

**RELATIONSHIPS BETWEEN MIXSMART PARAMETERS AND BREAD  
WHEAT QUALITY CHARACTERISTICS IN SOUTH AFRICAN DRY LAND  
CULTIVARS**

by

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## ABSTRACT

Ten hard red wheat genotypes from the National Cultivar Trials conducted in South Africa, were planted at three environments over two seasons in the eastern part of the summer rainfall area of South Africa. Wheat quality analysis, including hectolitre mass, falling number, grain and flour protein content, flour yield, corrected flour colour, farinograph analysis, alveograph analysis, mixograph analysis, loaf volume and corrected loaf volume were performed on these samples. The mixograph analysis was subjected to Mixsmart analysis and six midline Mixsmart parameters were selected based on their repeatability, coefficient of variation, coefficient of determination and their genotype contribution to variation. The selected parameters were midline left slope (MLS), midline peak time (MPT), midline peak value (MPV), midline right slope (MRS), midline right integral (MRI) and midline tail width (MTW). These parameters showed highly significant correlations with the fixed, non-negotiable primary quality criteria used during the cultivar classification process in South Africa. Therefore, they could be effective in assisting breeders during early selections for potential breeding lines that would exhibit acceptable primary criteria during the final evaluation phases for cultivar classification.

Stability for the parameters were investigated using AMMI and GGE-biplots. Genotypes varied in their stability. For only two of the Mixsmart parameters, MPT and MTW, the GGE-biplot indicated the most stable genotype also as the ideal genotype.

Over years scientists differed in total mixing time preferred for performing mixograph analysis (6, 8 or 10 min). This study investigated if differences in the mixing time would alter the relationships between the Mixsmart parameters and primary criteria. TimeX values were therefore set for 6, 8 and 10 min. ANOVA indicated envelope timeX slope (ETXS), envelope timeX width (ETXW), envelope timeX integral (ETXI), envelope timeX value (ETXV), midline timeX slope (MTXS), midline timeX width (MTXW), midline timeX integral (MTXI) and midline timeX value (MTXV) to show variation (causing differences at the

different time intervals), but when these parameters were eliminated from linear discriminant analyses (LDA), there were no variations in mixing time interval values for the three timeX times. Correlations did not differ significantly between the primary quality criteria and Mixsmart parameters at the different time intervals, indicating that relationships between Mixsmart parameters and primary quality criteria remained constant at the three timeX times.

Wheat quality analysis required for classification purposes of potential breeding lines, are conducted by the South African Grain Laboratory (SAGL) and breeders have to select the best representative samples, over a period of three years, of each breeding line over five environments for submission to SAGL. Breeders utilise white flour or whole meal when conducting pre-selection and it is of utmost importance that relationships with the primary criteria do not differ when different flour types are used. LDA confirmed that no differences existed between the white flour and whole meal used for mixograph analysis as indicators of acceptable quality.

**Keywords:** Wheat quality, Mixsmart<sup>®</sup> software, selection, stability, grain, milling, rheology, baking, mixograph.

## DECLARATION

I, Christina Wilhelmina Miles declare that this thesis, that I herewith submit for the Doctor of Philosophy degree in Plant Breeding at the University of the Free State, is my independent work and that I have not previously submitted it for a qualification at another institution of higher education. All sources of materials and financial assistance used for the study have been duly acknowledged. I also agree that the University of the Free State has the sole right to the publication of this thesis.

A handwritten signature in black ink, appearing to read 'Miles', enclosed within a circular scribble.

Signed on 16-05-2018 at ARC-Small Grain

## DEDICATION

I would like to dedicate this work to the most important people in my life:

- My late parents – Thank you for being a perfect example to me and for teaching me perseverance.
- My husband, Wayne – Thank you for bearing with me, for your patience and support.
- My two teenage daughters, Marisca and Chandré – Thank you for your support and understanding. I am sorry that I was not able to pay all my attention when it was required of me, so please take my advice – if you are fortunate to have the opportunity to study one day, please, complete your studies first and then you will be able to pay all your attention to your own children when they require that of you...

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- My family, Wayne, Marisca and Chandré
- God, since without His Grace, this would not have been possible

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## LIST OF ABBREVIATIONS

AEA	Average Environmental Axis
AMMI	Additive Main Effects and Multiplicative Interaction
ASV	AMMI stability value
BH	Bühler
BFLY	Break flour yield
Bhm	Bethlehem
Bult	Bultfontein
Cla	Clarens
CV%	Coefficient of variation
E	Environment
ELI	Envelope left integral
ELS	Envelope left slope
ELT	Envelope left time
ELV	Envelope left value
ELW	Envelope left width
EPI	Envelope peak integral
EPT	Envelope peak time
EPV	Envelope peak value
EPW	Envelope peak width
ERI	Envelope right integral
ERS	Envelope right slope
ERT	Envelope right time
ERV	Envelope right value
ERW	Envelope right width
ETI	Envelope tail integral
ETS	Envelope tail slope
ETV	Envelope tail value
ETW	Envelope tail width
ETXI	Envelope timeX integral
ETXS	Envelope timeX slope
ETXV	Envelope timeX value
ETXW	Envelope timeX width
E X Y	Environment year interaction
FABS	Farinograph water absorption

FAO	Food and Agriculture Organisation
FLN	Falling number
FLY	Flour yield
FPC	Flour protein content
GGE	Genotype main effects plus genotype environment interaction
G X E	Genotype environment interaction
GPC	Grain protein content
G-share	Genotype contribution to variation
G X Y	Genotype year interaction
HLM	Hectolitre mass
HMW-GS	High molecular glutenin subunits
$h^2$	Repeatability
IPCA	Interaction Principal Component Analysis
JQ	Junior Quadrumat
kg.h <sup>-1</sup>	Kilogram per hectolitre
KJ	Kent Jones
KJ76	Corrected flour colour
Lad	Ladybrand
LDA	Linear discriminant analysis
LFV	Loaf volume
LFV12%	Corrected loaf volume
LMW-GS	Low molecular weight glutenin subunits
Max	Maximum
m.b.	Moisture basis
Min	Minimum
MLI	Midline left integral
MLS	Midline left slope
MLT	Midline left time
MLV	Midline left value
MLW	Midline left width
MPI	Midline peak integral
MPT	Midline peak time
MPV	Midline peak value
MPW	Midline peak width
MRI	Midline right integral
MRS	Midline right slope

MRT	Midline right time
MRV	Midline right value
MRW	Midline right width
MTI	Midline tail integral
MTS	Midline tail slope
MTV	Midline tail value
MTW	Midline tail width
MTXI	Midline timeX integral
MTXS	Midline timeX slope
MTXV	Midline timeX value
MTXW	Midline timeX width
MS	Mean squares
PC	Principal Component
PCA	Principal Component Analysis
P/L	Alveograph stability/distensibility ratio
R <sup>2</sup>	Coefficient of determination
SAGL	Southern African Grain Laboratory
s	Seconds
SS	Sum of squares
Std.dev	Standard deviation
STR	Alveograph dough strength
WM	Whole meal
W-value	Alveograph W-value
Y	Year
%	Percentage
07	2007
09	2009

# CHAPTER 1

## INTRODUCTION

Bread wheat is one of the most important grain crops, forming 81% of the total winter cereal crop in South Africa (SAGL, 2017). South Africa has three major wheat production regions: winter rainfall area (Western Cape), summer rainfall area (Free State) and irrigation areas (Mpumalanga, Eastern Free State, Kwazulu-Natal and Eastern Cape), where planting occurs from mid-April until the end of July and harvesting occurs from late October until early January (Department of Agriculture, 2012; SAGL, 2017).

Until now, South Africa's breeding programmes focussed on quality and potential breeding lines could only be commercially classified if they exhibited better quality characteristics when compared to a biological standard for that specific area. Deviations from the biological standard's quality characteristics are allowed (a complete discussion of these deviations will follow in Chapter 2). Three years' data are required for final classification, and each year, the potential breeding line as well as the quality standard have to be evaluated across five environments (localities) (SAGL, 2013). Classification norms include primary and secondary criteria, where primary criteria are fixed and non-negotiable. Primary criteria include hectolitre mass (HLM), falling number (FLN), grain protein content (GPC), flour protein content (FPC), flour yield (FLY), corrected flour colour (KJ76), mixograph mixing time (MPT), farinograph water absorption (FABS), alveograph stability/distensibility ratio (P/L), alveograph dough strength (STR), loaf volume (LFV) and corrected loaf volume (LFV12%) (SAGL, 2013).

The world wheat crop is used for human consumption, animal fodder and industrial applications (FAO, 2013), like biofuel production and the demand for wheat is estimated to increase with 60% by 2050 (Singh et al., 2011). It is well known that yield and quality are negatively correlated, which makes it more challenging to satisfy the whole wheat chains' needs (Peña et al., 2002; Shewry, 2007; Saint Pierre et al., 2008). Producers want higher yields, millers want higher

flour yields, bakers want higher dough yields and higher LFV and consumers require a tasty end product.

Wheat breeding programmes in South Africa therefore, focus on higher yields, better quality, better processing properties and increased disease resistance. Thus, yield does not form part of the classification process yet, but amendments proposed include (i) yield to be incorporated as part of the classification process, (ii) relaxed quality criteria regarding certain quality parameters for high yielding lines, and (iii) regarding irrigation wheat, that only two years' data are required for final classification (SAGL, 2017).

Wheat quality is complex and is affected by environmental conditions (Mann et al., 2009; Castillo et al., 2012) resulting in difficulty to understand bread making quality of wheat flour (MacRitchie, 2016). Wheat breeders have the difficult task to supply millers and bakers with wheat having the required quality traits. Several quality analyses exist for breeders to assist with selection of breeding lines having the required traits (Peña et al., 2002). Mixograph analysis is commonly used in South Africa as an early indicator during the selection phases, of acceptable quality, although only mixing time (MPT) is used as one of the criteria during cultivar classification. With Mixsmart<sup>®</sup> software additional points can be measured on a mixogram. Other advantages of using a mixograph include: it requires small sample sizes, it is quick and easy to perform and is less expensive compared to alveograph analysis and baking tests (Peña et al., 2002; Groos et al., 2007).

Much is still unknown about mixograph parameters in South African wheat, especially all the parameters measured by Mixsmart<sup>®</sup> software. Unanswered questions include: How do they relate to quality characteristics, and could specific mixograph parameters be used for early generation quality selection, are they stably expressed over locations and seasons, are their relationships with quality characteristics altered at longer mixing times, and is their expression different when using different milling procedures?

The aim of this study was to investigate if Mixsmart parameters could be effectively applied as a research tool to assist wheat breeders with early generation selection of the primary quality criteria used during the classification process, by:

- Combining different ways to select the most appropriate Mixsmart parameters adhering to principles of being representative of the whole dough mixing process, being related to primary criteria during the classification process and exhibiting very few inter-correlations;
- Determining of the selected parameters' relationships with the primary classification criteria;
- Determining the stability of the selected parameters in assisting breeders in selecting stable breeding lines exhibiting superior quality;
- Determining if different timeX values (6, 8 or 10 min) would result in different relationships with the primary criteria, and;
- Determining if mixograms performed on flour obtained from different milling procedures would result in different relationships with the primary criteria.

