 days – Adam Tas expressed 11 protein bands (Table 1). The untreated sample expressed 16 protein bands indicating that treatment had a significant effect on the number of proteins produced by Adam Tas.

At 40 days cold treatment five less protein bands were expressed than in the control. Group 3 expressed an additional band under cold treatment, while groups 2, 4, 7, 8 and 14 showed a reduction of one band each, and both the bands at group 12, when treated with cold. Double bands were expressed in groups 8, 12 and 14 at the control, and at group 3 during cold treatment. Groups 5, 8, 11, 13 and 14 expressed bands of a higher intensity under treatment while seven bands showed high intensity in the control population. The number of bands with a high density varied from five in the treated material to seven in the control.

50 days – At 50 days cold treatment Adam Tas expressed 13 protein bands (Table 1). The untreated sample expressed 16 protein bands indicating that treatment had a significant effect on the number of proteins produced by Adam Tas.

At 50 days cold treatment three less protein bands were expressed than in the control. Groups 8, 9, 13 and 14 showed a reduction of one band each under cold treatment. Double bands were expressed in groups 8, 9 and 13 at the control, and in group 14 at both the control and cold treatment. Groups 9, 11 and 14 expressed bands of a higher intensity under treatment while seven bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to seven in the control.

60 days - Adam Tas expressed nine protein bands (Table 1). The untreated sample expressed 14 protein bands indicating that treatment had a significant effect on the number of proteins produced by Adam Tas.

At 60 days cold treatment five more protein bands were expressed than in the control. Group 5 expressed two additional bands under cold treatment, while groups 4, 8, 9 and 14 showed a reduction of one band each, and both the bands in group 13, when treated with cold. Double bands were expressed in groups 9, 12 and 14 at the control, and in group 5 during cold treatment. Group 11 expressed a band of a higher intensity under treatment while seven bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to seven in the control.

Betta

10 days - Betta expressed 14 protein bands (Table 2). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by Betta.

Although there were no significant differences between the total number of proteins produced, groups 5, 7 and 8 expressed additional bands under cold treatment, while group 6 showed a reduction of one band when treated with cold. Double bands were expressed in group 3 at the control, and in groups 5, 7 and 8 during cold treatment. Groups 5, 8, 13 and 14 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to four in the treated material.

20 days - Betta expressed 14 protein bands (Table 2). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by Betta.

Although there were no significant differences between the total number of proteins produced, groups 5 and 7 expressed additional bands under cold treatment, while groups 4 and 8 showed a reduction of one band each when treated with cold. Double bands were expressed in group 3 and 8 at the control, and in groups 3 and 5 during cold treatment. Groups 5, 8, 13 and 14 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to four in the treated material.

30 days - Betta expressed 12 protein bands (Table 2). The untreated sample expressed 14 protein bands indicating that treatment had an effect on the number of proteins produced by Betta.

At 30 days cold treatment two less protein bands were expressed than in the control. Group 4 expressed an additional band under cold treatment, while groups 3, 5 and 8 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6 and 8 at the control, and in groups 4 and 6 during cold treatment. Groups 4, 6, 8 and 12 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to four in the treated material.

40 days - Betta expressed 13 protein bands (Table 2). The untreated sample expressed 15 protein bands indicating that treatment had an effect on the number of proteins produced by Betta.

At 40 days cold treatment two less protein bands were expressed than in the control. Group 2 expressed an additional band under cold treatment, while groups 3 and 6 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4, 6, 8 and 11 at the control, and in groups 2, 4 and 11 during cold treatment. Groups 4, 7, 8 and 12 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to four in the treated material.

50 days - Betta expressed 13 protein bands (Table 2). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by Betta.

At 50 days cold treatment one more protein band was expressed than in the control. Groups 8, 10 and 11 expressed additional bands under cold treatment, while groups 1 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 2, 5, 7 and 12 at the control, and in groups 2, 6, 7 and 11 during cold treatment. Groups 2, 4, 7, 8 and 12 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to five in the treated material.

60 days - Betta expressed 11 protein bands (Table 2). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the number of proteins produced by Betta.

At 60 days cold treatment two less protein bands were expressed than in the control. Group 6 expressed an additional band under cold treatment, while groups 4, 5 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1, 5 and 11 at the control, and in group 6 during cold treatment. Groups 3, 5, 8, 9 and 13 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to five in the treated material.

Betta DN

10 days - Betta DN expressed 17 protein bands (Table 3). The untreated sample expressed 15 protein bands indicating that treatment had an effect on the number of proteins produced by Betta DN.

At 10 days cold treatment two more protein bands were expressed than in the control. Groups 5, 8, 10 and 12 expressed an additional band under cold treatment, while groups 6 and 9 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1, 6 and 9 in the control, and in groups 1, 5, 8 and 12 during cold treatment. Groups 3, 5, 8 and 12 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population. The number of bands with a high density varied from four in the treated material to five in the control.

20 days - Betta DN expressed 12 protein bands (Table 3). The untreated sample had the same number of protein bands indicating that treatment had no effect on the number of proteins produced by Betta DN.

Although there were no significant differences between the total number of proteins produced, groups 9 and 12 expressed an additional band under cold treatment, while groups 6 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6 at the control, and in group 12 during cold treatment. Groups 6, 8 and 12 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density was the same for the cold treatment and the control.

30 days - Betta DN expressed 14 protein bands (Table 3). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by Betta DN.

At 30 days cold treatment two more protein bands were expressed than in the control. Groups 1 and 10 expressed an additional band under cold treatment, while group 5 showed a reduction of one band each when treated with cold. Double bands were expressed in group 5 at the control, and in group 1 during cold treatment. Groups 3, 8, 9 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to four in the control.

40 days - Betta DN expressed 16 protein bands (Table 3). The untreated sample had the same number of bands indicating that treatment had no effect on the number of proteins produced by Betta DN.

Although there were no significant differences between the number of proteins produced, groups 5 and 14 expressed an additional band, and group 10 two additional bands, under cold treatment,

while groups 2, 6, 11 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1, 6, 11 and 12 at the control, and in groups 1, 10 and 11 during cold treatment. Groups 3, 8 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density was the same in both the control and cold treatement.

50 days - Betta DN expressed 14 protein bands (Table 3). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the number of proteins produced by Betta DN.

At 50 days cold treatment one more protein band was expressed than in the control. Groups 5 and 13 expressed an additional band, and group 1 two additional bands, under cold treatment, while group 7 showed a reduction of one band, and group 12 a reduction of two bands, when treated with cold. Double bands were expressed in groups 7 and 11 at the control, and in groups 1 and 13 during cold treatment. Groups 3, 8, 9 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to four in cold treatment.

60 days - Betta DN expressed 14 protein bands (Table 3). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by Betta DN.

At 60 days cold treatment two more protein bands were expressed than in the control. Groups 3, 7, 8 and 10 expressed an additional band under cold treatment, while groups 2 and 4 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3, 7, 8 and 12 at the control, and in group 12 during cold treatment. Groups 3, 8 and 10 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from three the treated material to four in the control.

Caledon

10 days - Caledon expressed 15 protein bands (Table 4). The untreated sample expressed 14 protein bands indicating that treatment had an effect on the number of proteins produced by Caledon.

At 10 days cold treatment one more protein band was expressed than in the control. Groups 1, 9 and 14 expressed additional bands under cold treatment, while groups 8 and 12 showed a

reduction of one band each when treated with cold. Double bands were expressed in groups 8 and 12 at the control, and in groups 9 and 14 during cold treatment. Groups 5, 6, 7, 8 and 11 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to five in the treated material.

20 days - Caledon expressed 13 protein bands (Table 4). The untreated sample expressed 17 protein bands indicating that treatment had a significant effect on the number of proteins produced by Caledon.

At 20 days cold treatment four more protein bands were expressed than in the control. Group 5 expressed an additional band under cold treatment, while groups 1, 2, 4, 8 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4, 8, 12 and 14 at the control, and in groups 5 and 14 during cold treatment. Groups 3, 8 and 11 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to five in the control.

30 days - Caledon expressed 14 protein bands (Table 4). The untreated sample had exactly the same number of bands indicating that treatment had no effect on the number of proteins produced by Caledon.

Although there were no significant differences between the total number of proteins produced, groups 1 and 13 expressed an additional band under cold treatment, while groups 2 and 4 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4 and 8 at the control, and in group 8 during cold treatment. Groups 5 and 10 expressed a band of a higher intensity under treatment while 4 bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to four in the treated material.

40 days - Caledon expressed 12 protein bands (Table 4). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by Caledon.

At 40 days cold treatment one more protein band was expressed than in the control. Groups 2, 10, 13 and 14 expressed an additional band under cold treatment, while groups 3, 4 and 5 showed a reduction of one band each when treated with cold. Double bands were expressed in

group 4 at the control, and in groups 10 and 14 during cold treatment. Groups 4 and 8 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control

50 days - Caledon expressed 14 protein bands (Table 4). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by Caledon.

At 50 days cold treatment two more protein bands were expressed than in the control. Groups 1, 2, 10 and 13 expressed an additional band, and group 12 two additional bands, under cold treatment, while groups 3 and 6 showed a reduction of one band each, and group 14 a reduction of two bands, when treated with cold. Double bands were expressed in groups 4 and 14 at the control, and in groups 4, 10 and 12 during cold treatment. Groups 7 and 8 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

60 days - Caledon expressed 16 protein bands (Table 4). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the number of proteins produced by Caledon.

At 60 days cold treatment three more protein bands were expressed than in the control. Groups 1, 4, 7, 9, 10 and 13 expressed additional bands under cold treatment, while group 12 showed a reduction of one band, and group 14 a reduction of two bands, when treated with cold. Double bands were expressed in group 14 at the control, and in groups 4, 7, 9 and 10 during cold treatment. Groups 3, 7 and 10 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to four in the control.

Chinese Spring

10 days - Chinese Spring expressed 11 protein bands (Table 5). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the number of proteins produced by Chinese Spring.

At 10 days cold treatment two less protein bands were expressed than in the control. Group 11 expressed an additional band under cold treatment, while groups 7, 9 and 13 showed a reduction

of one band each when treated with cold. Double bands were expressed in groups 7 and 9 at the control. Groups 4 and 7 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

20 days - Chinese Spring expressed 10 protein bands (Table 5). The untreated sample had the same number of protein bands indicating that treatment had no effect on the number of proteins produced by Chinese Spring.

Although there were no significant differences between the total number of proteins produced, group 6 expressed an additional band, and group 9 two additional bands, under cold treatment, while groups 5, 7 and 10 showed a reduction of one band each when treated with cold. Double bands were expressed in group 7 at the control, and in group 9 during cold treatment. Group 7 expressed a band of a higher intensity under treatment and in the control.

30 days - Chinese Spring expressed 11 protein bands (Table 5). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by Chinese Spring.

At 30 days cold treatment one protein band less was expressed than in the control. Group 3 expressed an additional band under cold treatment, while groups 9 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in group 9 at the control. Groups 7 and 12 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to four in the control.

40 days - Chinese Spring expressed 11 protein bands (Table 5). The untreated sample had the same number of protein bands indicating that treatment had no effect on the number of proteins produced by Chinese Spring.

Although there were no significant differences between the total number of proteins produced, groups 5, 8 and 9 expressed an additional band under cold treatment, while groups 7, 11 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4, 7 and 12 at the control, and in group 4 under cold treatment. Groups 6 and 7 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population.

50 days - Chinese Spring expressed 11 protein bands (Table 5). The untreated sample had the same number of protein bands indicating that treatment had no effect on the number of proteins produced by Chinese Spring.

Although there were no significant differences between the total number of proteins produced, groups 9 and 10 expressed an additional band under cold treatment, while groups 6 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6 and 12 at the control. Groups 7 and 8 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population.

60 days - Chinese Spring expressed nine protein bands (Table 5). The untreated sample had the same number of protein bands indicating that treatment had no effect on the number of proteins produced by Chinese Spring.

Although there were no significant differences between the total number of proteins produced, groups 5 and 8 expressed an additional band, and group 6 two additional bands, under cold treatment, while groups 4, 7, 10 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6 and 8 under cold treatment. Group 9 expressed a band of a higher intensity under treatment as well as in the control.

Gamtoos

10 days - Gamtoos expressed 13 protein bands (Table 6). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by Gamtoos.

At 10 days cold treatment one more protein band was expressed than in the control. Groups 8 and 12 expressed an additional band under cold treatment, while group 10 showed a reduction of one band when treated with cold. Double bands were expressed in groups 5 and 10 at the control, and in groups 5 and 12 during cold treatment. Group 9 expressed a band of a higher intensity both in the control and under treatment.

20 days - Gamtoos expressed 11 protein bands (Table 6). The untreated sample expressed nine protein bands indicating that treatment had an effect on the number of proteins produced by Gamtoos.

At 20 days cold treatment two more protein bands were expressed than in the control. Groups 4, 5, 7 and 10 expressed an additional band under cold treatment, while groups 1 and 8 showed a

reduction of one band each when treated with cold. Double bands were expressed in group 5 during cold treatment. Groups 5 and 9 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

30 days - Gamtoos expressed 11 protein bands (Table 6). The untreated sample had the same number of protein bands indicating that treatment had no effect on the number of proteins produced by Gamtoos.

Although there were no significant differences between the total number of proteins produced, group 13 expressed an additional band under cold treatment, while group 1 showed a reduction of one band when treated with cold. Double bands were expressed in groups 5, 10 and 13 during cold treatment, and in groups 5 and 10 in the control population. Groups 5 and 9 expressed a band of a higher intensity both under treatment and in the control.

40 days - Gamtoos expressed 10 protein bands (Table 6). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the number of proteins produced by Gamtoos.

At 40 days cold treatment three less protein bands were expressed than in the control. Group 3 expressed an additional band under cold treatment, while groups 2, 4 and 6 showed a reduction of one band each when treated with cold. Double bands were expressed in group 6 during the control. Groups 9 and 14 expressed a band of a higher intensity under the control. The number of bands with a high density varied from nothing in the treated material to two in the control.

50 days - Gamtoos expressed 11 protein bands (Table 6). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by Gamtoos.

At 50 days cold treatment one protein band less was expressed than in the control. Groups 6 and 12 expressed an additional band under cold treatment, while groups 2, 3 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 7 and 9 during cold treatment, and in groups 7, 9 and 11 in the control population. Groups 9, 13 and 14 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

60 days - Gamtoos expressed 13 protein bands (Table 6). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by Gamtoos.

At 60 days cold treatment one more protein band was expressed than in the control. Group 12 expressed an additional band, and group 7 two additional bands, under cold treatment, while groups 1 and 2 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 7 and 9 during cold treatment, and in group 9 in the control. Groups 5, 9, 13 and 14 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population.

Gariep

10 days - Gariep expressed 16 protein bands (Table 7). The untreated sample expressed ten protein bands indicating that treatment had a significant effect on the number of proteins produced by Gariep.

At 10 days cold treatment six more protein bands were expressed than in the control. Groups 1, 5, 6, 8, 10, 12 and 13 expressed an additional band each under cold treatment, while groups 7 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 5, 6, 8, 10 and 13 during cold treatment, and in Group 7 at the control population. Group 5 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to two in the control.

20 days - Gariep expressed 13 protein bands (Table 7). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by Gariep.

At 20 days cold treatment one more protein band was expressed than in the control. Groups 3, 7 and 13 expressed an additional band each under cold treatment, while groups 1, 4 and 10 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 7, 8 and 13 during cold treatment, and in groups 4 and 8 at the control population. Group 8 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to two in the control.

30 days - Gariep expressed 13 protein bands (Table 7). The untreated sample expressed 14 protein bands indicating that treatment had an effect on the number of proteins produced by Gariep.

At 30 days cold treatment one protein band less was expressed than in the control. Group 11 expressed an additional band under cold treatment, while groups 1 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 8 and 11 during cold treatment, and in Group 8 at the control population. Groups 3, 8, 9 and 14 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to four in the treated material.

40 days - Gariep expressed eight protein bands (Table 7). The untreated sample expressed 12 protein bands indicating that treatment had a significant effect on the number of proteins produced by Gariep.

At 40 days cold treatment four less protein bands were expressed than in the control. Group 2 expressed an additional band under cold treatment, while groups 1, 4, 5, 9 and 13 showed a reduction of one band each when treated with cold. Groups 8 and 14 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to two in the cold treated material.

50 days - Gariep expressed 13 protein bands (Table 7). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the number of proteins produced by Gariep.

At 50 days cold treatment two more protein bands were expressed than in the control. Groups 2, 3, 8 and 9 expressed an additional band each under cold treatment, while groups 5 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in group 8 during cold treatment, and in group 5 at the control population. Groups 8 and 14 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population.

60 days - Gariep expressed 16 protein bands (Table 7). The untreated sample expressed ten protein bands indicating that treatment had a significant effect on the number of proteins produced by Gariep.

At 60 days cold treatment six more protein bands were expressed than in the control. Groups 2, 3, 7, 9, 11 and 12 expressed an additional band each under cold treatment. Double bands were expressed in groups 3, 7, 11 and 12 during cold treatment. Groups 3, 4, 7, 10 and 14 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to five in the treated material.

Hugenoot

10 days - Hugenoot expressed 11 protein bands (Table 8). The untreated sample expressed 13 protein bands indicating that treatment had a significant effect on the number of proteins produced by Hugenoot.

At 10 days cold treatment two less protein bands were expressed than in the control. Groups 2 and 4 expressed an additional band each under cold treatment, while groups 1, 3 and 6 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3 and 6 during the control. Groups 4 and 5 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to two in the treated material.

20 days - Hugenoot expressed 13 protein bands (Table 8). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by Hugenoot.

At 20 days cold treatment one more protein band was expressed than in the control. Groups 7, 9 and 14 expressed an additional band each, and group 4 two additional bands, under cold treatment, while groups 3, 5, 10 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in group 4 during cold treatment, and in groups 5 and 10 in the control population. Group 5 expressed a band of a higher intensity under treatment while one band showed high intensity in the control population.

30 days - Hugenoot expressed 14 protein bands (Table 8). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the number of proteins produced by Hugenoot.

At 30 days cold treatment one more protein band was expressed than in the control. Groups 5 and 10 expressed an additional band each under cold treatment, while group 5 showed a reduction of one band when treated with cold. Double bands were expressed in groups 5 and 10

during cold treatment, and in group 9 in the control. Groups 4 and 12 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population.

40 days - Hugenoot expressed 13 protein bands (Table 8). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by Hugenoot.

Although there were no significant differences between the total number of proteins produced, group 4 expressed an additional band each under cold treatment, while group 7 showed a reduction of one band when treated with cold. Double bands were expressed in group 11 during cold treatment, and in groups 7 and 11 in the control. Groups 2, 4 and 7 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to four in the control.

50 days - Hugenoot expressed 14 protein bands (Table 8). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the number of proteins produced by Hugenoot.

At 50 days cold treatment three more protein bands were expressed than in the control. Groups 4, 7, 8 and 9 expressed an additional band each under cold treatment, while group 6 showed a reduction of one band when treated with cold. Double bands were expressed in groups 7 and 8 during cold treatment, and in group 6 in the control. Groups 2, 7 and 10 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to five in the control.

60 days - Hugenoot expressed ten protein bands (Table 8). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by Hugenoot.

At 60 days cold treatment one less protein band was expressed than in the control. Group 9 expressed an additional band, and two additional bands in group 4, under cold treatment, while groups 3 and 11 showed a reduction of one band each, and group 9 a reduction of two bands, when treated with cold. Double bands were expressed in groups 4 and 12 during cold treatment, and in groups 9 and 12 in the control. Groups 2, 7 and 10 expressed a band of a higher intensity under treatment while six bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to six in the control.

Letaba

10 days - Letaba expressed 12 protein bands (Table 9). The untreated sample expressed 14 protein bands indicating that treatment had a significant effect on the number of proteins produced by Letaba.

At 10 days cold treatment two less protein bands were expressed than in the control. Groups 3 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in group 6 during cold treatment, and in groups 6 and 14 in the control. Groups 4, 6 and 9 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to four in the control.

20 days - Letaba expressed 14 protein bands (Table 9). The untreated sample expressed ten protein bands indicating that treatment had a significant effect on the number of proteins produced by Letaba.

At 20 days cold treatment four more protein bands were expressed than in the control. Groups 1, 2, 4, 5 and 14 expressed an additional band each under cold treatment, while group 3 showed a reduction of one band when treated with cold. Double bands were expressed in groups 2 and 14 during cold treatment. Groups 2, 4, 6, 7, 9 and 14 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to six in the treated material.

30 days - Letaba expressed 12 protein bands (Table 9). The untreated sample expressed 13 protein bands indicating that treatment had a significant effect on the number of proteins produced by Letaba.

At 30 days cold treatment one less protein band was expressed than in the control. Groups 5 and 13 expressed an additional band each under cold treatment, while groups 6, 10 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6 and 10 during the control. Groups 6 and 7 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

40 days - Letaba expressed 12 protein bands (Table 9). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by Letaba.

At 40 days cold treatment one more protein band was expressed than in the control. Groups 1, 2 and 14 expressed an additional band each under cold treatment, while groups 5 and 6 showed a reduction of one band each when treated with cold. Double bands were expressed in group 3 during cold treatment, and in groups 3 and 6 in the control. Groups 6 and 9 expressed a band of a higher intensity under treatment and in the control.

50 days - Letaba expressed 12 protein bands (Table 9). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by Letaba.

At 50 days cold treatment one more protein band was expressed than in the control. Groups 4, 10 and 14 expressed an additional band each under cold treatment, while groups 1, 2, 6 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3 and 10 during cold treatment, and in groups 3 and 6 in the control. Groups 6 and 9 expressed a band of a higher intensity under treatment and in the control.

60 days - Letaba expressed ten protein bands (Table 9). The untreated sample expressed 13 protein bands indicating that treatment had a significant effect on the number of proteins produced by Letaba.

At 60 days cold treatment three less protein bands were expressed than in the control. Groups 7, 12 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6 and 10 during cold treatment and in the control. Groups 3 and 9 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

Limpopo

10 days - Limpopo expressed 12 protein bands (Table 10). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by Limpopo.

At 10 days cold treatment one more protein band was expressed than in the control. Groups 4 and 13 expressed an additional band each under cold treatment, while group 10 showed a reduction of one band when treated with cold. Double bands were expressed in group13 during cold treatment. Groups 3, 5 and 13 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to three in the treated material.

20 days - Limpopo expressed 11 protein bands (Table 10). The untreated sample expressed 13 protein bands indicating that treatment had a significant effect on the number of proteins produced by Limpopo.

At 20 days cold treatment two less protein bands were expressed than in the control. Groups 1, 5 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in group 13 during the control. Group 6 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population. There is no clear pattern with regard to the number of double bands between the control and treatment in the cultivar Limpopo at 20 days. The number of bands with a high density varied from one in the treated material to five in the control.

30 days - Limpopo expressed 11 protein bands (Table 10). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by Limpopo.

At 30 days cold treatment one less protein band was expressed than in the control. Groups 1, 3, 6 and 9 expressed an additional band each under cold treatment, while groups 7, 10, 11, 12 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in group 9 during cold treatment, and in groups 10 and 13 in the control. Groups 5, 8 and 13 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to three in the treated material.

40 days - Limpopo expressed 11 protein bands (Table 10). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by Limpopo.

At 40 days cold treatment one less protein band was expressed than in the control. Group 3 expressed an additional band in cold treatment, while groups 4 and 5 showed a reduction of one band each when treated with cold. Groups 6 and 8 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

50 days - Limpopo expressed 12 protein bands (Table 10). The untreated sample expressed eight protein bands indicating that treatment had a significant effect on the number of proteins produced by Limpopo.

At 50 days cold treatment four more protein bands were expressed than in the control. Groups 1, 3, 7, 9, 11 and 13 expressed an additional band each under cold treatment, while groups 6 and 10 showed a reduction of one band each when treated with cold. Double bands were expressed in group 13 during cold treatment. Groups 7 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

60 days - Limpopo expressed 11 protein bands (Table 10). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by Limpopo.

Although there were no significant differences between the total number of proteins produced, groups 1, 2 and 7 expressed an additional band each, and group 11 two additional bands, under cold treatment, while groups 3, 6, 9, 10 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in group 11 during cold treatment, and in groups 10 and 13 in the control. Group 7 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to three in the control.

Molen

10 days - Molen expressed 12 protein bands (Table 11). The untreated sample expressed 18 protein bands indicating that treatment had a significant effect on the number of proteins produced by Molen.

At 10 days cold treatment six less protein bands were expressed than in the control. Group 10 expressed an additional band under cold treatment, while groups 1, 2, 5, 6, 7, 8 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in group 10 during cold treatment, and in groups 6, 7, 8 and 11 in the control. Groups 8, 10 and 14 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to three in the treated material.

20 days - Molen expressed 13 protein bands (Table 11). The untreated sample expressed 16 protein bands indicating that treatment had a significant effect on the number of proteins produced by Molen.

At 20 days cold treatment three less protein bands were expressed than in the control. Groups 2, 4 and 8 expressed an additional band each under cold treatment, while groups 3, 5, 7, 9, 11

and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4 and 8 during cold treatment, and in groups 5, 7, 9, 11 and 14 in the control. Groups 4, 5, 8, 10 and 14 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to five in the treated material.

30 days - Molen expressed 15 protein bands (Table 11). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by Molen.

Although there were no significant differences between the total number of proteins produced, groups 4, 12 and 14 expressed an additional band each under cold treatment, while groups 5, 6 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 9 and 14 during cold treatment, and in groups 5, 9 and 11 in the control. Groups 5, 8. 10, 11 and 14 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to six in the treated material.

40 days - Molen expressed 15 protein bands (Table 11). The untreated sample expressed 16 protein bands indicating that treatment had a significant effect on the number of proteins produced by Molen.

At 40 days cold treatment one less protein band was expressed than in the control. Groups 2, 12 and 14 expressed an additional band each under cold treatment, while groups 3, 4, 9 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 10 and 14 during cold treatment, and in group 3, 4, 9, 10 and 11 in the control. Groups 4, 6, 8, 9, 10, 11 and 14 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to eight in the treated material.

50 days - Molen expressed 11 protein bands (Table 11). The untreated sample expressed 14 protein bands indicating that treatment had a significant effect on the number of proteins produced by Molen.

At 50 days cold treatment three less protein bands were expressed than in the control. Groups 4 and 14 expressed an additional band each under cold treatment, while groups 2, 3, 6, 7 and 8 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4, 9 and 14 during cold treatment, and in group 9 in the control. Groups 4, 5, 9, 11 and 14

expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to six in the treated material.

60 days - Molen expressed 14 protein bands (Table 11). The untreated sample expressed 15 protein bands indicating that treatment had an effect on the number of proteins produced by Molen.

At 60 days cold treatment one less protein band was expressed than in the control. Group 3 showed a reduction of one band when treated with cold. Double bands were expressed in groups 8 and 14 during cold treatment and in the control. Groups 4, 6, 8, 10, 11 and 14 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population. The number of bands with a high density varied from five in the control to seven in the treated material.

Molopo

10 days - Molopo expressed ten protein bands (Table 12). The untreated sample expressed 16 protein bands indicating that treatment had a significant effect on the number of proteins produced by Molopo.

At 10 days cold treatment six less protein bands were expressed than in the control. Groups 1, 2, 3, 9, 10 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1, 3 and 14 during the control. Groups 3, 7, 11 and 13 expressed a band of a higher intensity under treatment while six bands showed high intensity in the control population. The number of bands with a high density varied from four in the treated material to six in the control.

20 days - Molopo expressed 13 protein bands (Table 12). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by Molopo.

Although there were no significant differences between the total number of proteins produced, groups 2 and 6 expressed an additional band each under cold treatment, while groups 12 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1, 3 and 14 during cold treatment and in the control. Groups 3, 7 and 14 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to five in the control.

30 days - Molopo expressed 12 protein bands (Table 12). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by Molopo.

Although there were no significant differences between the total number of proteins produced, group 6 expressed an additional band under cold treatment, while group 1 showed a reduction of one band when treated with cold. Double bands were expressed in group 14 during cold treatment, and in groups 1 and 14 in the control. Group 7 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to two in the control.

40 days - Molopo expressed 13 protein bands (Table 12). The untreated sample expressed 14 protein bands indicating that treatment had an effect on the number of proteins produced by Molopo.

At 40 days cold treatment one less protein band was expressed than in the control. Group 12 expressed an additional band under cold treatment, while groups 3, 5 and 9 showed a reduction of one band each when treated with cold. Double bands were expressed in group 14 during cold treatment, and in groups 3 and 14 in the control. Groups 7 and 8 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

50 days - Molopo expressed 12 protein bands (Table 12). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by Molopo.

At 50 days cold treatment one more protein band was expressed than in the control. Groups 2, 4 and 10 expressed an additional band each under cold treatment, while groups 3 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in group 14 during the control. Groups 7, 8 and 11 expressed a band of a higher intensity under cold treatment and in the control.

60 days - Molopo expressed 11 protein bands (Table 12). The untreated sample expressed nine protein bands indicating that treatment had a significant effect on the number of proteins produced by Molopo.

At 60 days cold treatment two more protein bands were expressed than in the control. Groups 1, 4 and 6 expressed an additional band each under cold treatment, while group 14 showed a

reduction of one band when treated with cold. Double bands were expressed in group 1 during cold treatment, and in group 14 during the control. Groups 3, 5, 7, 8, 11 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to six in the treated material.

Nantes

10 days - Nantes expressed 16 protein bands (Table 13). The untreated sample expressed 17 protein bands indicating that treatment had an effect on the number of proteins produced by Nantes.

At 10 days cold treatment one less protein band was expressed than in the control. Groups 1 and 5 expressed an additional band each under cold treatment, while groups 2, 6 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1, 5, 9 and 13 during cold treatment, and in groups 6, 9, 11 and 13 during the control. Groups 5, 8, 12 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. There is no clear pattern with regard to the number of double bands between the control and treatment in the cultivar Nantes at 10 days.

20 days - Nantes expressed 15 protein bands (Table 13). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by Nantes.

Although there were no significant differences between the total number of proteins produced, groups 6 and 9 expressed an additional band each under cold treatment, while groups 4, 5 and 7 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6, 9, 11 and 13 during cold treatment, and in groups 5, 7, 11 and 13 in the control. Groups 5, 8 and 13 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to four in the control.

30 days - Nantes expressed 15 protein bands (Table 13). The untreated sample expressed 17 protein bands indicating that treatment had a significant effect on the number of proteins produced by Nantes.

At 30 days cold treatment two less protein bands were expressed than in the control. Groups 5 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 9 and 14 during cold treatment, and in groups 5, 9, 11 and 14 in the control.

Groups 5, 8 and 13 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to four in the control.

40 days - Nantes expressed 15 protein bands (Table 13). The untreated sample expressed 12 protein bands indicating that treatment had a significant effect on the number of proteins produced by Nantes.

At 40 days cold treatment three more protein bands were expressed than in the control. Groups 1, 4, 9, 10 and 12 expressed an additional band each under cold treatment, while groups 6 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 9 and 13 during cold treatment, and in groups 6, 11 and 13 in the control. Groups 5, 8 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

50 days - Nantes expressed 16 protein bands (Table 13). The untreated sample expressed 12 protein bands indicating that treatment had a significant effect on the number of proteins produced by Nantes.

At 50 days cold treatment four more protein bands were expressed than in the control. Groups 4, 6, 10 and 12 expressed an additional band each under cold treatment, while group 11 showed a reduction of one band when treated with cold. Double bands were expressed in groups 6, 12 and 13 during cold treatment, and in groups 11 and 13 in the control. Groups 5, 8 and 13 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to three in the treated material.

60 days - Nantes expressed 15 protein bands (Table 13). The untreated sample expressed 16 protein bands indicating that treatment had an effect on the number of proteins produced by Nantes.

At 60 days cold treatment one less protein band was expressed than in the control. Groups 5 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 12 and 13 during cold treatment, and in groups 5, 11 and 13 in the control. Groups 2, 7, 8 and 13 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population. The number of bands with a high density varied from four in the treated material to five in the control.

Palmiet

10 days - Palmiet expressed 13 protein bands (Table 14). The untreated sample expressed 14 protein bands indicating that treatment had an effect on the number of proteins produced by Palmiet.

At 10 days cold treatment one less protein band was expressed than in the control. Group 12 expressed an additional band under cold treatment, while groups 4 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in group 12 during cold treatment, and in group 4 in the control. Group 2 expressed a band of a higher intensity under treatment. The number of bands with a high density varied from none in the control to one in the treated material.

20 days - Palmiet expressed 13 protein bands (Table 14). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by Palmiet.

Although there were no significant differences between the total number of proteins produced, groups 1 and 4 expressed an additional band each under cold treatment, while groups 5 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1 and 4 during cold treatment, and in group 5 in the control. Groups 4, 5, 8 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to four in the treated material.

30 days - Palmiet expressed 13 protein bands (Table 14). The untreated sample expressed 14 protein bands indicating that treatment had an effect on the number of proteins produced by Palmiet.

At 30 days cold treatment one less protein band was expressed than in the control. Group 11 expressed an additional band under cold treatment, while groups 6 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 5, 10 and 11 during cold treatment, and in groups 5 and 10 in the control. Groups 4, 5, 8 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to five in the treated material.

40 days - Palmiet expressed 16 protein bands (Table 14). The untreated sample expressed 13 protein bands indicating that treatment had a significant effect on the number of proteins produced by Palmiet.

At 40 days cold treatment three more protein bands were expressed than in the control. Groups 1, 6 and 13 expressed an additional band each under cold treatment, while groups 5 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1, 6, 10, 11 and 13 during cold treatment, and in groups 5, 10 and 11 in the control. Groups 5, 8, 11 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to four in the treated material.

50 days - Palmiet expressed 15 protein bands (Table 14). The untreated sample had the same number bands indicating that treatment had no significant effect on the number of proteins produced by Palmiet.

Although there were no significant differences between the total number of proteins produced, groups 6 and 10 expressed an additional band each under cold treatment, while groups 9 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1, 6 and 10 during cold treatment, and in groups 1 and 9 in the control. Groups 5, 8, 13 and 14 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population. The number of bands with a high density varied from four in the treated material to five in the control.

60 days - Palmiet expressed 15 protein bands (Table 14). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by Palmiet.

Although there were no significant differences between the total numer of proteins produced, groups 1, 11 and 13 expressed an additional band each under cold treatment, while groups 2, 4, 10 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1, 5, 11 and 13 during cold treatment, and in groups 5 and 10 in the control. Groups 4 and 8 expressed a band of a higher intensity under treatment and in the control.

PAN 3211

10 days - PAN 3211 expressed 10 protein bands (Table 15). The untreated sample expressed 15 protein bands indicating that treatment had a significant effect on the number of proteins produced by PAN 3211.

At 10 days cold treatment five less protein bands were expressed than in the control. Groups 1, 3, 6, 8, 9, 11 and 13 expressed an additional band each under cold treatment, while groups 7 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3, 9, 11 and 13 during the control. Groups 5 and 9 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to two in the treated material.

20 days - PAN 3211 expressed 15 protein bands (Table 15). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by PAN 3211.

Although there were no significant differences between the total number of proteins produced, groups 9 and 13 expressed an additional band each under cold treatment, while groups 6 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 5, 9 and 13 during cold treatment, and in groups 5 and 12 in the control. Groups 5, 9, 13 and 14 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to four in the treated material.

30 days - PAN 3211 expressed 14 protein bands (Table 15). The untreated sample expressed 16 protein bands indicating that treatment had a significant effect on the number of proteins produced by PAN 3211.

At 30 days cold treatment two less protein bands were expressed than in the control. Groups 4 and 5 expressed an additional band each under cold treatment, while groups 2, 9, 11 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in group 5 during cold treatment, and in groups 9, 11 and 13 in the control. Groups 5, 6, 13 and 14 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to four in the treated material.

40 days - PAN 3211 expressed 12 protein bands (Table 15). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the number of proteins produced by PAN 3211.

At 40 days cold treatment one less protein band was expressed than in the control. Groups 2, 5, 11 and 13 expressed an additional band each under cold treatment, while groups 3, 8, 10 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 5, 11 and 13 during cold treatment, and in groups 3 and 13 in the control. Groups 5, 6 and 9 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to three in the treated material.

50 days - PAN 3211 expressed 12 protein bands (Table 15). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the number of proteins produced by PAN 3211.

At 50 days cold treatment one less protein band was expressed than in the control. Groups 3, 8 and 12 expressed an additional band each under cold treatment, while groups 1, 4, 5, 6 and 7 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3 and 12 during cold treatment, and in group 5 in the control. 5 and 9 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

60 days - PAN 3211 expressed 11 protein bands (Table 15). The untreated sample expressed 14 protein bands indicating that treatment had a significant effect on the number of proteins produced by PAN 3211.

At 60 days cold treatment three less protein bands were expressed than in the control. Groups 8, 11 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in group 9 during cold treatment, and in groups 9, 11 and 13 in the control. Groups 5 and 9 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

PAN 3349

10 days - PAN 3349 expressed 14 protein bands (Table 16). The untreated sample expressed 15 protein bands indicating that treatment had an effect on the number of proteins produced by PAN 3349.

At 10 days cold treatment one less protein band was expressed than in the control. Groups 2 and 4 expressed an additional band each under cold treatment, while groups 7, 11 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4 and 14 during cold treatment, and in groups 7, 11 and 14 in the control. Group 6 expressed a band of a higher intensity under treatment and in the control.

20 days - PAN 3349 expressed 15 protein bands (Table 16). The untreated sample expressed 13 protein bands indicating that treatment had a significant effect on the number of proteins produced by PAN 3349.

At 20 days cold treatment two more protein bands were expressed than in the control. Groups 1, 5 and 13 expressed an additional band each under cold treatment, while group 11 showed a reduction of one band when treated with cold. Double bands were expressed in groups 7 and 14 during cold treatment, and in groups 7, 11 and 14 in the control.

30 days - PAN 3349 expressed eight protein bands (Table 16). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the number of proteins produced by PAN 3349.

At 30 days cold treatment three less protein bands were expressed than in the control. Groups 4, 12 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4 and 14 during the control.

40 days - PAN 3349 expressed 12 protein bands (Table 16). The untreated sample expressed ten protein bands indicating that treatment had a significant effect on the number of proteins produced by PAN 3349.

At 40 days cold treatment two more protein bands were expressed than in the control. Groups 1, 10 and 13 expressed an additional band each under cold treatment, while group 8 showed a reduction of one band when treated with cold. Double bands were expressed in groups 10 and 14 during cold treatment, and in group 14 in the control.

50 days - PAN 3349 expressed 13 protein bands (Table 7). The untreated sample expressed five protein bands indicating that treatment had a significant effect on the number of proteins produced by PAN 3349.

At 50 days cold treatment eight more protein bands were expressed than in the control. Groups 1, 2, 8 and 13 expressed an additional band each, and groups 10 and 14 two additional bands each, under cold treatment. Double bands were expressed in groups 10 and 14 during cold treatment.

60 days - PAN 3349 expressed nine protein bands (Table 16). The untreated sample expressed seven protein bands indicating that treatment had an effect on the number of proteins produced by PAN 3349.

At 60 days cold treatment two more protein bands were expressed than in the control. Groups 1 and 13 expressed an additional band each under cold treatment.

PAN 3377

10 days - PAN 3377 expressed 10 protein bands (Table 17). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by PAN 3377.

At 10 days cold treatment one more protein band was expressed than in the control. Groups 7 and 11 expressed an additional band each under cold treatment, while groups 3, 6 and 9 showed a reduction of one band each when treated with cold. Double bands were expressed in group 11 during cold treatment, and in groups 3 and 9 in the control.

20 days - PAN 3377 expressed 10 protein bands (Table 17). The untreated sample expressed nine protein bands indicating that treatment had an effect on the number of proteins produced by PAN 3377.

At 20 days cold treatment one more protein band was expressed than in the control. Groups 7 and 9 expressed an additional band each under cold treatment, while group 6 showed a reduction of one band when treated with cold. Groups 5 expressed a band of a higher intensity under treatment and in the control.

30 days - PAN 3377 expressed eight protein bands (Table 17). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the number of proteins produced by PAN 3377.

At 30 days cold treatment three less protein bands were expressed than in the control. Group 7 expressed an additional band under cold treatment, while groups 2, 3, 6 and 9 showed a reduction of one band each when treated with cold. Double bands were expressed in group 12 during the control. Groups 5 and 8 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

40 days - PAN 3377 expressed 11 protein bands (Table 17). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by PAN 3377.

Although there were no significant differences between the total number of proteins produced, group 9 expressed an additional band under cold treatment, while group 3 showed a reduction of one band when treated with cold. Double bands were expressed in group 12 during cold treatment and the control. Groups 5, 8 and 10 expressed a band of a higher intensity under treatment. The number of bands with a high density varied from none in the control to three in the treated material.

50 days - PAN 3377 expressed nine protein bands (Table 17). The untreated sample expressed 12 protein bands indicating that treatment had a significant effect on the number of proteins produced by PAN 3377.

At 50 days cold treatment three less protein bands were expressed than in the control. Group 10 expressed an additional band under cold treatment, while groups 3 and 9 showed a reduction of one band each, and group 12 a reduction of two bands, when treated with cold. Double bands were expressed in groups 9 and 12 during the control. Groups 5, 6 and 8 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to three in the treated material.

60 days - PAN 3377 expressed nine protein bands (Table 17). The untreated sample expressed 12 protein bands indicating that treatment had a significant effect on the number of proteins produced by PAN 3377.

At 60 days cold treatment three less protein bands were expressed than in the control. Groups 3, 11 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in group 12 during the control. Groups 4, 5, 8, 10 and 12 expressed a band of a

higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to five in the treated material.

Scheepers 69

10 days - Scheepers 69 expressed 13 protein bands (Table 18). The untreated sample expressed ten protein bands indicating that treatment had a significant effect on the number of proteins produced by Scheepers 69.

At 10 days cold treatment three more less protein bands expressed than in the control. Groups 3, 4, 9 and 13 expressed an additional band each under cold treatment, while group 2 showed a reduction of one band when treated with cold. Double bands were expressed in groups 3 and 13 during cold treatment. Group 5 expressed a band of a higher intensity under treatment. The number of bands with a high density varied from none in the control to one in the treated material.

20 days - Scheepers 69 expressed 14 protein bands (Table 18). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the number of proteins produced by Scheepers 69.

At 20 days cold treatment one more protein band was expressed than in the control. Groups 4, 8 and 9 expressed an additional band each under cold treatment, while groups 6 and 7 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 8 and 13 during cold treatment, and in groups 7 and 13 in the control. Groups 5 and 7 expressed a band of a higher intensity under treatment. The number of bands with a high density varied from none in the control to two in the treated material.

30 days - Scheepers 69 expressed 14 protein bands (Table 18). The untreated sample expressed ten protein bands indicating that treatment had a significant effect on the number of proteins produced by Scheepers 69.

At 30 days cold treatment four more protein bands were expressed than in the control. Groups 5, 7, 8, 11 and 13 expressed an additional band each under cold treatment. Double bands were expressed in groups 8 and 11 during cold treatment.

40 days - Scheepers 69 expressed 13 protein bands (Table 18). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by Scheepers 69.

At 40 days cold treatment one more protein band was expressed than in the control. Groups 3, 9 and 13 expressed an additional band each under cold treatment, while groups 2 and 8 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3, 9 and 13 during cold treatment, and in group 8 in the control. Groups 5 and 6 expressed a band of a higher intensity under. The number of bands with a high density varied from none in the control to two in the treated material.

50 days - Scheepers 69 expressed 12 protein bands (Table 18). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the number of proteins produced by Scheepers 69.

At 50 days cold treatment one less protein band was expressed than in the control. Groups 4 and 7 expressed an additional band each under cold treatment, while groups 3, 6 and 8 showed a reduction of one band each when treated with cold. Double bands were expressed in group 7 during cold treatment, and in group 8 in the control. Group 4 expressed a band of a higher intensity under treatment. The number of bands with a high density varied from none in the control to one in the treated material.

60 days - Scheepers 69 expressed 12 protein bands (Table 18). The untreated sample expressed 15 protein bands indicating that treatment had a significant effect on the number of proteins produced by Scheepers 69.

At 60 days cold treatment three less protein bands were expressed than in the control. Groups 2, 4 and 10 expressed an additional band each under cold treatment, while groups 3, 5, 7, 9 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in group 10 during cold treatment, and in groups 3, 5, 9 and 11 in the control. Group 6 expressed a band of a higher intensity under treatment. The number of bands with a high density varied from none in the control to one in the treated material.

Snack

10 days - Snack expressed 12 protein bands (Table 19). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the number of proteins produced by Snack.

At 10 days cold treatment one less protein band was expressed than in the control. Groups 2, 3, 5 and 11 expressed an additional band each under cold treatment, while groups 1, 6, 7, 10 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3 and 5 during cold treatment, and in groups 7 and 12 in the control. Groups 3, 5, 7, 13

and 14 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to five in the treated material.

20 days - Snack expressed 13 protein bands (Table 19). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by Snack.

At 20 days cold treatment one more protein band was expressed than in the control. Groups 2, 6, 12 and 13 expressed an additional band each under cold treatment, while groups 1, 7 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in group 13 during cold treatment, and in groups 5, 7 and 11 in the control. Groups 3, 5, 13 and 14 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to four in the treated material.

30 days - Snack expressed 14 protein bands (Table 19). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the number of proteins produced by Snack.

At 30 days cold treatment three more protein bands were expressed than in the control. Groups 2, 8, 11 and 13 expressed an additional band each under cold treatment, while group 12 showed a reduction of one band when treated with cold. Double bands were expressed in groups 7, 8 and 13 during cold treatment, and in group 7 in the control. Groups 3, 5, 7, 13 and 14 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to five in the treated material.

40 days - Snack expressed 12 protein bands (Table 19). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by Snack.

At 40 days cold treatment one more protein band was expressed than in the control. Groups 2, 6 and 12 expressed an additional band each under cold treatment, while groups 5 and 7 showed a reduction of one band each when treated with cold. Double bands were expressed in group 8 during cold treatment, and in groups 5 and 8 in the control. Groups 3, 5, 7, 13 and 14 expressed a band of a higher intensity under treatment while three bands showed high intensity in the

control population. The number of bands with a high density varied from three in the control to five in the treated material.

50 days - Snack expressed 14 protein bands (Table 19). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the number of proteins produced by Snack.

At 50 days cold treatment one more protein band was expressed than in the control. Groups 1, 3, 7, 8 and 10 expressed an additional band each under cold treatment, while groups 2, 5, 12 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3 and 7 during cold treatment, and in groups 5, 12 and 13 in the control. Groups 3, 5, 7 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to four in the treated material.

60 days - Snack expressed 12 protein bands (Table 19). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by Snack.

At 60 days cold treatment one more protein band was expressed than in the control. Groups 2, 7, 8, 9 and 13 expressed an additional band each under cold treatment, while groups 1, 4 and 10 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 7 and 8 during cold treatment. Groups 3, 5, 8, 13 and 14 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to five in the treated material.

SST 363

10 days - SST 363 expressed 13 protein bands (Table 20). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by SST 363.

At 10 days cold treatment one more protein band was expressed than in the control. Groups 7 and 12 expressed an additional band each under cold treatment, while group 4 showed a reduction of one band when treated with cold. Double bands were expressed in groups 3, 6 and 12 during cold treatment, and in groups 3 and 6 in the control. Groups 9 and 12 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population.

20 days - SST 363 expressed nine protein bands (Table 20). The untreated sample expressed 13 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 363.

At 20 days cold treatment four less protein bands were expressed than in the control. Groups 1, 2, 4, 6 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1 and 6 during the control. Groups 8 and 11 expressed a band of a higher intensity in the control population. The number of bands with a high density varied from none in the treated material to two in the control.

30 days - SST 363 expressed 11 protein bands (Table 20). The untreated sample expressed 14 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 363.

At 30 days cold treatment three less protein bands were expressed than in the control. Group 10 expressed an additional band under cold treatment, while groups 2, 4, 7 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 2, 7 and 12 during the control. Group 11 expressed a band of a higher intensity in the control population. The number of bands with a high density varied from none in the treated material to one in the control.

40 days - SST 363 expressed 13 protein bands (Table 20). The untreated sample expressed 10 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 363.

At 40 days cold treatment three more protein bands were expressed than in the control. Groups 2, 3 and 6 expressed an additional band each under cold treatment. Double bands were expressed in groups 3 and 12 during cold treatment, and in group 12 in the control. Group 8 expressed a band of a higher intensity under. The number of bands with a high density varied from nothing in the control to one in the treated material.

50 days - SST 363 expressed eight protein bands (Table 20). The untreated sample expressed nine protein bands indicating that treatment had an effect on the number of proteins produced by SST 363.

At 50 days cold treatment one less protein band was expressed than in the control. Group 10 expressed an additional band under cold treatment, while groups 1 and 5 showed a reduction of one band each when treated with cold. Group 8 expressed a band of a higher intensity under

treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to two in the control.

60 days - SST 363 expressed eight protein bands (Table 20). The untreated sample expressed 12 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 363.

At 60 days cold treatment four less protein bands were expressed than in the control. Groups 1, 2, 5 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 12 during the control. Group 8 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to two in the control.

SST 367

10 days - SST 367 expressed 11 protein bands (Table 21). The untreated sample expressed 16 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 367.

At 10 days cold treatment five less protein bands were expressed than in the control. Groups 1, 4, 6 and 9 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4, 6 and 9 during the control. Group 12 expressed a band of a higher intensity under treatment. The number of bands with a high density varied from none in the control to one in the treated material.

20 days - SST 367 expressed 12 protein bands (Table 21). The untreated sample expressed 14 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 367.

At 20 days cold treatment two less protein bands were expressed than in the control. Groups 3 and 8 expressed an additional band each under cold treatment, while groups 2, 6, 9 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3 and 8 during cold treatment, and in groups 6 and 9 in the control.

30 days - SST 367 expressed 12 protein bands (Table 21). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by SST 367.

At 30 days cold treatment one more protein band was expressed than in the control. Group 6 expressed an additional band, and group 10 two additional bands, under cold treatment, while groups 8 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6 and 10 during cold treatment.

40 days - SST 367 expressed 12 protein bands (Table 21). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by SST 367.

At 40 days cold treatment six more protein bands were expressed than in the control. Groups 5, 9 and 11 expressed an additional band each under cold treatment, while groups 7 and 10 showed a reduction of one band each when treated with cold. Double bands were expressed in group 11 during cold treatment, and in group 7 in the control.

50 days - SST 367 expressed nine protein bands (Table 21). The untreated sample expressed 13 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 367.

At 50 days cold treatment four less protein bands were expressed than in the control. Groups 9 and 11 expressed an additional band each under cold treatment, while groups 4, 5, 6, 7 and 10 showed a reduction of one band each when treated with cold. Double bands were expressed in group 11 during cold treatment, and in group 7 and 10 in the control.

60 days - SST 367 expressed 11 protein bands (Table 21). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by SST 367.

At 60 days cold treatment six more protein bands were expressed than in the control. Group 5 expressed an additional band under cold treatment, while group 2 showed a reduction of one band when treated with cold. Double bands were expressed in group 8 during cold treatment and in the control.

SST 57

10 days - SST 57 expressed 14 protein bands (Table 22). The untreated sample expressed 13 protein bands indicating that treatment had an on the total number of proteins produced by SST 57.

At 10 days cold treatment one more protein band was expressed than in the control. Groups 7, 11 and 13 expressed an additional band each under cold treatment, while groups 4 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 7, 10 and 13 during cold treatment, and in groups 10 and 12 in the control. Groups 3, 5 and 13 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to four in the treated material.

20 days - SST 57 expressed 12 protein bands (Table 22). The untreated sample expressed 14 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 57.

At 20 days cold treatment two less protein bands were expressed than in the control. Group 6 expressed an additional band under cold treatment, while groups 2, 4 and 7 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6, 10 and 13 during cold treatment, and in groups 10 and 13 in the control. Groups 3, 6 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

30 days - SST 57 expressed 11 protein bands (Table 22). The untreated sample had the same number of bands indicating that treatment had no significant effect on the number of proteins produced by SST 57.

Although there were no significant differences between the total number of proteins produced, groups 6 and 10 expressed an additional band each under cold treatment, while groups 7 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6 and 13 during cold treatment and in the control. Groups 3, 6 and 13 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to four in the treated material.

40 days - SST 57 expressed nine protein bands (Table 22). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by SST 57.

At 40 days cold treatment two less protein bands were expressed than in the control. Groups 4 and 6 expressed an additional band each under cold treatment, while groups 1, 5 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in group 13

during cold treatment and in the control. Groups 3, 6, 7 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to five in the treated material.

50 days - SST 57 expressed ten protein bands (Table 22). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by SST 57.

At 50 days cold treatment one less protein band was expressed than in the control. Group 12 showed a reduction of one band when treated with cold. Double bands were expressed in group 13 during cold treatment and in the control. Groups 3, 5, 6 and 13 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to five in the treated material.

60 days - SST 57 expressed ten protein bands (Table 22). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 57.

At 60 days cold treatment one less protein band was expressed than in the control. Group 8 expressed an additional band under cold treatment, while groups 2 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 8 and 13 during cold treatment, and in group 13 in the control. Groups 7, 8 and 13 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population.

SST 66

10 days - SST 66 expressed 12 protein bands (Table 23). The untreated sample expressed 14 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 66.

At 10 days cold treatment two less protein bands were expressed than in the control. Groups 3, 7 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 9 and 13 during cold treatment, and in groups 3, 9 and 13 in the control. Group 5 expressed a band of a higher intensity under treatment. The number of bands with a high density varied from none in the control to one in the treated material.

20 days - SST 66 expressed 11 protein bands (Table 23). The untreated sample had exactly the sam e number bands indicating that treatment had no significant effect on the number of proteins produced by SST 66.

Although there were no significant differences between the total number of proteins produced, groups 6 and 11 expressed an additional band each under cold treatment, while groups 8 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in group 6 during cold treatment, and in group 12 in the control.

30 days - SST 66 expressed 11 protein bands (Table 23). The untreated sample expressed 13 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 66.

At 30 days cold treatment two less protein bands were expressed than in the control. Group 9 expressed an additional band under cold treatment, while groups 4, 6 and 7 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 9 and 14 during cold treatment, and in groups 7 and 14 in the control. Group 12 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to three in the control.

40 days - SST 66 expressed ten protein bands (Table 23). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by SST 66.

At 40 days cold treatment one less protein band was expressed than in the control. Groups 2, 9, 11 and 12 expressed an additional band each under cold treatment, while groups 4, 5, 8, 10 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in group 9 during cold treatment, and in group 13 in the control.

50 days - SST 66 expressed 12 protein bands (Table 23). The untreated sample had the same number bands indicating that treatment had no significant effect on the number of proteins produced by SST 66.

Although there were no significant differences between the total number of proteins produced, groups 4 and 11 expressed an additional band each under cold treatment, while groups 2 and 7 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 2 and 7 during cold treatment. Group 12 expressed a band of a higher intensity in the control population. The number of bands with a high density varied from none in the treated material to one in the control.

60 days - SST 66 expressed 15 protein bands (Table 23). The untreated sample expressed 12 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 66.

At 60 days cold treatment three more protein bands were expressed than in the control. Groups 3, 6, 10 and 12 expressed an additional band each under cold treatment, while group 11 showed a reduction of one band when treated with cold. Double bands were expressed in groups 6 and 10 during cold treatment, and in group 11 in the control.

