

6143 86975

U.O.V.S. BIBLIOTEK

HIERDIE EKSEMPLAAR MAG ONDER
GEEN OMSTANDIGHED E UIT DIE
BIBLIOTEK VERWYDER WORD NIE

University Free State



34300001319643

Universiteit Vrystaat

**GENETIC VARIABILITY AND ANALYSIS OF SCREENING
PROCEDURES OF PREHARVEST SPROUTING IN SOUTH
AFRICAN WINTER WHEAT (*Triticum aestivum*) CULTIVARS**

BY

ANNELIE BARNARD

Submitted in fulfillment of the
requirements for the degree

Philosophiae Doctor

in the Department of Plant Science
(Plant Breeding)
Faculty of Natural and Agricultural Sciences
University of the Free State
BLOEMFONTEIN

November 2002

**PROMOTER : PROF. C.S. VAN DEVENTER
CO-PROMOTER : DR. H. MAARTENS**

GENETIC VARIABILITY AND ANALYSIS OF SCREENING PROCEDURES OF
PREHARVEST SPROUTING IN SOUTH AFRICAN WINTER WHEAT
(*Triticum aestivum*) CULTIVARS

ACKNOWLEDGEMENTS	vi
LIST OF TABLES	vii
LIST OF FIGURES	ix
ABBREVIATION LIST	xi

CHAPTER 1

INTRODUCTION	1
REFERENCES	7

CHAPTER 2

LITERATURE REVIEW	10
2.1 INTRODUCTION	10
2.2 DORMANCY	11
2.3 ENVIRONMENTAL EFFECTS	16
2.4 α -AMYLASE ACTIVITY	18
2.5 WHEAT GRADING SYSTEMS	21
2.6 FALLING NUMBER (FN)	25
2.7 OXIDATIVE PENTOSE PHOSPHATE PATHWAY	28
2.8 CONCLUSIONS	32
REFERENCES	33

CHAPTER 3

ASSESSMENT OF PREHARVEST SPROUTING IN SOUTH AFRICAN WINTER AND INTERMEDIATE WHEAT CULTIVARS USING NON-PARAMETRIC ANALYSES	44
3.1 ABSTRACT	44

3.2	INTRODUCTION	45
3.3	MATERIALS AND METHODS	46
3.3.1	Wheat cultivars and trials	46
3.3.2	Simulation of sprouting conditions and assessment of tolerance	46
3.3.3	Statistical analyses	48
3.4	RESULTS AND DISCUSSION	49
3.4.1	Canonical variate analysis	49
3.4.2	AMMI model	51
3.5	CONCLUSIONS	56
	REFERENCES	57

CHAPTER 4

THE POSSIBLE ROLE OF THE OXIDATIVE PENTOSE PHOSPHATE PATHWAY IN SEED DORMANCY IN TWO WINTER WHEAT

CULTIVARS	59
4.1 ABSTRACT	59
4.2 INTRODUCTION	60
4.3 MATERIALS AND METHODS	62
4.3.1 Materials	62
4.3.2 Seed germination	62
4.3.3 Assay of G6PDH	63
4.3.4 Metabolism of radioactive glucose	63
4.3.5 Separation of isoenzymes by ion-exchange chromatography	64
4.3.6 Statistical analyses	64
4.4 RESULTS AND DISCUSSION	64
4.4.1 Seed germination and G6PDH activities	64
4.4.2 Metabolism of radioactive glucose	66

4.4.3	Separation of isoenzymes by ion-exchange chromatography	69
4.5	CONCLUSIONS	72
	REFERENCES	76

CHAPTER 5

GENETIC DIVERSITY OF SOUTH AFRICAN WINTER WHEAT CULTIVARS IN RELATION TO PREHARVEST SPROUTING AND FALLING

NUMBER	80
5.1 ABSTRACT	80
5.2 INTRODUCTION	81
5.3 MATERIALS AND METHODS	82
5.3.1 Falling Number Determination	82
5.3.2 Preharvest Sprouting Determination	82
5.3.3 Statistical analyses	83
5.4 RESULTS AND DISCUSSION	83
5.5 CONCLUSIONS	87
REFERENCES	89

CHAPTER 6

COMPARISON BETWEEN VARIOUS METHODS FOR ESTIMATING SPROUT DAMAGE IN WHEAT

6.1	ABSTRACT	91
6.2	INTRODUCTION	92
6.3	MATERIALS AND METHODS	93
6.3.1	Preharvest Sprouting	93
6.3.2	Percentage Sprouted Kernels	93
6.3.3	Falling Number (FN)	94
6.3.4	Stirring Number (SN)	95
6.3.5	α -Amylase	95

6.3.6	Diastatic Activity	96
6.3.7	WheatRite Method	96
6.3.8	Statistical analyses	97
6.4	RESULTS AND DISCUSSION	97
6.5	CONCLUSIONS	105
	REFERENCES	108

CHAPTER 7

THE GENETIC VARIABILITY OF PREHARVEST SPROUTING IN

WHEAT	110
7.1 ABSTRACT	110
7.2 INTRODUCTION	111
7.3 MATERIALS AND METHODS	112
7.3.1 Experimental Material	112
7.3.2 Characteristics measured	114
7.3.3 Statistical analyses	115
7.3.3.1 Analysis of variance	115
7.3.3.2 General and Specific Combining abilities	115
7.3.3.3 Phenotypic correlations	115
7.4 RESULTS AND DISCUSSION	116
7.4.1 Analysis of variance	116
7.4.2 Combining ability for preharvest sprouting and α -amylase activity	119
7.4.3 General combining ability (GCA)	119
7.4.4 Specific combining ability (SCA)	121
7.4.5 Phenotypic correlations	122
7.5 CONCLUSIONS	125
REFERENCES	127

CHAPTER 8

THE USE OF A PROTEIN MARKER TO FOLLOW PREARVEST SPROUTING

RESISTANCE IN WHEAT	129
8.1 ABSTRACT	129
8.2 INTRODUCTION	130
8.3 MATERIALS AND METHODS	131
8.3.1 Genetic material	131
8.3.2 Extraction of proteins	132
8.3.3 Electrophoresis	133
8.3.4 Simulation of sprouting conditions and assessment of tolerance	133
8.4 RESULTS AND DISCUSSION	133
8.5 CONCLUSIONS	138
REFERENCES	139

CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS	141
9.1 CONCLUSIONS	141
9.2 RECOMMENDATIONS	142

CHAPTER 10

SUMMARY	144
OPSOMMING	147

ACKNOWLEDGEMENTS

- Thank to my Heavenly Father for the privilege, strength and inspiration to complete this study.
- I hereby also gratefully acknowledge the contribution of the following personnel for their assistance in various techniques: Mr Barend Wentzel and Mss Sarita Carelzen, Maryke Craven and Chrissie Miles.
- My sincere appreciation also goes to Prof J C Pretorius for his valuable suggestions and comments for Chapter 4.
- My study leader, Prof. C van Deventer for his guidance, help and advice during this study, as well as Dr H Maartens for her valuable contributions.
- My sincere thanks and appreciation to the Director of Small Grain Institute, Dr J le Roux, as well as the Agricultural Research Council and the Winter Cereal Trust for financial support and the opportunity to undertake this study.
- Finally, my husband Johnny for moral support and encouragement and my children, Suané, Lourens and Hanru who had to sacrifice a lot and had to manage without the support and help of a mother while I completed the study.

LIST OF TABLES

Table 2.1	Wheat grading table used in the South African grading system	24
Table 3.1	Summary of the cultivars used to screen for preharvest sprouting	47
Table 3.2	Description of the nine growth environments	48
Table 3.3	The sprouting response of 17 winter and intermediate wheat cultivars commonly grown in the Free State over nine environments	51
Table 3.4	AMMI analysis of variance for 17 cultivars grown over nine environments	53
Table 3.5	The cultivar means and first interaction PCA scores (IPCA 1) of the 17 cultivars used in this study	54
Table 3.6	The environment means and first interaction PCA scores (IPCA 1) of the 9 environments used in this study, ranked according to IPCA scores	55
Table 5.1	The Falling Numbers (FN1) of twenty cultivars/lines determined at three localities in the Eastern Free State during 1998	84
Table 5.2	Falling number determinations (FN1 and FN2) and visual preharvest sprouting assessment at Bethlehem during 1999	86
Table 6.1	Summary of the cultivars used to determine FN and various other characteristics	94
Table 6.2	Correlation coefficient* between sprouting scale, FN, PLN, SN, sprouted kernels, α -amylase activity and maltose content of 16 wheat cultivars.....	102

Table 6.3	Analysis of variance results for sprouting scale, sprouted kernels, FN, SN, maltose content, α -amylase activity and Wheatrite method for 16 wheat cultivars	104
Table 7.1	The preharvest sprouting score and description of the five cultivars used as parents in the study	113
Table 7.2	Combined analysis of variance for preharvest sprouting resistance and α -amylase activity	116
Table 7.3	Genotype means for preharvest sprouting resistance and α -amylase activity	118
Table 7.4	Analysis of variance of general combining ability (GCA) and specific combining ability (SCA) effects for preharvest sprouting resistance and α -amylase activity in a 5 x 5 diallel	119
Table 7.5	General combining abilities (GCA) of five wheat cultivars for preharvest sprouting resistance and α -amylase activity	120
Table 7.6	Specific combining abilities (SCA) of ten wheat hybrids for preharvest sprouting resistance and α -amylase activity	121
Table 7.7	Phenotypic correlations between preharvest sprouting resistance, α -amylase activity, yield and yield components in a 5 x 5 diallel	124
Table 8.1	The preharvest sprouting score and description of the five cultivars used as parents in the study	132
Table 8.2	Crossing combinations to show the sequence used in the A-PAGE technique	132
Table 8.3	Sprouting score of the 5 parents, as well as of the ensuing progeny after 72 h of simulated rainfall	136

LIST OF FIGURES

Figure 2.1	The oxidative pentose phosphate (OPP) pathway	31
Figure 3.1	Plot of the first two canonical variates to show the interrelationships of preharvest sprouting responses of 17 cultivars grown in the winter wheat region	50
Figure 3.2	Biplot of the AMMI interaction PCA component scores <i>versus</i> the scale of sprouting for cultivars and environments (1 - 9)	55
Figure 4.1	Percentage germination of Betta-DN (HR), Betta-DN (AR), Tugela-DN (HR) and Tugela-DN (AR) seeds over a 72 h incubation period. Seeds where the radicle protruded the testa were regarded as germinated	65
Figure 4.2	Glucose-6-phosphate-dehydrogenase activity in Betta-DN (HR), Betta-DN (AR), Tugela-DN (HR) and Tugela-DN (AR) seeds over a 72 h germination period	66
Figure 4.3	Evolution of $^{14}\text{CO}_2$ from metabolized $[1-^{14}\text{C}]$ -glucose (A) and $[6-^{14}\text{C}]$ -glucose (B) by Betta-DN (HR), Betta-DN (AR), Tugela-DN (HR) and Tugela-DN (AR) seeds over a 72 h germination period	68
Figure 4.4	C6/C1 ratios calculated as evolved $^{14}\text{CO}_2$ from metabolized D- $[1-^{14}\text{C}]$ -glucose and D- $[6-^{14}\text{C}]$ -glucose by Betta-DN (HR), Betta-DN (AR), Tugela-DN (HR) and Tugela-DN (AR) seeds	69
Figure 4.5	Ion-exchange column chromatographic separation of isoenzymes of G6PDH in green and etiolated leaves of Tugela-DN (A) and Betta-DN (B)	70

Figure 4.6	Ion-exchange column chromatographic separation of isoenzymes of 6PGDH in green and etiolated leaves of Tugela-DN (A) and Betta-DN (B)	71
Figure 5.1	The relationship between FN values of 20 cultivars/lines and their respective visual preharvest sprouting scores	87
Figure 6.1	FN values <i>versus</i> relative sprouting response of 16 wheat cultivars	97
Figure 6.2	FN values <i>versus</i> percentage sprouted kernels in 16 wheat cultivars	98
Figure 6.3	The relation between the reciprocal of FN (i.e. PLN) and % sprouted kernels on 16 wheat cultivars	99
Figure 6.4	Relationship between sprouting scale and sprouted kernels (%)	99
Figure 6.5	α -Amylase activity <i>versus</i> percentage sprouted kernels for 16 wheat samples	100
Figure 6.6	α -Amylase activity <i>versus</i> FN of 16 wheat cultivars	100
Figure 6.7	Comparison of the FN method <i>versus</i> the SN method	101
Figure 6.8	FN method <i>versus</i> the WheatRite method in 16 wheat cultivars	103
Figure 8.1	A-PAGE of Neepawa, Clark's Cream, Betta-DN, Tugela-DN and RL4137. The arrows indicate the protein duplet which was detected in the cultivars with preharvest sprouting resistance	134
Figure 8.2	A-PAGE of gliadins extracted from seed of the parents (1-5), as well as the F2 seed of various crossings (6-24)	135
Figure 8.3	Percentage sprouted kernels of the F2 population which obtained a preharvest sprouting score of 1.0 to 2.9 in the rain simulator	138

ABBREVIATION LIST

ABA	:	Abscisic acid
ACA	:	ϵ -amino- <i>n</i> -caproic acid
AMMI	:	Additive main effects and multiplicative interaction method
ANOVA	:	Analysis of Variance
A-PAGE	:	Acid-polyacrylamide gel electrophoresis
AR	:	After ripened
ATP	:	Adenosine 5'-triphosphate
BHC	:	Benzamidine hydrochloride
CVA	:	Canonical variate analysis
DEAE	:	De-ethylamino-ethyl
E	:	Environment
EDTA	:	Ethylenediaminetetraacetic acid
FN	:	Falling Number
G	:	Genotype
GA	:	Gibberellic acid
GCA	:	General combining ability
G6PDH	:	Glucose-6-phosphate dehydrogenase
HC	:	Hierarchical classification
HR	:	Harvest ripe
IPCA	:	Interaction Principal Component Analysis
LSD	:	Least significant difference
NADP	:	Nicotinamide adenine dinucleotide phosphate
NADPH	:	Reduced nicotineamide adenine dinucleotide phosphate
OPP	:	Oxidative pentose phosphate
6-PGDH	:	6-phosphogluconate dehydrogenase
PLN	:	Perten Liquefaction Number
PMSF	:	Phnylmethyl-sulphonyl fluoride

PVP	:	Polyvinylpolypyrrolidone-Polyclear AT
RH	:	Relative humidity
rpm	:	Revolutions per minute
RVU	:	Rapid Visco Units
SCA	:	Specific combining ability
SE	:	Standard Errors
SKCS	:	Single Kernel Characterisation System
SN	:	Stirring Number
SS	:	Sum of squares
TRIS	:	Tris(hydroxymethyl)aminomethane
TRT	:	Treatment

CHAPTER 1

INTRODUCTION

Monocotyledons make a major contribution to man's economy and among these the *Gramineae* provide a principal source of food crops and crop-related weeds (Osborne, 1983). More wheat is consumed in the world than any other cereal, and by all indications, the demand for wheat will grow.

Wheat is the world's single most important food crop in terms of tons of grain produced each year. World output in the past decade was more than 590 million metric tons, an increase of about 30% over the average for the period 1975 to 1990. This increase in wheat production, more than any other crop, has allowed food supply to keep pace with population growth (Gooding & Davies, 1997). Of all the wheat grain consumed, it has been estimated that about 65% is used directly as food for humans, 21% as a feed for livestock, 8% as seed, and 6% for other uses including industrial raw material (Orth & Shellenberger, 1988).

South Africa is the largest producer of wheat in southern Africa and wheat production amounted to approximately 2 million metric tons per annum during the past 20 years, with significant year-to-year variation. The 2001/02 season was a highly favourable season with an estimated 2.5 million tons produced. Domestic consumption is currently approximately 2.65 million tons per annum and rising, making South Africa a net importer of wheat.

In South Africa, the average wheat yield was raised from a mere 0.66 ton/ha to 2.42 ton/ha during the past four decades. During this time, the area of wheat planted in South Africa decreased from 1 360 000 ha in 1965 to 959 000 ha in 2001 (Abstract of Agricultural Statistics, 2002).

Wheat is produced in almost all nine provinces in South Africa. The Free State province, however, is the largest producing area, contributing about 40 to 50% of the total wheat production in normal years. During the 2001/2002 season the Free State produced 878 000 tons (38% of the mean national wheat crop), followed by the Western Cape province with 767 000 tons. Together these two provinces account for approximately 70% of the total wheat production. The Northern Cape and North West produced 11.5% and 7.1% of the national wheat crop respectively, with smaller productions in Mpumalanga (4.5%), Limpopo (2.5%), KwaZulu-Natal (2.3%), Gauteng (0.6%) and the Eastern Cape (0.4%). This contribution to wheat production in South Africa, however, varies considerably due to the high-risk nature of production.

Efforts have recently intensified toward developing cultivars suited for domestic markets. For a cultivar to be released, it has to comply with a wide range of requirements, namely wide adaptability, high yield potential and stability, disease and pest resistance, as well as good quality characteristics (hectolitre mass, protein and falling number). Concern for sprouting susceptibility adds an additional hurdle to acceptance. Prospects for improving sprouting resistance were encouraging given the genetic resources available and the availability of protocols to evaluate them (Morris & Paulsen, 1987). Sprouting first becomes visible as the bran layer surrounding the embryo ruptures. The level of sprout damage is determined by the percentage of sprouted kernels in a harvested sample, classed as "damaged kernels" according to the SA Grain Standards.

Preharvest sprouting in wheat is a problem in many parts of the world, occurring three to four years out of 10 (Derera, 1989). The problem of grain sprouting in the field has been reported in the United States, Canada, Northern and Western Europe, New Zealand and Australia (Kulp *et al.*, 1983), as well as portions of central South America and the southern parts of Africa (Wahl & O'Rourke, 1993).

In South Africa, areas prone to preharvest sprouting are the winter rainfall regions of the Southern Cape, as well as the summer rainfall regions of the north eastern Free State and Kwazulu-Natal. The highest risk occurs in the winter wheat region where the probability of summer rainfall over the harvest period is highest.

Considerable preharvest sprouting damage occurred in South Africa during the past 10 years. During the 1993 season, preharvest sprouting damage was reported in the Riversdal, Albertinia, Humansdorp and Marble Hall regions. During the 1995/96, 1996/97 and 1997/98 seasons, damage due to sprouting occurred especially in the north western, central and eastern Free State.

The preceding two seasons were characterised by abnormally wet conditions during harvest, especially in the eastern and central parts of the Free State province, resulting in major preharvest sprouting problems. Due to widespread rainfall during harvest time, a significant number of hectares were not even harvested and many others were most probably damaged by sprouting which would have resulted in an even higher percentage of actual economic damage. Although no official statistics are available, mainly due to the withholding and unofficial trading of sprouted grain, the magnitude of sprouting was estimated at nearly 100 000 tons in the Free State.

In many areas around the world, cereal producers battle against the elements each year to harvest their crops before rain induces preharvest sprouting. Sprouting of grain in the field prior to harvest and the activation of systems which causes breakdown of starch and protein reserves, reduces seed quality and vigour (Tsunewaki *et al.*, 1983), lower the commercial value of grain and grain yield (Belderok, 1968), severely restricts the range of processing applications and adversely affects grain storage. This degradation causes major economic losses to grain producers worldwide, and the cereal product industries of milling, baking and brewing (Buchanan & Nicholas, 1980) are also adversely affected. Sprouting represents a major constraint to the reliable production of high-quality grain suitable for processing into food for human consumption (Mares, 1993).

Grain dormancy is the major factor responsible for conferring preharvest sprouting tolerance in harvest-ripe grains of wheat (Strand, 1983). Prolonged dormancy in wheat creates a difficulty when germinative response is required shortly after harvest. Lack of dormancy, on the other hand, results in losses from field sprouting when damp weather delays harvest.

The level and duration of dormancy are known to be affected by environmental factors such as temperature during seed development (Miyamoto & Everson, 1958; Walker-Simmons & Sesing, 1990). Other factors that affect the expression of dormancy include rainfall, humidity, evapotranspiration and radiation just prior to harvest (Clark *et al.*, 1984).

A number of physiological mechanisms, including hormonal (ABA, GA, ethylene) as well as other inhibitors (molybdenum, nitrate), which might play a regulatory role in controlling dormancy in developing grains have been postulated from time to time. Despite its importance, however, the mechanisms which control dormancy and subsequently sprouting in cereals are still only poorly understood (Mares, 1993).

The control of seed germination is also associated with an increase in the activity of the oxidative pentose phosphate pathway (OPP) at the expense of the glycolytic pathway (Swamy & Sanchyarani, 1986). The first tentative suggestion that the OPP pathway might be the oxidative process involved in the loss of seed dormancy was put forward by Major in 1966 (as cited by Roberts & Smith, 1977). It has since been suggested that, during the early stages of germination, an active OPP pathway is necessary and that this pathway is less operative when dormancy prevails (Gordon, 1980; Sánchez de Jiménez & Quiroz, 1983).

Sprout damage may also be assessed by Falling Number (FN), which serves as a gauge for α -amylase activity and starch degradation. FN values provide a snapshot of endosperm quality at harvest time (Hagemann & Ciha, 1984), but can fluctuate

widely depending on the degree of ripening and the amount of rainfall preceding harvest (Mares, 1993).

The FN method was officially introduced into the South African grading regulations during 1998. Prior to this, the degree of sprouting was determined by means of visual inspection during intake. A maximum limit of 2% visually sprouted kernels is still part of the South African grading regulations for wheat. The small grain industry of South Africa experienced various levels of difficulties in implementing FN as part of the new grading regulation during the harvesting seasons of 2000/2001 and 2001/2002.

Wheat producers in South Africa are confused with this new method which is included in the quality description of wheat sold by farmers. Apparently between 1 and 15% of wheat delivered in the Eastern Free State was degraded due to low FN (< 250 sec).

Because of the relative fast introduction into South African agriculture, several physiological aspects of sprouting seemed to have been neglected. No information existed for the effect of cultivar composition on FN. Even in this late stage of implementation, there seem to be different perceptions regarding the relationship between visual sprouting and FN.

To alleviate the possible sprouting risk, the development of cultivars that are able to tolerate or resist the damaging effects of rain during harvest time should be a major objective in a breeding programme. The multigenic inheritance of sprouting resistance, however, makes it a difficult characteristic to incorporate into new cultivars (Lukow *et al.*, 1989). The current screening method for preharvest sprouting resistance, involving a rain simulator, is a laborious process. Alternative screening methods, such as electrophoretic analysis, which requires less time, would be more desirable.

Globally speaking, sprouting of wheat occurs sporadically (two or three years out of 10) and as a result of this, research is often only focussed temporarily on sprouting. In South Africa, relatively little research was done in the past on preharvest sprouting.

The main objectives of preharvest sprouting research should be for a more complete understanding of the physiology and the genetics of the germination process, and to find technological solutions to reduce the negative effects of preharvest sprouting (Ringlund, 1993).

The objectives of this study were thus to:

- i) study the variability for sprouting resistance in South African winter wheats.
- ii) study the OPP pathway in seeds of a preharvest sprouting susceptible and preharvest sprouting resistant cultivar.
- iii) determine the ability of South African winter wheat cultivars to withstand a specified amount of simulated rainfall.
- iv) evaluate and compare the different methods of measuring preharvest sprouting resistance in a number of South African winter wheat cultivars.
- v) determine the genetic variability of preharvest sprouting, α -amylase activity, and various other traits in wheat.
- vi) to find a protein marker to screen for preharvest sprouting resistance in wheat.

REFERENCES

- ABSTRACT OF AGRICULTURAL STATISTICS. 2002. The Directorate, Agricultural Information Services. pp 10-12.
- BELDEROK, B. 1968. Seed dormancy problems in cereals. *Fld. Crop Abstr.* 21, 203-211.
- BUCHANAN, A.M. & NICHOLAS, E.M. 1980. Sprouting, α -amylase and breadmaking quality. *Cereal Res. Commun.* 8, 23-28.
- CLARKE, J.M., CHRISTENSEN, J.V. & DEPAUW, R.M. 1984. Effect of weathering on falling numbers of standing and windrowed wheat. *Can. J. Plant Sci.* 64, 457-463.
- DERERA, N.F., 1989. The effects of preharvest rain. In: N.F. Derera (ed). *Preharvest field sprouting in cereals*. University of Sydney, Narrabri, N.S.W. Australia, pp 2-14.
- GOODING, M.J. & DAVIES, W.P. 1997. An introduction to the utilization, development and production of wheat. In: M.J. Gooding and W.P. Davies (eds). *Wheat production and utilization*. CAB International, New York, USA, pp 1-59.
- GORDON, I.L. 1980. Germinability, dormancy and grain development. *Cereal Res. Commun.* 8(1), 115-129.
- HAGEMANN, M.G. & CIHA, A.J. 1984. Evaluation of methods used in testing winter wheat susceptibility to preharvest sprouting. *Crop Sci.* 24, 249-254.
- KULP, K., ROEWE-SMITH, P. & LORENZ, K. 1983. Preharvest sprouting of winter wheat. I. Rheological properties of flours and physicochemical characteristics of starches. *Cereal Chem.* 60, 355-359.
- LUKOW, O.M, DYCK, P.L. & BUSHUK, W. 1989. Possible linkage of falling number value with gliadin proteins in wheats with genes for improved sprouting resistance. *Cereal Chem.* 66(6), 531-532.
- MARES, D.J. 1993. Genetic studies of sprouting tolerance in red and white wheats. In: M.K. Walker-Simmons and J.L. Ried (eds.) *Preharvest Sprouting in Cereals*. AACC, Minnesota, USA, pp 21-29.

- MIYAMOTO, T. & EVERSON, E.H. 1958. Biochemical and physiological studies of wheat seed pigmentation. *Agron. J.* 50, 733-738.
- MORRIS, C.F. & PAULSEN, G.M. 1987. Development of preharvest sprouting-resistant germplasm from Clark's Cream hard white winter wheat. *Cereal Res. Commun.* 15, 229-235.
- ORTH, R.A. & SHELLENBERGER, J.A. 1988. Origin, production and utilization of wheat. In: Y. Pomeranz (ed). *Wheat Chemistry and Technology*. American Association of Cereal Chemists, Minnesota, pp 1-14.
- OSBORNE, D.J. 1983. Biochemical control systems operating in the early hours of germination. *Can. J. Bot.* 61, 3568-3577.
- RINGLUND, K. 1993. The importance of preharvest sprouting research. In: M.K. Walker-Simmons and J.L. Ried (eds). *Preharvest Sprouting in Cereals*. Pullman, Washington, pp 3-7.
- ROBERTS, E.H. & SMITH, R.D. 1977. Dormancy and the pentose phosphate pathway. In: K.K. Khan (ed). *The physiology and biochemistry of seed dormancy and germination*. Elsevier/North-Holland Biomedical Press, pp 385-411.
- SÀNCHEZ DE JIMÉNEZ, E. & QUIROZ, J. 1983. Role of glucose-6-phosphate dehydrogenase in corn seed germination. In: J.E. Kruger and D.E. LaBerge (eds). *Third International Symposium on Preharvest Sprouting in Cereals*. Westview Press. Boulder, Colorado, pp 197-203.
- STRAND, E. 1983. Effects of temperature and rainfall on seed dormancy of small grain cultivars. In: J.E. Kruger and D.E. LaBerge (eds). *Third International Symposium on Preharvest Sprouting in Cereals*. Westview Press, Boulder, Colorado. pp 260-266.
- SWAMY, P.M. & SANDHYARANI, C.K. 1986. Contribution of the pentose phosphate pathway and glycolytic pathway to dormancy breakage and germination of peanut (*Arachis hypogea* L.) seeds. *J. Exp. Bot.* 37, 80-88.

- TSUNEWAKI, K., YOSHIDA, T. & TSUJI, S. 1983. Genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. IX. The effect of alien cytoplasms on seed germination of common wheat. *Jpn. J. Genet.* 58, 33-41.
- WAHL, T.I. & O'ROURKE, A.D. 1993. The economics of sprout damage in wheat. In: M.K. Walker-Simmons and J.L. Ried (eds). *Preharvest Sprouting in Cereals*. Pullman, Washington, pp 10-17.
- WALKER-SIMMONS, M. & SESING, J. 1990. Temperature effects on embryonic abscisic acid levels during development of wheat grain dormancy. *J. Plant Growth Regul.* 9, 51-56.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Preharvest sprouting is defined as the germination of grains within the head prior to harvest. Preharvest rain on wheat coupled with warm temperatures create the right conditions for the wheat kernels to germinate in the wheat head (Mansour, 1993). In other words, the wheat starts to sprout before the farmer has a chance to get into the field to harvest the crop. The germination process is controlled by a complex chain of events involving hormones and enzymes and involves the *de novo* synthesis of hydrolytic enzymes that subsequently cause endosperm modification (DePauw & McCaig, 1991).

Sprout damage is relatively common in the major wheat producing areas, occurring in three to four years out of 10 (Derera, 1989). Preharvest sprouting reduces seed quality and vigour (Tsunewaki et al., 1983), milling and baking quality (Buchanan & Nicholas, 1980) and grain yield (Belderok, 1968). Many products prepared or baked from flours milled from sprouted wheat will suffer in quality. In fact, some products simply cannot be produced from such flours. Breads baked from sprouted hard wheat will have a decreased volume, a compact interior, and the crust will be considered too brown. Many soft wheat flour products are affected by high enzymatic activity. In formulations such as sponge cakes, it will result in cakes low in volume, with a compact grain and texture, sogginess, and browning of crust. Batters and breadings are affected by exhibiting too brown a colour when they are fried or baked. Certain cookies baked from sprouted-wheat flour exhibit unsatisfactory craze or surface structure. Sprouted wheat flour loses its thickening power, hence it cannot be used in cream soups and gravy mixes (Mansour, 1993). The market value of the resulting wheat is thus reduced, because of its lower value to end-users, which results in economic losses to farmers (Wahl & O'Rourke, 1993;

Verity et al., 1999). Reduction in grade lowers the price received by farmers and causes economic and marketing problems for the grain trade (Bettge & Pomeranz, 1993).

The main objectives of preharvest sprouting research should be a more complete understanding of the physiology and the genetics of the germination process, and to find technological solutions to reduce the negative effects of preharvest sprouting (Ringlund, 1993).

The primary means of increasing cultivar resistance to preharvest sprouting conditions is through the genetic manipulation of seed dormancy (Morris & Paulsen, 1988). It is generally accepted that the long-term solution to this problem lies in the development of cultivars which are able to tolerate or resist the damaging effects of rain during the period between ripeness of maturity and the completion of harvest (Mares, 1993). Breeders certainly have a challenge to breed for sprout resistant wheat varieties.

The major importance of sprouting research is to solve practical sprouting problems for the cereal producers and the cereal industry. However, because germination is one of the basic processes in plant biology, it is also important for the advancement of biological sciences to fully understand all aspects of the germination process.

2.2 DORMANCY

Whilst physical or mechanical mechanisms associated with spike structure and the seed coat can afford some protection, seed dormancy remains the primary source of tolerance. Seed dormancy is a cultivar characteristic which is important in order to avoid preharvest sprouting damage in the ears (Strand, 1989). It is, however, very important for the survival of many plant species. Some wild species can stay dormant for years and survive several seasons of unfavourable growing conditions. For the seed industry, dormancy is a negative characteristic of a seed lot or a variety.

The positive effects of seed dormancy, however, are more important than the negative ones for both cereal production and the cereal industry (Ringlund, 1993).

Seed dormancy is defined as the failure of viable embryos to germinate when subjected to favourable conditions of temperature, moisture and oxygen (DePauw & McCaig, 1991). This germination inhibition is caused by internal factors within the organ (caryopsis) and the period of afterripening necessary before dormancy breaks down (Derera *et al.*, 1977). With this definition in mind, it is obvious that the one major factor that will influence the occurrence of preharvest sprouting is the dormancy of the cultivar.

Primary dormancy is established during seed development and maturation on the mother plant. It prevents germination during development and usually also after the seeds are shed. The inability of dormant seeds to germinate under favourable conditions can be related to embryo dormancy or seed coat-imposed dormancy. Embryo dormancy may be eliminated through after-ripening by either stratification or dry storage.

Dormancy may have a great variety of causes, for example, an impervious seed coat in one species, a chemical inhibitor or an immature embryo in another. It could therefore be expected that the same environmental factors have different effects on dormancy in different species, depending on the endogenous mechanism involved (Fenner, 1991).

Many investigations have shown that there is considerable genetic variation in the dormancy period and that this character is under simple genetic control (Gale, 1976; Bhatt *et al.*, 1983). Although seed dormancy is genetic in character, there is much evidence that dormancy is also affected by many environmental and edaphic factors (Takahashi, 1980; Black *et al.*, 1987; Duffus, 1990). Lower dormancy (i.e., increased germinability) is generally associated with high temperatures, short days, red light, drought and high nitrogen levels (Fenner, 1991).

In many species the germinability of individual seeds is related to their position on the plant or within the fruit (Fenner, 1991). Differences in germinability due to position relative to other seeds is well known in the grasses where proximal grains in spikelets are usually the least dormant, for example in *Aegilops ovata* (Datta et al., 1970), *Avena lucoviciana* (Morgan & Berrie, 1970) and *Poa trivialis* (Froud-Williams & Ferris, 1987). In *Aegilops kotschy*, the lower grain is the more dormant one (Wurzbürger & Leshem, 1976). In the *Compositae*, the germination requirements of ray florets can differ markedly from those of disk florets as found for example in *Bidens pilosa* (Forsyth & Brown, 1982).

Where the plant produces two very distinct types of seed (i.e. clearly dimorphic), there is almost invariably a link between seed size and germinability. The larger, less dispersable morph is usually less dormant (Fenner, 1991).

The germinability of seeds from a population at a given location varies from year to year (Townsend, 1977). Germinability was higher in seeds which matured in the driest year, and vice versa. For plants which have an extended period of flowering and seed ripening, great variation in germinability can occur between seeds maturing at different times in the same growing season. Where seeds are gathered from individual plants through the season, any change in germinability could also be due to physiological changes connected with the ageing of the parent plant (Fenner, 1991).