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## CHAPTER 2

### MIXSMART PARAMETERS AND WHEAT QUALITY

Wheat has been a source of food for over 8500 years (Braun et al., 2010) and the demand for wheat is increasing due to its adaptability to different environments as well as its versatility (Porter et al., 2007). Wheat is planted across three production regions in South Africa: the summer rainfall region (winter and intermediate wheat types), winter rainfall region (spring wheat types) and irrigation region (spring wheat types).

MacRitchie (2016) stated that bread making quality is difficult to understand. It is complex, since what is observed from different technological tests for wheat quality, is only the expression of the reaction of a specific genotype's properties to a specific environment (Mann et al., 2009; Castillo et al., 2012). Wheat quality characteristics of importance during wheat trade include HLM, FLN and GPC. Wheat trade across the world is separated into trading for hard or soft wheat. According to the South African regulations (SAGL, 2017) the wheat grading table (Table 2.1) currently utilised in South Africa, is as follows:

**Table 2.1 Grading table for bread wheat (minimum values are indicated)**

<b>Grade</b>	<b>Hectolitre mass (kg.hl<sup>-1</sup>)</b>	<b>Protein content (%)</b>	<b>Falling number (s)</b>
<b>1</b>	77	12	220
<b>2</b>	76	11	220
<b>3</b>	74	10	220
<b>4</b>	72	9	200
<b>Utility</b>	70	8	150
<b>Other wheat</b>	<70	<8	<150

kg.hl<sup>-1</sup> = kilogram per hectolitre, s = seconds, % = percentage

Protein content (quantity) and protein quality (composition) are the main determinants of flour quality (Finney and Shogren, 1972; Finney et al., 1987; Graybosch et al., 1996; Wieser et al., 1998; Koekemoer et al., 1999; Branlard et al., 2001; DuPont and Altenbach, 2003; Rakszegi et al., 2005). Wheat protein is unique and can be divided into gluten, albumin and globulin. Gluten forms up to 85% of endosperm protein and is responsible for the rheological behaviour of dough (Anjum et al., 2007; Zhao et al., 2010). Gluten consists of glutenin and gliadin, where glutenin confers strength and elasticity to dough and gliadin confers viscosity and elasticity (Hoseney, 1994; Wieser et al., 2006).

Bread wheat has three genomes; A, B and D. Payne et al. (1987) named high molecular glutenin subunits (HMW-GS) null, 1 and 2\* on chromosome 1A, subunits 6+8, 7, 7+8, 7+9, 17+18, 14+15 on chromosome 1B and subunits 2+12, 5+10, 3+12, 4+12, 2+11 on chromosome 1D. HMW-GS 5 and HMW-GS 2 were found to be associated with good and poor bread making quality, respectively (Payne et al., 1987; Hoseney, 1994). HMW-GS 5+10 contributed more to dough characteristics than HMW-GS 17+18, where HMW-GS 1 contributed the least to dough properties (Uthayakumaran, 2002). HMW-GS 7 was reported to contribute more to higher dough strength characteristics than other subunits (Marchylo et al., 1992).

Gupta and MacRitchie (1994) as well as Khatkar et al. (1996) reported peak time, dough strength and bread making potential differences to be more acceptable when genotypes have subunits 5+10, 7+8, 17+18, 1 and 2\*, compared to having subunits 6+8, 2+12 and 20. Contrary to this, Khatkar et al. (1996) reported that subunits 2+12 produced desirable LFV with acceptable strong dough characteristics. Subunits 7+8 combined with 2\*, 1 or 5+10 exhibited LFV as well as higher peak values (heights). Uthayakumaran et al. (2001; 2002) stated that if gliadin fractions were higher, peak times were shorter and peak resistance as well as maximum resistance on a mixogram were lower, resulting in lower LFV.

Wieser et al. (1998) reported the existence of three main groups within gluten proteins and different types within each group. The HMW group includes x- and y-type HMW-GS. All hexaploid genotypes have six HMW subunits, but not all are

expressed. The medium molecular weight group includes  $\omega$ 5 and  $\omega$ 1, 2-type gliadins. The low molecular weight (LMW) group includes LMW-GS and  $\alpha$ - and  $\gamma$ -gliadins. Wieser and Kieffer (2001) reported x-type HMW-GS to include subunits 1 to 7 and y-type HMW-GS to include subunits 8 to 12. They also reported that the  $\gamma$ -types are less important than the x-types regarding dough rheology. Peak time differences, which reflect dough strength differences, were found to be the result of differences in glutenin proportions (Lundh and MacRitchie, 1989). The gliadins are represented by  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\omega$  fractions (Shewry et al., 1986).

Protein content is less affected by genetics and more by environmental conditions, whereas protein quality is determined more by genetics (Hoseney, 1994). Protein quality varies in terms of properties, structures and proportions between and within cultivars (Veraverbeke and Delcour, 2002; Shewry, 2003). Hoseney (1994) confirmed that protein content is determined by the environment, which explains the variation in protein content between 6 and 25%, depending on availability of nitrogen (Blackman and Payne, 1987).

The environment also influences the protein quality. Nitrogen fertiliser and temperature was reported to affect the ratio of HMW-GS to LMW-GS, the total HMW-GS per grain as well as the proportion available in flour. Higher temperatures allow for less time to accumulate HMW-GS but a higher rate of accumulation occurs (DuPont et al., 2007). If nitrogen fertiliser is applied after anthesis, the accumulation rate of HMW-GS is higher as well as the amount per grain (Wieser et al., 1998; DuPont et al., 2006; 2007). Compared to glutenins, gliadins increase more when nitrogen fertiliser is applied, resulting in an increase in flour protein content (Saint Pierre et al., 2008). Protein content is not a good indicator of LFV, but Dobraszczyk and Schofield (2002) stated that LFV could be predicted accurately when protein content is combined with some mixogram parameters.

When potential breeding lines are evaluated for classification purposes for commercial release, several quality characteristics are important.

The wheat classification process is as follows: biological standards exist for each production region and potential breeding lines are compared to these standards according to primary and secondary quality characteristics. Primary criteria are fixed and non-negotiable. The potential line is allowed to deviate from the biological standard regarding the different characteristics, within allowed tolerances. Evaluations are done for three years and the quality standard and the potential line must be planted and evaluated across five environments. The category of primary or secondary quality characteristics and the allowed deviations are summarised in Table 2.2.

**Table 2.2 Information on quality criteria being considered during the commercial cultivar classification process in South Africa**

<b>Characteristic</b>	<b>Category</b>	<b>Deviation</b>
<b>Hectoliter mass</b>	P	-1.8 unit
<b>Thousand kernel mass</b>	S	±4 g
<b>Falling number</b>	P	-15%
<b>Protein (12% m.b.)</b>	P	-1%
<b>Flour yield</b>	P	-1.5%
<b>Break flour yield</b>	S	±5%
<b>Flour colour</b>	P	+1 unit
<b>Farinogram water absorption</b>	P	±2.5%
<b>Farinogram development time</b>	S	±25%
<b>Farinogram stability</b>	S	+10 to -30%
<b>Alveogram strength</b>	P	±20% (+25% for winter rainfall area)
<b>Alveogram stability</b>	S	±20%
<b>Alveogram distensibility</b>	S	-10% to +20%
<b>Alveogram P/L value</b>	P	±25%
<b>Loaf volume/corrected loaf volume</b>	P	-10%
<b>Mixogram peak time</b>	P	Summer rainfall area: +15% to -25% Winter rainfall area: +45% to -5% Irrigation area: +35% to -10%

P = primary, S = secondary

The optimised 100 g straight dough bread making method is utilised for determining LFV, but the method does not reflect baking quality of the flour, it

rather serves as an indication of the relationship between protein content and LFV (SAGL, 2013).

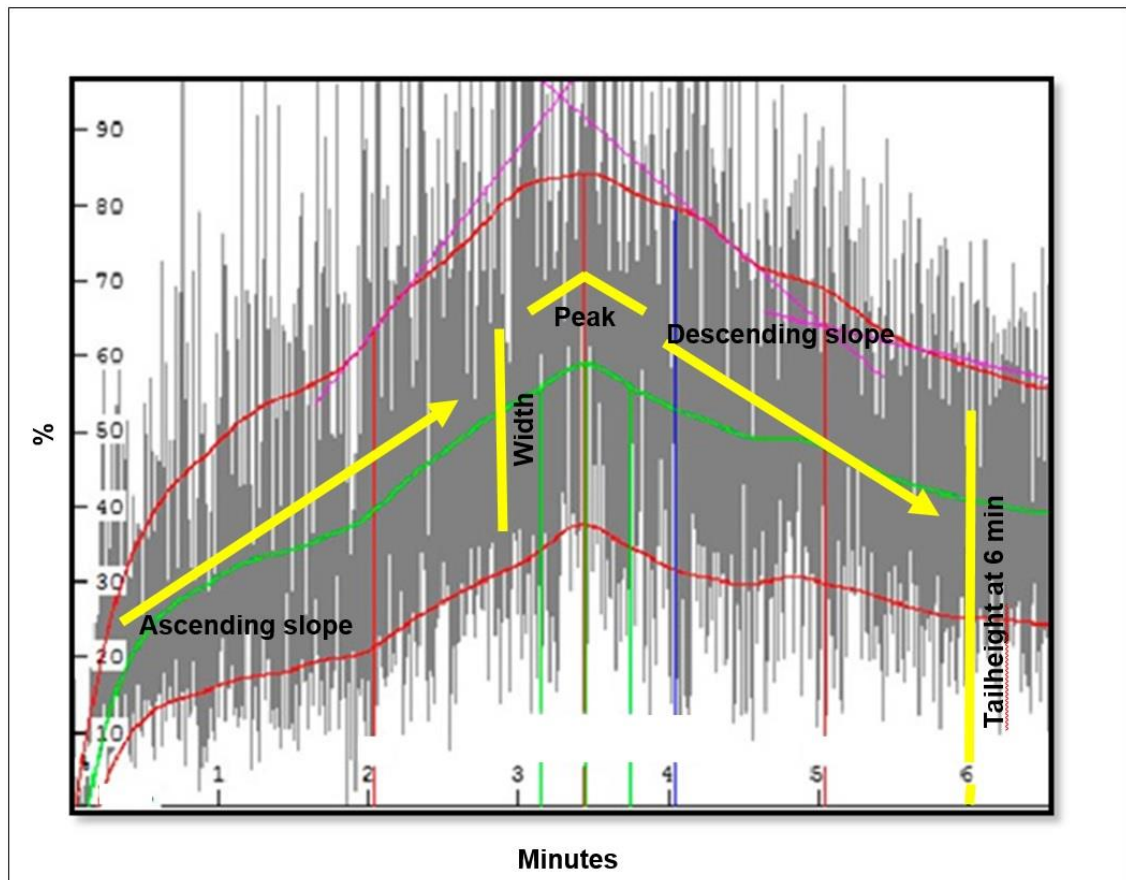
Yield has become more important for classification during the last few years and since a negative relationship exists between yield and quality, it was proposed that yield be incorporated as part of the classification process. Within South Africa it was recently recommended that relaxed quality criteria regarding certain quality parameters for high yielding lines be included, and regarding irrigation wheat, that only two years' data are required for final classification (SAGL, 2017).