SST 822

10 days - SST 822 expressed 13 protein bands (Table 24). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the number of proteins produced by SST 822.

Although there were no significant differences between the total number of proteins produced, groups 4, 7 and 9 expressed an additional band each under cold treatment, while groups 3, 8 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 5, 7 and 9 during cold treatment, and in groups 3, 5 and 8 in the control. Group 3 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to two in the control.

20 days - SST 822 expressed 12 protein bands (Table 24). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the number of proteins produced by SST 822.

At 20 days cold treatment one less protein band was expressed than in the control. Group 7 expressed an additional band under cold treatment, while groups 5 and 9 showed a reduction of one band each when treated with cold. Double bands were expressed in group 12 during cold treatment, and in groups 5, 9 and 12 in the control. Group 5 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to two in the control.

30 days - SST 822 expressed 13 protein bands (Table 24). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by SST 822.

At 30 days cold treatment one more protein band was expressed than in the control. Groups 2, 3, 5, 9 and 14 expressed an additional band each under cold treatment, while groups 4, 6 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3, 9 and 12 during cold treatment, and in groups 6 and 12 in the control. Groups 3 and 8 expressed a band of a higher intensity in the control population. The number of bands with a high density varied from no bands in the treated material to two in the control.

40 days - SST 822 expressed ten protein bands (Table 24). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the number of proteins produced by SST 822.

Although there were no significant differences between the total number of proteins produced, groups 1 and 7 expressed an additional band each under cold treatment, while groups 11 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in group 7 during cold treatment, and in group 12 in the control. Group 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to three in the control.

50 days - SST 822 expressed 12 protein bands (Table 24). The untreated sample expressed 14 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 822.

At 50 days cold treatment two less protein bands were expressed than in the control. Groups 2, 3 and 13 expressed an additional band each, and group 9 two additional bands, under cold treatment, while groups 5, 6, 7, 8, 10 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6, 7, 8 and 12 during cold treatment, and in groups 6, 9, 12 and 13 in the control. Group 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to three in the control.

60 days - SST 822 expressed 14 protein bands (Table 24). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the number of proteins produced by SST 822.

At 60 days cold treatment two more protein bands were expressed than in the control. Groups 6, 11 and 12 expressed an additional band each, and group 9 two additional bands, under cold treatment, while groups 2, 8 and 13 showed a reduction of one band each when treated with cold.

Double bands were expressed in groups 6, 9, 11 and 12 during cold treatment, and in groups 8 and 13 in the control. Group 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to three in the control.

SST 825

10 days - SST 825 expressed 11 protein bands (Table 25). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the number of proteins produced by SST 825.

Although there were no significant differences between the total number of proteins produced, groups 4 and 7 expressed an additional band each under cold treatment, while groups 1 and 3 showed a reduction of one band each when treated with cold. Double bands were expressed in group 4 during cold treatment. Groups 2, 7, 8, 9 and 10 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to five in the treated material.

20 days - SST 825 expressed 12 protein bands (Table 25). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by SST 825.

At 20 days cold treatment one more protein band was expressed than in the control. Group 8 expressed an additional band under cold treatment. Groups 5, 7, 8, 9 and 10 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to five in the treated material.

30 days - SST 825 expressed 14 protein bands (Table 25). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 825.

At 30 days cold treatment three more protein bands were expressed than in the control. Groups 8, 13 and 14 expressed an additional band each under cold treatment. Double bands were expressed in group 14 during cold treatment. Groups 2, 3, 5, 6, 7, 8, 9 and 10 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to eight in the treated material.

40 days - SST 825 expressed 15 protein bands (Table 25). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 825.

At 40 days cold treatment four more protein bands were expressed than in the control. Groups 3, 8, 13 and 14 expressed an additional band each under cold treatment. Double bands were expressed in groups 3 and 14 during cold treatment. Groups 2, 3, 5, 6, 7, 8, 9, 10 and 14 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to nine in the treated material.

50 days - SST 825 expressed 12 protein bands (Table 25). The untreated sample expressed nine protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 825.

At 50 days cold treatment three more protein bands were expressed than in the control. Groups 3, 8 and 14 expressed an additional band each under cold treatment, while groups 2 and 4 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3 and 8 during cold treatment. Groups 3, 5, 6, 7, 8, 9, 10 and 14 expressed a band of a higher intensity under treatment while hree bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to nine in the treated material.

60 days - SST 825 expressed 13 protein bands (Table 25). The untreated sample expressed seven protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 825.

At 60 days cold treatment six more protein bands were expressed than in the control. Groups 3, 7, 8, 11 and 14 expressed an additional band each under cold treatment, while group 2 showed a reduction of one band when treated with cold. Double bands were expressed in groups 3, 8 and 14 during cold treatment. Groups 3, 5, 6, 7, 8, 9, 10 and 14 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to ten in the treated material.

SST 966

10 days - SST 966 expressed 11 protein bands (Table 26). The untreated sample expressed nine protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 966.

At 10 days cold treatment two more protein bands were expressed than in the control. Groups 1, 2, 4, 10 and 12 expressed an additional band each under cold treatment, while groups 5, 8 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 10 and 12 during cold treatment. Groups 3, 4, 7 and 13 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to four in the treated material.

20 days - SST 966 expressed 12 protein bands (Table 26). The untreated sample expressed eight protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 966.

At 20 days cold treatment four more protein bands were expressed than in the control. Groups1, 4, 11 and 12 expressed an additional band each under cold treatment, while group 5 showed a reduction of one band when treated with cold. Double bands were expressed in groups 4 and 12 during cold treatment. Groups 3, 4, 7, 9 and 13 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to five in the treated material.

30 days - SST 966 expressed nine protein bands (Table 26). The untreated sample expressed ten protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 966.

At 30 days cold treatment one less protein band was expressed than in the control. Groups 1, 4 and 14 expressed an additional band each under cold treatment, while groups 2, 3, 5 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in group 13 during the control. Groups 4 and 7 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

40 days - SST 966 expressed 15 protein bands (Table 7). The untreated sample expressed 13 protein bands indicating that treatment had a significant effect on the number of proteins produced by SST 966.

At 40 days cold treatment two more protein bands were expressed than in the control. Groups 1, 2, 8 and 12 expressed an additional band each under cold treatment, while groups 3 and 7 showed a reduction of one band each when treated with cold. Double bands were expressed in group 8 during cold treatment, and in group 7 in the control. Groups 4, 5, 7, 10 and 14 expressed

a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to five in the treated material.

50 days - SST 966 expressed ten protein bands (Table 7). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the number of proteins produced by SST 966.

Although there were no significant differences between the total number of proteins produced, groups 9 and 14 expressed an additional band each under cold treatment, while groups 1 and 2 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 9 during cold treatment. Groups 4, 5, 7, 9 and 14 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population. The number of bands with a high density varied from five in the control to six in the treated material.

60 days – SST 966 expressed ten protein bands (Table 26). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the number of proteins produced by SST 966.

Although there were no significant differences between the total number of proteins produced, groups 9 and 10 expressed an additional band each under cold treatment, while groups 2 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in group 9 during cold treatment. Groups 4, 5, 7, 9 and 10 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to five in the treated material.

Tugela DN

10 days - Tugela DN expressed 11 protein bands (Table 27). The untreated sample expressed ten protein bands indicating that treatment had an effect on the number of proteins produced by Tugela DN.

At 10 days cold treatment one more protein band was expressed than in the control. Groups 6 and 8 expressed an additional band each under cold treatment, while group 11 showed a reduction of one band when treated with cold. Double bands were expressed in group 6 during cold treatment, and in group 11 in the control. Groups 4, 7, 10 and 11 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to four in the treated material.

20 days - Tugela DN expressed protein bands (Table 27). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by Tugela DN.

At 20 days cold treatment one less protein band was expressed than in the control. Group 5 expressed an additional band under cold treatment, while groups 1 and 9 showed a reduction of one band each when treated with cold. Groups 2, 4, 7, 10 and 11 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to five in the treated material.

30 days - Tugela DN expressed 12 protein bands (Table 27). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by Tugela DN.

At 30 days cold treatment one more protein band was expressed than in the control. Groups 2 and 9 expressed an additional band each under cold treatment, while group 12 showed a reduction of one band when treated with cold. Double bands were expressed in group 12 in the control. Groups 4, 7 and 12 expressed a band of a higher intensity under treatment and in the control.

40 days - Tugela DN expressed ten protein bands (Table 27). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the number of proteins produced by Tugela DN.

At 40 days cold treatment one less protein band was expressed than in the control.. Group 1 expressed an additional band under cold treatment, while groups 6 and 9 showed a reduction of one band each when treated with cold. Double bands were expressed in group 7 during cold treatment and in the control. Groups 4, 7 and 12 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to three in the treated material.

50 days - Tugela DN expressed 12 protein bands (Table 27). The untreated sample expressed ten protein bands indicating that treatment had an effect on the number of proteins produced by Tugela DN.

At 50 days cold treatment two more protein bands were expressed than in the control. Groups 1, 4 and 8 expressed an additional band each under cold treatment, while group 11 showed a reduction of one band when treated with cold. Double bands were expressed in group 10 during

cold treatment and in the control. Groups 2 and 7 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to five in control.

60 days - Tugela DN expressed 13 protein bands (Table 27). The untreated sample expressed nine protein bands indicating that treatment had a significant effect on the number of proteins produced by Tugela DN.

At 60 days cold treatment four more protein bands were expressed than in the control. Groups 2, 4, 10 and 12 expressed an additional band each under cold treatment. Double bands were expressed in groups 2, 7 and 11 during cold treatment, and in groups 7 and 11 in the control. Group 4 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to three in the control.

Each cultivar expressed a certain number of bands over the periods of cold treatment and controls. Some of these cultivars lost bands with cold treatment, while others gained bands The cultivar with the least number of bands expressed was PAN 3349 with only five bands in the 50 days control and seven bands in the 60 days control. SST 825 expressed seven bands in the 60 days control. Molen expressed 18 bands in the 10 days control, while Betta DN expressed 17 bands in the 10 days cold treatment. Caledon also expressed 17 bands in the 20 days control, and Nantes 17 bands in the 10 and 30 days control.

Number of bands expressed

The four most cold tolerant wheat cultivars and the four most cold sensitive or susceptible wheat cultivars according to studies done by Jacobs (1999) were selected. The four most cold tolerant cultivars were Caledon, Molen, SST 966 and Tugela DN and the four most cold susceptible cultivars were Adam Tas, Palmiet, Snack and SST 66. The number of bands expressed in each group in these respective cultivars are showed in the following tabels.

Tabel 4.3 The number of bands expressed in Adam Tas (susceptible to cold)

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	3	7	3	6	4	6	6	6	5	7	4	2	9
Control	3	4	3	5	4	5	7	8	10	6	7	5	6	12

Tabel 4.4 The number of bands expressed in Caledon (resistant to cold)

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	4	4	8	6	5	7	7	8	9	6	5	3	6
Control	1	4	10	6	6	6	6	9	6	6	6	6	0	9

Tabel 4.5 The number of bands expressed in Molen (resistant to cold)

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	4	3	8	4	3	5	7	8	8	6	5	5	10
Control	5	4	7	6	7	6	8	8	10	7	10	3	5	8

Tabel 4.6 The number of bands expressed in Palmiet (susceptible to cold)

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	10	1	6	6	7	7	3	6	6	9	7	5	8	4
Control	5	2	6	7	9	5	3	6	6	9	7	6	6	6

Tabel 4.7 The number of bands expressed in Snack (susceptible to cold)

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	1	5	8	1	7	4	9	9	5	4	6	5	8	5
Control	3	1	6	2	9	4	10	6	3	5	5	6	6	5

Tabel 4.8 The number of bands expressed in SST 66 (susceptible to cold)

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	1	5	6	2	4	7	7	4	9	6	5	5	7	5
Control	1	4	6	3	5	6	6	6	7	6	3	5	8	5

Tabel 4.9 The number of bands expressed in SST 966 (resistant to cold)

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	3	2	7	3	1	6	6	8	7	2	8	6	4
Control	1	4	4	3	6	1	7	6	6	5	3	5	7	2

Tabel 4.10 The number of bands expressed inTugela DN (resistant to cold

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	5	5	6	6	6	8	5	4	7	6	6	0	0
Control	2	4	5	4	5	6	8	3	5	6	8	6	0	0

When the number of bands gained and the number of bands lost in all the groups for the different cultivars were calculated, the following was observed:

- Adam Tas gained four bands, but 20 bands were lost across the 14 groups.
- Palmiet gained nine bands and lost eight bands across the 14 groups.
- Snack gained 12 bands, but lost 10 bands across 14 groups.
- SST 66 gained six bands, but lost eight bands across the 14 groups.

The more tolerant cultivars reacted as follows:

- Caledon gained 15 bands, but lost eight bands across 14 groups.
- Molen gained six bands, but lost 20 bands across the groups.
- SST 966 gained 16 bands, but lost nine bands across the groups.
- Tugela DN gained nine bands, but lost three bands across the 14 groups.

The tolerant cultivars (except Molen) gained more bands across the 14 groups. Two of the susceptible cultivars (Adam Tas and SST 66) lost more bands across the groups, while Palmiet and Snack gained more bands than they lost.

The differences between the number of bands expressed with the cold treatment and in the control were calculated for each group. The following groups showed clear differences between the cold tolerant and sensitive or susceptible cultivars.

Group 3

The cold sensitive cultivars gained bands with cold treatment, while the cold tolerant cultivars lost bands with cold treatment.

Group 4

The cold sensitive cultivars lost bands with cold treatment, while the cold tolerant cultivars gained bands with cold treatment. The only exception was Caledon (tolerant) that lost two bands

Group 10

The cold sensitive cultivars had no change in the number of bands or lost bands with cold treatment, while the cold tolerant cultivars gained bands with cold treatment.

Group 11

The cold sensitive cultivars had no change or gained bands with cold treatment, while the cold tolerant cultivars had no change or they lost bands with cold treatment.

Group 12

The cold sensitive cultivars had no change or they lost bands with cold treatment, while the cold tolerant cultivars had no change or they gained bands with cold treatment. Caledon was the only cold tolerant cultivar that lost a band with cold treatment.

Group 14

The cold sensitive cultivars had no change or they lost bands with cold treatment, while the cold tolerant cultivars gained bands with cold treatment. Caledon was again the only cold tolerant cultivar that lost two bands with cold treatment.

The other cultivars (with known cold tolerance) that were used in this study, were also evaluated to see how they reacted in groups 2, 3, 10, 11, 12 and 14. The cold susceptible cultivars according to Jacobs (1999) were PAN 3211, Scheepers 69, SST 363 and SST 825. The cold tolerant cultivars were Betta, Gariep, Limpopo, PAN 3349 and SST 822.

Group 3

SST 363 and SST 825 (cold susceptible) gained bands, while PAN 3211 and Scheepers 69 lost bands. If one looks at the results of Jacobs (1999) it is seen that PAN 3211 and Scheepers 69 fall just outside the more tolerant group of cultivars. PAN 3349 showed no change in the number of bands. Betta lost four bands. Gariep, Limpopo and SST 822 gained bands. According to the results of Jacobs (1999), Limpopo and SST 822 were just outside the group of more susceptible cultivars.

Group 4

Only SST 363 lost three bands in Group 4. PAN 3211 and SST 825 had no change in the number of bands Scheepers 69 gained bands. Of the cold tolerant cultivars, Limpopo gained one band and PAN 3349 and SST 822 had no change in the number of bands. Betta and Gariep lost bands. Betta falls just outside the group of very cold tolerant cultivars, and it could be that this cultivar reacted like Caledon.

Group 10

The cold sensitive cultivars had no change in the number of bands or they lost bands. Only Scheepers 69 gained one band. Betta and PAN 3349 gained bands, Gariep had no change in the number of bands and Limpopo and SST 822 lost bands

Group 11

SST 825 gained one band and Scheepers 69 and SST 363 had no change in the number of bands. PAN 3211 lost two bands. Betta had no change in the number of bands and PAN 3349 and SST 822 lost bands Gariep and Limpopo gained bands

Group 12

The cold susceptible cultivars had no change in the number of bands or they lost bands. Only PAN 3211 gained one band. Betta had no change in the number of bands in this group. Limpopo, PAN 3349 and SST 822 lost bands, while Gariep gained two bands

Group 14

Only SST 825 gained four bands, the other susceptible cultivars had no change in the number of bands. PAN 3349 and SST 822 gained bands, while the other tolerant cultivars had no change in the number of bands in this group.

The expression of the different bands

The four cold susceptible and four cold tolerant cultivars were compared with each other to see what groups were the most important for each cold treatment. The number of bands, the number of dark bands and the number of double bands were observed to see if there were any differences between the susceptible and the tolerant cultivars.

Tabel 4. 12 The bands expressed with 10 days cold treatment in four cold sensitive and four cold tolerant wheat cultivars

	Adam Tas	Palmiet	Snack	SST 66	Caledon	Molen	SST 966	Tugela DN
Group 1								
Group 2								
Group 3								
Group 4								
Group 5								
Group 6								
Group 7								
Group 8								
Group 9								
Group 10								
Group 11								
Group 12								
Group 13								
Group 14								

There were no clear difference between the average number of bands expressed for the cold susceptible and the cold tolerant cultivars. The average number of dark bands expressed were higher in the cold tolerant than the cold sensitive cultivars. The ratio of dark bands in the tolerant cultivars to the susceptible cultivars was three to one. The ratio of double bands (where one of the bands was dark) was also three to one for the cold tolerant to sold susceptible cultivars.

As dark bands are easy to select for, it was decided to see in which groups, more than two dark bands were observed for the susceptible and the tolerant cultivars. Group 5 and group 14 had two or more dark bands in the cold susceptible cultivars. Group 4, group 7, group 8, group 10 and group 11 had two or more bands in the cold tolerant cultivars after 10 days cold treatment (Tabel 4.12).

Tabel 4. 13 The bands expressed with 20 days cold treatment in four cold sensitive and four cold tolerant wheat cultivars.

	Adam Tas	Palmiet	Snack	SST 66	Caledon	Molen	SST 966	Tugela DN
Group 1								
Group 2								
Group 3								
Group 4								
Group 5								
Group 6								
Group 7								
Group 8								
Group 9								
Group 10								
Group 11								
Group 12								
Group 13								
Group 14								

There were no clear difference between the average number of bands expressed for the cold susceptible and the cold tolerant cultivars. The average number of dark bands were higher in the cold tolerant than the cold sensitive cultivars. The ratio of dark bands in the cold tolerant cultivars to the susceptible cultivars was two to one. The ratio of double bands of normal intensity and double bands where one band was dark was also two to one for the tolerant and susceptible cultivars. The average number of single bands expressed was higher in the cold sensitive than the cold tolerant cultivars.

Group 5, group 8 and group 13 had two or more dark bands in the cold susceptible cultivars. Group 3, group 4, group 7, group 8, group 10 and group 11 had two or more dark bands in the cold tolerant cultivars after 10 days of cold treatment (Table 4.13).

Tabel 4. 14 The bands expressed with 30 days cold treatment in four cold sensitive and four cold tolerant wheat cultivars

	Adam Tas	Palmiet	Snack	SST 66	Caledon	Molen	SST 966	Tugela DN
Group 1		—			—	—	—	—
Group 2	—		—			—		—
Group 3	—	—	—	—	—	—		—
Group 4		—			—	—	—	—
Group 5		=	—	—	—	—		—
Group 6	—		—		—			—
Group 7	—		—	—	—	—	—	—
Group 8	—	—	=	—	=	—	—	—
Group 9	—	—	—	=	—	=	—	—
Group 10	—	=	—	—	—	—	—	—
Group 11	—	=	—	—	—	—	—	—
Group 12								
Group 13		—	=	—	—	—	—	
Group 14	=	—	—	=	—	=	—	

There were no clear differences between the average number of bands expressed for the cold susceptible and the cold tolerant cultivars. The same number of dark bands were expressed in the cold tolerant and susceptible cultivars. The average number of single bands were higher in the cold tolerant than the cold sensitive cultivars.

Group 5, group 8, group 13 and group 14 had two or more dark bands in the cold susceptible cultivars. Group 4, group 5, group 7 and group 10 had two or more bands in the cold tolerant cultivars after 30 days cold treatment (Table 4.14).

Tabel 4. 15 The bands expressed with 40 days cold treatment in four cold sensitive and four cold resistant wheat cultivars

	Adam Tas	Palmiet	Snack	SST 66	Caledon	Molen	SST 966	Tugela DN
Group 1								
Group 2								
Group 3								
Group 4								
Group 5								
Group 6								
Group 7								
Group 8								
Group 9								
Group 10								
Group 11								
Group 12								
Group 13								
Group 14								

There were no clear differences between the average number of bands expressed for the cold susceptible and the cold tolerant cultivars. There were also no differences between the number of dark bands, double bands or single bands

Group 5, group 8, group 11, group 13 and group 14 had two or more dark bands in the cold susceptible cultivars. Group 4, group 7, group 8, group 10 and group 14 had two or more bands in the cold tolerant cultivars after 40 days cold treatment (Table 4.15).

Tabel 4. 16 The bands expressed with 50 days cold treatment in four cold sensitive and four cold resistant wheat cultivars

	Adam Tas	Palmiet	Snack	SST 66	Caledon	Molen	SST 966	Tugela DN
Group 1								
Group 2								
Group 3								
Group 4								
Group 5								
Group 6								
Group 7								
Group 8								
Group 9								
Group 10								
Group 11								
Group 12								
Group 13								
Group 14								

There were no clear differences between the average number of bands expressed for the cold susceptible and the cold tolerant cultivars. There were no differences between the number of dark or double bands. The average number of single bands were higher in the cold sensitive than the cold tolerant cultivars.

Group 5, group 13 and group 14 had two or more dark bands in the cold susceptible cultivars. Group 4, group 5, group 7, group 9 and group 14 had two or more bands in the cold tolerant cultivars after 50 days cold treatment (Table 4.16).

Tabel 4. 17 The bands expressed with 60 days cold treatment in four cold sensitive and four cold resistant wheat cultivars

	Adam Tas	Palmiet	Snack	SST 66	Caledon	Molen	SST 966	Tugela DN
Group 1								
Group 2								
Group 3								
Group 4								
Group 5								
Group 6								
Group 7								
Group 8								
Group 9								
Group 10								
Group 11								
Group 12								
Group 13								
Group 14								

There were no clear differences between the average number of bands expressed for the cold susceptible and the cold tolerant cultivars. The average number of dark double bands was higher in the cold tolerant than the cold sensitive cultivars. The ratio of the dark bands in the tolerant and susceptible cultivars was three to one. The ratio of double bands (where one of the bands were dark) of the tolerant to susceptible cultivars were three to one. The average number of dark bands was also higher in the cold tolerant than the cold sensitive cultivars.

Group 5 and group 8 had two or more dark bands in the cold susceptible cultivars. Group 4, group 7 and group 10 had two or more bands in the cold tolerant cultivars after 30 days cold treatment.

If all the groups are compared over all the cold treatments, four groups stand out for the presence of dark bands in the susceptibles cultivars. These groups are 6, 9, 10 and 13. In the tolerant cultivars, groups 5, 7,8 and 9 were the most important.

If one selects, however, for cold susceptibility, or cold tolerance by using these groups, it is important to note that:

- Group 6 is also expressed in the tolerant cultivars at 10 and 60 days cold treatment. It is expressed in all the susceptible cultivars after 20 days cold treatment.
- Group 9 is related to a cold gene and accumulated after cold treatment in the susceptible and tolerant cultivars.
- Group 10 is also correlated with cold treatment as it accumulates in most of the susceptibles after cold treatment and in the tolerant cultivars after 30, 40 and 60 days cold treatment.
- Group 13 can be used to screen for cold susceptibility after 20, 50 and 60 days cold treatment. It is not present in the tolerant cultivars.
- Group 8 is expressed in all the cold tolerant cultivars after cold treatment and also in the susceptible cultivars after 10 and 20 days cold treatment and also after 60 days cold treatment. The susceptible cultivars lose this group after cold treatments of 30, 40 and 50 days.
- Group 5 and group 7 can be used to screen for cold tolerance as they are only present in cultivars that are tolerant to cold.

Sarhan and Perras (1987), Perras and Sarhan (1989) and Abromeit *et al* (1992) found that the intensity of three protein bands (48, 47 and 42 kDa) increased during cold hardening. In this study it was found that the intensity of the protein bands of 48 and 47 kDa (group 6) increased only in the susceptible cultivars. They also found that the intensity of protein bands of 67 and 63 kDa decreased. This was also found in this study. However, after 40 and 50 days cold treatment, the intensity of the protein bands of 63 kDa (group 3) increased in the cold tolerant cultivars. There was also an increase in the protein bands of 67 kDa (group 2) only at the susceptible cultivars with 10 days cold treatment.

Zhou *et al* (1994) found a decrease in the proteins with a molecular weight of 22 – 31 kDa. This was, however, not found in this study. Proteins with a molecular weight of 22 kDa (group 10) increased in the susceptible cultivars after cold treatment. In the tolerant cultivars it increased after 40 and 50 days cold treatment, but it decreased in all the other cold treatments. The protein with the molecular weight of 31 kDa (group 9) increased in the susceptible and tolerant cultivars.

Jacobs (1999) found that proteins with a molecular weight of 44, 43, 38 and 20 kDa were produced in response to cold hardening. This was also found in this study. The susceptible cultivars produced proteins with a molecular weight of 44 kDa (group 6) in response to cold hardening. The tolerant cultivars produced proteins with a molecular weight of 43 kDa (group 7) and 38 kDa (group 8) in response to cold hardening.

Worzella (1947) observed that for one set of conditions genes for winter hardiness appeared to act in a dominant manner, while at other times they acted recessively. Sutka and Veisz (1988) found that at mild stress levels tolerance appeared to be a dominant trait, while under more extreme freezing stress conditions tolerance exhibited a recessive character. If one looks at the dark, double and double bands where one of the bands is dark, it is found that these bands are inherited in a 3:1 ration after 10 and 20 days cold treatment, where these bands are dominant in the tolerant cultivars. However, with longer cold treatments they reacted in a 1:1 ratio. After 60 days cold treatment, the double bands, where on of the bands was dark again, reacted in a dominant manner (3:1).

Chapter 5

COLD RESISTANCE IN THE ROOTS OF WHEAT SEEDLINGS

The protein subunits expressed after cold treatment in the roots of wheat seedlings

Twenty seven wheat cultivars were tested for the expression of protein bands after treatment with cold.

The proteins of the roots were also divided into 14 different groups that were the same as the protein groups of the coleoptiles. Please refer to Table 4.1, page 31 for the different groups and their respective molecular weights.

The occurrence of bands in the different groups

Table 5.1 gives a summary of the different groups, the migration distances of the proteins in the group, the occurrence of the bands in the groups in cold treatment, and the occurrence of the bands in the groups in the controls.

Table 5.1 The occurrence of bands in different groups of proteins in the seedling roots of the wheat cultivars studied

Group	Migration distance	Occurrence in Cold treatment	Occurrence in Controls
Group 1	10 – 20	90	102
Group 2	21 – 30	113	99
Group 3	31 – 40	150	152
Group 4	41 – 50	137	128
Group 5	51 – 60	129	142
Group 6	61 – 70	142	111
Group 7	71 – 80	166	163
Group 8	81 – 90	169	167
Group 9	91 – 100	174	154
Group 10	101 – 110	141	128
Group 11	111 – 120	135	138
Group 12	121 – 130	156	158
Group 13	131 – 140	142	135
Group 14	141 – 150	112	112

The protein group expressed the most in all the different wheat cultivars was group 8 (33.5 - 38 kDa) where the bands occurred 336 times. The group that was expressed the least was group 1 (72 – 76.5 kDa) where the bands occurred 192 times. Group 9 (28 – 32.5 kDa) occurred the

most in the cold treated cultivars with 174 bands and the group expressed the most in the controls was group 8 with 167 bands The protein group expressed the least in the cold treated cultivars was group 1 with 90 bands and the group expressed the least in the controls was group 2 (66.5 – 71 kDa) with 99 bands Group 2 (66.5 – 71 kDa) was expressed 113 times with the cold treatments and 99 times in the controls. Group 5 (50 – 54.5 kDa) was expressed 129 times in the cold treated cultivars and 142 times in their controls. Group 6 (44.5 – 49 kDa) was expressed 142 times with cold treatment and 111 times in their controls. Group 10 (22.5 – 27 kDa) was expressed 141 times with cold treatment and 128 times in the controls.

Please refer to Appendix B for all the banding patterns of the different cultivars.

Adam Tas

10 days - Adam Tas expressed 14 protein bands (Table 1). The untreated sample expressed the same number of bands indicating that treatment had no significant effect on the total number of proteins produced by Adam Tas.

Although there were no significant differences between the total number of proteins produced, groups 2, 6, 11 and 12 expressed an additional band each under cold treatment, while groups 3, 7, 10 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 2, 11 and 12 after cold treatment, and in group 7 and 14 in the control. Groups 8, 9 and 11 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to five in the control.

20 days - Adam Tas expressed 13 protein bands (Table 1). The untreated sample expressed 19 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Adam Tas.

At 20 days six less protein bands were expressed than in the control. Group 8 expressed an additional band under cold treatment, while groups 1, 2, 3, 5, 9, 11 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in group 8 after cold treatment, and in groups 1, 2, 9, 11 and 14 in the control. Groups 6, 8 and 10 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

30 days - Adam Tas expressed 11 protein bands (Table 1). The untreated sample expressed 19 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Adam Tas.

At 30 days eight less protein bands were expressed than in the control. Groups 8 and 12 expressed an additional band each under cold treatment, while groups 1, 3, 5, 7, 9, 11 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in group 8 after cold treatment, and in groups 1, 3, 7, 9, 11 and 14 in the control. Groups 6, 8 and 10 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

40 days - Adam Tas expressed 13 protein bands (Table 1). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by Adam Tas.

Although there were no significant differences between the total number of proteins produced, groups 2, 6 and 9 expressed an additional band each under cold treatment, while groups 4, 5, 7 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 2, 6, 9 and 14 after cold treatment, and in groups 13 and 14 in the control. Groups 6, 8 and 10 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

50 days - Adam Tas expressed 11 protein bands (Table 1). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by Adam Tas.

At 50 days one less protein band was expressed than in the control. Groups 6 and 7 expressed an additional band each under cold treatment, while groups 8, 13 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in group 7 after cold treatment, and in groups 8 and 13 in the control. Groups 7 and 11 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

60 days - Adam Tas expressed 10 protein bands (Table 1). The untreated sample expressed nine protein bands indicating that treatment had an effect on the total number of proteins produced by Adam Tas.

At 60 days one more protein band was expressed than in the control. Groups 1, 2 and 12 expressed an additional band each under cold treatment, while groups 3 and 11 showed a reduction of one band each when treated with cold. Groups 4, 8, 9, 10 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control

population. The number of bands with a high density varied from three in the control to six in the treated material.

Betta

10 days - Betta expressed 13 protein bands (Table 2). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by Betta.

At 10 days one more protein band was expressed than in the control. Groups 2, 8, and 11 expressed an additional band each, and group 10 two additional bands, under cold treatment, while groups 1, 3, 6 and 7 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 2, 5, 8 and 10 after cold treatment, and in group 5 in the control. Groups 5 and 8 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to four in the control.

20 days - Betta expressed 11 protein bands (Table 2). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by Betta.

At 20 days one protein less band were expressed than in the control. Groups 2 and 6 expressed an additional band each under cold treatment, while groups 1, 3 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in group 2. Groups 5, 8 and 13 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to five in the control.

30 days - Betta expressed 11 protein bands (Table 2). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by Betta.

Although there were no significant differences between the total number of proteins produced, groups 2 and 10 expressed an additional band each under cold treatment, while group 11 showed a reduction of one band when treated with cold. Double bands were expressed in group 5 after cold treatment, and in group 5 and 11 in the control. Groups 5, 8 and 13 expressed a band of a higher intensity under treatment and in the control population.

40 days - Betta expressed 12 protein bands (Table 2). The untreated sample expressed 14 protein bands indicating that treatment had an effect on the total number of proteins produced by Betta.

At 40 days two less protein bands were expressed than in the control. Groups 9, 10 and 12 expressed an additional band each under cold treatment, while groups 1, 3, 11 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 2, 8, 10 and 12 after cold treatment, and in groups 2 and 8 in the control. Groups 4, 6 and 8 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

50 days - Betta expressed 15 protein bands (Table 2). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Betta.

At 50 days four more protein bands were expressed than in the control. Groups 3, 8, 10 and 12 expressed an additional band each under cold treatment. Double bands were expressed in groups 4, 6 and 10 after cold treatment, and in groups 4 and 6 in the control. Groups 4, 6 and 7 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to five in the control.

60 days - Betta expressed 12 protein bands (Table 2). The untreated sample expressed ten protein bands indicating that treatment had an effect on the total number of proteins produced by Betta.

At 60 days two more protein bands were expressed than in the control. Groups 3 and 13 expressed an additional band each under cold treatment, while group 9 showed a reduction of one band when treated with cold. Double bands were expressed in group 3 after cold treatment. Groups 4 and 7 expressed a band of a higher intensity under treatment and in the control population.

Betta DN

10 days - Betta DN expressed 14 protein bands (Table 3). The untreated sample expressed eight protein bands indicating that treatment had a significant effect on the total number of proteins produced by Betta DN.

At 10 days six more protein bands were expressed than in the control. Groups 2, 4, 6, 9, 11 and 12 expressed an additional band each under cold treatment, while groups 1 and 5 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6, 9 and 11 after cold treatment, and in group 1 in the control. Group 1 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to two in the control.

20 days - Betta DN expressed 13 protein bands (Table 3). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the total number of proteins produced by Betta DN.

At 20 days two more protein bands were expressed than in the control. Groups 1, 4 and 6 expressed an additional band each under cold treatment, while group 5 showed a reduction of one band when treated with cold. Double bands were expressed in group 1 after cold treatment and in group 5 in the control. Groups 3 and 6 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population.

30 days - Betta DN expressed 14 protein bands (Table 3). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by Betta DN.

At 30 days two more protein bands were expressed than in the control. Groups 2, 6 and 8 expressed an additional band each under cold treatment, while groups 4 and 5 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 2 and 8 after cold treatment and in group 5 in the control. Group 5 expressed a band of a higher intensity under treatment. The number of bands with a high density varied from no bands in the control to one in the treated material.

40 days - Betta DN expressed 12 protein bands (Table 3). The untreated sample had the same number of protein bands indicating that treatment had no significant effect on the total number of proteins produced by Betta DN.

Although there were no significant differences between the total number of proteins produced, groups 1, 7 and 8 expressed an additional band each under cold treatment, while groups 11, 13 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 7 and 8 after cold treatment, and in group 11 in the control. Groups 5 and 11 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population.

50 days - Betta DN expressed 17 protein bands (Table 3). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by Betta DN.

Although there were no significant differences between the total number of proteins produced, groups 3, 10 and 12 expressed an additional band each under cold treatment, while groups 1, 4 and 7 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3, 6, 10 and 12 after cold treatment, and in groups 4, 6 and 7 in the control. Group 7 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from one band in the treated material to two in the control.

60 days - Betta DN expressed 15 protein bands (Table 3). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Betta DN.

At 60 days four more protein bands were expressed than in the control. Groups 3, 4, 11 and 13 expressed an additional band each under cold treatment, while group 1 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3, 4 and 7 after cold treatment, and in group 7 in the control. Group 7 expressed a band of a higher intensity under treatment. The number of bands with a high density varied from one in the treated material to two in the control.

Caledon

10 days - Caledon expressed 13 protein bands (Table 4). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the total number of proteins produced by Caledon.

At 10 days two more protein bands were expressed than in the control. Groups 6, 7, 10 and 11 expressed an additional band each under cold treatment, while groups 8 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 7 and 12 after cold treatment, and in group 8 in the control. Groups 5 and 7 expressed a band of a higher intensity in the control population. The number of bands with a high density varied from no bands in the treated material to two in the control.

20 days - Caledon expressed 13 protein bands (Table 4). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by Caledon.

Although there were no significant differences between the total number of proteins produced, groups 1 and 10 expressed an additional band each under cold treatment, while groups 6, 8 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1 and 10 after cold treatment, and in group 8 in the control. Groups 4, 5 and 7 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to three in the treated material.

30 days - Caledon expressed 11 protein bands (Table 4). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by Caledon.

At 30 days one less protein band was expressed than in the control. Groups 1, 4 and 9 expressed an additional band each under cold treatment, while groups 2, 7, 8 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4 and 9 after cold treatment, and in group 8 in the control. Groups 4, 5 and 8 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to three in the treated material.

40 days - Caledon expressed 14 protein bands (Table 4). The untreated sample expressed 10 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Caledon.

At 40 days four more protein bands were expressed than in the control. Groups 1, 2, 6, 7 and 9 expressed an additional band each under cold treatment, while groups 3 and 8 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 2, 7 and 9 after cold treatment, and in group 8 in the control. Groups 5, 8 and 10 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

50 days - Caledon expressed 15 protein bands (Table 4). The untreated sample expressed ten protein bands indicating that treatment had a significant effect on the total number of proteins produced by Caledon.

At 50 days five more protein bands were expressed than in the control. Groups 2, 3, 6 and 11 expressed an additional band each under cold treatment. Double bands were expressed in groups 2, 8 and 11 after cold treatment, and in group 8 in the control. Groups 5, 8 and 11 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to five in the treated material.

60 days - Caledon expressed 15 protein bands (Table 4). The untreated sample expressed 12 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Caledon.

At 60 days three more protein bands were expressed than in the control. Groups 2, 6, 7, 9 and 11 expressed an additional band each under cold treatment, while group 8 showed a reduction of two bands when treated with cold. Double bands were expressed in groups 2, 7 and 9 after cold treatment, and in group 8 in the control. Groups 5, 7 and 10 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to three in the treated material.

Chinese Spring

10 days - Chinese Spring expressed 11 protein bands (Table 5). The untreated sample expressed 12 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Chinese Spring.

At 10 days one less protein band was expressed than in the control. Groups 2 and 8 expressed an additional band each under cold treatment, while groups 1, 3, 6 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4 and 8 after cold treatment, and in groups 4 and 6 in the control. Groups 2 and 8 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population.

20 days - Chinese Spring expressed 10 protein bands (Table 5). The untreated sample expressed 12 protein bands indicating that treatment had an on the total number of proteins produced by Chinese Spring.

At 20 days two less protein bands were expressed than in the control. Groups 2, 4, 11 and 13 expressed an additional band each under cold treatment, while groups 1, 3, 5, 10 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in group 11 after cold treatment, and in groups 3, 5 and 12 in the control. Groups 2 and 8 expressed a band

of a higher intensity under treatment while two bands showed high intensity in the control population.

30 days - Chinese Spring expressed nine protein bands (Table 5). The untreated sample expressed 13 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Chinese Spring.

At 30 days four less protein bands were expressed than in the control. Group 13 expressed an additional band, and group 6 two additional bands, under cold treatment, while groups 1, 7, 10, 11, 12 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6 and 13 after cold treatment, and in group11 in the control. Group 3 and 8 expressed a band of a higher intensity under treatment while one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to two in the treated material.

40 days - Chinese Spring expressed eight protein bands (Table 5). The untreated sample expressed nine protein bands indicating that treatment had an effect on the total number of proteins produced by Chinese Spring.

At 40 days one less protein band was expressed than in the control. Groups 7 and 9 expressed an additional band each under cold treatment, while groups 8, 10 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in group 9 after cold treatment, and in group 8 in the control.

50 days - Chinese Spring expressed 14 protein bands (Table 5). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Chinese Spring.

At 50 days three more protein bands were expressed than in the control. Groups 6, 7, 9, 11 and 14 expressed an additional band each under cold treatment, while groups 5 and 10 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4, 9, 11 and 14 after cold treatment, and in groups 4 and 5 in the control. Groups 5, 7 and 9 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to three in the treated material.

60 days - Chinese Spring expressed 11 protein bands (Table 5). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by Chinese Spring.

Although there were no significant differences between the total number of proteins produced, groups 9 and 14 expressed an additional band each under cold treatment, while groups 5 and 8 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 9 and 14 after cold treatment. Group 9 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to three in the control.

Gamtoos

10 days - Gamtoos expressed 12 protein bands (Table 6). The untreated sample expressed ten protein bands indicating that treatment had an effect on the total number of proteins produced by Gamtoos.

At 10 days two more protein bands were expressed than in the control. Groups 4, 5, 9 and 12 expressed an additional band each under cold treatment, while groups 1 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 5 and 12 after cold treatment. Groups 3 and 8 expressed a band of a higher intensity under treatment and in the control.

20 days - Gamtoos expressed ten protein bands (Table 6). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by Gamtoos.

At 20 days six more protein bands were expressed than in the control. Groups 1 and 4 expressed an additional band each under cold treatment, while groups 3, 6, 9 and 10 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3 and 6 during the control. Groups 3 and 8 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

30 days - Gamtoos expressed 13 protein bands (Table 6). The untreated sample expressed 15 protein bands indicating that treatment had an effect on the total number of proteins produced by Gamtoos.

At 30 days two less protein bands were expressed than in the control. Groups 4 and 10 expressed an additional band each under cold treatment, while group 3 showed a reduction of one band, and group 1 the reduction of two bands, when treated with cold. Double bands were expressed in groups 5, 8 and 10 after cold treatment, and in groups 1, 3, 5 and 8 in the control.

Groups 5 and 8 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

40 days - Gamtoos expressed 13 protein bands (Table 6). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Gamtoos.

At 40 days two more protein bands were expressed than in the control. Groups 2, 3, 7 and 8 expressed an additional band each under cold treatment, while groups 10 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3, 7 and 8 after cold treatment, and in groups 10 and 12 in the control. Groups 5, 10, 13 and 14 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to four in the treated material.

50 days - Gamtoos expressed 11 protein bands (Table 6). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by Gamtoos.

At 50 days one less protein band was expressed than in the control. Group 3 showed a reduction of one band when treated with cold. Double bands were expressed in group 14 after cold treatment and in the control. Groups 5, 8 and 12 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

60 days - Gamtoos expressed 11 protein bands (Table 6). The untreated sample expressed 14 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Gamtoos.

At 60 days three less protein bands were expressed than in the control. Groups 2, 5, 11 and 13 expressed an additional band each under cold treatment, while groups 3, 4, 8, 10 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 5 and 13 after cold treatment, and in groups 4, 8 and 12 in the control. Groups 5, 7 and 9 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to three in the treated material.

Gariep

10 days - Gariep expressed 11 protein bands (Table 7). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by Gariep.

At 10 days one less protein band was expressed than in the control. Groups 5 and 12 expressed an additional band each, and group 3 two additional bands, under cold treatment, while group 9 showed a reduction of one band, and group 11 the reduction of two bands, when treated with cold. Double bands were expressed in groups 3 and 5 after cold treatment, and in groups 9 and 11 in the control. Groups 3, 5, 9 and 10 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to four in the treated material.

20 days - Gariep expressed 11 protein bands (Table 7). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by Gariep.

At 20 days two less protein bands were expressed than in the control. Groups 1, 2 and 13 expressed an additional band each under cold treatment, while groups 3, 5, 8 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3, 5, 8 and 14 during the control. Groups 3, 7, 9 and 13 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to four in the treated material.

30 days - Gariep expressed ten protein bands (Table 7). The untreated sample expressed 15 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Gariep.

At 30 days five less protein bands were expressed than in the control. Group 2 expressed an additional band under cold treatment, while groups 3, 5, 10, 11 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3, 11 and 13 during the control. Groups 3, 7 and 9 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to three in the treated material.

40 days - Gariep expressed 15 protein bands (Table 7). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Gariep.

At 40 days four more protein bands were expressed than in the control. Groups 2, 5, 7, 9, 12 and 14 expressed an additional band each under cold treatment, while groups 4 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 7 and 9 after cold treatment. Groups 2, 3, 6, 7 and 9 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to five in the treated material.

50 days - Gariep expressed 13 protein bands (Table 7). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the total number of proteins produced by Gariep.

At 50 days two more protein bands were expressed than in the control. Groups 1, 5, 8 and 11 expressed an additional band each under cold treatment, while groups 7 and 10 showed a reduction of one band each when treated with cold. Double bands were expressed in group 14 after cold treatment, and in groups 7 and 14 in the control. Groups 3, 5, 7 and 11 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to four in the treated material.

60 days - Gariep expressed 13 protein bands (Table 7). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by Gariep.

Although there were no significant differences between the total number of proteins produced, groups 1, 3 and 10 expressed an additional band each under cold treatment, while group 14 showed a reduction of one band when treated with cold. Double bands were expressed in groups 9 and 12 after cold treatment, and in groups 9, 12 and 14 in the control. Groups 3, 7 and 10 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to three in the treated material.

Hugenoot

10 days - Hugenoot expressed 13 protein bands (Table 8). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by Hugenoot.

At 10 days one more protein band was expressed than in the control. Groups 1 and 9 expressed an additional band each under cold treatment, while group 13 showed a reduction of one band when treated with cold. Double bands were expressed in groups 1 and 11 after cold treatment, and in group 11 in the control. Groups 2, 4, 7 and 9 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to four in the treated material.

20 days - Hugenoot expressed nine protein bands (Table 8). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the total number of proteins produced by Hugenoot.

At 20 days two less protein bands were expressed than in the control. Groups 5 and 8 expressed an additional band each under cold treatment, while groups 1, 7, 12 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in group 7 in the control. Groups 2, 4, 7 and 9 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to four in the treated material.

30 days - Hugenoot expressed 12 protein bands (Table 8). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by Hugenoot.

At 30 days one less protein band was expressed than in the control. Groups 1, 7 and 9 expressed an additional band each under cold treatment, while groups 2, 4, 5 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 7 and 9 after cold treatment, and in groups 2, 4 and 11 in the control. Groups 2, 6, 7 and 9 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to four in the treated material.

40 days - Hugenoot expressed nine protein bands (Table 8). The untreated sample expressed 13 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Hugenoot.

At 40 days four less protein bands were expressed than in the control. Groups 3 and 6 expressed an additional band each under cold treatment, while groups 1, 5, 10, 11 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in group 3 after cold treatment, and in groups 1, 5, 11 and 12 in the control. Groups 2, 6 and 7

expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

50 days - Hugenoot expressed 14 protein bands (Table 8). The untreated sample expressed 16 protein bands indicating that treatment had an effect on the total number of proteins produced by Hugenoot.

At 50 days two less protein bands were expressed than in the control. Group 3 expressed an additional band under cold treatment, while groups 6, 11 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in group 2 after cold treatment, and in groups 2, 6, 11 and 12 in the control. Groups 2, 4, 5, 7 and 8 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to five in the treated material.

60 days - Hugenoot expressed 12 protein bands (Table 8). The untreated sample expressed 14 protein bands indicating that treatment had an effect on the total number of proteins produced by Hugenoot.

At 60 days two less protein bands were expressed than in the control. Groups 1 and 13 expressed an additional band each under cold treatment, while groups 2, 5, 11 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1 and 9 after cold treatment, and in groups 9, 11 and 12 in the control. Group 6 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from one in the control to two in the treated material.

Letaba

10 days - Letaba expressed 14 protein bands (Table 9). The untreated sample expressed ten protein bands indicating that treatment had a significant effect on the total number of proteins produced by Letaba.

At 10 days four more protein bands were expressed than in the control. Groups 2, 4, 7, 9 and 11 expressed an additional band each under cold treatment, while groups 10 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 2, 5, 7 and 11 after cold treatment, and in group 5 in the control. Groups 2 and 7 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population.

20 days - Letaba expressed ten protein bands (Table 9). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by Letaba.

At 20 days two less protein bands were expressed than in the control. Groups 3, 6 and 10 expressed an additional band each under cold treatment, while groups 9, 12 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in group 10 after cold treatment, and in groups 5, 8 and 13 in the control. Groups 5, 8 and 13 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to three in the treated material.

30 days - Letaba expressed 13 protein bands (Table 9). The untreated sample expressed 14 protein bands indicating that treatment had an effect on the total number of proteins produced by Letaba.

At 30 days one less protein band was expressed than in the control. Groups 8, 10 and 11 expressed an additional band each under cold treatment, while groups 2, 4, 6, 9 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 8 and 11 after cold treatment, and in groups 6 and 9 in the control. Group 8 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to two in the control.

40 days - Letaba expressed 11 protein bands (Table 9). The untreated sample expressed 15 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Letaba.

At 40 days four less protein bands were expressed than in the control. Group 6 expressed two additional bands under cold treatment, while groups 1, 2, 5, 7, 10 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in group 6 after cold treatment, and in groups 2, 5 and 7 in the control. Groups 2, 6, 8, 9 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to five in the treated material.

50 days - Letaba expressed 12 protein bands (Table 9). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the total number of proteins produced by Letaba.

At 50 days one less protein band was expressed than in the control. Groups 8 and 11 expressed an additional band each under cold treatment, while group 12 showed a reduction of one band when treated with cold. Double bands were expressed in group 11 after cold treatment. Groups 5 and 7 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

60 days - Letaba expressed 12 protein bands (Table 9). The untreated sample expressed 10 protein bands indicating that treatment had an effect on the total number of proteins produced by Letaba.

At 60 days two more protein bands were expressed than in the control. Groups 1, 7, 9, 11 and 12 expressed an additional band each under cold treatment, while group 13 showed a reduction of two bands when treated with cold. Double bands were expressed in groups 7 and 12 after cold treatment, and in group 13 in the control. Group 5 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to four in the control.

Limpopo

10 days - Limpopo expressed 14 protein bands (Table 10). The untreated sample expressed 10 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Limpopo.

At 10 days four more protein bands were expressed than in the control. Groups 4, 8, 13 and 14 expressed an additional band each under cold treatment, while group 11 showed a reduction of one band when treated with cold. Double bands were expressed in groups 4, 7 and 13 after cold treatment, and in group 7 in the control. Group 7 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to four in the control.

20 days - Limpopo expressed 11 protein bands (Table 10). The untreated sample expressed 10 protein bands indicating that treatment had an effect on the total number of proteins produced by Limpopo.

At 20 days one more protein band was expressed than in the control. Groups 1, 4 and 9 expressed an additional band each under cold treatment, while groups 3, 6 and 8 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4 and 9 after cold treatment, and in group 3 in the control. Groups 7 and 11 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

30 days - Limpopo expressed 12 protein bands (Table 10). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by Limpopo.

Although there were no significant differences between the total number of proteins produced, group 8 expressed an additional band, and group 12 expressed two additional bands, under cold treatment, while groups 3, 7 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4, 8 and 12 after cold treatment, and in groups 3, 4 and 7 in the control. Groups 7 and 11 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in control.

40 days - Limpopo expressed eight protein bands (Table 10). The untreated sample expressed nine protein bands indicating that treatment had an effect on the total number of proteins produced by Limpopo.

At 40 days one less protein band was expressed than in the control. Groups 8 and 12 expressed an additional band each under cold treatment, while groups 1, 2, 9 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4 and 12 after cold treatment, and in group 4 in the control. Group 7 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from 1 in the treated material to two in the control.

50 days - Limpopo expressed ten protein bands (Table 10). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the total number of proteins produced by Limpopo.

At 50 days one less protein band was expressed than in the control. Groups 4, 6 and 11 expressed an additional band each under cold treatment, while groups 3, 5, 8 and 9 showed a reduction of one band each when treated with cold. Double bands were expressed in group 4

after cold treatment, and in group 9 in the control. Groups 7, 11 and 13 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to three in the treated material.

60 days - Limpopo expressed 12 protein bands (Table 10). The untreated sample expressed nine protein bands indicating that treatment had a significant effect on the total number of proteins produced by Limpopo.

At 60 days three more protein bands were expressed than in the control. Groups 2, 6, 10, 12 and 14 expressed an additional band each under cold treatment, while groups 3, 9 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4 and 12 after cold treatment, and in group 4 in the control. Group 7 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to four in the control.

Molen

10 days - Molen expressed 15 protein bands (Table 11). The untreated sample expressed 14 protein bands indicating that treatment had an effect on the total number of proteins produced by Molen.

At 10 days one more protein band was expressed than in the control. Groups 1, 6, 7 and 13 expressed an additional band each under cold treatment, while groups 3, 5, 8 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1, 6, 7 and 13 after cold treatment, and in groups 3, 8 and 12 in the control. Groups 4, 6, 9 and 12 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to four in the treated material.

20 days - Molen expressed 12 protein bands (Table 11). The untreated sample had exacely the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by Molen.

Although there were no significant differences between the total number of proteins produced, groups 2, 4, 6, 7 and 10 expressed an additional band each under cold treatment, while groups 1, 3, 5, 9 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in group 4 after cold treatment, and in groups 5 and 9 in the control. Groups 4, 5, 7, 9,

10 and 13 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to six in the treated material.

30 days - Molen expressed 13 protein bands (Table 11). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by Molen.

Although there were no significant differences between the total number of proteins produced, groups 2, 10 and 13 expressed an additional band each under cold treatment, while groups 1, 5 and 7 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 2, 12 and 13 after cold treatment, and in groups 1, 7 and 12. Groups 4, 8 and 10 expressed a band of a higher intensity under treatment while six bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to six in the control.

40 days - Molen expressed 11 protein bands (Table 11). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by Molen.

At 40 days two less protein bands were expressed than in the control. Groups 1 and 7 showed a reduction of one band each, and group 5 the reduction of two bands, when treated with cold. Double bands were expressed in group 8 after cold treatment, and in groups 1, 5, 7 and 8 in the control. Groups 3 and 6 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to four in the control.

50 days - Molen expressed 12 protein bands (Table 11). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by Molen.

Although there were no significant differences between the total number of proteins produced, groups 5, 7 and 10 expressed an additional band each under cold treatment, while group 1 showed a reduction of one band, and group 13 the reduction of two bands, when treated with cold. Double bands were expressed in group 7 after cold treatment, and in groups 1 and 13 in the control. Groups 3, 6, 8 and 9 expressed a band of a higher intensity under treatment and in the control population.

60 days - Molen expressed 14 protein bands (Table 11). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Molen.

At 60 days three more protein bands were expressed than in the control. Groups 2, 5, 6, 12 and 14 expressed an additional band each under cold treatment, while groups 3, 7 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6 and 12 after cold treatment, and in groups 3 and 13 in the control. Groups 8, 9 and 10 expressed a band of a higher intensity under treatment and in the control population.

Molopo

10 days - Molopo expressed 13 protein bands (Table 12). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the total number of proteins produced by Molopo.

At 10 days two more protein bands were expressed than in the control. Groups 4, 10 and 12 expressed an additional band each under cold treatment, while group 1 showed a reduction of one band when treated with cold. Double bands were expressed in groups 4 and 14 after cold treatment, and in group 14 in the control. Group 7 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to two in the control.

20 days - Molopo expressed 13 protein bands (Table 12). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by Molopo.

Although there were no significant differences between the total number of proteins produced, groups 3, 6, 8 and 9 expressed an additional band each under cold treatment, while groups 5, 7, 10 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4, 9 and 14 after cold treatment, and in groups 4, 7 and 14 in the control. Groups 7 and 11 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to four in the control.

30 days - Molopo expressed 11 protein bands (Table 12). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by Molopo.

At 30 days two less protein bands were expressed than in the control. Group 8 expressed an additional band under cold treatment, while groups 3, 5 and 10 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 7 and 14 after cold treatment, and in groups 3, 7 and 14 in the control. Groups 7, 11 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

40 days - Molopo expressed 11 protein bands (Table 12). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by Molopo.