In the majority of cases germinability is promoted by short-day regimes, while dormancy increases with day length (Fenner, 1991). This is well illustrated in the cases of *Portulaca oleracea* (Guttermann, 1974), *Beta vulgaris* var *rubra* (Heide et al., 1976), *Amaranthus retroflexus* (Kigel et al., 1977; Kigel et al., 1979), *Lactuca sativa* (Guttermann, 1973) and *Aegilops kotschy* (Wurzbürger & Koller, 1976).

Seasonal and year-to-year differences in seed dormancy ensure that the regeneration niche (as defined by Grubb, 1977) will be broader than would otherwise be the case (Fenner, 1991).

An interesting point about dormancy acquisition related to day length is that, at least in some cases, the day length is detected not by the seed itself, but by the parent plant. This was shown by Gutterman (1977) by covering the fruits of *Carrichtera annua* to exclude daylight. A similar effect is seen in *Trigonella arabica* (Gutterman, 1978) and *Datura ferox* (Sanchez et al., 1981). Clearly the stimulus is detected in the vegetative parts of the parent plant and some products are translocated to the seeds. Where dormancy is imposed by biochemical means, drought usually has the effect of reducing dormancy, possibly by interfering with the synthesis of a germination inhibitor or promoter (Fenner, 1991).

A number of research findings in wheat indicated that besides dormancy there are many factors which may contribute to different degrees of resistance to preharvest sprouting and these factors exhibit genotypic differences (Derera et al., 1977). Endosperm sensitivity to gibberellin has long ago been reported (Gale & Marshall, 1973; Derera et al., 1976; Gale, 1976; McMaster, 1976).

Reitan (1980) detected two mechanisms controlling dormancy, one associated with and one not associated with seed coat colour. The mechanism not associated with seed coat colour appeared to be controlled by recessive genes. The general characteristics of dormancy and the probable chromosomal location of dormancy genes appear to be similar in both red and white-grained cultivars (Mares & Ellison, 1990), although there appear to be significant differences in the mode of inheritance.

For both red and white-grained wheats the major factor being utilized in breeding programs is grain dormancy. Despite its importance, however, the mechanisms which control dormancy in cereals are still only poorly understood (Mares, 1993). Studies of dormancy in wheat have focussed attention on differences in the

sensitivity of the embryo to exogenously applied abscisic acid (ABA) or tissue extracts (Walker-Simmons *et al.*, 1989; Morris & Paulsen, 1988). Whereas cultivar variation in ABA concentrations are probably not sufficient to explain the observed variation in dormancy, differences in sensitivity of the embryo to ABA are greater and appeared to reflect the variability in the germination response of intact grains in water (Walker-Simmons *et al.*, 1989). The participation of ABA in the control of dormancy of developing seeds is well documented for a number of species (Benech-Arnold *et al.*, 1995).

Morris and Paulsen (1988) identified a water soluble germination inhibitor in wheat bran which had characteristics similar to ABA. Thus there is considerable evidence to suggest that at least part of the control of germination and preharvest sprouting resides in the embryo and is mediated by ABA and/or other endogenous inhibitors (Mares, 1993).

Cytokinins also interact against ABA and permit many enzyme activities when GA (gibberellic acid) is blocked. They appear to interact directly against ABA and often act synergistically with GA. The interaction between these two may involve the release of GA's from compartments by cytokinin effects on membranes. Cytokinins stimulate many catabolic and respiration enzymes, including α -amylase (Gordon, 1980). Cytokinins are particularly effective in breaking secondary dormancy.

Although the control of dormancy is poorly understood at the physiological level, the trait can be manipulated by conventional hybridization and progeny selection procedures (Morris & Paulsen, 1988). It was shown that the duration of dormancy was relatively independent of the level of dormancy in mature wheat grain (DeMacon & Morris, 1993). According to Morris & Paulsen (1988) the primary means of increasing cultivar resistance to preharvest sprouting conditions is through the genetic manipulation of seed dormancy.

2.3 ENVIRONMENTAL EFFECTS

Environmental conditions during seed development are known to strongly influence the level of sprouting tolerance (Takahashi, 1980). Resistance to preharvest sprouting is expressed as a quantitative character and is affected by environment and genotype x environment interaction (G X E) (Hagemann & Ciha, 1987). Field evidence of the influence of the environment during development on seed germinability is thus quite strong. Only a few of the studies attribute germinability to the effect of particular environmental factors, such as temperature changes as the season progresses (Chadoef-Hannel & Barralis, 1983), or annual variations in rainfall (Baskin & Baskin, 1975). Since day length, temperature and other climatic factors vary simultaneously through the season, experiments under controlled conditions in which individual factors are investigated separately are the only means of disentangling the effects seen in the field (Fenner, 1991). With very few exceptions germinability is positively correlated with temperature during seed maturation.

In a number of cases this has been demonstrated by correlating mean temperature in the field during maturation with the germinability (or dormancy) of the seeds. An early example of this approach is the work of Von Abrams & Hand (1956) on *Rosa*. They found a strong positive relationship between germination and the mean of the average daily temperature in the last 30 days of ripening, over a period of five years.

Dorne (1981) also demonstrated a similar relationship in the 30 days preceding harvest in *Chenopodium bonus-henricus*. In *Stellaria media*, seed collected from the field throughout the year germinated more readily if matured in summer than it did if matured in winter (Van der Vegte, 1978), and experiments under controlled conditions confirmed this difference in behavior to the temperature during development (Fenner, 1991). *Oxalis corniculata* also showed a marked difference in the dormancy levels between summer and winter seeds (Holt, 1987).

Tests by Reddy *et al.* (1985) found that for five cultivars of wheat, the effect of parental growth temperature on reducing dormancy was best detected at high germination temperatures. Clearly, the mechanism which imposes dormancy on seeds developed under cool temperatures expresses itself at different germination temperatures in different species.

Sawhney *et al.* (1985) found with wild oats that seed dormancy levels are affected by pre-anthesis temperature regimes. This was also seen in studies on tobacco (Thomas & Raper, 1975). This indicates that the effect in this case is not due to the environment operating directly on the developing seed, but is due to some influence transmitted by the parent which has experienced the relevant temperature while in the vegetative phase.

The mechanism whereby low parental temperatures promote dormancy is unknown, but may involve the synthesis of inhibitory substances at low temperatures or the synthesis of germination-promoting ones at higher temperatures (Fenner, 1991). In wheat and barley low temperatures during the seed-drying phase can delay the onset (and reduce the rate) of gibberellin-induced processes which result in the production of α -amylase. This may at least partly account for the poorer germination in seeds matured at lower temperatures (Nicholls, 1980).

Temperature can also affect the abscisic acid content of the seed during development. Each environmental factor affects the seeds in several different ways (Fenner, 1991). Higher temperatures reduce seed size (Downes & Gladstones, 1984; Wulff, 1986), alter the chemical composition of the seeds (Harris *et al.*, 1980; Green, 1986), as well as increase the germinability (Fenner, 1991).

These multiple effects of individual factors decrease the possible variation. It may be impossible, for example, for a plant to produce seeds which are only less dormant in a warm season. Because of the complex effects of even one environmental factor, it is impossible to prescribe any optimal set of conditions for reproduction by seed for

any one species. This is well illustrated by research on *Festuca arundinacea* where low parental temperatures produced larger seeds, intermediate temperatures optimised fertilisation of the ovaries and high temperatures produced seeds with the fastest germination rate (Bean, 1980).

2.4 α -AMYLASE ACTIVITY

Preharvest sprouting of grain during wet harvest conditions leads to high levels of α -amylase (EC 3.2.1.1), which are detrimental to end-use quality (McCaig & DePauw, 1992).

Excess α -amylase activity in wheat grain causes enzymatic starch hydrolysis during processing, which may disrupt manufacture and cause poor quality end-products. Hence, high quality grain must have a low α -amylase activity, which is usually measured by the FN test (Lunn *et al.*, 2001).

α -Amylase is an important determinant of wheat grain quality (Bhagwat & Bhatia, 1994). Excessive α -amylase in grains due to sprouting reduces quality resulting in sticky bread crumb (Derera, 1980). Preharvest sprouting of cereals results in elevated levels of α -amylase and consequent poor grain quality. Wheat with high concentrations of α -amylase is unsuitable for breadmaking and other end-uses (Henry, 1989).

Severe sprouting can depress yield and the viability of the grain. The primary problem, however, is the increase in α -amylase activity which occurs with the onset of germination (Flintham & Gale, 1982). This enzyme has deleterious effects on bread and noodle quality, causing hydrolytic loss of dough viscosity and high diastatic activity during processing (Buchanan & Nicholas, 1980; Moss, 1980; Mansour, 1993). The adverse effect of sprouting on rheological and baking properties

is attributed mainly to the increased enzymatic activity. Increased α -amylase activity is confined to the outer bran layers (Finney *et al.*, 1981).

α -Amylase is synthesized *de novo* in the scutella and aleurone layers of germinating grain in response to gibberellins. The discovery that the 'Tom Thumb' gene, *Rht3*, can inhibit the response of wheat aleurone to gibberellins (Gale & Marshall, 1973) provided a new genetic approach to the control of sprouting damage (Flintham & Gale, 1982). Gibbons (1979) has suggested that the scutellum rather than the aleurone is the primary site of production of hydrolases during germination. Embryos synthesize and release gibberellin, which induces transcription of α -amylase, while it also induces synthesis of other hydrolytic enzymes (Kusaba *et al.*, 1991).

A significant correlation between values for sprouting of kernels in intact spikes and α -amylase activity in the kernels has been reported by Derera *et al.* (1977), Gordon *et al.* (1977), Bhatt *et al.* (1981), Soper *et al.* (1989) and DePauw *et al.* (1990).

In wheat and barley low temperatures during the seed-drying phase can delay the onset (and reduce the rate) of gibberellin-induced processes which result in the production of α -amylase. This may at least partly account for the poorer germination in seeds matured at lower temperatures (Nicholls, 1980).

Endogenous proteinaceous inhibitors of cereal α -amylase have been reported (Weselake *et al.*, 1985). These or similar compounds may regulate α -amylase activity during sprouting and other processes (Warchalewski, 1977). The relationship between the level of inhibitor and α -amylase activity, however, is not clear (Abdul-Hussain & Paulsen, 1989).

The inhibitor is reported to increase during grain maturation, when α -amylase activity is increasing (Pace *et al.*, 1978). Other reports indicate that the inhibitor

remains active during germination (Weselake *et al.*, 1985). Susceptibility to preharvest sprouting and production of α -amylase enzyme differ markedly between red and white wheat classes and among genotypes within each class (McCrate *et al.*, 1981). Inhibition of α -amylase using specific enzyme inhibitors may reduce the effects of sprouting on grain quality (Henry & Blakeney, 1990).

Olered (1964) showed that there are two types of α -amylase, one that is mainly found in the pericarp of premature seeds, and another found in germinating seeds. Later it was shown that there were many isozymes for each of these two types of α -amylase (Marchylo & Kruger, 1983). The two types of α -amylase are coded for by two different genes, and dormancy is related to the gibberellic acid insensitivity found in semi-dwarf wheats (Gale, 1983).

During the early stages of development of wheat grains, α -amylase is synthesized in the green pericarp tissue of the ovule wall which eventually forms part of the dry seed coat. The activity of this enzyme declines rapidly in the middle stages of grain formation and in most cultivars only trace levels remain at harvest ripeness. These levels pose few problems in subsequent grain processing. Recently, however, a number of cultivars have been identified which, contrary to this accepted developmental pattern, produce unacceptably high levels of α -amylase during the later stages of ripening. The expression of this phenomenon, termed late maturity α -amylase (LMA), is influenced by the environment and is in some cultivars extremely variable and unpredictable (Mrva & Mares, 1996).

LMA refers to a genetic defect, which can result in high levels of α -amylase in ripe wheat grain in the absence of preharvest sprouting (Mares *et al.*, 1994). High levels of α -amylase (low FN) render wheat grain unsuitable for a wide range of end-product applications and result in the wheat being downgraded at receival.

The α -amylase isozymes observed in grains affected by LMA are very similar to the early stages of germination, i.e. predominantly high pl isozymes (Mrva & Mares,

1999). These isozymes are the result of new enzyme synthesis rather than abnormal retention of "green" or low *pl* pericarp α -amylase that is present in the early stages of grain development (Mrva & Mares, 2001). It has been found that certain cultivars sometimes produced high levels of enzyme during the later stages of grain development, and that the particular isozymes involved were controlled by the α -Amy-1 genes (malt amylase) (Gale *et al.*, 1983). The high *pl* isozymes are controlled by α -Amy-1 gene families located on the long arms of the homoeologous group 6 chromosomes of wheat. These isozymes are normally not detectable in developing grain, but are typical of germinated grains and those containing LMA (Mrva & Mares, 2001).

In LMA-affected grains, high *pl* α -amylase isozymes appear to be synthesised throughout the entire aleurone layer and are then released into the adjacent endosperm. This contrasts markedly with the pattern of enzyme production during germination or sprouting where initial enzyme synthesis is concentrated at the embryo end of the grain (Mrva & Mares, 1996), the scutellum being the site of α -amylase synthesis over the initial two to three days (Mrva & Mares, 1999). The aleurone is only activated later in germination. This difference in distribution of α -amylase activity within the grain can be used to differentiate LMA from preharvest sprouting (Mrva & Mares, 2001).

Irrespective of the affect of late maturity amylase on end product quality, varieties with this defect would present grain receival and marketing agents with a considerable problem.

2.5 WHEAT GRADING SYSTEMS

Wheat buyers and sellers have used various systems over the years to come to an agreement on the price to be paid for wheat on the basis of its quality. In the earliest days of wheat marketing, when small, local transactions were the rule, the buyer and seller could examine the lot of wheat together and come to a mutual agreement on

the price (Zeleny, 1971). The problem became more difficult when wheat was shipped considerable distances and some fair and equitable method of agreeing on price was required in advance of shipment. The problem was first solved by the advance submission of a representative sample of the wheat to the buyer. Marketing wheat on the basis of a sample from each lot led to transactions based on type samples that purported to portray the quality of the wheat offered to the buyer, but that were not, in fact, actual samples of it.

At first these samples were compared visually with the wheat delivered. It soon became apparent, however, that to minimize disputes, some disinterested party, an inspector experienced in judging wheat quality, was needed to determine whether or not a shipment was in fact equal to the sample that was used as a basis for the transaction. Later, such judgements were made in part by more objective tests. Grading systems, in which specifications based on both objective and subjective evaluations defining the grade, are currently in place in many countries around the world.

The visual system used in South Africa is a simple, effective way of assessing the level of sprout damage. Grain inspectors carefully detect sprouted kernels (along with those affected by other types of weather damage and by other grading factors) in a representative sample taken from the wheat lot. When 2% of the sample is sprouted, the wheat is downgraded to a lower grade. Different classes of wheat have different tolerances for sprouting, as indicated in Table 2.1. Tolerance levels are set to reflect the intrinsic quality of the wheat in relation to specific end-products.

The system has been effective in ensuring that top grades of wheat contain minimal amounts of α -amylase. It is a very tedious job to examine wheat for the number of sprouted kernels. Individual sprouted kernels also vary enormously in the amount of α -amylase they contain, depending on the severity of sprouting. This has been addressed recently in the South African grading system with the implementation of the FN method.

There are two other commercially important measurements of wheat quality in the South African grading regulations, namely hectolitre mass and protein concentration. These three quality measurements (FN, hectolitre mass and protein) are used by the milling and baking industries to determine whether wheat grain is suitable for baking and should be purchased. If any one of these three criteria is below threshold values the grain is unsuitable for milling and baking, and in years of generally low quality, the price of good quality grain rises relative to the price of wheat for animal feed.

At grain receival or during harvesting, mixing a small quantity of highly sprouted grain with larger amounts of sound grain can downgrade an entire batch of grain. The necessity for accurately discriminating sprouted from sound wheat highlights the need for a quick, easy and reliable test for preharvest sprouting. The most common method for detecting preharvest sprouting at an intake silo measures α -amylase activity using the FN method, in which the consequences of enzymatic hydrolysis of starch caused by amylase production is assessed as the time required for a plunger to fall through a heated slurry of whole meal and water (Verity *et al.*, 1999).

Table 2.1 The wheat-grading table used in the South African bread wheat grading system

Grade	Minimum hectolitre mass	Maximum percentage permissible deviation										Class D Only Mealy kernels
		A	B	C	D	E	F	G	H	I	J	
		Heavily frost damaged kernels	Screenings	Other grain and unthreshed ears	Gravel, stones, turf and glass	Foreign matter + D	Heat-damaged kernels	Damaged kernels + F	Sprouted kernels	G + H	Collective deviations	
Supergrade	79 kg	5	3	1	0.5	1	0.5	2	2	2	5	90
Grade 1	76 kg	5	3	1	0.5	1	0.5	2	2	2	5	80
Grade 2	74 kg	5	3	1	0.5	1	0.5	2	2	2	5	70
Utility grade	70 kg	10	10	4	0.5	3	0.5	5	5	5	10	-
Class Other	< 70 kg	> 10	> 10	> 4	> 0.5	> 3	> 0.5	> 5	> 5	> 5	> 10	-

2.6 FALLING NUMBER (FN)

Sprouting affects the grade of wheat. Grain inspectors determine the amount of sprout damage in wheat by visual examination of a sample. Such a procedure is subjective, and changes known as incipient sprouting often can occur in the wheat before visual damage is detectable (D'Appolonia *et al.*, 1982). Even if visible sprouting does not occur, the α -amylase level may be considerably elevated as a result of a wet harvesting season. Thus the α -amylase activity of wheat cannot be reliably estimated by determining the percentage of sprouted kernels visually. The fact that grain with none or a slight indication of sprouting might show a very high α -amylase activity, and thus prove to be of low baking quality, made visual inspection inadequate. A practical method to determine α -amylase activity of grain at an intake silo during harvesting was thus necessary.

The FN method, which is a rapid and accurate test for determining α -amylase activity, has been approved by the American Association of Cereal Chemists (2000) and is a standardised procedure applied in worldwide grain trade. It is widely used to estimate α -amylase activity in wheat grain and is utilized by flour millers all over the world as a grain quality measurement for bread making.

The success of this method is reflected in its wide acceptance and application in most countries where the occurrence of sprouting damage is a possibility. It was introduced and used as an official method connected to payment for all wheat and rye production in Australia, France, Finland, Norway, Sweden and Zimbabwe. Most countries (including South Africa) buy wheat according to a specified FN. High quality grain must have a low α -amylase activity, which is usually measured by the FN test (Lunn *et al.*, 2001).

The FN method was officially introduced into the South African grading regulations during 1998. Prior to this, the degree of sprouting was determined by

means of visual inspection. A maximum limit of 2% visually sprouted kernels is still used as an acceptable measure.

FN is defined as the time in seconds required to stir and to allow a viscometer stirrer to fall a measured distance through a hot aqueous meal, flour or starch gel undergoing liquefaction due to α -amylase activity.

The principle of the FN method is that α -amylase activity can be indirectly measured by using the starch in the sample as substrate. The method is based upon the rapid gelatinization of an aqueous suspension of flour or meal in a boiling water bath and subsequent measurement of the liquefaction of the starch paste by the α -amylase in the sample. The FN test measures primarily the change in viscosity of a heated flour or ground whole wheat-water suspension due to enzymatic breakdown of starch by α -amylase.

To obtain a representative sample, 300 g of wheat are ground and blended. From this a representative flour sample is weighed and transferred to the viscometer. Distilled water (25 ml) is added and the tube is vigorously shaken 20 to 30 times to obtain a uniform suspension. The tube and stirrer are placed into a boiling water bath and the motor automatically starts stirring after five seconds. After 60 seconds the stirrer is automatically released and allowed to drop by its own weight from the uttermost position. After falling the fixed distance, the FN value in seconds is presented on a display. The FN value is the total number of seconds from the time of immersion and start until the stirrer-viscometer has fallen the prescribed distance.

With increasing α -amylase activity, more starch will be broken down and the viscosity of the starch paste will decrease. This allows the stirrer to move more quickly through the suspension and decrease the FN value.

High α -amylase (low FN) can be caused by any one, or combination of, the following phenomena:

- ◆ *Retained pericarp α -amylase.* This results from a failure of the normal, maturation dependent destruction of low pl (pericarp, "green", developmental) α -amylase that is always present in the maternal seed coat of the developing grain (Olered & Jonsson, 1978). Although the mechanism is not well understood, it appears to be associated with environmental conditions such as frost, low temperature, low light intensity or with conditions that interfere with the normal course of grain development and ripening. Grains with retained pericarp enzyme often appear greenish rather than golden in colour (Mrva, 2001 – personal communication). Some wheat varieties produce α -amylase during the later stages of grain ripening in the absence of sprouting. This is sufficient to reduce the FN to below acceptable levels. In some varieties this only occurs some seasons, apparently in response to a specific set of environmental conditions. Others, however, produce late maturity amylase under all growing conditions although the effect is most dramatic in a cooler, humid environment.
- ◆ *Pre-ripeness sprouting.* This results from the premature germination of ripening wheat grain. This phenomenon has been observed several times in the past decade in the United Kingdom, (Kettlewell & Cashman, 1997; Flinham & Gale, 1998) and in trials conducted in northern Japan (Nakatsu et al., 1996), in grains that showed symptoms of black point. In controlled environmental studies the condition has been induced in the later stages of ripening by a combination of moisture and cool temperature.
- ◆ *Post ripeness sprouting* (usually referred to as preharvest sprouting). This is the most common cause of high amylase in wheat and results when ripe wheat is subjected to rain and remains wet for long enough that germination can commence.

- ◆ *Late maturity α -amylase (LMA)* is found in specific genotypes in some environments (Mrva & Mares, 2001).

Irrespective of the causes for low FN, high levels of α -amylase lead to starch breakdown which affects the quality of end-use products negatively.

2.7 OXIDATIVE PENTOSE PHOSPHATE (OPP) PATHWAY

The principal storage products of cereals and other *Graminaceous* plants are carbohydrates, mainly starch (Bewley & Black, 1978). The oxidation of carbohydrates involves enzymatic conversion to glucose-6-phosphate which is further metabolized *via* either glycolysis or the oxidative pentose phosphate (OPP) pathway, the two major pathways of carbohydrate oxidation to pyruvate in higher plants (Rumpho & Kennedy, 1983).

In plants, the OPP pathway is connected *inter alia* with fatty acid biosynthesis, nitrate reduction and the shikimate pathway (Schnarrenberger *et al.*, 1995). The pathway is known to occur in chloroplasts. Reactions of the OPP pathway are outlined in Figure 2.1. The pathway is normally presented as a cyclic pathway in which glucose-6-phosphate is converted by a series of reactions back to fructose-6-phosphate. In the process carbon dioxide is liberated and NADPH is formed.

The first reaction involves glucose-6-phosphate, which can arise either from starch breakdown by starch phosphorylase followed by phosphoglucomutase action in glycolysis, or from the addition of the terminal phosphate of ATP to glucose, or directly from photosynthetic reactions. It is immediately oxidized by glucose-6-phosphate dehydrogenase (G6PDH) to 6-phosphogluconic acid. The 6-phosphogluconate does not accumulate, but is both dehydrogenated and decarboxylated rapidly by 6-phosphoglucodehydrogenase (6-PGDH), yielding the

five-carbon compound ribulose-5-phosphate, NADPH and CO_2 (Salisbury & Ross, 1978).

The two major functions of this pathway are firstly to generate NADPH that is required for biosynthetic reactions. Hence, this segment of the pathway will be most active in plant tissues that are very actively growing. Secondly, various intermediates for the pathway itself are produced, including 5-carbon and 4-carbon compounds which may be needed as building blocks for various synthetic processes (Roberts & Smith, 1977).

Involvement of the OPP pathway in the regulation of seed dormancy has been reported in a number of studies based on the measurement of the activities of the two key enzymes of this pathway, namely G6PDH (E.C. 1.1.1.49) and 6PGDH (E.C. 1.1.1.44) (Kovacs & Simpson, 1976; Gosling & Ross, 1980, Swamy & Sandhyarani, 1986; Galais *et al.*, 2000). Since the enzymes controlling this pathway appear to occur in the cytosol (Averill *et al.*, 1998), the regulation of movement of glucose-6-phosphate into and through the pathway is achieved by modulating enzyme levels and the concentration of cofactors.

The above properties suggest that G6PDH is controlled *in vivo* by the ratio $\text{NADP}^+:\text{NADPH}$. Glucose-6-phosphate, the immediate precursor of the OPP pathway (Figure 2.1), is generated in the cytosol and imported into the plastids by the plastidic glucose-6-phosphate/phosphate translocator (Debnam & Emes, 1999).

Of all the metabolic pathways studied thus far, the one which has been related most to dormancy breaking and seed germination, is the OPP pathway (Pretorius & Small, 1992). Roberts (1973) postulated that the functioning of the OPP pathway is a prerequisite for dormancy-breaking and germination. Most studies based on C6/C1 ratios and activity measurements of the two initial enzymes of the pathway, appear to support this hypothesis (Hendricks & Taylorson, 1975; Kovacs & Simpson, 1976; Ashihara & Matsumura, 1977; Swamy & Sandhyarani, 1986). Recently, strong

support for the hypothesis in relation to dormancy in seeds of *Avena fatua*, has come from Galais et al. (2000). However, reports to the contrary have been made by Upadhyay et al., 1981 and Thevenot et al., 1989.

From results with barley (*Hordeum vulgare*) seeds, it has been proposed that the difference between dormant and non-dormant seeds lies in greater participation of the OPP pathway in the latter seeds prior to germination. Gordon (1980), as well as Sánchez de Jiménez & Quiroz (1983), suggested that an active OPP pathway is necessary during the early stages of germination and that this pathway is less operative when dormancy prevails. Gahan et al. (1986) reported that the changes in the activity of the OPP pathway enzymes during the processes leading to radicle emergence of *Avena fatua*, are due to OPP pathway activity. Similarly Gallais et al., (2000) reported that the dormancy experienced in seeds of *Avena sativa* is due to the low activity of the OPP pathway in the embryo. Pretorius & Small (1992) indicated that the lack of OPP pathway activity is part of the mechanism leading to a state of secondary dormancy and subsequent loss of germinative capacity in soak-injured bean seeds.

The role of the OPP pathway in seed germination is not known yet, but it seems improbable that the production of NADPH is important in reductive synthetic reactions. However, although its precise role is not known, it is possible that the OPP pathway may be involved in the initiation of cell elongation. The fact that carbohydrate oxidation in plants is compartmented should be taken into account in future investigations of glycolysis and the OPP pathway in plants.

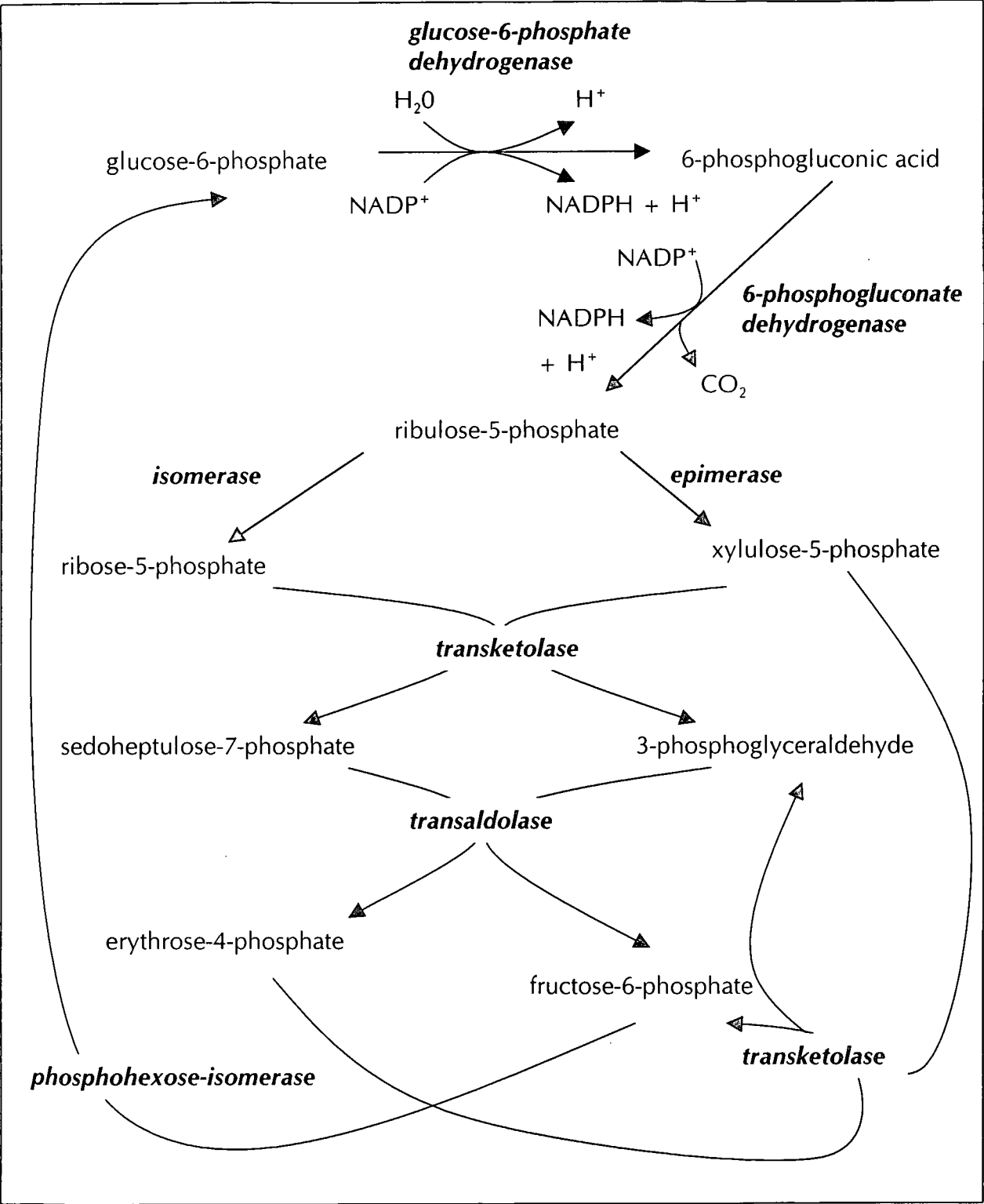


Figure 2.1 The oxidative pentose phosphate (OPP) pathway (Salisbury & Ross, 1978).

2.8 CONCLUSIONS

It would appear that protection derived from a single component of already complex sprouting resistance might not be adequate under certain environmental conditions. Therefore, in order to provide adequate resistance to sprouting, efforts should be made to combine different components into a variety through extensive crossing programmes, involving varieties known to be promising with regard to one or more components. The segregating populations should then be thoroughly screened for a combination of as many components as possible in an effort to evolve varieties with multiple resistance to preharvest sprouting (Derera *et al.*, 1977).

In seeking to breed cultivars with resistance to preharvest germination in the ear, rapid progress could be expected if the aspects of inheritance of all the characters contributing to resistance are appreciated and selection is made for the desired combination of these characters (Bhatt *et al.*, 1977).

However, both breeding for sprouting resistance and development of other methods to reduce the sprouting problem, will be much easier when the germination process is fully understood. To successfully breed for preharvest sprouting tolerance, it is necessary to both identify variation for individual components of tolerance and to develop methods by which this variation can be combined.

REFERENCES

- ABDUL-HUSSAIN, S. & PAULSEN, G.M. 1989. Role of proteinaceous α -amylase enzyme inhibitors in preharvest sprouting of wheat grain. *J. Agric. Food Chem.* 37(2), 295-299.
- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 2000. Approved Methods of the AACC, 10th ed. Vol II. Falling Number Determination. Method 56-81B. The Association: St. Paul, Minnesota.
- ASHIHARA, H. & MATSUMURA, H. 1977. Changes in the activity and function of the pentose phosphate pathway during germination of black gram *Phaseolus mungo* seeds. *International J. of Biochem.* 72, 248-254.
- AVERILL, R.H., BAILEY-SERRES, J. & KRUGER, N.J. 1998. Co-operation between cytosolic and plastidic oxidative pentose phosphate pathways revealed by 6-phosphogluconate dehydrogenase-deficient genotypes of maize. *Plant Journal* 14 (4), 449-457.
- BASKIN, J.M. & BASKIN, C.C. 1975. Year-to-year variation in the germination of freshly-harvested seeds of *Arenaria patula* var. *robusta* from the same site. *J. Tennessee Acad. Sci.* 50, 106-108.
- BEAN, E.W. 1980. Factors affecting the quality of herbage seeds. In: P.D. Hebblethwaite (ed). *Seed production*. London, Butterworth. pp. 593-604.
- BELDEROK, B. 1968. Seed dormancy problems in cereals. *Fld. Crop Abstr.* 21, 203-211.
- BENECH-ARNOLD, R.L., FENNER, M. & EDWARDS, P.J. 1995. Influence of potassium nutrition on germinability, abscisic acid content and sensitivity of the embryo to abscisic acid in developing seeds of *Sorghum bicolor* (L.) Moench. *New Phytol.* 130, 207-216.
- BETTGE A.D. & POMERANZ, Y. 1993. Air-aspirated cleaning to separate sound from preharvest sprouted wheat. *Cereal Chem.* 70(1), 36-41.
- BEWLEY, D. & BLACK, M. 1978. *Physiology and biochemistry of seeds in relation to germination*. Vol. 1. Development, Germination and Growth. Springer-Verlag, Berlin/New York. pp 133-176.