## **2.1 Short history of the mixograph**

The mixograph was originally developed to simulate high-speed mixers utilised in the USA and, Swanson and Working (1926; 1933) were the first scientists to describe the mixograph. The first models required 100 g of flour and the mixing bowls had no pins (Walker and Hazelton, 1997). A mixograph requiring 35 g of flour was built during 1939 by Working (Shogren, 1997). The development of a 10 g mixograph resulted from studies conducted in the Hard Winter Wheat Quality Laboratory (Finney and Shogren, 1972). Finney modified it into a 5 g mixograph (Finney, 1989) and Rath et al. (1990) developed a mixograph requiring only 2 g of flour.

The mixograph is a valuable tool to indicate bread wheat quality. A visco-elastic dough, which resists the mixing action forms and this resistance is recorded on a mixogram (Figure 2.1) (Bruinsma et al., 1978; Gras et al., 1990; Walker and Hazelton, 1997). The dough mixing process consists of three stages; the water absorption phase, the dough development phase and the dough breakdown phase (Wikström and Bohlin, 1996). Mixing tolerance is indicated by the width after peak time as well as the stability of the slopes before and after peak time (Finney, 1997). Dough development and dough breakdown can be visualised in the ascending and descending slopes, respectively. The descending slope is related to the genotype and the protein content of the flour. The larger the angle formed by the slopes, the flatter the mixogram and the higher the mixing tolerance (Hazelton and Walker, 1997). Lukow (1997) confirmed that protein

content, water absorption and dough strength are the most important contributors to peak height.



**Figure 2.1** Indications of measurements on a mixogram

Peak time is largely controlled by genetics (Hoseney, 1994; Van Lill, 1992) and heritability for peak time is medium to high and for mixing tolerance, heritability is medium (Gras and O'Brien, 1992). European countries prefer the farinograph to the mixograph, since the farinograph has been used in Europe long before the mixograph (Lukow, 1997; Weipert, 1997). Ingelin (1997) found the mixograph to be a better predictor of mixing times during baking.

Electronic data collection and analysis of mixograms result in less human error, reduced labour, effective time management and more measured data points per mixogram (Pon et al., 1989; Buckley et al., 1990; Gras et al., 1990; Lukow, 1997). The software divides the mixogram into midline and envelope curves, making measurements at certain times, heights, slopes, widths and areas on the

mixograms (Walker and Walker, 1992; Dobraszczyk and Schofield, 2002). Martinant et al. (1998) reported that midline parameters have better repeatability and that strong correlations exist between top envelope and midline parameters. Height values are indicative of dough consistency, widths are indicative of mixing tolerance and areas are indicative of dough strength. Small slope values indicate a flatter curve, which is preferable to large slope values, being indicative of poor tolerance to mixing (Walker et al., 1997). The software selects the peak automatically, but it can be manually manipulated (Hazelton and Walker, 1997). A total of 44 measurement points can be made on a mixogram using Mixsmart® software. The 44 Mixsmart parameters, descriptions, abbreviations thereof and units of measurement are summarised in Table 2.3.

**Table 2.3 Mixsmart parameters, descriptions, abbreviations and units of measurement**

Parameter	Description	Abbreviation	Unit
1) Envelope left time	Time from starting point until 1 min before envelope peak time	ELT	min
2) Envelope left value	Envelope curve height at 1 min before envelope peak time	ELV	%
3) Envelope left slope	Envelope curve slope from beginning until 1 min before envelope peak time	ELS	%/min
4) Envelope left width	Envelope curve-width at 1 min before envelope peak time	ELW	%
5) Envelope left integral	Envelope area under envelope curve from beginning until 1 min before envelope peak time	ELI	%Torque*min
6) Envelope peak time	Time where envelope curve reaches a peak	EPT	min
7) Envelope peak value	Envelope curve height at envelope peak time	EPV	%
8) Envelope peak width	Envelope curve-width at envelope peak time	EPW	%
9) Envelope peak integral	Envelope area under envelope curve from beginning until envelope peak time	EPI	%Torque*min
10) Envelope right time	Envelope time from beginning until 2 min after envelope peak time	ERT	min
11) Envelope right value	Envelope curve height at 2 min after envelope peak time	ERV	%
12) Envelope right slope	Envelope slope from envelope peak time until 2 min after envelope peak time	ERS	%/min
13) Envelope right width	Envelope curve-width at 2 min after envelope peak time	ERW	%
14) Envelope right integral	Envelope area under envelope curve from beginning until 2 min after envelope peak time	ERI	%Torque*min
15) Envelope tail value	Envelope curve height at end of mixing process measured on envelope curve (e.g. 6.5 min)	ETV	%
16) Envelope tail slope	Envelope slope from envelope peak time until end of mixing process measured on envelope curve	ETS	%/min
17) Envelope tail width	Envelope curve-width at end of mixing process measured on envelope curve	ETW	%
18) Envelope tail integral	Envelope area under curve from beginning until end of mixing process measured on envelope curve	ETI	%Torque*min
19) Envelope timeX value	Envelope curve height at 6 min	ETXV	%
20) Envelope timeX slope	Envelope slope from envelope peak time until 6 min measured on envelope curve	ETXS	%/min

**Table 2.3 Mixsmart parameters, descriptions, abbreviations and units of measurement (continued)**

21) Envelope timeX width	Envelope curve-width at 6 min	ETXW	%
22) Envelope timeX integral	Envelope area under envelope curve from starting point until 6 min	ETXI	%Torque*min
23) Midline left time	Time from starting point until 1 min before peak time measured on midline curve	MLT	min
24) Midline left value	Midline curve height at 1 min before midline peak time	MLV	%
25) Midline left slope	Midline curve slope from beginning until 1 min before midline peak time	MLS	%/min
26) Midline left width	Midline curve-width at 1 min before midline peak time	MLW	%
27) Midline left integral	Midline area under curve from beginning until 1 min before midline peak time	MLI	%Torque*min
28) Midline peak time	Time where midline curve reaches a peak – optimum dough development	MPT	min
29) Midline peak value	Midline curve height at midline peak time	MPV	%
30) Midline peak width	Midline curve-width at midline peak time	MPW	%
31) Midline peak integral	Midline area under curve from beginning until midline peak time	MPI	%Torque*min
32) Midline right time	Midline time from beginning until 2 min after midline peak time	MRT	min
33) Midline right value	Midline curve height at 2 min after midline peak time	MRV	%
34) Midline right slope	Slope measured from midline peak time until 2 min after midline peak time	MRS	%/min
35) Midline right width	Midline curve-width at 2 min after midline peak time	MRW	%
36) Midline right integral	Midline area under curve from beginning until 2 min after midline peak time	MRI	%Torque*min
37) Midline tail value	Midline curve height at end of mixing process (e.g. 6.5 min)	MTV	%
38) Midline tail slope	Slope from midline peak time until end of mixing process measured on midline curve	MTS	%/min
39) Midline tail width	Midline curve-width at end of mixing process	MTW	%
40) Midline tail integral	Midline area under curve from beginning until end of mixing process	MTI	%Torque*min
41) Midline timeX value	Midline curve height at 6 min	MTXV	%
42) Midline timeX slope	Slope from midline peak time till 6 min measured on midline curve	MTXS	%/min
43) Midline timeX width	Midline curve-width at 6 min	MTXW	%
44) Midline timeX integral	Midline area under curve from beginning until 6 min	MTXI	%Torque*min

## 2.2 Factors affecting mixogram parameters

Known factors having an effect on the mixogram characteristics include genotype (protein quality), environment, flour protein content (protein quantity), water absorption and temperature (Finney, 1997). Mixing time for a specific genotype may vary with as little as 10-20% over a wide range of environments, but it may also vary by as much as 50-75% from the average expected mixing time, depending on climatic conditions (Finney, 1997). When protein quantity decreases below 12%, mixing time increases, because of the greater effort to develop the dough. Peak height and mixing time in a genotype generally

decreases and increases, respectively, with increasing water absorption (Finney, 1997). Curve width and ascending slopes are also affected by water absorption (Hazelton and Walker, 1997). When insufficient water is added when performing mixograph analyses, short mixing times, increased peak heights, areas and band widths are the result (Finney and Shogren, 1972). Baig and Hosney (1977) reported that mixograms are routinely performed at 25°C, and the flour, water and formed dough are only in contact with the mixograph bowl at the start of the mixing process, it is therefore difficult to control temperature during the whole mixing process. Baig and Hosney (1977) and Shelke and Walker (1990) reported that higher dough temperatures resulted in longer mixing times and lower peak heights.

The mixograph has been used widely to evaluate the quality of wheat flour for food-production, to classify wheat, to determine the effects of ingredients and additives on dough rheology and to predict the influence of these effects on end-products and to predict water absorption in several processing systems (Lang et al., 1992; Van Lill and Purchase, 1995; Khatkar et al., 1996; Lukow, 1997; Ponte and Ingelin, 1997; Dobraszczyk and Schofield, 2002). When Lang et al. (1992) added additional gluten to flour, peak height and work input increased, but peak times became shorter. Weak et al. (1977) reported that added ascorbic acid, resulted in flatter mixograms. When salt is present while mixing, the dough will take longer to reach a peak. Lang et al. (1992) and He et al. (1992) also reported higher peak values (heights) in the presence of salt, but the latter also stated that different types of salt caused differences in their results. When bran was added, water absorption became higher (Halim and Lorenz, 1985; Haridas and Malini, 1991), but mixing times shortened. The addition of sucrose esters resulted in longer mixing times but no effect was noticed on peak values (heights) (Lang et al., 1992). Hosney and Brown (1983) reported that higher dough pH caused more stable dough characteristics. When onion and garlic flavourants were added to dough, mixograms were flatter, resulting from either shorter mixing times or lower peak values (Indrani et al., 1992; Venkateswara et al., 1992).

Lorenz (1974) reported that an increase in elevation resulted in an increase in peak values and mixing time. Uprety et al. (1976) reported nitrogen application

to have no effect on mixing time and area under the mixogram, although tolerance decreased, while other characteristics increased when nitrogen application was increased for Mexican genotypes. Indian genotypes were less affected. Kosmolak and Crowle (1980) reported a decrease in mixing time and an increase in water absorption when protein content was higher due to higher nitrogen applications in Canadian genotypes. Vanhamel et al. (1993) reported that added pentosans caused an increase in peak height as well as the area under the mixogram.

### **2.3 Grain characteristics and Mixsmart parameters**

HLM, otherwise known as test weight, indicates grain-soundness (Czarnecki and Evans, 1986) and it can be defined as the kernel density and packing efficiency of a wheat cultivar (Jalaluddin and Harrison, 1989; Koen, 2006), where density is the result of environmental conditions and packing efficiency is part of the genetics of the cultivar. Some cultivars always have a higher HLM compared to others growing in the same environment (Gaines et al., 1996; Bordes et al., 2008). Seed conditions resulting in lower HLM include kernel shrivelling and insect damage (Carver, 1996; Wrigley and Batey, 2003). Rain occurring during the harvesting season may result in the grain absorbing moisture, making the kernels less dense, resulting in lower HLM (Carver, 1996). Kernel morphology also affects HLM, since short, plump kernels pack more uniformly compared to long kernels which pack more randomly (Dick and Matsuo, 1988), meaning that short plump kernels usually have higher HLM. The HLM has a direct impact on the costs involved during wheat-trading (Bordes et al., 2008) and is also an important factor during wheat grading (Donelson et al., 2002).