Although there were no significant differences between the total number of proteins produced, groups 2, 9 and 13 expressed an additional band each under cold treatment, while groups 1, 3, 8 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in group 13 after cold treatment, and in groups 3 and 14 in the control. Groups 7 and 11 expressed a band of a higher intensity under treatment and in the control population.

50 days - Molopo expressed 11 protein bands (Table 12). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by Molopo.

At 50 days two less protein bands were expressed than in the control. Groups 1, 3 and 13 expressed an additional band each under cold treatment, while groups 2, 4, 5, 12 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3, 7 and 13 after cold treatment, and in groups 4 and 14 in the control. Group 7 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to two in the control.

60 days - Molopo expressed 12 protein bands (Table 12). The untreated sample had the same number bands indicating that treatment had no significant effect on the total number of proteins produced by Molopo.

Although there were no significant differences between the total number of proteins produced, groups 1 and 6 expressed an additional band each under cold treatment, while groups 5 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 7 and 14 after cold treatment and in the control. Groups 7 and 11 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

The number of bands with a high density varied from two in the treated material to three in the control.

Nantes

10 days - Nantes expressed 15 protein bands (Table 13). The untreated sample expressed 14 protein bands indicating that treatment had an effect on the total number of proteins produced by Nantes.

At 10 days one more protein band was expressed than in the control. Groups 6, 8, 13 and 14 expressed an additional band each under cold treatment, while groups 2, 4 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6 and 8 after cold treatment, and in groups 2, 4 and 12 in the control. Groups 3, 5 and 8 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

20 days - Nantes expressed 15 protein bands (Table 13). The untreated sample expressed 14 protein bands indicating that treatment had an effect on the total number of proteins produced by Nantes.

At 20 days one more protein band was expressed than in the control. Groups 10, 12 and 14 expressed an additional band each under cold treatment, while groups 5 and 7 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1, 10 and 12 after cold treatment, and in group 1 in the control. Groups 4, 8 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

30 days - Nantes expressed 13 protein bands (Table 13). The untreated sample expressed 14 protein bands indicating that treatment had an effect on the total number of proteins produced by Nantes.

At 30 days one less protein band was expressed than in the control. Groups 3, 7, 8 and 10 expressed an additional band each under cold treatment, while groups 2, 5, 9, 11 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3, 8 and 12 after cold treatment, and in groups 2, 11 and 12 in the control. Groups 1, 4 and 8 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to three in the treated material.

40 days - Nantes expressed 14 protein bands (Table 13). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by Nantes.

At 40 days two more protein bands were expressed than in the control. Groups 2, 3, 6, 9 and 12 expressed an additional band each under cold treatment, while groups 4, 11 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 2, 3 and 12 after cold treatment, and in group 4 in the control. Groups 4 and 8 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population.

50 days - Nantes expressed 15 protein bands (Table 13). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Nantes.

At 50 days four more protein bands were expressed than in the control. Groups 1, 2, 4, 10 and 14 expressed an additional band each under cold treatment, while group 7 showed a reduction of one band when treated with cold. Double bands were expressed in groups 1, 4 and 10 after cold treatment. Groups 4 and 8 expressed a band of a higher intensity under treatment and in the control population.

60 days - Nantes expressed 14 protein bands (Table 13). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by Nantes.

At 60 days one more protein band was expressed than in the control. Groups 2, 4 and 10 expressed an additional band each under cold treatment, while groups 1, 3 and 8 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4 and 10 after cold treatment, and in group 1 and 8 in the control. Groups 4 and 8 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to two in the treated material.

Palmiet

10 days - Palmiet expressed 14 protein bands (Table 14). The untreated sample expressed 15 protein bands indicating that treatment had an effect on the total number of proteins produced by Palmiet.

At 10 days one less protein band was expressed than in the control. Groups 9 and 10 expressed an additional band each under cold treatment, while groups 6, 7 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 2 and 5 after cold treatment, and in groups 2, 5, 6 and 13 in the control. Groups 5 and 8 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to four in the control.

20 days - Palmiet expressed 13 protein bands (Table 14). The untreated sample expressed 15 protein bands indicating that treatment had an effect on the total number of proteins produced by Palmiet.

At 20 days two more protein bands were expressed than in the control. Groups 2 and 10 expressed an additional band each under cold treatment, while groups 2, 4, 6 and 9 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 5 and 13 after cold treatment, and in groups 2, 5 and 13 in the control. Groups 5, 8 and 12 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to four in the control.

30 days - Palmiet expressed 12 protein bands (Table 14). The untreated sample expressed 16 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Palmiet.

At 30 days four less protein bands were expressed than in the control. Groups 1, 2 and 7 expressed an additional band each under cold treatment, while groups 2, 5, 6, 8 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3 and 14 after cold treatment, and in groups 4, 6, 8, 11 and 14 in the control. Groups 6, 9 and 14 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to three in the treated material.

40 days - Palmiet expressed 16 protein bands (Table 14). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Palmiet.

At 40 days five more protein bands were expressed than in the control. Groups 5, 10, 11 and 12 expressed an additional band each under cold treatment. Double bands were expressed in

groups 4, 11, 12 and 14 after cold treatment, and in groups 4 and 14 in the control. Groups 6, 9 and 14 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

50 days - Palmiet expressed 14 protein bands (Table 14). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by Palmiet.

At 50 days one more protein band was expressed than in the control. Groups 3, 4, 10 and 13 expressed an additional band each under cold treatment, while group 14 showed a reduction of one band, and group 11 the reduction of two bands, when treated with cold. Double bands were expressed in groups 4 and 13 after cold treatment, and in groups 11 and 14 in the control. Groups 6 and 9 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

60 days - Palmiet expressed 12 protein bands (Table 14). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by Palmiet.

At 60 days one less protein band was expressed than in the control. Groups 8, 11, 12 and 14 expressed an additional band each under cold treatment, while groups 1, 3, 10 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 8 and 14 after cold treatment, and in group 1 and 13 in the control. Groups 6 and 14 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

PAN 3211

10 days - PAN 3211 expressed 12 protein bands (Table 15). The untreated sample expressed 15 protein bands indicating that treatment had a significant effect on the total number of proteins produced by PAN 3211.

At 10 days three less protein bands were expressed than in the control. Groups 3 and 8 expressed an additional band each under cold treatment, while groups 2, 4, 7, 9, 12 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3, 8 and 14 after cold treatment. Groups 3, 5 and 8 expressed a band of a higher

intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to three in the treated material.

20 days - PAN 3211 expressed 11 protein bands (Table 15). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by PAN 3211.

At 20 days one less protein band was expressed than in the control. Groups 8, 9, 11 and 13 expressed an additional band each under cold treatment, while groups 3, 7, 10, 12 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in group 9 after cold treatment, and in groups 3, 7, 12 and 14 in the control. Groups 5, 9 and 14 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to four in the treated material.

30 days - PAN 3211 expressed 11 protein bands (Table 15). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by PAN 3211.

Although there were no significant differences between the total number of proteins produced, group 7 expressed an additional band under cold treatment, while group 2 showed a reduction of one band when treated with cold. Double bands were expressed in group 14 after cold treatment and in the control. Groups 5 and 9 expressed a band of a higher intensity under treatment and in the control population.

40 days - PAN 3211 expressed 11 protein bands (Table 15). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by PAN 3211.

At 40 days one less protein band was expressed than in the control. Groups 3, 7 and 8 expressed an additional band each under cold treatment, while groups 2, 6 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in group 3 after cold treatment, and in group 14 in the control. Groups 5 and 9 expressed a band of a higher intensity under treatment and in the control population.

50 days - PAN 3211 expressed 11 protein bands (Table 15). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by PAN 3211.

At 50 days one less protein band was expressed than in the control. Group 10 expressed an additional band, and group 6 two additional bands, under cold treatment, while groups 2, 4, 7 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6 and 14 after cold treatment, and in group 14 in the control. Groups 5 and 9 expressed a band of a higher intensity under treatment and in the control population.

60 days - PAN 3211 expressed 12 protein bands (Table 15). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the total number of proteins produced by PAN 3211.

At 60 days six more protein bands were expressed than in the control. Groups 7 and 8 expressed an additional band each under cold treatment, while group 1 showed a reduction of one band when treated with cold. Double bands were expressed in groups 3 and 14 after cold treatment and in the control. Group 7 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to two in the control.

PAN 3349

10 days - PAN 3349 expressed nine protein bands (Table 16). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the total number of proteins produced by PAN 3349.

At 10 days two less protein bands were expressed than in the control. Group 14 expressed an additional band under cold treatment, while groups 6, 11 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in group14 after cold treatment, and in groups 6 and 11 in the control. The number of bands with a high density varied from 3 in the control to 5 in the treated material.

20 days - PAN 3349 expressed nine protein bands (Table 16). The untreated sample expressed 13 protein bands indicating that treatment had a significant effect on the total number of proteins produced by PAN 3349.

At 20 days four less protein bands were expressed than in the control. Group 2 expressed an additional band under cold treatment, while groups 1, 3, 4 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1, 3 and 12 in the control. Group 6 expressed a band of a higher intensity under treatment and in the control population.

30 days - PAN 3349 expressed 12 protein bands (Table 16). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by PAN 3349.

At 30 days one less protein band was expressed than in the control. Groups 7, 10 and 11 expressed an additional band each under cold treatment, while groups 1, 2 and 8 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3, 7 and 13 after cold treatment, and in groups 3, 6 and 13 in the control. Groups 3 and 6 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population.

40 days - PAN 3349 expressed eight protein bands (Table 16). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the total number of proteins produced by PAN 3349.

At 40 days three less protein bands were expressed than in the control. Group 9 expressed an additional band under cold treatment, while groups 1, 8, 13 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in group 6 after cold treatment, and in groups 6, 8 and 13 in the control. Groups 6, 9 and 13 expressed a band of a higher intensity under treatment while only one band showed high intensity in the control population. The number of bands with a high density varied from one in the control to three in the treated material.

50 days - PAN 3349 expressed 12 protein bands (Table 16). The untreated sample expressed nine protein bands indicating that treatment had a significant effect on the total number of proteins produced by PAN 3349.

At 50 days three more protein bands were expressed than in the control. Groups 2, 5, 6, 7 and 10 expressed an additional band each under cold treatment, while groups 1, 8 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6 and 7 after cold treatment, and in group 8 in the control. Group 6 expressed a band of a higher intensity in the control population. The number of bands with a high density varied from no bands in the treated material to one in the control.

60 days - PAN 3349 expressed six protein bands (Table 16). The untreated sample expressed nine protein bands indicating that treatment had a significant effect on the total number of proteins produced by PAN 3349.

At 60 days three less protein bands were expressed than in the control. Groups 7 and 8 expressed an additional band each under cold treatment, while groups 2, 4, 5, 9 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6 and 7 after cold treatment, and in groups 6 and 9 in the control. Group 6 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

PAN 3377

10 days - PAN 3377 expressed 11 protein bands (Table 17). The untreated sample expressed ten protein bands indicating that treatment had an effect on the total number of proteins produced by PAN 3377.

At 10 days one more protein band was expressed than in the control. Groups 2 and 3 expressed an additional band each under cold treatment, while group 1 showed a reduction of one band when treated with cold. Double bands were expressed in group 12 after cold treatment and in the control. Groups 5 and 8 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

20 days - PAN 3377 expressed 12 protein bands (Table 17). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the total number of proteins produced by PAN 3377.

At 20 days one more protein band was expressed than in the control. Groups 2 and 6 expressed an additional band each under cold treatment, while group 1 showed a reduction of one band when treated with cold. Double bands were expressed in group 12 after cold treatment and in the control. Group 8 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to two in the control.

30 days - PAN 3377 expressed 11 protein bands (Table 17). The untreated sample expressed eight protein bands indicating that treatment had a significant effect on the total number of proteins produced by PAN 3377.

At 30 days three more protein bands were expressed than in the control. Groups 2, 3, 6, 10 and 14 expressed an additional band each under cold treatment, while groups 1 and 11 showed a reduction of one band each when treated with cold. Groups 5, 8, 12 and 13 expressed a band of

a higher intensity under treatment. The number of bands with a high density varied from no bands in the control to four in the treated material.

40 days - PAN 3377 expressed 11 protein bands (Table 17). The untreated sample expressed nine protein bands indicating that treatment had an effect on the total number of proteins produced by PAN 3377.

At 40 days two more protein bands were expressed than in the control. Groups 2, 6 and 10 expressed an additional band each under cold treatment, while group 1 showed a reduction of one band when treated with cold. Groups 5 and 8 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to three in the control.

50 days - PAN 3377 expressed 11 protein bands (Table 17). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by PAN 3377.

At 50 days one less protein band was expressed than in the control. Groups 2, 6, 8 and 11 expressed an additional band each under cold treatment, while groups 1, 5, 10, 12 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in group 11 after cold treatment, and in groups 5 and 12 in the control. Group 8 expressed a band of a higher intensity under treatment while one band showed high intensity in the control population.

60 days - PAN 3377 expressed 14 protein bands (Table 17). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the total number of proteins produced by PAN 3377.

At 60 days three more protein bands were expressed than in the control. Groups 3, 6, 7, 9 and 11 expressed an additional band each under cold treatment, while groups 5 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 7, 9 and 11 after cold treatment, and in group 5 and 14 in the control. Groups 8, 9 and 13 expressed a band of a higher intensity. The number of bands with a high density varied from no bands in the control to four in the treated material.

Scheepers 69

10 days - Scheepers 69 expressed 13 protein bands (Table 18). The untreated sample expressed nine protein bands indicating that treatment had a significant effect on the total number of proteins produced by Scheepers 69.

At 10 days four more protein bands were expressed than in the control. Groups 6, 7, 9 and 13 expressed an additional band each under cold treatment. Double bands were expressed in groups 7 and 13 after cold treatment.

20 days - Scheepers 69 expressed 11 protein bands (Table 18). The untreated sample expressed ten protein bands indicating that treatment had an effect on the total number of proteins produced by Scheepers 69.

At 20 days one more protein band was expressed than in the control. Groups 6, 8 and 9 expressed an additional band each under cold treatment, while groups 2 and 10 showed a reduction of one band each when treated with cold. Double bands were expressed in group 8 after cold treatment.

30 days - Scheepers 69 expressed 12 protein bands (Table 18). The untreated sample expressed ten protein bands indicating that treatment had an effect on the total number of proteins produced by Scheepers 69.

At 30 days two more protein bands were expressed than in the control. Groups 2, 6, 9, 11 and 13 expressed an additional band each under cold treatment, while groups 8 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in group 7 after cold treatment, and in groups 7 and 14 in the control.

40 days - Scheepers 69 expressed 11 protein bands (Table 18). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by Scheepers 69.

At 40 days two less protein bands were expressed than in the control. Group 8 expressed an additional band, and group 5 two additional bands, under cold treatment, while groups 7, 9 and 10 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 5 and 8 after cold treatment, and in groups 7 and 13 in the control.

50 days - Scheepers 69 expressed 15 protein bands (Table 18). The untreated sample expressed 14 protein bands indicating that treatment had an effect on the total number of proteins produced by Scheepers 69.

At 50 days one more protein band was expressed than in the control. Group 14 expressed an additional band under cold treatment. Double bands were expressed in groups 10 and 14 after cold treatment, and in group 10 in the control.

60 days - Scheepers 69 expressed 10 protein bands (Table 18). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by Scheepers 69.

Although there were no significant differences between the total number of proteins produced, group 8 expressed an additional band under cold treatment, while group 11 showed a reduction of one band when treated with cold.

Snack

10 days - Snack expressed 15 protein bands (Table 19). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by Snack.

At 10 days two more protein bands were expressed than in the control. Groups 9, 10, 12 and 13 expressed an additional band each under cold treatment, while group 11 showed a reduction of two band when treated with cold. Double bands were expressed in groups 3, 9, 10 and 12 after cold treatment, and in groups 3 and 11 in the control. Groups 6, 9, 13 and 14 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to four in the treated material.

20 days - Snack expressed 15 protein bands (Table 19). The untreated sample expressed 12 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Snack.

At 20 days three more protein bands were expressed than in the control. Groups 2, 9, 11 and 12 expressed an additional band each under cold treatment, while group 6 showed a reduction of one band when treated with cold. Double bands were expressed in groups 9 and 12 after cold treatment, and in group 6 in the control. Groups 6, 9, 13 and 14 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population.

30 days - Snack expressed 14 protein bands (Table 19). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Snack.

At 30 days three more protein bands were expressed than in the control. Groups 3, 5, 11 and 13 expressed an additional band each under cold treatment, while group 6 showed a reduction of one band when treated with cold. Double bands were expressed in groups 3, 10 and 13 after cold treatment. Groups 6, 9, 13 and 14 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population.

40 days - Snack expressed 14 protein bands (Table 19). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by Snack.

Although there were no significant differences between the total number of proteins produced, groups 1, 7 and 13 expressed an additional band each under cold treatment, while groups 3, 6 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 7, 10 and 13 after cold treatment, and in groups 3, 6, 10 and 12 in the control. Groups 4, 6, 9, 13 and 14 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to five in the treated material.

50 days - Snack expressed 14 protein bands (Table 19). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by Snack.

At 50 days one more protein band was expressed than in the control. Groups 1, 4 and 6 expressed an additional band each under cold treatment, while groups 3 and 10 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 6 and 13 after cold treatment, and in groups 3, 10 and 13 in the control. Groups 6, 9, 13 and 14 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population. The number of bands with a high density varied from four in the treated material to five in the control.

60 days - Snack expressed 13 protein bands (Table 19). The untreated sample expressed eight protein bands indicating that treatment had a significant effect on the total number of proteins produced by Snack.

At 60 days five more protein bands were expressed than in the control. Groups 1, 3, 6, 10, 11 and 13 expressed an additional band each under cold treatment, while group 5 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3 and 10 after cold treatment. Groups 6, 9, 13 and 14 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population.

SST 363

10 days - SST 363 expressed nine protein bands (Table 20). The untreated sample expressed eight protein bands indicating that treatment had an effect on the total number of proteins produced by SST 363.

At 10 days one more protein band was expressed than in the control. Groups 7 and 9 expressed an additional band each under cold treatment, while groups 8 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in group 7 after cold treatment, and in group 12 in the control. Groups 4, 6 and 9 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population.

20 days - SST 363 expressed seven protein bands (Table 20). The untreated sample expressed nine protein bands indicating that treatment had an effect on the total number of proteins produced by SST 363.

At 20 days two less protein bands were expressed than in the control. Group 12 expressed an additional band under cold treatment, while groups 1, 2 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in group 7 after cold treatment and in the control. Groups 3, 7 and 9 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to four in the treated material.

30 days - SST 363 expressed seven protein bands (Table 20). The untreated sample expressed 13 protein bands indicating that treatment had a significant effect on the total number of proteins produced by SST 363.

At 30 days six less protein bands were expressed than in the control. Group 9 expressed an additional band under cold treatment, while groups 3, 6, 8, 10 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in group 9 after cold treatment, and in groups 3, 8 and 11 in the control. Groups 3 and 7 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from two in the treated material to four in the control.

40 days - SST 363 expressed 13 protein bands (Table 20). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 363.

At 40 days one more protein band was expressed than in the control. Groups 1, 2 and 10 expressed an additional band each under cold treatment, while groups 3, 5 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1, 2, 9 and 10 after cold treatment, and in groups 9 and 11 in the control. Groups 2, 4 and 6 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to three in the treated material.

50 days - SST 363 expressed 11 protein bands (Table 20). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 363.

At 50 days one less protein band was expressed than in the control. Group 2 showed a reduction of band each when treated with cold. Double bands were expressed in group 4 after cold treatment and in the control. Groups 4 and 7 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population.

60 days - SST 363 expressed 13 protein bands (Table 20). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 363.

At 60 days one more protein band was expressed than in the control. Groups 1, 4, 5, 6 and 11 expressed an additional band each under cold treatment, while groups 2, 3 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4 and 13 after cold treatment, and in groups 2, 12 and 13 in the control. Groups 4, 6, 7 and 8 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to four in the treated material.

SST367

10 days - SST 367 expressed 12 protein bands (Table 21). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 367.

At 10 days one more protein band was expressed than in the control. Groups 8 and 11 expressed an additional band each under cold treatment, while group 9 showed a reduction of one band when treated with cold. Double bands were expressed in groups 6, 8 and 11 after cold treatment, and in group 6 in the control. The number of bands with a high density varied from 3 in the control to 5 in the treated material.

20 days - SST 367 expressed 13 protein bands (Table 21). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 367.

At 20 days two more protein bands were expressed than in the control. Group 4 expressed an additional band under cold treatment. Double bands were expressed in group 4 after cold treatment. Group 7 expressed a band of a higher intensity under treatment. The number of bands with a high density varied from no bands in the treated material to one in the control.

30 days - SST 367 expressed 13 protein bands (Table 21). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 367.

At 30 days two more protein bands were expressed than in the control. Groups 4, 6, 13 and 14 expressed an additional band each under cold treatment, while groups 3 and 9 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 4, 8 and 12 after cold treatment, and in group 8 in the control. Group 7 expressed a band of a higher intensity under treatment. The number of bands with a high density varied from no bands in the treated material to one in the control.

40 days - SST 367 expressed 15 protein bands (Table 21). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 367.

At 40 days two more protein bands were expressed than in the control. Groups 7, 9 and 12 expressed an additional band each under cold treatment, while group 6 showed a reduction of one band when treated with cold. Double bands were expressed in groups 4, 7, 9 and 12 after cold treatment, and in group 4 in the control. Group 7 expressed a band of a higher intensity in the control population. The number of bands with a high density varied from no bands in the treated material to one in the control.

50 days - SST 367 expressed 14 protein bands (Table 21). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by SST 367.

Although there were no significant differences between the total number of proteins produced, groups 1, 2 and 10 expressed an additional band each under cold treatment, while groups 8, 11 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 2 and 7 after cold treatment, and in groups 7, 8 and11 in the control. Group 9 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from one in the treated material to three in the control.

60 days - SST 367 expressed 11 protein bands (Table 21). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 367.

At 60 days one less protein band was expressed than in the control. Groups 1 and 2 expressed an additional band each under cold treatment, while groups 5, 9 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in group 2 after cold treatment, and in group 12 in the control. Groups 6 and 8 expressed a band of a higher intensity under treatment while only one bands showed high intensity in the control population. The number of bands with a high density varied from one in the control to two in the treated material.

SST 57

10 days - SST 57 expressed 11 protein bands (Table 22). The untreated sample expressed ten protein bands indicating that treatment had an effect on the total number of proteins produced by SST 57.

At 10 days one more protein band was expressed than in the control. Groups 8 and 10 expressed an additional band each under cold treatment, while group 12 showed a reduction of one band when treated with cold. Double bands were expressed in group 8 after cold treatment, and in group 12 in the control. Groups 1, 5, 6, 8, 12 and 13 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to six in the treated material.

20 days - SST 57 expressed 11 protein bands (Table 22). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by SST 57.

Although there were no significant differences between the total number of proteins produced, groups 5 and 11 expressed an additional band each under cold treatment, while groups 1 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 5 and 11 after cold treatment. Groups 3, 5, 6, 8 and 12 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to five in the treated material.

30 days - SST 57 expressed 14 protein bands (Table 22). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by SST 57.

Although there were no significant differences between the total number of proteins produced, groups 8 and 10 expressed an additional band each under cold treatment, while group 6 showed a reduction of two bands when treated with cold. Double bands were expressed in groups 7, 8, 10 and 12 after cold treatment, and in groups 6, 7 and 12. Groups 2, 5, 7 and 12 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population.

40 days - SST 57 expressed 12 protein bands (Table 22). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by SST 57.

Although there were no significant differences between the total number of proteins produced, group 10 expressed two additional bands under cold treatment, while groups 7 and 9 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 10 and 12 after cold treatment, and in group 7 in the control. Groups 2, 3, 5, 7, 8, 10 and 12 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to seven in the treated material.

50 days - SST 57 expressed ten protein bands (Table 22). The untreated sample expressed eight protein bands indicating that treatment had an effect on the total number of proteins produced by SST 57.

At 50 days two more protein bands were expressed than in the control. Groups 9, 10 and 12 expressed an additional band each under cold treatment, while group 1 showed a reduction of one band when treated with cold. Double bands were expressed in group 12 after cold treatment.

Groups 2, 3, 5, 7 and 12 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to five in the treated material.

60 days - SST 57 expressed 12 protein bands (Table 22). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 57.

At 60 days one less protein band was expressed than in the control. Group 10 expressed an additional band under cold treatment, while groups 1 and 3 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 8 and 12 after cold treatment, and in groups 3, 8 and 12 in the control. Groups 2, 3, 5, 7 and 12 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to five in the treated material.

SST 66

10 days - SST 66 expressed 11 protein bands (Table 23). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 66.

At 10 days two less protein bands were expressed than in the control. Groups 7 and 12 expressed an additional band each under cold treatment, while groups 1, 8, 9 and 11 showed a reduction of one band each when treated with cold. Double bands were expressed in group 10 after cold treatment, and in groups 8, 9 and 10 in the control.

20 days - SST 66 expressed 13 protein bands (Table 23). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 66.

At 20 days one more protein band was expressed than in the control. Groups 2, 9 and 11 expressed an additional band each under cold treatment, while groups 7 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3 and 11 after cold treatment, and in group 3 in the control.

30 days - SST 66 expressed nine protein bands (Table 23). The untreated sample expressed ten protein bands indicating that treatment had an effect on the total number of proteins produced by SST 66.

At 30 days one less protein band was expressed than in the control. Groups 7 and 13 expressed an additional band each under cold treatment, while groups 8, 9 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 11 and 13 after cold treatment, and in groups 8 and 11 in the control.

40 days - SST 66 expressed 12 protein bands (Table 23). The untreated sample expressed ten protein bands indicating that treatment had an effect on the total number of proteins produced by SST 66.

At 40 days two more protein bands were expressed than in the control. Groups 1, 5, 9 and 14 expressed an additional band each under cold treatment, while groups 11 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in group 9 after cold treatment, and in group 12 in the control. Group 8 expressed a band of a higher intensity in the control population. The number of bands with a high density varied from no bands in the treated material to one in the control.

50 days - SST 66 expressed ten protein bands (Table 23). The untreated sample expressed nine protein bands indicating that treatment had an effect on the total number of proteins produced by SST 66.

At 50 days one more protein band was expressed than in the control. Groups 1, 7 and 10 expressed an additional band each under cold treatment, while group 11 showed a reduction of one band when treated with cold.

60 days - SST 66 expressed 11 protein bands (Table 23). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 66.

At 60 days one less protein band was expressed than in the control. Group 1 expressed an additional band under cold treatment, while groups 10 and 12 showed a reduction of one band each when treated with cold. Group 5 expressed a band of a higher intensity under treatment while one band showed high intensity in the control population.

SST822

10 days - SST 822 expressed 16 protein bands (Table 24). The untreated sample expressed ten protein bands indicating that treatment had a significant effect on the total number of proteins produced by SST 822.

At 10 days six more protein bands were expressed than in the control. Groups 1, 2, 4, 5, 6, 12 and 13 expressed an additional band each under cold treatment. Double bands were expressed in groups 4, 5 and 12 after cold treatment. Groups 1, 3, 6, 12 and 14 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to five in the treated material.

20 days - SST 822 expressed 11 protein bands (Table 24). The untreated sample expressed 13 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 822.

At 20 days two more protein bands were expressed than in the control. Groups 5 and 10 expressed an additional band each under cold treatment, while groups 2, 6, 8 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 5 and 7 after cold treatment, and in group 7 in the control. Groups 1, 3, 7 and 14 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to four in the treated material.

30 days - SST 822 expressed 15 protein bands (Table 24). The untreated sample expressed ten protein bands indicating that treatment had a significant effect on the total number of proteins produced by SST 822.

At 30 days five more protein bands were expressed than in the control. Groups 1, 3, 4, 5, 11 and 13 expressed an additional band each under cold treatment, while groups 8, 10 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 1, 5 and 11 after cold treatment, and in group 8 and 12 in the control. Groups 3, 5 and 14 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to three in the treated material.

40 days - SST 822 expressed 13 protein bands (Table 24). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by SST 822.

Although there were no significant differences between the total number of proteins produced, groups 4 and 8 expressed an additional band each under cold treatment, while groups 1, 2 and 6 showed a reduction of one band each when treated with cold. Double bands were expressed in

groups 7 and 8 after cold treatment, and in groups 6 and 7 in the control. Groups 3, 5, 12 and 14 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population.

50 days - SST 822 expressed 11 protein bands (Table 24). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 822.

At 50 days one less protein band was expressed than in the control. Groups 4 and 7 expressed an additional band each under cold treatment, while groups 5 and 8 showed a reduction of one band each when treated with cold. Double bands were expressed in group 7 after cold treatment, and in group 8 in the control. Groups 3, 6, 7 and 14 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to four in the treated material.

60 days - SST 822 expressed ten protein bands (Table 24). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 822.

At 60 days two less protein bands were expressed than in the control. Groups 5 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 5 and 12 in the control. Groups 6 and 8 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population.

SST 825

10 days - SST 825 expressed 12 protein bands (Table 25). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 825.

At 10 days one more protein band was expressed than in the control. Groups 4, 8 and 13 expressed an additional band each under cold treatment, while groups 1 and 2 showed a reduction of one band each when treated with cold. Double bands were expressed in group 4 after cold treatment. Groups 3, 7, 8, 9 and 10 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to five in the treated material.

20 days - SST 825 expressed 13 protein bands (Table 25). The untreated sample expressed six protein bands indicating that treatment had a significant effect on the total number of proteins produced by SST 825.

At 20 days seven more protein bands were expressed than in the control. Groups 2, 3, 5, 6, 8, 12 and 13 expressed an additional band each under cold treatment. Groups 5, 7, 8, 9 and 10 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to five in the treated material.

30 days - SST 825 expressed 14 protein bands (Table 25). The untreated sample expressed six protein bands indicating that treatment had a significant effect on the total number of proteins produced by SST 825.

At 30 days eight more protein bands were expressed than in the control. Groups 2, 3, 5, 6, 8, 12, 13 and 14 expressed an additional band each under cold treatment. Double bands were expressed in group 14 after cold treatment. Groups 2, 3, 5, 6, 7, 8, 9 and 10 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to eight in the treated material.

40 days - SST 825 expressed 12 protein bands (Table 25). The untreated sample expressed six protein bands indicating that treatment had a significant effect on the total number of proteins produced by SST 825.

At 40 days six more protein bands were expressed than in the control. Groups 3, 12, 13 and 14 expressed an additional band each under cold treatment. Double bands were expressed in groups 3 and 14 after cold treatment. Groups 4, 7, 8, 9 and 10 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to five in the treated material.

50 days - SST 825 expressed 13 protein bands (Table 25). The untreated sample expressed eight protein bands indicating that treatment had a significant effect on the total number of proteins produced by SST 825.

At 50 days five more protein bands were expressed than in the control. Groups 3, 5, 8, 13 and 14 expressed an additional band each under cold treatment, while group 1 showed a reduction of one band when treated with cold. Double bands were expressed in group 3 after cold treatment.

Groups 3, 4, 5, 7, 9, 10, 11 and 14 expressed a band of a higher intensity under treatment while three bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to nine in the treated material.

60 days - SST 825 expressed 12 protein bands (Table 25). The untreated sample expressed six protein bands indicating that treatment had a significant effect on the total number of proteins produced by SST 825.

At 60 days six more protein bands were expressed than in the control. Groups 3, 5, 6, 7, 13 and 14 expressed an additional band each under cold treatment, while group 1 showed a reduction of one band when treated with cold. Double bands were expressed in group 14 after cold treatment. Groups 3, 5, 8, 9, 10, 11 and 14 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to seven in the treated material.

SST 966

10 days - SST 966 expressed 12 protein bands (Table 26). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by SST 966.

Although there were no significant differences between the total number of proteins produced, groups 9 and 11 expressed an additional band each under cold treatment, while groups 7 and 10 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 9 and 11 after cold treatment. Groups 4, 5, 8 and 9 expressed a band of a higher intensity under treatment while six bands showed high intensity in the control population. The number of bands with a high density varied from five in the treated material to six in the control.

20 days - SST 966 expressed ten protein bands (Table 26). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 966.

At 20 days two less protein bands were expressed than in the control. Groups 1 and 9 expressed an additional band each under cold treatment, while groups 7 and 10 showed a reduction of one band each, and group 13 the reduction of two bands, when treated with cold. Double bands were expressed in group 9 after cold treatment, and in group 13 in the control. Groups 4, 8, 9 and 11 expressed a band of a higher intensity under treatment while six bands showed high intensity in the control population. The number of bands with a high density varied from five in the treated material to six in the control.

30 days - SST 966 expressed 11 protein bands (Table 26). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by SST 966.

At 30 days one less protein band was expressed than in the control. Groups 1 and 9 expressed an additional band each under cold treatment, while groups 3 and 7 showed a reduction of one band each when treated with cold. Double bands were expressed in group 9 after cold treatment. Groups 4, 8 and 9 expressed a band of a higher intensity under treatment while six bands showed high intensity in the control population. The number of bands with a high density varied from four in the treated material to six in the control.

40 days - SST 966 expressed 11 protein bands (Table 26). The untreated sample expressed ten protein bands indicating that treatment had an effect on the total number of proteins produced by SST 966.

At 40 days one more protein band was expressed than in the control. Groups 1 and 6 expressed an additional band each under cold treatment, while group 13 showed a reduction of one band when treated with cold. Double bands were expressed in group13 in the control. Groups 4, 7, 8, 9 and 12 expressed a band of a higher intensity under treatment while six bands showed high intensity in the control population. The number of bands with a high density varied from five in the treated material to six in the control.

50 days - SST 966 expressed nine protein bands (Table 26). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by SST 966.

At 50 days six more protein bands were expressed than in the control. Groups 5, 9 and 10 expressed an additional band each under cold treatment, while groups 1, 4 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in group 9 after cold treatment, and in group 4 in the control. Groups 4, 5, 7, 8 and 9 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from four in the control to six in the treated material.

60 days - SST 966 expressed eight protein bands (Table 26). The untreated sample expressed ten protein bands indicating that treatment had an effect on the total number of proteins produced by SST 966.

At 60 days two less protein bands were expressed than in the control. Group 9 expressed an additional band under cold treatment, while groups 3 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in group 9 after cold treatment, and in group 12 in the control. Groups 4, 5, 7, 8 and 9 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population. The number of bands with a high density varied from five in the control to six in the treated material.

Tugela DN

10 days - Tugela DN expressed 11 protein bands (Table 27). The untreated sample had the same number protein bands indicating that treatment had no significant effect on the total number of proteins produced by Tugela DN.

Although there were no significant differences between the total number of proteins produced, groups 4 and 14 expressed an additional band each under cold treatment, while groups 3 and 5 showed a reduction of one band each when treated with cold. Double bands were expressed in group 3 in the control. Groups 3, 6 and 8 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population. The number of bands with a high density varied from three in the control to five in the treated material.

20 days - Tugela DN expressed 14 protein bands (Table 27). The untreated sample expressed 12 protein bands indicating that treatment had an effect on the total number of proteins produced by Tugela DN.

At 20 days two more protein bands were expressed than in the control. Groups 3, 6, 12 and 14 expressed an additional band each under cold treatment, while groups 2, 7, 8 and 13 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 3, 6 and 12 after cold treatment, and in groups 7, 8 and 13 in the control. Groups 3, 5, 6, 7, 8 and 12 expressed a band of a higher intensity under treatment while two bands showed high intensity in the control population. The number of bands with a high density varied from two in the control to six in the treated material.

30 days - Tugela DN expressed 12 protein bands (Table 27). The untreated sample had the same number bands indicating that treatment had no significant effect on the total number of proteins produced by Tugela DN.

Although there were no significant differences between the total number of proteins produced, groups 8 and 11 expressed an additional band each under cold treatment, while groups 2 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in

groups 5, 8 and 10 after cold treatment, and in groups 5 and 10 in the control. Groups 3, 5, 9 and 13 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population.

40 days - Tugela DN expressed 14 protein bands (Table 27). The untreated sample expressed 11 protein bands indicating that treatment had a significant effect on the total number of proteins produced by Tugela DN.

At 40 days three more protein bands were expressed than in the control. Groups 2, 6, 10 and 13 expressed an additional band each under cold treatment, while groups 8 and 14 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 5, 10 and 13 after cold treatment, and in groups 5 and 8 in the control. Groups 3 and 13 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population. The number of bands with a high density varied from three in the treated material to four in the control.

50 days - Tugela DN expressed 12 protein bands (Table 27). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the total number of proteins produced by Tugela DN.

At 50 days one more protein band was expressed than in the control. Groups 8 and 9 expressed an additional band each under cold treatment, while group 10 showed a reduction of one band each when treated with cold. Double bands were expressed in group 8 after cold treatment, and in group 10 in the control. Groups 3, 5, 13 and 14 expressed a band of a higher intensity under treatment while four bands showed high intensity in the control population.

60 days - Tugela DN expressed ten protein bands (Table 27). The untreated sample expressed 11 protein bands indicating that treatment had an effect on the total number of proteins produced by Tugela DN.

At 60 days one less protein band was expressed than in the control. Groups 5, 6 and 8 expressed an additional band each under cold treatment, while groups 2, 7, 9 and 12 showed a reduction of one band each when treated with cold. Double bands were expressed in groups 5 and 6 after cold treatment, and in group 9 in the control. Groups 3, 6, 8, 13 and 14 expressed a band of a higher intensity under treatment while five bands showed high intensity in the control population.

Number of bands expressed

The four most cold tolerant wheat cultivars and the four most cold sensitive or susceptible wheat cultivars according to studies done by Jacobs (1999) were selected. The four most cold tolerant cultivars were Betta Caledon, Molen, and Tugela DN and the four most cold susceptible cultivars were Palmiet, Snack, SST 825 and Tugela DN. The number of bands expressed in each group in these respective cultivars are showed in the following tabels.

Tabel 5.2 The number of bands expressed in Betta (tolerant to cold)

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	2	9	4	5	6	6	5	8	5	9	3	7	5	0
Control	4	6	5	5	7	6	6	6	5	4	5	5	5	1

Tabel 5.3 The number of bands expressed in Caledon (tolerant to cold)

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	8	5	7	6	4	8	6	9	8	5	4	6	1
Control	0	6	5	5	6	1	6	12	6	6	2	6	6	1

Tabel 5.4 The number of bands expressed in Gariep (tolerant to cold)

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	6	5	7	0	5	5	6	6	8	3	2	8	5	7
Control	3	3	6	3	5	5	6	6	8	5	5	6	6	8

Tabel 5.5 The number of bands expressed in Molen (tolerant to cold)

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	6	5	7	3	8	6	7	6	5	5	6	6	3
Control	8	2	8	6	6	4	6	8	7	2	6	6	6	2

Tabel 5.6 The number of bands expressed in Palmiet (susceptible to cold)

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	6	6	4	7	7	5	5	6	5	5	5	7	6	9
Control	6	8	1	8	5	8	5	7	5	2	6	5	8	9

Tabel 5.7 The number of bands expressed in Snack (susceptible to cold)

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	2	6	3	4	6	6	6	6	6	4	5	6	6
Control	0	1	6	2	4	5	6	6	6	6	2	5	4	6

Tabel 5.8 The number of bands expressed in SST 825 (susceptible to cold)

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	2	6	6	5	3	6	6	6	6	6	5	6	6
Control	3	2	1	6	1	0	5	3	6	6	6	1	0	3

Tabel 5.9 The number of bands expressed in Tugela DN (susceptible to cold)

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	2	6	1	5	6	4	6	6	6	5	3	6	5
Control	0	4	6	0	6	4	5	5	5	5	4	4	6	4

When the number of bands gained and the number of bands lost in all the groups for the different cultivars were calculated, the following were observed:

Palmiet gained 10 bands, but two bands were lost across the 14 groups.
Snack gained 16 bands and lost one bands across the 14 groups.
SST 825 gained 37 bands, but lost three bands across 14 groups.
Tugela DN gained 14 bands, but lost two bands across the 14 groups.

The more tolerant cultivars reacted as follows:

Betta gained 11 bands, but lost nine bands across the 14 groups.
Caledon gained 23 bands, but lost eight bands across 14 groups.
Gariep gained eight bands, but lost 10 bands across the 14 groups.
Molen gained 11 bands, but lost nine bands across the 14 groups.

The susceptible cultivars (except Palmiet) gained more bands across the 14 groups. Palmiet gained and lost the same number of bands across the groups. The tolerant cultivars (except Molen) also gained more bands across the groups. Molen lost and gained the same number of bands across the groups.

The differences between the number of bands expressed with the cold treatment and in the control were calculated for each group. The following groups showed clear differences between the cold tolerant and sensitive or susceptible cultivars.

Group 3
The cold sensitive cultivars had no change or gained bands with cold treatment, while the cold tolerant cultivars had no change or they lost bands with cold treatment. The only exception was Gariep (tolerant) that gained one band.

Group 5
The cold sensitive cultivars had no change or gained bands with cold treatment, while the cold tolerant cultivars had no change or they lost bands with cold treatment.

Group 13
The cold sensitive cultivars had no change or gained bands with cold treatment, while the cold tolerant cultivars had no change or they lost bands with cold treatment. The only exception was Palmiet (susceptible) that lost one band, and Caledon (tolerant) that gained three bands

The other cultivars (with known cold tolerance) that were used in this study, were also evaluated to see how they reacted in groups 3, 5 and 13. The cold susceptible cultivars according to Jacobs (1999) were Adam Tas, PAN 3211, Limpopo and SST 822. The cold tolerant cultivars were SST 363, SST 66 and PAN 3349.

Group 3

PAN 3211 and SST 822 (cold susceptible) gained bands, while Adam Tas and Limpopo lost bands. If one looks at the result of Jacobs (1999) it is seen that SST 363 and PAN 3349 fall just outside the more tolerant group of cultivars, and both these cultivars lost bands SST 66 showed no change in the number of bands

Group 5

All four the cold susceptible cultivars lost bands, except PAN 3211 which showed no change in the number of bands. SST 363 and PAN 3349 also showed no change in the number of bands and SST 66 gained bands. SST 363 and PAN 3349 fall just outside the more tolerant group of cultivars and it could be that this cultivars reacted more like the cold susceptible cultivars.

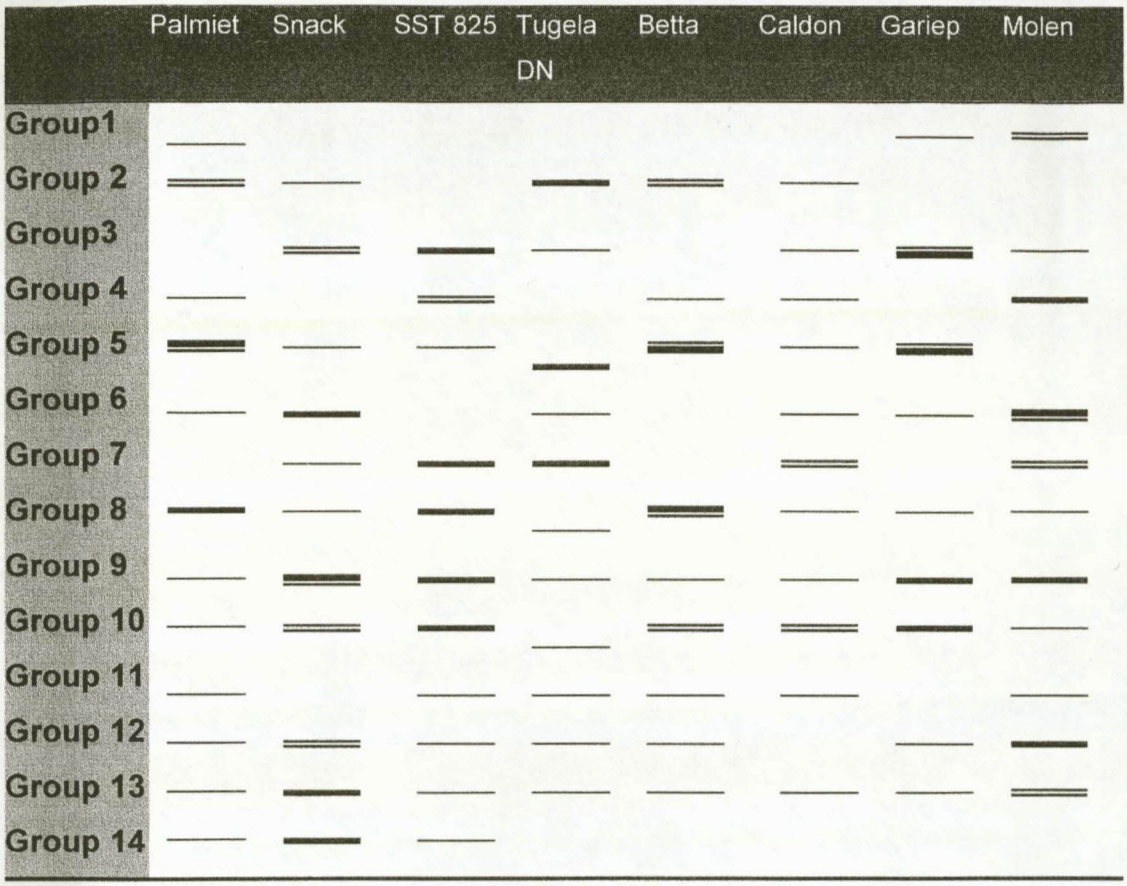
Group 13

The cold susceptible cultivars had no change in the number of bands or they lost bands except for SST 822 which gained bands. SST 66 had no change in the number of bands, SST 363 gained a band and PAN 3349 lost one band.

The expression of the different bands

The four cold susceptible and four cold tolerant cultivars were compared with each other to see what group or groups were the most important for each cold treatment. The number of bands, the number of dark bands and the number of double bands were observed to see if there were any differences between the susceptible and the tolerant cultivars.

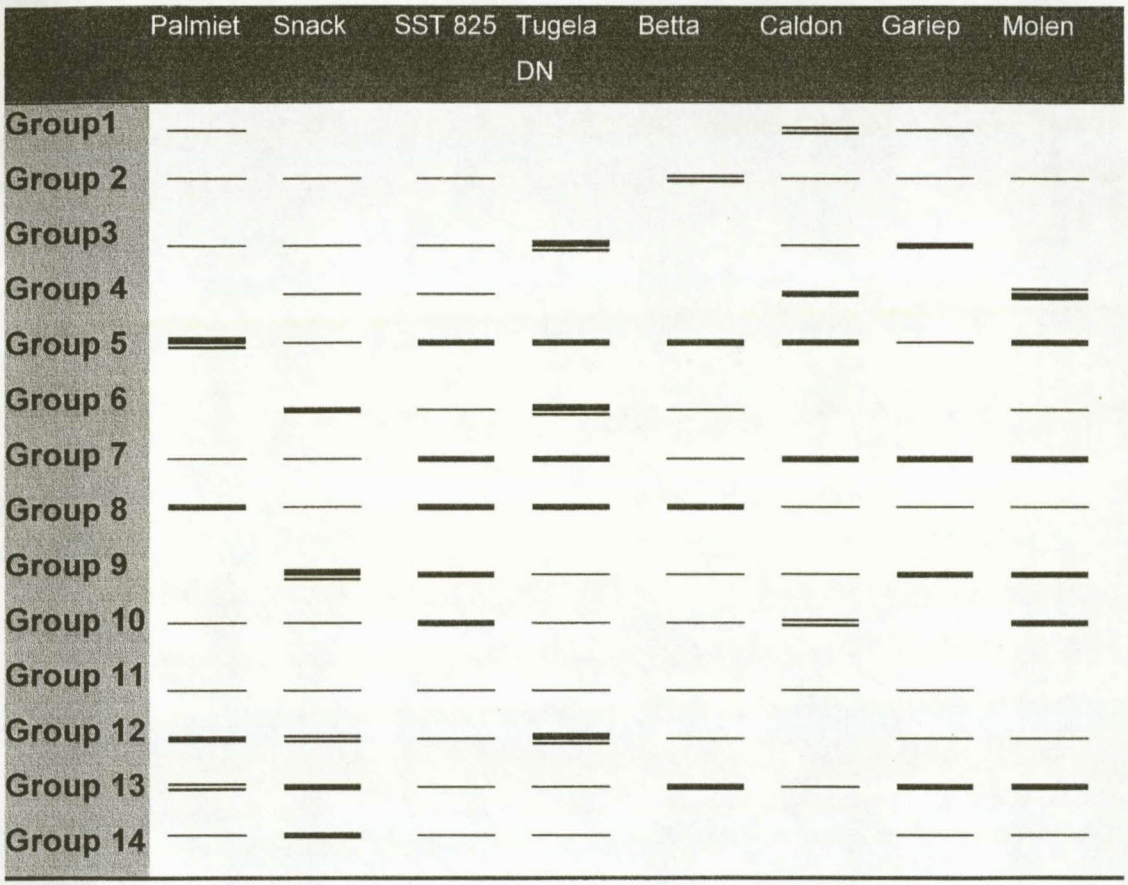
Tabel 5.10 The bands expressed with 10 days cold treatment in four cold sensitive and four cold tolerant wheat cultivars



There were no differences between the average number of bands expressed for the cold susceptible and the cold tolerant cultivars. The average number of dark bands expressed were higher in the cold sensitive than the cold tolerant cultivars.

As dark bands are easy to select for, it was decided to see in which group or groups, more than two dark bands were observed for the susceptible and the tolerant cultivars. Group 3, group 6, group 8 and group 9 had two or more dark bands in the cold susceptible cultivars. Group 5 and group 9 had two or more bands in the cold tolerant cultivars after 10 days cold treatment.

Tabel 5.11 The bands expressed with 20 days cold treatment in four cold sensitive and four cold tolerant wheat cultivars



There were no clear differences between the average number of bands expressed for the cold susceptible and the cold tolerant cultivars

Groups 5, 6, 7, 8, 9 and 12 had two or more dark bands in the cold susceptible cultivars. Groups 4, 5, 7, 9 and 13 had two or more bands in the cold tolerant cultivars after 20 days cold treatment.

Tabel 5.12 The bands expressed with 30 days cold treatment in four cold sensitive and four cold tolerant wheat cultivars

There were no clear differences between the average number of bands expressed for the cold susceptible and the cold tolerant cultivars. The average number of dark bands expressed were higher in the cold sensitive than the cold tolerant cultivars.

Groups 3, 5, 6, 9, 13 and 14 had two or more dark bands in the cold susceptible cultivars. Group 4 and group 8 had two or more bands in the cold tolerant cultivars after 30 days cold treatment.

Tabel 5.13 The bands expressed with 40 days cold treatment in four cold sensitive and four cold tolerant wheat cultivars

There were no clear differences between the average number of bands expressed for the cold susceptible and the cold tolerant cultivars.

Group 4, group 6 and group 10 had two or more dark bands in the cold susceptible cultivars. Group 3, group 5 and group 6 had two or more bands in the cold tolerant cultivars after 40 days cold treatment.

Tabel 5.14 The bands expressed with 50 days cold treatment in four cold sensitive and four cold tolerant wheat cultivars

There were no clear differences between the average number of bands expressed for the cold susceptible and the cold tolerant cultivars. The average number of dark bands expressed were higher in the cold sensitive than the cold tolerant cultivars. The average number of single bands expressed were higher in the cold tolerant than the cold sensitive cultivars.

Groups 3, 5, 9, 13 and 14 had two or more dark bands in the cold susceptible cultivars. Groups 3, 6, 7, 9 and 11 had two or more bands in the cold tolerant cultivars after 50 days cold treatment.

Tabel 5.15 The bands expressed with 60 days cold treatment in four cold sensitive and four cold tolerant wheat cultivars

There were no clear differences between the average number of bands expressed for the cold susceptible and the cold tolerant cultivars. The average number of single bands expressed were higher in the cold tolerant than the cold sensitive cultivars.

Groups 3, 6, 8, 9, 13 and 14 had two or more dark bands in the cold susceptible cultivars. Group 7 and group 10 had two or more bands in the cold tolerant cultivars after 60 days cold treatment.

The following groups had two or more dark bands in the susceptible or tolerant cultivars: 3, 4, 5, 6, 7, 8, 9, 13 and 14. It is better to select for cold susceptibility, because of the more abundant presence of dark bands in the susceptible cultivars. If one looks at the presence of dark bands, it is important to note the following:

- Group 3 accumulated in the susceptible and the tolerant cultivars after long periods (longer than 30 days) cold treatment.
- Group 4 accumulated after short periods of cold treatment (shorter than 30 days) in the tolerant cultivars. The susceptible cultivars had this band only after 40 days of cold treatment.

- Group 5 accumulated after all the cold treatments in the tolrant cultivars. It is, however, only present in the susceptible cultivars after 20, 30 and 50 days cold treatment. It is thus possible to use this band to select for tolerance, but only at certain periods of cold treatment.
- Group 6 is present in all the susceptible cultivars and can thus be used to select for cold susceptibility. At 40 days cold treatment it is also present in the cold tolrant cultivars and only this specific treatment can therefore not be used to select for susceptibility.
- Group 7 accumulated in the susceptible and tolerant cultivars after short periods of cold treatment. After long periods of cold treatment (50 and 60 days) it is only present in the cold tolrant cultivars. It is therefore possible to use this band to select for cold tolrance after long periods of cold treatment.
- Group 8 was present in the susceptible cultivars after 10, 20 and 60 days cold treatment and in the tolerant cultivars after 30 and 50 days cold treatment.
- Group 9 was also correlated with cold treatment, as it is present in all the susceptible cultivars and also in the tolrant cultivars after 10, 20 and 50 days cold treatment.
- Group 13 can be used to screen for susceptibility to cold, as it is present in the susceptible cultivars. It is only present after 20 days cold treatment in the tolerant cultivars.
- Group 14 can also be used to screen for susceptibility to cold, as it is only present in the susceptible cultivars.

Sarhan and Perras (1987), Perras and Sarhan (1989) and Abromeit *et al* (1992) found that the intensity of three protein bands (48, 47 and 42 kDa) increased during cold hardening. In this study it was found that the intensity of the protein bands of 48 and 47 kDa (group 6) increased in the susceptible cultivars. After 40 days of cold treatment, there were also an increase in the intensity of this protein bands in the tolerant cultivars. The intensity of group 7 (42 kDa) increased in the susceptible and tolerant cutivars. After long periods of cold treatment, the intensity increased only in the tolerant cultivars. They also found that the intensity of protein bands of 67 and 63 kDa decreased. In this study it was found that the number of bands of group 2 (67 kDa) decreased in the susceptible cultivars after cold treatment, while the number of bands increased in the cold tolerant cultivars. There were however, no change in the intensity of the bands The intensity of the protein bands of 63 kDa (group 3) increased in the cold susceptible and the tolerant cultivars after long periods of cold treatment.

Zhou *et al* (1994) found a decrease in the proteins with a molecular weight of 22 - 31 kDa. This was, however, not found in this study. Proteins with a molecular weight of 28 - 32.5 kDa (group 9) increased in the susceptible cultivars after cold treatment and there were also an increase in the protein band intensity in the tolerant cultivars at 10, 20 and 50 days. It is therefore correlated with cold treatment. There were no definite increase or decrease in the intensities of the protein

bands with a molecular weight of 22 – 27 kDa, as in some cutivars an increase was observed and in other cultivars a decrease was observed.