- BHAGWAT, S.G. & BHATIA, C.R. 1994. Quantitative differences in the gibberellin induced α -amylase activity from aleurone layers of tall and semi-dwarf wheat cultivars. *Cereal Res. Commun.* 22(1-2), 129-134.
- BHATT, G.M., DERERA, N.F. & McMASTER, G.J. 1977. Utilization of Tom Thumb source of preharvest sprouting tolerance in a wheat breeding programme. *Euphytica* 26, 565-572.
- BHATT, G.M., PAULSEN, G.M., KULP, K. & HEYNE, E.G. 1981. Preharvest sprouting in hard winter wheats: assessment of methods to detect genotypic and nitrogen effects and interactions. *Cereal Chem.* 58, 300-302.
- BHATT, G.M., ELLISON, F.W. & MARES, D.J. 1983. Inheritance studies in dormancy in three wheat crosses. In: J.E. Kruger & D.E. LaBerge (eds). *Third International Symposium on Preharvest Sprouting in Cereals*. Westview Press, Boulder, Colorado. pp 274-278.
- BLACK, M., BUTLER, J. & HUGHES, M. 1987. Control and development of dormancy in cereals. In: D.J. Mares (ed). *Proceedings of the Fourth International Symposium on Preharvest Sprouting in Cereals*. Westview Press, Boulder, Colorado. pp 379-392.
- BUCHANAN, A.M. & NICHOLAS, E.M. 1980. Sprouting, α -amylase and breadmaking quality. *Cereal Res. Commun.* 8, 23-28.
- CHADOEF-HANNEL, R. & BARRALIS, G. 1983. Evolution de l'aptitude à germer des graines d'*Anaranthus retroflexus* L. récoltées dans différentes conditions, au cours de leur conservation. *Weed Res.* 23, 109-117.
- D'APPOLONIA, B.L., McARTHUR, L.A, PISESOOKBUNTERNG, W. & CIACCO, C.F. 1982. Comparison of the grain amylase analyzer with the amylograph and falling number method. *Cereal Chem.* 59, 254-257.
- DATTA, S.C., EVENARI, M. & GUTTERMAN, Y. 1970. The heteroblasty of *Aegilops ovata* L. *Israel J. Bot.* 19, 463-483.
- DEBNAM, P.M. & EMES, M.J. 1999. Subcellular distribution of enzymes of the oxidative pentose phosphate pathway in root and leaf tissues. *J. Exp. Bot.* 50 (340), 1653-1661.

- DEMACON, V.L. & MORRIS, C.F. 1993. Rate of after-ripening among diverse hexaploid wheat genotypes. In: M.K. Walker-Simmons and J.L. Ried (eds). *Preharvest Sprouting in Cereals*. St Paul, Minnesota. pp 61-68.
- DEPAUW, R.M. & McCAIG, T.N. 1991. Components of variation, heritabilities and correlations for indices of sprouting tolerance and seed dormancy in *Triticum* spp. *Euphytica* 52, 221-229.
- DEPAUW, R.M., McCAIG, T.N., MARES, D., BRENNAN, P., HENRY, R.J., KING, R., McEWAN, J.M. & GORDON, I. 1990. Interrelationships among assays for germination of kernels from threshed spikes, unthreshed spikes and α -amylase in wheat. In: K. Ringlund, E. Mosleth and D.J. Mares (eds). *Fifth International Symposium on Preharvest Sprouting in Cereals*. Loen, Norway. pp 195-205.
- DERERA, N.F. 1980. Sprouting damage. *Cereal Res. Commun.* 8, 15-22.
- DERERA, N.F., 1989. The effects of preharvest rain. In: N.F. Derera (ed). *Preharvest field sprouting in cereals*. University of Sydney, Narrabri, N.S.W. Australia. pp 2-14.
- DERERA, N.F., BHATT, G.M. & McMASTER, G.J. 1977. On the problem of preharvest sprouting in wheat. *Euphytica* 26, 299-308.
- DERERA, N.F., McMASTER, G.J. & BALAAM, L.N. 1976. Preharvest sprouting resistance and associated components in 12 wheat cultivars. *Cereal Res. Commun.* 4, 173-179.
- DORNE, A.J. 1981. Variation in seed germination inhibition of *Chenopodium bonus-henricus* in relation to altitude of plant growth. *Can. J. Bot.* 59, 1893-1901.
- DOWNES, R.W. & GLADSTONES, J.S. 1984. Physiology of growth and seed production in *Lupinus angustifolius* L. II. Effect of temperature before and after flowering. *Aus. J. Agric. Res.* 35, 501-509.
- DUFFUS, C.M. 1990. Recent progress in the physiology and biochemistry of cereal grains in relation to preharvest sprouting. In: K. Ringlund, E. Mosleth and D.J. Mares (eds). *Fifth International Symposium on Preharvest Sprouting in Cereals*. Westview Press, Boulder, Colorado. pp 47-56.

- FENNER, M. 1991. The effects of the parent environment on seed germinability. *Seed Sci. Res.* 1, 75-84.
- FINNEY, K.F., NATSUAKI, O., BOLTE, L.C., MATHEWSON, R. & POMERANZ, Y. 1981. α -Amylase in field-sprouted wheats: Its distribution and effect on Japanese-type sponge cake and related physical and chemical tests. *Cereal Chem.* 58, 355-363.
- FLINTHAM, J.E. & GALE, M.D. 1982. The Tom Thumb dwarfing gene, Rht3 in wheat. I. Reduced preharvest damage to breadmaking quality. *Theor. Appl. Genet.* 62, 121-126.
- FLINTHAM, J.E. & GALE, M.D. 1998. Plant height and yield components of inbred isogenic and F1 hybrid Rht dwarf wheats. *J. Applied Genetics* 39 (1), 73-83.
- FORSYTH, C. & BROWN, N.A.C. 1982. Germination of the dimorphic fruits of *Bidens pilosa* L. *New Phytologist* 90, 151-164.
- FROUD-WILLIAMS, R.J. & FERRIS, R. 1987. Germination of proximal and distal seeds of *Poa trivialis* L. from contrasting habitats. *Weed Res.* 27, 245-250.
- GAHAN, P.B., DAYSON, A.L., BLACK, M. & CHAPMAN, J.M. 1986. Localization of glucose-6-phosphate dehydrogenase activity in seeds and its possible involvement in dormancy breakage. *Ann. Bot.* 57, 791-799.
- GALE, M.D. 1976. High α -amylase - breeding and genetical aspects of the problem. *Cereal Res. Commun.* 4, 231-243.
- GALE, M.D. 1983. α -Amylase genes in wheat. In: J.E. Kruger and D.E. LaBerge (eds). *Proceedings of the Third International Symposium on Preharvest sprouting in Cereals*. Westview Press, Boulder, Colorado. pp 105-110.
- GALE, M.D. & MARSHALL, G.A. 1973. Insensitivity to gibberellin in dwarf wheats. *Ann. Bot.* 37, 729-735.
- GALE, M.D., FLINTHAM, J.E. & ARTHUR, E.D. 1983. α -Amylase production in the late stages of grain development – an early sprouting damage risk period? In: J.E. Kruger and D.E. LaBerge (eds). *Proceedings of the Third International Symposium on Preharvest Sprouting in Cereals*. Westview Press, Boulder, Colorado. pp 29-35.

- GALLAIS, S., DE CRESCENZO, M.A.P. & LAVAL-MARTIN, D.L. 2000. Evidence of active NADP⁺ phosphatase in dormant seeds of *Avena sativa* L. *J. Exp. Bot.* 51(349), 1389-1394.
- GIBBONS, G. 1979. On the localization and transport of α -amylase during germination and early seedling growth of *Hordeum vulgare*. *Carlsberg Res. Commun.* 44, 353-366.
- GORDON, I.L. 1980. Germinability, dormancy and grain development. *Cereal Res. Commun.* 8(1), 115-129.
- GORDON, I.L., DERERA, N.F. & BALAAM, L.M. 1977. Selection against sprouting damage in wheat. I. Germination of unthreshed grain with a standard wetting procedure. *Aust. J. Agric. Res.* 28, 583-596.
- GOSLING, P.G. & ROSS, J.D. 1980. Pentose phosphate metabolism during dormancy breakage in *Corylus avellana* L. *Planta.* 148, 362-366.
- GREEN, A.G. 1986. Effect of temperature during seed maturation on the oil composition of low-linolenic genotypes of flax. *Crop Sci.* 26, 961-965.
- GRUBB, P.J. 1977. The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biol. Reviews* 52, 107-145.
- GUTTERMAN, Y. 1973. Differences in progeny due to daylength and hormone treatment of the mother plant. In: W. Heydecker, (ed). *Seed ecology*. London, Butterworth. pp 59-80
- GUTTERMAN, Y. 1974. The influence of the photoperiodic regime and red-far red light treatments of *Portulaca oleracea* L. plants on the germinability of their seeds. *Oecologia* (Berlin). 17, 27-38.
- GUTTERMAN, Y. 1977. Influence of environmental conditions and hormonal treatments of the mother plants during seed maturation, on the germination of their seed. In: C.P. Malik (ed). *Advances in Plant Reproductive Physiology*. New Dehli, Kalyani Publishers. pp 288-294
- GUTTERMAN, Y. 1978. Seed coat permeability as a function of photoperiodical treatments of the mother plants during seed maturation in the desert annual plant: *Trigonella arabica*, del. *J. Arid Environ.* 1, 141-144.

- HAGEMANN, M.G. & CIHA, A.J. 1987. Environmental x genotype effects on seed dormancy and after-ripening in wheat. *Agron. J.* 79, 192-196.
- HARRIS, H.C., MCWILLIAM, J.R. & BOFINGER, V.J. 1980. Prediction of oil quality of sunflower from temperature probabilities in eastern Australia. *Aus. J. Agric. Res.* 31, 477-488.
- HEIDE, O.M., JUNTILA, O. & SAMUELSEN, R.T. 1976. Seed germination and bolting in red beet as affected by parent plant environment. *Physiol. Plant.* 36, 343-349.
- HENDRICKS, S.B. & TAYLORSON, R.B. 1975. Breaking of seed dormancy by catalase inhibition. *Proceedings of the National Academy of Sciences, U.S.A.* 72, 306-309.
- HENRY, R.J. 1989. Rapid α -amylase assays for assessment of preharvest sprouting damage in wheat. *J. Sci. Food Agric.* 49, 15-23.
- HENRY, R.J. & BLAKENEY, A.B. 1990. Post harvest management of α -amylase. *Aspects App. Biol.* 25, 387-393.
- HOLT, J.S. 1987. Factors affecting germination in greenhouse-produced seeds of *Oxalis corniculata*, a perennial weed. *American J. Bot.* 74, 429-436.
- KETTLEWELL, P.S. & CASHMAN, M.M. 1997. α -Amylase activity of wheat grain from crops differing in grain drying rate. *J. Agric. Sci.* 128, 127-134.
- KIGEL, J., GIBLY, A. & NEGBI, M. 1979. Seed germination in *Amaranthus retroflexus* L. as affected by the photoperiod and age during flower induction of the parent plants. *J. Exp. Bot.* 30, 997-1002.
- KIGEL, J., OFIR, M. & KOLLER, D. 1977. Control of the germination responses of *Amaranthus retroflexus* L. seeds by their parental photothermal environment. *J. Exp. Bot.* 28, 1125-1136.
- KOVACS, M.I.P. & SIMPSON, G.M. 1976. Dormancy and enzyme levels in seeds of wild oats. *Phytochem.* 15, 455-458.
- KUSABA, M., KOBAYASHI, O., YAMAGUCHI, I., TAKAHASHI, N & TAKEDA, G. 1991. Effects of gibberellin on genetic variations in α -amylase production in germinating barley seeds. *J. Cereal Sci.* 14, 151-160.

- LUNN, G.D., KETTLEWELL, P.S., MAJOR, B.J. & SCOTT, R.K. 2001. Effects of pericarp α -amylase activity on wheat (*Triticum aestivum*) Hagberg Falling Number. *Ann. Appl. Biol.* 138, 207-214.
- MANSOUR, K. 1993. Sprout damage in wheat and its effect on wheat flour products. In: M.K. Walker-Simmons and J.L. Ried (eds). *Preharvest Sprouting in Cereals*. Pullman, Washington, pp 8-9.
- MARCHYLO, B.A. & KRUGER, J.E. 1983. Separation of wheat α -amylase isozymes by chromatofocussing. In: J.E. Kruger and D.E. LaBerge (eds). *Proceedings of the Third International Symposium on Preharvest Sprouting in Cereals*. Westview Press, Boulder, Colorado. pp 96-104.
- MARES, D.J. 1993. Genetic studies of sprouting tolerance in red and white wheats. In: M.K. Walker-Simmons & J.L. Ried (eds). *Preharvest Sprouting in Cereals*. Pullman, Washington, pp 21-29.
- MARES, D.J. & ELLISON, F.W. 1990. Dormancy and preharvest sprouting tolerance in white-grained and red-grained wheats. In: K. Ringlund, E. Mosleth and D.J. Mares (eds). *Fifth International Symposium on Preharvest Sprouting in Cereals*. Westview Press, Boulder, Colorado. pp 75-84.
- MARES, D.J., MRVA, K & PANOZZO, J.F. 1994. Characterization of the high α -amylase levels in grain of the wheat cultivar BD 159. *Aus. J. Agric. Res.* 45, 1003-1011.
- McCAIG, T.N. & DEPAUW, R.M. 1992. Breeding for preharvest sprouting tolerance in white-seed-coat spring wheat. *Crop Sci.* 32, 19-23.
- McCRATE, A.J., NIELSEN, M.T., PAULSEN, G.M. & HEYNE, E.G. 1981. Preharvest sprouting and α -amylase activity in hard red and hard white winter wheat cultivars. *Cereal Chem.* 58, 424-428.
- McMASTER, G.J. 1976. Response to GA3 in α -amylase synthesis of four wheat cultivars. *Cereal Res. Commun.* 4, 227-230.
- MORGAN, S.F. & BERRIE, A.M.M. 1970. Development of dormancy during seed maturation in *Avena ludoviciana* winter wild oat. *Nature.* 228, 1225-1226.

- MORRIS, C.F. & PAULSEN, G.M. 1988. Localization and physical properties of endogenous germination inhibitors in white wheat grain. *Cereal Chem.* 65(5), 404-408.
- MOSS, H.J. 1980. The pasting properties of some wheat starches free of sprouting damage. *Cereal Res. Commun.* 8, 297-302.
- MRVA, K. & MARES, D.J. 1996. Control of late maturity α -amylase synthesis compared to enzyme synthesis during germination. In: K. Noda and D.J. Mares (eds). *Proceedings of the Seventh International Symposium on Preharvest Sprouting in Cereals*. Center for Academic Societies Japan, Osaka. pp 419-426.
- MRVA, K. & MARES, D.J. 1999. Regulation of high pl α -amylase synthesis in wheat aleurone by a gene(s) located on chromosome 6B. *Euphytica* 109, 17-23.
- MRVA, K. & MARES, D.J. 2001. Induction of late maturity α -amylase in wheat by cool temperature. *Aust. J. Agric.Res.* 52, 477-484.
- NAKATSU, S., MIYAMOTO, H. & AMANO, Y. 1996. Variation for α -amylase activity and dormancy in Hokkaido wheat varieties. In: K.Noda and D.J. Mares (eds). *Proceedings of the Seventh International Symposium on Preharvest Sprouting in Cereals*. Center for Academic Societies Japan, Osaka. pp 411-418.
- NICHOLLS, P.B. 1980. Development of responsiveness to gibberellic acid in the aleurone layer of immature wheat and barley caryopses: effect of temperature. *Aus. J. Plant Physiol.* 7, 645-653.
- OLERED, R. 1964. Studies on the development of α -amylase activity in ripening wheat. *Ark. Kemi.* 22:175-183.
- OLERED, R. & JONSSON, G. 1978. Electrophoretic studies of α -amylase in wheat II. *J. Sci. Fd. Agric.* 21, 385-392.
- PACE, W., PARLAMENTI, R., URRAB, A., SILANO, V. & VITTOZZI, L. 1978. Protein α -amylase inhibitor from wheat. *Biochem. Biophys. Acta.* 422, 159-169.

- PRETORIUS, J.C. & SMALL, J.G.C., 1992. The effect of soaking injury in bean seeds on aspects of the oxidative pentose phosphate pathway in embryonic axes. *Seed Sci. Res.* 2, 33-39.
- REDDY, L.V., METZGER, R.J. & CHING, T.M. 1985. Effect of temperature on seed dormancy of wheat. *Crop Sci.* 25, 455-458.
- REITAN, L. 1980. Genetical aspects of seed dormancy in wheat related to seed coat color in an 8 x 8 diallele cross. *Cereal Res. Commun.* 8, 275-282.
- RINGLUND, K. 1993. The importance of preharvest sprouting research. In: M.K. Walker-Simmons and J.L. Ried (eds). *Preharvest Sprouting in Cereals*. Pullman, Washington. pp 3-7.
- ROBERTS, E.H. 1973. Oxidative processes and the control of seed germination. In: W. Heydecker (ed). *Seed Ecology*. PA, USA, Pennsylvania State University Press, University Park. pp 189-218.
- ROBERTS, E.H. & SMITH, R.D. 1977. Dormancy and the pentose phosphate pathway. In: K.K. Khan (ed). *The physiology and biochemistry of seed dormancy and germination*. Elsevier/North-Holland Biomedical Press. pp 385-411.
- RUMPHO, M.E. & KENNEDY, R.A. 1983. Activity of the pentose phosphate and glycolytic pathways during anaerobic germination of *Echinochloa crus-galli* (barnyard grass) seeds. *J. Exp. Bot.* 34, 893-902.
- SALISBURY, F.B. & ROSS, C.W. 1978. In: W.A. Jensen (ed). 2nd Ed. *Plant Physiology*. Wadsworth Publishing Company, USA. pp 174-191.
- SANCHEZ, R.A., EYHERABIDE, G. & DE MIGUEL, L. 1981. The influence of irradiance and water deficit during fruit development on seed dormancy in *Datura ferox* L. *Weed Res.* 21, 127-132.
- SÀNCHEZ DE JIMÉNEZ, E & QUIROZ, J. 1983. Role of Glucose-6-phosphate dehydrogenase in corn seed germination. In: J.E. Kruger and D.E. LaBerge (eds). *Third International Symposium on Preharvest Sprouting in Cereals*. Westview Press. Boulder, Colorado. pp 197-203.

- SAWHNEY, R., QUICK, W.A. & HSIAO, A.I. 1985. The effect of temperature during parental vegetative growth on seed germination of wild oats (*Avena fatua* L.). *Ann. Bot.* 55, 25-28.
- SCHNARRENBARGER, C., FLECHNER, A. & MARTIN, W. 1995. Enzymatic evidence for a complete oxidative pentose phosphate pathway in chloroplast and an incomplete pathway in the cytosol of spinach leaves. *Plant Physiol.* 108, 609-614.
- SOPER, J.F., CANTRELL, R.G. & DICK, J.W. 1989. Sprouting damage and kernel color relationships in durum wheat. *Crop Sci.* 29, 895-898.
- STRAND, E. 1989. Studies on seed dormancy in small grain species. II. Wheat. *Norwegian J. Agric. Sci.* 3, 101-115.
- SWAMY, P.M. & SANDHYARANI, C.K. 1986. Contribution of the pentose phosphate pathway and glycolytic pathway to dormancy breakage and germination of peanut (*Arachis hypogea* L.) seeds. *J. Exp. Bot.* 37, 80-88.
- TAKAHASHI, N. 1980. Effect of environmental factors during seed formation on preharvest sprouting. *Cereal Res. Commun.* 8, 175-183.
- THEVENOT, C., SIMOND-COTE, E., PERINO, C. & DAUSSANT, J. 1989. Pentose phosphate pathway dehydrogenases in relation to the dormancy, germination and growth of apple embryos. *Israel Journal of Botany* 38, 193-198.
- THOMAS, J.F. & RAPER, C.D. 1975. Seed germinability as affected by the environmental temperature of the mother plant. *Tobacco Sci.* 19, 98-100.
- TOWNSEND, C.E. 1977. Germination of polycross seed of cicer milkvetch as affected by year of production. *Crop Sci.* 17, 909-912.
- TSUNEWAKI, K., YOSHIDA, T. & TSUJI, S. 1983. Genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. IX. The effect of alien cytoplasm on seed germination of common wheat. *Jpn. J. Genet.* 58, 33-41.
- UPADHYAY, M.K., SIMPSON, G.M. & NAYLOR, J.M. 1981. Levels of glucose-6-phosphate and 6-phosphogluconate dehydrogenases in the embryos and endosperms of some dormant and non-dormant lines of *Avena fatua* during germination. *Can. J. Bot.* 59, 1640-1646.

- VAN DER VEGTE, F.W. 1978. Population differentiation and germination ecology in *Stellaria media* (L.) Vill. *Oecologia* (Berl.) 37, 231-245.
- VERITY, J.C.K., HAC, L. & SKERRIT, J.H. 1999. Development of a field Enzyme-Linked Immunosorbent Assay (ELISA) for detection of α -amylase in preharvest sprouted wheat. *Cereal Chem.* 76 (5), 673-681.
- VON ABRAMS, G.J. & HAND, M.E. 1956. Seed dormancy in *Rosa* as a function of climate. *American J. Bot.* 43, 7-12.
- WAHL, T.I. & O'ROURKE, A.D. 1993. The economics of sprout damage in wheat. In: M.K. Walker-Simmons and J.L. Ried (eds). *Preharvest Sprouting in Cereals*, Pullman, Washington, pp 10-17.
- WALKER-SIMMONS, M.K., KUDRNA, D.A. & WARNER, R.L. 1989. Reduced accumulation of ABA during water stress in a molybdenum cofactor mutant of barley. *Plant Physiol.* 90, 728-733.
- WARCHALEWSKI, J.R. 1977. Isolation and purification of native α -amylase inhibitors from winter wheat. *Bull. Acad. Pol. Sci.* XXV, 725-729.
- WESELAKE, R.J., MCGREGOR, A.W. & HILL, R.D. 1985. Endogenous α -amylase inhibitor in various cereals. *Cereal Chem.* 62, 120-123.
- WULFF, R.D. 1986. Seed size variation in *Desmodium paniculatum*. I. Factors affecting seed size. *J. Ecol.* 74, 87-97.
- WURZBURGER, J. & KOLLER, D. 1976. Differential effects of the parental photothermal environment on development of dormancy in caryopses of *Aegilops kotschyi*. *J. Exp. Bot.* 27, 43-48.
- WURZBURGER, J. & LESHEM, Y. 1976. Correlative aspects of imposition of dormancy in caryopses of *Aegilops kotschyi*. *Plant Physiol.* 57, 670-671.
- ZELENY, L. 1971. Criteria of Wheat Quality. In: Y. Pomeranz (ed). *Wheat: Chemistry and Technology*. Vol. 1. AACC, Minnesota, USA. pp19-49.

CHAPTER 3

ASSESSMENT OF PREHARVEST SPROUTING IN SOUTH AFRICAN WINTER AND INTERMEDIATE WHEAT CULTIVARS USING NON-PARAMETRIC ANALYSES

3.1 ABSTRACT

Preharvest sprouting significantly reduces the quality of the South African wheat crop. This study classified the preharvest sprouting resistance of 17 South African winter and intermediate wheat cultivars sampled over a range of typical winter wheat growth environments. Sampling was performed under field conditions and sprouting responses were evaluated under controlled conditions. Variation in sprouting response between cultivars was predominantly genetically determined and varied from 1.1 to 7.2 on a scale from 1 (no visual sprouting) to 8 (fully sprouted). Canonical variate analysis and AMMI analysis, as well as the use of hierarchical clustering of cultivars over environments using the AMMI estimates, identified three distinct groups, ranging from resistant to susceptible. The AMMI model was used as it combines the additive main effects of the analysis of variance with the interaction effects of principal components analysis. In general Betta-DN, Elands, Limpopo, Caledon and PAN 3235 showed good resistance to preharvest sprouting, while the hybrids Carina, Caritha and Carol, as well as the pureline Tugela-DN, were highly susceptible to sprouting. Betta-DN and Elands had the best stability over environments and years. It became evident that the sprouting responses of cultivars were in some cases also triggered by different environmental factors. Genotypic variation appeared to be dominant, indicating that progress in the development of cultivars with sprouting tolerance is feasible. The occurrence of extreme climatic conditions had a significant effect on cultivar responsiveness, whereas mild temperature differences during grain filling were secondary.

3.2 INTRODUCTION

Extended periods of rainfall with subsequent periods of high humidity occurring after grain maturation can cause seed to germinate prior to harvest. Preharvest sprouting degrades starch and protein reserves in mature grains through a complex sequence of events (Derera, 1982). Sprouting leads to excessive α -amylase activity and occurs when wet conditions occur prior to harvesting (Derera, *et al.*, 1977). This enzymic presence is associated with low FNs and collapsed loaves (Ibrahim & D'Appolonia, 1979; Derera, 1989). In South Africa, areas prone to preharvest sprouting are the Southern Cape (Marais & Kruis, 1983), North Eastern Free State and Kwazulu-Natal. The highest risk occurs in the winter wheat region where the probability of summer rainfall over the harvest period is the highest.

Preharvest sprouting is inhibited by the inherent failure of viable embryos to germinate when subjected to favourable conditions of temperature, moisture and oxygen (DePauw & McCaig, 1991). This tendency, called dormancy, is responsible for 49 to 64% of the variance which occurs in preharvest sprouting (Strand, 1983).

The level and duration of dormancy are known to be affected by environmental factors such as temperature during seed development (Miyamoto & Everson, 1958). Cool conditions are associated with high levels of dormancy, whereas warmer temperatures have an inverse effect (Walker-Simmons & Sesing, 1990). Other factors that affect dormancy include rainfall, humidity, evapotranspiration and radiation just prior to harvest (Belderok, 1968).

Considering the environmental impact on dormancy, the assessment of resistance to preharvest sprouting of breeding lines may be influenced by growth environment. Very little, however, is known about either the magnitude or the nature of the genotype by environment interaction for preharvest sprouting found in South Africa (Marais & Kruis, 1983).

This study was therefore undertaken to assess the level of resistance of South African winter and intermediate wheat cultivars to preharvest sprouting under a wide range of environmental conditions using non-parametric analyses.

3.3 MATERIALS AND METHODS

3.3.1 Wheat cultivars and trials

Four winter and 13 intermediate wheat cultivars were used in this study. Detail about these cultivars is given in Table 3.1. The cultivars were planted in field trials conducted at Bethlehem and Clocolan during 1996 and 1997. An additional trial was planted during 1998 at Bethlehem. A randomized block design with four replicates were used. Each plot consisted of five rows with an intra-row spacing of 45 cm. A description of the planting dates and climate during grain filling is given in Table 3.2. Weather data during grain filling (rainfall, humidity, maximum temperatures) were collected from neighbouring meteorological stations.

Fifty spikes per cultivar were individually tagged at anthesis. Anthesis was regarded as the stage where 50% of the spikes reached anther extrusion. When the spikes reached harvest maturity ($< 15\%$ moisture content), intact spikes were hand-harvested. The peduncles were cut 10 cm below the base of the spike. These spikes were placed in a chest freezer (-20°C) to maintain seed dormancy at harvest-time levels (Noll & Czarnecki, 1980).

3.3.2 Simulation of sprouting conditions and assessment of tolerance

Within two months after sampling, the sprouting response of intact spikes to a uniform wetting treatment was determined in a rain simulator (McMaster & Derera, 1976). According to this technique, 10 erect spikes were placed on perforated trays. Thereafter a misty spray was applied overhead, while the trays rotated at a uniform speed. After a 72 hour period, during which day/night temperatures of $25/15^{\circ}\text{C}$

(RH = 98%) were maintained, the spikes were evaluated on a revised method of McMaster & Derera (1976) on a scale from 1 (no visible sprouting) to 8 (fully sprouted).

Table 3.1 Summary of the cultivars used to screen for preharvest sprouting

Cultivar	Release date	Growth Habit and Type	Origin
Betta-DN	1993	Intermediate pureline	Small Grain Institute
Elands	1998	Intermediate pureline	Small Grain Institute
Gariep	1994	Intermediate pureline	Small Grain Institute
Tugela-DN	1992	Intermediate pureline	Small Grain Institute
Karee	1982	Intermediate pureline	Small Grain Institute
Limpopo	1994	Intermediate pureline	Small Grain Institute
Caledon	1996	Intermediate pureline	Small Grain Institute
Carina	1985	Winter Hybrid	Former Carnia
Carol	1986	Winter Hybrid	Former Carnia
Caritha	1986	Winter Hybrid	Former Carnia
Hugenoot	1989	Intermediate pureline	Monsanto
SST 124	1987	Intermediate pureline	Monsanto
SST 363	1996	Intermediate pureline	Monsanto
SST 367	1996	Intermediate pureline	Monsanto
SST 936	1993	Winter hybrid	Monsanto
PAN 3211	1993	Intermediate pureline	Pannar
PAN 3235	1995	Intermediate pureline	Pannar

Table 3.2 Description of the nine growth environments

Env no.	Year	Location	Planting date	Julian day		Climate during grain filling ^a		
				Plant	Anthesis ^b	Rain (mm)	Max T (°C)	Hum (%)
1	1996	Bethlehem	1996-05-20	140	296	75.3	21.4	78.7
2	1996	Bethlehem	1996-06-03	154	299	72.2	22.5	76.1
3	1996	Bethlehem	1996-07-20	201	321	53.8	24.9	75.0
4	1996	Clocolan	1996-06-11	162	301	61.5	24.2	67.3
5	1997	Bethlehem	1997-06-09	160	302	53.3	22.9	67.6
6	1997	Bethlehem	1997-07-03	187	306	57.0	24.2	71.8
7	1997	Bethlehem	1997-07-17	198	316	36.5	25.4	73.5
8	1997	Clocolan	1997-07-23	204	325	44.3	25.6	72.4
9	1998	Bethlehem	1998-06-11	162	285	160.2	23.5	68.5

Env = Environment

^aRain = Total precipitation during grain filling

Max = Average maximum temperature during grain filling

Hum = Average maximum humidity during grain filling

^bAnthesis = Average anthesis Julian day

3.3.3 Statistical analyses

Each trial was considered as a separate environment, amounting to a total of nine environments representative of the range normally found in the eastern Free State.

Canonical variate analysis (CVA) was first performed on the sprouting scale and the environmental factors (temperature, humidity and rainfall) as it was of more interest to show differences between cultivars than between environments. The variability in a large number of variables was firstly reduced to a smaller set of variables which accounted for most of the variability. The new set of variables, called canonical variates, are linear combinations of the original measurements, and are thus given as vectors of loadings for the original measurements. With this approach, a set of directions are obtained in such a way that the ratio of between-group variability to within-group variability in each direction is maximised. In this study two groupings were considered from available data, namely climatic groups and planting date.

The AMMI statistical model combines the additive main effects of the analysis of variance (ANOVA) with the interaction effects of principal components analysis (PCA), and it is very useful for the exploratory analysis of two-way data, such as genotype by environment data. It applies the additive ANOVA model to the two-way data averaged over the replicates and then performs a PCA on the residuals obtained from the ANOVA (Zobel *et al.*, 1988; Smith, 1991; Annicchiarico, 1992; Gauch, 1992). This combination of the two methods is more powerful than each of these methods performed individually, since the ANOVA, as an additive model, describes only main effects and provides no insight into the particular patterns of the interaction.

After the AMMI analysis, a hierarchical classification (HC) analysis, using the average linkage method, was performed on the AMMI estimates to group the cultivars over environments into groups which were most similar with respect to sprouting. It was found by Crossa *et al.* (1991) and Smith & Gauch (1992) that clustering of the AMMI estimates resulted in more cohesive groups.

The statistical analyses were performed using the GENSTAT 5 statistical programme (GENSTAT 5 Committee, 1993).

3.4 RESULTS AND DISCUSSION

3.4.1 Canonical variate analysis

The first two canonical variates accounted for 81% of the total variation among cultivars. The plot of the second *versus* the first canonical variate means is shown in Figure 3.1. In such a plot, points close together are similar and points further apart are dissimilar with respect to the variates that discriminate between them. This plot indicates three distinct groupings of the cultivars. Betta-DN, Elands, Limpopo,

Caledon and PAN 3235 had the least sprouting, while Tugela-DN, Carol, Carina and Caritha had the highest sprouting score (Table 3.3).

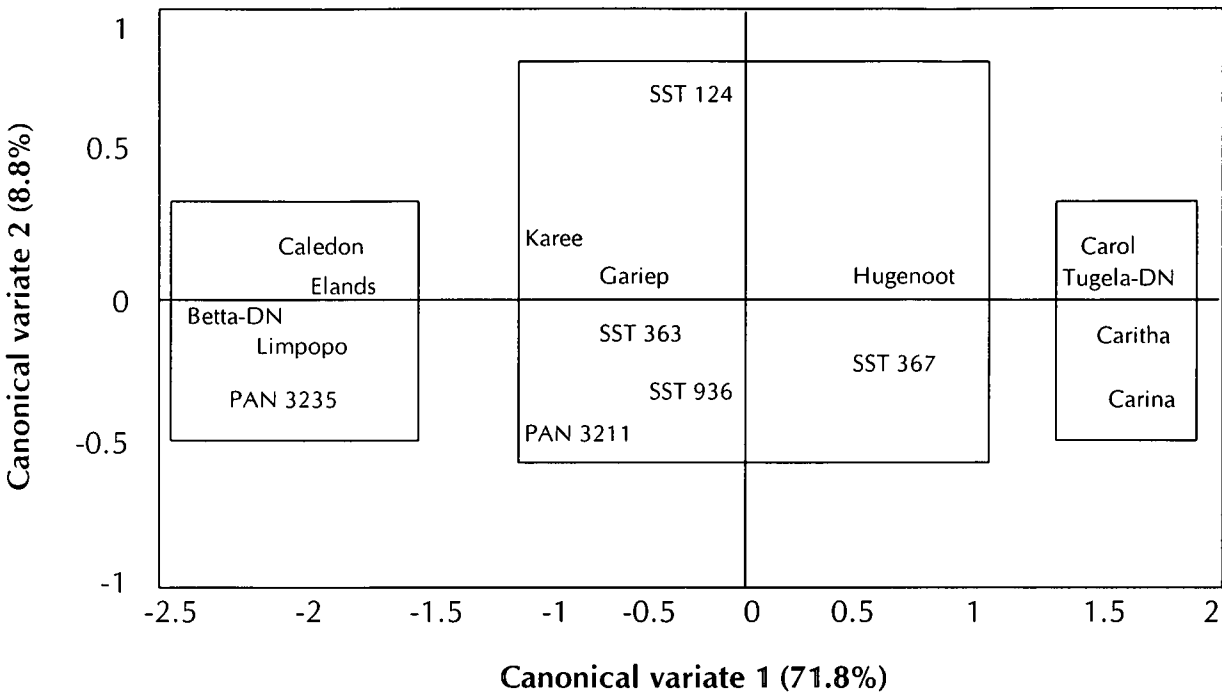


Figure 3.1 Plot of the first two canonical variates to show the interrelationships of preharvest sprouting responses of 17 cultivars grown in the winter wheat region.