Dobraszczyk and Schofield (2002) reported strong positive correlations between protein content and mixogram height parameters envelope peak value (EPV), midline peak value (MPV) as well as tail height. Chung et al. (2001) reported strong positive correlations between protein content and MPV as well as midline peak width (MPW), and between protein content and midline left slope (MLS) and envelope peak integral (EPI). Souza et al. (1993) reported a negative correlation between protein content and midline right slope (MRS). Millers require

a stable flour quality despite the influence of the environment on protein (DuPont et al., 2007).

Labuschagne et al. (2016) reported highly significant correlations between FPC and the following Mixsmart parameters: envelope left time (ELT), envelope peak time (EPT), envelope peak value (EPV), envelope peak time (EPT), midline left value (MLV), MPV, midline right value (MRV), midline right integral (MRI) and midline tail integral (MTI) for the irrigation area of South Africa. The study also found highly significant correlations between FPC and envelope peak width (EPW), EPT, envelope right value (ERV), envelope tail value (ETV), envelope tail integral (ETI), envelope timeX value (ETXV), envelope timeX integral (ETXI), MPV, MRV, MRI, midline tail value (MTV), MTI and midline timeX value (MTXV) for the summer rainfall region of South Africa. No correlations were observed between FPC and Mixsmart parameters for the winter rainfall region of South Africa. Miles et al. (2012; 2014) reported correlations between FPC and MPV, MLS and MTI, although GPC correlated only with MLS. Dobraszczyk and Schofield (2002) reported positive correlations between FPC and MPV, MTI, EPV, MTV and MPW. Martinant et al. (1998) reported significant correlations between protein content and midline left width (MLW), MPV, MPW and midline timeX integral (MTXI) at 8 min. Khatkar et al. (1996) reported correlations between protein content and MPT, MPV and MPW.

FLN measures the alpha-amylase activity in flour and is low when pre-harvest sprouting occurs, resulting in high levels of alpha-amylase, thus having a negative effect on bread quality however, it does not affect milling characteristics as such (Hagberg, 1960; Kaldy and Rubenthaler, 1987; Edwards et al., 1989; Posner and Hibbs, 1997). Low FLN values are the result of excessive sugar and low starch content, resulting loaves of bread will have sticky crumbs, poor texture and will cause difficulty during mechanical cutting (Chamberlain et al., 1981; Posner and Hibbs, 1997). Other effects reported for flour having low FLN values include lower water absorption, which, in turn, might result in lower LFV (Dowell et al., 2008). Kulp et al. (1983) reported low FLN values to have an effect on dough weakening, but no significant effects on peak times and water absorption were observed.

Kernel hardness is not included as a characteristic during the cultivar classification process in South Africa, but only medium-hard to hard breeding lines are allowed to be submitted for classification (SAGL, 2013). The milling process is affected by kernel hardness, since harder wheat types break into larger pieces when milled, having higher FLY compared to softer kernels, which break into smaller pieces, making the sieving process difficult and resulting in lower FLY (Malouf et al., 1992). Harder wheat has higher water absorption levels, resulting from damaged starch (Bass, 1988; Bettge et al., 1995). Van Lill and Smith (1997) reported that harder wheat types exhibited higher protein content and higher FLY values. Huebner and Gaines (1992) as well as Lyon and Shelton (1999) also reported correlations between hardness and protein content, although Pomeranz et al. (1985) found no correlation between these two parameters. A strong relationship was reported by Martinant et al. (1998) between hardness and MPV, MPW, midline timeX value (MTXV) and midline timeX width (MTXW) respectively.

Thousand kernel mass refers to the weight of a thousand kernels, therefore plumper kernels have more endosperm and will have higher thousand kernel mass (Bordes et al., 2008). Millers use it as an indication of expected FLY (Posner and Hibbs, 1997). Contradiction exists in literature where some researchers reported relationships between thousand kernel mass and protein content (Löffler and Busch, 1982) and others reported no correlations between these two parameters (Pomeranz et al., 1985).

According to Tsilo et al. (2010), kernel diameter and HLM were correlated and smaller kernels showed no relationship with the brightness of flour. Kernel size indicated FLY within a genotype but not between genotypes as reported by Marshall et al. (1986). Posner and Hibbs (1997) indicated differences in kernel size within a genotype to be the result of environmental influence. Miles et al. (2014) reported MTV, MPW, MTW, MRI and MTI to correlate with kernel diameter.

Grain hardness and vitreous kernels are characteristics that are often confused. Grain hardness is the hardness of the kernels (Bettge et al., 1995) and vitreousness has a glass-like appearance caused by a lack of air spaces in the

kernels. When the crop dries, protein shrinking occurs, but the kernels stay intact (Dobraszczyk, 1994). Pomeranz and Williams (1990) reported that vitreousness is caused by nitrogen and high temperatures, therefore environment plays a large role in vitreousness. Soft wheat genotypes may also be vitreous if grown under perfect conditions (Hoseney, 1994), although hardness, high protein content and vitreousness are often used in the same context (Dexter et al., 1988).

## **2.4 Milling characteristics and Mixsmart parameters**

FLY potential is determined by the grain's morphology. Required morphological characteristics include plump, uniform kernels with a spherical shape (Fowler and Priestly, 1991), since uniformity results in even milling delivering higher FLY yield with lower ash contents (Gaines et al., 1997), resulting in whiter flour. Favourable kernel characteristics include smooth surfaces with narrow creases, small to medium protruding embryos, small brushes that are less dense and kernels must not be too long. Berman et al. (1996) also stated that harder wheat kernels should possess sufficient protein and be vitreous. FLY is important to millers, since their profit is determined by milling cultivars, which have higher FLY potential (Bass, 1988). Bran contamination is limited during the milling process by conditioning of grain prior to milling, since conditioning results in separation of the bran and the endosperm (Marais and D'Appolonia, 1981). Miles et al. (2014) reported significant correlations between FLY and MLS.

Break flour yield (BFLY) is the total flour obtained from the break rollers of the Bühler experimental mill (Bass, 1988). Softer wheat has higher BFLY and lower protein content (Rogers et al., 1993; Labuschagne et al., 1997). A negative correlation between BFLY and protein content was reported by Gaines (1991) and Kosmolak and Dyck (1981) reported a positive correlation between large kernels and BFLY.

Flour colour is a combined function of yellowness and brightness, where yellowness is influenced by carotenoid pigments and brightness is influenced by the milling process (Oliver et al., 1993). Flour colour is determined by focusing on the bran-contamination in the flour after milling, by measuring the reflectance

of a light source in the green band of the light spectrum (Mailhot and Patton, 1988). Variation in flour colour can be the result of genotype (G), environment (E), GXE interaction or different milling processes. Bran contamination, black point, frost damage and immature kernels might result in darker flour colour, which is undesirable for white bread production (Bass, 1998; Posner and Hibbs, 1997).

## **2.5 Rheological characteristics and Mixsmart parameters**

Dough rheology results from mixing flour and water where gluten in the flour and water interact to form dough. Different apparatuses can be utilised to study dough rheology including the mixograph, farinograph and alveograph (Walker and Hazelton, 1996). Hosney (1994) reported peak time control to be related to glutenin and that peak time was influenced by protein content. He stated that flour having less than 12% protein, took longer to reach a peak, but when protein content was higher than 12%, no effect was noticed regarding peak times. He stated that flour with protein contents higher than 12% also exhibited acceptable tolerance to mixing and when peak time increased, extensibility decreased and stability, elasticity and tolerance increased. Finney and Shogren (1972) stated that dough with peak times of 5 min and longer have too much tolerance, resulting in too little extensibility and unwanted elasticity which is undesirable for bread production. Short peak times result in less elasticity and more extensibility, which is undesirable for stable dough characteristics.

Peak height is reached when optimum hydration has occurred, therefore peak height is a function of protein content and water absorption (Hosney, 1994). Khatkar et al. (1996) reported strong positive relationships between right slope and right curve width Mixsmart parameters and peak height and curve width at the peak height. They concluded that high values obtained for these parameters are indicative of poor mixing tolerance.

Labuschagne et al. (2016) reported highly significant positive correlations between alveograph dough strength (STR) and EPT, EPV, EPI, envelope right integral (ERI), midline left time (MLT), MLV, midline left integral (MLI), MPT, MPV, midline peak integral (MPI), midline right time (MRT), MRV, MRI, MTI and

MTXV respectively for the irrigation area of South Africa. The study also reported correlations between STR and EPW, EPI, ERV, ERI, ETV, ETI, ETXV, ETXI, MPV, MPI, MRV, midline right width (MRW), MRI, MTV, midline tail slope (MTS), MTI, MTXV and MTXS respectively for the summer rainfall area and between STR and EPV, ERV, ETXV, MPV, MRV and MTI respectively for the winter rainfall region. Miles et al. (2014) reported correlations between alveograph P/L value and MPT, MTV, MRS, MRW, MTW, MLI, MPI and MRI as well as between alveograph dough strength and MPV, MLS, MLW, MPW and MTI. Branlard et al. (1991) reported correlations between alveograph dough strength and MPT, MPV, MTV (at 7 min), MPW and MTW (at 7 min).

Labuschagne et al. (2016) also reported highly significant positive correlations between alveograph P/L value and EPW, EPT, ERV, envelope right width (ERW), ERI, envelope right slope (ERS), ERW, ERI, ETXW, ETXI, MLT, MLI, MPT, MPI, MRT, MRI, MTW, MTXV and MTXW for the irrigation area. This study also reported highly significant positive correlations between alveograph P/L value and ETXW and MTXW for the summer rainfall area as well as between alveograph P/L value and ERI, ETI, ETXI, MLT, MLI, MPT, MPI, MRT and MRI respectively for the winter rainfall area.

Water absorption determined on a mixograph and a farinograph differs, because the mixing actions and dough consistency of these apparatuses differ, and the water content used in a mixograph depends on protein content (Wikström and Bohlin, 1996; Ingelin, 1997). Water absorption, determined by the farinograph, is a primary quality characteristic (Table 2.2) used during the cultivar classification process in South Africa and values of 62-64% are favoured (Koekemoer, 2003; SAGL, 2013). MacRitchie (1984) and Van Lill et al. (1995) defined water absorption as the potential of the protein to absorb water. They reported significant correlations between gliadin and glutenin with FPC, dough mixing time, dough stability, water absorption and LFV. Finney et al. (1997) reported a linear relationship between water absorption and protein content in a cultivar. Significant correlations were reported by Zounis and Quail (1997) between FABS and MPW as well as between water absorption and MPV. Finney (1997) stated that longer mixing times and lower peak heights were associated with higher

water absorption. Miles et al. (2014) reported correlations between FABS and MPV, MTV, MLS, MLW, MPW and MTI.