Jacobs (1999) found that proeins with a molecular weight of 44 (group 6), 43 (group 7), 38 (group 8) and 20 (group 11) kDa were produced in response to cold hardening. In this study it was found that group 3, group 4, group 5, group 7 and group 9 were produced in the roots of wheat cutivars in response to cold hardening in both the susceptible and the tolerant cultivars. The susceptible cultivars furthermore, produced proteins in group 6, group 13 and group 14 in response to cold hardening.

The presence of dark bands (thus bands with a higher intensity) was more abundant in the susceptible cultivars. In most of the treatments the ratio of dark bands in the susceptible cultivars to the tolerant cultivars, was two to one. It can be that the proteins in the roots of susceptible cultivars reacted more severely than the proteins in the roots of tolerant cultivars. It is also possible that the susceptible cultivars produce more proteins in their roots, while tha tolerant cultivars produce only proteins that make themmore tolerant to cold conditions, thus proteins in group 5, group 7 and group 9. It is further also possible that the production of certain proteins can alter the effect of other more cold tolerant proteins.

Chapter 6

CORRELATIONS BETWEEN PROTEIN BANDS IN THE COLEOPTILES AND ROOTS OF WHEAT SEEDLINGS

7.1 Correlations between protein bands in the coleoptiles of wheat seedlings

Correlations between protein bands expressed at 10 days cold treatment

The following significant correlations were observed between the protein bands ($p<0.05$):

With 10 days cold treatment, groups 2, 8, 9. 10 and 12 had a change in the amount of dark or double bands (where one of the bands was dark) in the susceptible cultivars. Groups 4, 6, 7, 8 and 9 had an increase in these bands in the cold tolerant cultivars after cold treatment.

- Cold treatment was positively correlated with group 3 ($r=0.5$).
- Group 1 was positively correlated with group 8 ($r=0.4$) and with group 12 ($r=0.42$).
- Group 3 was positively correlated with group 13 ($r=0.39$).
- Group 4 was negatively correlated with group 13 ($r=-0.44$).
- Group 5 was positively correlated with group 13 ($r=0.45$).
- Group 12 was negatively correlated with group 14 ($r=-0.4$).

Correlations between protein bands expressed at 20 days cold treatment

The following significant correlations were observed between the protein bands ($p<0.05$):

- Group 2 was positively correlated with group 7 ($r=0.4$).
- Group 3 was negatively correlated with group 4 ($r=-0.45$).
- Group 4 was positively correlated with group 10 ($r=0.4$) and negatively correlated with group 13 ($r=-0.43$).
- Group 5 was negatively correlated with group 6 ($r=-0.43$) and positively correlated with group 13 ($r=0.45$).
- Group 7 was negatively correlated with group 13 ($r=-0.41$).

Correlations between protein bands expressed at 30 days cold treatment

The following significant correlations were observed between the protein bands ($p<0.05$):

- Group 1 was positively correlated with group 4 ($r=0.45$).
- Group 2 was positively correlated with group 3 ($r=0.4$) and with group 9 ($r=0.4$).
- Group 3 was negatively correlated with group 4 ($r=-0.5$).
- Group 5 was positively correlated with group 10 ($r=0.5$) and with group 13 ($r=0.51$).
- Group 8 was positively correlated with group 9 ($r=0.42$) and with group 11 ($r=0.44$).
- Group 12 was negatively correlated with group 13 ($r=-0.46$).

Correlations between protein bands expressed at 40 days cold treatment

The following significant correlations were observed between the protein bands ($p<0.05$):

Cold treatment was positively correlated with group 4 ($r=0.46$) and with group 10 ($r=0.47$).

- Group 1 was positively correlated with group 11 ($r=0.57$).
- Group 2 was negatively correlated with group 13 ($r=-0.53$).
- Group 3 was negatively correlated with group 4 ($r=-0.49$).
- Group 4 was positively correlated with group 7 ($r=0.48$) and with group 10 ($r=0.42$).
- Group 5 was positively correlated with group 11 ($r=0.44$).
- Group 6 was positively correlated with group 9 ($r=0.66$).
- Group 7 was negatively correlated with group 9 ($r=-0.4$).
- Group 8 was positively correlated with group 10 ($r=0.48$).
- Group 10 was positively correlated with group 14 ($r=0.4$)
- Group 12 was negatively correlated with group 13 ($r=-0.45$) and with group 14 ($r=-0.48$)

Correlations between protein bands expressed at 50 days cold treatment

The following significant correlations were observed between the protein bands ($p<0.05$):

Cold treatment was positively correlated with group 1 ($r=0.45$).

- Group 2 was negatively correlated with group 5 ($r=-0.39$) and positively correlated with group 12 ($r=0.4$).
- Group 3 was negatively correlated with group 4 ($r=-0.56$).
- Group 5 was negatively correlated with group 12 ($r=-0.4$) and positively correlated with group 13 ($r=0.41$).
- Group 8 was negatively correlated with group 9 ($r=-0.38$).
- Group 9 was positively correlated with group 14 ($r=0.54$).
- Group 10 was negatively correlated with group 11 ($r=-0.4$).

Correlations between protein bands expressed at 60 days cold treatment

The following significant correlations were observed between the protein bands ($p<0.05$):

- Group 1 was negatively correlated with group 9 ($r=-0.39$).
- Group 3 was negatively correlated with group 4 ($r=-0.5$).
- Group 4 was positively correlated with group 10 ($r=0.46$).
- Group 6 was negatively correlated with group 7 ($r=-0.44$).
- Group 8 was positively correlated with group 13 ($r=0.4$)

The correlations indicated that the presence of some bands supressed the expression of other bands.

With the coleoptiles, it was found that certain groups were more important in the cold susceptible cultivars, while others were more important in the tolerant cultivars.

With 10 days cold treatment, groups 5 and 14 had a change in the amount of dark or double bands (where one of the bands was dark) in the susceptible cultivars. Groups 4, 7, 8, 10 and 11 had an increase in these bands in the cold tolerant cultivars after cold treatment. It was found that group 5 could be used to screen for susceptability in 10 days. If one looks at the correlations, group 5 was positively correlated with group 13. That means that if a cultivar does not have protein bands in group 5 at 10 days cold treatment, one could screen for susceptability by looking at the presence of group 13. Group 4 and 7 could be used to screen for cold tolerance at 10 days. If one looks at the correlations, group 4 was negatively correlated with group 13.

With 20 days cold treatment, groups 5, 8 and 13 had a change in the amount of dark or double bands (where one of the bands was dark) in the susceptible cultivars. Groups 3, 4, 7, 8, 10 and 11 had an increase in these bands in the cold tolerant cultivars after cold treatment. It was found that group 5 and 13 could be used to screen for susceptability in 20 days. If one looks at the correlations, group 5 was negatively correlated with group 6. That means that if a cultivar does not have protein bands in group 5 at 10 days cold treatment, one could screen for tolerance by looking at the presence of group 6. Group 13 was negatively correlated with group 4 and group 7 thus one could screen for tolerance by looking at the presence of groups 4 and 7 at 10 days. It was found that group 4, 7 and 10 could be used to screen for tolerance in 20 days. Group 4 was negatively correlated with group 3, which means that in the absence of group 4 group 3 could be used to screen for susceptability at 10 days. Group 7 was positively correlated with group 2, which means that in the absence of group 7 group 2 could be used to screen for tolerance at 10 days.

With 30 days cold treatment, groups 5, 8, 13 and 14 had a change in the amount of dark or double bands (where one of the bands was dark) in the susceptible cultivars. Groups 4, 5, 7, 10 and 11 had an increase in these bands in the cold tolerant cultivars after cold treatment. It was found that group 13 could be used to screen for susceptability, and groups 4 and 7 to screen for tolerance at 30 days. If one looks at the correlations, group 13 was positively correlated with group 5 and negatively correlated with group 12. This could mean that in the absence of group 13, group 5 could be used to screen for susceptability and group 12 could be used to screen for tolerance at 30 days. Group 4 was positively correlated with group 1 and negatively correlated with group 3. This could mean that in the absence of group 4 group 1 could be used to screen for tolerance and group 3 to screen for susceptability at 30 days.

With 40 days cold treatment, groups 5, 8, 11 and 13 had a change in the amount of dark or double bands (where one of the bands was dark) in the susceptible cultivars. Groups 4, 7, 8, 10 and 14 had an increase in these bands in the cold tolerant cultivars after cold treatment. It was found that group 5 and 13 could be used to screen for susceptability, and groups 4, 7 and 10 to screen for tolerance, at 40 days. If one looks at the correlations, group 5 was positively correlated with group 11, which means that group 11 could be used to screen for susceptability in the absence of group 5. Group 13 was negatively correlated with group 2, which means that group 2 could be used to screen for tolerance in the absence of group 13. Group 4 was negatively correlated with group 3 and positively correlated with group 10. This indicates that group 3 could be used to screen for susceptability, and group 10 to screen for tolerance, in the absence of group 4. Group 7 was negatively correlated with group 9, and thus group 9 could be used to screen for susceptability in the absence of group 7 at 40 days. Group 10 was positively correlated with group 14 which means that group 14 could be used to screen for tolerance at 40 days in the absence of group 10.

With 50 days cold treatment, groups 5, 13 and 14 had a change in the amount of dark or double bands (where one of the bands was dark) in the susceptible cultivars. Groups 4, 5, 7, 9 and 14 had an increase in these bands in the cold tolerant cultivars after cold treatment. It was found that group 5 and 13 could be used to screen for susceptability, and group 4 and group 7 to screen for tolerance, at 50 days. If one looks at the correlations, group 5 was negatively correlated with group 2 and group 12, and positively correlated with group 13. This indicates that groups 2 and 12 could be used to screen for tolerance, and group 13 to screen for susceptability, in the absence of group 5. Group 4 was negatively correlated with group 3 which means that group 3 could be used to screen for susceptability in the absence of group 4.

With 60 days cold treatment, groups 5 and 8 had a change in the amount of dark or double bands (where one of the bands was dark) in the susceptible cultivars. Groups 4, 7 and 10 had an increase in these bands in the cold tolerant cultivars after cold treatment. It was found that group 5 could be used to screen for susceptability, and groups 4, 7 and 10 to screen for tolerance, at 60 days. If one looks at the correlations, group 4 was negatively correlated with group 3, and positively correlated with group 10 at 60 days. This indicates that group 3 could be used to screen for susceptability, and group 10 to screen for tolerance, in the absence of group 4. Group 7 was negatively correlated with group 6 which means that in the absence of group 7 group 6 could be used to screen for susceptability to cold at 60 days.

According to the correlations calculated by the Spearman-rank, there are four different protein bands in the coleoptiles of wheat seedlings, that is positively correlated with cold. The expression of these four bands in the different wheat cultivars is summarised in Tabel 7.1.

Tabel 7.1 The expression of possitively correlated protein bands in the coleoptiles of different wheat cultivars.

	Group 3 at 10 days cold	Group 4 at 40 days cold	Group 10 at 40 days cold	Group 1 at 50 days cold
Adam Tas	SB	X	SB	X
Betta	X	DDB	SB	X
Betta DN	DB	SB	BB	BB
Caledon	SB	DB	BB	SB
Chinese Spring	SB	BB	SB	X
Gamtoos	X	X	SB	X
Gariep	X	X	SB	SB
Hugenoot	SB	DDB	SB	SB
Letaba	X	X	SB	X
Limpopo	DB	X	SB	SB
Molen	SB	DB	DDB	SB
Molopo	DB	SB	SB	SB
Nantes	SB	SB	SB	SB
Palmiet	SB	SB	BB	BB
PAN3211	SB	X	X	X
PAN3349	SB	SB	BB	SB
PAN3377	SB	SB	DB	X
Scheepers 69	BB	X	SB	X
Snack	DDB	X	SB	SB
SST 363	BB	SB	SB	X
SST 367	SB	SB	SB	SB
SST 57	BB	SB	SB	X
SST 66	SB	X	X	X
SST 822	DB	SB	SB	X
SST 875	X	SB	DB	X
SST 966	BB	BB	BB	X
Tugela DN	X	DB	SB	SB

SB – single band

BB – double band

DB – dark band

DBB – dark double band

It can be seen from Tabel 7.1 that the protein bands expressed the most in the different winter wheat cultivars are Group 4 at 40 days cold (83%), and Group 10 at 40 days cold (100%). This protein bands could be 55.5 - 60 kDa and 22.5 - 27 kDa respectively. The protein bands expressed the most in the different spring wheat cultivars are Group 3 at 10 days cold (80%), and group 10 at 40 days cold (90%). These bands could be 61 – 65.5 kDa and 22.5 - 27 kDa respectively. The protein bands expressed the most in the different facultative wheat cultivars were also Group 3 at 10 days cold (80%), and Group 10 at 40 days cold (80%). The protein bands correlated with cold that were expressed the most in all the different wheat cultivars was Group 3 at 10 days (77%) and Group 10 at 40 days cold.

7.2 Correlations between protein bands in the roots of wheat seedlings

Correlations between protein bands expressed at 10 days cold treatment

The following significant correlations were observed between the protein bands ($p<0.05$):

- Cold treatment was positively correlated with group 11 ($r=0.42$).
- Group 2 was negatively correlated with group 3 ($r=-0.55$).
- Group 3 was positively correlated with group 6 ($r=0.42$).
- Group 5 was negatively correlated with group 7 ($r=-0.62$) and positively correlated with group 8 ($r=0.47$).
- Group 7 was negatively correlated with group 8 ($r=-0.63$).
- Group 10 was negatively correlated with group 11 ($r=-0.53$).

Correlations between protein bands expressed at 20 days cold treatment

The following significant correlations were observed between the protein bands ($p<0.05$):

Cold treatment was negatively correlated with group 13 ($r=-0.44$) and with group 14 ($r=-0.53$).

- Group 2 was negatively correlated with group 3 ($r=-0.62$) and with group 14 ($r=-0.51$).
- Group 4 was positively correlated with group 9 ($r=0.41$) and negatively correlated with group 12 ($r=-0.42$).
- Group 6 was negatively correlated with group 7 ($r=-0.39$).
- Group 7 was negatively correlated with group 8 ($r=-0.53$) and with group 11 ($r=-0.39$).
- Group 8 was positively correlated with group 11 (r-0.38) and negatively correlated with group 14 ($r=-0.39$).

Correlations between protein bands expressed at 30 days cold treatment

The following significant correlations were observed between the protein bands ($p<0.05$):

- Group 2 was negatively correlated with group 3 (r=-0.39) and with group 14 (r=-0.54).
- Group 3 was negatively correlated with group 8 (r=-0.45).
- Group 4 was negatively correlated with group 7 (r=-0.41) and group 13 (r=-0.38), and positively correlated with group 8 (r=0.41).
- Group 7 was negatively correlated with group 8 (r=-0.66).
- Group 9 was negatively correlated with group 12 (r=-0.4).
- Group 10 was positively correlated with group 12 (r=0.39).

Correlations between protein bands expressed at 40 days cold treatment

The following significant correlations were observed between the protein bands ($p<0.05$):

- Cold treatment was negatively correlated with group 3 (r=-0.46) and positively correlated with group 8 (r=0.53).
- Group 2 was positively correlated with group 6 (r=0.54), group 8 (r=0.47) and with group 10 (r=0.45). It is also negatively correlated with group 14 (r=-0.42).
- Group 3 was negatively correlated with group 4 (r=-0.49).
- Group 4 was negatively correlated with group 5 (r=-0.4).
- Group 7 was positively correlated with group 12 (0.55).

Correlations between protein bands expressed at 50 days cold treatment

The following significant correlations were observed between the protein bands ($p<0.05$):

- Cold treatment was negatively correlated with group 1 (r=-0.44), group 3 (r=-0.46) and with group 6 (r=-0.42).
- Group 2 was positively correlated with group 8 (r=0.58) and negatively correlated with group 14 (r=-0.45).
- Group 3 was negatively correlated with group 4 (r=-0.51).
- Group 4 was positively correlated with group 10 (r=0.41).
- Group 5 was negatively correlated with group 6 (r=-0.5).
- Group 8 was negatively correlated with group 14 (r=-0.39).
- Group 13 was positively correlated with group 14 (r=0.42).

Correlations between protein bands expressed at 60 days cold treatment

The following significant correlations were observed between the protein bands ($p<0.05$):

- Cold treatment was positively correlated with group 5 (r=0.4).
- Group 2 was negatively correlated with group 6 (r=-0.43).

- Group 3 was negatively correlated with group 4 (r=-0.56).
- Group 4 was positively correlated with group 12 (r=0.4).
- Group 5 was negatively correlated with group 6 (r=-0.5).
- Group 7 was negatively correlated with group 8 (r=-0.38) and with group 14 (r=-0.39).
- Group 9 is positively correlated with group 13 (r=0.5).
- Group 10 is positively correlated with group 12 (r=0.52).

The correlations revealed that some groups are always expressed in the presence of another group, and there are groups that is only expressed when another group is not present.

With 10 days cold treatment, groups 2, 8, 9, 10 and 12 had a change in the amount of dark or double bands (where one of the bands was dark) in the susceptible cultivars. Groups 4, 7, 8, 10 and 11 had an increase in these bands in the cold tolerant cultivars after cold treatment. It was found that group 10 and 12 could be used to screen for susceptability, and group 7 to screen for tolerance, at 10 days. If one looks at the correlations, group 10 was negatively correlated with group 11, which means that group 11 could be used to screen for tolerance at 20 days in the absence of group 10. Group 7 was negatively correlated with groups 5 and 8, which indicated that groups 5 and 8 could be used to screen for susceptability at 20 days in the absence of group 7.

With 30 days cold treatment, groups 6, 9, 13 and 14 had a change in the amount of dark or double bands (where one of the bands was dark) in the susceptible cultivars. Groups 4, 5, 8, 10 and 13 had an increase in these bands in the cold tolerant cultivars after cold treatment. It was found that group 6 could be used to screen for susceptability, and group 5 to screen for tolerance, at 30 days.

With 40 days cold treatment, groups 4, 6, 9, 10 and 14 had a change in the amount of dark or double bands (where one of the bands was dark) in the susceptible cultivars. Groups 3, 5, 7, 8, 9 and 10 had an increase in these bands in the cold tolerant cultivars after cold treatment. It was found that group 6 could be used to screen for susceptability, and group 5 and 7 to screen for tolerance, at 40 days. If one looks at the correlations, group 6 was positively correlated with group 2, which indicated that group 2 could be used to screen for susceptability at 40 days cold treatment in the absence of group 6. Group 5 was negatively correlated with group 4, thus group 4 could be used to screen for susceptability in the absence of group 5 at 40 days. Group 7 was positively correlated with group 12 and indicates that group 12 could be used to screen for tolerance in the absence of group 7 at 40 days cold treatment.

With 50 days cold treatment, groups 6, 9 and 13 had a change in the amount of dark or double bands (where one of the bands was dark) in the susceptible cultivars. Groups 3, 5, 7, 8 and 9 had an increase in these bands in the cold tolerant cultivars after cold treatment. It was found that group 6 and 13 could be used to screen for susceptability, and groups 5 and 7 to screen for tolerance, at 50 days cold treatment. If one looks at the correlations, group 6 was negatively correlated with group 5 which indicated that group 5 could be used to screen for tolerance in the absence of group 6. Group 13 was positively correlated with group 14, thus in the absence of group 13, group 14 could be used to screen for tolerance at 50 days cold treatment.

With 60 days cold treatment, groups 6, 8, 9, 10, 13 and 14 had a change in the amount of dark or double bands (where one of the bands was dark) in the susceptible cultivars. Groups 5, 6, 7, 8, 9, 10 and 14 had an increase in these bands in the cold tolerant cultivars after cold treatment. It was found that group 13 could be used to screen for susceptability, and group 5 and 7 to screen for tolerance, in 60 days. If one looks at the correlations, group 13 was positively correlated with group 9 which indicated that group 9 could be used to screen for susceptability to cold in the absence of group 13 at 60 days. Group 5 was negatively correlated with group 6, thus in the absence of group 5, group 6 could be used to screen for susceptability to cold. Group 7 was negatively correlated with groups 8 and 14 which means that groups 8 and 14 could be used to screen for susceptability in the absence of group 7 at 60 days cold treatment.

According to the correlations calculated by the Spearman-rank, there are 9 different protein bands in the roots of wheat seedlings that can be correlated with cold resistance. The expression of these 9 bands in the different wheat cultivars is summarised in Tabel 4.3.

Tabel 4.3 The expression of the protein bands in the root of wheat seedlings correlated with cold resistance in different wheat cultivars

	Group 11 at 10 days cold	Group 13 at 20 days cold	Group 14 at 20 days cold	Group 3 at 40 days cold	Group 8 at 40 days cold	Group 1 at 50 days cold	Group 3 at 50 days cold	Group 6 at 50 days cold	Group 5 at 60 days cold
Adam T	DDB	SB	SB	X	BB	X	X	SB	SB
Betta	SB	DB	X	X	DDB	SB	SB	DDB	SB
Betta DN	BB	X	X	SB	BB	X	BB	BB	SB
Caledon	SB	SB	X	X	DB	X	SB	SB	DB
Chinese S	X	SB	X	SB	X	X	X	SB	X
Gamtoos	X	SB	SB	BB	BB	SB	SB	X	DDB
Gariep	X	DB	SB	DB	SB	SB	DB	SB	X
Hugenoot	BB	X	X	BB	SB	SB	SB	SB	X
Letaba	BB	DB	X	DB	DB	SB	SB	X	DB
Limpopo	DB	SB	SB	X	SB	SB	X	SB	X
Molen	SB	DB	SB	DB	BB	SB	DB	DB	SB
Molopo	SB	SB	BB	SB	X	SB	BB	X	X
Nantes	SB	DB	SB	BB	DB	BB	SB	SB	SB
Palmiet	SB	BB	SB	X	SB	SB	SB	DB	X
PAN 3211	X	SB	DB	BB	SB	SB	SB	BB	SB
PAN 3349	SB	SB	SB	SB	X	X	SB	BB	X
PAN 3377	SB	SB	X	X	DB	X	X	SB	X
Scheeper	SB	SB	SB	SB	BB	X	SB	SB	SB
Snack	X	DB	DB	SB	SB	SB	SB	DDB	X
SST 363	X	X	X	X	DB	SB	X	SB	SB
SST 367	BB	X	X	SB	SB	SB	SB	X	X
SST 57	SB	X	X	DB	DB	X	DB	X	DB
SST 66	X	SB	SB	SB	SB	SB	SB	SB	DB
SST 822	SB	X	DB	DB	BB	X	DB	DB	SB
SST 875	SB	SB	SB	BB	DB	X	BB	X	DB
SST 966	BB	X	X	X	DB	X	X	X	DB
Tugela DN	SB	SB	SB	DB	SB	X	DB	SB	BB

SB – single band
BB – double band
DB – dark band
DBB – dark double band

It can be seen from Tabel 7.2 that the protein bands expressed the most in the different winter wheat cultivars were Group 11 at 10 days cold (100%), and Group 8 at 40 days cold (83%) which were both positively correlated with cold resistance. The molecular weights of these bands were 17 – 21.5 kDa and 33.5 - 38 kDa respectively. The protein bands expressed the most in the different spring wheat cultivars were Group 8 at 40 days cold (90%) which was positively correlated with cold, and Group13 (6 – 10.5 kDa) at 20 days cold (80%), Group 14 (0 – 6 kDa) at 20 days cold (80%), Group 3 (61 – 65.5 kDa) at 40 days cold (80%), and Group 3 at 50 days cold (80%), which were all negatively correlated with cold. The protein bands expressed the most in the different facultative wheat cultivars were Group 8 (33.5 – 38 kDa) at 40 days cold (100%) which was positively correlated with cold, and Group 3 (61 – 65.5 kDa) at 40 days (80%), Group 1 (72 – 76.5 kDa) at 50 days cold (80%), Group 3 at 50 days cold (80%), and Group 6 (44.5 – 49 kDa) at 50 days cold (80%), which were all negatively correlated with cold. Group 1 and Group 14, which were both negatively correlated with cold, were expressed in 56% and 59% of all the different cultivars. Group 14 at 20 days was expressed in 42 % of the winter wheat cultivars, and Group 1 at 50 days in 50% of the spring wheat cultivars. The protein band expressed the most in all the different wheat cultivars was Group 8 (33.5 – 38 kDa) at 40 days (89%) which was positively correlated with cold.

From Tabel 7.1 and Table 7.2 it can be seen that there were eight different protein bands expressed at five different treatments correlated with cold in the roots of wheat seedlings, while there were only four bands expressed at three different treatments correlated with cold in the coleoptiles. All the protein bands of the coleoptiles were possitively correlated with cold, but only three of the eight protein bands of the roots were possitively correlated with cold, the rest were negatively correlated. .Two different protein bands in the coleoptiles were expressed at 40 days cold treatment, while there were two protein bands expressed at 20 and 30 days cold treatment of the roots, and three protein bands expressed at 50 days cold treatment of the roots of wheat seedlings. The roots of wheat seedlings responded with more protein bands after longer cold treatment than the coleoptiles did. Group 3, with protein bands of 61 – 65.5 kDa, is possitively correlated with 10 days cold treatment of the coleoptiles, but negatively correlated with 40 and 60 days cold treatment of the roots of wheat seedlings. Group 10 (22.5 – 27 kDa) were expressed in all the coleoptiles of winter cultivars at 40 days cold treatment, and Group 11 (17 – 21.5 kDa) were expressed in all the roots of winter cultivars at 10 days cold treatment. Group 10 were expressed in 92% of the coleoptiles of all the wheat cultivars, and Group 11 in 98% of the roots of all the different wheat cultivars.

Conclusion

The correlations calculated by the Spearman-rank showed that four different protein bands was associated with cold treatments in the coleoptiles of wheat seedlings. All four proteins were possitively correlated with cold resistance. There were two different bands expressed at 40 days cold treatment, Group 4 and Group 10, and these two bands were also expressed in the coleoptiles of all the winter wheat cultivars at 40 days cold. This suggested that the coleoptiles of wheat seedlings responded the most at 40 days in cold conditions. Group 4 was expressed at 40 days cold in 83% of winter cultivars, 60% of the spring cultivars, and 40% of the facultative cultivars, therefore it can be concluded that this protein with a molecular weight of 55.5 - 60 kDa plays a significant role in the cold resistance of the coleoptiles of wheat seedlings. Group 10 were expressed at 40 days cold treatment in 92% of all the different wheat cultivars, and this protein with a molecular weight of 22.5 - 27 kDa can be seen to play a major role in cold resistance in the coleoptiles of different wheat cultivars.

The correlation study by the Spearman-rank showed that eight different protein bands were associated with cold treatment in the roots of wheat seedlings. Of these different bands only three were possitively correlated with cold, while the other five were negatively correlated with cold. One band, Group 3, was negatively correlated with cold at 40 days and at 50 days, and it could be said that the absence of this protein, with a molecular weight of 61 – 65.5 kDa, could contribute to cold resistance when the seedlings are exposed to cold longer than 30 days. The protein bands of Group 1 and Group 14, which were both negatively correlated with cold, showed cold sensitivity and were expressed the least in the roots of the wheat seedlings when exposed to cold treatment. There were two different bands expressed at 20 days, another two at 40 days, and three different bands at 50 days. This suggested that the roots of wheat seedlings responded the most to 50 days cold treatment. Group 11 and Group 8, both possitively correlated with cold resistance, were expressed in 100% (at 10 days cold) and 83% (at 40 days cold) respectively in the roots of winter wheat cultivars. Group 11 is expressed at 10 days cold in 60% of the spring cultivars and in 40% of the facultative wheat cultivars. Group 8 is expressed at 40 days in 90% of the spring cultivars and in 100% of the facultative wheat cultivars. It could be therefore said that these two proteins with molecular weights of 17 – 21.5 and 33.5 - 38 kDa contributed to cold resistance in the roots of wheat seedlings.

Chapter 7
CONCLUSION

The induction of specific proteins in the leaf, crown and tissues of wheat indicated that the regulation of some freezing tolerance genes was tissue specific. Thes finding was supported by the fact that the synthesis rate of some proteins, detected in more than one tissue, was differently regulated in one different tissues during cold hardening (Sarhan and Perras, 1989).

Zhou *et al* (1994) identified proteins that were induced by cold acclimation in wheat. Two cultivars with different levels of cold tolerance were used. Levels of polypeptides of 52 and 23 kDa increased specifically in the shoots of winter wheats.

The results of this study confirmed different reactions of HMW-proteins in the coleoptiles and the roots to cold treatment. Proteins with the molecular weights of 54.5 – 50 kDa, 43.5 – 39 kDa, 38 – 33.5 kDa and 32.5 – 28 kDa were expressed in the coleoptiles of cold tolerant wheat cultivars in response to cold treatment. The intensity of proteins with molecular weights of 49 – 44.5 kDa, 32.5 – 28 kDa, 27 – 22.5 kDa and 10.5 – 6 kDa increased during cold treatment in the cold susceptible cultivars.

Proteins with the molecular weights of 60 – 55.5 kDa, 43.5 – 39 kDa and 27 – 22.5 kDa were expressed in the roots of cold tolerant wheat cultivars in response to cold treatment. The intensity of proteins with molecular weights of 54.5 – 50 kDa and 10.5 – 6 kDa increased during cold treatment in the cold susceptible cultivars.

Soil takes longer to cool off than the air surrounding the plant. The soil is therefore, warmer for a longer period. It is possible that tolerant cultivars have, through evolution, produced more proteins in their coleoptiles, so that they would have a better chance to survive cold temperatures.

Thus, the proteins with molecular weights of 54, 5 – 50 kDa (group 5) and 43.5 – 39 kDa (group 7) could be used to screen for tolerance to cold in the coleoptiles of wheat cultivars, while the proteins with molecular weights of 60 – 55.5 kDa (group 4), 43.5 – 39 kDa (group 7) and 27 – 22.5 kDa (group 10) could be used to screen for tolerance to cold in the roots of wheat cultivars.

Growth habit and vernalisation requirement influenced the production and accumulation of some of the proteins. Roots were more sensitive to the cold treatment. The intensity of the protein bands in especially the roots was very low, other extraction and colouring methods should be tested in order to obtain more accurate results.

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

Chapter 8
SUMMARY

Cultivars produce proteins in their normal life cycle. Some of these proteins are produced in greater quantities in reaction to cold temperatures in certain cultivars. In this study, 27 wheat cutivars were tested for their protein composition. The proteins produced could be divided into 14 different groups with each group having a certain molecular weight range. These groups were the same for the proteins in the coleoptiles and roots of wheat cultivars.

Cold susceptible and tolerant cultivars were compared with each other to evaluate their differences in response to cold. The following was found:

In the coleoptiles, the tolerant cultivars gained bands across the 14 groups in reaction to cold temperatures. This was also found in the roots of tolerant cultivars. Two of the susceptible cultivars tested (Adam Tas and SST 66), lost more bands in their coleoptiles across the different groups than they gained. The other two susceptible cultivars tested (Palmiet and Snack) gained more bands than they had lost. All the susceptible cultivars (except Palmiet) gained more bands in their roots in response to cold hardening. Palmiet gained and lost the same number of bands. Most cultivars produced more proteins in response to cold.

Although proteins are produced in response to cold, not all the cultivars were able to produce the necessary proteins to make them more tolerant to cold. It was clear that some proteins were produced more in all or most cultivars in reaction to cold, but the susceptible and tolerant cultiavrs produced different proteins. In the coleoptiles, the susceptible cultivars had no change or gained bands in group 3 and group 11 while the tolerant cultivars had no change or lost bands in these groups. The susceptible cultivars lost bands (or there were no change in the number of bands produced) in group 4, group 10, group 12 and group 14. The tolerant cultivars gained bands in these groups in response to cold. In the roots, the susceptible cultivars gained bands (or there were no change in the number of bands produced) in group 5, group 11 and group 14. The cold tolerant cultivars lost bands in these groups. Only in group 2, did the cold susceptible cultivars lose bands, while the tolerant cultivars gained bands. The tolerant cultivars thus gained bands in their coleoptiles, whiel they produced fewer proteins in their roots in response to cold temperatures. The susceptible cultivars, however, produced more proteins in their roots than in their coleoptiles in response to cold. Jacobs (1999) found that some cultivars are susceptible to cold in their coleoptiles, but they are tolerant in their roots. SST 66 is an example of a cultivars that is susceptible to cold in its coleoptiles, but it is tolerant to cold in its roots. If one looks at SST

66, it is seen that this cultivars does not produce proteins in reaction to cold temperatures, thus reacting like a susceptible cultivar in its coleoptiles and like a tolerant cultivar in its roots.

The susceptible cultivars produced more dark bands in their roots. Thus, the intensity of proteins increased in the roots of susceptible cultivars. In tolerant cultivars however, the intensity of proeins increased in the coleoptiles. Tolerant cultivars produced three times more dark bands in their coleoptiles than susceptible cultivars.

If one looks at the production of certain groups, it is found that more proteins were produced in the coleoptiles of susceptible cultivars in group 6, group 8, group 9, group 10 and group 13. Only group 13 was produced in greater amounts in susceptible cultivars and not in the tolerant cultivars. In the coleoptiles of the tolerant cultivars, group 5 and group 7 were produced in greater amounts. It is however, not present in high amounts in susceptible cultivars. In the roots of cultivars, group 3, group 4, group 5 and group 9 can be correlated with cold temperature as it is produced in greater amounts in both the susceptible and tolerant cultivars. A higher intensity of proteins in group 6, group 13 and group 14 are correlated with susceptibility to cold, as it was only detected in susceptible cultivars. Tolerant cultivars produced these proteins in normal or low amounts in response to cold temperatures.

Thus, the proteins with molecular weights of 54,5 – 50 kDa (group 5) and 43.5 – 39 kDa (group 7) could be used to screen for tolerance to cold in the coleoptiles of wheat cultivars, while the proteins with molecular weight of 60 – 55.5 kDa (group 4), 43.5 – 39 kDa (group 7) and 27 – 22.5 kDa (group 10) could be used to screen for tolerance to cold in the roots of wheat cultivars. It is further recommended that breeders discard any lines that show an increase in the production of proteins in their roots in the range of 49 – 44.5 kDa (group 6), 10. 5 – 6 kDa (group 13) and 5 – 0 kDa (group 14).

OPSOMMING

Cultivars produseer proteïene in hulle normale lewens-siklus. Sommige proteine word in groter hoeveelhede geproduseer in koue temperature in sekere cultivars. In hierdie studie is 27 koring-cultivars getoets vir hulle proteïen-samestelling. Die proteïene kon ingedeel word in 14 verskillende groepe met elke groep in `n spesifieke molekulêre gewig-indeling. Die groepe was dieselfde vir proteine van koleoptiele en wortels van die plante.

Koue vatbare en tolerante cultivars is vergelyk met mekaar om hulle reaksies op koue-stres te toets. Die volgende is gevind:

In die koleoptiele het tolerante cultivars bande bygekry oor die 14 groepe in reaksie op koue. Dieselfde is gevind vir wortels van tolerante cultivars. Twee van die vatbare cultivars wat getoets is (Adam Tas en SST 66) het meer bande verloor in hulle koleoptiele oor die groepe as wat hulle bygekry het. Die ander twee vatbare cultivars wat getoets is (Palmiet en Snack) het meer bande bygekry as wat hulle verloor het. Al die vatbare cultivars, behalwe Palmiet, het meer bande bygekry in hulle wortels as wat hulle verloor het met koue. Palmiet het dieselfde hoeveelheid bande bygekry en verloor. Meeste cultivars het meer proteine geproduseer in reaksie op koue.

Alhoewel proteine geproduseer is in reaksie op koue, kon nie al die cultivars genoeg proteine produseer om hulle tolerant te maak vir koue nie. Dit was duidelik dat sommige proteïene meer of in al die cultivars geproduseer is in reaksie op koue, maar die vatbare en tolerante cultivars het verskillende proteïene geproduseer. In die koleoptiele het die vatbare cultivars geen verandering gehad nie, of hulle het bande bygekry in groep 3 en groep 11, terwyl die tolerante cultivars geen verandering gehad het nie, of hulle het bande verloor in hierdie groepe. Die vatbare cultivars het bande verloor (of daar was geen verandering in aantal bande nie) in groepe 5, 11 en 14. Die koue tolerante cultivars het bande verloor in hierdie groepe. Net in groep 2 het die koue vatbare cultivars bande verloor, terwyl die tolerante cultivars bande bygekry het. Die tolerante cultivars het dus bande bygekry in hulle koleoptiele, terwyl hulle minder proteïene in hulle wortels geproduseer het met koue. Die vatbare cultivars het bande bygekry met koue. Die tolerante cultivars het dus bande bygekry in hulle koleoptiele, terwyl hulle minder proteïene in hulle wortels geproduseer het met koue. Jacobs (1999) het gevind dat sekere cultivars vatbaar is vir koue in hulle koleoptiele, maar dat hulle tolerant is in hulle wortels. SST 66 is `n voorbeeld van `n cultivar wat vatbaar is in die koleoptiele, maar tolerant is in die wortels. As mens kyk na SST 66, dan kan dit gesien word dat die cultivar nie proteïene produseer in reaksie op koue nie, dus reageer dit soos `n vatbare cultivar in sy koleoptiele en tolerant in die wortels.

Die vatbare cultivars produseer meer donker bande in hulle wortels. Dus, die intensiteit van die proteïene neem toe in die wortels van vatbare cultivars. In die tolerante cultivars neem die intensiteit van die proteïene toe in die koleoptiele. Tolerante cultivars het drie keer meer donker bande in hulle koleoptiele geproduseer as in vatbare cultivars.

As mens kyk na die produksie van sekere groepe, is daar gevind dat meer proteïene geproduseer is in die koleoptiele van vatbare cultivars in groepe 6, 8, 9, 10 en 13. Net groep 13 is in groter hoeveelhede geproduseer in vatbare cultivars maar nie in tolerante cultivars nie. In die koleoptiele van tolerante cultivars is grope 5 en 7 in groter hoeveelhede geproduseer. Dit is nie in groot hoeveelhede in vatbare cultivars nie. In die wortels van cultivars was groepe 3, 4, 5 en 9 gekorreleer met koue temperature, omdat dit in groter hoeveelhede geproduseer is in vatbare en tolerante cultivars. 'n Hoër intensiteit van proteïene in groepe 6, 13 en 14 is gekorreleer met koue vatbaarheid, omdat dit net in vatbare cultivars teenwoordig is. Tolerante cultivars het hierdie proteïene in normale of lae konsentrasies geproduseer met koue behandeling.

Dus kan proteïene met molekulêre gewigte van 54.5 – 50 kDa (groep 5) en 43.5 – 39 kDa (groep 7) gebruik word om te toets vir koue toleransie in die koleoptiele van koring-cultivars, terwyl proteïene van of 60 – 55.5 kDa (groep 4), 43.5 – 39 kDa (groep 7) en 27 – 22.5 kDa (groep 10) gebruik kan word om te toets vir toleransie vir koue in die wortels van koring-cultivars. Daar word aanbeveel dat telers enige lyne moet uitgooi wat proteïene produseer in die gebied van 49 – 44.5 kDa (groep 6), 10.5 – 6 kDa (groep 13) en 5 – 0 kDa (groep 14).