The scores found for each of the canonical variates were then correlated with the original variates to find those that were the most important in discriminating between the cultivars. In this case only sprouting correlated highly ($r=0.92$) with the first canonical variate scores. The other variates were uncorrelated (correlation coefficients (r) ranging from 0.04 to 0.28). It could therefore be suggested to rather use the AMMI model for grouping as it removes the main effects of cultivars and environments, before a multivariate analysis of the interaction effects is performed.

Table 3.3 The sprouting response of 17 winter and intermediate wheat cultivars commonly grown in the Free State over nine environments. A scale from 1 (no visible sprouting) to 8 (fully sprouted) was used

Cultivar	Preharvest sprouting response									
	Environment									Average
	1	2	3	4	5	6	7	8	9	
Betta-DN	2.3	1.0	1.7	2.6	1.0	1.4	1.7	1.2	1.0	1.5
Elands	a	a	a	a	1.0	1.0	1.3	1.2	1.0	1.1
Gariep	3.1	3.4	4.1	3.8	3.9	3.2	3.1	3.8	3.5	3.5
Tugela-DN	7.6	6.5	7.4	6.9	7.5	7.2	7.1	7.5	6.9	7.2
Karee	a	1.2	3.5	a	a	3.0	1.2	1.0	2.9	2.1
Limpopo	2.1	2.6	3.1	2.6	3.2	2.4	1.6	1.9	1.6	2.3
Caledon	1.1	1.2	1.1	2.7	1.4	2.5	2.7	1.1	2.8	1.8
Carina	6.3	5.3	a	6.1	7.0	6.9	a	6.1	6.7	6.3
Carol	6.2	7.0	a	6.9	7.3	a	a	6.6	7.6	6.9
Caritha	7.3	7.0	7.4	6.5	6.5	6.7	7.2	7.6	6.7	7.0
Hugenoot	3.7	2.2	4.9	4.7	6.1	4.8	a	5.6	5.9	4.7
SST 124	a	6.5	4.1	a	a	2.8	4.0	1.9	4.3	3.9
SST 363	a	3.8	3.7	a	a	4.2	2.6	3.4	3.6	3.6
SST 367	4.3	2.0	3.8	4.6	3.9	5.2	3.7	6.3	4.5	4.3
SST 936	4.1	1.9	3.0	6.2	1.4	4.3	a	5.6	3.5	3.8
PAN 3211	5.3	2.3	1.9	a	a	5.4	2.6	2.1	6.0	3.7
PAN 3235	1.4	1.2	a	a	a	2.4	2.3	2.7	2.2	2.0
Average	4.2	3.4	3.8	4.9	4.2	4.0	3.2	3.9	4.2	3.9

a Not included in the trial

3.4.2 AMMI model

The whole principle behind the AMMI model is to summarise the genotype by environment interaction by means of a small number of 'components'. In this study it was found that only a few components were needed to explain the interaction, but as the biplot of the genotype and environment scores *versus* the means accounted for

77% of the treatment variation, it was restricted to one, as the first is always the most important component.

In the standard ANOVA procedure, a series of experiments having G genotypes in E environments would result in an interaction sum of squares (SS) with $(G-1)(E-1)$ degrees of freedom, which could be problematic to interpret. Not only is the interpretation complex, but it is also difficult to predict the responses in the different environments.

With the AMMI model, each environment is characterised with an environment 'score'. Likewise the genotype performance is characterised for each component with a genotype score. The weighted sum of products of such scores enables one to predict, via the model, how cultivars would respond in their differing environments.

Although the assumption of homogeneity of the nine environmental error variances had to be rejected, the AMMI analysis of the sprouting data was performed on the original data, as a weighted ANOVA performed prior to the AMMI analysis indicated a true significant interaction ($P < 0.001$) by the MATMODEL Version 2.0 program (Gauch, 1990). The analysis of variance for the AMMI1 model using four replicates is presented in Table 3.4. The partitioning of the treatment SS into cultivar, environment and interaction are 63%, 3% and 32% of the TRT SS, respectively. Contrary to yield where the environment SS is usually more than 90%, the cultivars accounted for 63% of the total TRT SS. Genotypic variation appeared thus to be dominant with respect to sprouting. The first interaction PCA component alone captures 29% of the interaction SS in only 18% of the interaction degrees of freedom (df). The AMMI1 model thus partitions the TRT SS and df into a model which contains 77% of the treatment SS and a discarded residual of 23%. It is this discarding (of noise in the data), as a result of the multiplicative partitioning of the interaction, that causes AMMI estimates to differ from treatment means. The ratio of genotype to genotype by environment effects indicated that cultivars differed regarding responsiveness to environmental effects.

Table 3.4 AMMI analysis of variance for 17 cultivars grown over nine environments

Source ^a of variation	df	SS	MS
Total	583	3387	5.8
Trt	152	3328	21.9***
E	8	112	14.0***
C	16	2148	134.2***
E x C	128	1069	8.4***
IPCA1	23	307	13.4***
Residual	105	762	7.3***
Error	438	59	0.1

*** Significant at P = 0.01

CV for Trt means is 3.9% of the grand mean

^a E = environment, C = cultivar, E x C = Interaction of environment by cultivar,

The cultivar means and first interaction PCA scores of the 17 cultivars are presented in Table 3.5 and those for environments in Table 3.6. The most stable cultivars, or least sensitive to environmental factors for low sprouting, were Betta-DN and Elands as their scores were virtually zero, whereas the most sensitive was SST 936 with a score of 2.2. A biplot of these cultivar scores *versus* the cultivar means, as well as the environment scores *versus* the environment means, indicates the main effects (cultivar and environment mean sprouting) on the horizontal axis and the interaction effects on the vertical axis (Figure 3.2). The hierarchical classification (HC) analysis at level 80.0 identified three groups (indicated on the biplot of Figure 3.2). It is interesting to note that the groups identified by the CVA plot in Figure 3.1 were very similar to those found by the AMMI biplot (Figure 3.2) and the subsequent HC of the AMMI estimates over environments.

Environments 3, 5 and 6 were the most similar in sprouting response, while environments 7 and 9 were totally different from the others. Environment 7 had the lowest rainfall, while environment 9 had a very high rainfall during the grain filling period, and the Julian anthesis date was also much earlier than for the other environments, as indicated in Table 3.2.

Table 3.5 The cultivar means and first interaction PCA scores (IPCA 1) of the 17 cultivars used in this study

Cultivars	Sprouting scale means	IPCA1 score
Tugela-DN	7.2	-0.149
Caritha	7.0	0.027
Carol	6.9	-0.310
Carina	6.3	-0.235
Hugenoot	4.7	-1.024
SST 367	4.1	0.958
SST 124	3.9	1.366
SST 936	3.8	2.146
PAN 3211	3.7	-1.398
SST 363	3.6	0.206
Gariep	3.5	0.793
Limpopo	2.3	0.598
Karee	2.1	-0.814
PAN 3235	2.0	-0.415
Caledon	1.8	0.335
Betta-DN	1.5	0.018
Elands	1.1	-0.011

Table 3.6 The environment means and first interaction PCA scores (IPCA 1) of the nine environments used in this study, ranked according to IPCA scores

Environments	Sprouting scale means	IPCA1 score
1	4.0	0.969
2	4.2	0.817
3	3.8	0.749
4	4.2	0.061
5	3.9	-0.341
6	3.4	-0.537
7	4.9	-0.820
8	4.2	-1.968
9	3.2	-2.014

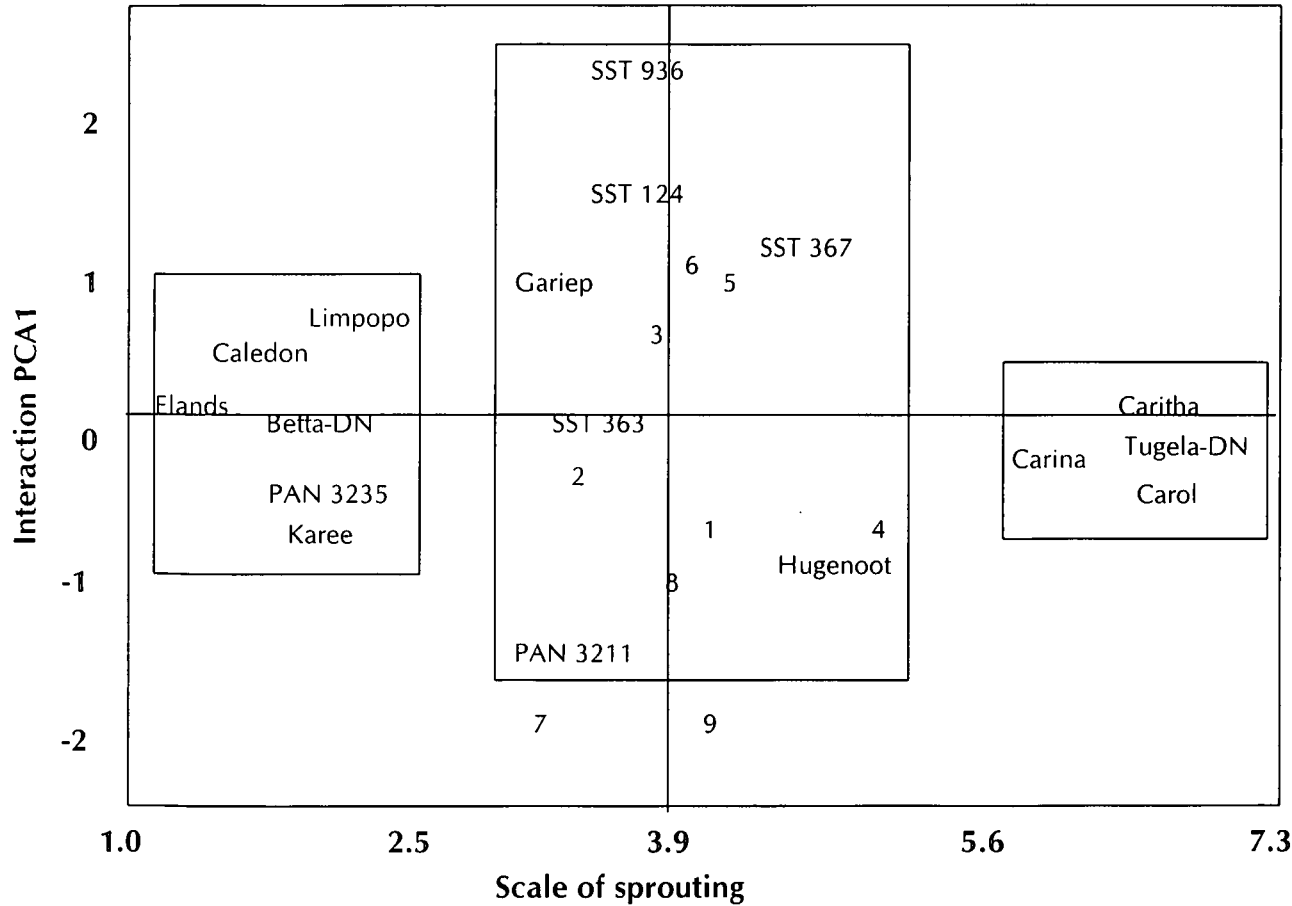


Figure 3.2 Biplot of the AMMI interaction PCA component scores *versus* the scale of sprouting for cultivars and environments (1 - 9).

3.5 CONCLUSIONS

On the basis of the findings of the canonical variate analysis, the AMMI analysis and the clustering of groups by means of hierarchical analysis, cultivar tendencies towards preharvest sprouting were arranged into three distinct groups: group I (minimal, 1.0 – 2.9), group II (moderate, 3.0 – 4.5) and group III (high, 4.6 – 8.0 sprouted). It is recommended that the AMMI model is rather be used for the evaluation and classification of cultivars, since the AMMI model, other than canonical variate analysis, makes use of a total study of the genotype by environment interaction. Betta-DN and certain cultivars derived from Betta-DN, such as Limpopo and Karee, showed good resistance to preharvest sprouting. However, other Betta-types such as Hugenoet showed only moderate resistance to preharvest sprouting. The hybrids Caritha, Carina and Carol, as well as the pureline Tugela-DN, tended to be distinctly susceptible to preharvest sprouting. Genotypic variation appeared to be dominant, indicating that progress in the development of cultivars with sprouting tolerance is feasible. Moreover, the methodology used in this study appeared sufficient in support of the evaluation and classification of breeding lines. It also became evident that the sprouting responses of cultivars were triggered by different environmental factors. The occurrence of extreme climatic conditions had a significant effect on cultivar responsiveness, whereas mild temperature differences during grain filling were secondary.

REFERENCES

- ANNICCHIARICO, P. 1992. Cultivar adaptation and recommendation from alfalfa trials in Northern Italy. *J. Genet. & Breed.* 46, 269-278.
- BELDEROK, B. 1968. Seed dormancy problems in cereals. *Field Crop Abstracts* 21(3), 203-211.
- CROSSA, J., FOX, P.N, PFEIFFER, W.H., RAJARAM, S. & GAUCH, H.G. 1991. AMMI adjustment for statistical analysis of an international wheat yield trial. *Theor. Appl. Genet.* 81, 27-37.
- DEPAUW, R.M. & McCAIG, T.N. 1991. Components of variation, heritabilities and correlations for indices of sprouting tolerance and seed dormancy in *Triticum* spp. *Euphytica*. 52, 221-229.
- DERERA, N.F. 1982. The harmful harvest rain. *J. Aust. Inst. Agr. Sci.* 48, 67-75.
- DERERA, N.F. 1989. The effects of preharvest rain. In: N.F. Derera (ed). *Preharvest field sprouting in cereals*. University of Sydney, Narrabri, N.S.W. Australia. pp 2-14.
- DERERA, N.F., BHATT, G.M. & McMASTER, G.J. 1977. On the problem of preharvest sprouting of wheat. *Euphytica* 26, 299-308.
- GAUCH, H.G. 1990. *MATMODEL Version 2.0: AMMI and Related Analyses for Two-way Data Matrices*. Ithaca, New York: Department of Soil, Crop and Atmospheric Sciences, Cornell University.
- GAUCH, H.G. 1992. *Statistical Analysis of Regional Yield trials: AMMI Analysis of Factorial Designs*. Amsterdam: Elsevier.
- GENSTAT 5 Committee. 1993. Statistics Dept. Rothamsted experimental station. Oxford: Clarendon Press.
- IBRAHIM, Y. & D'APPOLONIA, B.L. 1979. Sprouting of hard red spring wheat. *Bakers Dig.* 53(5), 17-23.
- MARAIS, G.F. & KRUIS, W.J.G. 1983. Preharvest sprouting - The South African Situation for seed dormancy in barley. In: J.E. Kruger and D.E. LaBerge (eds). *Third International Symposium on Preharvest Sprouting in Cereals*. Westview Press, Boulder, Colorado. pp 267-273.

- McMASTER, G.J. & DERERA, N.F. 1976. Methodology and sample preparation when screening for sprouting damage in cereals. *Cereal Res. Commun.* 4(2), 251-254.
- MIYAMOTO, T. & EVERSON, E.H. 1958. Biochemical and physiological studies of wheat seed pigmentation. *Agron. J.* 50, 733-738.
- NOLL, J.S. & CZARNECKI, E. 1980. Methods for extending the testing period for harvest-time dormancy in wheat. *Cereal Res. Commun.* 8, 233-238.
- SMITH, M.F. 1991. The AMMI model in Agriculture: A comparison with standard procedures. Unpublished M.Sc. dissertation. University of South Africa.
- SMITH, M.F. & GAUCH, H.G. 1992. Effects of noise on AMMI and Hierarchical classification analyses. *South African Statist. J.* 26, 121-142.
- STRAND, E. 1983. Effects of temperature and rainfall on seed dormancy of small grain cultivars. In: J.E. Kruger and D.E. LaBerge (eds). *Third International Symposium on Preharvest Sprouting in Cereals*. Westview Press, Boulder, Colorado. pp 260-266.
- WALKER-SIMMONS, M. & SESING, J. 1990. Temperature effects on embryonic abscisic acid levels during development of wheat grain dormancy. *J. Plant Growth Regul.* 9, 51-56.
- ZOBEL, R.W., WRIGHT, M.J. & GAUCH, H.G. 1988. Statistical analysis of a yield trial. *Agron. J.* 80, 388-393.

CHAPTER 4

THE POSSIBLE ROLE OF THE OXIDATIVE PENTOSE PHOSPHATE PATHWAY IN SEED DORMANCY IN TWO WINTER WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS

4.1 ABSTRACT

Seed dormancy is an important characteristic in small grain cultivars, because of its potential to prevent preharvest sprouting and the subsequent loss of quality. In this study the oxidative pentose pathway (OPP) activity in seeds of a preharvest sprouting susceptible winter wheat cultivar, Tugela-DN and that of a preharvest sprouting resistant cultivar, Betta-DN, were compared. Carbon flux through the OPP pathway was determined both indirectly (total *in vitro* activity of the first regulatory enzyme, glucose-6-phosphate dehydrogenase (G6PDH) and directly ($^{14}\text{CO}_2$ evolution from metabolized D-[1- ^{14}C]- and D-[6- ^{14}C]-glucose, including C6/C1 ratios). G6PDH activity increased twofold in both the harvest ripe (HR) and after ripened (AR) seeds of the susceptible cultivar during the germination phase (first 24 h of incubation) and threefold during the post germinative phase up to 72 h of incubation. The latter coincided with a high germination percentage. However, the G6PDH activity remained constant in both the HR and AR seeds of the resistant cultivar during the first 24 h of incubation and increased only slightly in the HR seeds over the remaining incubation time. Moreover, a C6/C1 ratio of below unity in seeds of Tugela-DN during the germination phase, confirmed that the OPP pathway was far more active in the preharvest sprouting susceptible cultivar than in the resistant cultivar, Betta-DN, where the C6/C1 ratio remained above unity during the germination phase.

4.2 INTRODUCTION

The quality decrease associated with preharvest sprouting in wheat has received considerable attention over the past 20 years. Probably because of the complexity of the preharvest sprouting phenomenon, this problem has been approached from different perspectives, including factors triggering the onset of preharvest sprouting (Derera *et al.*, 1977; McCrate *et al.*, 1981; Upadhyay & Paulsen, 1988), the effect on FN (Ibrahim & D'Appolonia, 1979), degradation of grain starch and protein reserves (Derera, 1982), seed dormancy (Strand, 1991) and the effect of environmental factors (Barnard *et al.*, 1997). It is surprising that most of the published results deal with causative factors while little is known about the underlying metabolic differences between preharvest sprouting of susceptible and resistant wheat cultivars.

Seed dormancy is defined as the failure of embryos to germinate when subjected to favourable conditions of temperature, moisture and oxygen (Simpson, 1990; DePauw & McCaig, 1991). According to Strand (1983), dormancy is responsible for at least 49 to 64% of the variance which occurs in preharvest sprouting of small grain seeds. Strand (1991) concluded that seed dormancy was an important characteristic in small grain cultivars, because of its potential to prevent preharvest sprouting and the subsequent loss of quality.

Dormancy may have a great variety of causes, e.g. an impervious seed coat in some species, a chemical inhibitor or an immature embryo in others. The same environmental factor may thus have different effects on dormancy in different species, depending on the endogenous mechanism involved (Fenner, 1991). Plant growth regulators (e.g. ABA) is present in relatively high concentrations in developing seeds of many species, and it has been shown to have an important role in regulation seed maturation and germinability (Benech-Arnold *et al.*, 1995). Despite its importance, however, the mechanisms which control dormancy in cereals are still only poorly understood (Mares, 1993).

From results with barley (*Hordeum vulgare*) and oats (*Avena sativa*) seeds, it has been proposed that the difference between dormant and non-dormant seed lies in greater participation of the oxidative pentose phosphate (OPP) pathway in the latter seeds prior to germination (Gallais et al., 2000). The first suggestion that the OPP pathway might be the process involved in the loss of seed dormancy was put forward by Major in 1966 (cited by Roberts & Smith, 1977). Roberts (1969) also proposed that the difference between dormant and non-dormant two-rowed barley (*Hordeum distichum*) seeds lies in greater participation of the OPP pathway in non-dormant seeds prior to germination.

Gordon (1980), as well as Sánchez de Jiménez & Quiroz (1983), suggested that an active OPP pathway is necessary during the early stages of germination and that this pathway is less operative when dormancy prevails. Gahan et al. (1986) reported that the changes in the activity of the OPP pathway enzymes during the processes leading to radicle emergence of *Avena fatua*, are due to OPP pathway activity. Similarly Gallais et al. (2000) reported that the dormancy experienced in seeds of *Avena sativa* is due to the low activity of the OPP pathway in the embryo. Pretorius & Small (1992) indicated that the lack of OPP pathway activity is part of the mechanism leading to a state of secondary dormancy and subsequent loss of germinative capacity in soak-injured bean seeds.

In order to explain the differences between preharvest sprouting susceptible and preharvest sprouting resistant cultivars, some of the mechanisms regulating dormancy was investigated. In this study the OPP pathway activity in seeds of a preharvest sprouting susceptible cultivar, Tugela-DN, was compared to that of a preharvest sprouting resistant cultivar, Betta-DN. Considering the environmental impact on dormancy, the assessment of resistance to preharvest sprouting by cultivars may be influenced by the growth environment. However, Tugela-DN and Betta-DN showed the least sensitivity to environmental factors which influenced preharvest sprouting over a four year period (Barnard et al., 1997) and were therefore chosen for this comparative study.

The aim was to use differences in G6PDH activities, metabolism of D-[1-¹⁴C]- and D-[6-¹⁴C]-glucose, as well as C6/C1 ratios, which occurred between the susceptible and resistant cultivars as direct and indirect indicators to ascertain whether OPP pathway activities occurred. The plastidic and cytosolic isoenzymes of both G6PDH and 6PGDH were additionally identified by using green and etiolated leaves with the aim of comparing the isoenzyme profiles of the preharvest sprouting susceptible and resistant cultivars for these two regulatory enzymes of the OPP pathway.

4.3 MATERIALS AND METHODS

4.3.1 Materials

Wheat seeds (*Triticum aestivum* cv. Tugela-DN and Betta-DN) were harvested at physiological ripeness and stored at -20°C before use. Physiological ripeness was regarded as the stage when the plant discoloured to beneath approximately 10 cm of the ear. These seeds are referred to as harvest ripe seeds (HR). Some seeds of both cultivars were set aside to after ripen in dry storage at room temperature for 12 months prior to this study. These seeds were 100% viable and non-dormant and are referred to as after ripened seeds (AR).

Biochemicals for enzyme assays, PMSF (phenylmethyl-sulphonyl fluoride), ACA (ϵ -amino-*n*-caproic acid), BHC (benzamidine hydrochloride) and DEAE Sephacel resin were from Sigma Chemical Company, St Louis, USA; Panacide from British Drug House Chemicals and radioactively labeled glucose from Amersham, UK. All other chemicals used were of the highest purity available.

4.3.2 Seed germination

Seeds (HR, as well as AR) were surface-sterilized in 0.5% (w/v) aqueous Panacide solution for 5 min, followed by thorough rinsing with distilled water. Seeds in batches of 25 were incubated in 90 mm petri dishes on a single layer of Whatman nr

1 filter paper, moistened with 5 ml water at 20°C for 0, 2, 4, 8, 16, 20, 24, 48 and 72 h. The petri dishes were sealed with parafilm to retain moisture.

4.3.3 Assay of Glucose-6-Phosphate Dehydrogenase (G6PDH)

G6PDH activity was assayed at 0, 24, 48 and 72 h of incubation, according to the method of Pretorius and Small (1992). Seeds were frozen in liquid nitrogen and homogenized by hand in 4 ml g⁻¹ ice-cold extraction buffer (0.1 M Tris-HCl, pH 7.5, 2 mM EDTA, 10 mM 2-mercaptoethanol, 10% (v/v) glycerol). The homogenate was centrifuged (2°C) at 12000 rpm for 10 min. G6PDH activity was determined in the supernatant following NADP⁺ reduction as a function of time at 340 nm. An aliquot (20 µl) of enzyme extract was added to a reaction cuvette which contained, in a final volume of 1 ml, 50 mM Tris-HCl, pH 7.5, 0.1 mM NADP, 10 mM MgCl₂, 2.5 mM glucose-6-phosphate and 0.044 U commercial 6PGDH.

4.3.4 Metabolism of radioactive glucose

A modified method of Nicolas & Aldasoro (1979) was used for the trapping and measurement of ¹⁴CO₂ evolved from metabolized [1-¹⁴C]- and [6-¹⁴C]-glucose, from which C6/C1 ratios were calculated. Five seeds, incubated separately for 0, 2, 4, 8, 12, 24 or 72 h, were placed in 250 µl distilled water, containing 2 µCi [1-¹⁴C]-glucose (specific activity 55,0 mCi mmol⁻¹) or [6-¹⁴C]-glucose (specific activity 56.0 mCi mmol⁻¹) in a Warburg vessel with 0.3 ml 12% KOH in the centre well. In addition, 0.4 µg ml⁻¹ streptomycin was added to the incubation medium. A pulse labeling time of 120 minutes was chosen as the optimal incubation time for all experiments.

At the end of the incubation period, 20 µl KOH was immediately withdrawn from the centre well and the dissolved ¹⁴CO₂ was trapped in 100 µl of a saturated Ba(OH)₂ solution in a scintillation vial. The latter was dissolved in 4 ml Instagel II scintillation cocktail. In this way the potential of seeds to metabolise both isotopes was measured

during the germination phase (0 – 24h) as well as during the post germinative phase (24 – 72h).

4.3.5 Separation of isoenzymes by ion-exchange chromatography

For obtaining leaf tissue and to identify the cytosolic and plastidic isoenzymes, wheat seedlings were grown at 25°C in the light or dark for 4 weeks. Crude extracts were prepared as described for the extraction of G6PDH. However, the extraction buffer contained in addition 0.1% (w/v) PVP (polyvinylpolypyrrolidone-Polyclar AT) to prevent phenolic action, and PMSF, ACA and BHC each at 1 mM to prevent protease activity.

Plastic syringes (10 ml) were used as ion-exchange columns by packing with DEAE Sephacel resin and equilibrating with 4 bed volumes of 100 mM Tris-HCl, pH 8.0, containing 10 mM 2-mercaptoethanol and 10% (v/v) glycerol. After application of the extracts, the columns were washed with two bed volumes of equilibration buffer. The isoenzymes of G6PDH (EC 1.1.1.49) and 6-PGDH (EC 1.1.1.44) were eluted in a linear gradient of KCl (0-0.5M) in equilibration buffer containing 25 μ M NADP and 600 μ M MgCl₂. The percentage recovery of both enzymes was higher than 80%.

4.3.6 Statistical analyses

Treatments and experiments were repeated three times and the standard errors (SE) of means were calculated.

4.4 RESULTS AND DISCUSSION

4.4.1 Seed germination and G6PDH activities

More than 60% of the Tugela-DN (HR and AR) and Betta-DN (AR) seeds germinated during the first 24 h of incubation at 20°C, while less than 10% Betta-DN (HR) seeds

germinated in the same period. The final germination percentage for the former three seed types was higher than 80% over a 72 h incubation period, while that of Betta-DN (HR) was less than 15% (Figure 4.1).

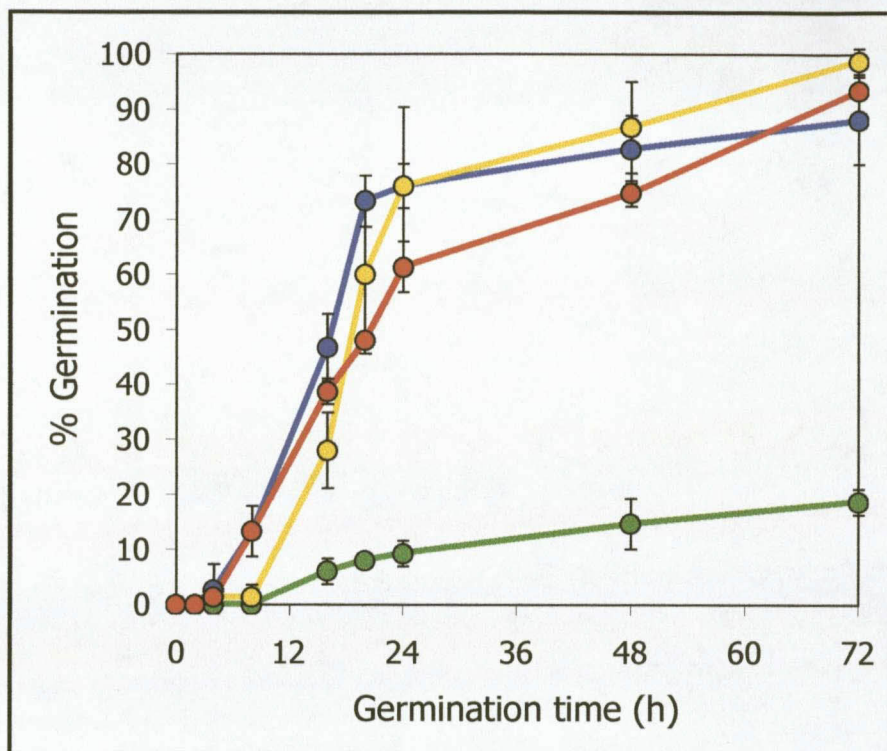


Figure 4.1 Percentage germination of Betta-DN *HR* (—●—), Betta-DN *AR* (—●—), Tugela-DN *HR* (—●—) and Tugela-DN *AR* (—●—) seeds over a 72 h incubation period. Seeds where the radicle protruded the testa were regarded as germinated.

A similar amount of G6PDH activity was present in both the dry HR and AR seeds of the two cultivars (Figure 4.2). During the germination phase over the first 24 h of incubation the G6PDH activity increased significantly (32%) in Tugela-DN (HR and AR) and in Betta-DN (AR) (27%) seeds, while remaining unchanged in Betta-DN (HR) seeds. During the post germinative phase the increase in G6PDH activity was threefold in Tugela-DN (HR and AR) as well as Betta-DN (AR) seeds over 72 h of incubation while remaining at a much lower level in seeds of the resistant cultivar, Betta-DN (HR). The

total G6PDH activity remained constant in the latter seeds between 48 h and 72 h of incubation. The increase in G6PDH activity (Figure 4.2) in Tugela-DN (HR and AR), as well as in Betta-DN (AR) seeds coincided with the high germination percentage (Figure 4.1) measured over the first 24 h of incubation. On the other hand, the much lower G6PDH activity in Betta-DN (HR) seeds coincided with the much lower germination percentage measured during the germination phase.

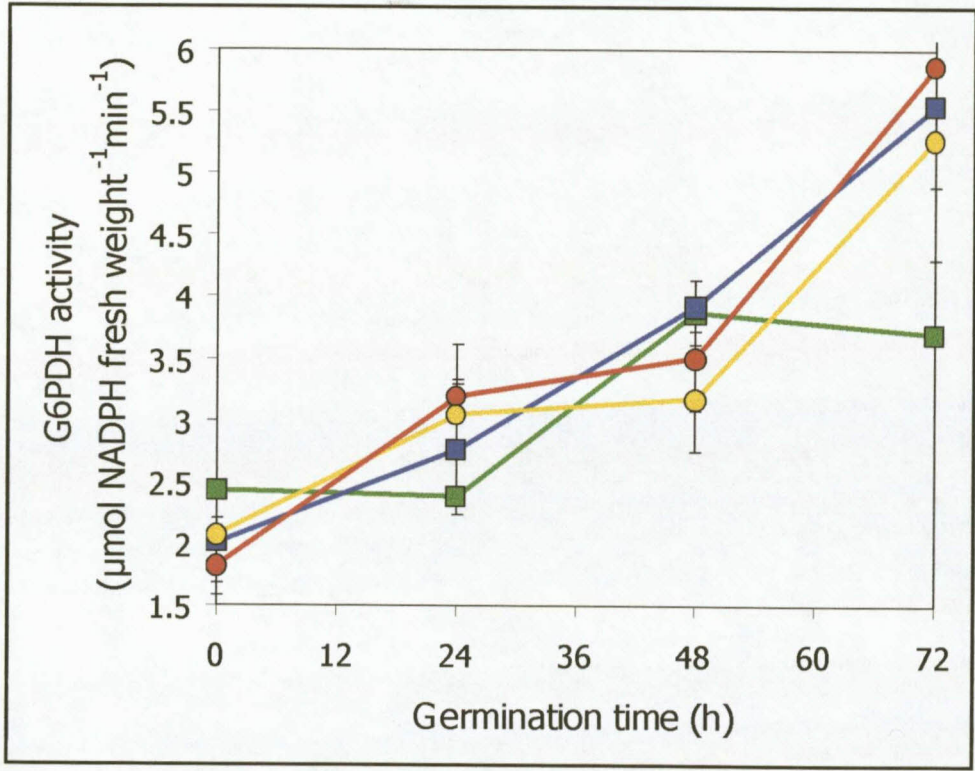


Figure 4.2 Glucose-6-phosphate-dehydrogenase activity in Betta-DN *HR* (—●—), Betta-DN *AR* (—●—), Tugela-DN *HR* (—●—) and Tugela-DN *AR* (—●—) seeds over a 72 h germination period.

4.4.2 Metabolism of radioactive glucose

Because of the differences in the magnitude of isotope uptake by plant tissue, it is not feasible to make a direct comparison of absolute $^{14}\text{CO}_2$ release when considering

metabolic rates. However, by plotting the $^{14}\text{CO}_2$ output as a percentage of the absorbed isotope (radioactivity) against labeling time, the slope of the obtained straight line provides a comparable measure of the rate at which isotopes were metabolised by the tissue (Pretorius & Small, 1992).