The alveograph blows a bubble from a thin dough piece while measuring air pressure and volume required until the dough bubble bursts. It provides information on dough stability (P), distensibility (L), strength and then the ratio between P/L. Dough strength and P/L value as determined by the alveograph are the two alveograph parameters of importance during the classification process in South Africa. Dough strength is determined by dividing the W value by 2.54 as the guidelines set by the American Association of Cereal Chemists (AACC, 2000).

HMW-GS 2\* correlated with P as well as P/L value and HMW-GS 5+10 correlated with W value and HMW-GS 1 correlated with the L value (Branlard and Dardevet, 1985; Hou et al., 1996; Payne et al., 1987). Miralbés (2004) reported that the W value is the most important alveograph characteristic as indicator of bread making quality and Bordes et al. (2008) stated that the W value is a summation of all alveograph parameters. Sadouki et al. (2006) reported correlations between W value and mixograph peak times, mixograph peak areas and mixing dough tolerance (right slopes on the mixogram). Færgestad et al. (2000) also reported a positive correlation between MPT, dough strength and HMW-GS.

## **2.6 Baking characteristics and Mixsmart parameters**

LFV is the final test a breeding line must pass before it can be assumed to have good bread making potential. Cauvain (2003) stated that protein is the most important ingredient to determine bread making quality. Finney and Shogren (1972) reported that peak times longer than 3 min combined with protein content higher than 13%, had no effect on LFV. Dobraszcyk and Schofield (2002) reported that mixogram parameters alone are not sufficiently indicative of LFV, but they identified three mixogram parameters, MPW, EPV and tail height (at 10 min) to be the best indicators of LFV. Chung et al. (2001) reported strong positive correlations between EPI and LFV.

Flour containing strong gluten, has mixograms with wider bandwidths (Pitz, 1997), higher peak values and higher work input is required to fully develop the dough (Lang et al., 1992). Labuschagne et al. (2016) reported highly significant correlations between wet gluten content and EPV, MLV, MPV, MRV and MTI respectively for the South African irrigation area and between wet gluten content and EPV, EPW, EPI, ERV, ERI, ETV, ETI, ETXV, ETXI, MRV and MTXV for the summer rainfall region but no correlations between wet gluten content and Mixsmart parameters for the winter rainfall region were recorded. The study also reported highly significant correlations between LFV and MPV, MRV and MTI (irrigation area) and LFV and EPI, ERV, ERI, ETI, ETXV, ETXI, MPI, MRV, MRI, MTXV and none for the winter rainfall region. Miles et al. (2014) reported correlations between LFV and MPV, MLS and MTI. The same correlations applied to LFV12%. Neacșu et al. (2009) reported correlations between LFV and MPT and MPV. Chung et al. (2001) reported significant positive correlations between LFV and EPI, MPW and MPV. Khatkar et al. (1996) reported correlations between LFV and MPV as well as MPW. Branlard et al. (1991) reported correlations between LFV and MPT, MPV, MTV, MPW and MTW.

## **2.7 Mixsmart parameters used as wheat quality predictors**

Wikström and Bohlin (1996) found five parameters to explain 92.8% of the variance observed in LFV and these parameters were left slope, peak time, initial width, area below the mixogram and peak height. They also reported that high left slope values, area below the curve and peak time and height, indicated strong dough. They also reported peak time to relate to left slope and FABS and that if higher values for area below the graph and high peak heights combined with low mixing time values, it will result in high LFV. Finney et al. (1987), Dong et al. (1992), Preston et al. (1992), Khatkar et al. (1996), Martinant et al. (1998), Lukow (1997) and Labuschagne et al. (2016) agreed that MPV and not MPT was more effective in predicting LFV. Neacșu et al. (2009) identified five parameters as predictors of quality, namely left slope, peak time, peak height, tail width and right slope and they found that these five parameters explained 91% of the variance in loaf volumes obtained.

Labuschagne et al. (2016) reported MPV and MRV to be good predictors of flour protein content, gluten content and loaf volume in the irrigation area of South Africa.

## **2.8 Criteria used for selection of Mixsmart parameters**

In order to reduce the 44 Mixsmart parameters (Table 2.3), scientists have applied different ways of selecting Mixsmart parameters. Branlard et al. (1991) stated that a selected parameter has to be highly heritable and highly correlated with the specific quality character that needs improvement.

Martinant et al. (1998), Labuschagne and Moloï (2015) and Labuschagne et al. (2016) used repeatability as selection basis and selected 10, 31 and 26 Mixsmart parameters respectively. Elangovan et al. (2008) and Miles et al. (2012; 2014) selected 10 and 13 parameters based on parameters representing the complete dough mixing process. Caffè-Treml et al. (2010) selected six parameters based on repeatability, the ability to discriminate among genotypes and having no or little inter-correlations between the selected parameters. Neacșu et al. (2009) selected five parameters being indicative of all basic rheological aspects of mixing characteristics using the criteria: low coefficients of variation (CV), high heritability and parameters exhibiting fewer correlations with the other selected parameters. Pelsler et al. (2016) used repeatability and low CV's as criteria and selected 17 parameters. Only two parameters occurred in all these studies, namely MPT and MPV. MTI and MLW also occurred frequently, but scientists overall seemed to select parameters that cover the whole dough mixing process. They included integral values (parameters descriptive of work input to develop the dough), slope values (parameters indicative of tolerance to mixing), curve height values (parameters describing dough consistency), values representative of the area under the curve (parameters describing dough strength), curve width values (describing dough elasticity and extensibility) and curve time values.

## 2.9 Environmental factors affecting wheat quality

Breeders have to consider the needs of millers and bakers by incorporating the genetic potential in breeding lines, while the product, which reaches the millers and bakers after harvesting, depends on the producers' crop management skills as well as environmental influences during the growing season.

Drought will result in high protein contents and low yield. Available nutrition during the growing phase will have an effect on plant and grain development, since protein content as well as yield depend on sufficient nitrogen (Wrigley and Batey, 2003). If protein contents are too low (<8%), dough strength will be too low and poor LFV will be delivered to consumers. Temperatures above 35°C will result in poor dough properties, since higher temperatures go hand-in-hand with higher CO<sub>2</sub> concentrations, leading to higher yield and lower protein content in the absence of sufficient nitrogen required for acceptable protein content (Blumenthal et al., 1996). Rain during the harvesting period, may cause pre-harvest sprouting, resulting in unacceptable flour characteristics for bread making purposes.

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## CHAPTER 3

### A COMBINATION OF DIFFERENT WAYS TO SELECT MIXSMART PARAMETERS FOR EFFICIENT QUALITY SELECTION

#### Abstract

Scientists have access to Mixsmart<sup>®</sup> software parameters to assist with selection for good quality during early generations of the wheat breeding programmes. Ten genotypes from the National Cultivar Trials, planted for two seasons at three sites in the eastern part of the summer rainfall region in South Africa were used for this study. Mixsmart parameters were ranked according to four criteria including: repeatability ( $h^2$ ), coefficient of variation (CV), coefficient of determination ( $R^2$ ) and genotype contribution to variation (G-share). Rankings for the 44 Mixsmart parameters were inconsistent across locations and seasons, except for MRI, MTV, MLI, ETW and MTXW. MRI consistently ranked ninth for  $h^2$ , CV and G-share, MTV ranked first for CV and  $R^2$  and ETW ranked sixth for  $h^2$  and  $R^2$ . MLI and MTXW ranked, respectively, third and sixth for  $R^2$  and G-share. Six midline parameters were selected (MLS, MRS, MPT, MPV, MRI and MTW) for further analyses based on adherence to the four criteria, and they exhibited significant correlations with primary criteria used during the cultivar classification process for commercialisation in South Africa. Although inter-relationships between the selected Mixsmart parameters existed, the selected parameters were representative of the different parts of the mixogram; the slopes, time, height, area and width of the curve.

#### 3.1 Introduction

Wheat breeders have access to several technological tests to assist with early generation selection for the end-use target, being high LFV (Branlard et al., 1992). Primary criteria during the commercial cultivar classification process, which include HLM, FLN, FLY, flour colour, grain and FPC, mixing time, FABS, alveograph dough strength and the ratio between dough stability and dough distensibility (P/L-value), LFV and LFV12%, are also a focus for wheat breeders

in South Africa (SAGL, 2013). Some of these tests can only be utilised during the advanced breeding phases, since they require larger sample sizes, are expensive, require highly trained technical operators and are time-consuming.

The dough mixing process consists of different stages: the water absorption phase, the dough development phase and the dough breakdown phase (Wikström and Bohlin, 1996). The mixograph is a widely utilised rheological apparatus (Bordes et al., 2008), with a simple procedure requiring small sample sizes (Rath et al., 1990; Khatkar et al., 1996), being inexpensive and with results available within 6 min. MPT is the only mixogram parameter taken into account during the cultivar classification process in South Africa (SAGL, 2013). With applying Mixsmart® software, 44 mixogram parameters are available (Pon et al., 1989; Martinant et al., 1998). The software divides a mixogram into two envelope curves (upper and lower) by construction of a midline curve (Walker and Walker, 1992; Dobraszczyk and Schofield, 2002). The 44 parameters are a result from measurements on a mixogram at different times, heights, widths, slopes and areas (Pon et al., 1989). Height, determined as a percentage of the full scale, gives information on dough consistency. Width is the difference between the top and the bottom envelope curve, where midline-width incorporates information from the top envelope curve. Slope-values are determined by dividing the value by the specific time, where small slope values are indicative of flat, stable curves and large slope values are indicative of poor tolerance to mixing, where the curve rises or drops quickly. Areas measured at a specific time under the top envelope or midline curve gives information about dough strength. Short peak times are undesirable for bread production, since it exhibits too little elasticity and too much extensibility, with medium to medium-long peak times being more desirable for bread production due to exhibiting acceptable tolerance to mixing (Finney et al. 1987).

Several scientists have applied different ways of selecting Mixsmart parameters, to enable them to focus on less Mixsmart parameters, that are more meaningful and representative of the whole dough mixing process. First Branlard et al. (1991) stated that a selected parameter has to be highly heritable and highly correlated with the character that needs to be improved. Wikström and Bohlin

(1996) stated that five parameters, namely build-up until peak time, peak time, initial width, area below the mixogram and peak height, in combination with LFV, were effective in predicting LFV.

Martinant et al. (1998), Labuschagne and Moloji (2015) and Labuschagne et al. (2016) selected 10, 31 and 26 Mixsmart parameters respectively, based on repeatability. Elangovan et al. (2008) selected 10 parameters and Miles et al. (2012; 2014) selected 13 parameters; both used using parameters that represented the whole dough mixing process. Caffè-Treml et al. (2010) selected six parameters based on repeatability, the ability to discriminate among genotypes and the selected parameters had to have no or little inter-correlations. Neacșu et al. (2009) identified five parameters being indicative of all basic rheological aspects of mixing characteristics. Their selection criteria were, parameters having low coefficients of variation (CV), high heritability and exhibiting less correlations with the other selected parameters. Pelsler et al. (2016) selected 17 Mixsmart parameters, based on repeatability and having acceptable CV's. The only parameters that were selected by all these scientists were MPT and MPV (midline peak value), although Khatkar et al. (1996), Martinant et al. (1998) and Labuschagne et al. (2016) indicated that MPV was a better predictor of LFV than MPT. Parameters MTI and MLW also occurred frequently, but scientists overall seemed to select parameters that cover the whole dough mixing process by including parameters descriptive of work input to develop the dough (integral values), parameters indicative of tolerance to mixing (slopes), parameters describing dough consistency (curve height), dough strength (area under the curve), dough elasticity and extensibility (width of the curve) and the time it takes for the dough to reach a peak.