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APPENDIX A

10 – 10 days cold treatment

10 K – 10 days control

20 – 20 days cold treatment

20 K – 20 days control

30 – 30 days cold treatment

30 K – 30 days control

40 – 40 days cold treatment

40 K – 40 days control

50 – 50 days cold treatment

50 K – 50 days control

60 – 60 days cold treatment

60 K – 60 days control

Table 1. The protein bands in Adam Tas -coleoptiles

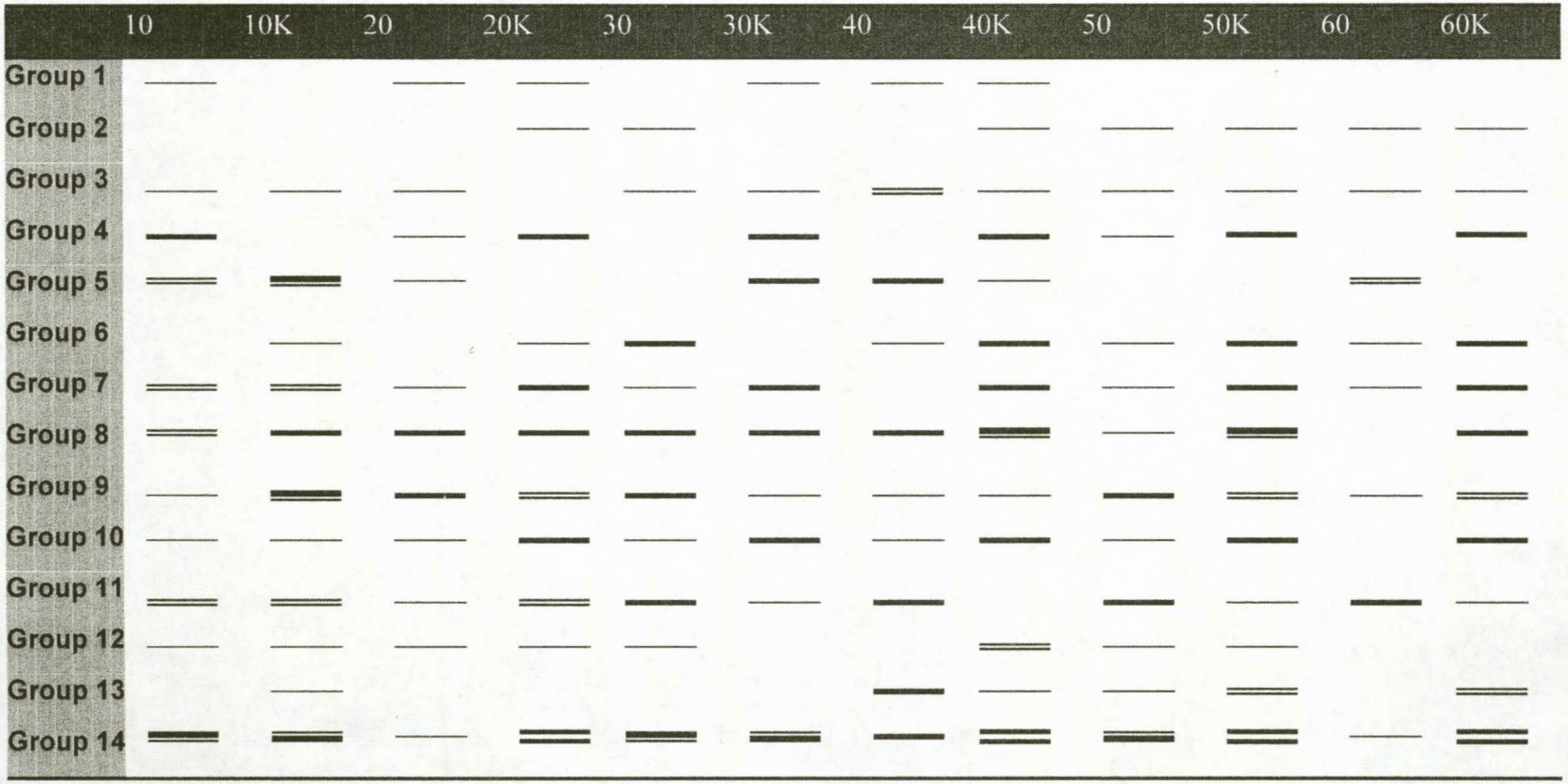


Table 2. The protein bands in Betta – coleoptiles

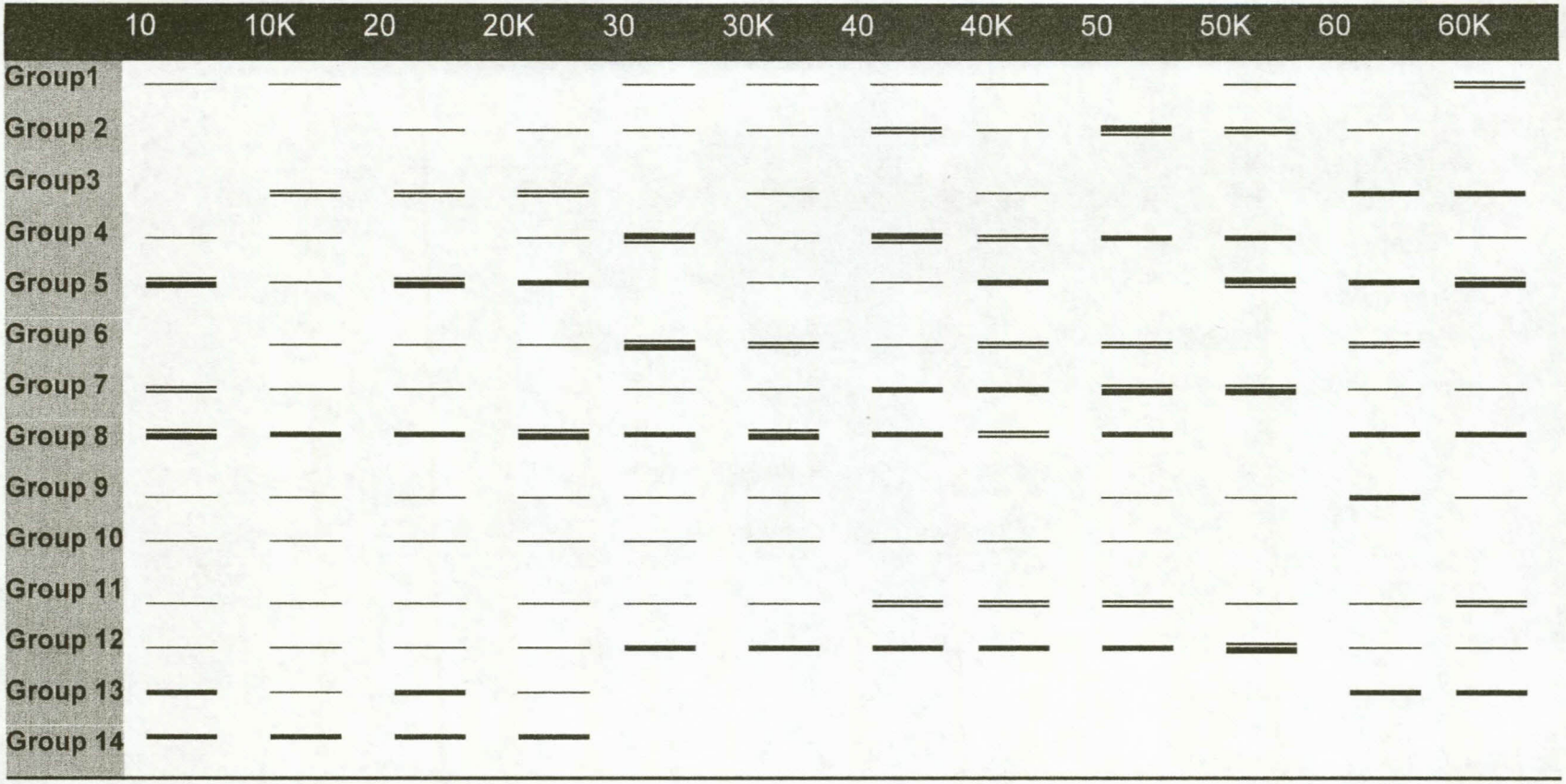


Table 3. The protein bands in Betta DN - coleoptiles

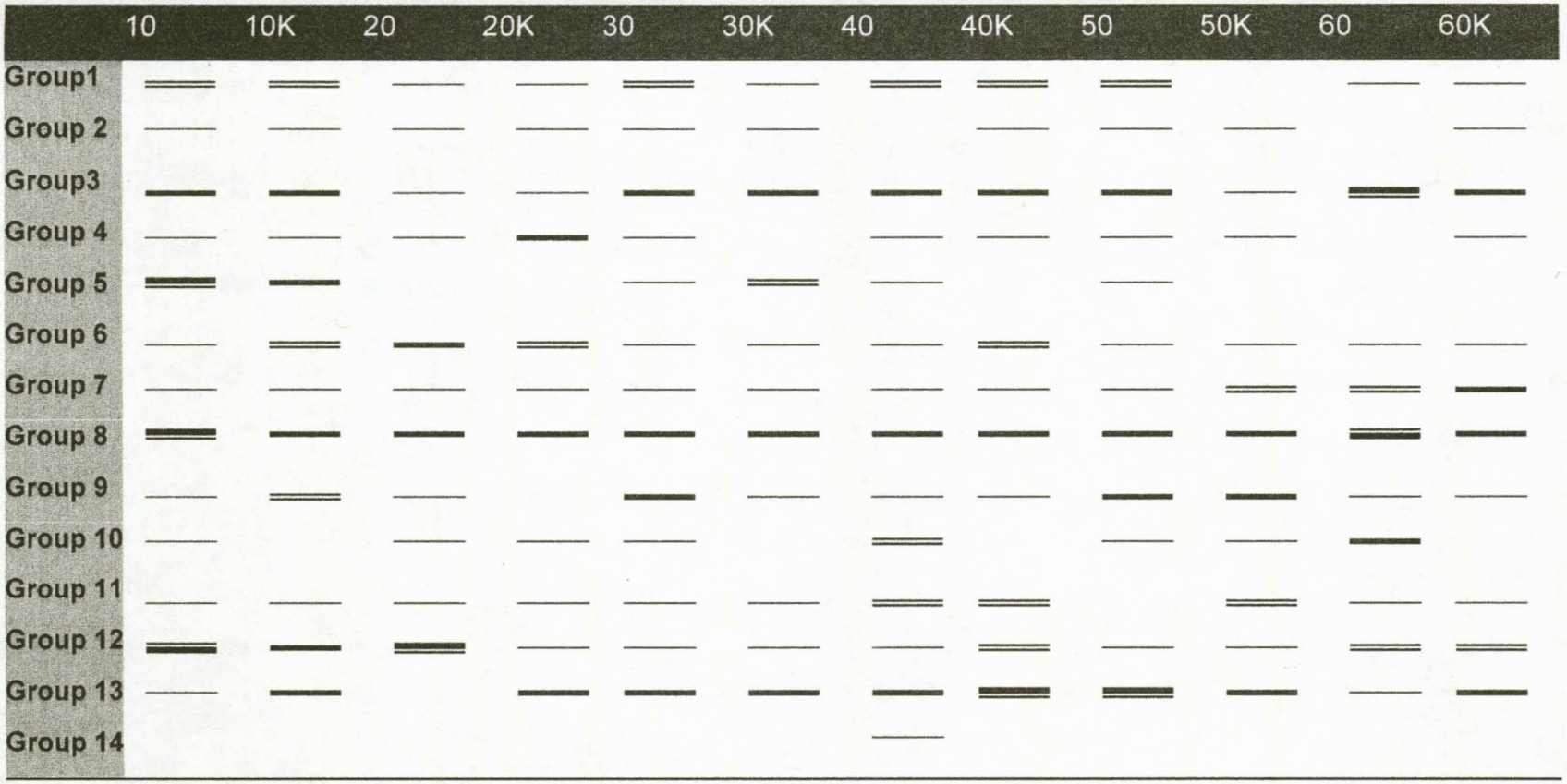


Table 4. The protein bands in Caledon -coleoptiles

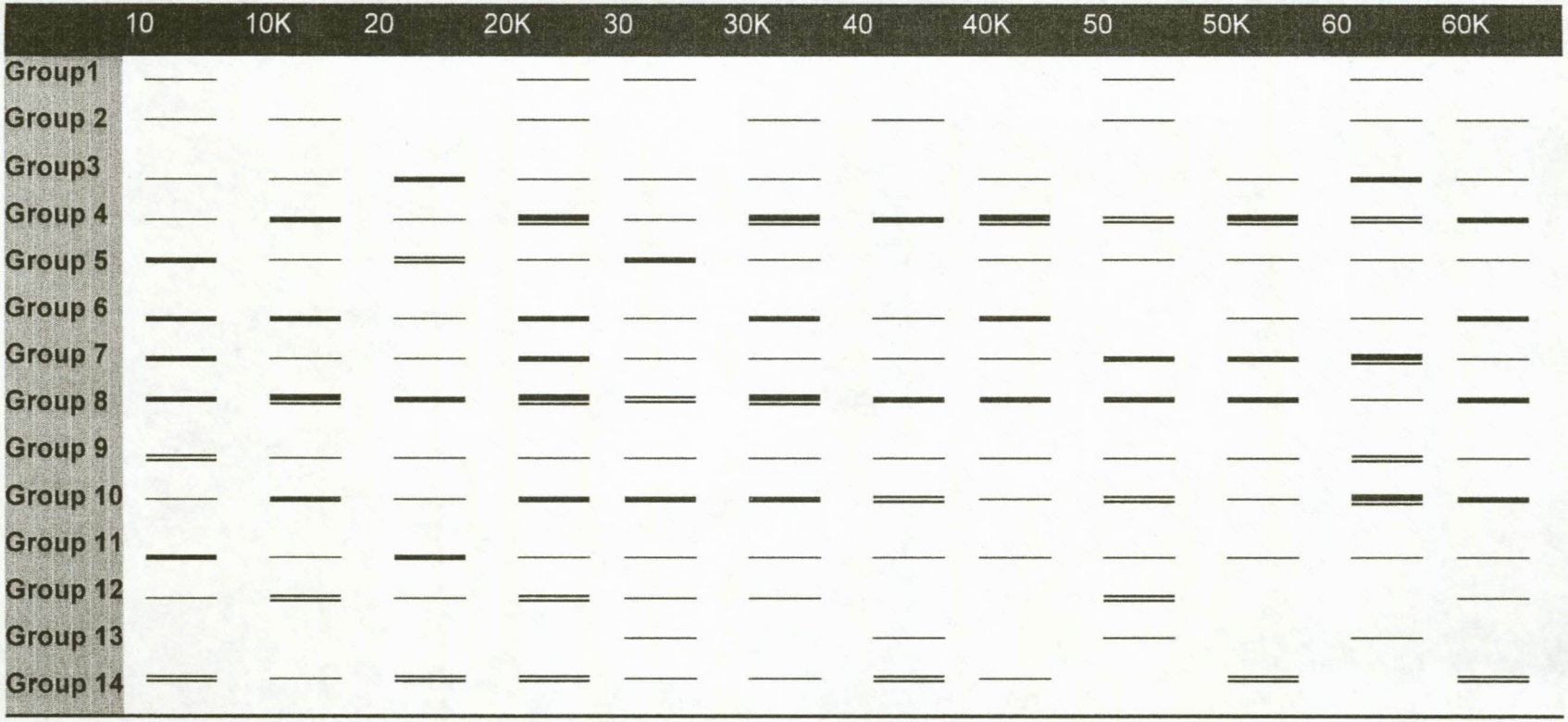


Table 5. The protein bands in Chinese Spring - coleoptiles

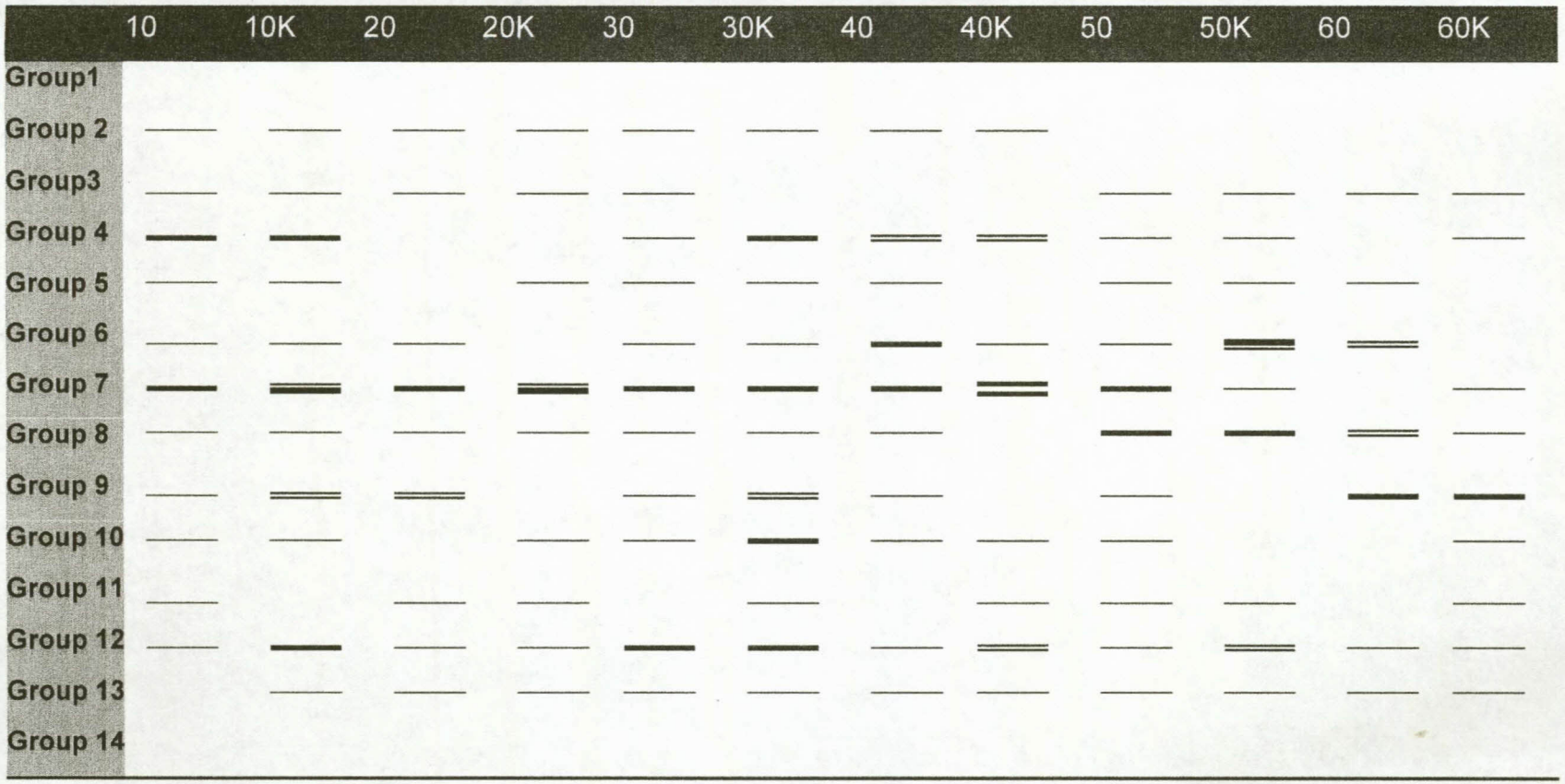


Table 6. The protein bands in Gamtoos – coleoptiles

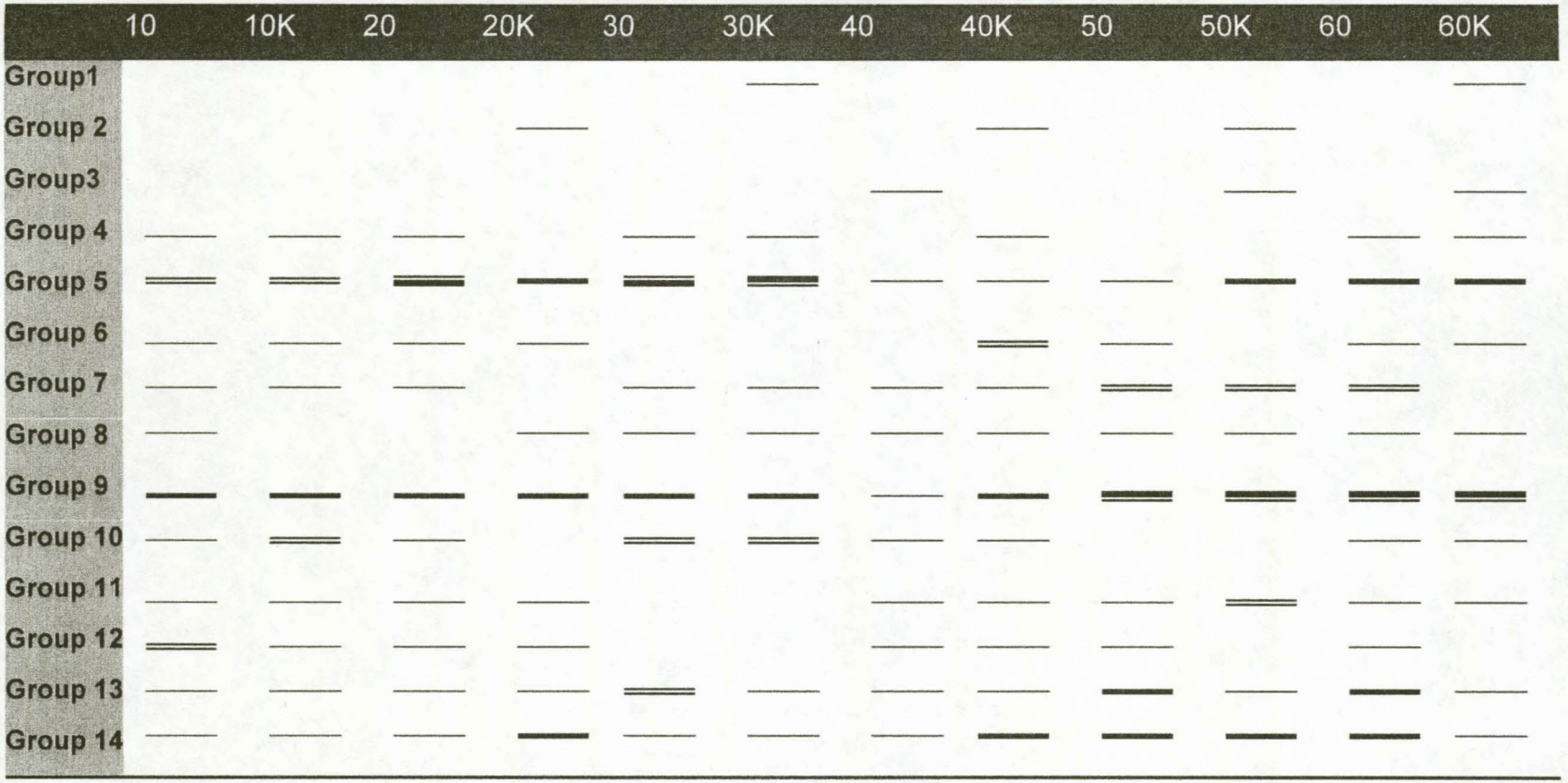


Table 7. The protein bands in Gariep - coleoptiles

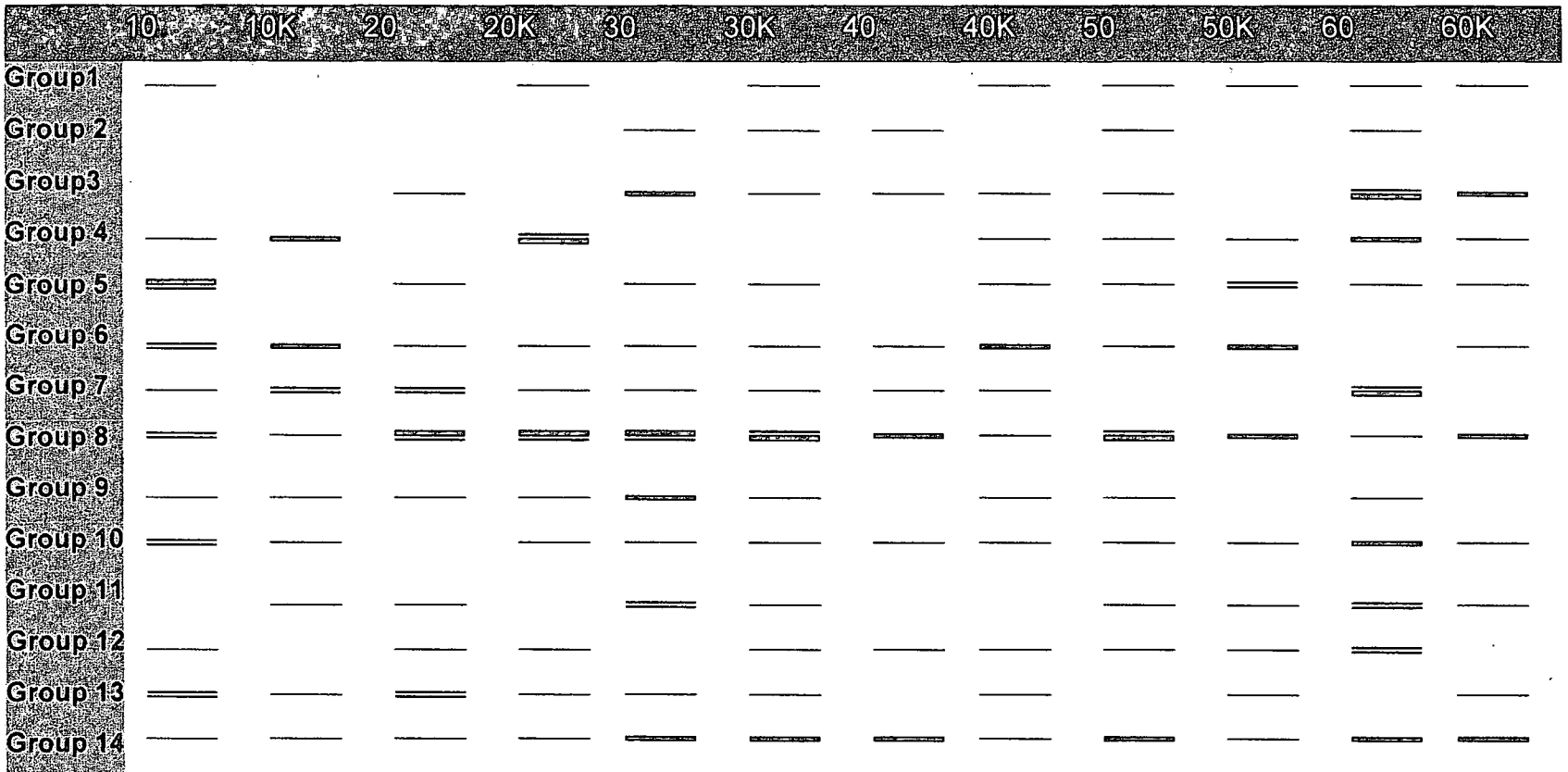


Table 8. The protein bands in Hugenoot - coleoptiles

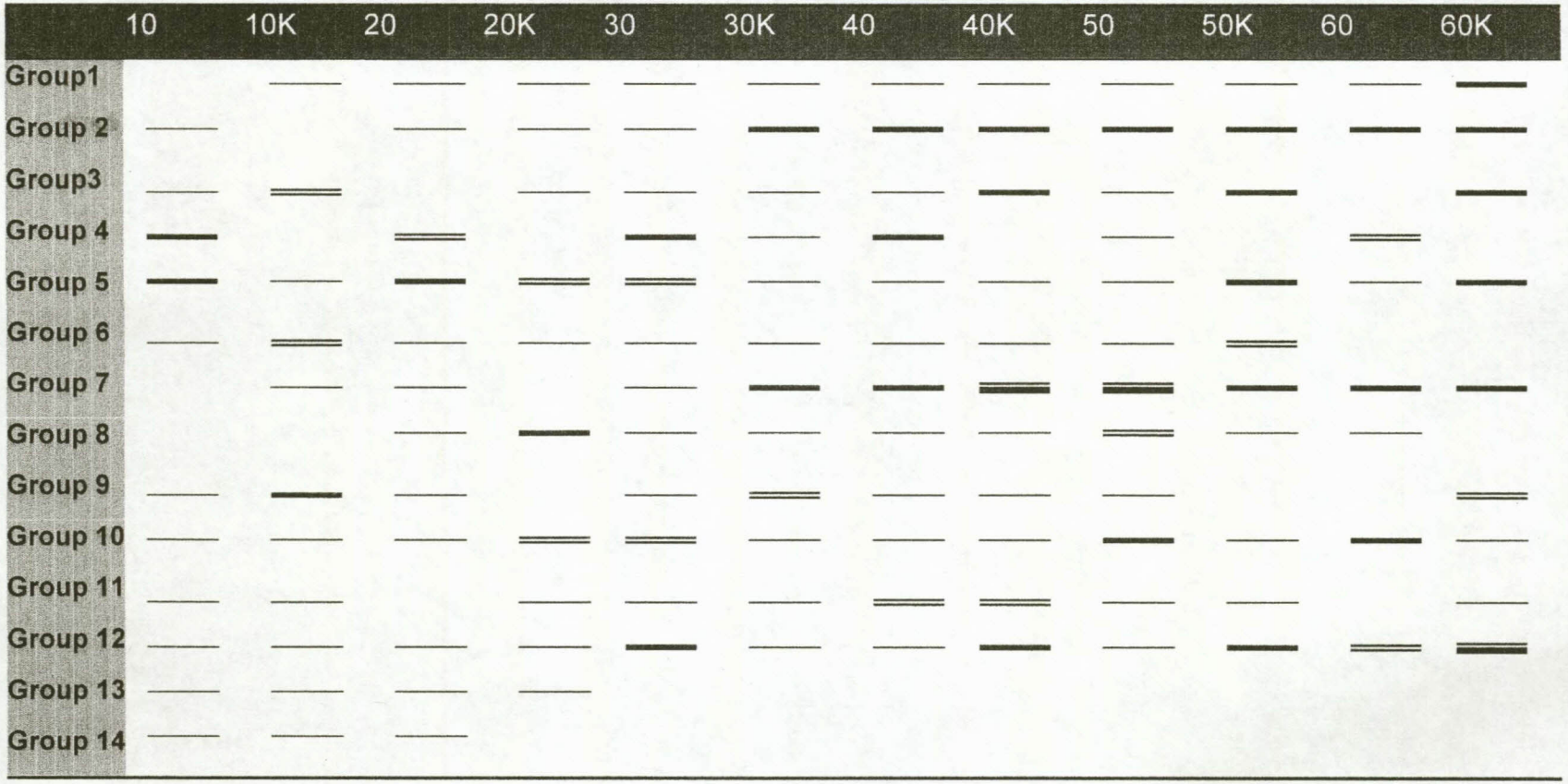


Table 9. The protein bands in Letaba - coleoptiles

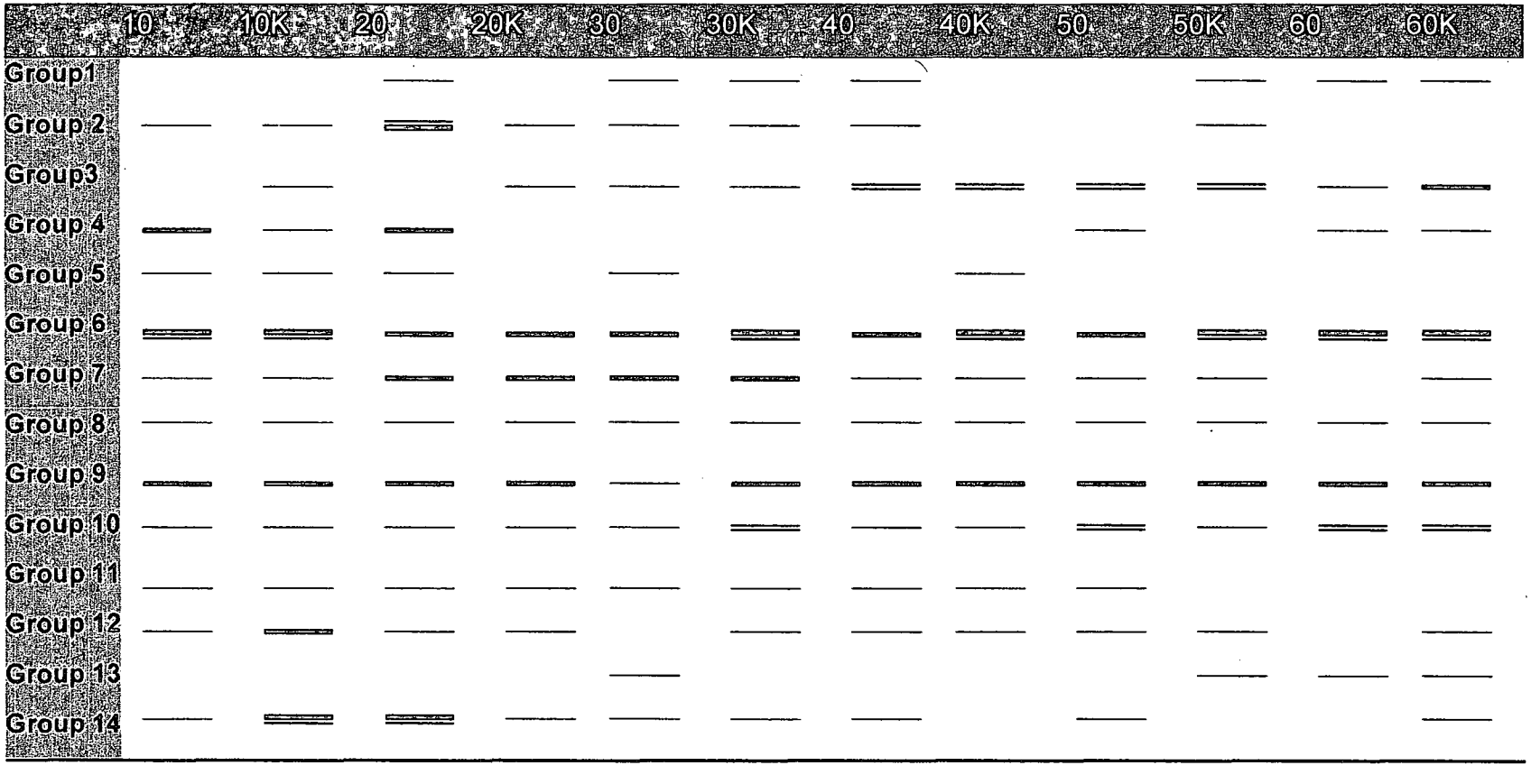


Table 10. The protein bands in Limpopo - coleoptiles

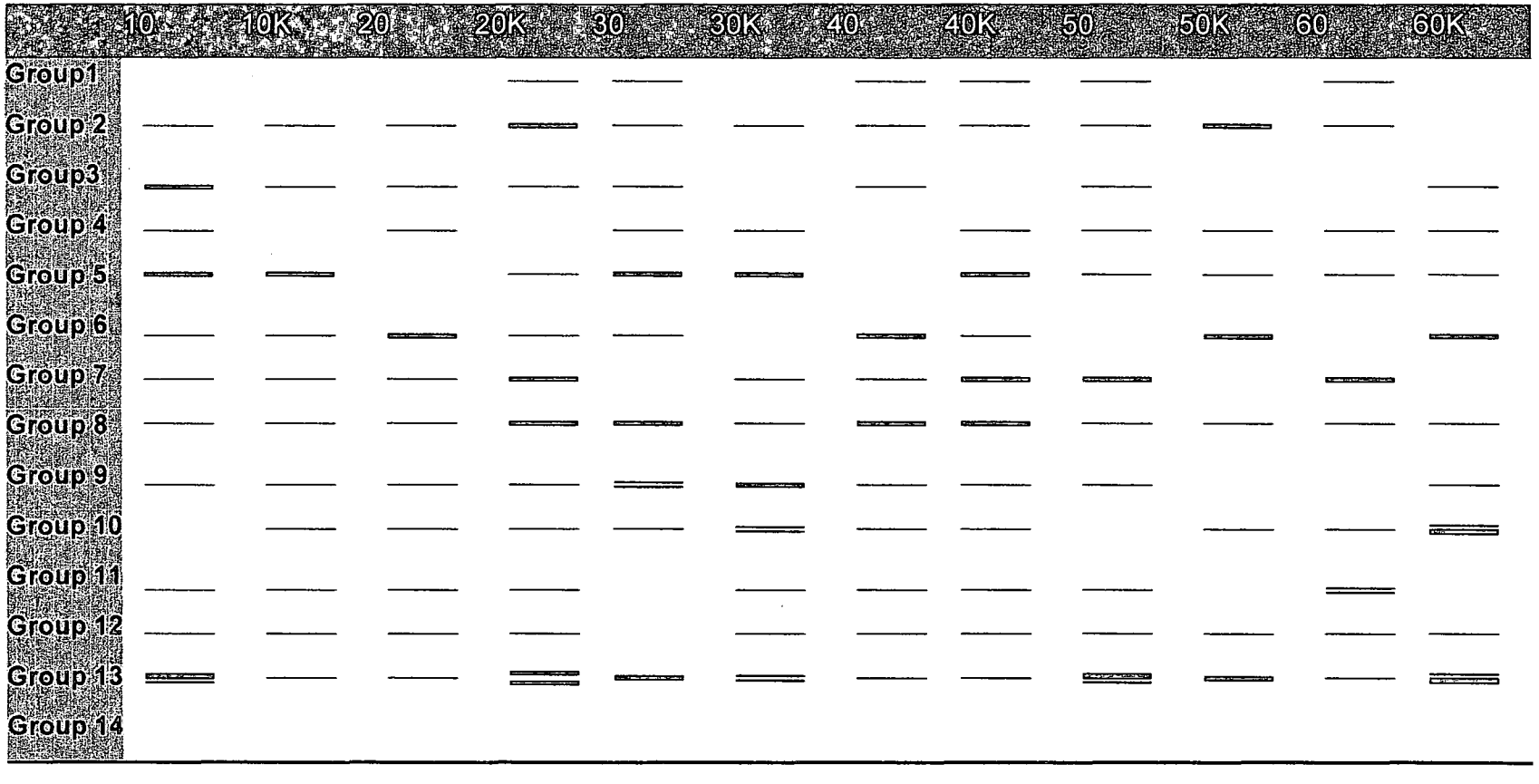


Table 11. The protein bands in Molen -coleoptiles

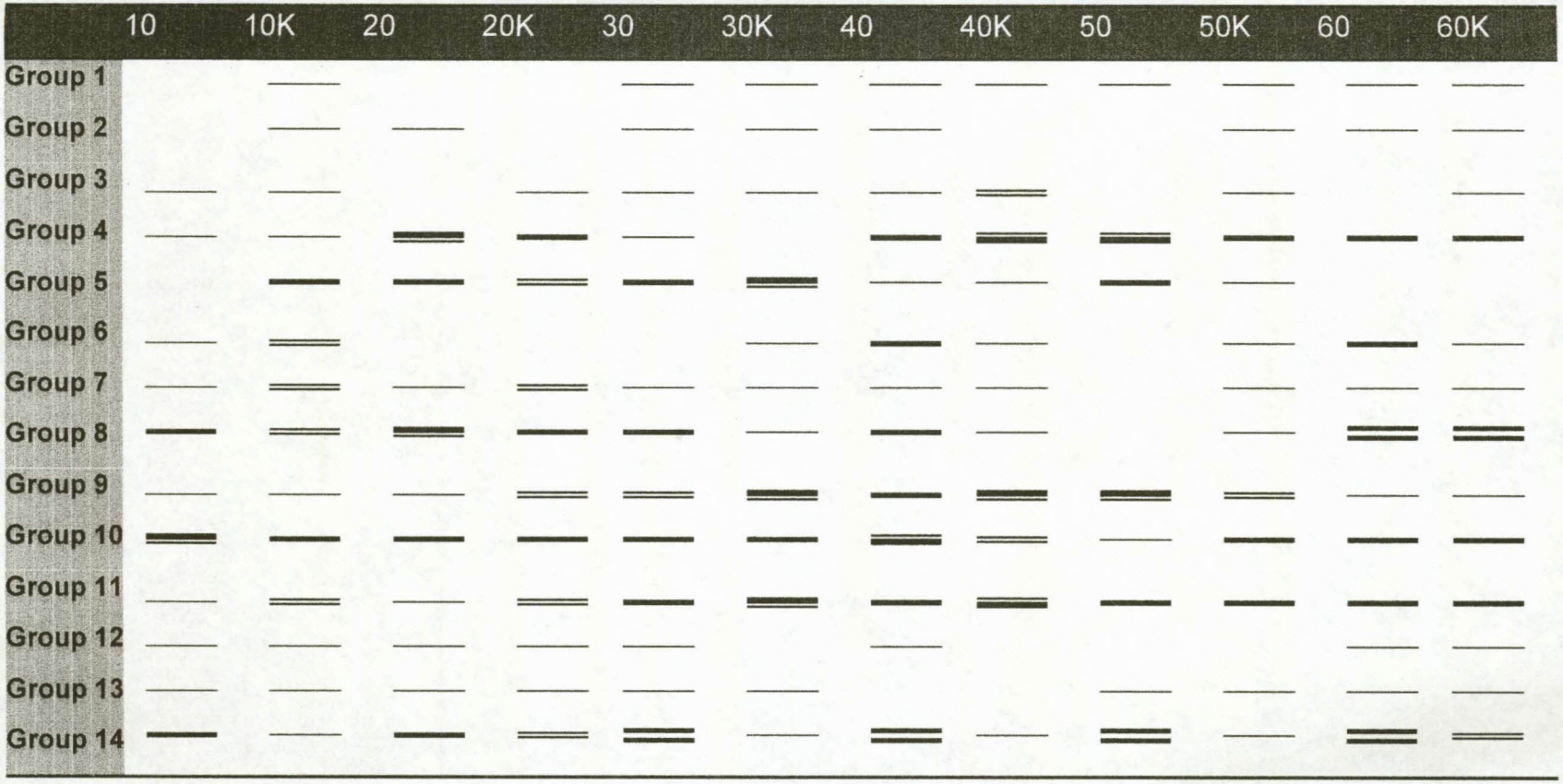


Table 12. The protein bands in Molopo – coleoptiles

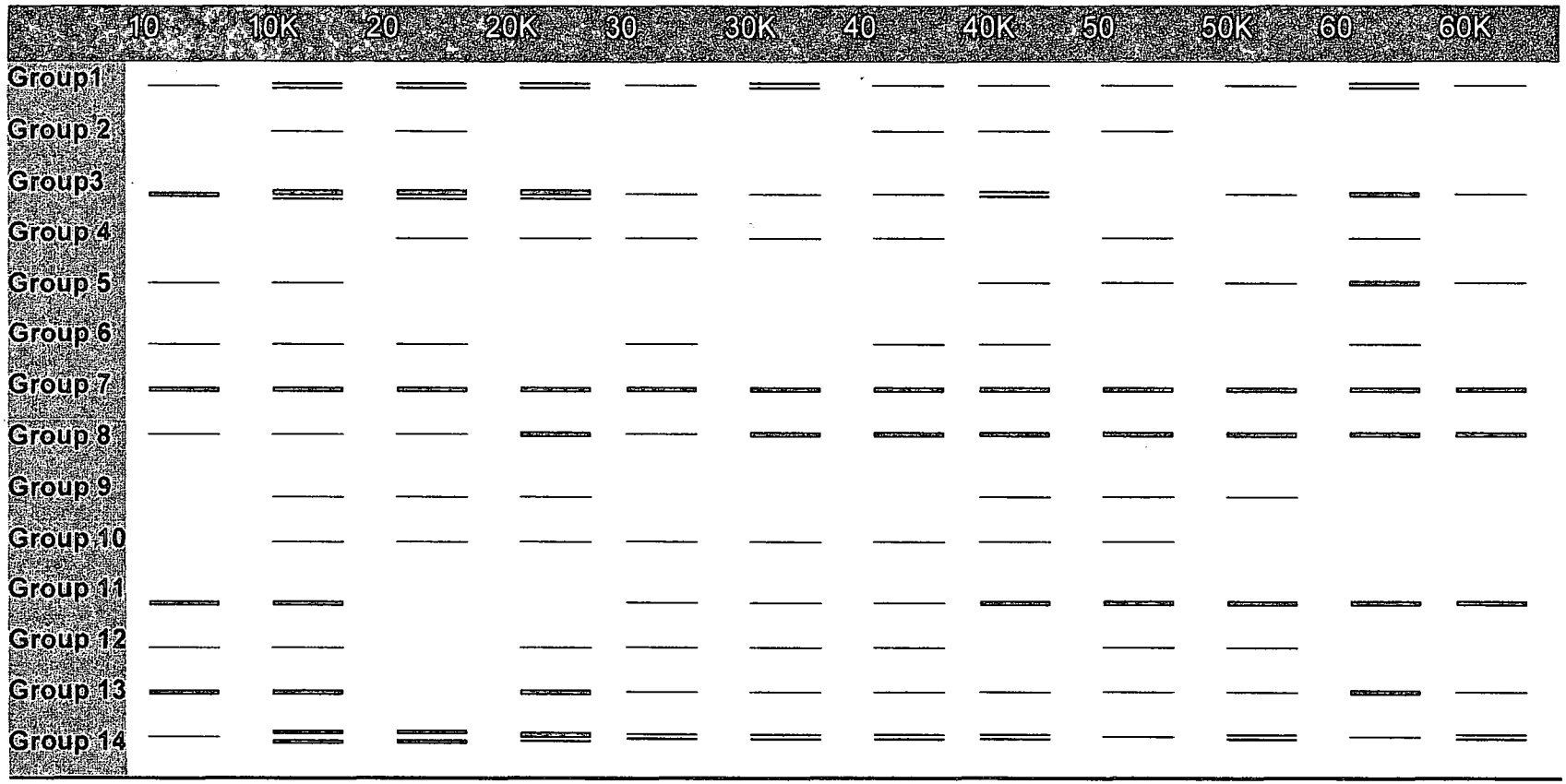


Table 13. The protein bands in Nantes - coleoptiles

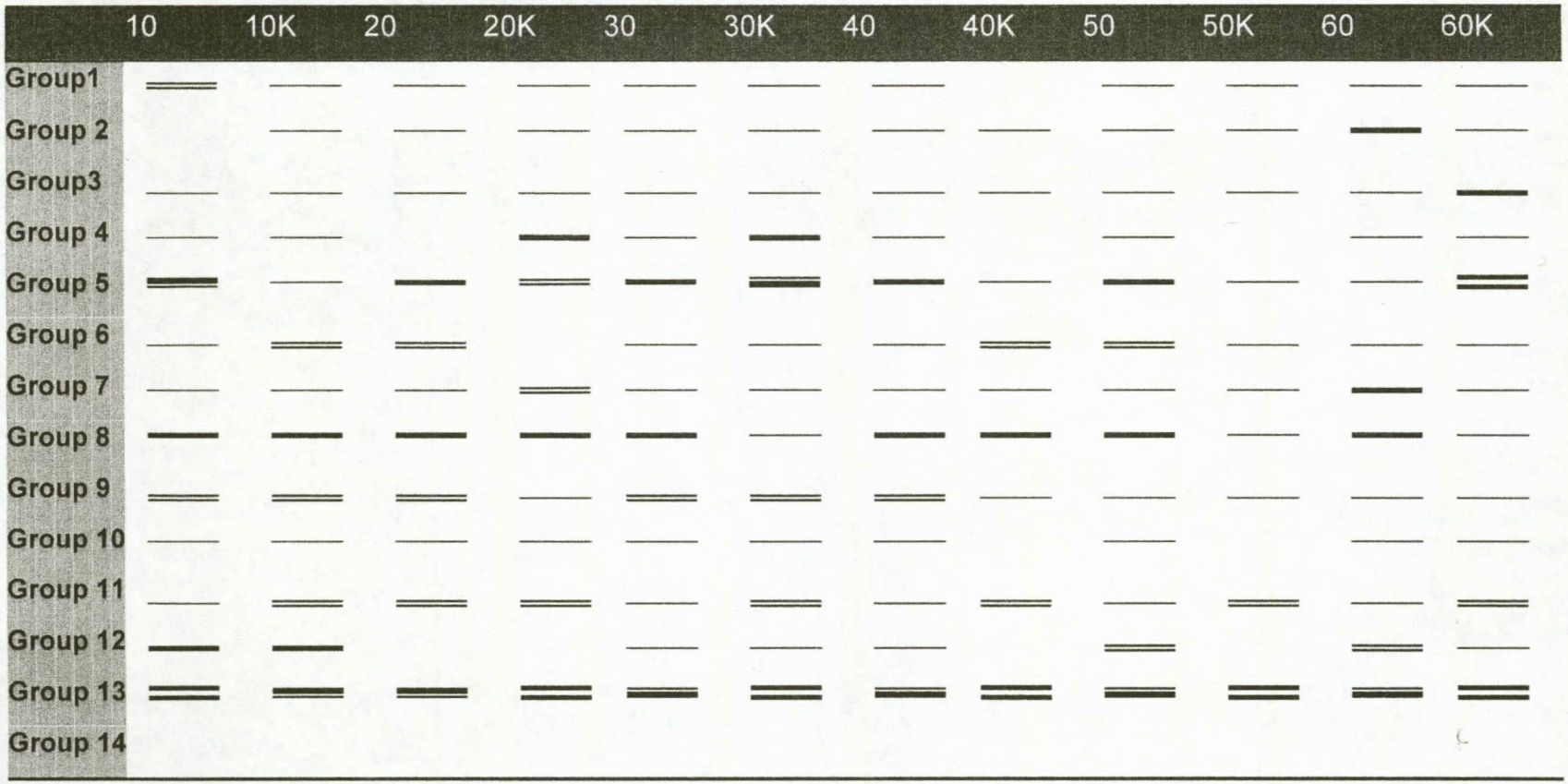


Table 14. The protein bands in Palmiet - coleoptiles

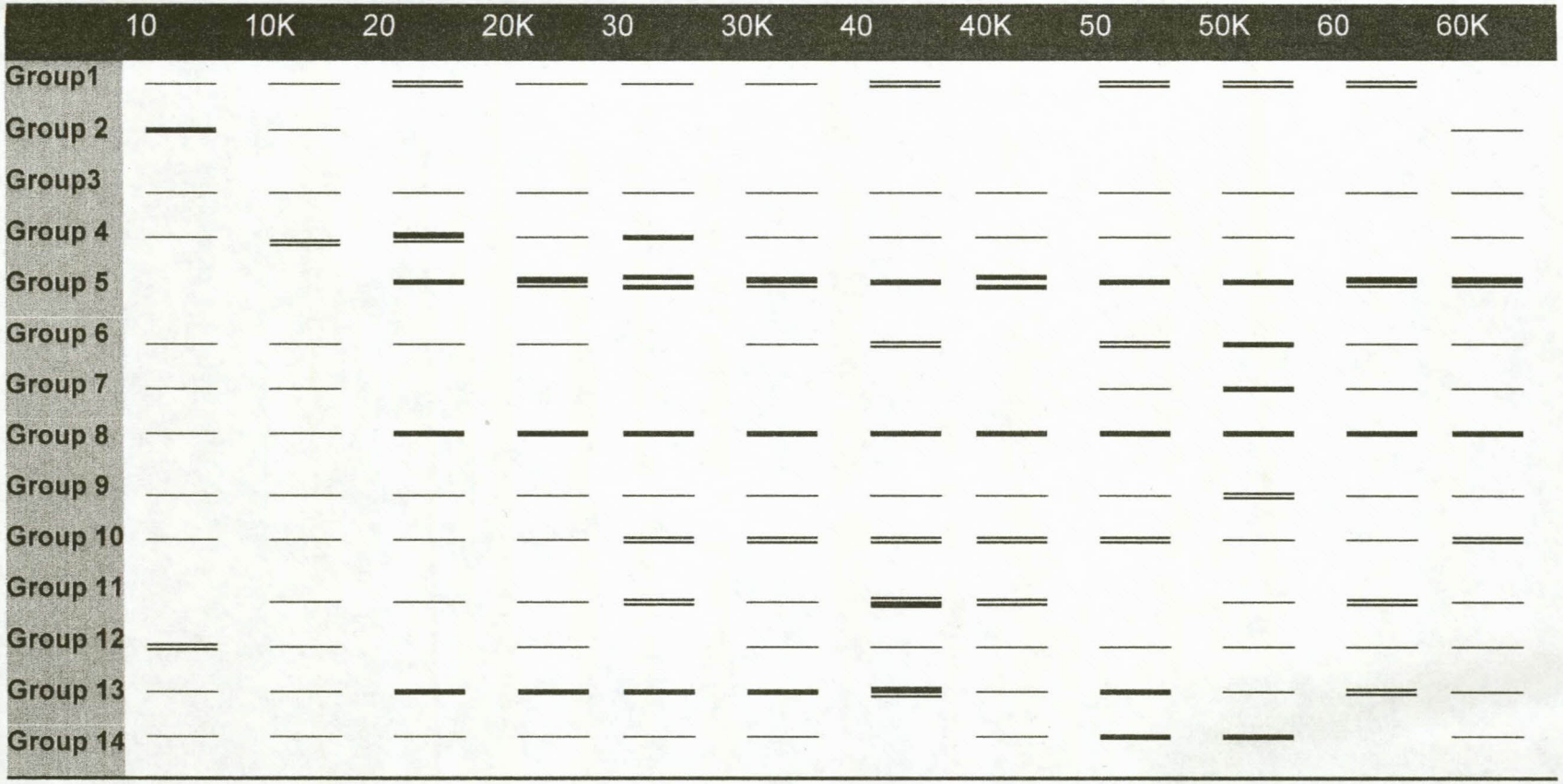


Table 15. The protein bands in PAN3211 -coleoptiles

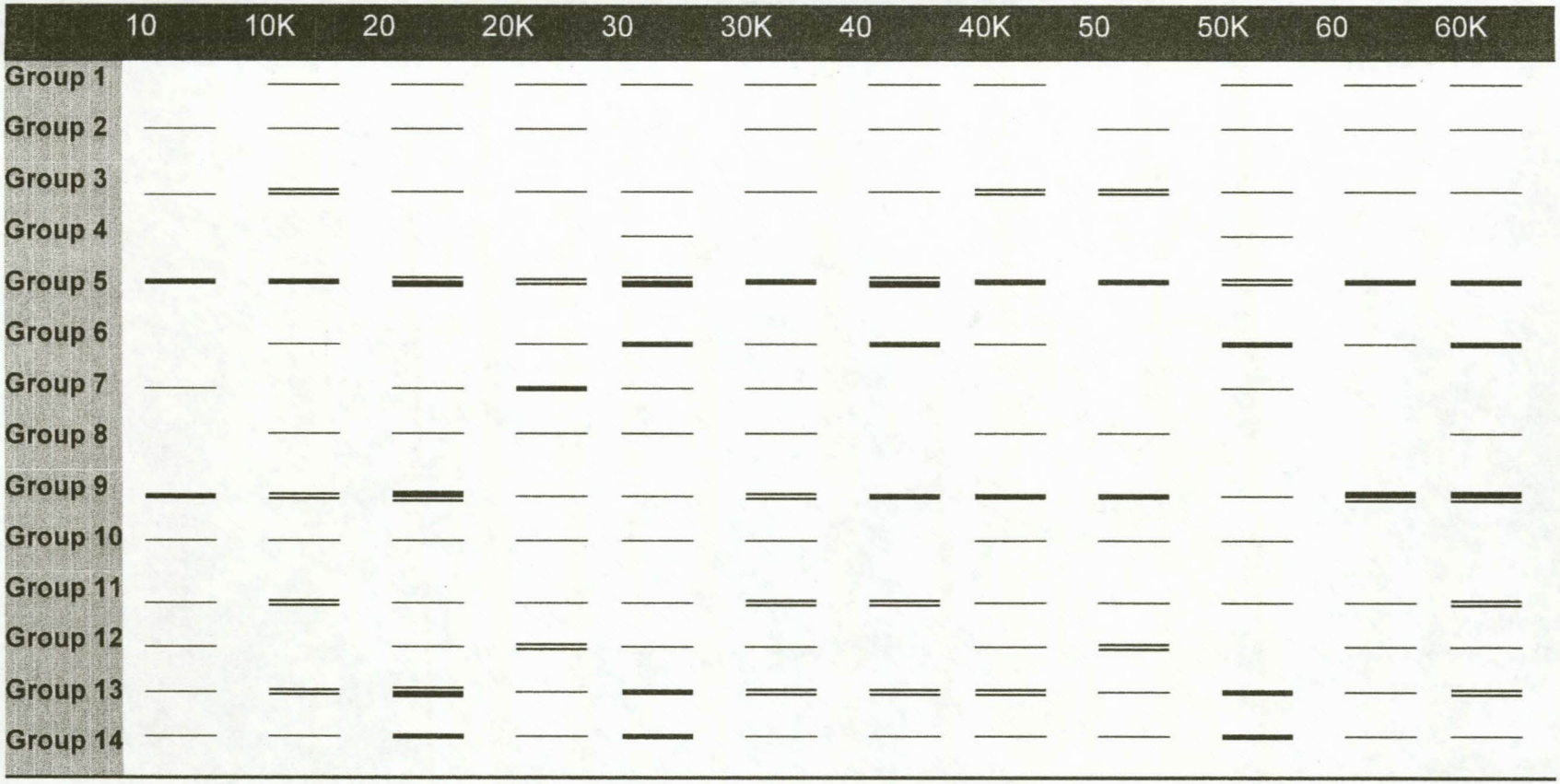


Table 16. The protein bands in PAN3349 -coleoptiles

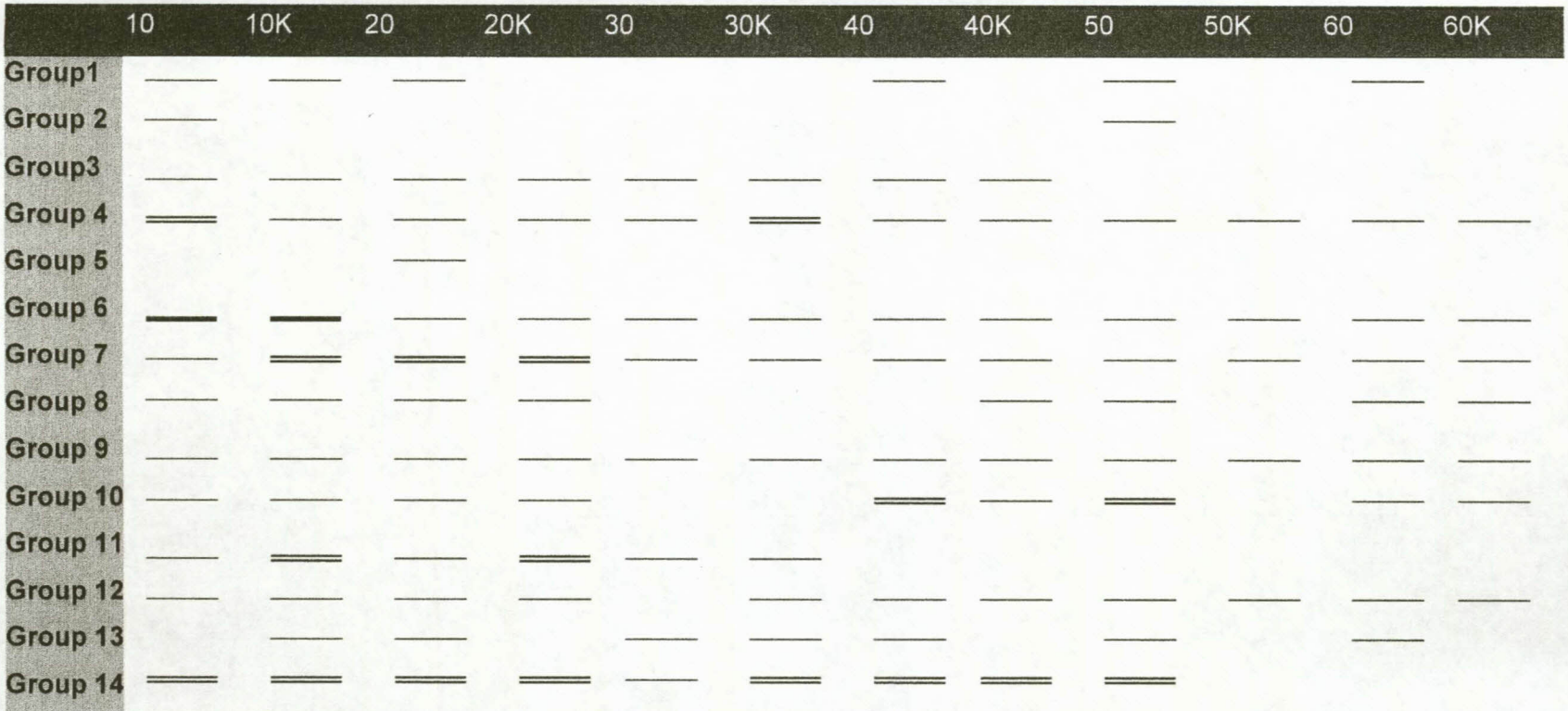


Table 17. The protein bands in PAN 3377 - coleoptiles

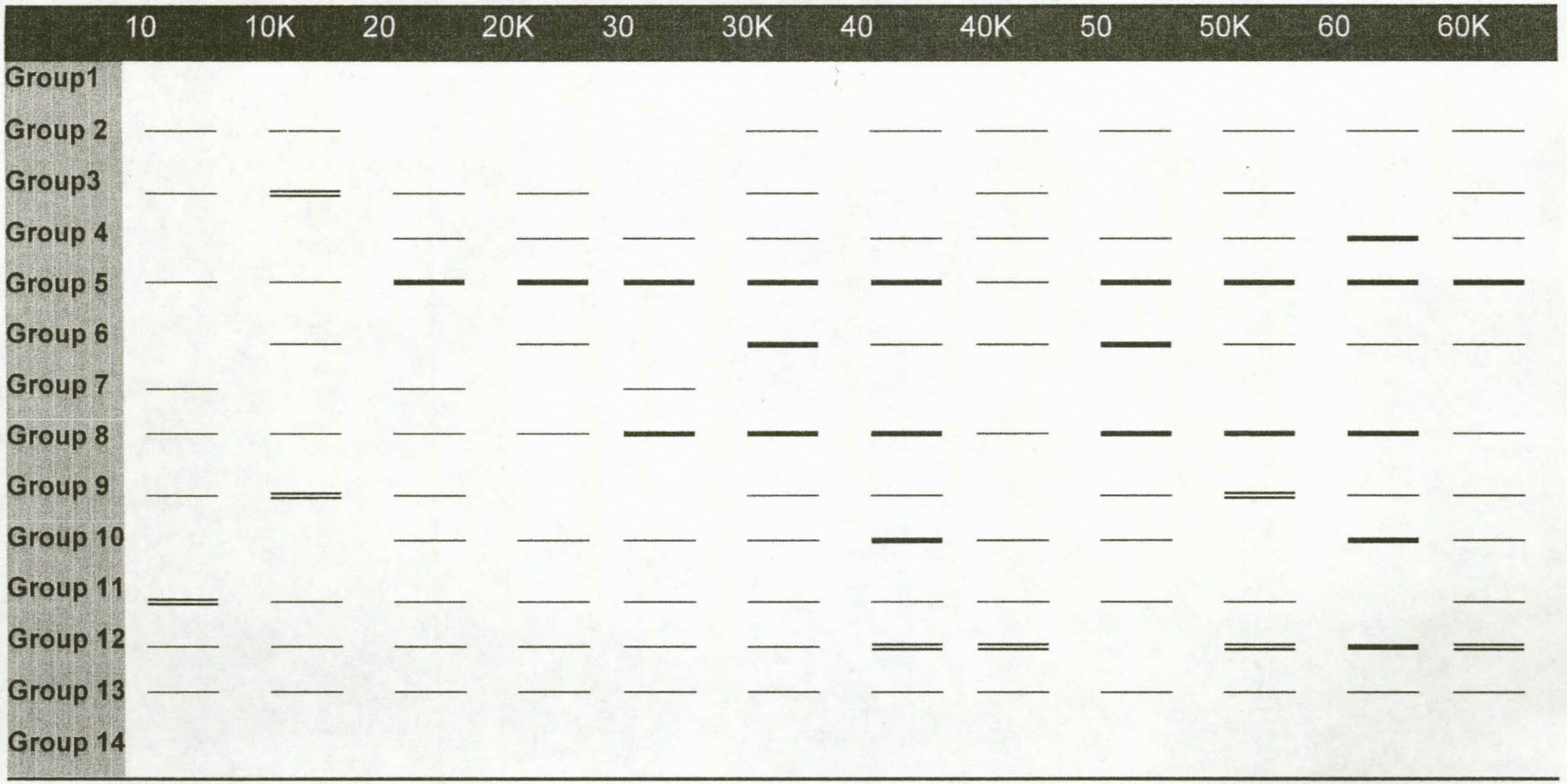


Table 18. The protein bands in Scheepers69 -coleoptiles

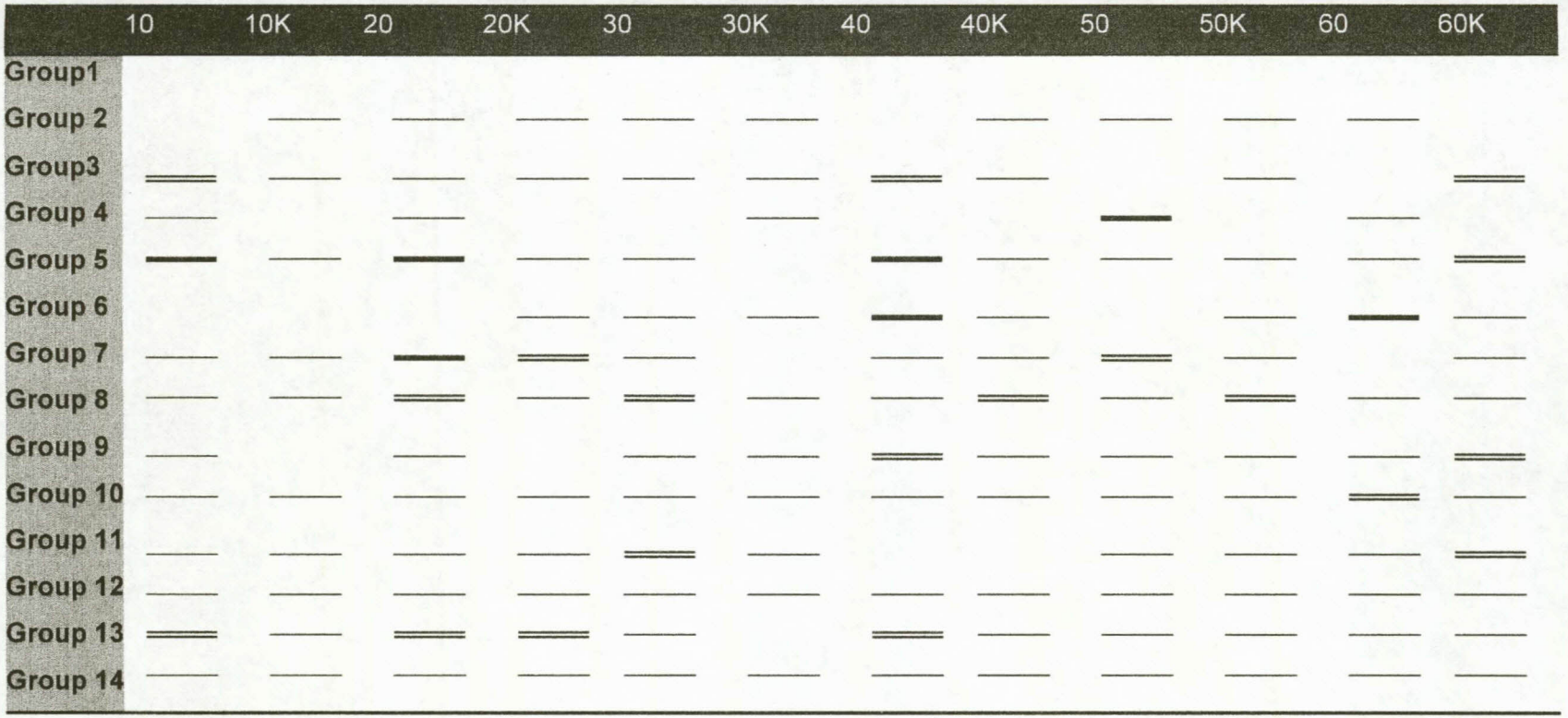


Table 19. The protein bands in Snack – coleoptiles

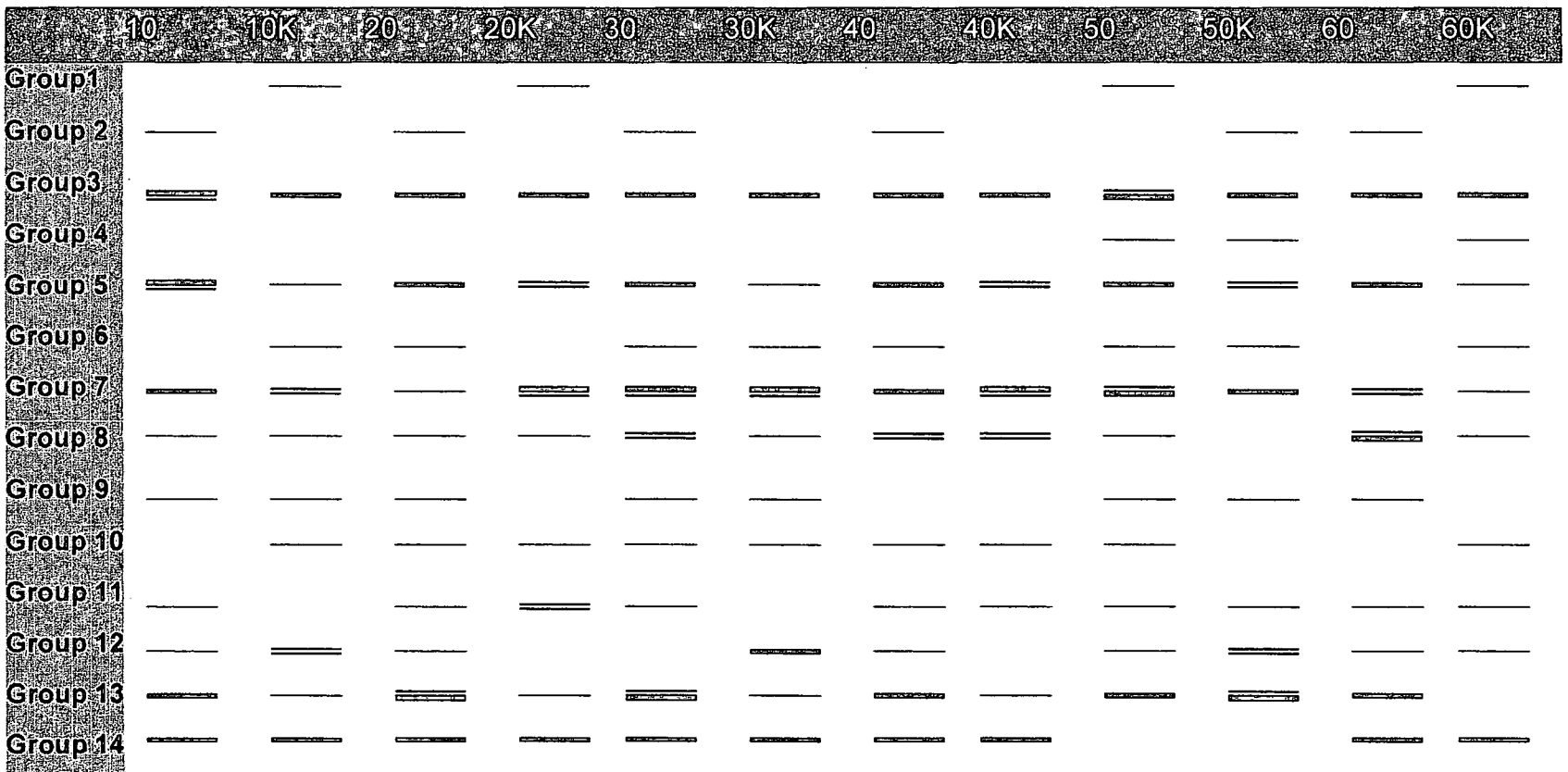
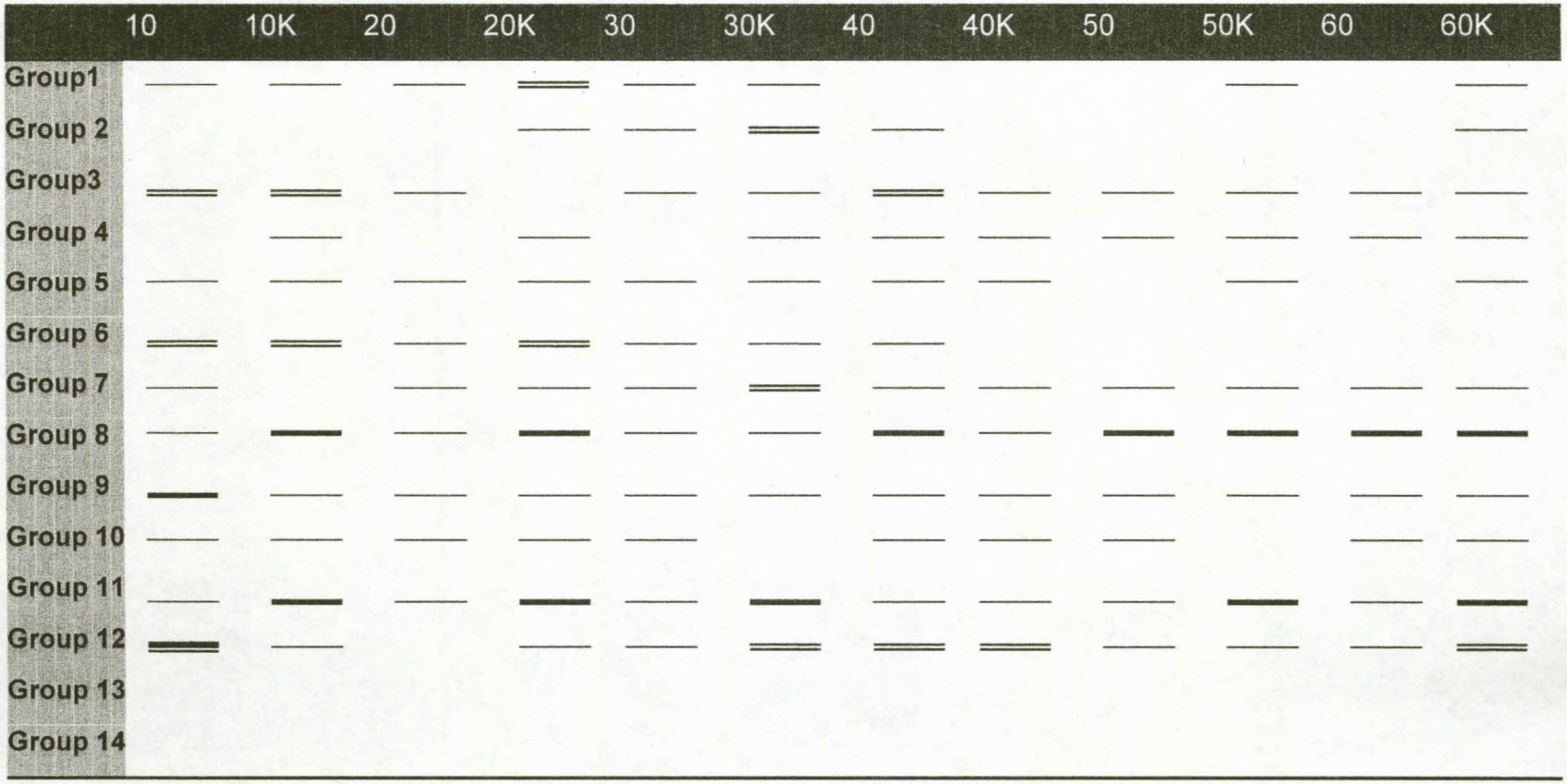
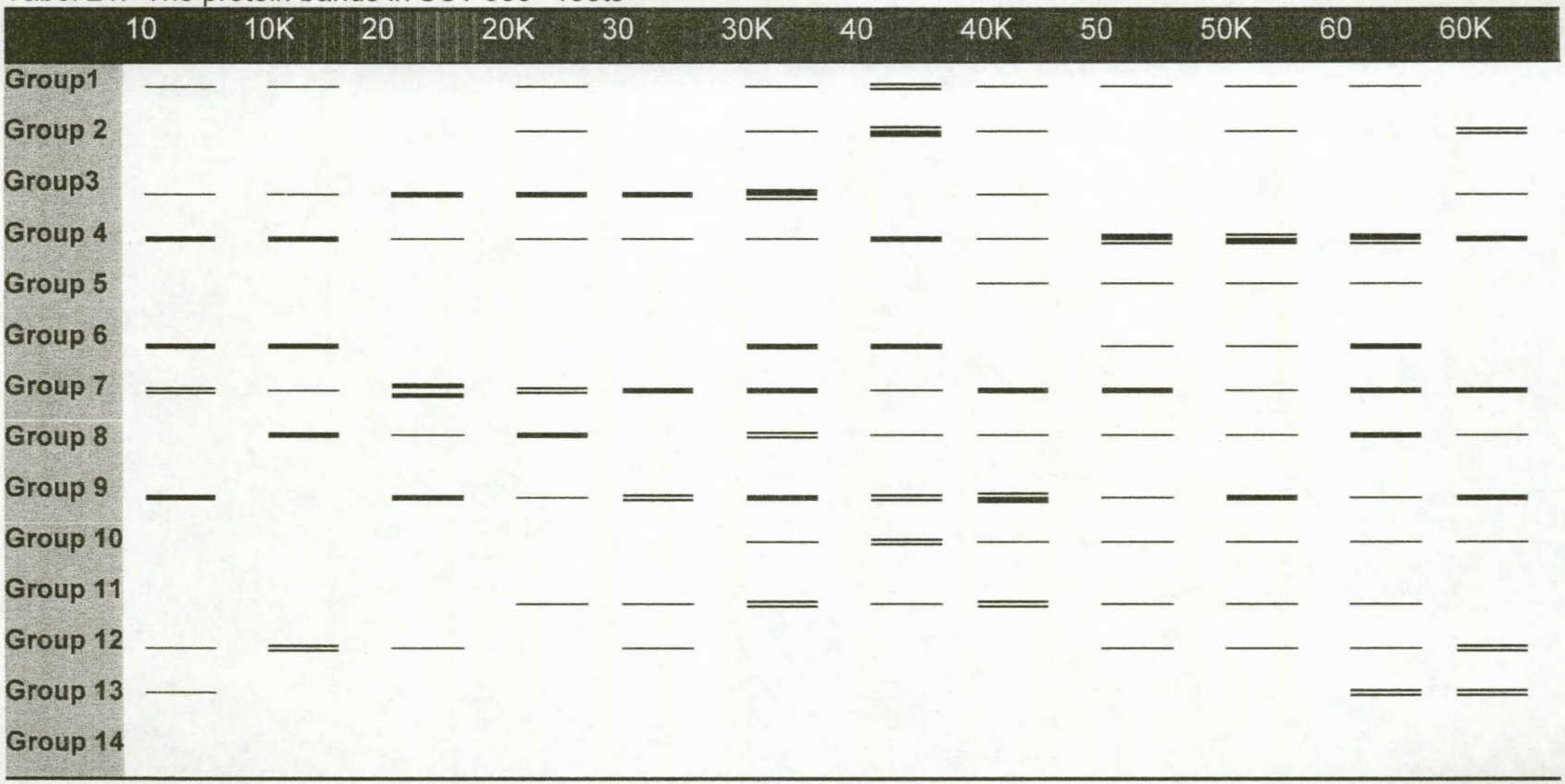


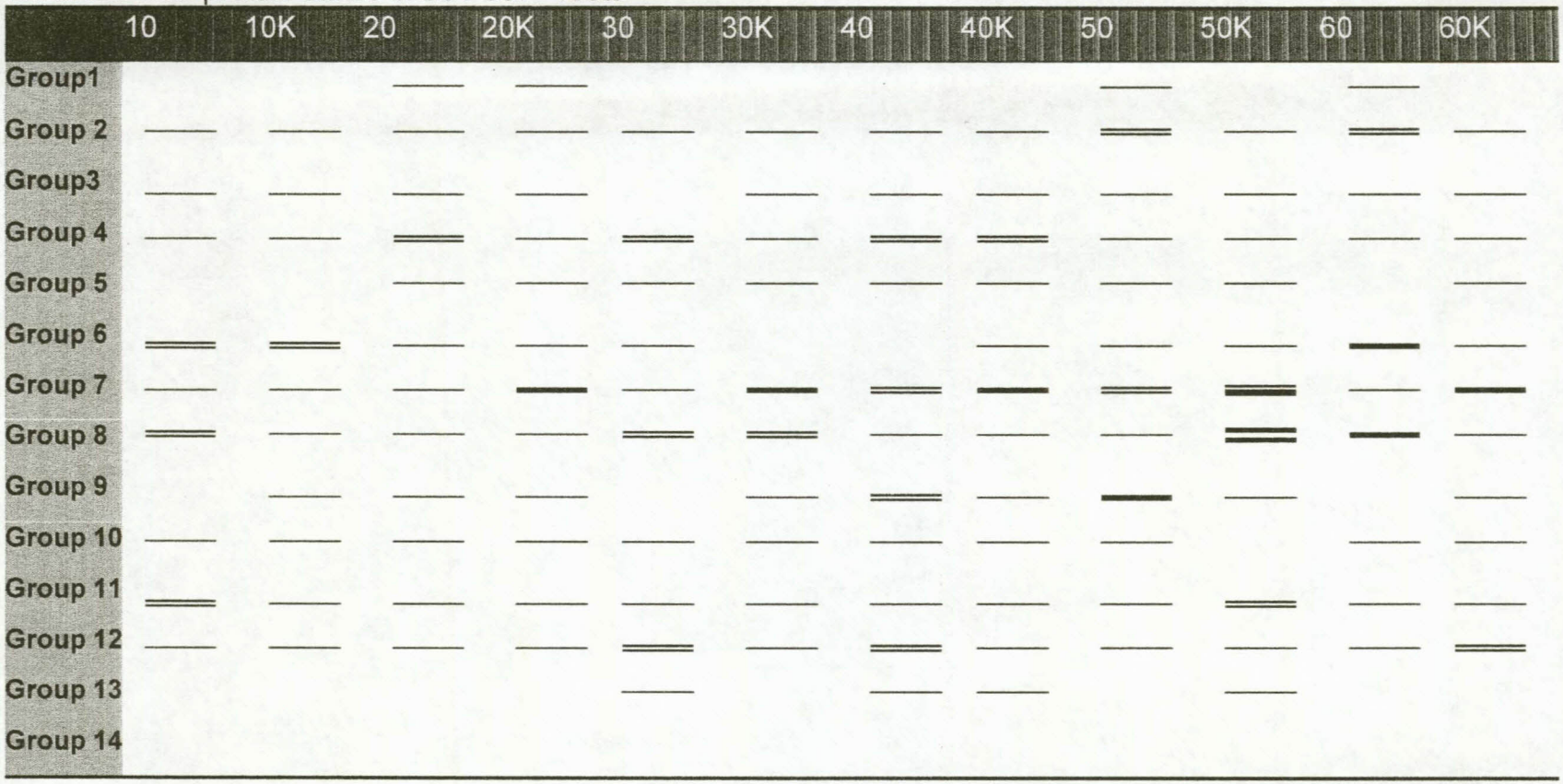
Table 20. The protein bands in SST 363 - coleoptiles



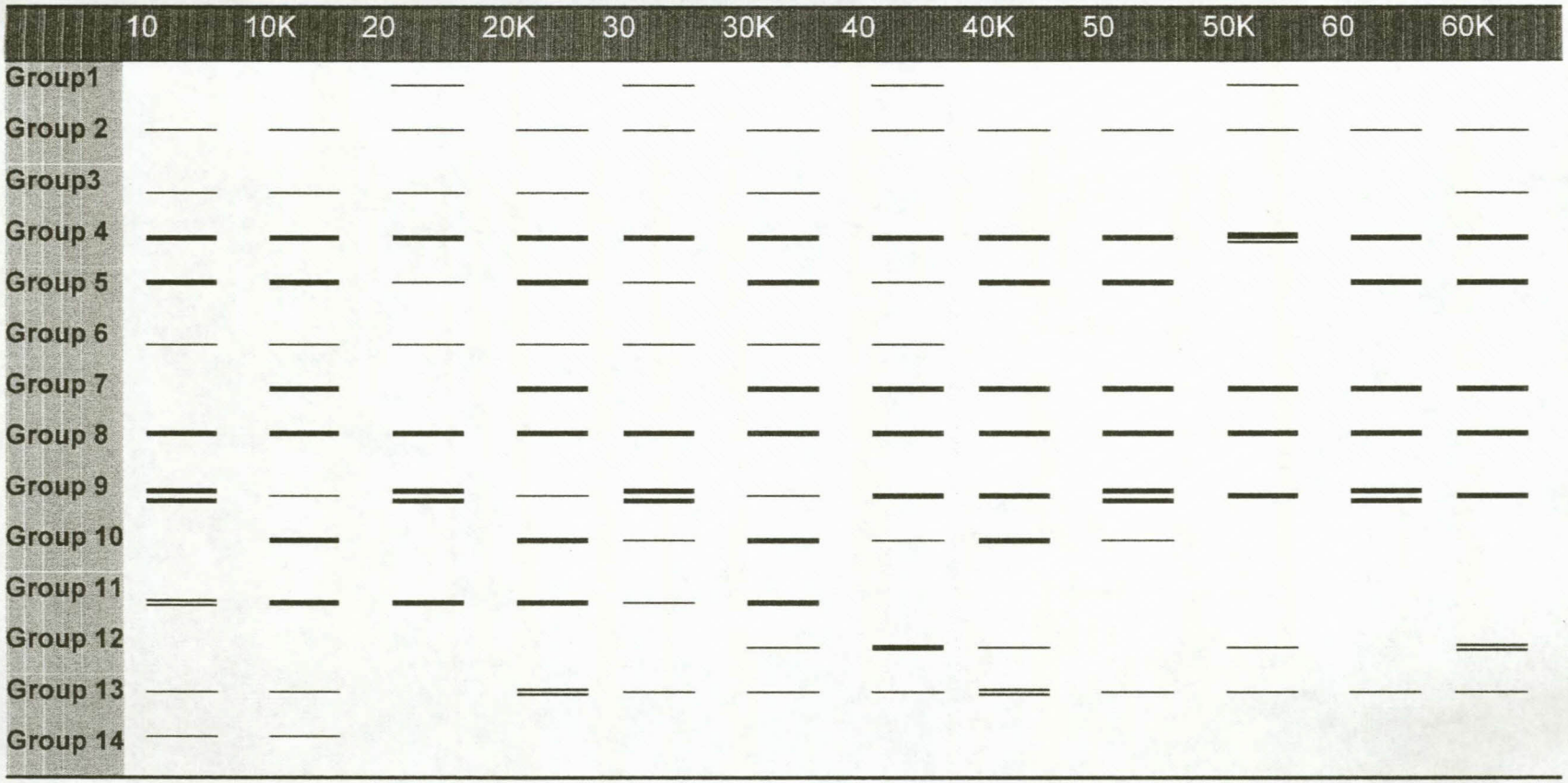
Tabel 21. The protein bands in SST 363 - roots



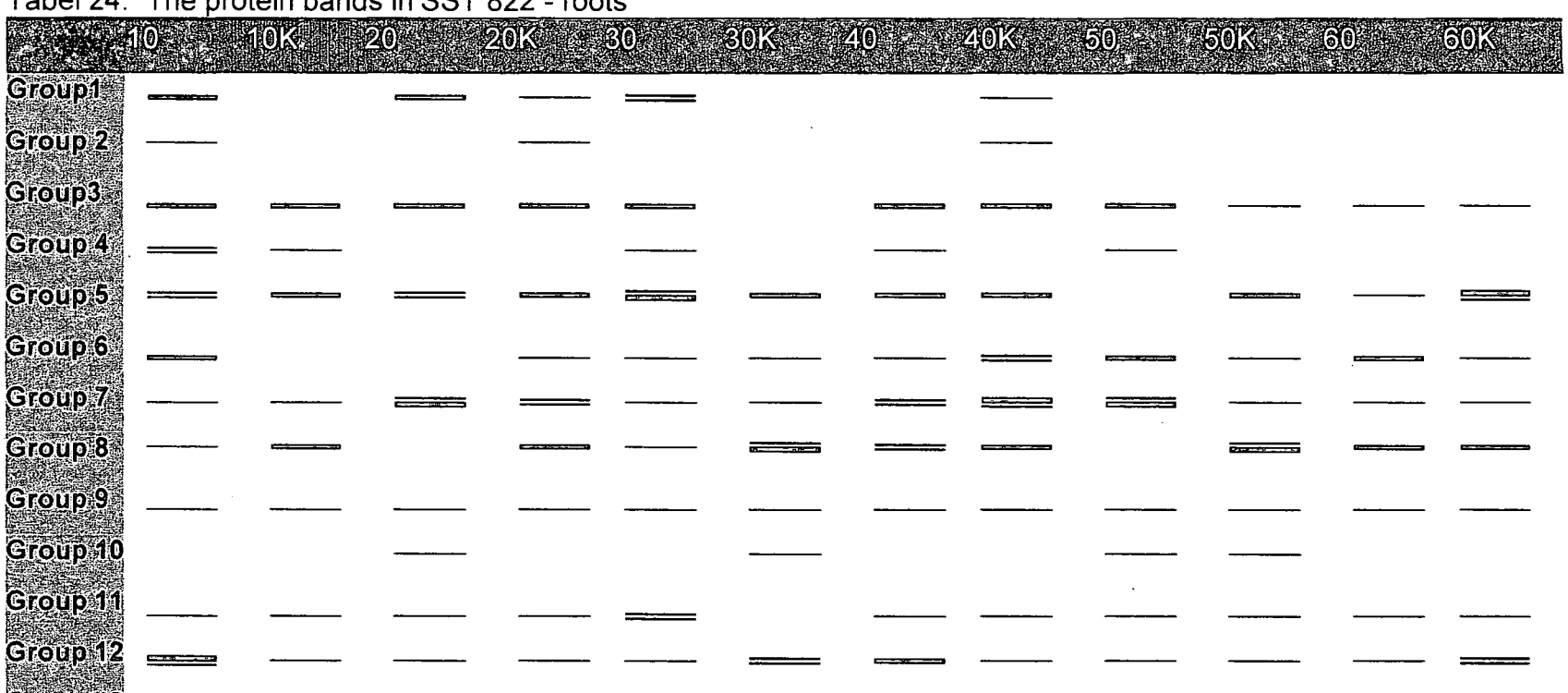
Tabel 22. The protein bands in SST 367 - roots



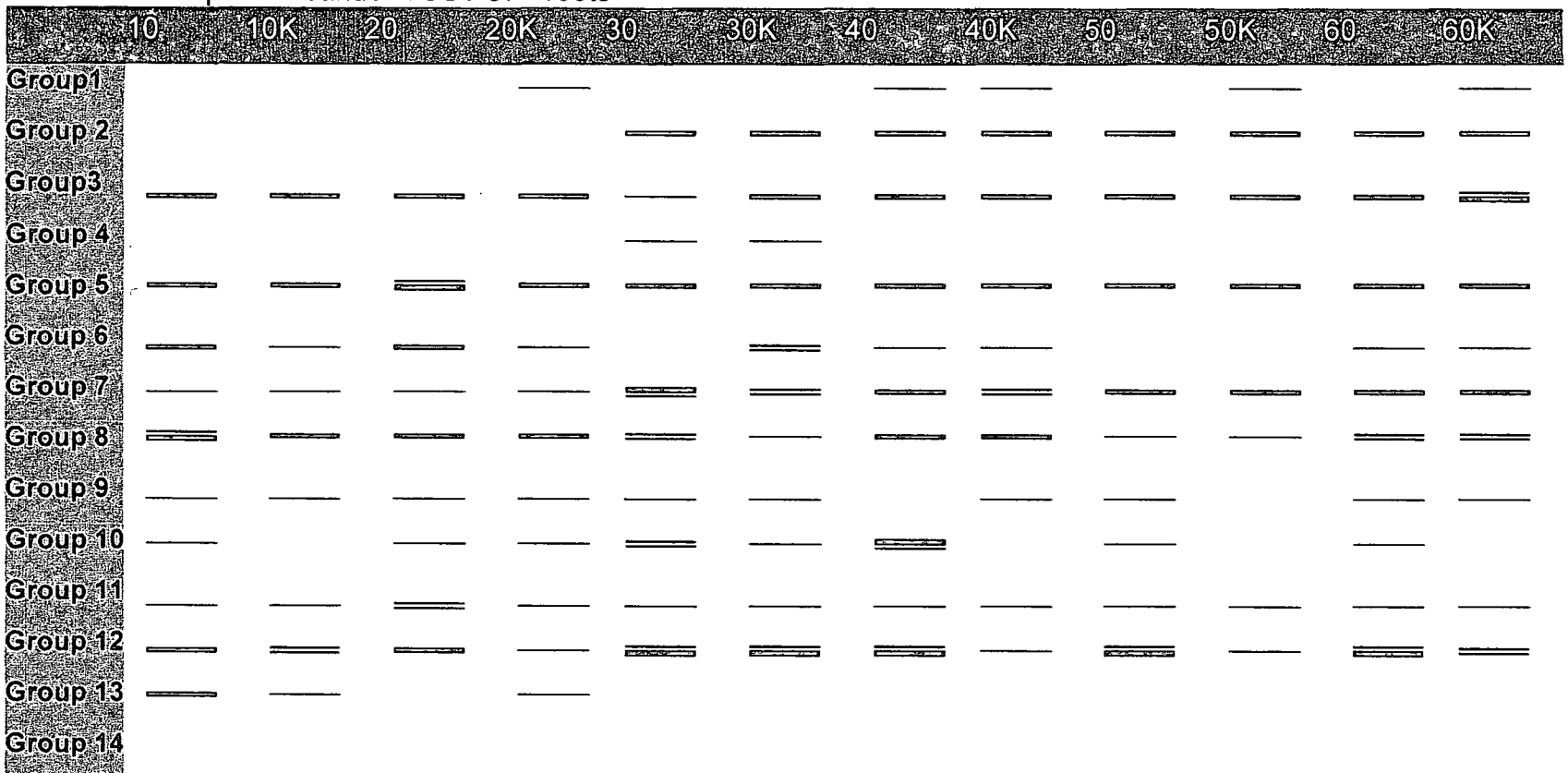
Tabel 23. The protein bands in SST 966 - roots



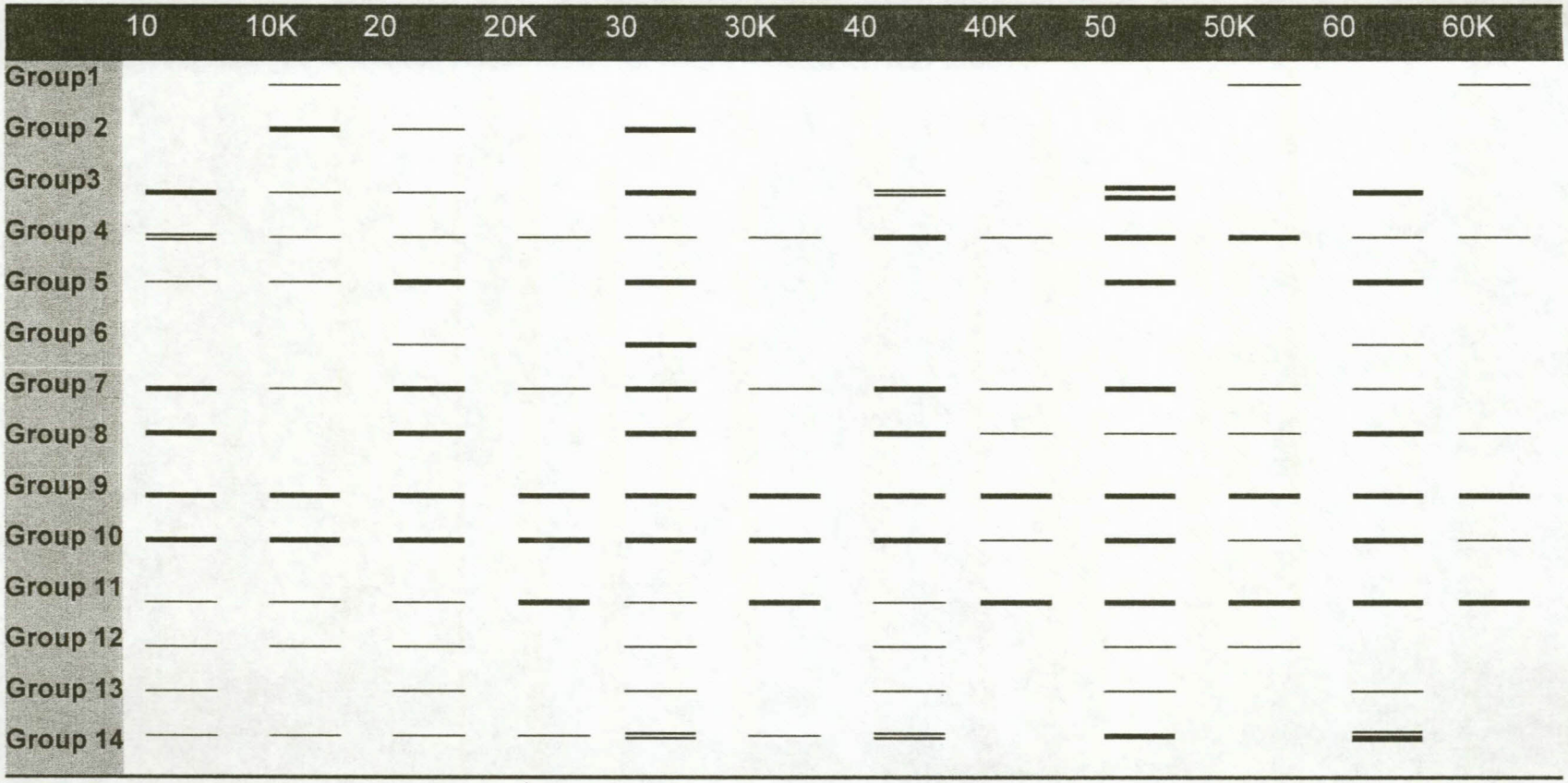
Tabel 24. The protein bands in SST 822 - roots



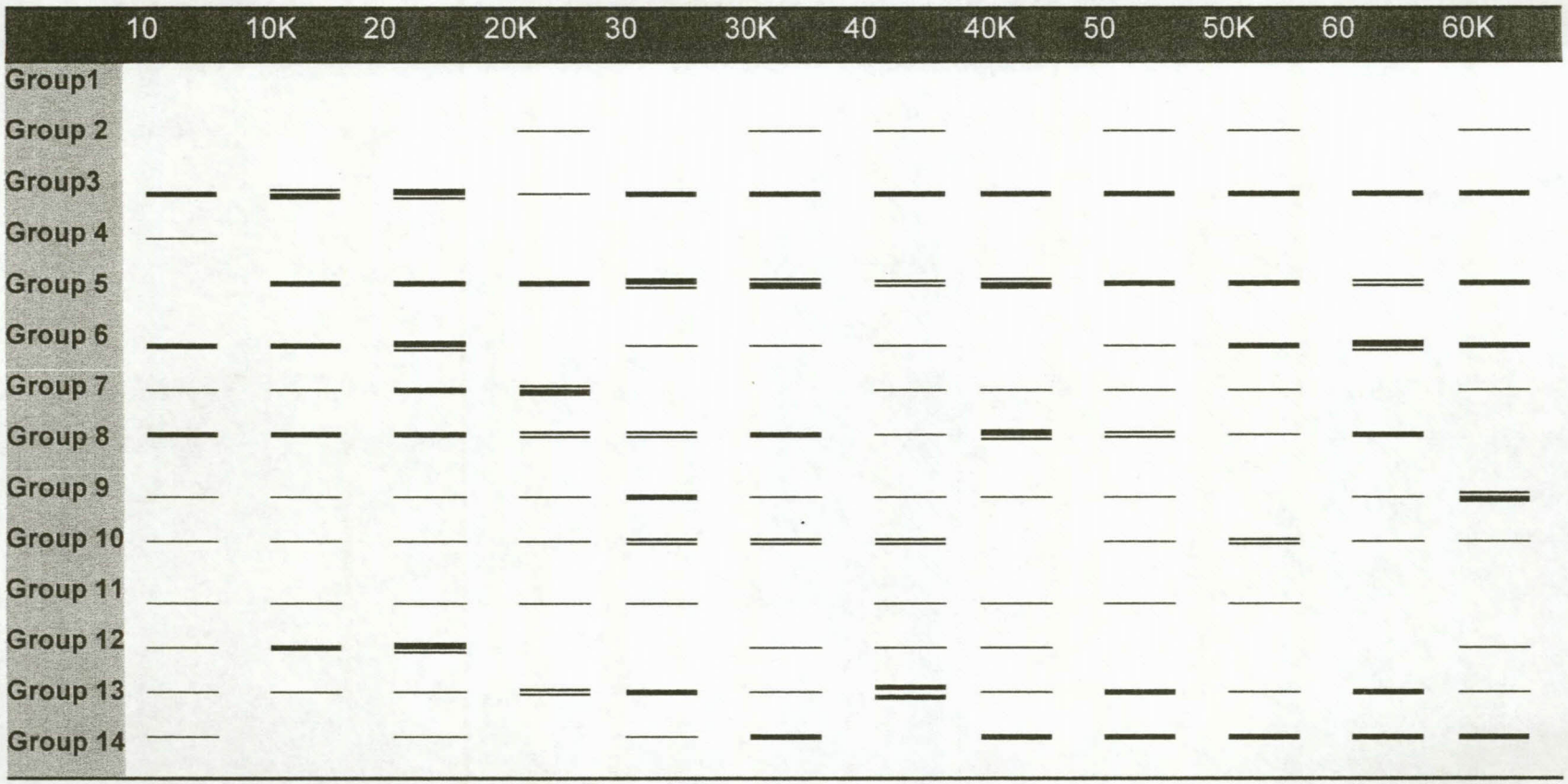
Tabel 25. The protein bands in SST 57 - roots



Tabel 26. The protein bands in SST 825 - roots



Tabel 27. The protein bands in Tugela DN - roots



Tabel 28. The number of bands expressed in Adam Tas

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	7	0	5	3	6	4	8	7	5	6	6	6	5
Control	6	5	5	6	6	2	7	7	8	6	8	2	8	10

Tabel 29. The number of bands expressed in Betta

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	2	9	4	5	6	6	5	8	5	9	3	7	5	0
Control	4	6	5	5	7	6	6	6	5	4	5	5	5	1

Tabel 30. The number of bands expressed in Betta DN

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	5	6	8	6	5	8	8	8	7	5	8	7	4	1
Control	6	3	5	5	8	4	8	6	6	5	5	4	3	2

Tabel 31. The number of bands expressed in Caledon

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	8	5	7	6	4	8	6	9	8	5	4	6	1
Control	0	6	5	5	6	1	6	12	6	6	2	6	6	1

Tabel 32. The number of bands expressed in Chinese Spring

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	2	3	8	3	6	5	5	6	2	4	4	7	5
Control	3	0	6	7	6	4	4	7	8	5	5	7	5	4

Tabel 33. The number of bands expressed in Gamtoos

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	2	5	4	9	3	6	7	4	5	2	6	5	7
Control	6	0	9	2	7	4	6	8	4	7	1	8	5	7

Tabel 34. The number of bands expressed in Gariep

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	6	5	7	0	5	5	6	6	8	3	2	8	5	7