Evolution of $^{14}\text{CO}_2$ from metabolized $[1-^{14}\text{C}]$ -glucose by seeds of both Betta-DN and Tugela-DN (AR) was four times lower than the HR seeds of both cultivars over the first 12 h of incubation. However, between 12 and 24 h, when more than 60% of the preharvest sprouting susceptible cultivar's seeds had germinated, the $^{14}\text{CO}_2$ release from metabolised $[1-^{14}\text{C}]$ -glucose increased in both cultivars (AR). The $^{14}\text{CO}_2$ evolution by seeds of Tugela-DN (HR and AR) and Betta-DN (AR) showed a threefold increase between 24 h and 72 h of germination (Figure 4.3A). In HR seeds of the preharvest sprouting resistant cultivar Betta-DN, the rate at which the $^{14}\text{CO}_2$ was released from $[1-^{14}\text{C}]$ -glucose remained unchanged during the first 24 h (germination phase) and up to 72 h of incubation (post germination phase).

The release of $^{14}\text{CO}_2$ from metabolized $[6-^{14}\text{C}]$ -glucose by all the different seeds of both the resistant and susceptible wheat cultivars, over the first 12 h of incubation, was almost two times lower than from $[1-^{14}\text{C}]$ -glucose (Figure 4.3B). Moreover, the rate of $^{14}\text{CO}_2$ release from metabolized $[6-^{14}\text{C}]$ -glucose over the following incubation period and up to 72 h of incubation was very similar in the seeds of both cultivars.

The calculated C6/C1 ratios (Figure 4.4) differed significantly for the two treatments of both cultivars during the germination phase (first 24 h of incubation), remaining below unity for Tugela-DN (HR and AR) and Betta-DN (AR) seeds, indicating an active OPP pathway. However, a C6/C1 ratio close to or above unity in Betta-DN (HR) seeds at 24 h and 72 h, showed that the OPP pathway was largely inactive at a longer incubation period.

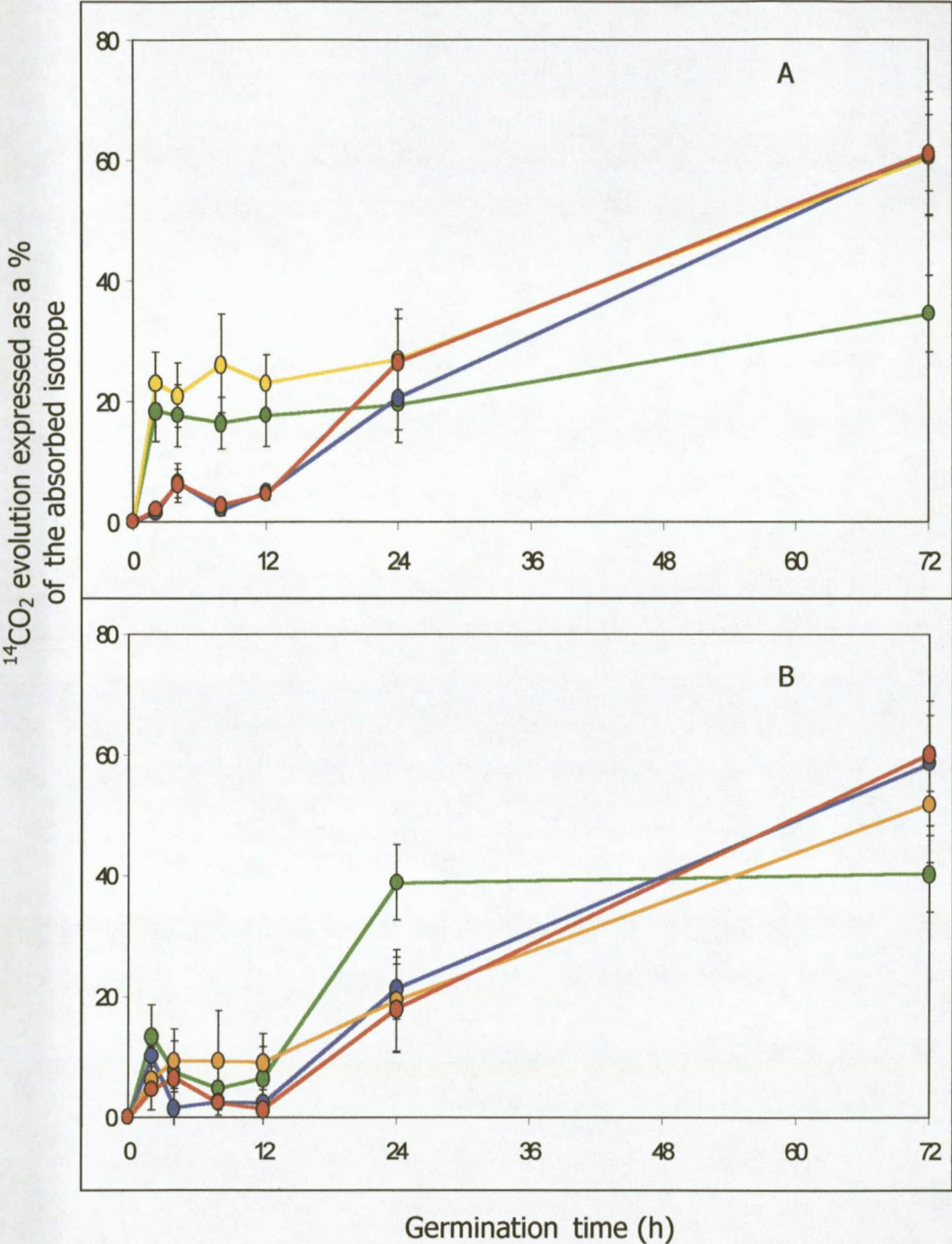


Figure 4.3 Evolution of $^{14}\text{CO}_2$ from metabolized [1- ^{14}C]-glucose (A) and [6- ^{14}C]-glucose (B) by Betta-DN HR (—●—), Betta-DN AR (—●—), Tugela-DN HR (—●—) and Tugela-DN AR (—●—) seeds over a 72 h germination period.

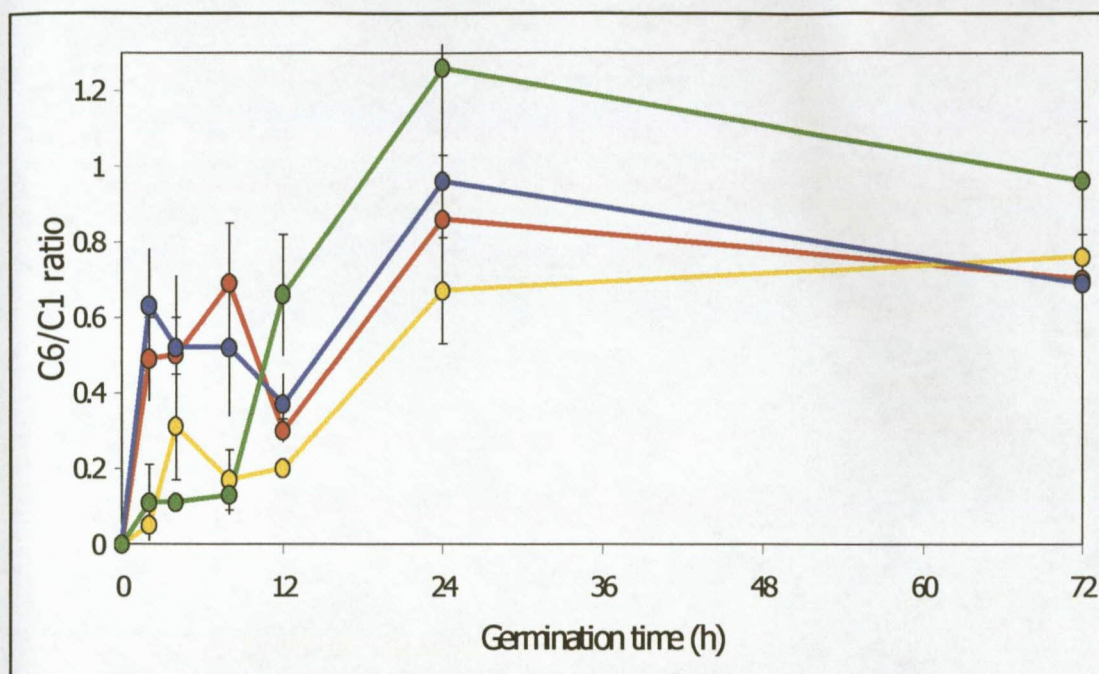


Figure 4.4 C6/C1 ratios calculated as evolved $^{14}\text{CO}_2$ from metabolized D-[1- ^{14}C]-glucose and D-[6- ^{14}C]-glucose by Betta-DN HR (—●—), Betta-DN AR (—●—), Tugela-DN HR (—○—) and Tugela-DN AR (—●—) seeds.

4.4.3 Separation of isoenzymes by ion-exchange chromatography

Plastid and cytosolic isoenzymes of G6PDH (Figure 4.5) and 6PGDH (Figure 4.6) were identified by comparing elution profiles of green and etiolated leaf material obtained by ion-exchange chromatography. The assumption was made that green leaves contained high levels of both cytosolic and plastid isoenzymes whereas etiolated leaves contained primarily cytosolic isoenzymes (Pretorius & Small, 1992).

Three G6PDH peaks were obtained from green leaves of Tugela-DN (Figure 4.5A) whereas only peak 1, albeit smaller, was obtained from etiolated leaves. Since plastids are underdeveloped in etiolated leaves, this indicated that Tugela-DN possesses one

cytosolic isoenzyme (peak 1) and two plastidic isoenzymes (peaks 2 and 3) in the leaf material. Only one coinciding peak was obtained from both green and etiolated leaves of Betta-DN (Figure 4.5B) indicating that only one cytosolic and no plastidic isoenzymes of G6PDH were present in the leaves of this preharvest sprouting resistant cultivar.

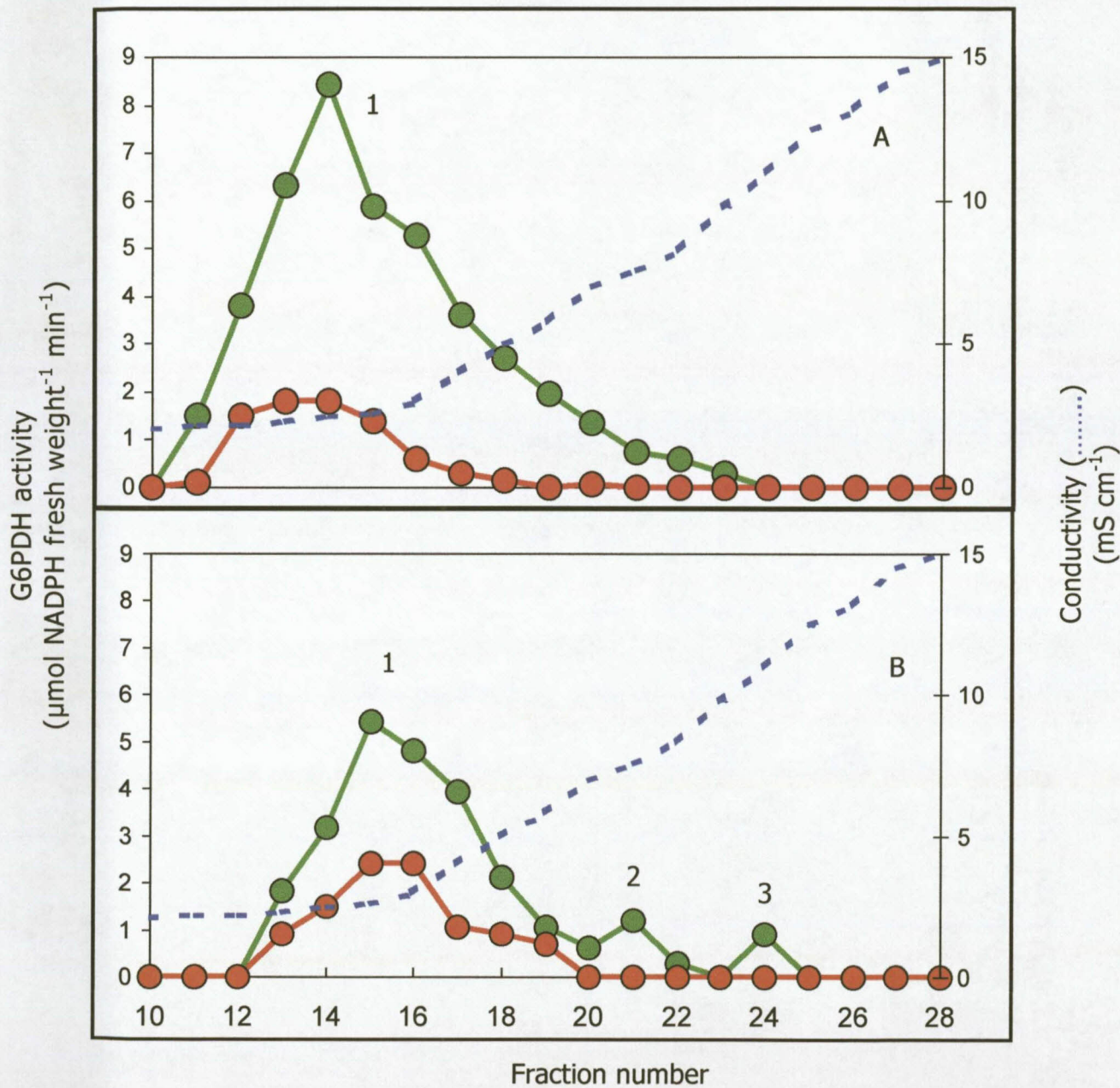


Figure 4.5 Ion-exchange column chromatographic separation of isoenzymes of G6PDH in green (—●—) and etiolated (—●—) leaves of Tugela-DN (A) and Betta-DN (B).

With 6PGDH, three isoenzymes were identified for both Tugela-DN (Figure 4.6A) and Betta-DN (Figure 4.6B). The absence of only peak 3 in etiolated leaves of both cultivars implicated peaks 1 and 2 as two cytosolic isoenzymes and peak 3 as a single plastidic isoenzyme.

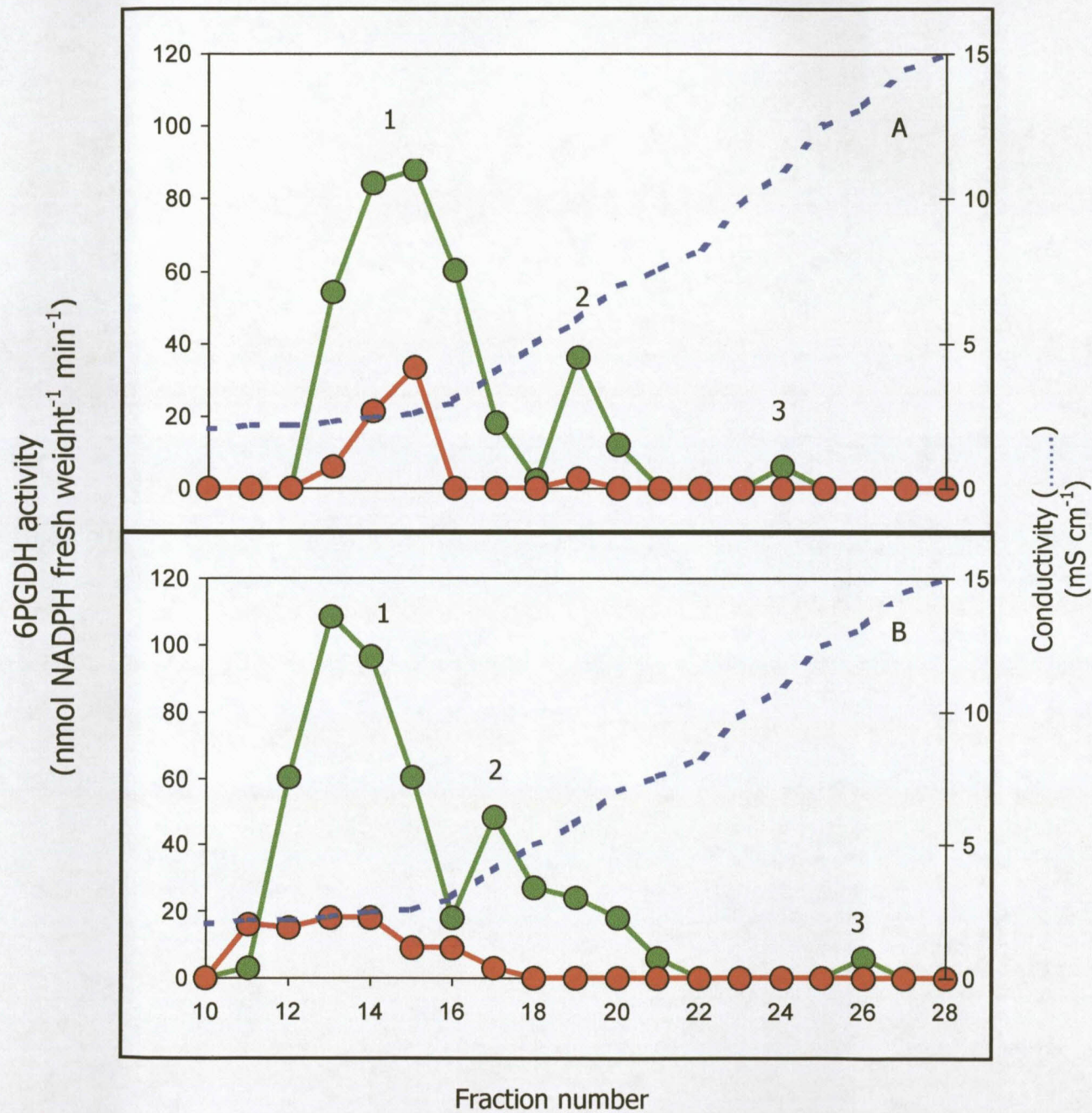


Figure 4.6 Ion-exchange column chromatographic separation of isoenzymes of 6PGDH in green (—●—) and etiolated (—●—) leaves of Tugela-DN (A) and Betta-DN (B).

4.5 CONCLUSIONS

More than two decades ago, it had been suggested that the OPP pathway played an important regulatory role in the germination of certain gramineous seeds (Roberts & Smith, 1977). Moreover, as far back as 1975, the activity of the OPP pathway has *inter alia* been believed to play an important role in the breaking of seed dormancy during early seed germination (Hendricks & Taylorson, 1975 ; Nicolas & Aldasoro, 1979). However, according to Fuerst *et al.* (1983), it seems unlikely that an increase in the activity of the OPP pathway is specifically associated with the loss of dormancy in wild oats. In contrast, Gahan *et al.* (1986) reported that the changes in the activity of the OPP pathway enzymes during the processes leading to radicle emergence of *Avena fatua*, are due to OPP pathway activity. Similarly Gallais *et al.*, (2000) reported that the dormancy experienced in seeds of *Avena sativa* is due to the low activity of the OPP pathway in the embryo.

Although absolute rates of metabolic pathways are difficult to prove (Ap Rees, 1980), the activity of this pathway can, however, be estimated by changes in the activity of G6PDH, which regulates entry of glucose-6-phosphate into the OPP pathway (Bewley & Black, 1978). Since the enzymes controlling this pathway appear to occur in the cytosol (Averill *et al.*, 1998), the regulation of movement of glucose-6-phosphate into and through the pathway is achieved by modulating enzyme levels and the concentration of cofactors. Glucose-6-phosphate, the immediate precursor of the OPP pathway, is generated in the cytosol and imported into the plastids by the plastidic glucose-6-phosphate/phosphate translocator (Debnam & Emes, 1999). In the present study, G6PDH-activity was similar in dry seeds of both the dormant and non-dormant varieties of wheat. Apparently, G6PDH is synthesized and stored during seed development, as was demonstrated for barley endosperm (Duffus & Rosie, 1977), castor beans (Simcox *et al.*, 1979) and wild oats (Upadhyay *et al.*, 1981). However, during the first 24 h of incubation (germination phase), the G6PDH activity increased almost two-fold in the preharvest sprouting susceptible

Tugela-DN seeds, while remaining at the same rate as was measured in dry seeds of the resistant cultivar, Betta-DN (HR).

Roberts & Smith (1977) reported that it did not seem likely that the relatively low activity of the OPP pathway in dormant seeds was due to a lack of dehydrogenase activity. In the present study, G6PDH-activity was similar at the start of imbibition in seeds of dormant and non-dormant varieties of wheat. Moreover, no significant differences in G6PDH activity were observed between the dry seeds of both the susceptible and resistant cultivars and also not during germination over the first 24 h of incubation. If it is considered that more than 60% of the susceptible cultivar's seed already germinated at 24 h compared to the 15% of the resistant cultivar, while no significant differences in G6PDH were observed, the findings of this thesis support the view of Roberts & Smith (1977).

During the post germination phase, especially at 72 h of incubation, the G6PDH-activity in the seeds of the non-dormant preharvest sprouting susceptible cultivar, Tugela-DN, was very similar to that of the after ripened (AR) seeds of the resistant cultivar, Betta-DN, while the activity was markedly lower in the harvest ripe (HR) seeds of Betta-DN. Concomitantly, the final germination percentage determined for Tugela-DN (HR and AR) and Betta-DN (AR) seeds was above 80% while that of the dormant Betta-DN (HR) seeds was below 15% over a 72 h incubation period. It is possible that the difference in G6PDH activity between the dormant and non-dormant preharvest sprouting resistant cultivars, could rather be ascribed to the difference in germination than the cause of it.

However, the distribution of label from metabolized [1- ^{14}C]- and [6- ^{14}C]-glucose, and the resulting C6/C1 ratio calculations, has frequently been used in the past as an indication of OPP pathway activity (Ashihara & Matsumura, 1977; Ap Rees, 1980; Rumpho & Kennedy, 1983). It is believed that increased participation of the OPP pathway decreases the C6/C1 ratio (Nicolas & Aldasoro, 1979) because of $^{14}\text{CO}_2$ being released directly from D-[1- ^{14}C]-glucose during this pathway. Direct evidence

of an increase in activity of the OPP pathway associated with the loss of dormancy has only been reported in two other species besides barley, i.e. *Prunus cerasus* (La Croix & Jarwal, 1967) and *Avena fatua* (Simmonds & Simpson, 1971; Gallais *et al.*, 2000).

In this study, the C6/C1 ratio of above unity (1.26) calculated for Betta-DN (HR) seeds during the germination phase (first 24 h of incubation), strongly indicated that the OPP pathway was inactive in the harvest ripe seeds of this dormant variety. In contrast, a C6/C1 ratio of below unity calculated for the non-dormant cultivar Tugela-DN (0.8) as well as the after ripened (AR) seeds of Betta-DN (0.6) over the same period, indicated that the OPP pathway was active in these seeds. It could therefore be speculated that the OPP pathway may play a role in the loss of dormancy and the subsequent initiation of premature germination of wheat seeds in the ears, especially in preharvest sprouting susceptible cultivars. From this data, it is the difference in OPP pathway activity which distinguished between the preharvest sprouting susceptible (Tugela-DN) and resistant (Betta-DN) cultivars.

Although the mechanism by which the increased participation of the OPP pathway breaks dormancy is still to be determined, it seems that this metabolic pathway is at least a likely candidate. Future studies should probably concentrate on contemplating the *in vivo* activation and/or induction of regulatory enzymes in both the cytosolic and plastidic fractions.

As a preliminary approach it was attempted to identify the isoenzyme forms of the two regulatory enzymes of the OPP pathway, namely G6PDH and 6PGDH (Turner & Turner, 1980), by comparing the enzyme activities in green and etiolated leaves (Pretorius & Small, 1992). In this protocol, the assumption was that undeveloped plastids in etiolated tissue would not contain any iso-forms of these two enzymes. Moreover, according to Brownleader *et al.* (1997), the cytosol of both non-photosynthetic and photosynthetic cells is believed to contain all the enzymes involved in both oxidative and non-oxidative components of the pentose phosphate

pathway. This was also unequivocally shown by Schnarrenberger *et al.* (1995) that chloroplast/cytosol isoenzymes exist for G6PDH and 6PGDH both in green leaf cells and in non-green tissues.

In this study, only one cytosolic isoenzyme of G6PDH was found in leaf material of the resistant cultivar, Betta-DN, but three isoenzymes were recorded for the preharvest sprouting susceptible cultivar, Tugela-DN, with one being cytosolic and two plastidic. For 6PGDH, three isoenzyme forms, of which two cytosolic and one plastidic, were present in the leaf material of both cultivars. Schnarrenberger *et al.* (1995) have shown a predominant localization of the enzyme activities of the pentose phosphate pathway in the chloroplast fraction of spinach leaves, but activity was also found in the cytosolic fraction.

As sufficient material was not available, the isoenzyme composition of G6PDH and 6PGDH in seeds of the susceptible and resistant cultivars could not be studied. A follow up investigation in this regard is envisaged. Future studies should concentrate on contemplating the *in vivo* activation and/or induction of regulatory enzymes in both the cytosolic and plastidic fractions of seeds.

REFERENCES

- ASHIHARA, H. & MATSUMURA, H. 1977. Changes in the activity and function of the pentose phosphate pathway during germination of black gram *Phaseolus mungo* seeds. *International J. of Biochem.* 72, 248-254.
- AP REES, T. 1980. Integration of pathways of synthesis and degradation of hexose phosphates. In: P.K. Stumpf and E.E. Conn (eds). *The Biochemistry of Plants*, Vol. 3. New York, USA, Academic. pp 1-42.
- AVERILL, R.H., BAILEY-SERRES, J. & KRUGER, N.J. 1998. Co-operation between cytosolic and plastidic oxidative pentose phosphate pathways revealed by 6-phosphogluconate dehydrogenase-deficient genotypes of maize. *Plant Journal*. 14 (4), 449-457.
- BARNARD, A., PURCHASE, J.L., SMITH, M.F. & VAN LILL, D. 1997. Determination of the preharvest sprouting resistance of South African winter wheat (*Triticum aestivum* L.) cultivars. *S.A. J. Plant Soil* 14(1), 4-8.
- BENECH-ARNOLD, R.L., FENNER, M. & EDWARDS, P.J. 1995. Influence of potassium nutrition on germinability, abscisic acid content and sensitivity of the embryo to abscisic acid in developing seeds of *Sorghum bicolor* (L.) Moench. *New Phytol.* 130, 207-216.
- BEWLEY, D. & BLACK, M. 1978. Physiology and biochemistry of seeds in relation to germination. Vol. 1. Development, Germination and Growth. Springer-Verlag, Berlin/New York. pp 133-176.
- BROWNLEADER, M.D., HARBORNE, J.B. & DEY, P.M. 1997. Carbohydrate metabolism: primary metabolism of monosaccharides. In: P.M. Dey and J.B. Harbone (eds). *Plant Biochem.* New York, Academic Press. pp 111-141.
- DEBNAM, P.M. & EMES, M.J. 1999. Subcellular distribution of enzymes of the oxidative pentose phosphate pathway in root and leaf tissues. *J. Exp. Bot.* 50 (340), 1653-1661.
- DEPAUW, R.M. & MCCAIG, T.N. 1991. Components of variation, heritabilities and correlations for indices of sprouting tolerance and seed dormancy in *Triticum* spp. *Euphytica* 52, 221-229.

- DERERA, N.F. 1982. The harmful harvest rain. *J. Austr. Inst. Agric. Sci.* 48, 67-75.
- DERERA, N.F., BHATT, G.M. & MCMASTER, G.J. 1977. On the problem of preharvest sprouting of wheat. *Euphytica* 26, 299-308.
- DUFFUS, C.M. & ROSIE, R. 1977. Carbohydrate oxidation in developing barley endosperm. *New Phytology* 78, 391-395.
- FENNER, M., 1991. The effects of the parent environment on seed germinability. *Seed Sci. Res.* 1, 75-84.
- FUERST, F.P., UPADHYAY, M.K., SIMPSON, G.M., NAYLOR, J.M. & ADKINS, S.W. 1983. A study of the relationship between seed dormancy and the pentose phosphate pathway activity in *Avena fatua*. *Can. J. Bot.* 61, 667-670.
- GAHAN, P.B., DAYSON, A.L., BLACK, M. & CHAPMAN, J.M. 1986. Localization of glucose-6-phosphate dehydrogenase activity in seeds and its possible involvement in dormancy breakage. *Ann. Bot.* 57, 791-799.
- GALLAIS, S., DE CRESCENZO, M.A.P. & LAVAL-MARTIN, D.L. 2000. Evidence of active NADP⁺ phosphatase in dormant seeds of *Avena sativa* L. *J. Exp. Bot.* 51(349), 1389-1394.
- GORDON, I.L. 1980. Germinability, dormancy and grain development. *Cereal Res. Commun.* 8(1), 115-129.
- HENDRICKS, S.B. & TAYLORSON, R.B. 1975. Breaking of seed dormancy by catalase inhibition. *Proceedings of the National Academy of Sciences, U.S.A.* 72, 306-309.
- IBRAHIM, Y. & D'APPOLONIA, B.L. 1979. Sprouting in hard red spring wheat. *Baker's Dig.* 53(5), 17-19.
- LA CROIX, L.J. & JARWAL, A.S. 1967. Metabolic changes in after ripening seed of *Prunus coasus*. *Plant Physiol.* 42, 479-480.
- MARES, D.J. 1993. Genetic studies of sprouting tolerance in red and white wheats. In: M.K. Walker-Simmons and J.L. Ried (eds). *Sixth International Symposium on Preharvest Sprouting in Cereals*. AACC, Minnesota, USA. pp 21-29.

- MCCRATE, A.J., NIELSEN, M.T., PAULSEN, G.M. & HEYNE, E.G., 1981. Preharvest sprouting and α -amylase activity in hard red and hard white winter wheat cultivars. *Cereal Chem.* 58(5), 424-428.
- NICOLAS, G. & ALDASORO, J.J. 1979. Activity of the pentose phosphate pathway and changes in nicotinamide nucleotide content during germination of seeds of *Cicer arietinum* L. *J. Exp. Bot.* 30 (119), 1163-1170.
- PRETORIUS, J.C. & SMALL, J.G.C. 1992. The effect of soaking injury in bean seeds on aspects of the oxidative pentose phosphate pathway in embryonic axes. *Seed Sci. Res.* 2, 33-39.
- ROBERTS, E.H. 1969. Seed dormancy and oxidation processes. *Symposium of the Society of Experimental Biologists* 23, 161-192.
- ROBERTS, E.H. & SMITH, R.D. 1977. Dormancy and the pentose phosphate pathway. In K.K. Khan (ed). *The physiology and biochemistry of seed dormancy and germination*. Elsevier/North-Holland Biomedical Press. pp 385-411.
- RUMPHO, M.E. & KENNEDY, R.A. 1983. Activity of the pentose phosphate and glycolytic pathways during anaerobic germination of *Echinochloa crus-galli* (barnyard grass) seeds. *J. Exp. Bot.* 34, 893-902.
- SÀNCHEZ DE JIMÉNEZ, E & QUIROZ, J. 1983. Role of glucose-6-phosphate dehydrogenase in corn seed germination. In: J.E. Kruger and D.E. LaBerge (eds). *Third International Symposium on Preharvest Sprouting in Cereals*. Westview Press. Boulder, Colorado. pp 197-203.
- SCHNARRENBARGER, C., FLECHNER, A. & MARTIN, W. 1995. Enzymatic evidence for a complete oxidative pentose phosphate pathway in chloroplast and an incomplete pathway in the cytosol of spinach leaves. *Plant Physiol.* 108, 609-614.
- SIMCOX, P.D., GARLAND, W., DELUCA, V., CANVIN, D.T. & DENNIS, D.T. 1979. Respiratory pathways and fat synthesis in developing castor oil seeds. *Can. J. Bot.* 57, 1008-1014.

- SIMMONDS, J.A. & SIMPSON, G.M. 1971. Increased participation of pentose phosphate pathway in response to after ripening and gibberellic acid treatment in caryopses of *Avena fatua*. *Can. J. Bot.* 49, 1833-1840.
- SIMPSON, G.M. 1990. Terminology and definitions of dormancy. In: G.M. Simpson, (ed). *Seed dormancy in grasses*. Cambridge University Press, New York. pp 43-48.
- STRAND, E. 1983. Effects of temperature and rainfall on seed dormancy of small grain cultivars. In: J. E. Kruger and D.E. LaBerge (eds). *Third International Symposium on Preharvest Sprouting in Cereals*. Westview Press, Boulder, Colorado. pp 260-266.
- STRAND, E. 1991. The intensity of seed dormancy in small grain cultivars. *Norwegian J. Agric. Sci.* 5(1), 45-49.
- TURNER, J.P & TURNER, D.H. 1980. The regulation of glycolysis and the pentose phosphate pathway. In: D.D. Davies, (ed). Vol. 2. *The biochemistry of plants*. New York, USA, Academic. pp 279-316.
- UPADHYAY, M.P. & PAULSEN, G.M. 1988. Heritabilities and genetic variation for preharvest sprouting in progenies of Clark's Cream white winter wheat. *Euphytica* 38, 93-100.
- UPADHYAY, M.K., SIMPSON, G.M. & NAYLOR, J.M. 1981. Levels of glucose-6-phosphate and 6-phosphogluconate dehydrogenases in the embryos and endosperms of some dormant and non-dormant lines of *Avena fatua* during germination. *Can. J. Bot.* 59, 1640-1646.

CHAPTER 5

GENETIC DIVERSITY OF SOUTH AFRICAN WHEAT CULTIVARS IN RELATION TO PREHARVEST SPROUTING AND FALLING NUMBER

5.1 ABSTRACT

The FN method, which is widely used to estimate α -amylase activity in wheat grain, was officially introduced as part of the South African grading regulations in 1998. The preceding two seasons had been characterised by abnormally wet conditions during harvest time, especially in the eastern parts of the Free State, resulting in major preharvest sprouting problems. Downgrading due to a low FN is an intermittent problem in South Africa. In this study South African winter and intermediate wheat cultivars were screened for their preharvest sprouting resistance in comparison with their inherent FN. Wheat cultivars varied substantially in their ability to withstand moist harvest conditions. The FN of cultivars which were exposed to optimal conditions for preharvest sprouting by using a rain simulator, dropped drastically in most cases. The genotype by environment interactions for sprouting resistance were significant which indicate that more stable cultivars should be selected by breeders. It is generally accepted that the long-term solution to this problem lies in the development of cultivars which are able to tolerate or resist the damaging effects of rain during the period between ripeness of maturity and the completion of harvest. By using the data generated from this study, it will be possible to select more tolerant types which can be used in the development of cultivars with an inherent higher FN.