The aim of this study was to combine different criteria used in previous studies to select Mixsmart parameters for inclusion in selection for baking quality characteristics (repeatability, CV, coefficient of determination and genotype contribution to variation), to establish how these criteria determine ranking of Mixsmart characteristics as selection tools for quality. A prerequisite for inclusion of Mixsmart parameters was that they had to be representative of the whole dough mixing process, be related to the primary selection criteria used in South

Africa during the commercial classification of bread wheat cultivars and had to exhibit no significant inter-correlations.

### 3.2 Materials and methods

#### 3.2.1 Field trials

Ten genotypes with four replicates from the National Cultivar Trials, planted over two seasons in three localities (Bethlehem, Clarens and Ladybrand), in the eastern part of the summer rainfall region in South Africa, were used for this research. Planting dates for this region was June/July for both seasons (Table 3.1). Trials were planted according to a randomised complete block design with four replicates, using a precision planter. Plots consisted of five rows, five metres in length and inter-row spacing was 45 cm, except at Clarens where inter-row spacing was 50 cm. Seeds were planted 5 cm apart and fertiliser, 6:2:1 (31) was applied. Total N, P and K were 50 kg, 17 kg and 9 kg respectively. To eliminate side row effect, only the three middle rows were harvested. Harvesting dates for both seasons were December/January (Table 3.1).

**Table 3.1 Information on the environments, planting and harvesting dates in the eastern part of the summer rainfall region**

Locality	GPS coordinates	Altitude	Planting dates	Harvesting dates
<b>Bethlehem</b>	28° 09' 17 25" S	1721	05-06-2007 & 22-06-2009	08-01-2008 & 14-12-2009
	28° 17' 45 15" E			
<b>Clarens</b>	28° 24' 35 37" S	1714	20-06-2007 & 25-06-2009	11-01-2008 & 31-12-2009
	28° 23' 49 77" E			
<b>Ladybrand</b>	29° 14' 30 02" S	1500	13-07-2007 & 17-06-2009	03-01-2008 & 29-12-2009
	27° 19' 27 89" E			

Low rainfall occurred during the first season before planting (Table 3.2). Although planting conditions were unfavourable during 2007 due to low rainfall, significant rainfall was recorded during late October. For the rest of the growing period during 2007, above normal rainfall resulted in yields that were above the long term averages. Insufficient seed for quality analyses were obtained during 2008, due to very dry conditions. Therefore, the next season's seed was used for analyses. The conditions during 2009 were more desirable before planting. Little

rain occurred during springtime, and average rainfall occurred just before and during harvesting. Lower yields were obtained due to less rain during the growing period, and late rain resulted in regrowth.

**Table 3.2 Information on rainfall and temperatures for the three environments in the eastern part of the summer rainfall region over two seasons**

Month	2007 Bethlehem				2007 Clarens				2007 Ladybrand			
	Min °C	Max °C	Days >30°C	Rainfall (mm)	Min °C	Max °C	Days >30°C	Rainfall (mm)	Min °C	Max °C	Days >30°C	Rainfall (mm)
				130.20 <sup>(a)</sup>				Error				151.00 <sup>(a)</sup>
<b>June</b>	-0.85	16.01	0	27.60	0.72	14.91	0	33.50				
<b>July</b>	-2.23	16.89	0	0.00	-0.73	16.23	0	3.00	-2.58	17.04	0	2.40
<b>Aug</b>	-0.06	19.87	0	0.00	1.68	18.72	0	0.10	0.58	20.22	0	3.40
<b>Sept</b>	6.89	25.59	0	0.00	8.67	24.18	0	47.90	7.31	26.40	6	47.30
<b>Oct</b>	9.87	20.05	0	158.70	8.92	19.23	0	208.00	9.88	22.37	0	80.20
<b>Nov</b>	10.57	23.08	0	96.70	10.40	22.42	0	129.50	10.52	25.96	4	118.60
<b>Dec</b>	12.29	24.02	0	86.30	11.78	21.92	0	99.50	13.20	25.61	3	81.30
<b>Jan</b>	10.23	26.89	0	16.00	12.19	24.12	0	23.90	16.13	31.67	3	4.10
				385.30 <sup>(b)</sup>				545.40 <sup>(b)</sup>				337.30 <sup>(b)</sup>
	2009 Bethlehem				2009 Clarens				2009 Ladybrand			
				315.40 <sup>(a)</sup>				522.40 <sup>(a)</sup>				256.70 <sup>(a)</sup>
<b>June</b>	2.49	14.90	0	58.70	2.49	14.53	0	57.20	1.77	15.56	0	28.00
<b>July</b>	0.43	15.06	0	0.00	0.43	15.05	0	0.00	-3.15	15.34	0	2.30
<b>Aug</b>	1.74	18.41	0	20.10	1.74	18.36	0	0.80	2.04	19.60	0	15.70
<b>Sept</b>	6.61	23.29	0	8.40	6.61	22.70	0	3.80	6.07	24.13	0	0.30
<b>Oct</b>	9.13	22.58	0	42.40	9.13	22.61	0	65.40	10.10	23.97	0	109.00
<b>Nov</b>	9.95	23.39	0	74.80	9.95	23.54	0	55.80	10.73	24.95	4	64.30
<b>Dec</b>	12.51	26.26	0	37.10	12.51	27.60	2	46.60	14.05	30.67	23	15.20
				241.50 <sup>(b)</sup>				229.60 <sup>(b)</sup>				234.70 <sup>(b)</sup>

Min=average minimum temperature, Max=average maximum temperature, <sup>a</sup>=measured rainfall from January till planting month, <sup>b</sup>=measured rainfall from planting month till harvesting month

### 3.2.2 Laboratory analyses

HLM was determined by means of a two-level funnel according to AACC method 55-10. The obtained weight was divided by five, and expressed as kg.hl<sup>-1</sup>.

Conditioning of the wheat samples, to 16%, were done 18 hrs before milling (AACC-method 26-95). Samples were milled on a laboratory, pneumatic mill, Bühler model MLU-202 (Bühler Bros., Inc., Uzwil, Switzerland). FLY was determined as described by Bass (1988):

$$\% \text{ FLY} = \left[ \frac{\text{Total flour obtained}}{\text{Total (flour + bran)}} \right] * 100$$

Protein content (AACC method 39-11.01, using a FOSS Grain Analyser 1241, with NIR-technology) and moisture content (AACC method 44-15A, using a Brabender moisture oven) were determined on the white flour samples.

The flour weight and water volume required for a 35 g mixograph analyses (AACC method 54-40A) were determined by means of formulas, developed by Walker et al. (1997) and were as follows:

Firstly, protein content was converted to 14% moisture basis (m.b.):

$$= [\text{protein (as is)} * 86] / (100 - \text{moisture content})$$

Secondly, the required weight of flour was determined:

$$= [86 / (100 - \text{moisture content})] * 35$$

Lastly, the required volume of water was determined:

$$= [(1.5 * \text{protein 14\% m.b.}) + 43.6] * 0.35$$

Mixsmart<sup>®</sup> software (National Manufacturer, Lincoln, Nebraska, USA) was applied and the mixing process was recorded for 6.5 min, with TimeX value being

6.0 min. A description of the 44 Mixsmart parameters, abbreviations and the units of measurement are given in Chapter 2, Table 2.1.

AACC methods listed in the American Association of Cereal Chemists manual (AACC, 2000) were used, as this is the standard methodology used at the Small Grain Institute quality laboratory. FLN was determined on the white flour applying AACC method 56-81B and altitude-corrected values were used. The FLN is an indication of alpha-amylase activity and it is measured as the time it takes a metallic stirrer to fall through a flour-water suspension while the suspension is being heated in a boiling water-bath.

Flour colour was determined according to the AACC method 14-30, using a Martin series III colour grader. The readings were done at a wavelength of 540 nm. Higher values indicated darker flour colour, compared to negative values indicative of whiter flour colour. Flour colour expressed on a 76% flour yield basis was determined as follows: for each 1% flour yield above 76%, 0.4 Kent Jones (KJ) units are subtracted from the obtained flour colour value and for each 1% flour yield below 76%, 0.4 KJ units are added to the measured flour colour value. The reason being to eliminate discrimination against higher flour-yielding genotypes that might result in flour having darker colour.

FABS was determined on a constant flour weight (300 g), applying AACC method 54-21. Water absorption is the volume of water needed for dough to reach a certain consistency, namely 500 Brabender units. The volume of water added was expressed as a percentage of the flour mass and was reported on a 14% moisture basis.

Alveograph analyses were performed according to AACC method 54-30A, where 250 g of white flour was used and a 2.5% NaCl solution was added, depending on the sample's moisture content. Alveograph parameters computed by and Alveolink used for this study, were dough strength ( $W$ -value/5.64) and the P/L-value, which is an indication of the ratio between dough stability and dough distensibility.

The baking test was performed according to the optimised, straight-dough baking procedure (AACC method 10-10B) and LFV was determined by means of rapeseed displacement (AACC method 10-05). LFV expressed on a 12% protein basis (corrected LFV), was determined as follows: for each 1% of protein content above 12%, 40 cm<sup>3</sup> was subtracted from the measured loaf volume and for each 1% protein content below 12%, 40 cm<sup>3</sup> were added to the measured loaf volume (SAGL, 2013).

### **3.2.3 Statistical analyses**

A Shapiro-Wilk test for normality was performed on the standardised residuals from the model before results could be assumed as reliable (Shapiro and Wilk, 1965). Levene's test was used to verify homogeneity of genotype and locality variances (Levene, 1960).

Analysis of variance (ANOVA) was performed using General Linear Models Procedure (PROC GLM) of SAS statistical software version 9.2 (SAS Institute Inc., Cary, USA). The primary method to analyse multi-environment trials is based on ANOVA, which is a fixed effects model and it requires homogenous variance-covariance of data. Therefore, sources of variation were divided into years, localities, replications within years and localities, genotypes and interactions of genotypes, years and localities. The statistical model is given by:

$$Y_{ijkl} = \mu + Y_i + L_j + YL_{ij} + B(YL_{ijk}) + G_k + GY_{ik} + GL_{ik} + GYL_{ijk} + \epsilon_{ijkl}$$

Where:  $Y_{ijkl}$  = observed parameter or characteristic

$\mu$  = general mean

$Y_i$  = effect of the year

$L_j$  = effect of the locality

$YL_{ij}$  = interaction effect of the year and locality

$B(YL_{ijk})$  = effect of block within year and locality

$G_k$  = effect of the genotype

$GY_{ik}$  = interaction effect of the genotype and year effect

$GL_{ik}$  = interaction effect of the genotype and locality

GYL<sub>ijk</sub> = interaction effect of the genotype, year and locality

€<sub>ijkl</sub> = error or residual effect

€<sub>ijkl</sub> ~ NID(0, σ<sup>2</sup>)

The Pearson product-moment correlation coefficients were also computed using PROC CORR in SAS software version 9.2 (SAS Institute, 2012). It measures the strength of a linear relationship between two variables. For response variables X and Y, it is denoted as r<sub>xy</sub> and was computed as:

$$r_{xy} = \frac{\sum_{i=1}^n \left( (x_i - \bar{x})(y_i - \bar{y}) \right)}{\sqrt{\sum_{i=1}^n (x_i - \bar{x})^2 \sum_{i=1}^n (y_i - \bar{y})^2}}$$

Repeatability was calculated according to formulae proposed by Falconer and MacKay (1996) and Piepho and Möhring (2007).