Control	3	3	6	3	5	5	6	6	8	5	5	6	6	8

Tabel 35. The number of bands expressed in Hugenoot

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	7	6	7	6	3	4	7	5	7	5	5	4	3	0
Control	6	8	4	7	6	4	6	4	5	7	10	8	4	0

Tabel 36. The number of bands expressed in Letaba

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	6	5	3	7	5	8	7	5	5	9	3	5	1
Control	3	7	4	3	9	4	7	6	5	5	4	6	8	1

Tabel 37. The number of bands expressed in Limpopo

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	5	1	3	12	0	3	6	5	5	3	5	8	5	5
Control	5	1	7	9	1	2	6	4	7	1	6	3	4	3

Tabel 38. The number of bands expressed in Molen

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	6	5	7	3	8	6	7	6	5	5	6	6	3
Control	8	2	8	6	6	4	6	8	7	2	6	6	6	2

Tabel 39. The number of bands expressed in Molopo

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	1	7	7	1	2	9	5	7	2	6	2	8	10
Control	4	1	6	8	5	0	10	4	5	3	6	4	5	12

Tabel 40. The number of bands expressed in Nantes

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	8	7	7	8	3	7	3	7	4	9	4	9	6	3
Control	8	6	6	7	5	5	4	8	4	5	7	8	5	1

Tabel 41. The number of bands expressed in Palmiet

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	6	6	4	7	7	5	5	6	5	5	5	7	6	9
Control	6	8	1	8	5	8	5	7	5	2	6	5	8	9

Tabel 42. The number of bands expressed in PAN 3211

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	5	1	9	0	6	5	4	5	7	4	3	6	4	10
Control	6	6	7	3	6	4	5	0	6	4	2	8	5	12

Tabel 43. The number of bands expressed in PAN 3349

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	1	3	6	1	1	9	7	2	6	4	3	4	4	4
Control	6	2	8	3	1	10	4	5	6	2	4	6	6	4

Tabel 44. The number of bands expressed in PAN 3377

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	6	4	6	5	5	4	6	7	5	7	8	4	3
Control	5	1	1	6	8	0	3	4	6	4	5	9	5	4

Tabel 45. The number of bands expressed in Scheepers 69

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	3	6	1	7	5	8	7	6	5	5	6	7	7
Control	0	3	6	2	5	2	8	5	3	7	4	6	6	7

Tabel 46. The number of bands expressed in Snack

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	2	9	3	4	7	7	6	8	10	4	7	9	6
Control	0	1	9	2	4	8	6	6	6	9	3	6	4	6

Tabel 47. The number of bands expressed in SST 363

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	5	2	3	8	2	4	8	4	8	4	4	5	3	0
Control	5	6	6	7	2	3	7	7	6	4	6	5	2	0

Tabel 48. The number of bands expressed in SST367

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	8	5	9	4	5	8	8	4	6	7	8	2	0
Control	1	6	6	7	5	6	7	8	6	5	7	7	2	0

Tabel 49. The number of bands expressed in SST 57

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	1	4	6	1	7	4	7	9	5	8	7	11	1	0
Control	4	4	7	1	6	6	8	7	5	2	6	10	2	0

Tabel 50. The number of bands expressed in SST 66

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	2	7	3	5	6	5	6	6	5	5	3	7	4
Control	1	1	7	3	4	6	3	8	6	5	7	5	6	4

Tabel 51. The number of bands expressed in SST 822

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	1	6	5	8	5	9	5	6	2	6	7	5	6
Control	2	2	5	1	7	6	8	8	6	2	5	8	3	6

Tabel 52. The number of bands expressed in SST 825

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	2	8	7	5	3	6	7	6	6	6	5	6	9
Control	3	1	1	6	1	0	5	3	6	6	6	1	0	3

Tabel 53. The number of bands expressed in SST 966

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	6	2	6	6	4	3	6	11	3	4	1	6	1
Control	1	6	4	7	5	3	6	6	6	4	3	5	8	1

Tabel 54. The number of bands expressed in Tugela DN

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	2	7	1	8	8	4	8	6	8	5	4	7	5
Control	0	4	7	0	7	4	6	7	6	7	4	4	7	4

Table 21. The protein bands in SST 367 - Coleoptiles

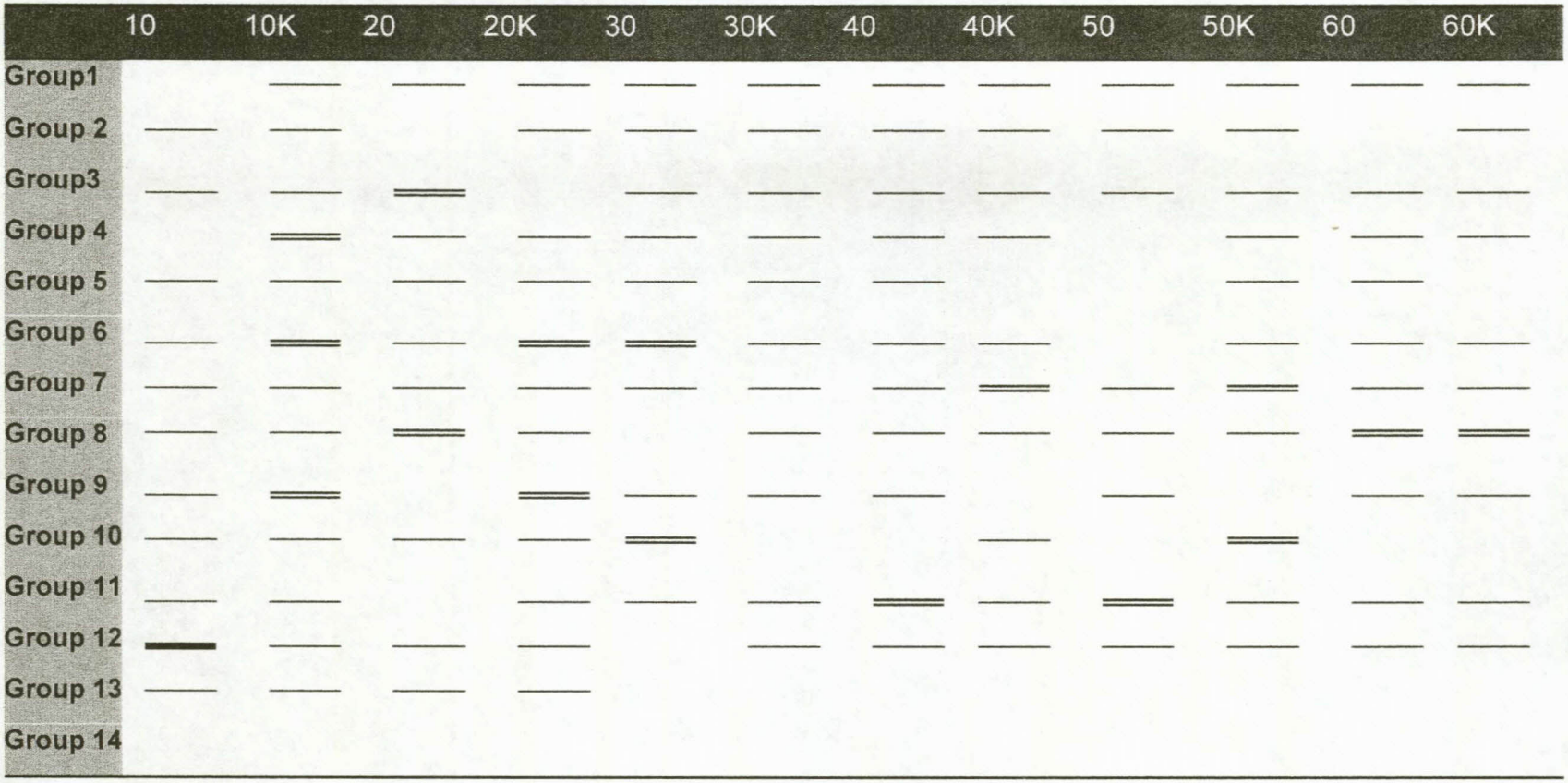


Table 22. The protein bands in SST 57 - coleoptiles

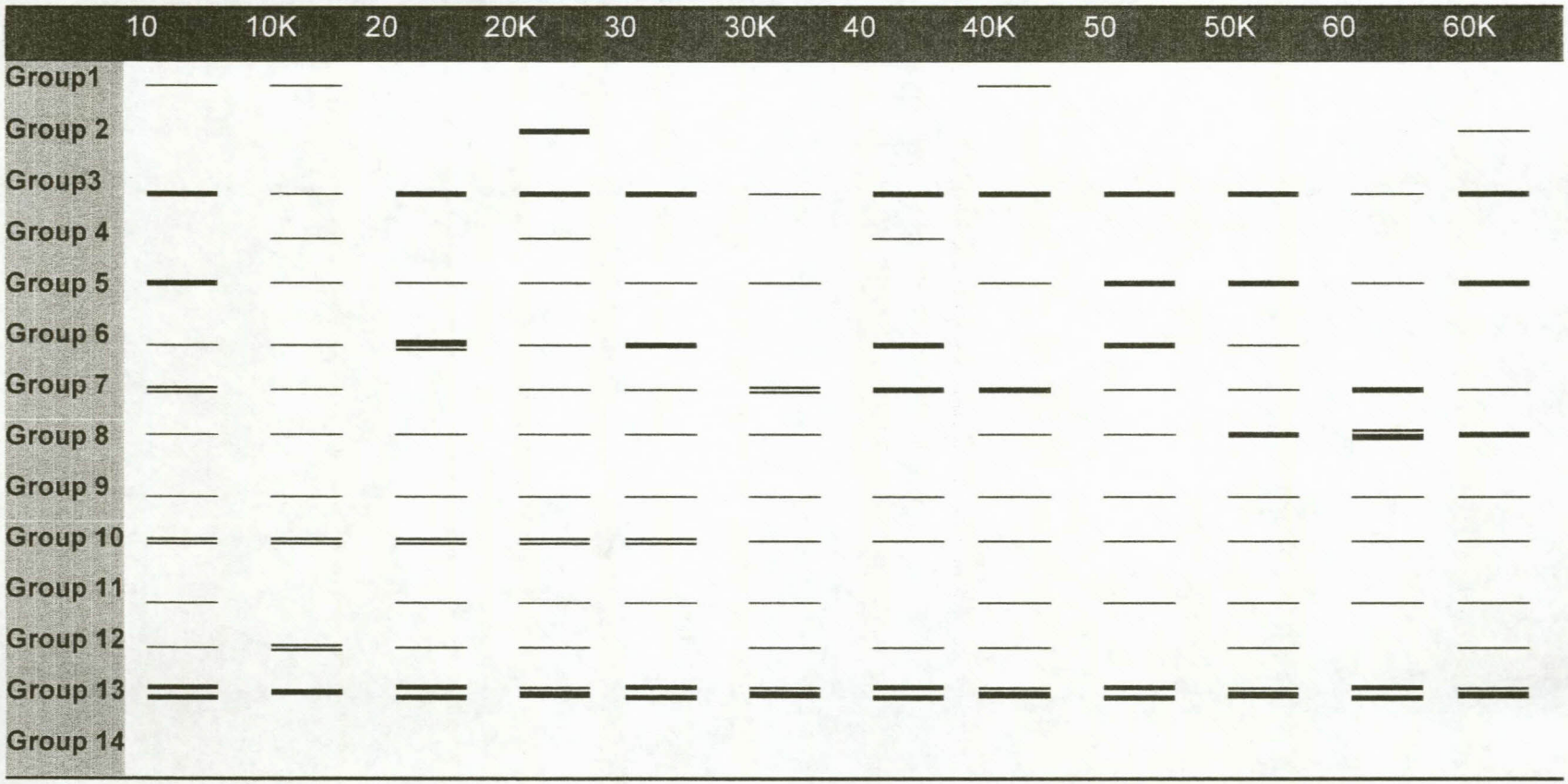


Table 23. The protein bands in SST 66 -coleoptiles

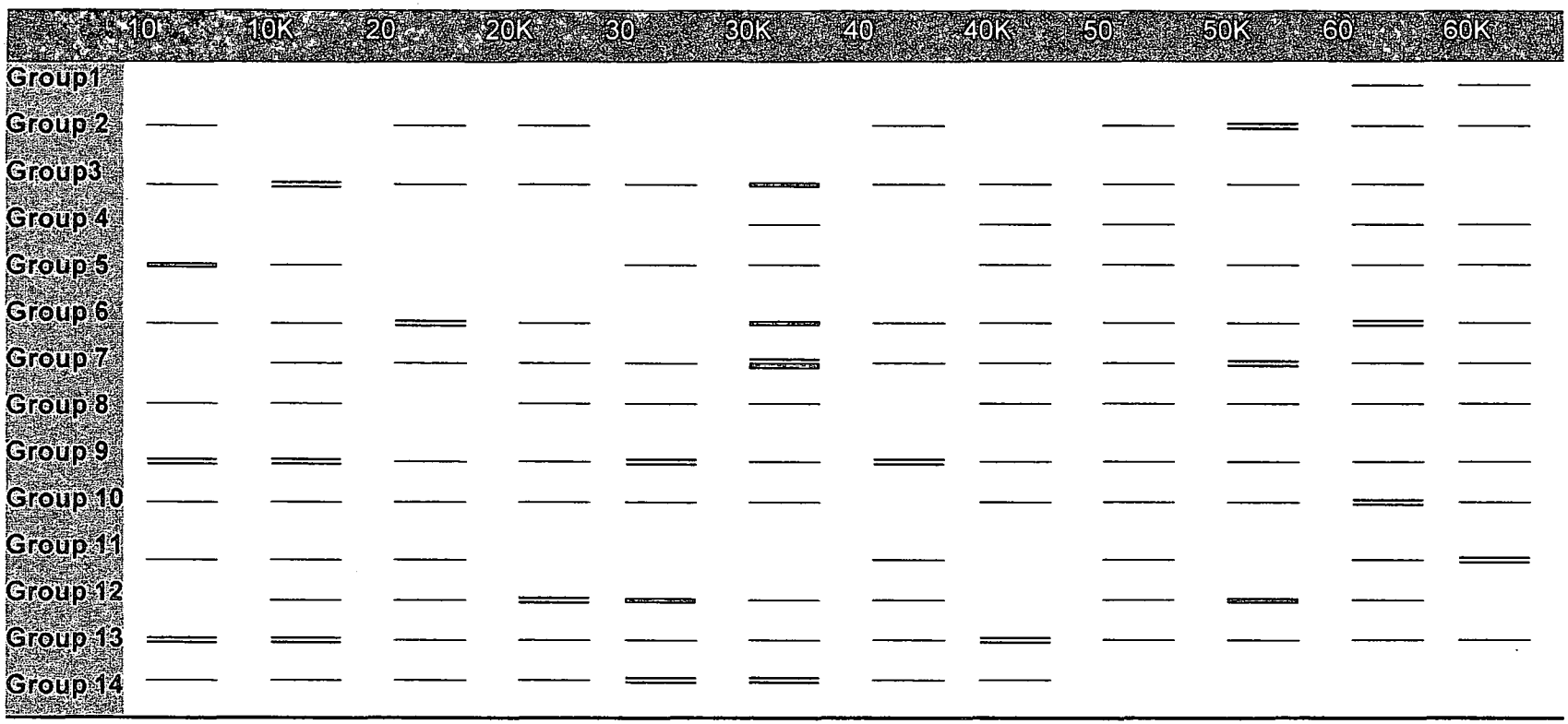


Table 24. The protein bands in SST 822 -coleoptiles

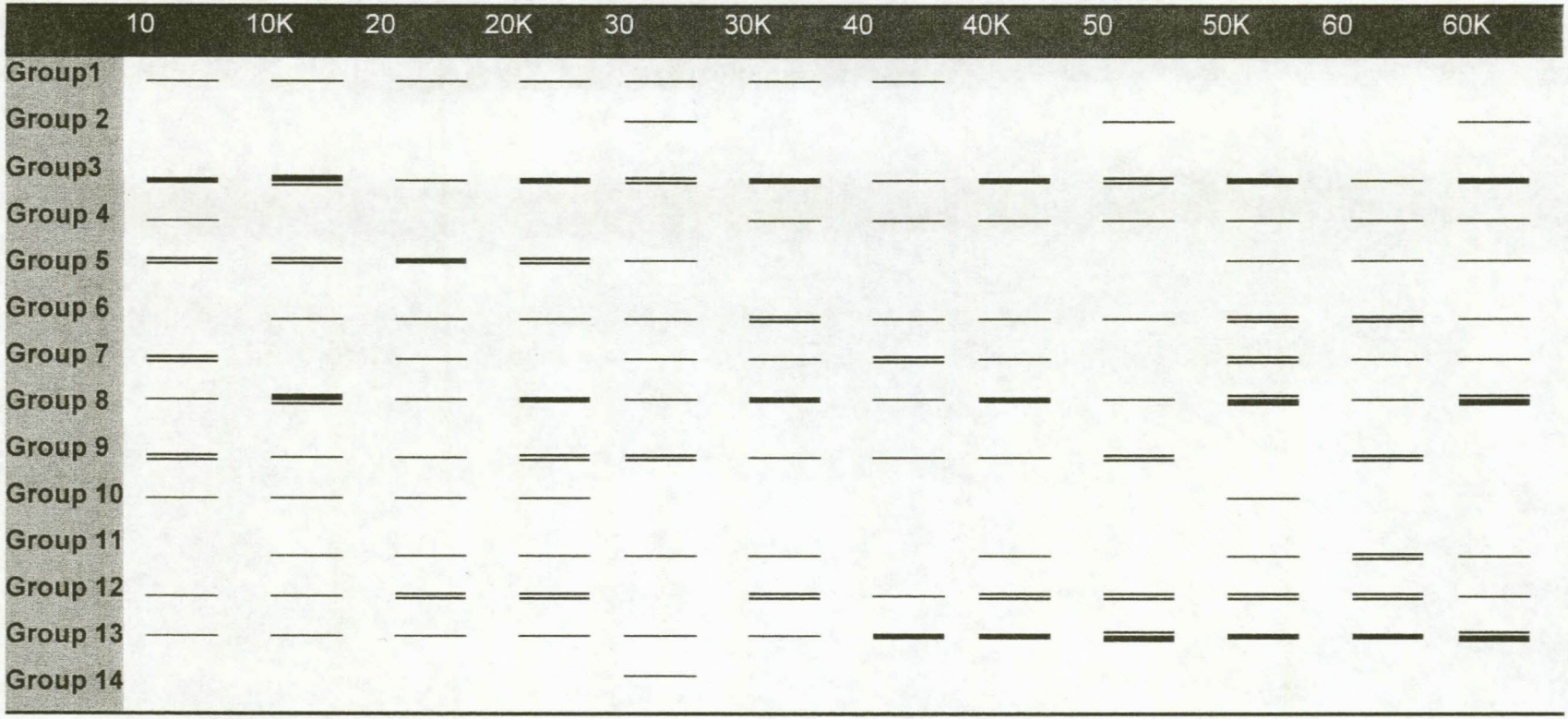


Table 25. The protein bands in SST825 -coleoptiles

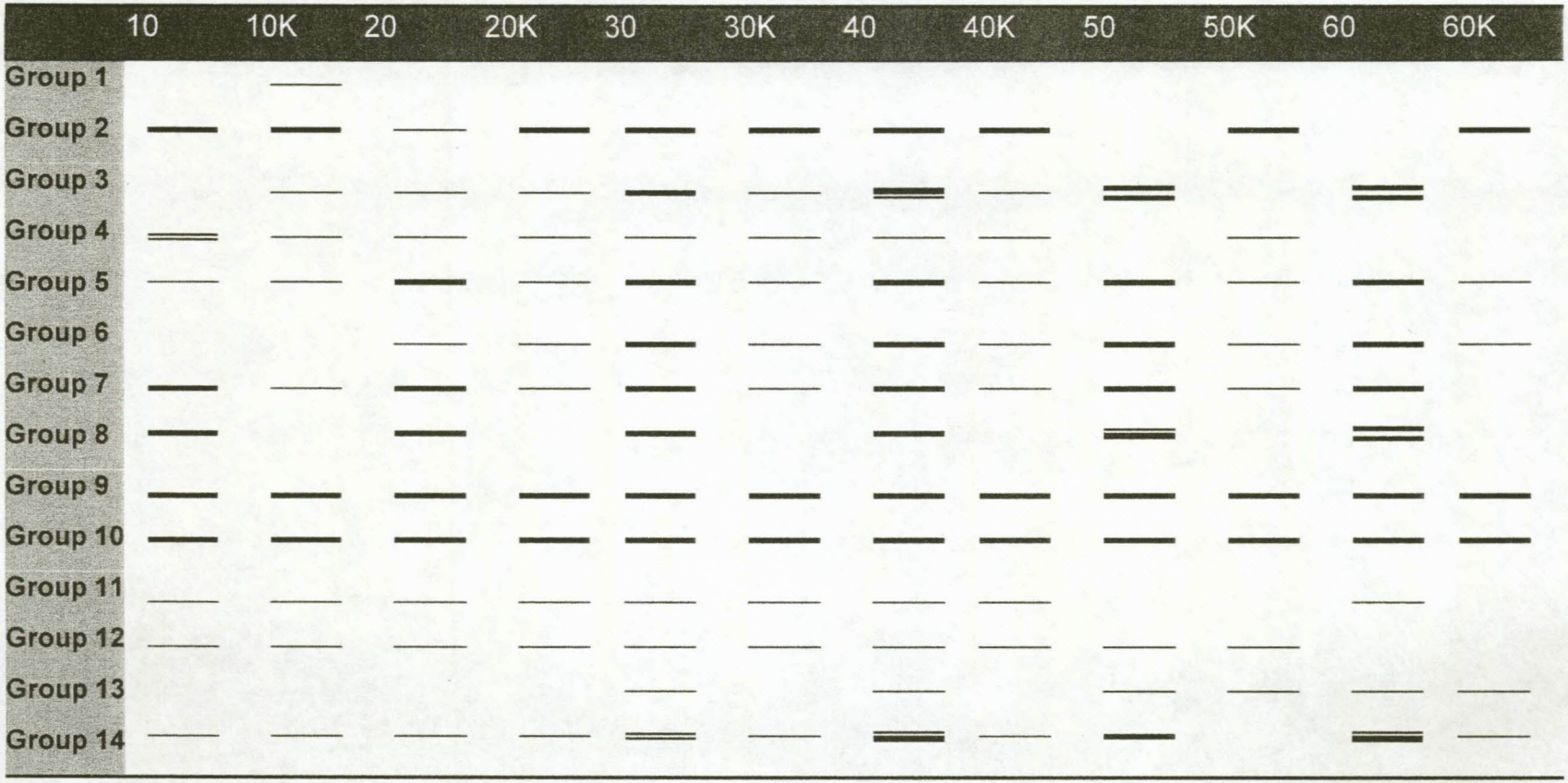


Table 26. The protein bands in SST966 -coleoptiles

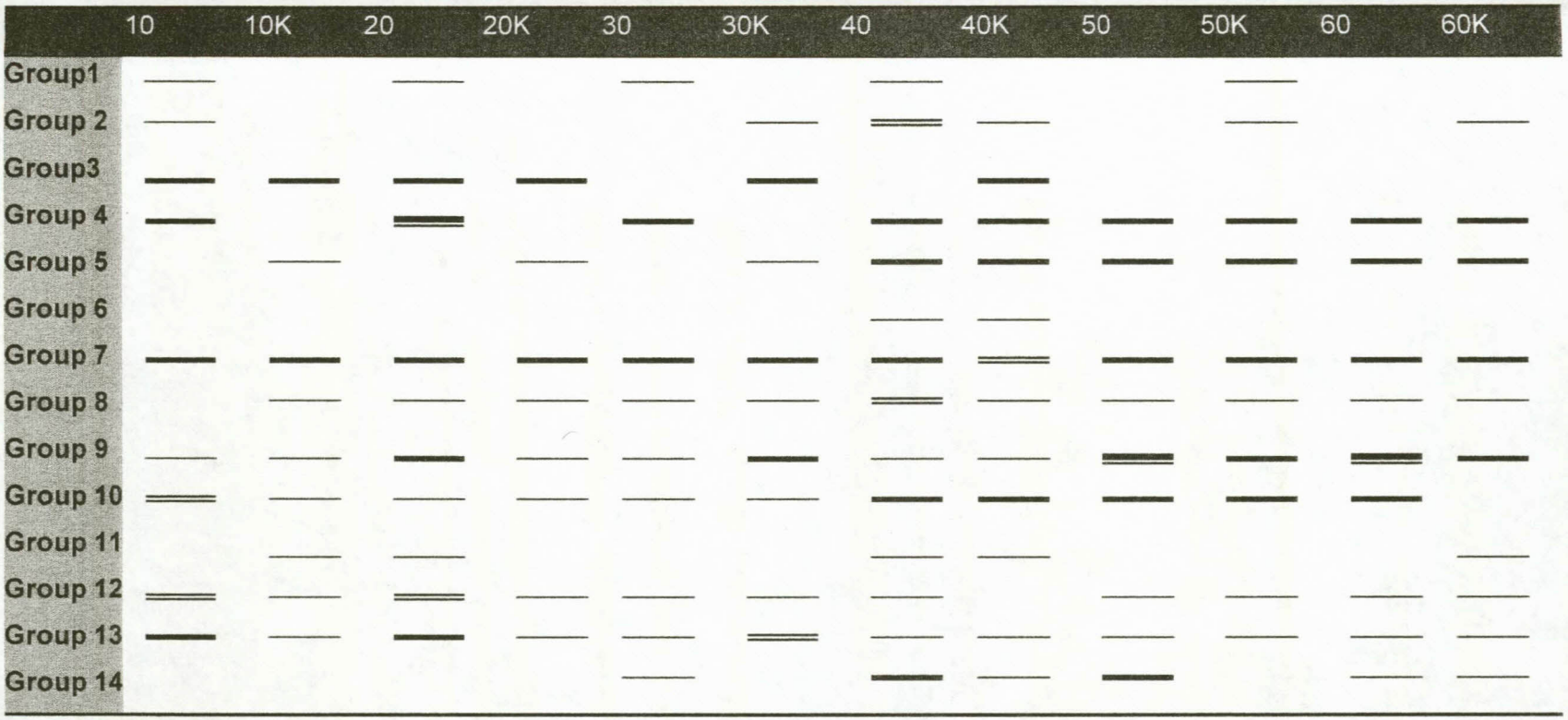
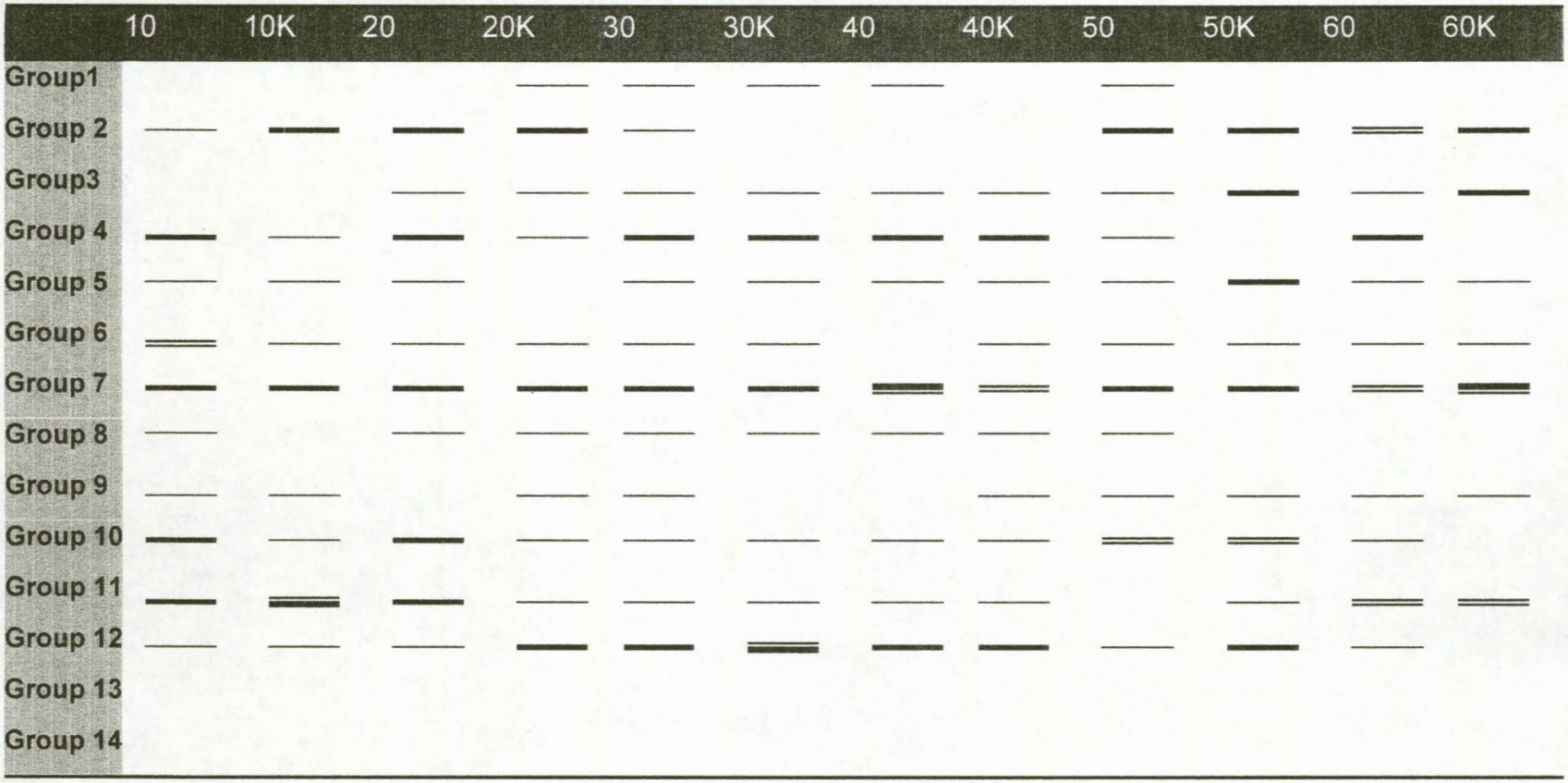