5.2 INTRODUCTION

Preharvest sprouting of wheat results in large economic losses around the world each year in countries that experience wet conditions prior to harvest (Kruger, 1990). Sprouting results in large increases in the amounts of certain germinative enzymes such as α -amylase (EC 3.2.1.1) and consequent poor grain quality. This can adversely affect the quality of end-products in various ways (Henry, 1989). Excess α -amylase results in bread with a wet, sticky crumb.

Even if visible sprouting does not occur, the α -amylase level may be considerably elevated as a result of a wet harvest season. Thus the α -amylase activity of wheat cannot be reliably estimated by determining the percentage of sprouted kernels (Zeleny, 1988). The fact that grain with none or a slight indication of sprouting might show a very high α -amylase activity and thus prove to be of low baking quality, made the visual inspection hazardous and unreliable. A practical method to determine α -amylase activity of grain at an intake silo during harvest, was thus necessary. The FN method was officially introduced into the South African grading rules during 1998. Prior to this, sprouting degree has been determined by visual inspection. A limit of 2% sprouted kernels is still used as an acceptable amount.

According to Kettlewell (1993) there is relatively little possibility for agronomic techniques to reduce this problem before harvest. A primary objective of wheat breeding programs is to develop wheat varieties that resist sprouting during wet harvest seasons.

In this study South African winter and intermediate wheat cultivars were screened for their preharvest sprouting resistance in comparison with their inherent FN, as well as their ability to withstand a specified amount of simulated rainfall.

5.3 MATERIALS AND METHODS

In 1998, 18 commercially released winter and intermediate wheat cultivars, representing most of the wheat planted in the dryland winter wheat production areas of South Africa, as well as two advanced breeding lines, were grown at three localities in the Eastern Free State, namely Bethlehem, Petrus Steyn and Clarens. A randomised complete block design with four replicates was used. Ears were hand-harvested at physiological maturity. The FN of each of these cultivars and lines at each of the three localities was determined. During 1999, only trials planted at Bethlehem were used. The ears were treated in exactly the same way as in 1998, but visual preharvest sprouting was also determined on a scale from 1 (no visible sprouting) to 8 (fully sprouted) (Barnard *et al.*, 1997).

5.3.1 Falling Number Determination

The method described by the American Association of Cereal Chemists (AACC) (2000) was followed using duplicate samples equivalent to 7 g on a 14% moisture basis. The FN instrument used throughout the study was a Falling Number FN1800 model. The instrument was equipped with two slots for duplicate simultaneous measurements. Each reported FN value was an average of the two readings. A 300 g sample was first ground in a Model KT120 Falling Number Mill. FN was determined at harvest ripeness (hereafter referred to as FN1). After three days of simulated rainfall, the spikes were bundled and dried at room temperature, whereafter FN was again determined (hereafter referred to as FN2). FN2 was only determined in 1999. Results are reported in seconds.

5.3.2 Preharvest Sprouting Determination

Following harvest, spikes were subjected to simulated rainfall for 72 h in a rain simulator (McMaster & Derera, 1976). The entries were randomly placed on perforated trays. According to this technique, 10 erect spikes per replicate were

placed upright on perforated trays. Samples were loaded in quadruplicate, ensuring that each replicate of 10 spikes was assigned to different trays. After 72 h, spikes were evaluated for preharvest sprouting on a scale from 1 to 8 (Barnard *et al.*, 1997).

5.3.3 Statistical analyses

All statistical analyses were performed using the GENSTAT 5 statistical program (GENSTAT 5 Committee, 1993).

5.4 RESULTS AND DISCUSSION

The Eastern Free State was chosen for the experiment, since the highest risk for preharvest sprouting occurs in this part of the wheat production area of South Africa as the probability of summer rainfall over the harvest period is very high.

The results of all the FN determinations, measured during 1998, are shown in Table 5.1. Analysis of variance for FN was performed and highly significant ($P < 0.01$) interactions between cultivars and localities were found. FNs varied from an average of 331 (Bethlehem) to 334 (Petrus Steyn). FNs of individual cultivars varied from 165 (Caritha – Petrus Steyn) to 407 (SST 124 – Clarens). The only cultivar which probably had an inherent high amount of amylase activity (coupled with an inherent high FN), was Caritha with FNs which varied from 165.5 to 229.6 over the three localities and was significantly lower than the others. According to Mares & Mrva (1992) some varieties produce α -amylase during the later stages of grain ripening in the absence of sprouting.

Table 5.1 The Falling Numbers (FN1) of 20 cultivars and lines determined at three localities in the Eastern Free State during 1998

Cultivar	Falling Number (seconds)			
	Petrus Steyn	Clarens	Bethlehem	Average
Betta-DN	329.0	302.5	332.0	321.2
Gariep	286.8	299.3	315.3	300.4
Tugela-DN	390.0	359.8	371.5	373.8
PAN 3377	311.3	384.3	327.5	341.0
SST 124	382.0	406.8	383.3	390.7
Limpopo	290.5	323.5	314.3	309.4
Caledon	322.8	366.5	336.0	341.8
T95/7	309.5	316.0	321.8	315.8
Hugenoot	396.3	399.0	386.0	393.8
T96/6	365.3	387.0	373.8	375.3
SST 936	292.8	260.3	300.0	284.3
Carol	343.3	327.0	354.8	341.7
Carina	359.8	317.0	317.0	331.3
Caritha	165.5	229.6	187.0	194.0
Elands	316.8	335.0	323.8	325.2
PAN 3232	307.8	370.0	333.8	337.2
PAN 3235	326.5	348.3	392.3	355.7
PAN 3349	370.8	370.0	321.0	353.9
SST 367	309.5	279.3	289.8	292.8
SST 966	274.5	305.0	337.5	305.7
Average	322.5	334.3	331.1	328.6

LSD (0.05) Cult = 34.03

LSD (0.05) Loc = 8.72

LSD (0.05) Cult x Loc = 67.78

FNs were determined in 1999 only at the Bethlehem locality. FNs were determined at harvest ripeness (FN1) as well as after a 72 h simulated rain treatment (FN2). Results are shown in Table 5.2. FNs (FN1) varied from 168 (Caritha) to 355 (SST 124). Caritha again showed a significant lower FN compared to the others, indicating that it may have an inherent high amylase activity. Visual preharvest sprouting determination indicated that Betta-DN, Limpopo, Caledon, T96/6, Elands, PAN 3232 and PAN 3235 were tolerant to preharvest sprouting, while Tugela-DN, Carol, Carina and Caritha were susceptible to sprouting. This was also reported earlier by Barnard *et al.* (1997).

FN determination (FN2) after rain simulation showed, however, that visual determination could be hazardous and unreliable. Although Limpopo, Caledon, PAN 3232 and PAN 3235 showed a high tolerance to preharvest sprouting after a 72 h simulation rain treatment in the visual preharvest sprouting assessment, their FNs (FN2) dropped to between 78 and 162. Only Betta-DN, Elands and the advanced breeding line T96/6 showed FNs higher than 250 seconds. Wheat grain with a Hagberg FN below 250 seconds is unsuitable for baking (Major & Kettlewell, 1999).

Figure 5.1 shows the relationship between the FN method and the visual assessment of sprouting damage. Large variations between the methods occurred, although the overall trend indicated that a lower FN occurred with increasing sprout damage. This was also observed by Kruger & Tipples (1982). Some examples of differences are that samples with a 1 to 3 score visual sprout damage (good tolerance to preharvest sprouting), ranged in FN from 278 to a low of 78 seconds and samples with a 3 to 7 visual sprout damage (medium to low tolerance to preharvest sprouting), ranged from 111 to 62 seconds. It must be said, however, that these cultivars were subjected to an optimum rain simulation treatment and can therefore be used as a selection criteria for breeding cultivars with high FNs.

Table 5.2 Falling number determinations (FN1 and FN2) and visual preharvest sprouting assessment at Bethlehem during 1999. Visual sprouting was conducted on a scale from 1 (no visible sprouting) to 8 (fully sprouted)

Cultivar	FN1	Sprouting Scale	FN2
Betta-DN	302	1.3	260
Gariép	301	3.8	62
Tugela-DN	299	7.0	62
PAN 3377	355	2.3	91
SST 124	355	4.0	97
Limpopo	331	2.5	78
Caledon	319	1.8	93
T95/7	316	4.2	86
Hugenoot	345	3.7	62
T96/6	319	1.0	278
SST 936	333	3.8	62
Carol	316	6.8	111
Carina	295	6.7	101
Caritha	168	6.9	69
Elands	309	1.1	250
PAN 3232	291	2.8	162
PAN 3235	327	2.9	159
PAN 3349	339	5.5	65
SST 367	337	4.0	77
SST 966	228	4.2	62
Average	306.8	3.9	115

LSD (0.05) FN1 = 22.7

LSD (0.05) FN2 = 90.77

LSD (0.05) Scale = 0.92

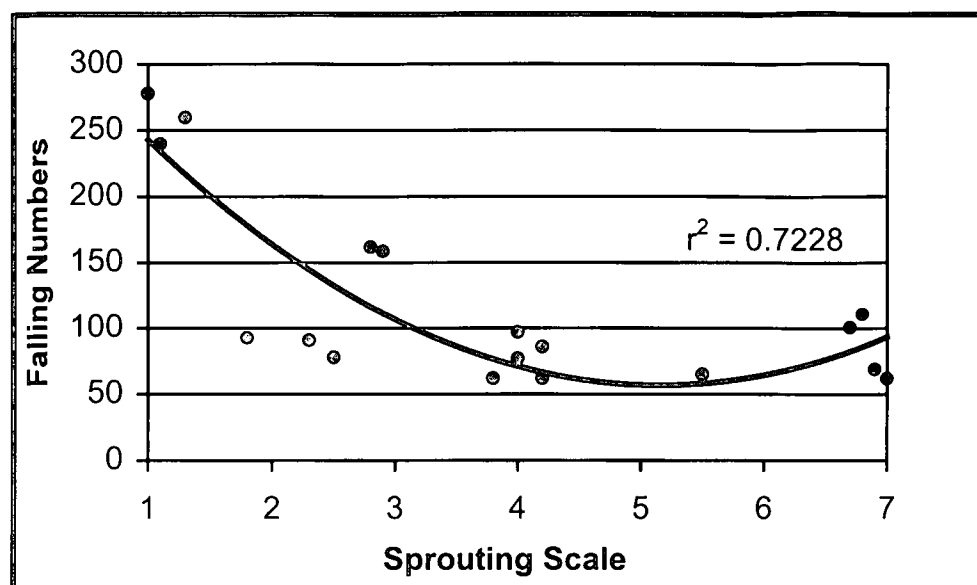


Figure 5.1 The relationship between FN values of 20 cultivars and lines and their respective visual preharvest sprouting scores.

5.5 CONCLUSIONS

The poor correlation between visual sprouting damage and FN can be explained in a number of ways. Firstly, germination may occur without visual determination. In this case, amylase activity has already taken place. Some kernels may also have undergone incipient sprouting (Kruger & Tipples, 1982) in which germination has proceeded without visible sprouting. The level of α -amylase activity in sprouted wheat kernels can vary quite widely depending upon the severity of sprouting (Kruger & Tipples, 1979). Similarly, a very small percentage of severely sprouted kernels in a parcel of wheat may result in a greater overall α -amylase level than when the entire sample had been very lightly sprouted.

There is relatively little opportunity for agronomic techniques to reduce the problem of preharvest sprouting coupled with low FNs before harvest. It is generally accepted that the long-term solution to this problem lies in the development of cultivars which are able to tolerate or resist the damaging effects of rain during the period between

ripeness of maturity and the completion of harvest. Previously the problem has been considered to be a sporadic failure of certain varieties and, as such, has received little attention.

By using the data generated from this study, it will be possible to select for more tolerant types which can be used in the development of cultivars with an inherently higher FN. Reducing the unpredictable effect of sprouting damage will help wheat producers in South Africa to produce a consistently good quality crop and thus enable them to compete on the international market.

REFERENCES

- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 2000. Approved Methods of the AACC, 10th ed. Vol II. Falling Number Determination. *Method 56-81B*. The Association: St. Paul, Minnesota.
- BARNARD, A., PURCHASE, J.L., SMITH, M.F. & VAN LILL, D. 1997. Determination of the preharvest sprouting resistance of South African winter wheat (*Triticum aestivum* L.) cultivars. *S. Afr. J. Plant & Soil*. 14(1), 4-8.
- GENSTAT 5 COMMITTEE. 1993. Statistics Dept., Rothamsted experimental station. Clarendon Press, Oxford.
- HENRY, R.J. 1989. Rapid α -amylase assays for assessment of preharvest sprouting damage in wheat. *J. Sci. Food Agri*. 49, 15-23.
- KETTLEWELL, P.S. 1993. Preharvest prediction of Hagberg falling number of wheat: some possible methods for the United Kingdom. In: P.S. Kettlewell, J.R. Garstang, C.M. Duffus, N. Magan, W.T.B. Thomas and N.D. Paveley (eds). *Cereal Quality III*. Association of Applied Biologists, Warwick UK. pp 257-266.
- KRUGER, J.E. 1990. Instrumental assessment of sprout-damage in wheat at primary or terminal receival points. *Cereal Foods World* 35(9), 935-939.
- KRUGER, J.E. & TIPPLES, K.H. 1979. Relationships between falling number, amylograph viscosity and α -amylase activity in Canadian wheat. *Cereal Res. Commun.* 8, 97-105.
- KRUGER, J.E. & TIPPLES, K.H. 1982. Comparison of the Hagberg Falling Number and modified grain amylase analyzer methods for estimating sprout damage in rye. *Can. J. Plant Sci.* 62, 839-844.
- MARES, D.J. & MRVA, K. 1992. Late maturity α -amylase in wheat. In: M.K. Walker-Simmons and J.L. Ried (eds). *Preharvest Sprouting in Cereals*. St. Paul, Minnesota. pp 178-184.
- MAJOR, B.J. & KETTLEWELL, P.S. 1999. The effectiveness of a terpenoid polymer in preserving the Hagberg Falling Number of winter wheat. *Ann. Appl. Biol.* 134, 38-39.

- MCMASTER, G.J. & DERERA, N.F. 1976. Methodology and sample preparation when screening for sprouting damage in cereals. *Cereal Res. Commun.* 4(2), 251-254.
- ZELENY, L. 1988. Criteria of wheat quality. In: Pomeranz, Y (ed), *Wheat: Chemistry and Technology*. American Association of Cereal Chemists, St. Paul, Minnesota. pp 32-38.

CHAPTER 6

COMPARISON BETWEEN VARIOUS METHODS FOR ESTIMATING SPROUT DAMAGE IN WHEAT

6.1 ABSTRACT

Sprouting in wheat is common when rain occurs during harvest. This causes the moisture content of the grain to increase to a level at which germination takes place and the α -amylase increases. Various methods can be used to determine α -amylase in cereals. The FN method is currently widely used in the milling and baking industry. As well as being time-tested and reliable, this method is very sensitive to the presence of low levels of enzyme and is often influenced by factors other than α -amylase, such as the nature of the starch, starch damage and environment. In this study various methods to determine preharvest sprouting and α -amylase activity were compared. All the methods evaluated showed significant correlations and any of the methods tested could therefore be used for the determination of sprouting. However, results from this study indicated that the Stirring Number and FN methods are the most reliable methods to use for the determination of preharvest sprouting and α -amylase activity.

6.2 INTRODUCTION

A significant risk factor in winter cereal farming in certain environments is preharvest sprouting due to significant rain on ripe grain prior to harvest. The price paid to the grower is usually significantly reduced because the market value of the grain is lowered. This is because even mild sprouting affects the suitability of wheat for most food uses (Kruger, 1994).

The extent of sprouting is very hard to predict. Preharvest sprouting susceptibility/tolerance is usually determined by means of a rain simulator (Barnard et al., 1997). Visual estimation of the percentage of sprouted kernels is used by a number of agencies, including the U.S. Federal Grain Inspection Service (Skerritt & Heywood, 2000). A maximum limit of 2% visually sprouted kernels is still part of the South African grading regulations for wheat. However, several studies have shown that visual estimation of sprouting can be unreliable (Mares, 1989), in part because much of the commercially relevant damage occurs before germination of the grain is visible (Jensen et al., 1984).

The standard method for quantification of sprouting is the FN test, a viscosity test in which the time required for a plunger to fall through a heated slurry of whole meal and water in a large glass test tube is measured (Hagberg, 1960.) Although the method is simple in concept, the equipment is usually considered too expensive for the farmer market, and since it requires a special balance and sample grinder, the method is aimed at the mill laboratory.

The Rapid Visco Analyser (RVA, Newport Scientific, Australia), is a promising instrument, being capable of a variety of rapid or comprehensive recordings regarding starch damage. The stirring number presents a rapid viscosity measurement of a flour water suspension after 3 minutes which is closely related to FN.

Due to the confusions and problems surrounding FN which were experienced during the previous two years, alternative methods for the determination of sprouting damage, were investigated. Research organisations in Australia have developed a simple field test (called WheatRite TM) to detect preharvest sprouting by measuring α -amylase in the grain.

This study compares the FN method with these and other methods for determining low levels of α -amylase in wheat.

6.3 MATERIALS AND METHODS

Sixteen winter and intermediate wheat cultivars, representative of the wheat grown in the Free State, were included in this study. Detail about these cultivars is given in Table 6.1.

6.3.1 Preharvest Sprouting

Following harvest, spikes were subjected to simulated rainfall for 72 h in a rain simulator (McMaster & Derera, 1976) as described in detail in Chapter 5. All the measurements were determined on samples which were subjected to simulated rainfall.

6.3.2 Percentage Sprouted Kernels

After subjecting the spikes to simulated rainfall and preharvest sprouting determination, spikes were individually examined for sprouted kernels. The sprouted kernels were counted and calculated as a percentage. Kernels were considered visually sprouted with the emergence of the radicle.

Table 6.1 Summary of the cultivars used to determine FN and various other characteristics

Cultivar	Release date	Growth Habit and Type	Origin
Betta-DN	1993	Intermediate pureline	Small Grain Institute
Elands	1998	Intermediate pureline	Small Grain Institute
Gariep	1994	Intermediate pureline	Small Grain Institute
Tugela-DN	1992	Intermediate pureline	Small Grain Institute
Limpopo	1994	Intermediate pureline	Small Grain Institute
Caledon	1996	Intermediate pureline	Small Grain Institute
Hugenoot	1989	Intermediate pureline	Monsanto
SST 124	1987	Intermediate pureline	Monsanto
SST 363	1996	Intermediate pureline	Monsanto
SST 367	1996	Intermediate pureline	Monsanto
SST 936	1993	Winter hybrid	Monsanto
SST 966	1996	Winter hybrid	Monsanto
PAN 3211	1993	Intermediate pureline	Pannar
PAN 3235	1995	Intermediate pureline	Pannar
PAN 3377	1998	Intermediate pureline	Pannar
PAN 3349	1997	Intermediate pureline	Pannar

6.3.3 Falling Number (FN)

The FNs of each of the cultivars were determined as described in Chapter 5. After three days of simulated rainfall, the spikes were bundled and dried at room temperature, whereafter FN was determined. Results are reported in seconds. Perten Liquefaction Numbers (PLN) were calculated by using the following formula:

$$\text{PLN} = \frac{6000}{(\text{FN} - 50)}$$

PLN values, unlike FNs, are additive and can therefore be used in calculations to determine average values if required.

6.3.4 Stirring Number (SN)

A Rapid Visco Analyzer – Mini 3 was used for Stirring Number analysis according to the AACC Method No 22-08 (ICC Std No 161) (2000). This included a sample size of ± 4.0 g (dry matter), water volume of 25 ml and a block temperature of 91°C. All procedures were followed according to regulation to ensure that the samples were homogeneous after grinding. The samples were weighed according to their moisture content by making use of a moisture adaptation table. Four replications were conducted and the results, which were determined in Rapid Visco Units (RVU's), were transformed to seconds by the following formula:

$$Y \text{ (FN)} = [1.2868 \times \text{SN(RVU's)}] + 35.792$$

6.3.5 α -Amylase

α -Amylase activity was assayed according to a revised method of Barnes & Blakeney (1974). Flour (5 g) was added to 0.09 M NaCl and shaken continuously for 15 minutes. The contents were filtered through filter paper (Whatman No 4). The filtrate and 0.09 M NaCl were added to a centrifuge tube and incubated in a 50° C waterbath for 10 min. The extracts were incubated with a highly specific dye-labelled substrate (Phadebas tablets from Pharmacia). These tablets consist of a substrate made by cross-linking partially hydrolyzed potato starch. The substrate is labelled with Cibacron blue by covalent bonds. α -Amylase hydrolyses the blue starch polymer into a water-soluble blue dye which absorbs light at 620 nm. The reaction was terminated by adding 1 ml of 0.5 M NaOH to each test tube. The samples were centrifuged for 10 min at 20000 rpm.

A 5 ml aliquot of the supernatant was made up to 1 ml with a buffer containing 58.4 M sodium chloride and 1.14 mM calcium acetate and the absorbance was read at 620 nm.

Results are given in enzyme units per litre. An enzyme unit is defined as the amount of enzyme catalyzing the hydrolysis of 1 μ m glucosidic linkage at 50°C.

6.3.6 Diastatic Activity

Diastatic activity was determined according to the AACC Method (Method 22-15) of 2000. Flour (5 g) were mixed with 5 ml ignited quartz sand and heated to 30°C. A buffer containing 0.3% (v/v) acetic acid and 0.03 M sodium acetate (pH 4.7) were added and mixed until all flour was in suspension. The solution was kept at a constant temperature of 30°C for 1 h. H₂SO₄ (3.68 N) and 12% sodium tungstate were added and mixed thoroughly. The solution was filtered, by using Whatman No 4 filter papers and the amount of maltose was determined in the filtrate. Results are reported in % maltose produced.

6.3.7 WheatRite Method

Wheat samples were ground by using a Model KT120 Falling Number Mill. The samples were transferred to individual tubes. Salt solution (which is included in the WheatRite kit), was added and the tubes were briefly shaken by hand. Two drops of wheat extract were applied to a disposable test card, which was closed and left for 5 min. If amylases were present, a purple/pink band would appear on the card. The worse the sprouting, the darker the purple/pink band. Four replications were done on these samples. The results on the card were estimated by visual inspection. Twelve participants, consisting of senior researchers and qualified grain handlers, were used in this study. Each of the replications was visually inspected by three participants. The value identified by two or more of the participants was used as a replication.

6.3.8 Statistical analyses

All statistical analyses were performed by using the Agrobase 2000 statistical program.

6.4 RESULTS AND DISCUSSION

Figures 6.1 to 6.3 show the results obtained by using visual assessment and FN to measure preharvest sprouting. Figure 6.1 shows the relationship between the FN and the visual assessment of sprout damage by means of a sprouting scale. There are large variations between these methods, although the overall trend indicates that a lower FN occurs with increasing sprout damage. Some examples of the differences are that samples with a scale value of 2 to 3 ranged in FN from 153 to 62 seconds.

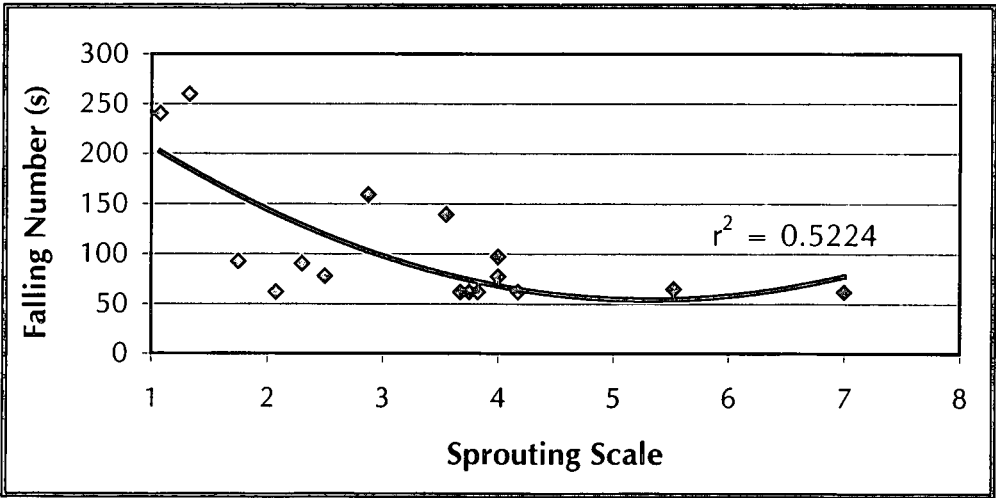


Figure 6.1 FN values *versus* relative sprouting response of 16 wheat cultivars.

The variations in the percentage sprouted kernels compared to FN values is shown in Figure 6.2. FNs varied between 62 seconds and 260 seconds. The results obtained showed a curvilinear relationship with FN.

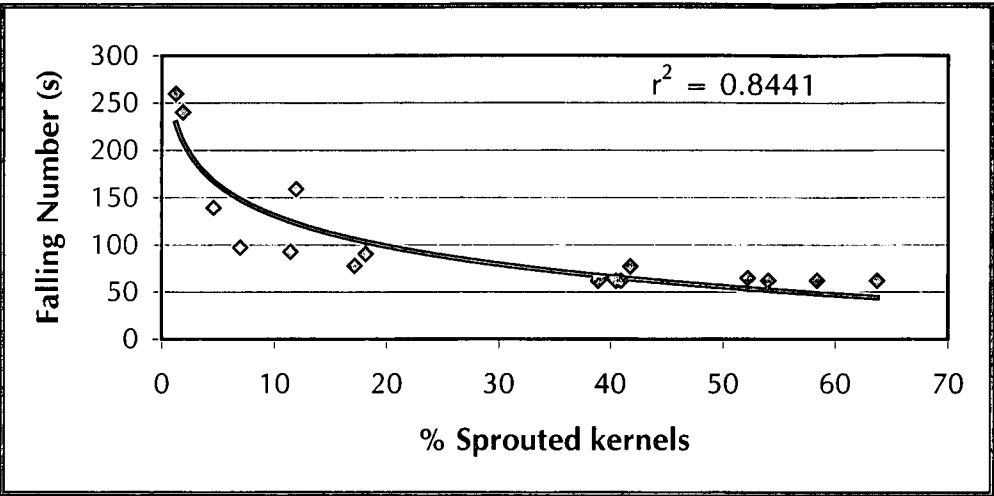


Figure 6.2 FN values *versus* percentage sprouted kernels in 16 wheat cultivars.

The FN results were transformed to Perten Liquefaction Numbers (PLN) since PLN is a linear relationship in contrast to FN. It is therefore better to calculate correlation coefficients on PLN than on FN. When the percentage sprouted kernels was plotted *versus* the PLN, a straight line relationship with a correlation coefficient of 0.82 was found (Figure 6.3). This indicates that FN can give a good prediction of preharvest sprouting in wheat.

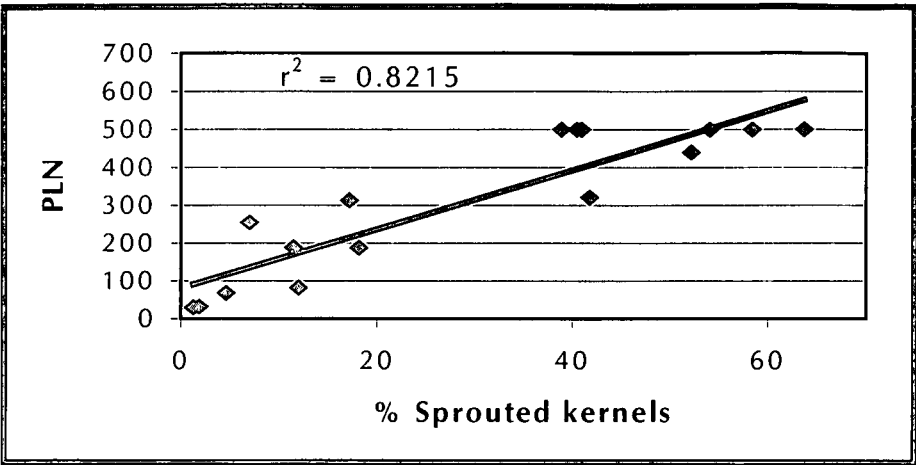


Figure 6.3 The relation between the reciprocal of FN (i.e. PLN) and percentage sprouted kernels on 16 wheat cultivars.

There is, however, not a clear linear correlation between the sprouting scale and the percentage sprouted kernels (Figure 6.4). This can be explained by the fact that although the kernels had already sprouted and thus were counted as sprouted kernels, the radicle had not emerged through the chaff of the wheat spike and thus received a low sprouting value.

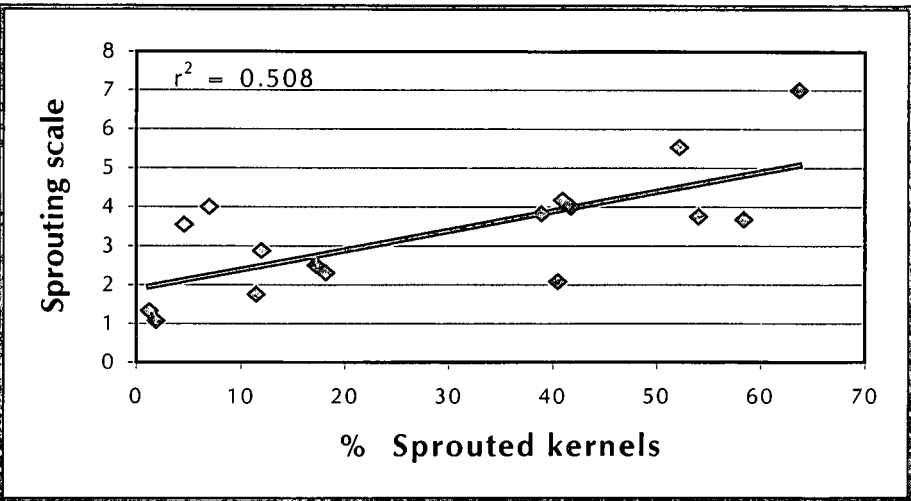


Figure 6.4 Relationship between sprouting scale and sprouted kernels (%).

As shown in Figure 6.5, visual assessment of sprout damage agrees poorly with the amount of α -amylase present. Thus, samples with 10% or less sprout damage may have more α -amylase than samples containing around 20% sprouted kernels. α -Amylase values were also compared with values obtained by the FN procedure (Figure 6.6). A curvilinear behaviour was found which is typical of the relationship between the two methods (Kruger & Tipples, 1982).

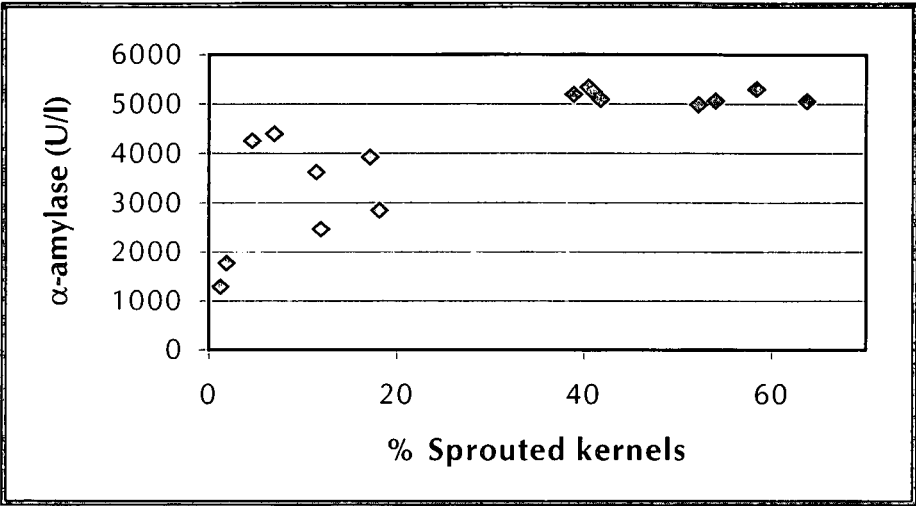


Figure 6.5 α -Amylase activity *versus* percentage sprouted kernels for 16 wheat samples.

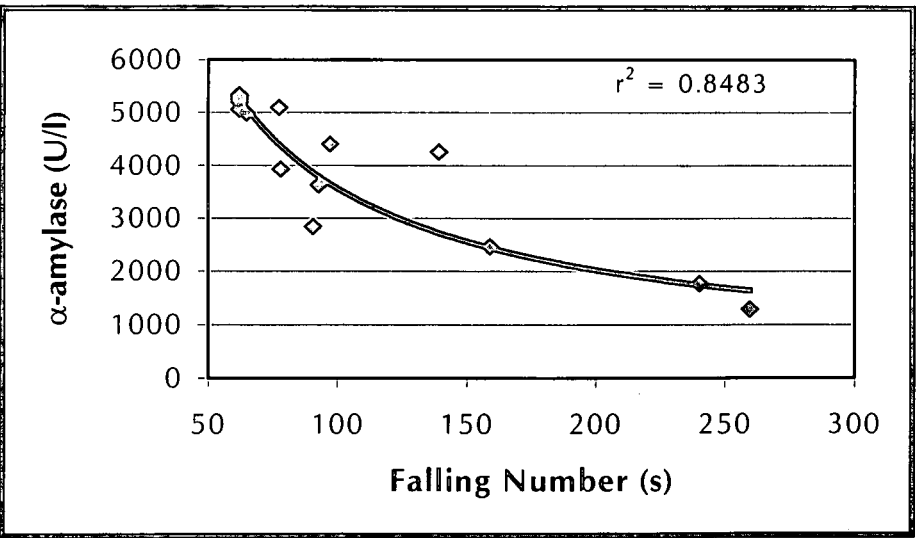


Figure 6.6 α -Amylase activity *versus* FN of 16 wheat cultivars.