Heritability (h<sup>2</sup>) or repeatability = VG/VP,

where VG is the variation in genetic values

VP is the proportion of phenotypic variation.

Coefficient of variation (CV) was calculated as follows:

CV = (Standard deviation/Mean) \* 100

Coefficient of determination (R<sup>2</sup>) was calculated as follows:

$$R^2 = 1 - \frac{SS \text{ res}}{SS \text{ tot}}$$

Genotype contribution to variation (G-share) was calculated as follows:

= (Genotype sum of squares)/total SS) \* 100

### **3.3 Results**

#### ***3.3.1 Mixsmart parameters and primary quality characteristics***

MPT, the only mixogram parameter used during commercial classification of cultivars (SAGL, 2013), was higher for all three localities during the first season compared to the second season (Table 3.3). HLM and FLN were acceptable for all the localities during both growing seasons, indicative that the seed were well filled and no pre-harvest sprouting has occurred. Ladybrand had the lowest grain and FPC for both seasons, which could be that the P/L-values were higher for Ladybrand in both seasons, resulting from less distensibility. FABS was higher for Ladybrand during the second season (Table 3.3).

**Table 3.3 Mixsmart parameters and primary quality characteristics for the three environments over two seasons**

Parameter	First season			Second season		
	Bethlehem	Clarens	Ladybrand	Bethlehem	Clarens	Ladybrand
ELT	2.51 ± 0.91	1.90 ± 1.07	1.15 ± 0.88	1.95 ± 0.65	1.86 ± 0.64	1.40 ± 0.81
ELV	67.40 ± 7.23	59.93 ± 16.06	57.88 ± 17.05	55.42 ± 9.87	59.04 ± 10.32	51.02 ± 11.79
ELS	15.55 ± 6.51	24.26 ± 25.96	46.36 ± 43.23	19.44 ± 7.95	13.42 ± 11.14	25.28 ± 27.58
ELW	30.45 ± 5.03	27.65 ± 6.60	34.04 ± 11.52	23.00 ± 4.32	24.72 ± 4.81	27.01 ± 9.40
ELI	66.49 ± 24.51	49.22 ± 33.32	33.94 ± 29.32	37.12 ± 13.94	40.03 ± 15.60	31.63 ± 20.43
EPT	3.76 ± 0.88	3.38 ± 0.88	2.45 ± 1.24	3.01 ± 0.52	2.92 ± 0.55	2.35 ± 0.99
EPV	77.01 ± 8.33	74.10 ± 9.48	77.31 ± 6.35	63.48 ± 9.30	66.37 ± 6.91	61.90 ± 8.62
EPW	32.47 ± 4.52	31.55 ± 6.76	43.15 ± 14.25	23.88 ± 4.52	25.65 ± 3.78	32.74 ± 11.78
EPI	106.50 ± 23.48	94.43 ± 29.81	81.63 ± 38.68	62.15 ± 10.35	66.75 ± 11.20	57.22 ± 19.09
ERT	6.27 ± 1.114	5.99 ± 1.583	4.64 ± 2.237	5.08 ± 1.203	5.40 ± 1.05	5.01 ± 2.140
ERV	59.74 ± 4.91	56.42 ± 8.07	62.99 ± 7.13	48.28 ± 4.91	50.48 ± 3.58	52.84 ± 8.85
ERS	-12.49 ± 4.01	-14.03 ± 8.04	-15.95 ± 8.39	-13.20 ± 5.41	-12.14 ± 4.07	-10.22 ± 4.28
ERW	15.28 ± 3.87	15.08 ± 6.95	25.11 ± 13.45	10.12 ± 3.85	10.94 ± 2.64	20.53 ± 9.09
ERI	163.40 ± 32.21	148.20 ± 42.00	140.30 ± 51.20	93.55 ± 19.04	108.50 ± 19.18	112.10 ± 37.63
ETV	48.41 ± 4.78	44.32 ± 8.48	47.10 ± 4.72	37.46 ± 3.17	39.69 ± 2.58	40.00 ± 2.69
ETS	-1.28 ± 1.01	-2.20 ± 6.43	-1.99 ± 1.31	-1.00 ± 0.68	-0.92 ± 0.65	-1.27 ± 1.25
ETW	10.08 ± 4.33	9.08 ± 4.29	10.59 ± 3.35	6.04 ± 2.56	5.96 ± 2.45	9.34 ± 2.55
ETI	211.90 ± 34.22	199.40 ± 35.67	242.00 ± 42.06	132.30 ± 23.40	145.60 ± 21.27	191.90 ± 28.23
ETXV	60.91 ± 7.16	57.35 ± 7.97	61.79 ± 6.72	44.57 ± 4.06	47.83 ± 3.31	49.76 ± 4.60
ETXS	-4.74 ± 2.82	-5.83 ± 3.55	-4.88 ± 2.74	-2.82 ± 1.28	-3.32 ± 1.59	-3.57 ± 2.15
ETXW	16.59 ± 5.95	15.01 ± 4.94	18.21 ± 5.57	8.86 ± 4.02	9.78 ± 3.92	16.24 ± 2.91
ETXI	156.10 ± 22.99	149.70 ± 24.62	180.30 ± 27.96	100.30 ± 13.38	113.00 ± 10.72	136.70 ± 24.77
MLT	2.92 ± 0.91	2.73 ± 0.70	2.87 ± 0.66	2.13 ± 0.50	2.14 ± 0.47	2.36 ± 0.64
MLV	54.91 ± 4.74	53.82 ± 6.21	54.14 ± 4.06	44.92 ± 4.95	48.44 ± 3.47	44.33 ± 4.22
MLS	9.96 ± 3.80	8.60 ± 3.47	7.02 ± 3.08	8.74 ± 4.44	7.92 ± 3.41	4.13 ± 2.68
MLW	30.40 ± 4.38	29.85 ± 5.71	30.92 ± 3.98	23.79 ± 4.05	25.48 ± 3.47	27.29 ± 7.21
MLI	120.10 ± 37.29	112.30 ± 33.98	124.20 ± 31.98	71.53 ± 18.00	79.54 ± 18.15	83.98 ± 22.82
MPT	3.92 ± 0.91	3.73 ± 0.70	3.87 ± 0.66	3.13 ± 0.50	3.14 ± 0.47	3.36 ± 0.64
MPV	60.52 ± 6.28	58.89 ± 7.72	58.56 ± 5.42	51.32 ± 7.50	53.63 ± 5.29	46.94 ± 5.43
MPW	30.22 ± 5.27	27.55 ± 4.49	27.79 ± 3.60	22.01 ± 4.36	23.16 ± 3.59	22.46 ± 3.87
MPI	178.80 ± 36.68	169.40 ± 37.43	181.20 ± 32.61	120.30 ± 16.97	131.20 ± 16.96	129.90 ± 21.55
MRT	4.92 ± 0.91	4.73 ± 0.70	4.87 ± 0.66	4.13 ± 0.50	4.14 ± 0.47	4.36 ± 0.64
MRV	57.59 ± 5.79	55.16 ± 6.51	56.68 ± 4.914	46.58 ± 5.51	49.29 ± 3.86	45.33 ± 5.06
MRS	-4.39 ± 2.22	-5.20 ± 2.71	-2.61 ± 1.88	-5.40 ± 2.35	-5.19 ± 1.91	-2.53 ± 1.19
MRW	23.53 ± 5.14	20.26 ± 4.22	23.99 ± 4.11	13.60 ± 3.23	16.01 ± 2.85	19.72 ± 3.77

**Table 3.3 Mixsmart parameters and primary quality characteristics for the three environments over two seasons (continued)**

<b>MRI</b>	238.20 ± 36.69	226.80 ± 41.66	239.00 ± 33.81	169.60 ± 18.20	183.10 ± 16.83	176.20 ± 21.17
<b>MTV</b>	43.14 ± 3.24	39.62 ± 7.05	41.69 ± 3.53	34.34 ± 2.77	36.61 ± 1.72	35.06 ± 2.21
<b>MTS</b>	-0.78 ± 0.31	-1.49 ± 4.53	-1.08 ± 0.70	-0.57 ± 0.14	-0.62 ± 0.17	-0.62 ± 0.31
<b>MTW</b>	10.08 ± 4.32	9.08 ± 4.29	10.59 ± 3.35	6.04 ± 2.56	5.96 ± 2.45	9.34 ± 2.55
<b>MTI</b>	508.30 ± 41.37	488.60 ± 47.65	507.60 ± 35.93	414.80 ± 39.91	443.80 ± 25.84	417.00 ± 36.15
<b>MTXV</b>	52.28 ± 4.96	49.45 ± 6.58	52.34 ± 4.77	39.90 ± 3.52	42.68 ± 2.26	40.87 ± 3.53
<b>MTXS</b>	-3.17 ± 1.34	-3.74 ± 1.82	-3.48 ± 1.40	-2.11 ± 0.44	-2.31 ± 0.47	-2.14 ± 0.76
<b>MTXW</b>	16.59 ± 5.95	15.01 ± 4.94	18.21 ± 5.57	8.86 ± 4.02	9.78 ± 3.92	16.24 ± 2.91
<b>MTXI</b>	297.60 ± 30.04	291.10 ± 28.27	300.50 ± 20.85	250.00 ± 27.53	267.80 ± 19.35	248.10 ± 25.11
<b>HLM</b>	79.31 ± 1.96	76.90 ± 4.03	77.13 ± 1.01	79.33 ± 0.80	76.29 ± 1.45	78.94 ± 1.24
<b>FLN</b>	523 ± 75.13	432 ± 16.74	452 ± 26.55	471 ± 44.57	362 ± 72.47	394 ± 41.67
<b>FLY</b>	73.22 ± 1.02	74.21 ± 0.99	72.73 ± 1.51	74.59 ± 0.73	73.40 ± 1.02	72.33 ± 0.71
<b>KJ76</b>	-1.71 ± 0.87	-1.20 ± 1.01	-0.90 ± 1.15	-2.18 ± 0.80	-3.07 ± 0.82	-3.01 ± 0.73
<b>GPC</b>	12.58 ± 0.50	13.16 ± 0.90	12.16 ± 0.88	13.12 ± 0.58	12.86 ± 0.74	10.08 ± 0.63
<b>FPC</b>	11.12 ± 0.63	12.40 ± 1.03	10.87 ± 0.88	12.41 ± 0.60	11.98 ± 0.81	9.17 ± 0.76
<b>FABS</b>	57.74 ± 1.21	60.35 ± 1.63	58.82 ± 1.87	64.44 ± 2.08	62.32 ± 1.52	62.77 ± 2.12
<b>P/L</b>	0.69 ± 0.28	0.83 ± 0.33	1.22 ± 0.55	0.70 ± 0.18	0.81 ± 0.21	2.03 ± 0.88
<b>STR</b>	42.65 ± 5.79	54.83 ± 11.14	45.95 ± 6.95	58.68 ± 7.31	53.71 ± 7.34	41.42 ± 6.48
<b>LFV</b>	888.10 ± 46.78	894.38 ± 52.11	808.56 ± 48.2	950.50 ± 44.75	952.13 ± 53.78	793.75 ± 52.57
<b>LFV12%</b>	921.30 ± 39.55	876.10 ± 38.18	855.60 ± 36.76	891.30 ± 54.33	907.00 ± 55.87	878.00 ± 43.72