Table 27. The protein bands in Tugela DN - coleoptiles



Tabel 28. The number of protein bands expressed in Adam Tas

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	3	7	3	6	4	6	6	6	5	7	4	2	9
Control	3	4	3	5	4	5	7	8	10	6	7	5	6	12

Tabel 29. The number of protein bands expressed in Betta

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	7	3	6	6	8	8	7	5	5	8	6	3	2
Control	6	5	7	7	8	6	6	8	5	4	8	6	3	2

Tabel 30. The number of protein bands expressed in Betta DN

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	10	4	7	5	5	6	7	8	6	7	6	9	6	1
Control	7	6	6	5	3	9	7	6	6	2	8	8	7	0

Tabel 31. The number of protein bands expressed in Caledon

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	4	4	8	6	5	7	7	8	9	6	5	3	6
Control	1	4	10	6	6	6	6	9	6	6	6	6	0	9

Tabel 32. The number of protein bands expressed in Chinese Spring

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	4	5	5	5	7	5	7	7	4	3	6	5	0
Control	0	4	4	6	4	5	9	5	5	5	5	8	6	0

Tabel 33. The number of protein bands expressed in Gamtoos

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	0	1	4	9	4	8	5	8	6	5	5	7	6
Control	2	3	2	4	8	5	5	5	8	6	6	3	6	6

Tabel 34. The number of protein bands expressed in Gariep

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	4	6	3	6	6	7	10	5	6	6	6	5	6
Control	5	1	3	6	5	6	5	8	4	6	4	4	6	6

Tabel 35. The number of protein bands expressed in Hugenoot

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	5	6	4	8	7	5	6	6	5	7	5	7	2	2
Control	5	5	7	1	7	7	6	4	6	7	6	7	2	1

Tabel 36. The number of protein bands expressed in Letaba

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	5	6	4	3	12	5	6	6	8	5	4	2	6
Control	3	4	8	2	2	11	6	6	6	8	4	6	2	5

Tabel 37. The number of protein bands expressed in Limpopo

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	6	5	5	4	4	5	6	6	4	6	5	8	0
Control	2	5	3	4	6	5	4	6	5	8	4	6	9	0

Tabel 38. The number of protein bands expressed in Molen

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	4	3	8	4	3	5	7	8	8	6	5	5	10
Control	5	4	7	6	7	6	8	8	10	7	10	3	5	8

Tabel 39. The number of protein bands expressed in Molopo

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	8	3	6	5	3	5	6	6	2	4	5	4	5	9
Control	9	2	9	3	4	2	6	6	4	4	5	4	6	12

Tabel 40. The number of protein bands expressed in Nantes

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	7	5	6	5	7	7	6	6	10	6	7	7	12	0
Control	5	6	6	4	9	7	7	6	8	4	12	3	12	0

Tabel 41. The number of protein bands expressed in Palmiet

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	10	1	6	6	7	7	3	6	6	9	7	5	8	4
Control	5	2	6	7	9	5	3	6	6	9	7	6	6	6

Tabel 42. The number of protein bands expressed in PAN 3211

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	5	7	1	9	3	3	3	8	4	7	6	8	6
Control	6	5	8	1	8	6	3	5	9	5	9	5	10	6

Tabel 43. The number of protein bands expressed in PAN 3349

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	5	2	4	7	1	6	7	4	6	7	3	5	5	9
Control	1	0	4	7	0	6	8	4	6	4	5	6	2	8

Tabel 44. The number of protein bands expressed in PAN 3377

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	4	2	5	6	3	3	6	5	5	6	6	6	0
Control	0	5	7	5	6	6	0	6	6	4	6	9	6	0

Tabel 45. The number of protein bands expressed in Scheepers 69

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	4	6	4	6	3	6	8	7	7	6	6	9	6
Control	0	5	7	1	6	5	6	8	5	6	6	6	6	6

Tabel 46. The number of protein bands expressed in Snack

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	1	5	8	1	7	4	9	9	5	4	6	5	8	5
Control	3	1	6	2	9	4	10	6	3	5	5	6	6	5

Tabel 47. The number of protein bands expressed in SST 363

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	2	8	3	4	5	6	6	6	4	6	6	0	0
Control	6	4	6	6	6	5	6	6	6	4	6	8	0	0

Tabel 48. The number of protein bands expressed in SST 367

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	5	4	7	4	5	6	6	7	5	3	7	5	2	0
Control	6	6	5	7	4	8	8	7	6	6	6	6	2	0

Tabel 49. The number of protein bands expressed in SST 57

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	1	0	6	1	5	6	6	6	6	8	5	3	12	0
Control	2	2	6	2	6	4	7	6	6	8	5	7	11	0

Tabel 50. The number of protein bands expressed in SST 66

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	1	5	6	2	4	7	7	4	9	6	5	5	7	5
Control	1	4	6	3	5	6	6	6	7	6	3	5	8	5

Tabel 51. The number of protein bands expressed in SST 822

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	2	8	4	5	6	6	6	10	2	4	8	7	1
Control	3	1	7	4	5	8	5	9	5	3	6	10	7	0

Tabel 52. The number of protein bands expressed in SST 875

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	4	8	5	6	5	6	8	6	6	5	5	4	9
Control	0	6	4	5	6	5	5	0	6	6	4	5	2	5

Tabel 53. The number of protein bands expressed in SST 966

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	3	2	7	3	1	6	6	8	7	2	8	6	4
Control	1	4	4	3	6	1	7	6	6	5	3	5	7	2

Tabel 54. The number of protein bands expressed inTugela DN

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	5	5	6	6	6	8	5	4	7	6	6	0	0
Control	2	4	5	4	5	6	8	3	5	6	8	6	0	0

APPENDIX B

10 – 10 days cold treatment

10 K – 10 days control

20 – 20 days cold treatment

20 K – 20 days control

30 – 30 days cold treatment

30 K – 30 days control

40 – 40 days cold treatment

40 K – 40 days control

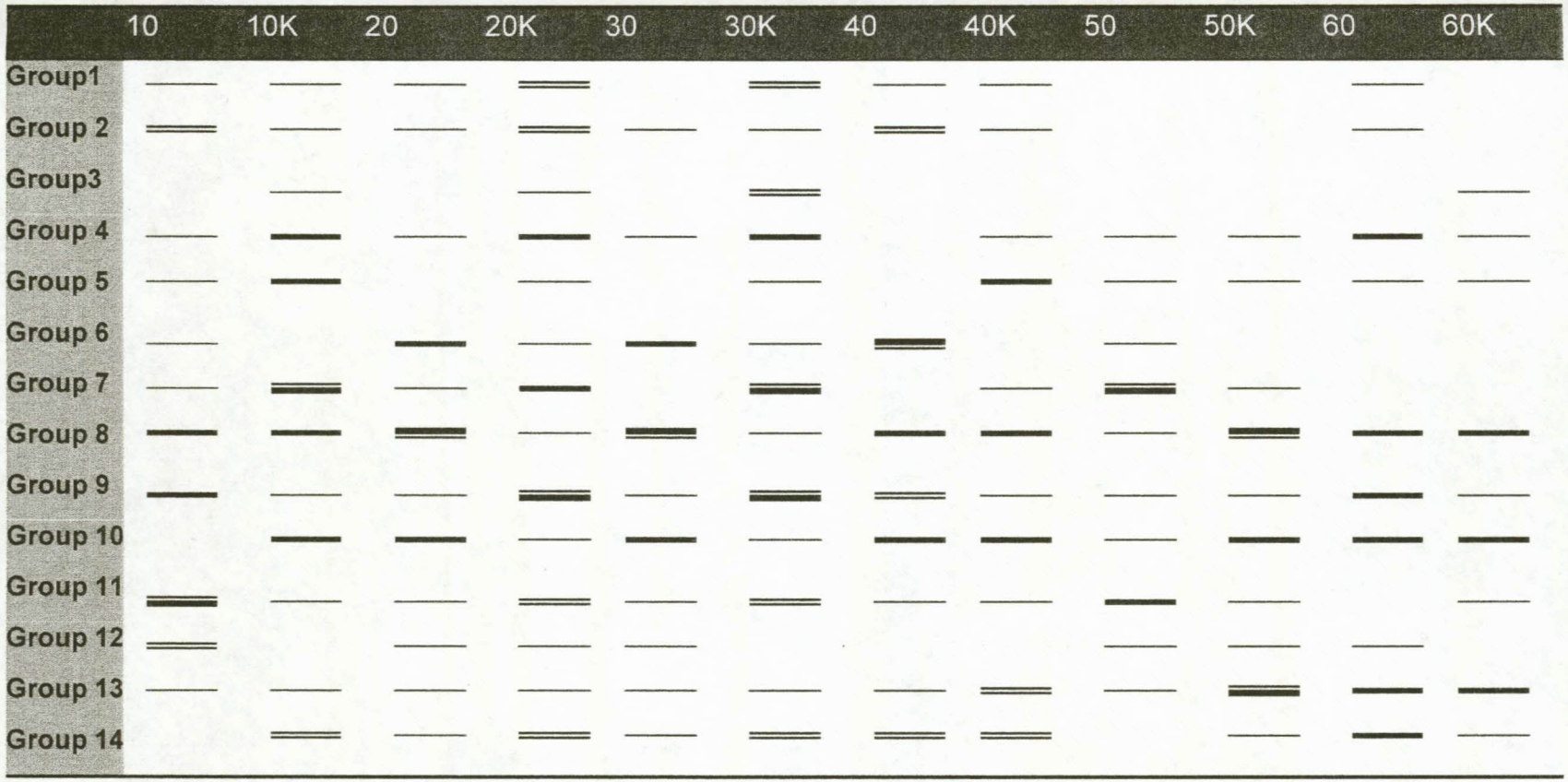
50 – 50 days cold treatment

50 K – 50 days control

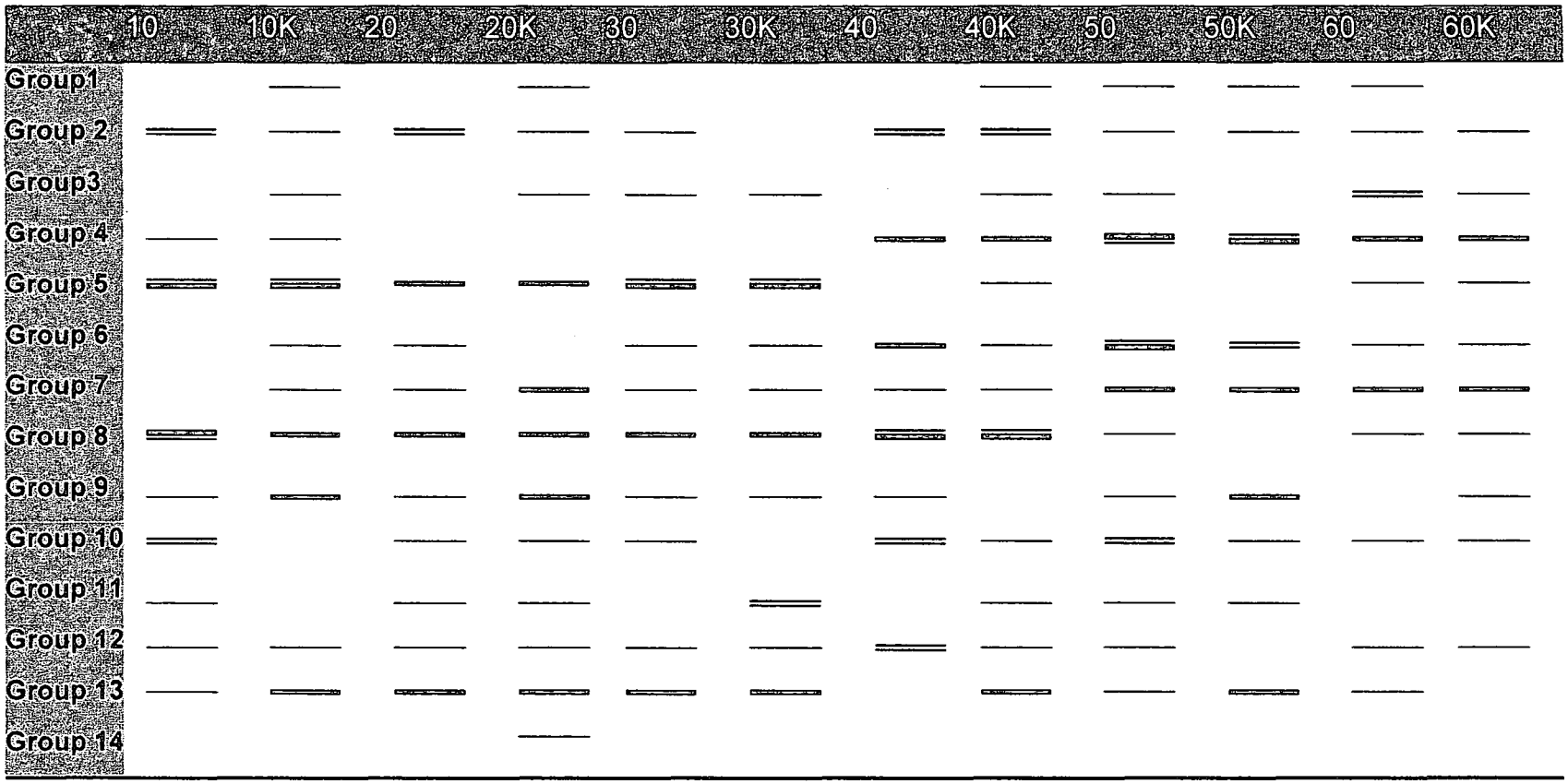
60 – 60 days cold treatment

60 K – 60 days control

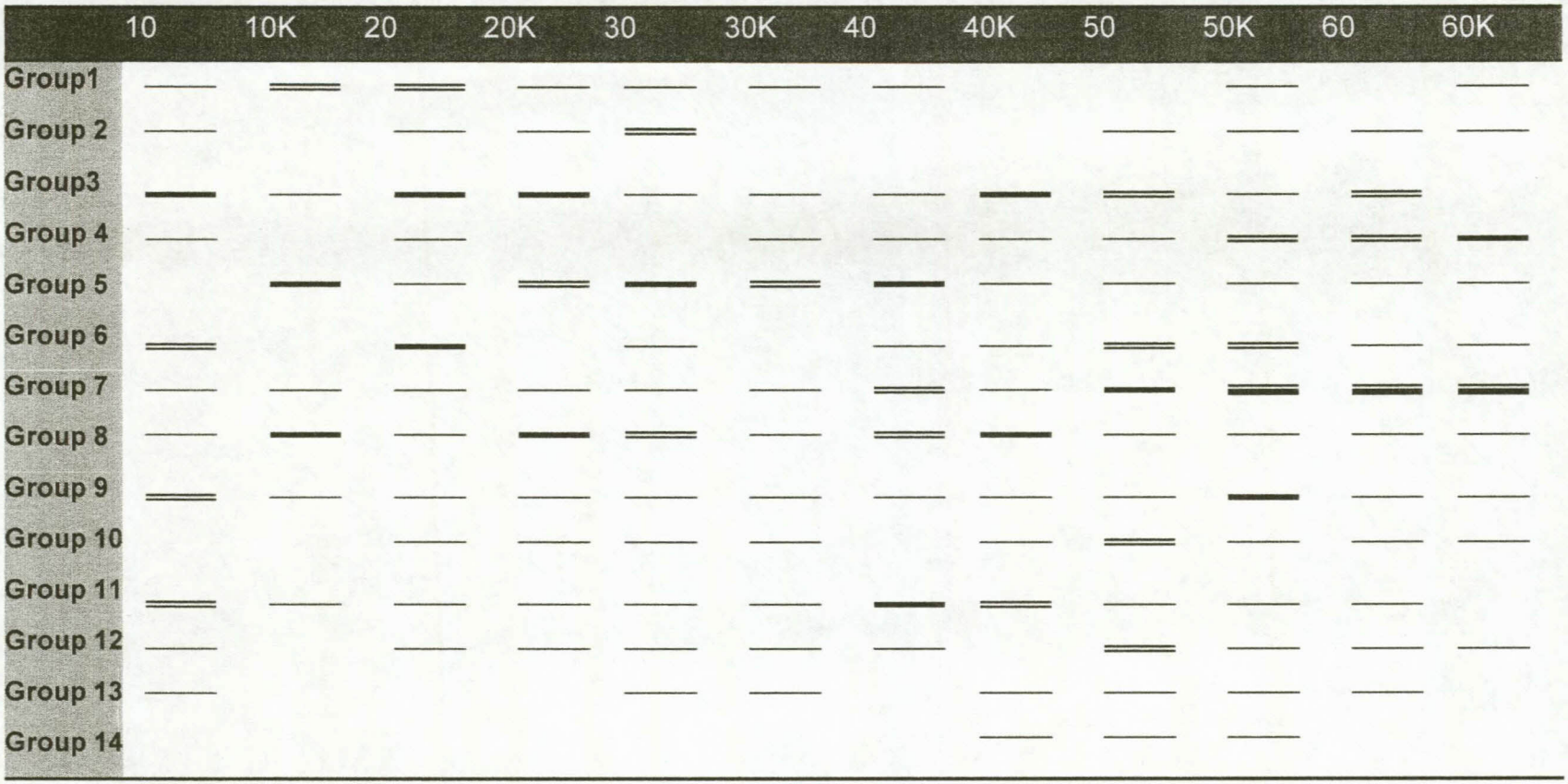
Tabel 1. The protein bands in Adam Tas – roots



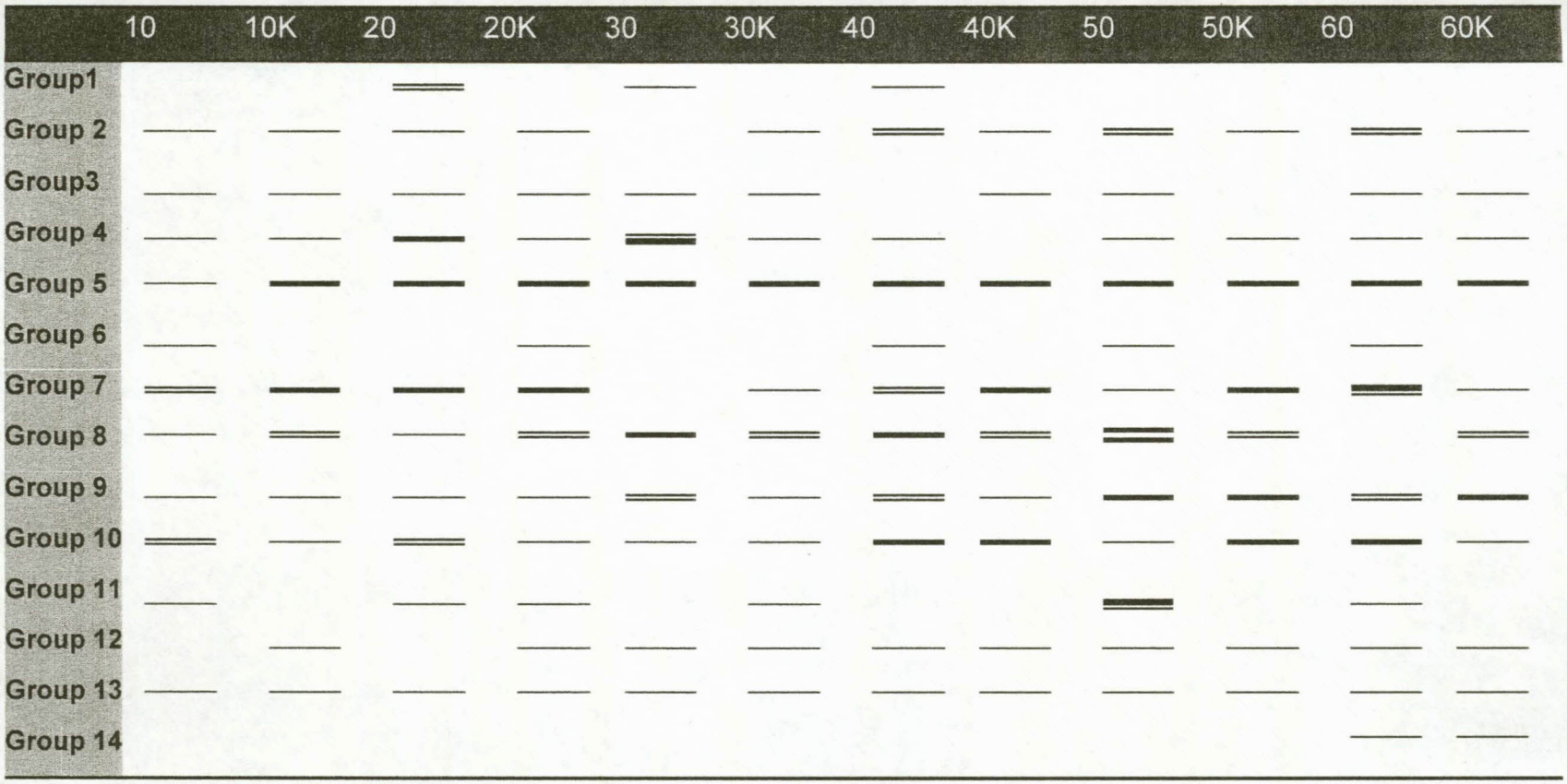
Tabel 2. The protein bands in Betta - roots



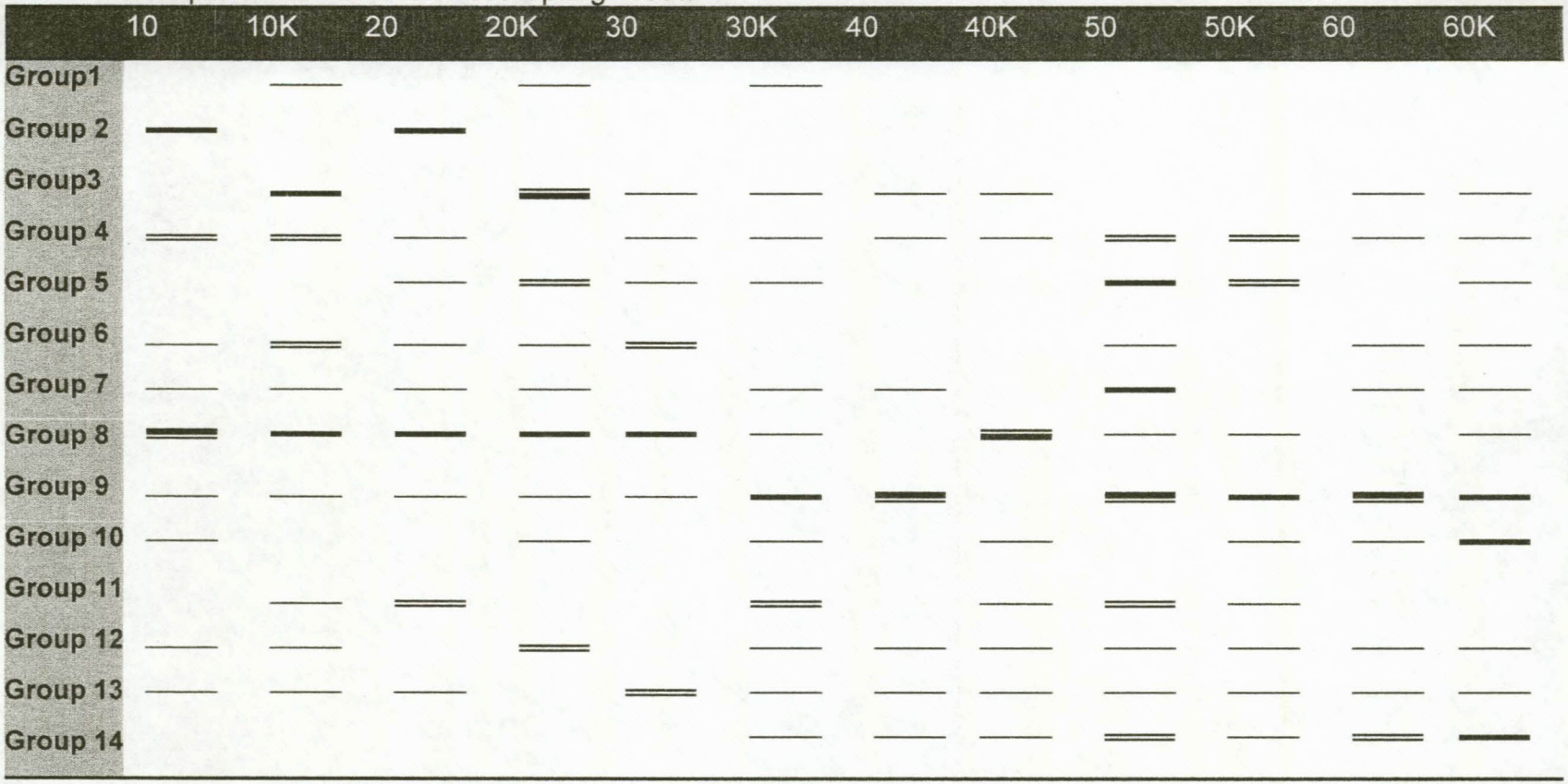
Tabel 3. The protein bands in Betta DN - roots



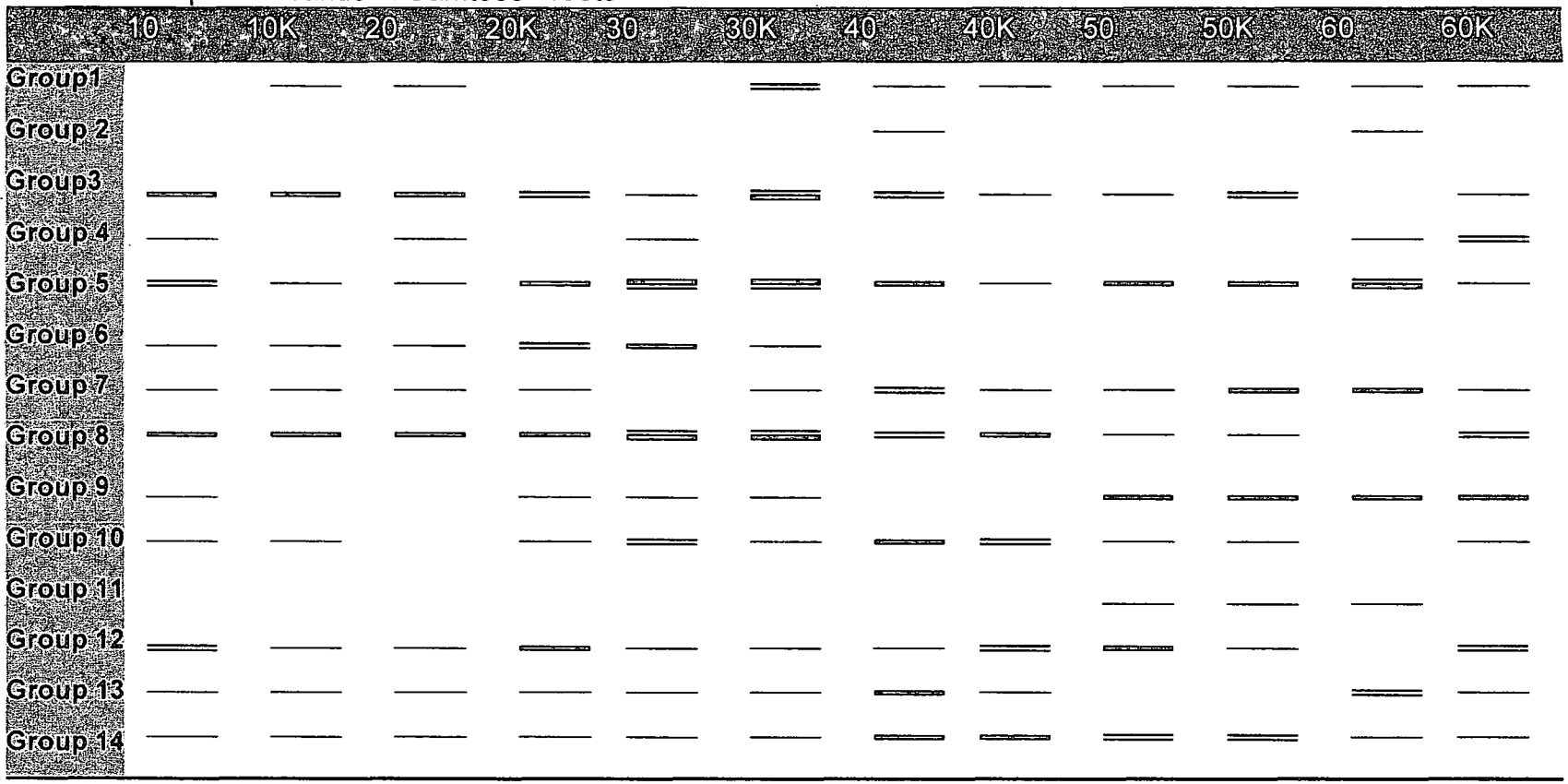
Tabel 4. The protein bands in Caledon - roots



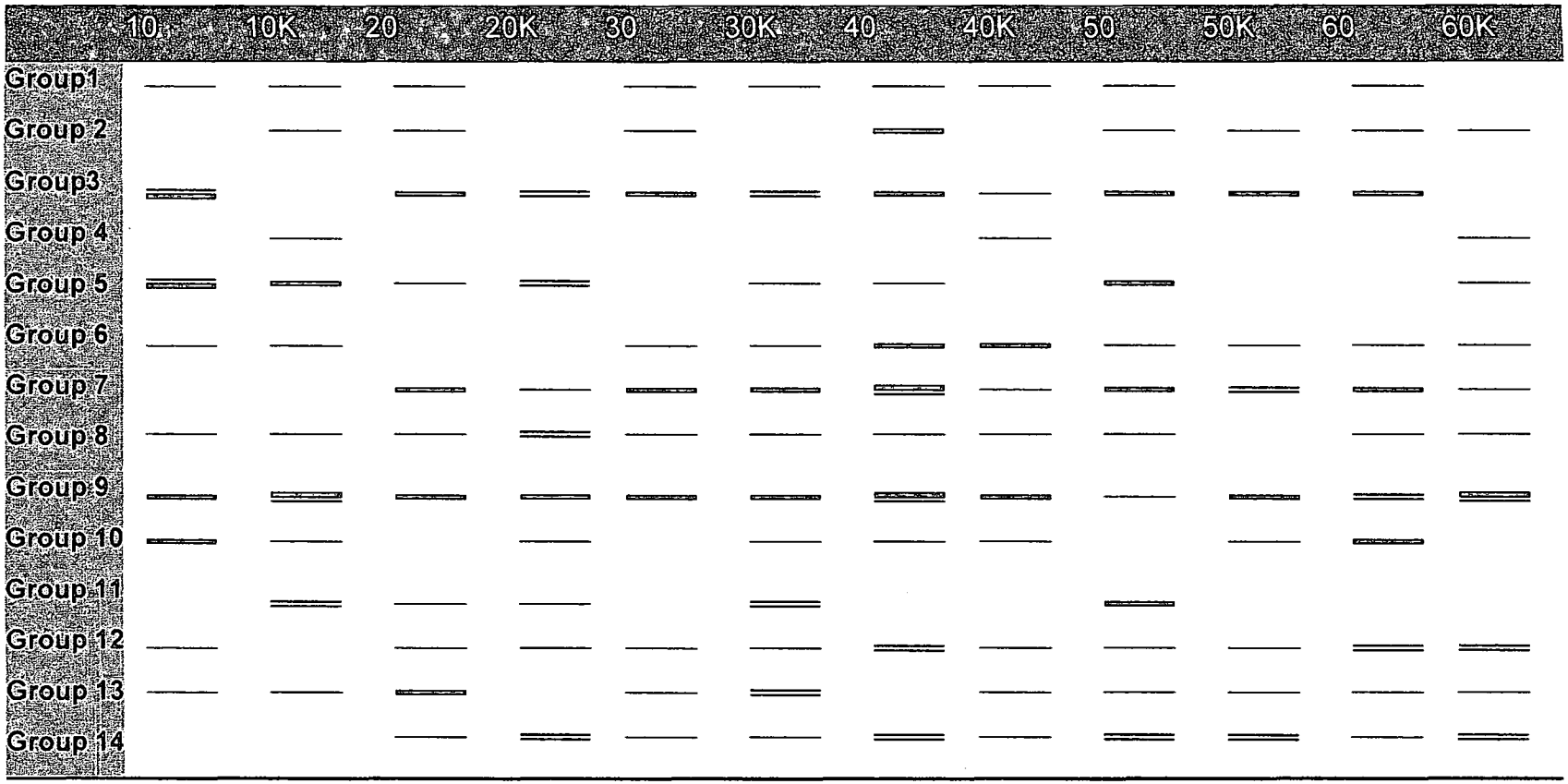
Tabel 5. The protein bands in Chinese Spring - roots



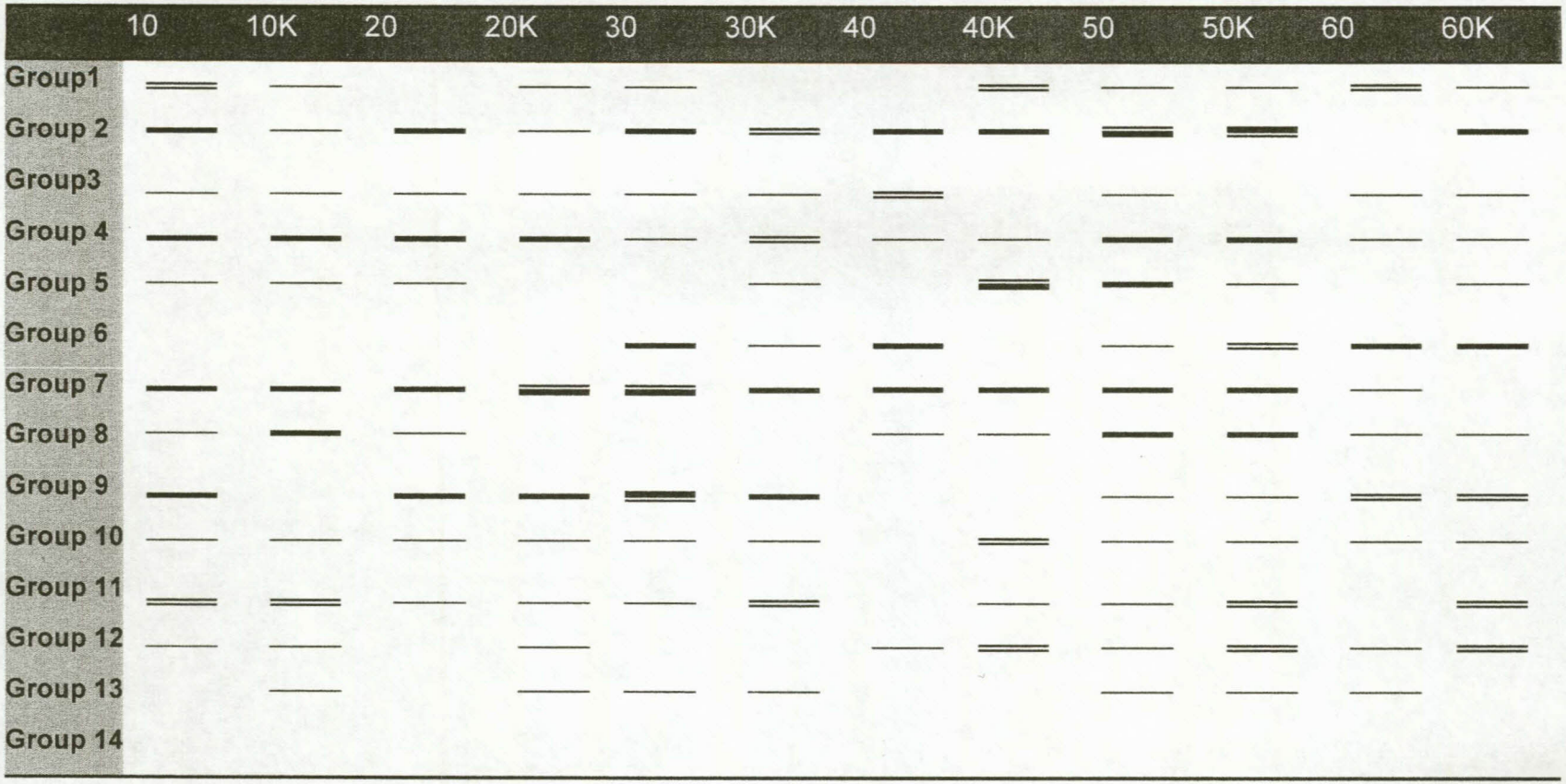
Tabel 6. The protein bands in Gamtoos - roots



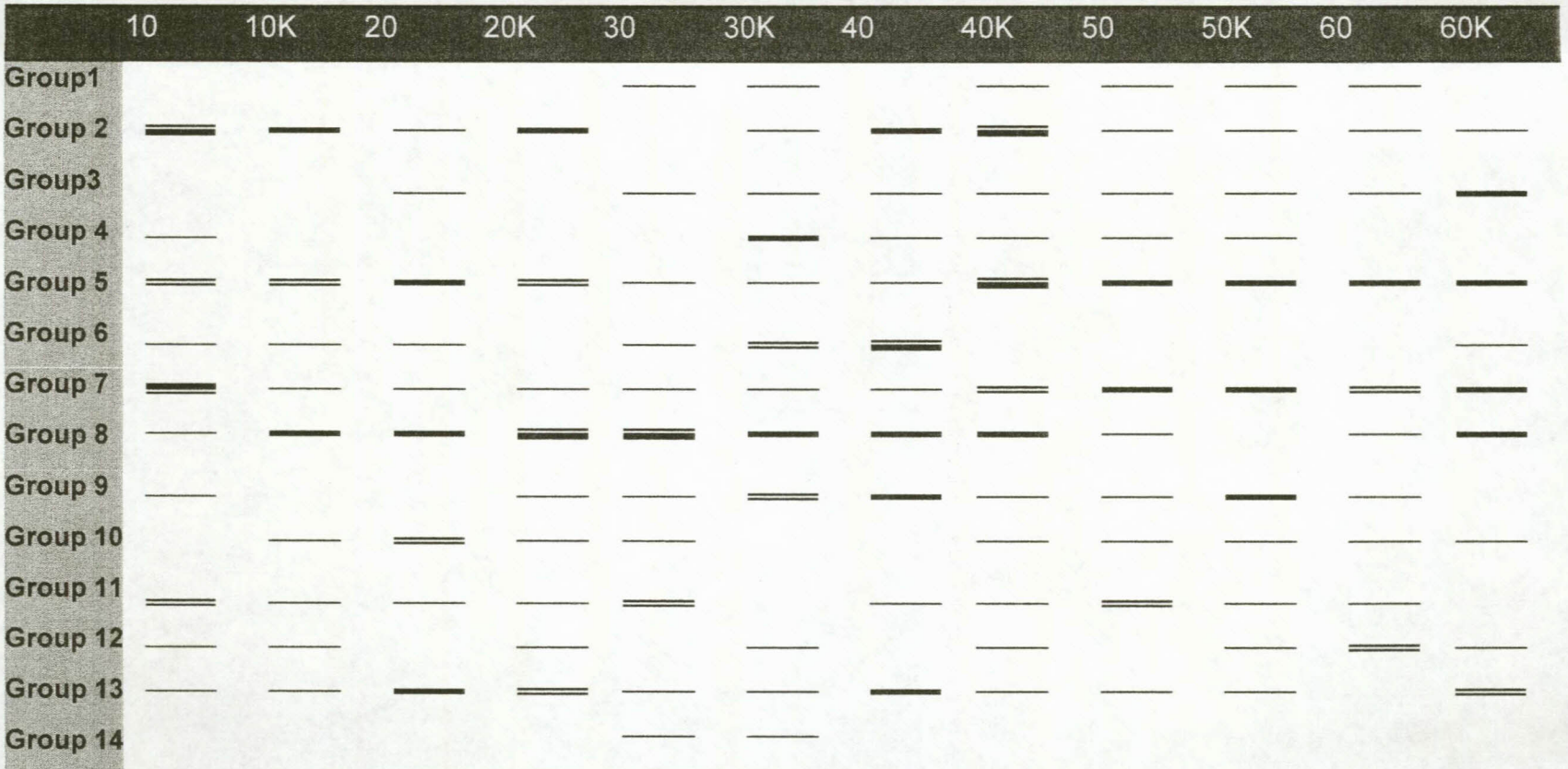
Tabel 7. The protein bands in Gariep - roots



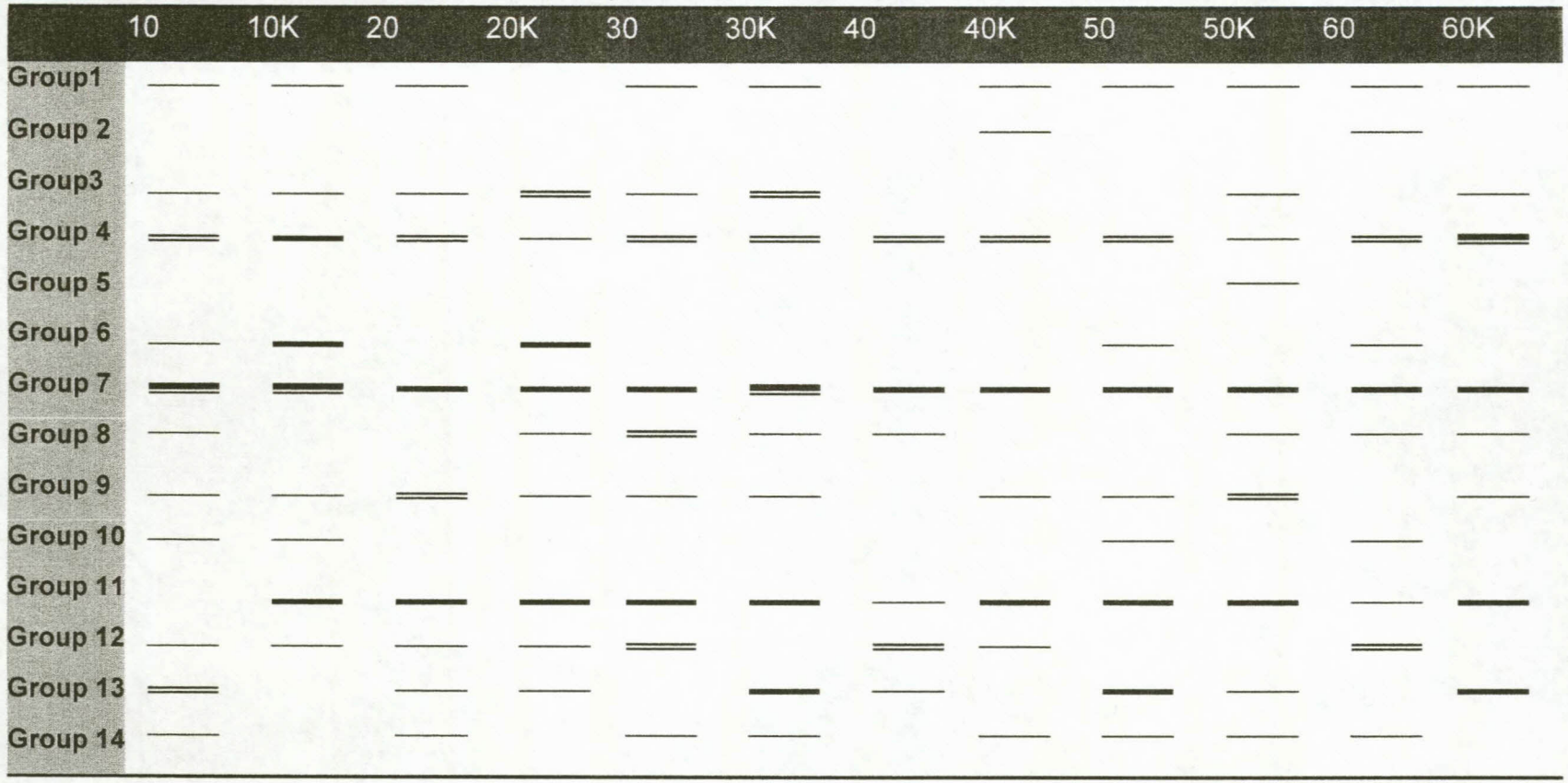
Tabel 8. The protein bands in Hugenoot - roots



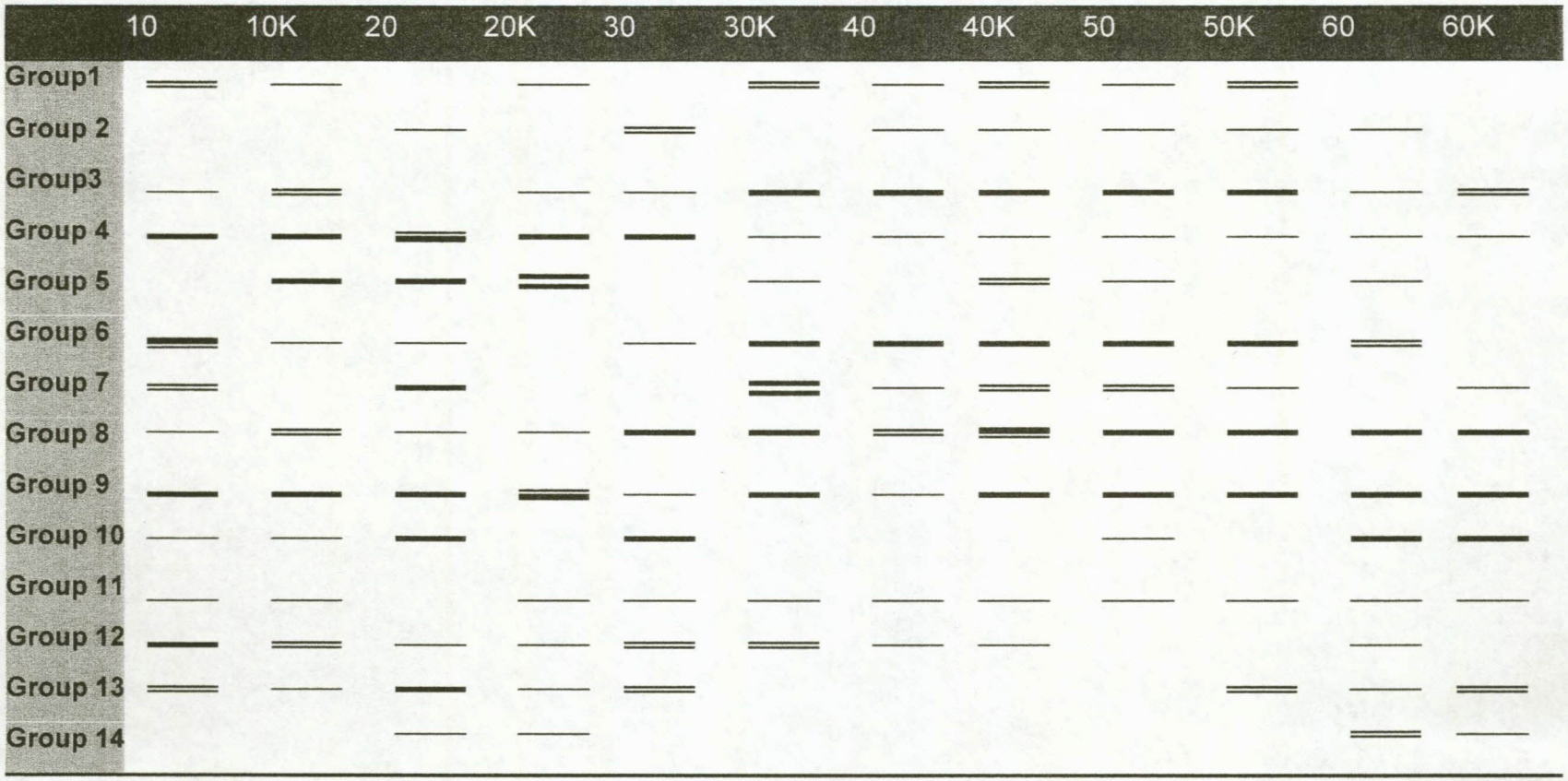
Tabel 9. The protein bands in Letaba - roots