There is a strong correlation between FN and SN values as determined on the same samples (Figure 6.7). The excellent reproducibility of the SN method (CV of 3.40%), lack of or no requirement for test tubes and its user friendly nature should make it an attractive tool for monitoring sprout damage at receival points.

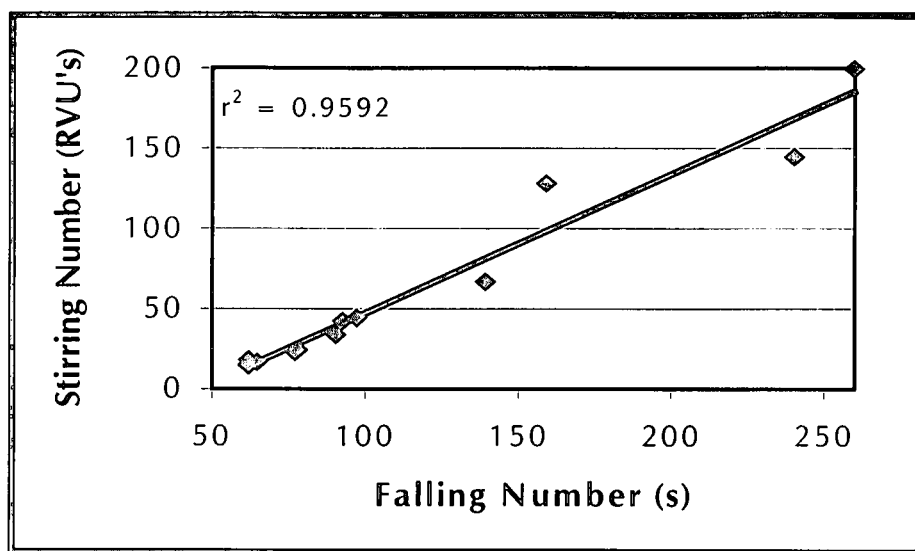


Figure 6.7 Comparison of the FN method *versus* the SN method.

Results obtained in this study showed a high correlation between PLN and SN, % sprouted kernels, % maltose and the WheatRite method. Table 6.2 shows the various correlation coefficients obtained with the sixteen cultivars. Although the WheatRite method correlated well with the other methods, the coefficient of variance was high (24.4%) and the significance was low (46,6%). This method is based on immunochromatography. The appearance of a purple/pink-coloured band on a small test card points to the presence of the enzyme and thus to preharvest sprouting in the sample.

Table 6.2 Correllation coefficient* between sprouting scale, FN, PLN, SN, sprouted kernels, α -amylase activity and maltose content of 16 wheat cultivars

	Sprouting scale	FN	PLN	SN	Sprouted kernels	α-amylase	Maltose
FN	-0.5321						
PLN	0.5101	-0.8072					
SN	-0.5442	0.9267	-0.7377				
Sprouted kernels	0.6488	-0.6660	0.7753	-0.6789			
α-amylase	0.6457	-0.8386	0.7780	-0.8912	0.7489		
Maltose	0.6620	-0.7384	0.5045	-0.7669	0.5356	0.7142	
WheatRite	-0.4274	0.5562	-0.4585	0.5689	-0.4583	-0.4861	-0.5471

* All values are highly significant at P=0.01

Figure 6.8 shows the relationship between FN and the WheatRite method. Although there is a linear correlation between these two methods ($r^2=0,7765$), the band intensity values were similar for wheat with FNs of 250 seconds and higher. Similarly, no differences in band intensity could be observed for FN values of between 60 and 200 seconds.

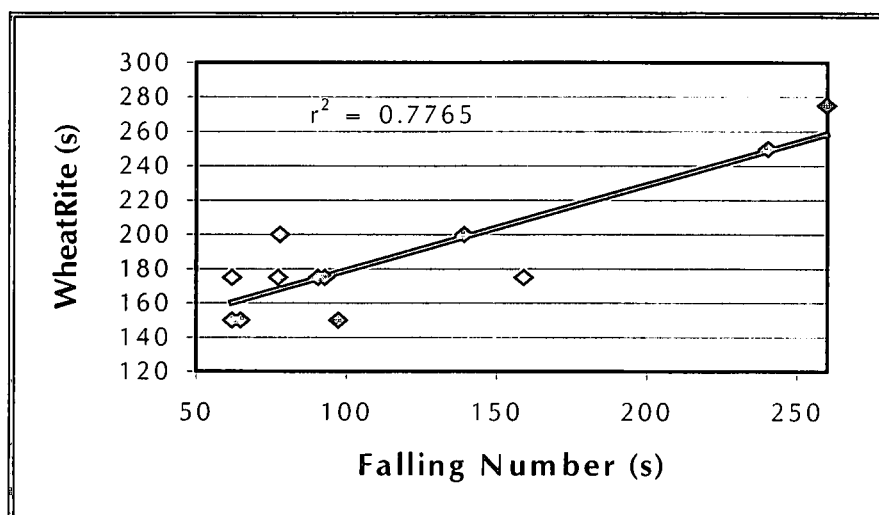


Figure 6.8 FN method *versus* the WheatRite method in 16 wheat cultivars.

Table 6.3 indicates the analyses of variance results for the sprouting scale, sprouted kernels, FN, SN, maltose content, α -amylase and WheatRite methods respectively. All the ANOVA's indicated highly significant differences. The cultivars Betta-DN and Elands were ranked first (i.e. highest tolerance to sprout damage) in all the methods.

Table 6.3 Analysis of variance results for sprouting scale, sprouted kernels, FN, SN, maltose content, α -amylase activity and Wheatrite method for 16 wheat cultivars

Source	Mean Squares						
	Sprouting scale	Sprouted kernels	Falling Number	Stirring Number	Maltose content	α -amylase activity	Wheatrite
Total							
Rep	0.054	15.438	284.557	1.814	0.266	122 011.4	729.167
Entries	9.531***	1 926.663***	16 255.291***	12 719.051***	16.882***	7 226 107.0***	5 166.667**
Residual	0.176	45.204	625.791	2.994	0.199	45 457.410	1 951.389

*** Significant at $P = 0.01$

** Significant at $P = 0.05$

The correlation coefficient between sprouting scale, FN, PLN, SN, sprouted kernels, α -amylase activity and maltose content of the 16 cultivars, were all highly significant.

The results indicated that the SN and FN methods were respectively the most reliable for the determination of preharvest sprouting and subsequent α -amylase activity. The SN method showed a high correlation with sprouted kernels, α -amylase activity, as well as maltose content. The FN method also had a high correlation with α -amylase activity, as well as with PLN, SN and maltose content. Therefore anyone of these methods can be used as an indication of preharvest sprouting.

6.5 CONCLUSIONS

The results of this study indicated that a highly significant correlation exists between FN and all the other methods used in this study. Similar correlations were observed by D'Appolonia *et al.*, 1982.

The FN method does not entirely reflect levels of α -amylase activity as other factors such as the gelatinization properties of the starch and fiber, can have noticeable influences (Kruger & Tipples, 1982). This might have been responsible for some of the variation that exists.

Hagberg (1960) and Perten (1964) described chemical methods for determining α -amylase activity, but the FN test, also devised by Hagberg, has proved to be more practical for measuring α -amylase activity than the conventional chemical methods. It is apparent that visual sprout damage assessment cannot accurately predict the level of α -amylase activity.

The poor correlation between α -amylase and sprout damage levels can be explained in a number of ways. Some kernels may have undergone "incipient" sprouting in which germination has proceeded without visible sprouting. Secondly, kernels

damaged in ways other than by sprouting may have elevated levels of enzyme associated with them. Finally, the level of α -amylase activity in sprouted wheat kernels can vary quite widely depending upon the severity of sprouting (Kruger & Tipples, 1980). Similarly, a very small percentage of severely sprouted kernels in a parcel of wheat, may result in a greater overall α -amylase level than if the entire sample had been very lightly sprouted.

For the evaluation of preharvest sprouting at the silo's, either the FN or SN methods would appear to be the methods of choice, since there is less operator expertise required, as is the case for the determination of α -amylase or maltose content. Ongoing costs are less, as substrate does not need to be continuously purchased. The reproducibility and repeatability of these methods were tested intensively (results not shown) and are considered satisfactorily high.

Plant breeders, on the other hand, would probably find that the determination of preharvest sprouting by means of a sprouting scale or visual determination is preferable, since these methods are a direct indication of the inherent capability of a cultivar or line to maintain tolerance to preharvest sprouting.

Regarding the WheatRite method, the various participants indicated that they could not clearly differentiate between the different samples visually. Since FN 150 being the lowest standard provided in the kit, the actual FNs could not be estimated. This poses a potential problem for producers who are supposed to use this kit in the field to determine their FNs. Since no differences could be obtained between FNs of 150 and 250 seconds in many cases, this is not a suitable option for the determination of FN for producers who want to confirm their FN before delivering to the silo's. If this test is used with a suitable reflectance reader, it might show different results.

There would be significant economic gain by harvesting sound and damaged grain separately. Research has consistently shown that estimates of FN on the basis of visual assessment alone are unreliable as a basis for commercial decisions. Sound

grain should be harvested first to avoid storage or harvesting with damaged grain, or the risk of damage by subsequent rain.

Currently growers may harvest grain across their whole property, and tests are done at silo level on the whole parcel of grain. However, except in very bad harvests, the extent and presence of preharvest sprouting can vary quite markedly between and within fields, being dependent on the rate of drying of the crop after rainfall has occurred, on the wheat cultivar sown, and on the time of sowing (Skerrit & Heywood, 2000). Growers usually have an intimate knowledge of the behaviour of different parts of their own property. If they were able to test the grain from different fields and parts of fields before harvest, it should be possible to harvest the damaged grain separately from sound grain and avoid the financial losses that result from downgrading the whole crop (Verity *et al.*, 1999).

REFERENCES

- AGROBASE. 2000. Agronomic Software Inc. Winnipeg, Manitoba, Canada.
- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 2000. Approved Methods of the AACC, 10th ed. Vol I. Measurement of α -amylase activity with the Rapid Visco Analyzer. *Method 22-08*. The Association: St. Paul, Minnesota.
- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 2000. Approved Methods of the AACC, 10th ed. Vol I. Measurement of Diastatic Activity of flour or Semolina. *Method 22-15*. The Association: St. Paul, Minnesota.
- BARNARD, A., PURCHASE, J.L., SMITH, M.F. & VAN LILL, D. 1997. Determination of the preharvest sprouting resistance of South African winter wheat (*Triticum aestivum* L.) cultivars. *S.A. J. Plant Soil* 14(1), 4-8.
- BARNES, W.C. & BLAKENEY, A.B. 1974. Determination of cereal α -amylase using a commercially available dye-labelled substrate. *Die Stärke*. 26 (6), 193-197.
- D'APPOLONIA, B.L., MCARTHUR, L.A., PISEOOKBUNTERNG, W. & CIACCO, C.F. 1982. Comparison of the grain amylase analyser with the amylograph and falling number method. *Cereal Chem.* 59, 254-257.
- HAGBERG, S., 1960. A rapid method for determining α -amylase activity. *Cereal Chem.* 37, 218-228.
- JENSEN, S.A., MUNCK, L. & KRUGER, J.E. 1984. A rapid fluorescence method for assessment of preharvest sprouting of cereal grains. *J. Cereal Sci.* 2, 187-201.
- MCMASTER, G.J. & DERERA, N.F. 1976. Methodology and sample preparation when screening for sprouting damage in cereals. *Cereal Res. Commun.* 4(2), 251-254.
- KRUGER, J.E. 1994. Enzymes of sprouted grain and possible technological significance. In: W. Bushuk and V. Rasper (eds). *Wheat: Production, properties and quality*. Blackie, Glasgow, UK. pp 143-153.
- KRUGER, J.E. & TIPPLES, K.H. 1980. Relationships between Falling Number, amylograph viscosity and α -amylase activity in Canadian wheat. *Cereal Res. Commun.* Vol 8(1), 97-105.

- KRUGER, J.E. & TIPPLES, K.H. 1982. Comparison of the Hagberg Falling Number and modified grain amylase analyser methods for estimating sprout damage in rye. *Can. J. Plant Sci.* 62, 839-844.
- MARES, D.J. 1989. Preharvest sprouting damage and sprouting tolerance: Assay methods and instrumentation. In: N.F. Derera (ed). *Preharvest field sprouting in cereals*. CRC Press, Boca Raton, FL. pp 129-170.
- PERTEN, H. 1964. Application of the falling number method for evaluating α -amylase activity. *Cereal Chem.* 41, 127-140.
- SKERRIT, J.H. & HEYWOOD, R.H. 2000. A five-minute field test for on-farm detection of preharvest sprouting in wheat. *Crop Sci.* 40, 742-756.
- VERITY, J.C.K., HAC, L. & SKERRIT, J.H. 1999. Development of a field Enzyme-Linked Immunosorbent Assay (ELISA) for detection of α -amylase in preharvest sprouted wheat. *Cereal Chem.* 76 (5), 673-681.

CHAPTER 7

THE GENETIC VARIABILITY OF PREHARVEST SPROUTING IN WHEAT

7.1 ABSTRACT

Preharvest sprouting resistance is a major breeding criterion in many regions. Screening for preharvest sprouting tolerance is difficult owing to the low heritability of the trait and its tendency to be expressed as a quantitatively inherited character. The screening procedure currently in use at the ARC-Small Grain Institute is designed to assess variation to sprouting in intact heads. Five winter wheat cultivars were crossed in a complete diallel fashion to generate two sets of F1 hybrids which were used in this study. The objectives of the study were to determine the genetic variability of sprouting resistance in the progeny of five winter wheat cultivars, including Elands, a cultivar with excellent sprouting resistance. The preharvest sprouting response and α -amylase activity of these cultivars and hybrids were compared. Elands was identified as contributing positively towards preharvest sprouting tolerance in hybrid combinations. Elands also had the best overall combining ability for the improvement of sprouting tolerance.

7.2 INTRODUCTION

Seed dormancy is the main factor responsible for conferring sprouting resistance to wheat. It is a desirable grain characteristic, especially in areas where humid harvest conditions are of frequent occurrence.

Sprouting of seed in wetted spikes is affected by seed dormancy, but also by other traits such as awns (Pool & Paterson, 1958; King & Richards, 1984), club spike (King & Richards, 1984), erectness of spike, openness of florets, tenacity of glumes (Paterson *et al.*, 1989) and the level of germination-inhibiting compounds in the bracts (Derera & Bhatt, 1980).

Heritabilities and genetic variation must be known to effectively exploit traits in breeding programs. Upadhyay & Paulsen (1988) suggested that these parameters must be measured in more than one cross in the case of preharvest sprouting resistance.

During the past decade, the development of wheat cultivars with harvest-time seed dormancy has been a breeding objective in many countries where moist harvest conditions can occur. The inheritance of preharvest sprouting resistance and many of its associated traits, particularly in the major genetic sources, however, is largely unknown.

Protocols to identify genotypes with improved sprouting resistance have evolved as two basic approaches: (i) sprouting tests of intact spikes subjected to a wetting treatment and (ii) germination tests of threshed seed. The first approach is intended to parallel field conditions but with greater control of rainfall-induced weathering immediately following physiological maturity. The second approach, based on germination tests, provides a direct measurement of seed dormancy, controlled by the embryo (Wu & Carver, 1999).

Intact-spike sprouting tests and germination tests usually produce results with strong genetic control and a high degree of interrelation, provided that spikes are sampled at a consistent maturity stage, usually physiological maturity, to minimise effects of differential dormancy (DePauw & McCaig, 1991; Hucl, 1994; Trethowan, 1995).

Numerous cultivars grown in Australia and Canada have been evaluated as potential sources of preharvest sprouting tolerance and breeding programs at the ARC-Small Grain Institute are currently involved in transferring the sources of tolerance identified into adapted South African varieties.

Screening for preharvest sprouting tolerance is difficult owing to the low heritability of the trait and its tendency to be expressed as a quantitatively inherited character. Further, the preharvest sprouting phenotype is complex in that the genes involved may be expressed in any one of three distinct tissues, the maternal plant, the endosperm and the embryo, of which the latter two belong genetically to the next generation (Lawson *et al.*, 1997).

The objective of this study was to determine the genetic variation of preharvest sprouting, α -amylase activity, and various other traits in five winter wheat cultivars with a wide spectrum of preharvest sprouting resistance as parents and the F₂ population.

7.3 MATERIALS AND METHODS

7.3.1 Experimental Material

Five winter wheat cultivars varying in preharvest sprouting tolerance (Table 7.1), namely Elands, Karee, PAN 3211, SST 363 and Tugela-DN were utilised in this study.

- Elands is an intermediate wheat cultivar with excellent adaptation to the Eastern Free State and is also well adapted to the Western and Central Free State. Elands has excellent quality characteristics and also excellent preharvest sprouting tolerance.
- Karee was a very popular cultivar for many years due to its stable yield production and good quality.
- PAN 3211 is a popular drought tolerant cultivar with excellent baking quality and good hectolitre mass.
- SST 363 is a short grower with wide adaptation.
- Tugela-DN has made a huge contribution to the total winter wheat production in South Africa, but due to its quality characteristics and poor preharvest sprouting resistance, it is not required by the milling and baking industries anymore.

These cultivars were crossed in all possible combinations to determine the combining abilities of the parents.

Table 7.1 The preharvest sprouting score and description of the five cultivars used as parents in this study

Cultivar	Preharvest sprouting score	Description of preharvest sprouting resistance
Elands	1.1	Excellent
Karee	2.7	Good
PAN 3211	3.1	Good to moderate
SST 363	3.6	Moderate
Tugela-DN	7.1	Poor

7.3.2 Characteristics measured

The inheritance of preharvest sprouting is extremely complex. It is necessary to quantify the following components and to analyse the inheritance of each component separately. The following characteristics were measured:

- Preharvest sprouting resistance – The sprouting response of intact spikes to a uniform wetting treatment was determined in a rain simulator (McMaster & Derera, 1976).
- α -Amylase activity – α -Amylase activity was assayed according to a revised method of Barnes and Blakeney (1974) – see Chapter 6.
- Hectolitre mass – Hectolitre mass is an indication of the soundness in grains. This method determines the weight of grain required to fill a level hectolitre measure. For experimental purposes, the weight of the wheat required to fill a 100 ml measure, was determined and converted to kg hl⁻¹.
- Hardness – Wheat kernel texture (degree of hardness or softness) was determined by instrumental measurement (Single Kernel Characterisation System) of the force required to crush wheat kernels.
- Grain yield – Grain mass was calculated from the total mass of all the kernels of the primary and secondary tillers.
- Spikes per plant – The number of productive spikes per plant was counted.
- Kernels per spike – The total number of kernels of the primary and secondary spikes was counted.
- Thousand kernel mass – Kernel size is usually reported as mass per 1000 kernels.
- Diameter – The diameter of the kernels was determined by the Single Kernel Characterisation System (SKCS).
- Grain protein content – Protein content in wheat grain was determined by means of an Infralyser 260 whole grain analyser on a 12% moisture base.

7.3.3 Statistical analyses

7.3.3.1 Analysis of variance (ANOVA)

Prior to the commencement of the analysis of general and specific combining ability, differences between genotypes were investigated. The randomised block analysis of variance, mean squares, standard errors and coefficients of variance (CV) was calculated by the Agrobase 2000 computer program for the experimental material in a randomised block design. Griffing's (1956) Model 1 was used as the five wheat cultivars used in this study were regarded as the population about which conclusions were to be made.

Significant differences between genotypes, identified by this analysis of variance, allowed for the analysis of general and specific combining ability to proceed.

7.3.3.2 General and Specific Combining Abilities

Griffing's analysis (1956) provides information concerning general and specific combining abilities of the parents, maternal effects and the genetic basis of characteristics. In this study only Method 1 (parents, single-crosses and reciprocal crosses), as described by Griffing (1956), was used. This method was used to obtain the mean squares of the parents and crosses for the calculation of high-parent heterosis.

7.3.3.3 Phenotypic correlations

The Agrobase 2000 computer program was used to compute phenotypic correlations (r) between the characteristics measured in this study. Phenotypic correlations were determined using the computational formula:

$$r_A = \frac{Cov_{ij}}{\sqrt{\sigma_i \sigma_j}}$$

to determine the phenotypic correlation between two characteristics *i* and *j*. Least significant value for P was also determined by the Agrobase 2000 program.

7.4 RESULTS AND DISCUSSION

7.4.1 Analysis of variance

The results of the analysis of variance done on preharvest sprouting resistance and α-amylase activity are summarised in Table 7.2. Differences between genotypes were highly significant (P<0.01) for both characteristics.

Table 7.2 Combined analysis of variance for preharvest sprouting resistance and α-amylase activity

Source	df	Preharvest Sprouting Resistance	α-amylase activity
Total	99		
Rep	3	0.397	721.067
Entry	24	4.631**	129 332.669**
Residual	72	0.371	310.963

** significant at P=0.01

The genotype means for preharvest sprouting and α -amylase are given in Table 7.3. The sprouting resistance of the parents varied considerably, as described in Table 7.1. Elands, as well as the hybrid Elands x PAN 3211, performed significantly better ($P < 0.05$) than the rest of the hybrids evaluated. The hybrids with the highest sprouting resistance, were Elands x PAN 3211, Karee x Elands, SST 363 x Elands and Tugela-DN x Elands, while PAN 3211 x Elands, Elands x Karee and Elands x SST 363 also showed good preharvest sprouting resistance (sprouting scale 2.1 to 2.9). None of the progeny, however, exceeded the resistance of Elands, although some had statistically similar resistance. These results show the positive contribution of Elands towards increasing preharvest sprouting resistance.

Tugela-DN had the poorest sprouting resistance with the hybrid Tugela-DN x SST 363 also showing moderate to poor sprouting resistance (sprouting scale 4.0 to 4.5). However, resistance to sprouting was higher in the progeny than in the susceptible parents.

The genotype means for α -amylase gave similar results. Tugela-DN again had the highest value (772.8), indicating a high α -amylase activity, while Elands and the Elands hybrids had low values (100 – 200), which is indicative of a low enzyme activity.

Table 7.3 Genotype means for preharvest sprouting resistance and α -amylase activity

Cultivar	Preharvest Sprouting Resistance	α -amylase
Tugela-DN	7.10	772.8
Karee	2.65	203.5
Elands	1.13	175.0
SST 363	3.55	518.5
PAN 3211	3.08	174.3
Tugela-DN/Karee	3.63	154.5
Tugela-DN/Elands	2.45	253.3
Tugela-DN/SST 363	4.08	646.0
Tugela-DN/PAN 3211	2.83	270.3
Karee/SST 363	2.55	201.0
Karee/PAN 3211	2.85	155.8
Karee/Tugela-DN	2.55	488.0
Karee/Elands	2.20	137.0
PAN 3211/Karee	2.70	260.3
PAN 3211/Tugela-DN	3.28	473.8
PAN 3211/Elands	2.68	208.5
PAN 3211/SST 363	3.10	313.0
Elands/Karee	2.73	103.5
Elands/PAN 3211	1.75	106.3
Elands/SST 363	2.68	347.0
Elands/Tugela-DN	3.40	135.0
SST 363/PAN 3211	3.13	181.5
SST 363/Karee	3.13	191.3
SST 363/Tugela-DN	4.23	152.8
SST 363/Elands	2.38	499.5
LSD _(0.05)	0.7177	20.777
CV (%)	20.1	6.19

7.4.2 Combining ability for preharvest sprouting and α -amylase activity

An analysis of variance was performed for preharvest sprouting and α -amylase activity. The mean squares for GCA, SCA, reciprocal effects and error are listed in Table 7.4. GCA mean squares, as well as SCA mean squares, were highly significant ($P < 0.01$) for both characteristics. No significant reciprocal mean squares ($P > 0.01$) were recorded for these characteristics.

Table 7.4 Analysis of variance of general combining ability (GCA) and specific combining ability (SCA) effects for preharvest sprouting resistance and α -amylase activity in a 5 x 5 diallel

Source	df	Preharvest Sprouting Resistance	α -amylase activity
Total	24		
GCA	4	4.556**	86 995.823**
SCA	10	0.763**	19 148.560**
Reciprocal	10	0.193	23 652.713
Residual	72	0.093	77.741

** significant at $P = 0.01$

7.4.3 General combining ability (GCA)

General combining ability effects were determined for preharvest sprouting and α -amylase and are presented in Table 7.5. Positive general combining ability is an indication of an increase for the specific characteristic evaluated, while a negative combining ability indicates a reduction in the characteristics measured. In this case, due to the fact that the sprouting scale is structured in such a way that a low value indicates a high sprouting resistance, while a high value is an indication of poor resistance to preharvest sprouting, the opposite is true. This also applies to α -

amylase where the absence of the enzyme indicates good preharvest sprouting resistance and *vice versa*.

The GCA for preharvest sprouting of Elands was significantly better ($P < 0.05$) than that of the other parents. Tugela-DN, being highly susceptible, should not be used as a parent. In terms of combining ability, Elands can be regarded as the best parent, followed by Karee and PAN 3211.

The GCA for α -amylase of Karee and Elands were significantly better ($P < 0.05$) than that of Tugela-DN and SST 363. The GCA of PAN 3211 was also significantly better than that of Tugela-DN and SST 363, but due to the low inherent FNs of PAN 3211, this cultivar should not be used as a parent.

The parent with the best overall combining ability for these two traits, was Elands, with the highest combining abilities for preharvest sprouting and α -amylase.

Table 7.5 General combining abilities (GCA) of five wheat cultivars for preharvest sprouting resistance and α -amylase activity

Parents	Preharvest Sprouting Resistance	α -Amylase activity
Tugela-DN	1.0315	127.0200
Karee	-0.2685	-75.0550
Elands	-9.7810	-70.8800
SST 363	0.2040	72.0200
PAN 3211	-0.1860	-53.1050
LSD _(0.05)	0.925	77.35

7.4.4 Specific combining ability (SCA)

Specific combining ability for a given characteristic is probably the most important aspect to consider when combining lines for that characteristic. The specific combining ability mean squares for preharvest sprouting resistance and α -amylase are presented in Table 7.6.

Significant differences ($P < 0.05$) for preharvest sprouting resistance were observed for Tugela-DN x Karee, Tugela-DN x Elands, Tugela-DN x SST 363, Tugela-DN x PAN 3211 and Karee x SST 363. For α -amylase, Tugela-DN x Elands, Tugela-DN x SST 363 and Karee x SST 363 were significantly different ($P < 0.05$) from the other hybrids. SST 363 x PAN 3211, Tugela-DN x Karee and Karee x Elands showed good specific combining abilities.

Table 7.6 Specific combining abilities (SCA) of ten wheat hybrids for preharvest sprouting resistance and α -amylase activity

Hybrid	Preharvest Sprouting Resistance	α -amylase activity
Tugela-DN X Karee	-0.7065	-15.6
Tugela-DN X Elands	-0.3563	-146.9
Tugela-DN X SST 363	-0.1165	-84.5
Tugela-DN X PAN 3211	-0.8265	13.2
Karee X Elands	0.4810	-18.7
Karee X SST 363	-0.1290	-85.7
Karee X PAN 3211	0.1985	51.3
Elands X SST 363	0.0710	137.2
Elands X PAN 3211	0.1485	-3.5
SST 363 X PAN 3211	0.0635	-56.5
LSD _(0.05)	0.925	77.35

7.4.5 Phenotypic correlations

Phenotypic correlations were calculated between all the traits measured to determine the influence of the different characteristics on one another. A phenotypic correlation matrix is presented in Table 7.7.

Although only 52%, highly significant correlations were observed between preharvest sprouting resistance and α -amylase activity ($P < 0.01$). A significant positive correlation between values for sprouting of kernels in intact spikes and α -amylase activity in the kernels has also been reported by Derera *et al.* (1977), Gordon *et al.* (1977), Bhatt *et al.* (1981), Soper *et al.* (1989) and DePauw *et al.* (1990).

The moderate correlation that was found, is typical of complex inherited traits. According to Upadhyay & Paulsen (1988) α -amylase is probably not an appropriate selection criterion for preharvest sprouting resistance because of the relatively low correlation with visual sprouting after simulated rain.

Sprouting resistance was also significantly correlated with hardness ($P < 0.05$), kernels per spike and diameter. α -Amylase was found to be significantly correlated with hardness ($P < 0.01$), yield ($P < 0.05$) and grain protein content ($P < 0.05$).

The significant correlation between preharvest sprouting resistance, α -amylase and kernel hardness implies that when breeding for an increase in kernel hardness, preharvest sprouting resistance will also be increased.

This correlation contrasts with the results of Rajaram *et al.* (1992), who observed no significant correlation between preharvest sprouting and grain hardness. However, comparison is difficult because they used FN as a measure of sprout damage, whereas this study used the sprouting response (measured as a sprouting scale) and

α -amylase activity. They also evaluated the natural sprouting response, whereas preharvest sprouting was simulated in a rain simulator in this study.

Strong significant correlations were observed for yield and spikes per plant and kernels per spike ($P < 0.01$), as well as thousand kernel mass and diameter ($P < 0.05$). Thousand kernel mass also correlated significantly ($P < 0.01$) with kernel diameter.

Table 7.7 Phenotypic correlations between preharvest sprouting resistance, α -amylase activity, yield and yield components in a 5 x 5 diallel

	PHS	α -Amylase	HLM	Hardness	Yield	Spikes/ plant	Kernels/ spike	TKM	Kernel Diameter
α -Amylase	0.5236**								
HLM	-1.823	0.0776							
Hardness	0.3447*	0.4243**	0.0288						
Yield	0.0893	0.2022*	-0.0150	0.0388					
Spikes/plant	-0.0687	0.1597	0.0517	0.2406	0.6775**				
Kernels/spike	0.3001*	0.1464	-0.1802	-0.0628	0.2782**	-0.1802			
TKM	0.1814	0.1892	0.2095*	-0.2217	0.2568*	0.0017	0.1347		
Diameter	0.2612*	0.2628	0.1840	-0.0444	0.2109*	-0.0093	0.1099	0.9530**	
GPC	0.2347	0.3200*	0.0094	-0.2194	0.0280	-0.0739	0.2855*	0.2440	0.1623

* Significant at P = 0.05

** Significant at P = 0.01

7.5 CONCLUSIONS

The primary objective of this study was to determine the combining abilities and heritabilities of five winter wheat cultivars for preharvest sprouting resistance and α -amylase activity and to identify the best combinations in the diallel. Preharvest sprouting of wheat is troublesome in most regions, even after the considerable attention was given to the problem (Lawson *et al.*, 1997). Lack of strong genetic resistance, incomplete understanding of its mechanism, emphasis on other attributes and the sporadic occurrence of sprouting have all undoubtedly contributed to the situation (Upadhyay & Paulsen, 1988). The availability of genetic sources of resistance, combined with suitable selection schemes, should however, speed progress toward a solution.

Present results indicate that incorporating resistance to preharvest sprouting into desirable wheat phenotypes, is an attainable objective. Lower mean preharvest sprouting scores and α -amylase activity in the hybrids showed that genes for resistance were effectively transferred from Elands.

It would appear that protection derived from a single component of already complex sprouting resistance might not be adequate under certain environmental conditions. Therefore, in order to provide adequate resistance to sprouting, efforts should be made to combine different components into a variety through extensive crossing programmes involving varieties known to be promising with regard to one or more components (Derera *et al.*, 1977).

The segregating populations should then be thoroughly screened for a combination of as many components as possible in an effort to evolve varieties with multiple resistance to preharvest sprouting (Derera *et al.*, 1977). Upadhyay & Paulsen (1988) suggested that screening for preharvest sprouting resistance should be based directly on sprouting of kernels in intact spikes.

In seeking to breed cultivars with resistance to preharvest germination in the ear, rapid progress could be expected if the aspects of inheritance of all the characteristics contributing to resistance are appreciated and selection is made for the desired combination of these characters (Bhatt *et al.*, 1977).

REFERENCES

- AGROBASE, 2000. Agronomic Software Inc. Winnipeg, Manitoba, Canada.
- BHATT, G.M., DERERA, N.F. & McMASTER, G.J. 1977. Utilization of Tom Thumb source of preharvest sprouting tolerance in a wheat breeding programme. *Euphytica* 26, 565-572.
- BHATT, G.M., PAULSEN, G.M., KULP, K. & HEYNE, E.G. 1981. Preharvest sprouting in hard winter wheats: assessment of methods to detect genotypic and nitrogen effects and interactions. *Cereal Chem.* 58, 300-302.
- BARNES, W.C. & BLAKENEY, A.B. 1974. Determination of cereal α -amylase using a commercially available dye-labeled substrate. *Die Stärke*. 26 (6), 193-197.
- DEPAUW, R.M. & McCAIG, T.N. 1991. Components of variation, heritabilities and correlations for indices of sprouting tolerance and seed dormancy in *Triticum* spp. *Euphytica* 52, 221-229.
- DEPAUW, R.M., McCAIG, T.N., MARES, D., BRENNAN, P., HENRY, R.J., KING, R., McEWAN, J.M. & GORDON, I. 1990. Interrelationships among assays for germination of kernels from threshed spikes, unthreshed spikes and α -amylase in wheat. In: K. Ringlund, E. Mosleth and D.J. Mares (eds). *Fifth International Symposium on Preharvest Sprouting in Cereals*. Loen, Norway. pp 195-205.
- DERERA, N.F. & BHATT, G.M. 1980. Germination inhibition of the bracts in relation to preharvest sprouting tolerance of wheat. *Cereal Res. Commun.* 8, 199-201.
- DERERA, N.F., BHATT, G.M. & McMASTER, G.J.. 1977. On the problem of preharvest sprouting in wheat. *Euphytica* 26, 299-308.
- GORDON, I.L., DERERA, N.F. & BALAAM, L.M.. 1977. Selection against sprouting damage in wheat. I. Germination of unthreshed grain with a standard wetting procedure. *Aust. J. Agric. Res.* 28, 583-596.
- GRIFFING, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.* 9, 463-493.