Tabel 10. The protein bands in Limpopo – roots

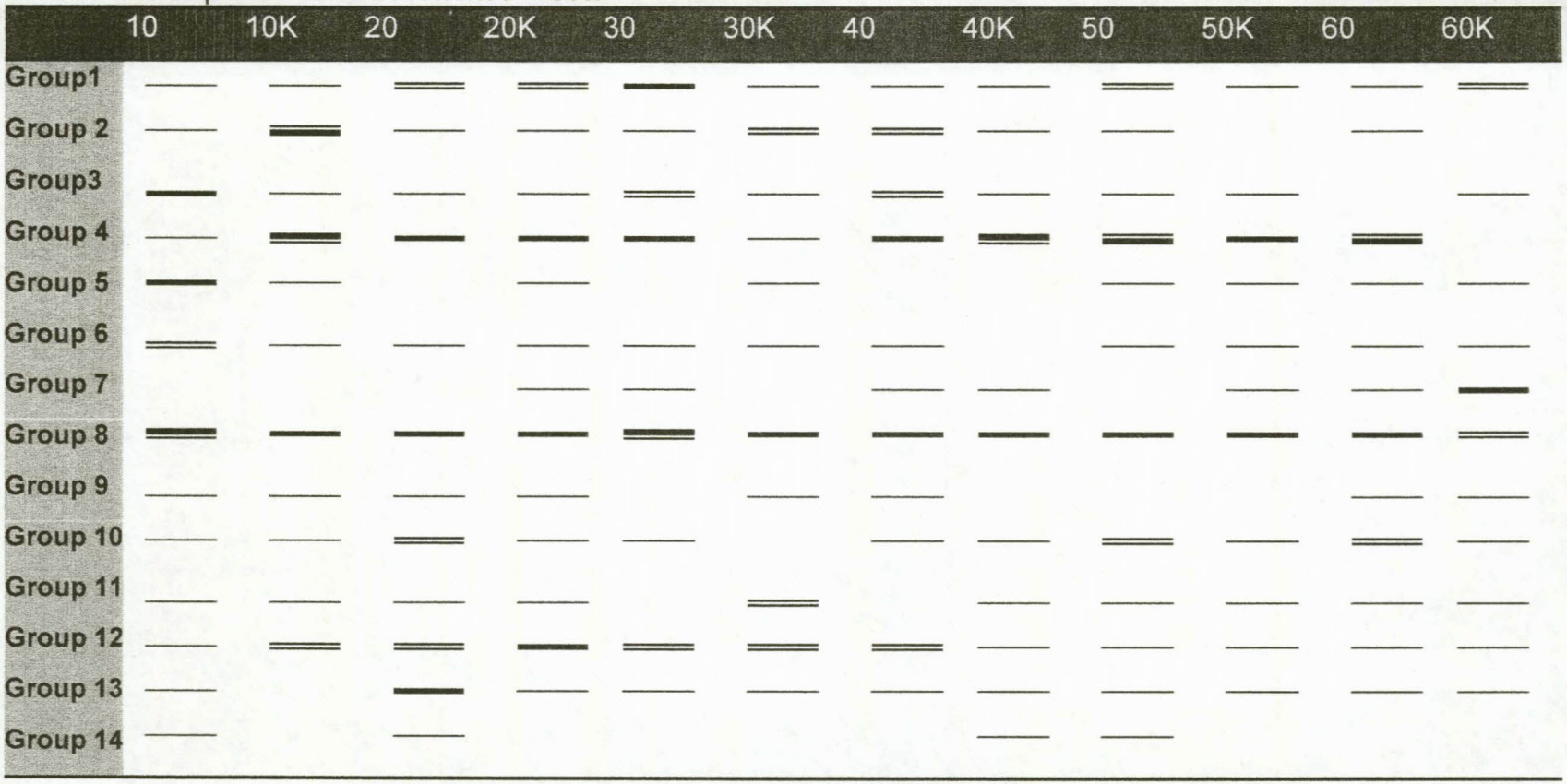


Tabel 11. The protein bands in Molen – roots

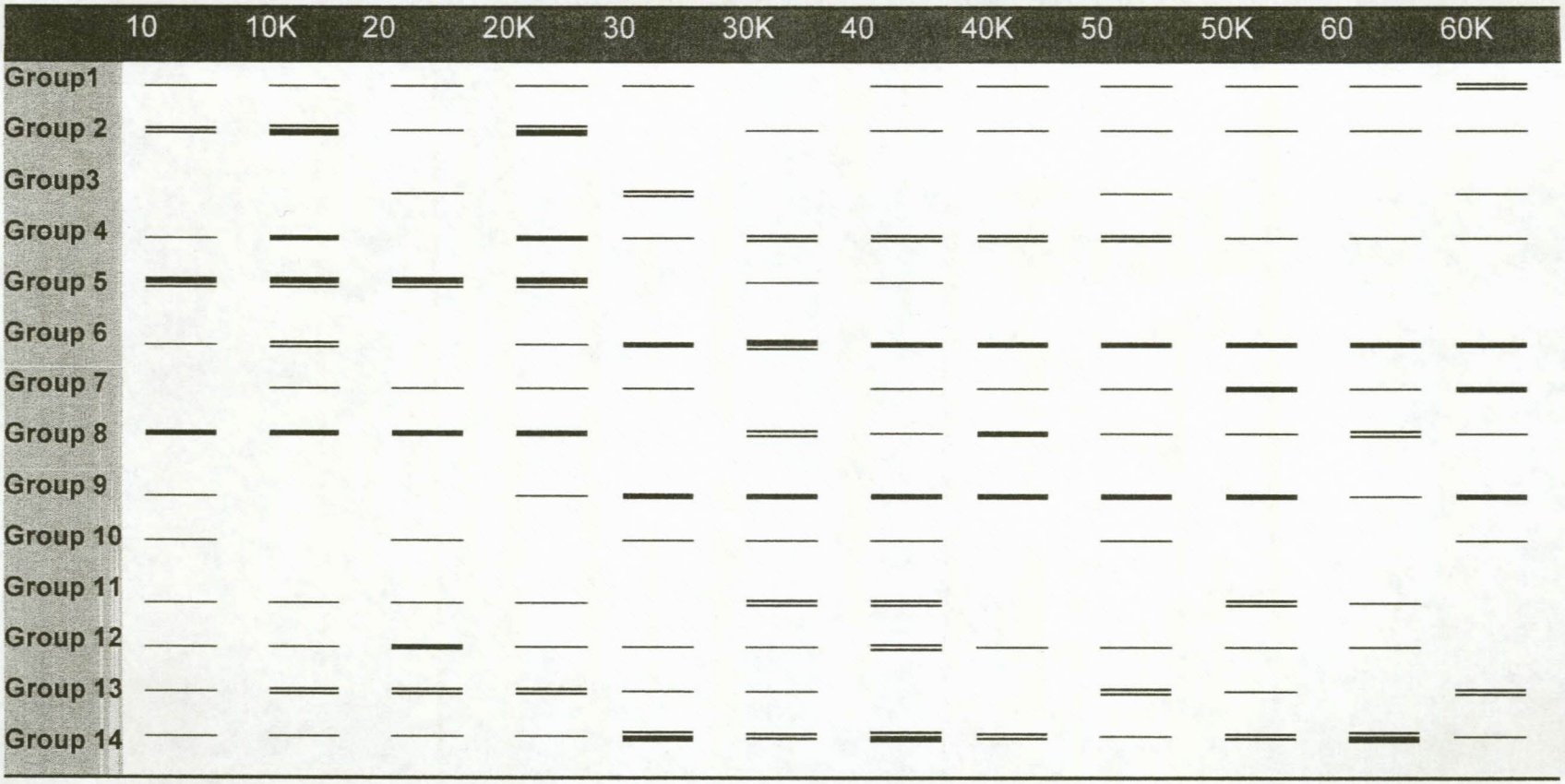


Tabel 12. The protein bands in Molopo - roots

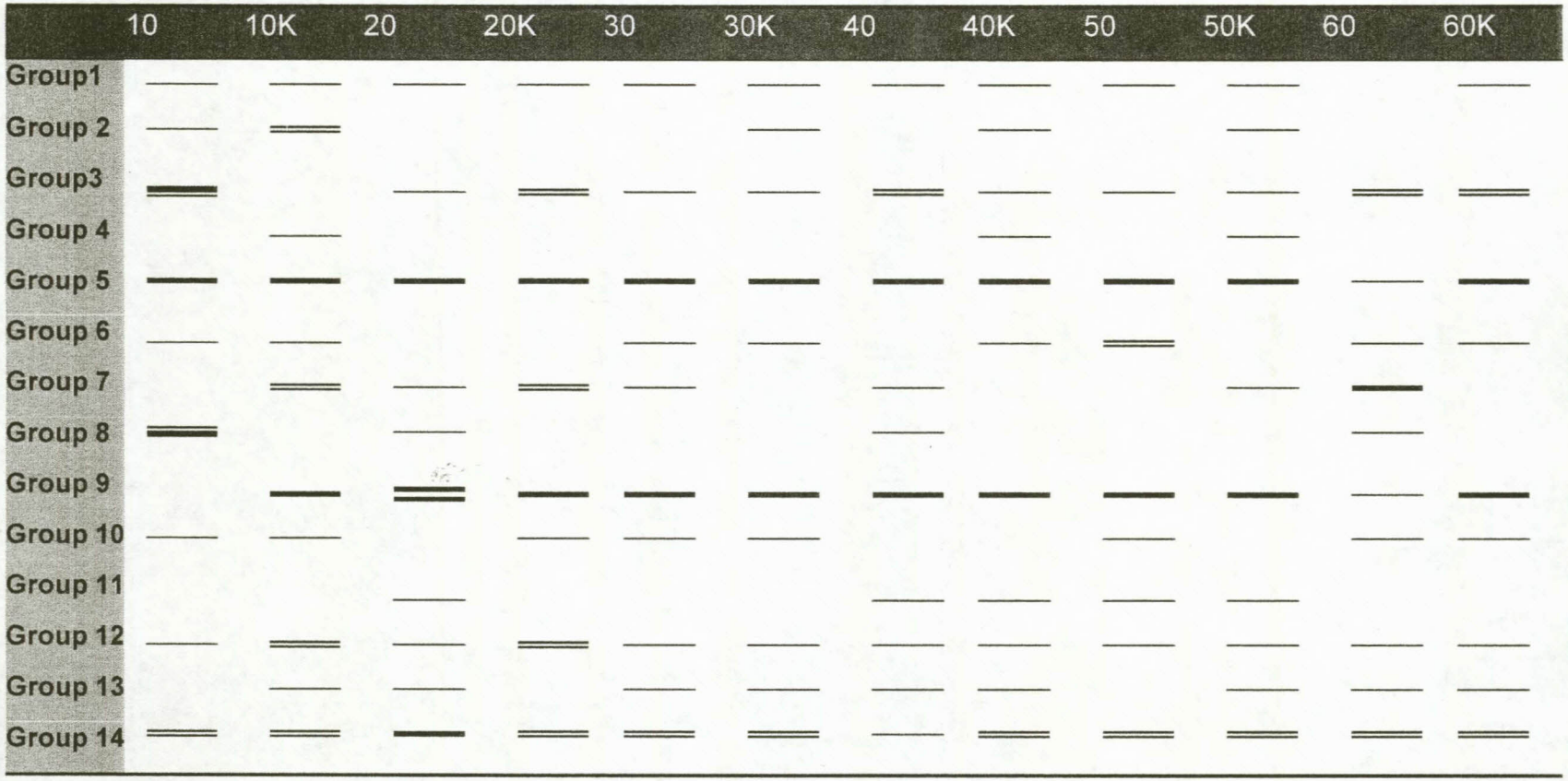
Tabel 13. The protein bands in Nantes - roots



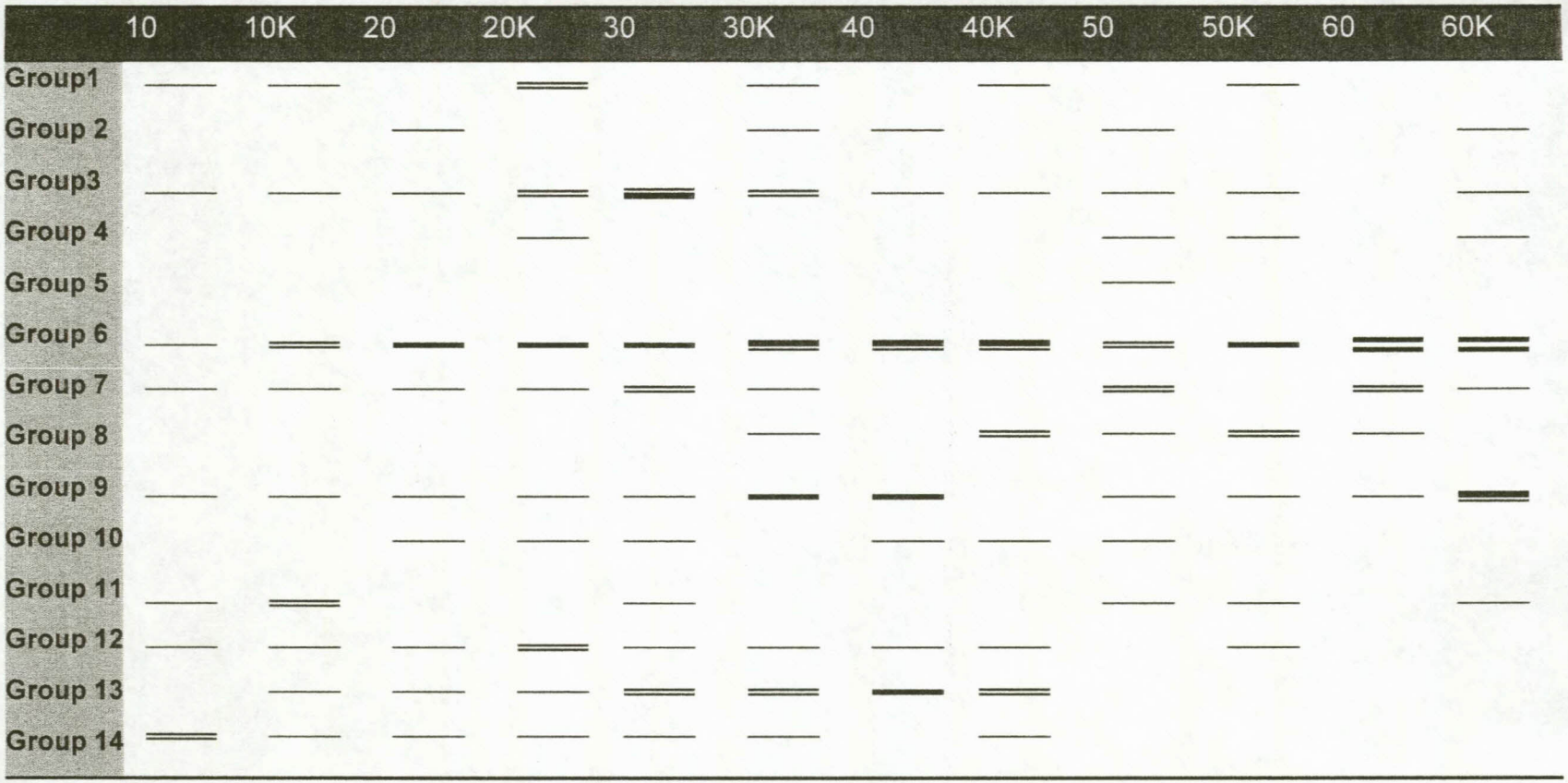
Tabel 14. The protein bands in Palmiet - roots



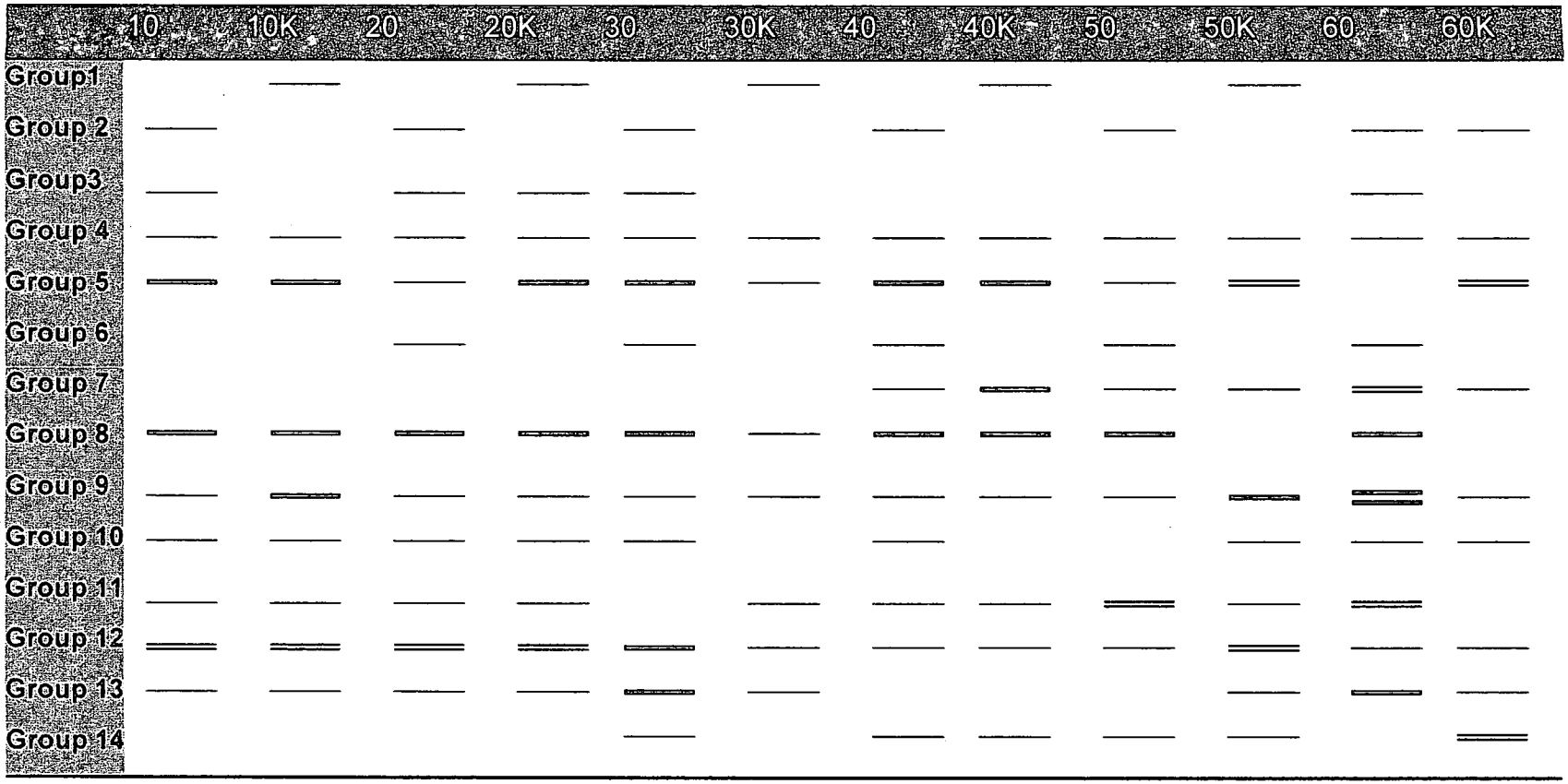
Tabel 15. The protein bands in PAN 3211 – roots



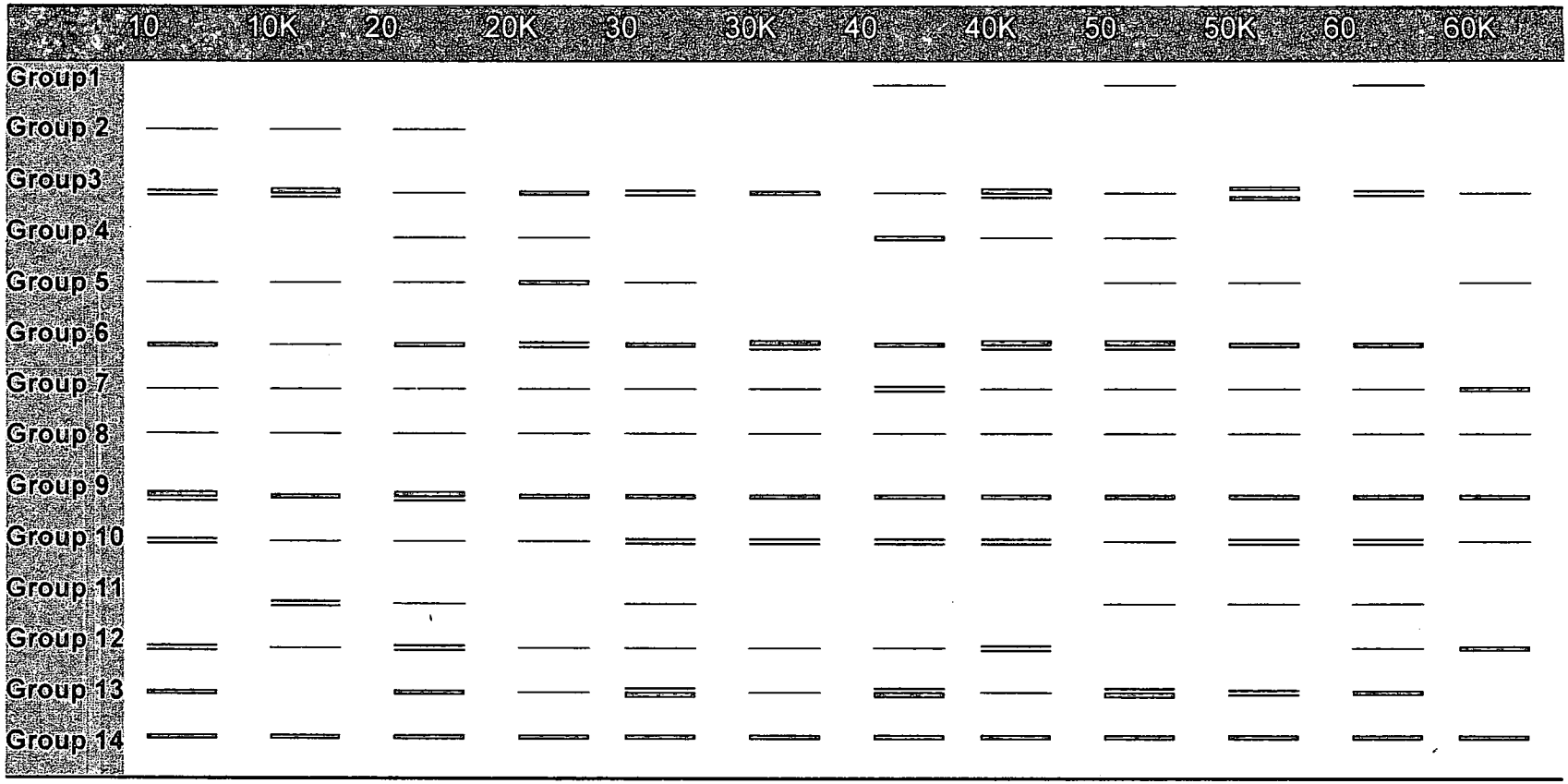
Tabel 16. The protein bands in PAN 3349 – roots



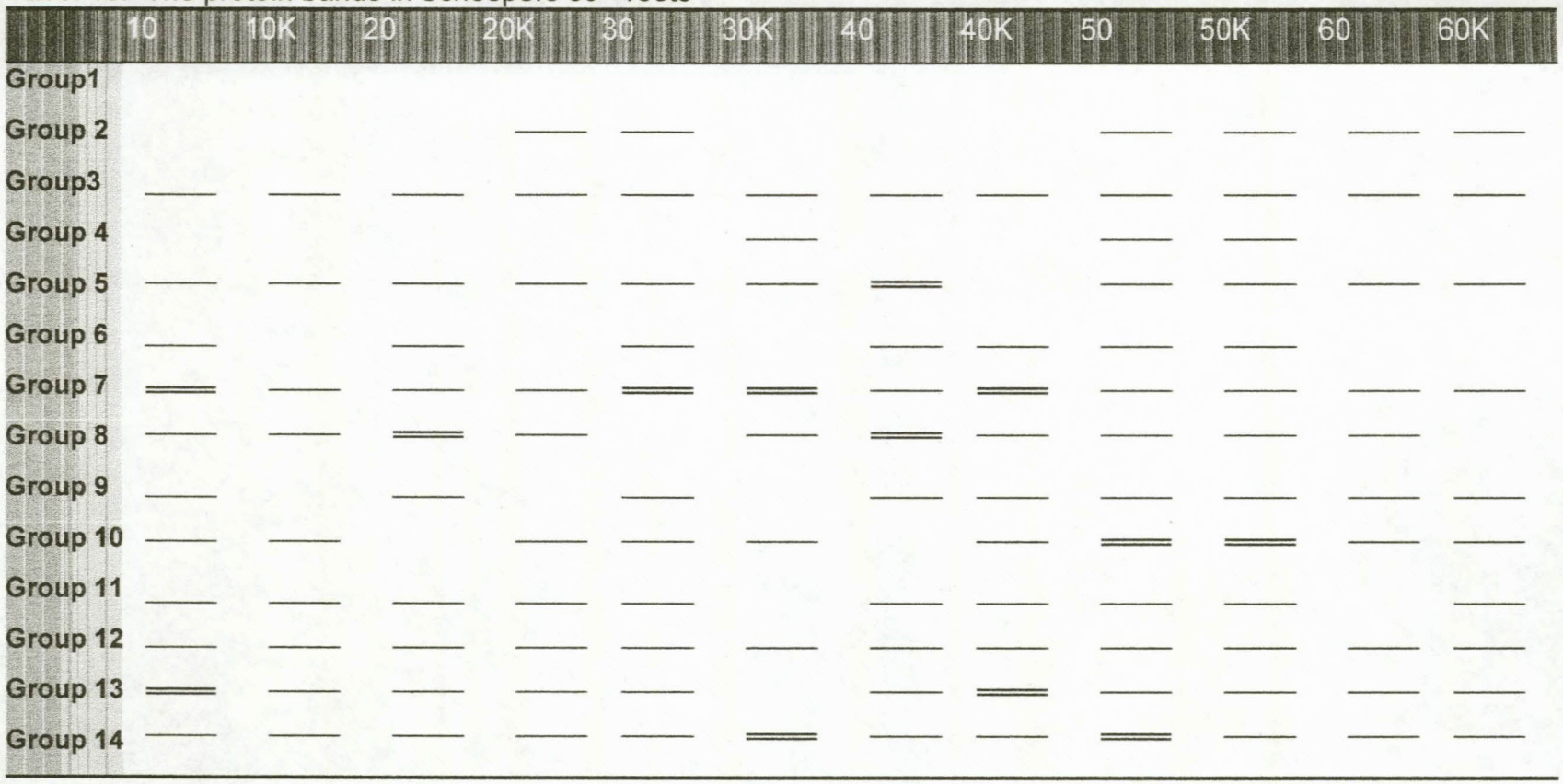
Tabel 17. The protein bands in PAN 3377 - roots



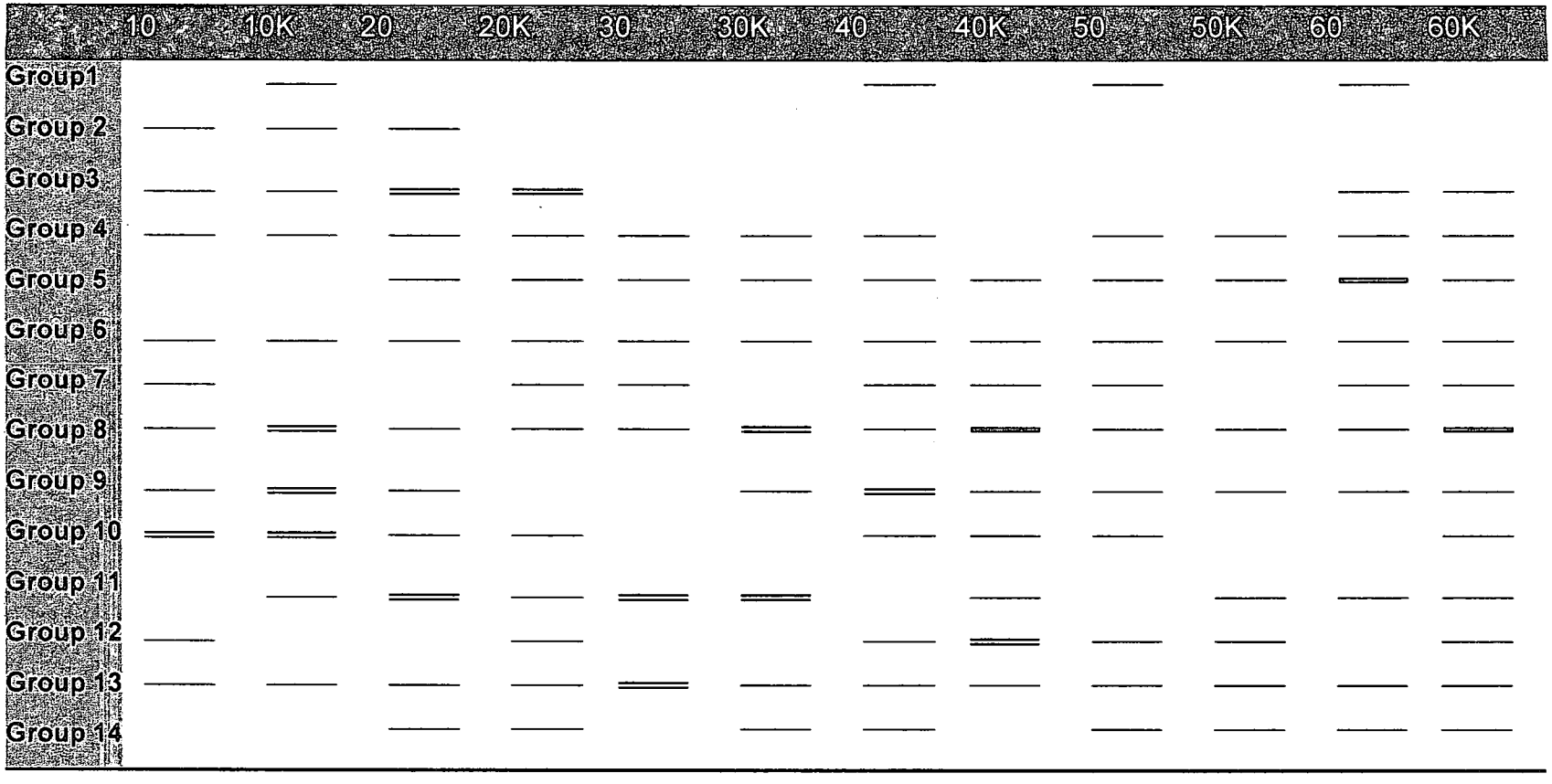
Tabel 19. The protein bands in Snack - roots



Tabel 18. The protein bands in Scheepers 69 - roots



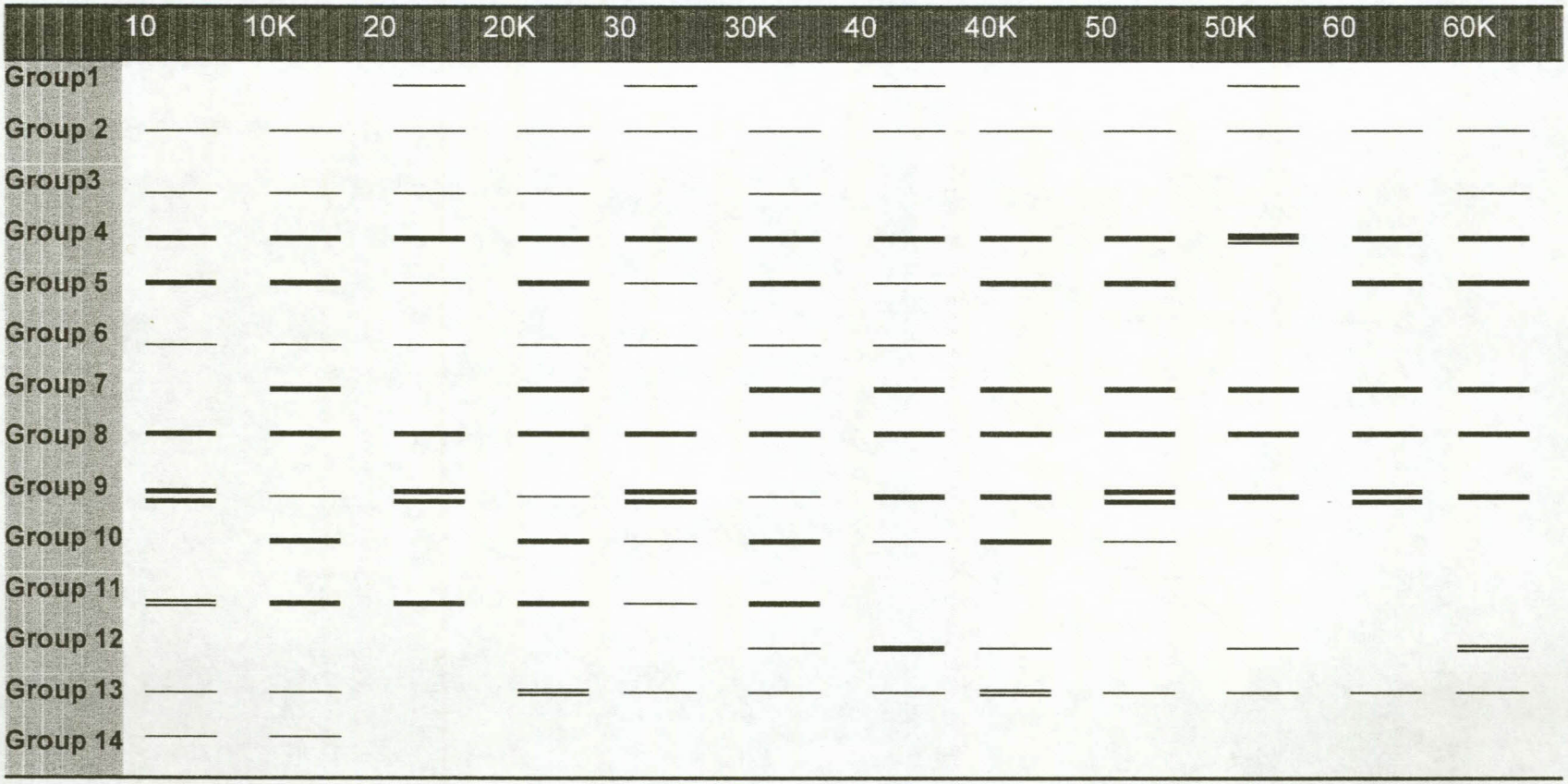
Tabel 20. The protein bands in SST 66 - roots



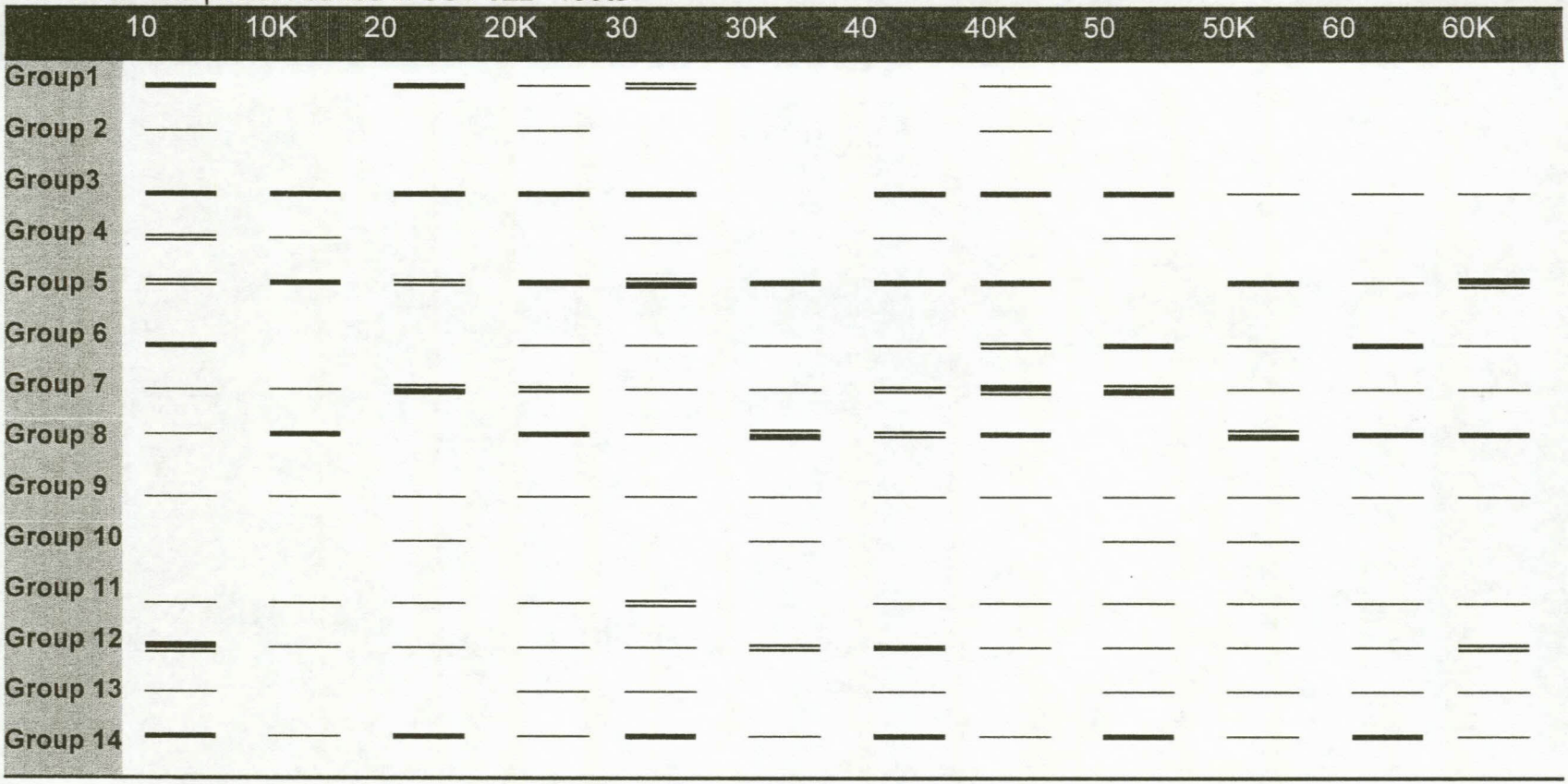
Tabel 21. The protein bands in SST 363 - roots

Tabel 22. The protein bands in SST 367 - roots

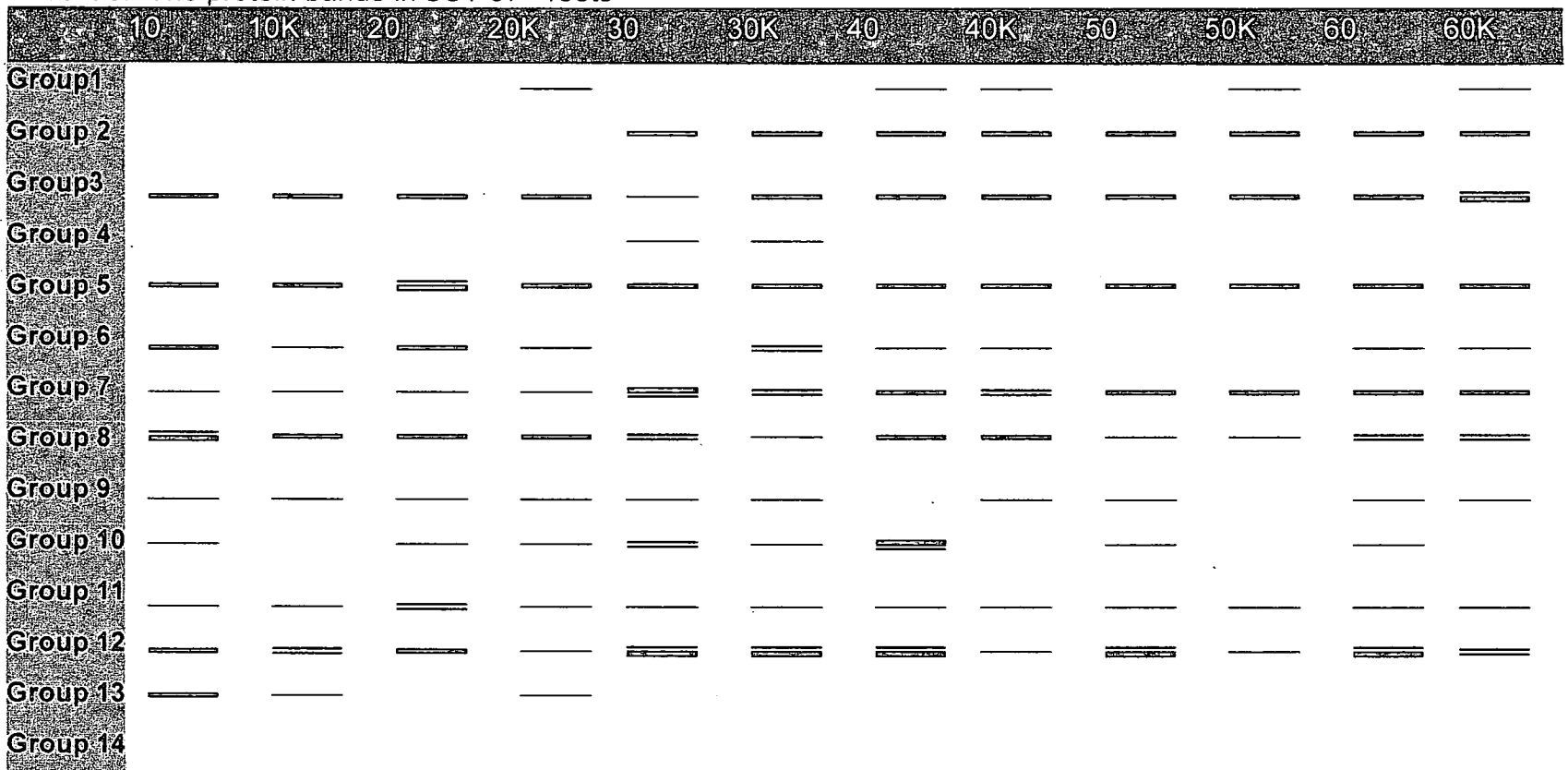
Tabel 23. The protein bands in SST 966 - roots



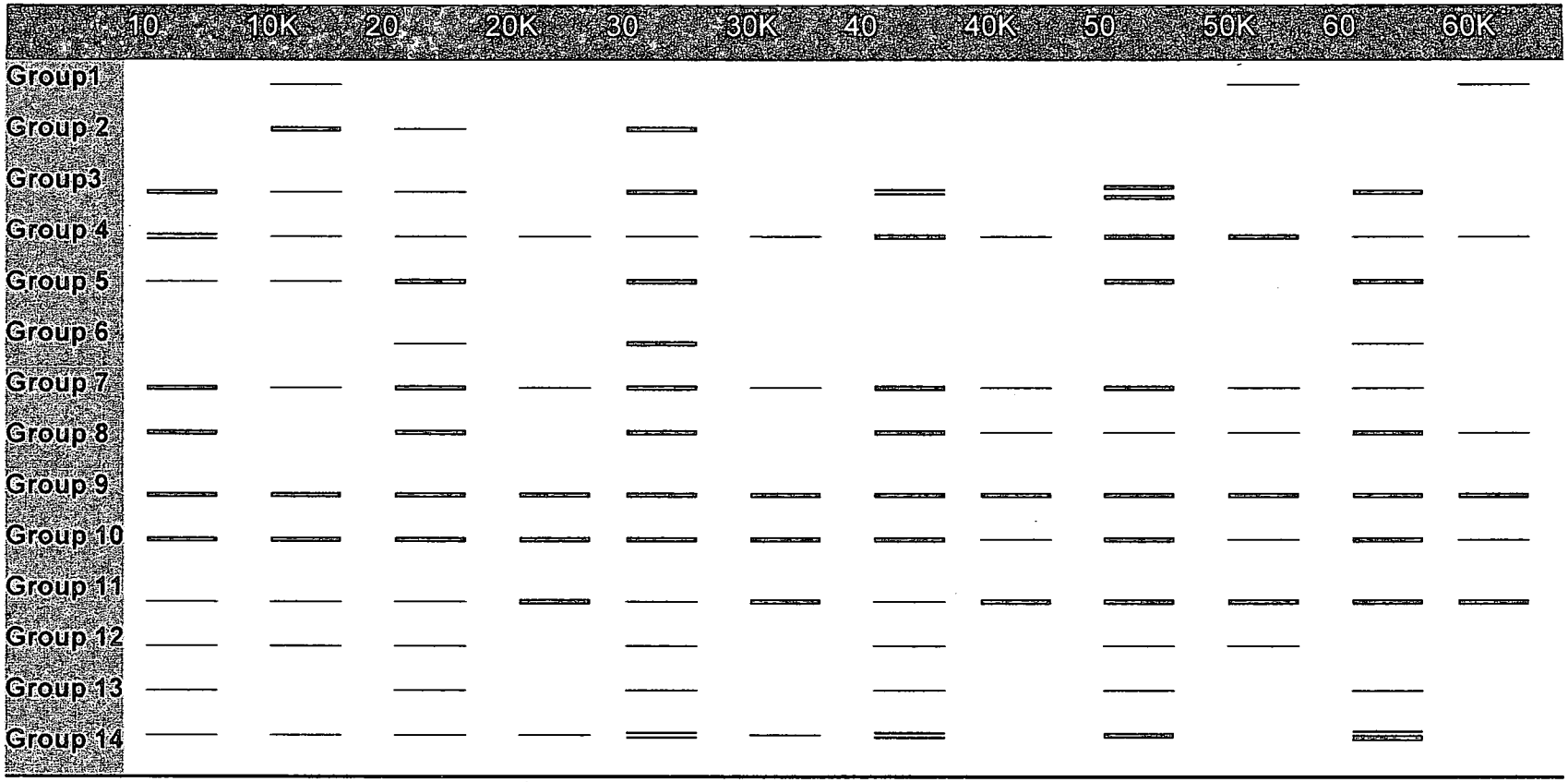
Tabel 24. The protein bands in SST 822 - roots



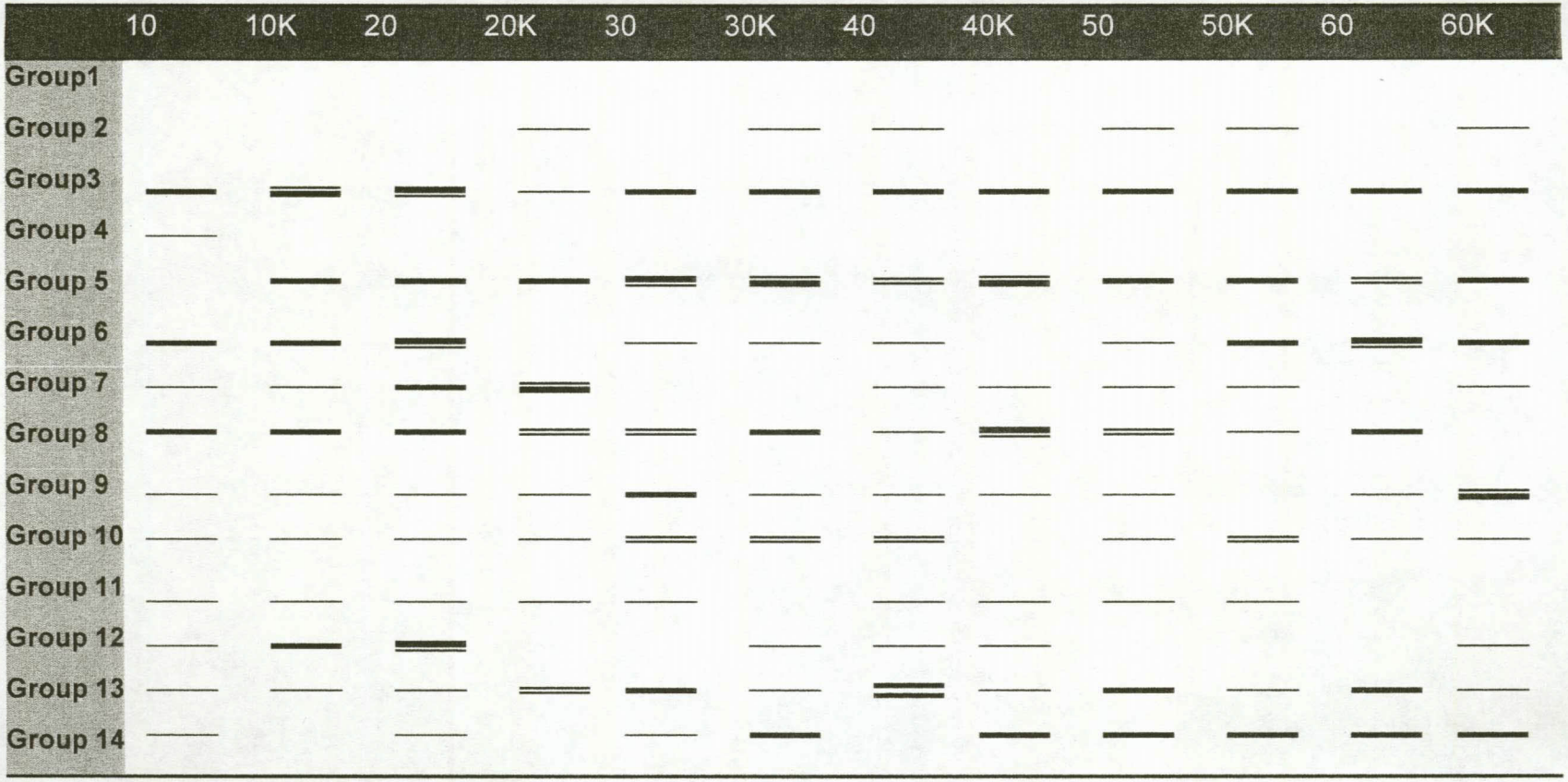
Tabel 25. The protein bands in SST 57 - roots



Tabel 26. The protein bands in SST 825 - roots



Tabel 27. The protein bands in Tugela DN - roots



Tabel 28. The number of bands expressed in Adam Tas

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	7	0	5	3	6	4	8	7	5	6	6	6	5
Control	6	5	5	6	6	2	7	7	8	6	8	2	8	10

Tabel 29. The number of bands expressed in Betta

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	2	9	4	5	6	6	5	8	5	9	3	7	5	0
Control	4	6	5	5	7	6	6	6	5	4	5	5	5	1

Tabel 30. The number of bands expressed in Betta DN

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	5	6	8	6	5	8	8	8	7	5	8	7	4	1
Control	6	3	5	5	8	4	8	6	6	5	5	4	3	2

Tabel 31. The number of bands expressed in Caledon

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	8	5	7	6	4	8	6	9	8	5	4	6	1
Control	0	6	5	5	6	1	6	12	6	6	2	6	6	1

Tabel 32. The number of bands expressed in Chinese Spring

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	2	3	8	3	6	5	5	6	2	4	4	7	5
Control	3	0	6	7	6	4	4	7	8	5	5	7	5	4

Tabel 33. The number of bands expressed in Gamtoos

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	2	5	4	9	3	6	7	4	5	2	6	5	7
Control	6	0	9	2	7	4	6	8	4	7	1	8	5	7

Tabel 34. The number of bands expressed in Gariep

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	6	5	7	0	5	5	6	6	8	3	2	8	5	7
Control	3	3	6	3	5	5	6	6	8	5	5	6	6	8

Tabel 35. The number of bands expressed in Hugenoot

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	7	6	7	6	3	4	7	5	7	5	5	4	3	0
Control	6	8	4	7	6	4	6	4	5	7	10	8	4	0

Tabel 36. The number of bands expressed in Letaba

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	6	5	3	7	5	8	7	5	5	9	3	5	1
Control	3	7	4	3	9	4	7	6	5	5	4	6	8	1

Tabel 37. The number of bands expressed in Limpopo

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	5	1	3	12	0	3	6	5	5	3	5	8	5	5
Control	5	1	7	9	1	2	6	4	7	1	6	3	4	3

Tabel 38. The number of bands expressed in Molen

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	6	5	7	3	8	6	7	6	5	5	6	6	3
Control	8	2	8	6	6	4	6	8	7	2	6	6	6	2

Tabel 39. The number of bands expressed in Molopo

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	1	7	7	1	2	9	5	7	2	6	2	8	10
Control	4	1	6	8	5	0	10	4	5	3	6	4	5	12

Tabel 40. The number of bands expressed in Nantes

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	8	7	7	8	3	7	3	7	4	9	4	9	6	3
Control	8	6	6	7	5	5	4	8	4	5	7	8	5	1

Tabel 41. The number of bands expressed in Palmiet

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	6	6	4	7	7	5	5	6	5	5	5	7	6	9
Control	6	8	1	8	5	8	5	7	5	2	6	5	8	9

Tabel 42. The number of bands expressed in PAN 3211

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	5	1	9	0	6	5	4	5	7	4	3	6	4	10
Control	6	6	7	3	6	4	5	0	6	4	2	8	5	12

Tabel 43. The number of bands expressed in PAN 3349

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	1	3	6	1	1	9	7	2	6	4	3	4	4	4
Control	6	2	8	3	1	10	4	5	6	2	4	6	6	4

Tabel 44. The number of bands expressed in PAN 3377

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	6	4	6	5	5	4	6	7	5	7	8	4	3
Control	5	1	1	6	8	0	3	4	6	4	5	9	5	4

Tabel 45. The number of bands expressed in Scheepers 69

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	3	6	1	7	5	8	7	6	5	5	6	7	7
Control	0	3	6	2	5	2	8	5	3	7	4	6	6	7

Tabel 46. The number of bands expressed in Snack

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	2	9	3	4	7	7	6	8	10	4	7	9	6
Control	0	1	9	2	4	8	6	6	6	9	3	6	4	6

Tabel 47. The number of bands expressed in SST 363

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	5	2	3	8	2	4	8	4	8	4	4	5	3	0
Control	5	6	6	7	2	3	7	7	6	4	6	5	2	0

Tabel 48. The number of bands expressed in SST367

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	8	5	9	4	5	8	8	4	6	7	8	2	0
Control	1	6	6	7	5	6	7	8	6	5	7	7	2	0

Tabel 49. The number of bands expressed in SST 57

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	1	4	6	1	7	4	7	9	5	8	7	11	1	0
Control	4	4	7	1	6	6	8	7	5	2	6	10	2	0

Tabel 50. The number of bands expressed in SST 66

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	2	7	3	5	6	5	6	6	5	5	3	7	4
Control	1	1	7	3	4	6	3	8	6	5	7	5	6	4

Tabel 51. The number of bands expressed in SST 822

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	4	1	6	5	8	5	9	5	6	2	6	7	5	6
Control	2	2	5	1	7	6	8	8	6	2	5	8	3	6

Tabel 52. The number of bands expressed in SST 825

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	2	8	7	5	3	6	7	6	6	6	5	6	9
Control	3	1	1	6	1	0	5	3	6	6	6	1	0	3

Tabel 53. The number of bands expressed in SST 966

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	3	6	2	6	6	4	3	6	11	3	4	1	6	1
Control	1	6	4	7	5	3	6	6	6	4	3	5	8	1

Tabel 54. The number of bands expressed in Tugela DN

group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cold	0	2	7	1	8	8	4	8	6	8	5	4	7	5
Control	0	4	7	0	7	4	6	7	6	7	4	4	7	4