- HUCL, P. 1994. Repeatability of a simplified method for determining sprouting resistance in wheat. *Plant Varieties Seeds* 7,79-84.
- KING, R.W. & RICHARDS, R.A. 1984. Water uptake in relation to preharvest sprouting in wheat: Ear characteristics. *Aust. J. Agric. Res.* 35, 327-336.
- LAWSON, W.R., GODWIN, I.D., COOPER, M. & BRENNAN, P.S. 1997. Genetic analysis of preharvest sprouting tolerance in three wheat crosses. *Aust. J. Agric. Res.* 48, 215-221.
- McMASTER, G.J. & DERERA, N.F., 1976. Methodology and sample preparation when screening for sprouting damage in cereals. *Cereal Res. Commun.* 4(2), 251-254.
- PATERSON, A.H., SORRELLS, M.E. & OBENDORF, R.L. 1989. Methods of evaluation for preharvest sprouting resistance in wheat breeding programs. *Can. J. Plant Sci.* 69, 681-689.
- POOL, M. & PATERSON, F.L. 1958. Moisture relations in soft red wheats. II. Awned versus awnless and waxy versus nonwaxy glumes. *Agron. J.* 50, 158-160.
- RAJARAM, S., ALBARRAN, M., PENA, R.J. & AMAYA, A. 1992. Advances in breeding for sprouting tolerance in wheat. In: M.K. Walker-Simmons and J.L. Ried (eds). *Preharvest sprouting in Cereals*. Pullman, Washington, pp 104-112.
- SOPER, J.F., CANTRELL, R.G. & DICK, J.W. 1989. Sprouting damage and kernel colour relationships in durum wheat. *Crop Sci.* 29, 895-898.
- TRETHOWAN, R.M. 1995. Evaluation and selection of bread wheat (*Triticum aestivum* L.) for preharvest sprouting tolerance. *Aust. J. Agric. Res.* 46, 463-474.
- UPADHYAY, M.P. & PAULSEN, G.M. 1988. Heritabilities and genetic variation for preharvest sprouting in progenies of Clarke's Cream white winter wheat. *Euphytica* 38, 93-100.
- WU, J. & CARVER, B.F. 1999. Sprout damage and preharvest sprout resistance in hard white winter wheat. *Crop Sci.* 39, 441-447.

CHAPTER 8

THE USE OF A PROTEIN MARKER TO FOLLOW PREHARVEST SPROUTING RESISTANCE IN WHEAT

8.1 ABSTRACT

Preharvest sprouting resistance (i.e. high FN values) is a major objective in the breeding programme of the ARC-Small Grain Institute. Sprouting is expressed as a quantitative character and is affected by environmental conditions. Polyacrylamide gel electrophoresis (PAGE) has been used extensively for the study of gliadins. PAGE has been employed to a more limited extent for characterisation of soluble wheat protein patterns. The objectives of this study were to explore PAGE as a means for establishing profiles of soluble proteins of five wheat cultivars varying in their resistance to preharvest sprouting and their ensuing progeny. A protein duplet, which could possibly be associated with resistance to preharvest sprouting, was detected in five of the F2 combinations. Although the protein duplet correlated well with the preharvest sprouting scores of the cultivars and their progeny, the association is not strong enough to be of practical value.

8.2 INTRODUCTION

Wheat is one of the most widely grown food grains. Rain just prior to or during harvest can induce germination in the ear. Sprouting, as this is referred to, consists of a complex sequence of events, and eventually degrades starch and protein reserves in mature grains (Derera, 1982). In addition, the preharvest rain crop may give an inferior yield and grain with a reduced hectolitre mass.

To alleviate the possible sprouting risk, the development of cultivars that are able to tolerate or resist the damaging effects of rain during harvest time should be a major objective in a breeding programme. The poligenic inheritance of sprouting resistance, however, makes it a difficult characteristic to incorporate into new cultivars (Lukow *et al.*, 1989). Current breeding strategies involve selection of sprouting resistant lines by using labour-intensive assessment of grain subjected to simulated rainfall. Alternative selection techniques, such as electrophoretic analysis, which require less time and grain (half a kernel per test can be used), would be desirable. As early as 1981, researchers identified markers of baking quality that could be used as selection criteria in breeding programs (Payne *et al.*, 1981). These results were confirmed and extended to other quality tests using other genotypes in several different countries (Morel, 1994).

Several researchers (Payne *et al.*, 1981, 1984, Campbell *et al.*, 1987, Lawrence *et al.*, 1987, 1988) have studied high molecular weight (HMW) glutenin subunits and their relation to the breadmaking potential of a flour. Individual gliadins have also been found to be associated with desirable and undesirable breadmaking characteristics (Campbell, *et al.*, 1987), although there is apparently less agreement on which specific gliadins are influential. Polyacrylamide gel electrophoresis (PAGE) has been used extensively for studies of gliadins (Clements, 1990). However, studies by Gupta *et al.* (1991) and Khelifi & Branlard (1992) have indicated that a more effective predictive model of dough properties should include the composition of both the low and high molecular weight subunits of glutenin.

Lukow *et al.* (1989) indicated in their study that four gliadin bands, which were probably linked to improved FN, were transferred to the cultivar Columbus through a crossing program between RL 4137 and Neepawa. Such an association would make PAGE a useful method for selection for sprouting resistance in segregating populations of a breeding program.

The aim of this study was to explore the possibility of using a protein marker to follow preharvest sprouting resistance in wheat. A-PAGE was used to determine the presence or absence of genetic material associated with preharvest sprouting.

8.3 MATERIALS AND METHODS

8.3.1 Genetic material

Five cultivars, namely Neepawa (susceptible), Clark's Cream (tolerant), Betta-DN (tolerant), RL 4137 (tolerant) and Tugela-DN (susceptible), were included in the initial screening. Five wheat cultivars varying in their preharvest sprouting tolerance (Table 8.1), namely Elands, Karee, PAN 3211, SST 363 and Tugela-DN were also screened by means of A-PAGE. These five cultivars were crossed in all combinations. The material generated was planted and the ensuing progeny (Table 8.2) was tested for possible markers.

Table 8.1 The preharvest sprouting score and description of the five cultivars used as parents in the study

Cultivar	Preharvest sprouting score	Description of preharvest sprouting resistance
Elands	1.1	Excellent
Karee	2.7	Good
PAN 3211	3.1	Good to moderate
SST 363	3.6	Moderate
Tugela-DN	7.1	Poor

Table 8.2 Crossing combinations to show the sequence used in the A-PAGE technique

		Female parents				
		Tugela-DN	Karee	Elands	SST 363	PAN 3211
1	Tugela-DN		6	7	8	9
2	Karee	12		13	10	11
3	Elands	21	18		19	20
4	SST 363	24	23	25		22
5	PAN 3211	15	14	16	17	

8.3.2 Extraction of proteins

The extraction of alcohol soluble proteins (gliadins) was performed according to Cooper (1987). Single seeds were crushed and transferred to 1,5 ml polypropylene centrifuge tubes. An ice cold extraction buffer, containing 0.05% pyronine G in 25% 2-chloroethanol, was added to the crushed seeds and thoroughly mixed. The tubes were allowed to stand overnight at room temperature. The tubes were centrifuged at 18 000 *rpm* and the supernatant was used for electrophoresis.

8.3.3 Electrophoresis

Composition of gels and electrode buffers were as described by Cooper (1987). Polyacrylamide gels (12% acrylamide – 0.375% bis-acrylamide) contained 2 M urea, 0.1% ascorbic acid, and 0.014% ferrous sulphate. Freshly prepared 1% (m/v) ammonium persulphate and 0.03% TEMED was added to the mixture prior to pouring the gel. The running buffer consisted of 0.4% glacial acetic acid and 5 mM glycine. Clean and dry gel cassettes were assembled according to the design of the equipment. Samples were loaded into the wells and the gel was placed in the electrophoresis tank, ensuring that the sample wells were completely filled. Electrophoresis was performed at 500 V (constant voltage). Water was circulated through the buffer tank to maintain the temperature at 15 – 20 °C. The gels were stained in 10% trichloroacetic acid with 1% (w/v) PAGE G90.

8.3.4 Simulation of sprouting conditions and assessment of tolerance

Intact ears were collected from the individual entries of the F2 population and were subjected to a uniform wetting treatment in a rain simulator as described in detail in Chapter 5.

8.4 RESULTS AND DISCUSSION

Typical results obtained by A-PAGE fractionation of extracts are presented in Figure 8.1. Five cultivars of which the preharvest sprouting tolerance was known, were initially screened. Two distinct protein bands, which could possibly be linked to sprouting resistance, were observed in the cultivars Clark's Cream, Betta-DN and RL 4137, which were all three resistant to preharvest sprouting. The bands were absent in the protein profiles of Neepawa and Tugela-DN, which were both highly susceptible to preharvest sprouting.

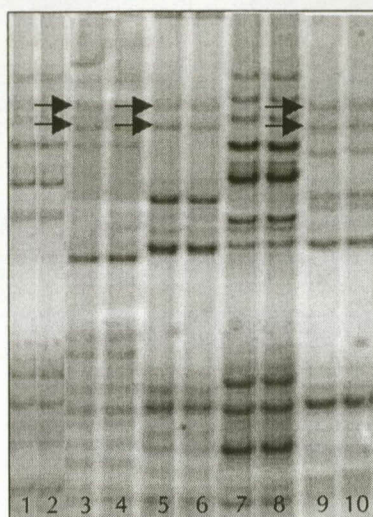


Figure 8.1 A-PAGE of the varieties Neepawa (1 & 2), Clark's Cream (3 & 4), Betta-DN (5 & 6), Tugela-DN (7 & 8) and RL 4137 (9 & 10). The arrows indicate the protein duplet which was detected in the cultivars with preharvest sprouting resistance.

The endosperm of the seed of the F1 plants of the different crossing combinations, as well as the parents, was evaluated according to the A-PAGE technique. The results obtained by A-PAGE fractionation of alcohol-soluble gliadin extracts are presented in Figure 8.2.

The protein duplet which was detected in the cultivars with preharvest sprouting resistance (Figure 8.1), was also observed in the four parents (Karee, Elands, SST 363 and PAN 3211) with good to moderate sprouting resistance. The duplet was also present in five of the F2 combinations, namely Karee/SST 363, Elands/Karee, Elands/PAN 3211, Elands/SST 363 and SST 363/Elands (Figure 8.2).

The preharvest sprouting score on a scale from 1 to 8 of the parents, as well as of the F2 population, are shown in Table 8.3.

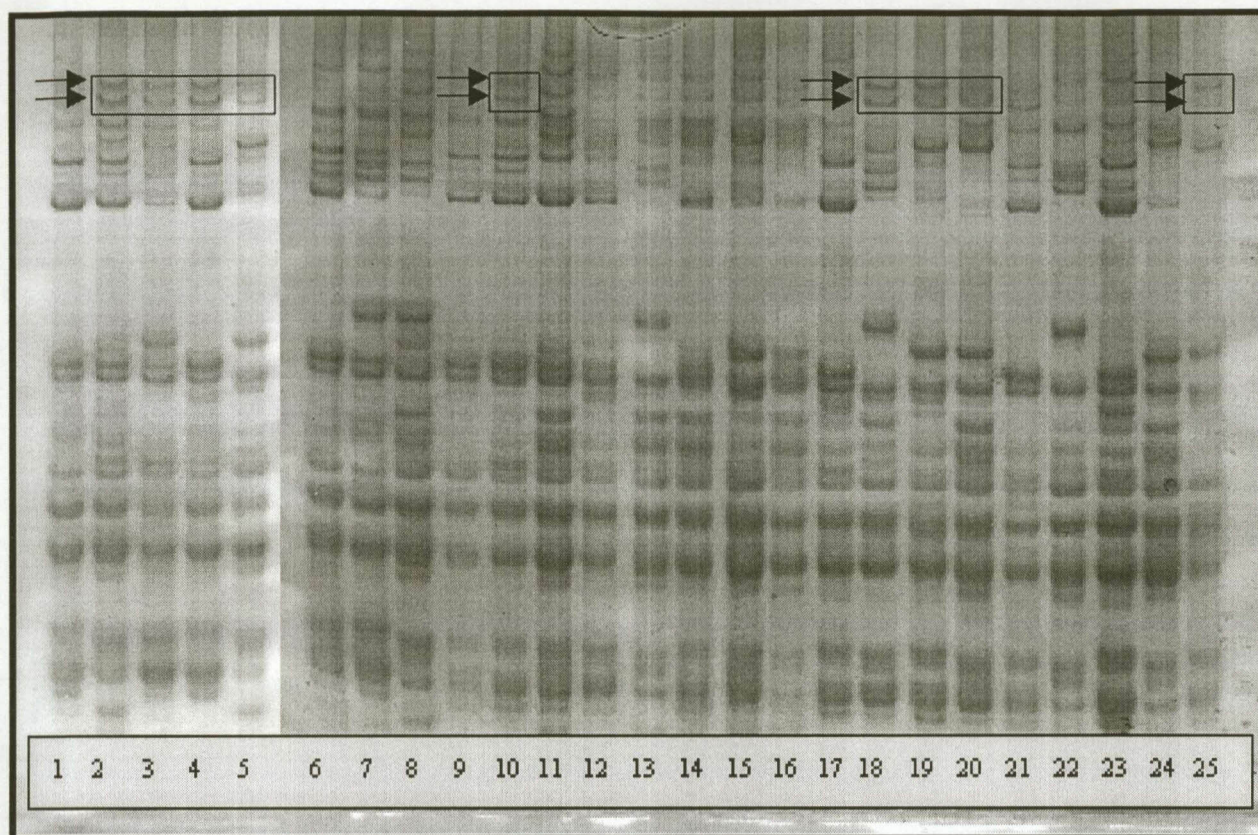


Figure 8.2 A-PAGE of gliadins extracted from seed of the parents (1-5), as well as the F2 seed of various crossings (6-25). The parents are (1) Tugela-DN, (2) Karee, (3) Elands, (4) SST 363 and (5) PAN 3211. The F2 progenies are (6) Tugela-DN/Karee, (7) Tugela-DN/Elands, (8) Tugela-DN/SST 363, (9) Tugela-DN/PAN 3211, (10) Karee/SST 363, (11) Karee/PAN 3211, (12) Karee/Tugela-DN, (13) Karee/Elands, (14) PAN 3211/Karee, (15) PAN 3211/Tugela-DN, (16) PAN 3211/Elands, (17) PAN 3211/SST 363, (18) Elands/Karee, (19) Elands/PAN 3211, (20) Elands/SST 363, (21) Elands/Tugela-DN, (22) SST 363/PAN 3211, (23) SST 363/Karee, (24) SST 363/Tugela-DN and (25) SST 363/Elands.

Table 8.3 Sprouting score of the five parents, as well as of the ensuing progeny after 72 h of simulated rainfall

Cultivar / cross	Preharvest sprouting score	Description of preharvest sprouting
Tugela-DN	7.1	Poor
Karee	2.7	Good
Elands	1.1	Excellent
SST 363	3.6	Moderate
PAN 3211	3.1	Good to moderate
Tugela-DN/Karee	3.6	Moderate
Tugela-DN/Elands	2.5	Good
Tugela-DN/SST 363	4.1	Moderate to poor
Tugela-DN/PAN 3211	2.8	Good
Karee/SST 363	2.6	Good
Karee/PAN 3211	2.9	Good
Karee/Tugela-DN	2.6	Good
Karee/Elands	2.2	Good
PAN 3211/Karee	2.7	Good
PAN 3211/Tugela-DN	3.3	Good to moderate
PAN 3211/Elands	2.7	Good
PAN 3211/SST 363	3.1	Good to moderate
Elands/Karee	2.7	Good
Elands/PAN 3211	1.8	Excellent
Elands/SST 363	2.7	Good
Elands/Tugela-DN	3.4	Good to moderate
SST 363/PAN 3211	3.1	Good to moderate
SST 363/Karee	3.1	Good to moderate
SST 363/Tugela-DN	4.2	Moderate to poor
SST 363/Elands	2.4	Good

Neither one of the F2 plants showed the same poor tolerance to sprouting as the susceptible parent, Tugela-DN. Sixty percent of the F2 population which were screened in the rain simulator, had a sprouting score between 1 and 2.9, which indicated excellent to good sprouting resistance. The crosses which consisted of the protein duplet (Figure 8.2), also scored relatively low preharvest sprouting values. Karee/SST 363, Elands/Karee, Elands/SST 363 and SST 363/Elands had good sprouting values (2.0 to 2.9), while the Elands/PAN 3211 cross had an excellent sprouting value (1.8).

Seven of the crosses, namely Tugela-DN/Elands, Tugela-DN/PAN 3211, Karee/PAN 3211, Karee/Tugela-DN, Karee/Elands, PAN 3211/Karee and PAN 3211/Elands also scored good preharvest sprouting values (2.1 to 2.9), although these crosses did not contain the protein duplet (Figure 8.2).

Due to the fact that the protein duplet was not present in all combinations with low sprouting scores, the visual percentage of sprouted kernels were determined on the proportion of the population which obtained preharvest sprouting scores of 1.0 to 2.9. This was conducted in order to determine if genetic variation occurred. Kernels were considered sprouted with the emergence of the radicle.

The results of the percentage sprouted kernels are shown in Figure 8.3. It is evident that 42% of the population (which obtained sprouting scores of 1.0 to 2.9) had a germination percentage of 0 to 20%, while 50% had a germination percentage of 21 to 50% and only 8% showed a germination percentage of higher than 51%.

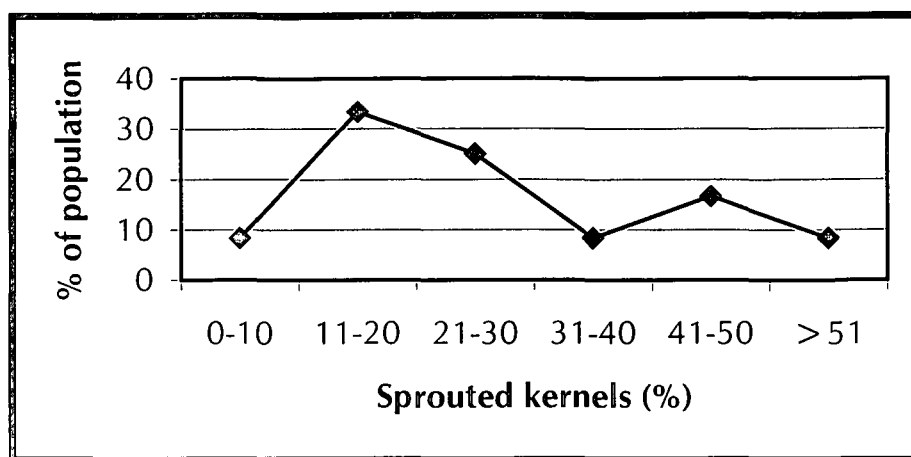


Figure 8.3 Percentage sprouted kernels of the F2 population which obtained a preharvest sprouting score of 1.0 to 2.9 in the rain simulator.

To obtain an overview of the genetic variation of the total F2 populations results, a combined data set was prepared from the visual sprouting resistance scores and the percentage sprouted kernels. It is evident that 25.2% of the F2 population consisted of the complete genetic source for preharvest sprouting resistance with a sprouting scale of 1.0 to 2.9 and percentage sprouted kernels less than 20%.

8.5 CONCLUSIONS

Although there is a correlation between the protein duplet present and the sprouting scores of the various cultivars and crosses, the association is not strong enough to be of practical value in selecting for sprouting resistance in wheat breeding programs based on the used parents as the source of resistance. Nevertheless, pre-screening of early generation lines for these bands, followed by a submission to simulated rainfall, and perhaps the determination of FN of the selected lines, would increase the relative selection efficiency of lines with improved sprouting resistance. Whether or not the apparent linkage of some gliadin bands to sprouting resistance may be a useful marker in wheat breeding programs, remains to be investigated with other crosses.

REFERENCES

- CAMPBELL, W.P., WRIGLEY, C.W., CRESSEY, P.J. & SLACK, C.R. 1987. Statistical correlations between quality attributes and grain-protein composition for 71 hexaploid wheats used as breeding parents. *Cereal Chem.* 64, 293-299.
- CLEMENTS, R.L. 1990. Polyacrylamide gel electrophoresis of salt-soluble proteins of soft wheats from the Eastern United States. *Cereal Chem.* 67(3), 264-267.
- COOPER, S R. 1987. Report of the Rules Committee 1983-1986. *Seed Sci & Technol.* 15, 555-575.
- DERERA, N.F. 1982. The harmful harvest rain. *Aust. J. Agric. Res.* 48, 67-75.
- GUPTA, R. B., BEKES, F. & WRIGHLEY, C.W. 1991. Prediction of physical dough properties from glutenin subunit composition in bread wheats: Correlation studies. *Cereal Chem.* 68, 328-333.
- KHELIFI, D. & BRANLARD, G. 1992. The effects of HMW and LMW subunits of glutenin and of gliadin on the technological quality of progeny from four crosses between poor breadmaking quality and strong wheat cultivars. *J. Cereal Sci.* 16, 195-209.
- LAWRENCE, G.J., MOSS, H.J., SHEPHERD, K.W. & WRIGLEY, C.W. 1987. Dough quality of biotypes of eleven Australian cultivars that differ in high-molecular-weight glutenin subunit composition. *J. Cereal Sci.* 6, 99-101.
- LAWRENCE, G.J. MACRITCHIE, F. & WRIGLEY, C.W. 1988. Dough and baking quality of wheat lines deficient in glutenin subunits controlled by the Glu-A1, Glu-B1 and Glu-D1 loci. *J. Cereal Sci.* 7, 109-112.
- LUKOW, O.M, DYCK, P.L. & BUSHUK, W. 1989. Possible linkage of falling number value with gliadin proteins in wheats with genes for improved sprouting resistance. *Cereal Chem.* 66(6), 531-532.
- MOREL, M.H. 1994. Acid-polyacrylamide gel electrophoresis of wheat glutenins: A new tool for the separation of high and low molecular weight subunits. *Cereal Chem.* 71(3), 238-242.

- PAYNE, P.I., HOLT, L.M. & LAW, C.N. 1981. Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. Part 1. Allelic variation in subunits amongst varieties of wheat (*Triticum aestivum*). *Theor. Appl. Genet.* 60, 229-236.
- PAYNE, P.I., JACKSON, E.A. & HOLT, L.M. 1984. The association between gliadin 45 and gluten strength in durum wheat varieties: A direct causal effect or the result of genetic linkage? *J. Cereal Sci.* 2, 73-81.

CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS

9.1 CONCLUSIONS

In this study it became evident that the sprouting responses of cultivars were to an extent triggered by different environmental factors. The occurrence of extreme climatic conditions had a major effect on cultivar responsiveness, whereas mild temperature differences during grain filling were secondary. Many South African cultivars showed good resistance to sprouting. By reducing the unpredictable effect of sprouting damage, wheat producers in South Africa will be able to produce a consistently good quality crop and compete on the international market.

Many problems are encountered with the grading of sprouted wheat and visual inspection of sprouting damage is not the preferred solution. Either the FN or SN methods would appear to be the preferred methods of choice, since there is less operator expertise required, as is the case for the determination of α -amylase or maltose content, and a much improved indication of α -amylase activity is attained.

The availability of genetic sources of resistance, combined with suitable selection protocols, should speed progress toward a solution. In seeking to breed cultivars with resistance to preharvest germination in the ear, rapid progress could be expected if the aspects of inheritance of all the characteristics contributing to resistance are taken into account and selection is made for the desired combination of these characters.

9.2 RECOMMENDATIONS

1. The G X E interactions were significant for preharvest sprouting. The response of the various cultivars differed from one region to another. It is therefore important that cultivar recommendations should be made per region. The more stable cultivars, in other words cultivars with a good tolerance to preharvest sprouting in every region, should be selected.
2. A relatively poor correlation exists between visual sprouting damage and Falling Number. Certain cultivars have a better ability to maintain a high FN during continuous rainfall than others. Previously cultivars were selected on the basis of tolerance to visual sprouting. With the implementation of the Falling Number method into the South African grading regulations, this selection criteria has to be revisited. Preliminary releases should be evaluated for their inherent Falling Number, as well as their ability to withstand wet and humid conditions during harvest ripe conditions. It is recommended that the α -amylase content of these lines is determined after exposure to simulated rainfall.
3. The Falling Number (FN) and Stirring Number (SN) methods showed good correlations with α -amylase content. Regulation states that the FN of a consignment wheat can be determined with any suitable method, on condition that the results may not vary more than 5% from the results obtained by the ICC Standard No 107/1. SN differs from FN in the expression of mixture viscosity, but complies with the ICC Standard regulations with regard to the 5% maximum deviation permitted. The SN method could therefore be recommended for the determination of FN and subsequent amylase activity. Both the FN and SN methods are good indications of the α -amylase activity in the wheat.

4. Elands is an intermediate wheat cultivar with excellent preharvest sprouting resistance. This cultivar was identified as contributing positively towards preharvest sprouting tolerance and also showed the best general combining ability. Elands can therefore be recommended for future breeding purposes to enhance sprouting resistance.

CHAPTER 10

SUMMARY

1. This study classified the preharvest sprouting resistance of 17 South African winter wheat cultivars sampled over a range of typical winter wheat growth environments. Variation in sprouting response between cultivars was predominantly genetically determined and varied from 1.1 to 7.2 on a scale from 1 to 8. In general Betta-DN, Elands, Limpopo, Caledon and PAN 3235 showed good resistance to preharvest sprouting, while the hybrids Carina, Caritha and Carol, as well as the pureline Tugela-DN, tended to be highly susceptible to sprouting. Betta-DN and Elands had the best stability over environments and years. Genotypic variation appeared to be dominant, indicating that progress in the development of cultivars with sprouting tolerance is feasible.
2. In this study the oxidative pentose pathway (OPP) activity in seeds of a preharvest sprouting susceptible winter wheat cultivar, Tugela-DN and that of a preharvest sprouting resistant cultivar, Betta-DN, were compared. G6PDH activity increased twofold in both the harvest ripe (HR) and after ripened (AR) seeds of Tugela-DN during the germination phase and threefold during the post germinative phase up to 72 h of incubation. However, the G6PDH activity remained constant in both the HR and AR seeds of Betta-DN during the first 24 h of incubation and increased only slightly in the HR seeds over the remaining incubation time. Moreover, a C6/C1 ratio of below unity in seeds of Tugela-DN during the germination phase, confirmed that the OPP pathway was far more active in this preharvest sprouting susceptible cultivar than in the resistant cultivar, Betta-DN, where the C6/C1 ratio remained above unity during the germination phase.

3. Downgrading of wheat stocks due to a low falling number is an intermittent problem in South Africa. This study determined the ability of South African winter wheat cultivars to withstand a specified amount of simulated rainfall. The falling number of cultivars that were exposed to optimal conditions for preharvest sprouting by using a rain simulator, dropped drastically in most cases. Betta-DN and Elands had a greater ability to maintain high FN during continuous rainfall than others. Limpopo, Caledon, PAN 3232 and PAN 3235 showed a high tolerance to preharvest sprouting after 72 h of simulated rain treatment, but their FN dropped to between 78s and 162s. FN determination after 72 h of rain simulation showed that visual determination could be inadequate. The G x E interactions for sprouting resistance were significant, which indicate that the more stable cultivars should be selected by breeders.
4. This study describes the findings in comparing various methods to determine preharvest sprouting and α -amylase activity. All the methods evaluated showed significant correlations and therefore any of these methods could be used for the determination of preharvest sprouting. However, results from this study indicated that the Stirring Number and Falling Number methods are the most reliable methods to use for the determination of preharvest sprouting and α -amylase activity in wheat.
5. Screening for preharvest sprouting tolerance is difficult due to the relatively low heritability of the trait and its tendency to be expressed as a quantitatively inherited character. The objectives of this study were to determine heritabilities and genetic variability of sprouting resistance in the progeny of five winter wheat cultivars, including Elands, a cultivar with excellent sprouting resistance. Five winter wheat cultivars were crossed in a complete diallel fashion to generate two sets of F1 hybrids that were used in this study. The preharvest sprouting response and α -amylase activity of these cultivars and hybrids were compared. Elands was identified as contributing positively

towards preharvest sprouting tolerance in hybrid combinations. Elands also had the best overall combining ability for improvement of sprouting tolerance.

6. Preharvest sprouting resistance (i.e. high falling number values) is a major objective in the breeding programme of the ARC-Small Grain Institute. Current breeding strategies involve selection of sprouting resistant lines by using labour-intensive assessment of grain subjected to simulated rainfall. Alternative selection techniques, such as electrophoretic analysis, which require less time and grain (half a kernel per test can be used), would be desirable. The objectives of this study were to explore PAGE as a means for establishing profiles of soluble proteins of five wheat cultivars varying in their resistance to preharvest sprouting and their ensuing progeny. A protein duplet, which could possibly be associated with resistance to preharvest sprouting, was detected in five of the F2 combinations. Although the protein duplet correlated well with the preharvest sprouting scores of the cultivars and their progeny, the association is not sufficiently strong to be of practical value.

OPSOMMING

1. In hierdie studie is die uitloopweerstand van 17 Suid-Afrikaanse winterkoringcultivars oor 'n wye reeks omgewingstoestande gekarakteriseer. Die variasie in die onderskeie uitloopreaksies is hoofsaaklik toe te skryf aan die genetiese eienskappe van die cultivars en het gevarieer tussen 1.1 en 7.2 op 'n skaal van 1 tot 8. Oor die algemeen het Betta-DN, Elands, Limpopo, Caledon en PAN 3235 die beste weerstand teen uitloop getoon, terwyl die basters Carina, Caritha en Carol, sowel as die suiwertelende Tugela-DN die swakste weerstand teen uitloop gehad het. Betta-DN en Elands het ook die beste stabiliteit oor omgewings en jare getoon. Genotipiese variasie was dominant, wat daarop dui dat dit moontlik is om cultivars met 'n verbeterde uitloopweerstand te teel.
2. Die werking van die oksidatiewe pentose fosfaatweg is in die saad van 'n uitloopvatbare cultivar, Tugela-DN, en 'n tolerante cultivar, Betta-DN, bestudeer. Glukose-6-fosfaat dehidrogenase (G6PDH) aktiwiteit het tweevoudig verhoog in beide die oesryp en naryp sade van Tugela-DN gedurende die ontkiemingsfase en drievoudig gedurende die na-ontkiemingsfase tot en met 72 h na inkubering. Die G6PDH-aktiwiteit het egter konstant gebly in beide die oesryp en naryp sade van Betta-DN gedurende die eerste 24 h van ontkieming en het slegs minimaal verhoog in die oesryp sade oor die res van die tyd. Die C6/C1 verhouding was onder 1 in saad van Tugela-DN, wat daarop dui dat die OPP-weg baie meer aktief was in die vatbare cultivar, as in die tolerante cultivar, Betta-DN, waar die C6/C1 verhouding bo 1 was gedurende die ontkiemingsperiode.
3. Die afgradering van graan as gevolg van lae valgetalle, is 'n geweldige probleem in Suid-Afrika. In hierdie studie is die vermoë van Suid-Afrikaanse winterkoringcultivars om vogtige oestoestande te weerstaan, bepaal. In die meeste gevalle het die valgetal drasties verlaag wanneer cultivars aan

optimale ontkiemingtoestande blootgestel is. Betta-DN en Elands kon hulle valgetalle konstant hou selfs na 72 h van gesimuleerde reënval. Limpopo, Caledon, PAN 3232 en PAN 3235 het 'n goeie toleransie teen uitloop getoon, maar na 72 h in 'n reënsimuleerder, het hulle valgetalle tussen 78s en 162s varieer. Die bepaling van valgetalle na 72 h gesimuleerde reënval het aangetoon dat visuele bepalings alleen onvoldoende is. Die G x E interaksie vir uitloopweerstand was betekenisvol en dui daarop dat die meer stabiele cultivars geselekteer moet word vir teeldoelindes.

4. Verskeie metodes vir die bepaling van uitloopskade en α -amilase aktiwiteit is in hierdie studie vergelyk. Al die metodes wat gebruik is, het baie goeie korrelasies getoon en derhalwe kan enigeen gebruik word vir die bepaling van uitloopskade. Resultate het egter aangetoon dat beide die valgetal- en roergetal-metodes die betroubaarste is en ook die beste herhaalbaarheid getoon het vir die bepaling van uitloopskade en ensiemaktiwiteit in koring.
5. Die karakterisering van uitloopweerstand is moeilik, aangesien dit 'n kwantitatiewe eienskap is en 'n relatief lae oorerflikheid het. Die doel van hierdie studie was om die oorerflikheid en genetiese variasie van uitloopweerstand in die nageslag van die vyf winterkoringcultivars te bepaal. Vyf winterkoringcultivars is in 'n volledige dialleel gekruis om twee stelle F1 basters te genereer wat in hierdie studie gebruik is. Die uitloopweerstand en α -amilase aktiwiteit van die cultivars en die basters is met mekaar vergelyk. Elands is geïdentifiseer as die cultivar met die beste kombineervermoë, asook die cultivar wat positief bygedra het tot die verbetering van uitloopweerstand.
6. Die verbetering van die uitloopweerstand van cultivars is 'n belangrike doelwit in die teelprogramme van LNR-Kleingraaninstituut. Huidiglik word vir uitloopweerstand geselekteer deur gebruik te maak van arbeidsintensiewe tegnieke met behulp van gesimuleerde reënval. Alternatiewe tegnieke, soos elektroforese wat vinniger resultate lewer, asook minder plantmateriaal

benodig, is wenslik. In hierdie studie is poliakrielamied-gel-elektroforese ondersoek as 'n moontlikheid om profiele van oplosbare proteïene saam te stel van vyf cultivars met 'n variasie in uitloopweerstand, asook hulle nageslag. 'n Proteïen-duplet, wat moontlik met uitloopweerstand geassosieer kan word, is in vyf van die F2 kombinasies gevind. Alhoewel hierdie duplet baie goed korreleer met die uitloopweerstandwaardes van die cultivars en hulle nageslag, is hierdie assosiasie nie sterk genoeg om prakties gebruik te word nie.

U.S.V.S. BIBLIOTHEK