Values ± standard deviation, ELT = envelope left time, ELV = envelope left value, ELS = envelope left slope, ELW = envelope left width, ELI = envelope left integral, EPT = envelope peak time, EPV = envelope peak value, EPW = envelope peak width, EPI = envelope peak integral, ERT = envelope right time, ERV = envelope right value, ERS = envelope right slope, ERW = envelope right width, ERI = envelope right integral, ETV = envelope tail value, ETS = envelope tail slope, ETW = envelope tail width, ETI = envelope tail integral, ETXV = envelope timeX value, ETXS = envelope timeX slope, ETXW = envelope timeX width, ETXI = envelope timeX integral, MLT = midline left time, MLV = midline left value, MLS = midline left slope, MLW = midline left width, MLI = midline left integral, MPT = midline peak time, MPV = midline peak value, MPW = midline peak width, MPI = midline peak integral, MRT = midline right time, MRV = midline right value, MRS = midline right slope, MRW = midline right width, MRI = midline right integral, MTV = midline tail value, MTS = midline tail slope, MTW = midline tail width, MTI = midline tail integral, MTXV = midline timeX value, MTXS = midline timeX slope, MTXW = midline timeX width, MTXI = midline timeX integral, HLM = Hectolitre mass, FLN = Falling number, FLY = Flour yield, KJ76 = Corrected flour colour, GPC = Grain protein content, FPC = Flour protein content, FABS = Farinograph water absorption, P/L = Alveograph stability/distensibility ratio, STR = Alveograph dough strength, LFV = Loaf volume, LFV12% = Corrected loaf volume

### **3.3.2 Ranking of the Mixsmart parameters**

All 44 Mixsmart parameters were ranked for  $h^2$ , CV,  $R^2$  and G-share. For  $h^2$  values above 0.70 were selected, for CV values below or equal to 15% were selected, for  $R^2$  values above 80 were selected and for G-share values above 20% were selected (selections are indicated in green; Table 3.4).

MRI was the only parameter ranking in the same position (ninth) for  $h^2$ , CV and G-share. MTV ranked first for CV and  $R^2$ , MLI ranked third for  $R^2$  and G-share, envelope tail width (ETW) ranked sixth for CV and  $R^2$  and MTXW ranked sixth for  $R^2$  and G-share.

CV varied from -41.32% (all slope-parameters had negative values) to 68.23% for envelope left slope (ELS). Parameters with the lowest CV's were MTV, ETV, MTI, MTXV and MTXI. Of the selected parameters, 41% were envelope parameters and 77% were midline parameters. More midline parameters had CV's below and equal to 15% compared to envelope parameters.

Repeatability or  $h^2$  for the parameters ranged from 0.00 to 0.86, with MRS having the highest repeatability (0.86), higher values being more desirable. ETI and MLS both had values of 0.80, EPV and ERS ranked third, MLW was fourth and ETXV and MPV ranked fifth with values of 0.77, 0.75 and 0.74 respectively. Six envelope and 11 midline parameters (27% and 50% respectively) had repeatability values above 0.70.

The highest  $R^2$  values ( $R^2=0.96$ ) were obtained by ETV, ETXV, MRI, MTV and MTXV. MLV had the lowest  $R^2$  value ( $R^2= 0.53$ ). Eight envelope and 19 midline parameters (36% and 86% respectively) had  $R^2$  values above 80%, meaning that the overall ANOVA model explained more than 80% of the variation in those parameters, which suggest that the selected model adequately described the observed variability in those parameters.

**Table 3.4 Rankings of Mixsmart parameters for  $h^2$ , CV,  $R^2$  and genotype contribution to variation**

Parameter	$h^2$	Ranking	CV	Ranking	$R^2$	Ranking	G-share	Ranking
ELI	0.12	28	37.55	40	0.73	16	9.53	34
ELS	0.02	30	68.23	42	0.76	13	10.73	31
ELT	0.00	31	31.37	37	0.72	17	4.83	39
ELV	0.68	11	13.77	22	0.74	15	19.22	15
ELW	0.54	19	16.72	26	0.74	15	18.06	16
EPI	0.32	24	21.46	34	0.77	12	12.45	27
EPT	0.13	27	17.96	27	0.78	11	15.75	22
EPV	0.77	3	4.79	10	0.92	5	26.24	10
EPW	0.39	22	18.22	28	0.77	12	11.62	30
ERI	0.32	23	18.98	30	0.77	12	8.92	35
ERS	0.77	3	-24.21	35	0.75	14	30.66	8
ERT	0.00	31	20.68	33	0.63	18	8.33	36
ERV	0.45	21	6.63	13	0.85	10	12.16	29
ERW	0.00	31	35.05	38	0.73	16	8.92	35
ETI	0.80	2	8.24	17	0.93	4	17.15	20
ETS	0.00	31	-41.32	41	0.63	18	5.05	38
ETV	0.64	14	3.03	2	0.96	1	19.67	13
ETW	0.73	6	15.09	24	0.91	6	40.12	2
ETXI	0.54	19	7.81	16	0.93	4	10.39	33
ETXS	0.24	25	-37.11	39	0.72	17	10.40	32
ETXV	0.74	5	3.81	6	0.96	1	19.32	14
ETXW	0.72	7	14.91	23	0.91	6	33.30	6
MLI	0.69	10	9.93	18	0.94	3	36.13	3
MLS	0.80	2	20.29	31	0.88	9	34.89	4
MLT	0.71	8	10.34	19	0.91	6	43.07	1
MLV	0.56	18	15.44	25	0.53	19	13.52	25
MLW	0.75	4	11.36	20	0.77	12	21.72	12
MPI	0.69	10	6.3	12	0.95	2	30.69	7
MPT	0.71	8	7.38	14	0.91	6	43.07	1
MPV	0.74	5	4.43	8	0.93	4	25.21	11
MPW	0.66	13	7.68	15	0.89	8	17.95	17
MRI	0.70	9	4.69	9	0.96	1	26.35	9
MRS	0.86	1	-20.63	32	0.90	7	34.42	5
MRT	0.71	8	5.74	11	0.91	6	43.07	1
MRV	0.67	12	4.2	7	0.93	4	17.55	18
MRW	0.11	29	12.56	21	0.85	10	7.04	37
MTI	0.59	17	3.21	3	0.95	2	13.42	26
MTS	0.22	26	-27.74	36	0.76	13	13.91	24
MTV	0.62	15	2.87	1	0.96	1	12.31	28
MTW	0.73	6	15.09	24	0.92	5	40.12	2
MTXI	0.61	16	3.64	5	0.93	4	16.69	21
MTXS	0.53	20	-18.71	29	0.89	8	17.51	19
MTXV	0.73	6	3.49	4	0.96	1	14.41	23
MTXW	0.72	7	14.91	23	0.91	6	33.30	6

Green = selections,  $h^2$  = heritability or repeatability, CV = coefficient of variation,  $R^2$  = coefficient of determination, G-share = genotype contribution to variation, ELI = envelope left integral, ELS = envelope left slope, ELT = envelope left time, ELV = envelope left value, ELW = envelope left width, EPI = envelope peak integral, EPT = envelope peak time, EPV = envelope peak value, EPW = envelope peak width, ERI = envelope right integral, ERS = envelope right slope, ERT = envelope right time, ERV = envelope right value, ERW = envelope right width, ETI = envelope tail integral, ETS = envelope tail slope, ETV = envelope tail value, ETW = envelope tail width, ETXI = envelope timeX integral, ETXS = envelope timeX slope, ETXV = envelope timeX value, ETXW = envelope timeX width, MLI = midline left integral, MLS = midline left slope, MLT = midline left time, MLV = midline left value, MLW = midline left width, MPI = midline peak integral, MPT = midline peak time, MPV = midline peak value, MPW = midline peak width, MRI = midline right integral, MRS = midline right slope, MRT = midline right time, MRV = midline right value, MRW = midline right width, MTI = midline tail integral, MTS = midline tail slope, MTV = midline tail value, MTW = midline tail width, MTXI = midline timeX integral, MTXS = midline timeX slope, MTXV = midline timeX value, MTXW = midline timeX width

Parameters with the highest G-share were the midline time parameters (MLT, MPT and MRT), followed by ETW and MTW, showing a G-share of 40.12%. The parameter having the lowest G-share (4.83%), was ELT, which was also the parameter having the lowest repeatability. A total of 18% for envelope and 55% for midline parameters had a G-share above 20%.

### **3.3.3 Selection of Mixsmart parameters**

When the four selection criteria ( $h^2$ , CV,  $R^2$  and G-share) were applied, the slope parameters, MLS and MRS, qualified for  $h^2$ ,  $R^2$  and G-share. Other slope parameters, MTS and MTXS, did not qualify. MTS did not adhere to any of the selection criteria and MTXS only qualified for genotype contribution to variation.

The three midline time parameters, MPT, MLT and MRT were comparable (they adhered to all four selection criteria), but MPT was selected since it is the only mixogram parameter used in South Africa (SAGL, 2013) during the classification process.

Regarding the midline value parameters (MPV, MLV, MRV, MTV and MTXV), MPV was selected, since it qualified for all four selection criteria ( $h^2$ , CV,  $R^2$  and G-share). MLV did not qualify at all while MRV and MTV only adhered to two of the criteria and MTXV adhered to four of the criteria, but had a low G-share.

MRI was the only midline integral parameter that adhered to all four selection criteria. MLI and MPI adhered to CV,  $R^2$  and genotype contribution to variation. MTI and MTXI adhered to CV and  $R^2$ . Rankings were inconsistent for the parameters (Table 3.4).

Regarding the midline width parameters (MLW, MPW, MRW, MTW and MTXW), MTW and MTXW both qualified for all four selection criteria, but MTW was selected since it indicated the width of the curve at six minutes (TimeX) and in South Africa 6 min is the standard time for a mixograph analyses. MLW adhered to three of the criteria ( $h^2$ , CV% and G-share) and MPW as well as MRW adhered to CV and  $R^2$ .

Based on selected parameters having to cover the whole dough mixing process and that more midline parameters adhered to the selection criteria, the following midline parameters were selected (Figure 3.1): MLS and MRS (covering the opposite slopes of the mixogram), MPT (covering mixing time), MPV (covering the height of the curve), MRI (covering the area under the curve) and MTW (covering the width of the curve).

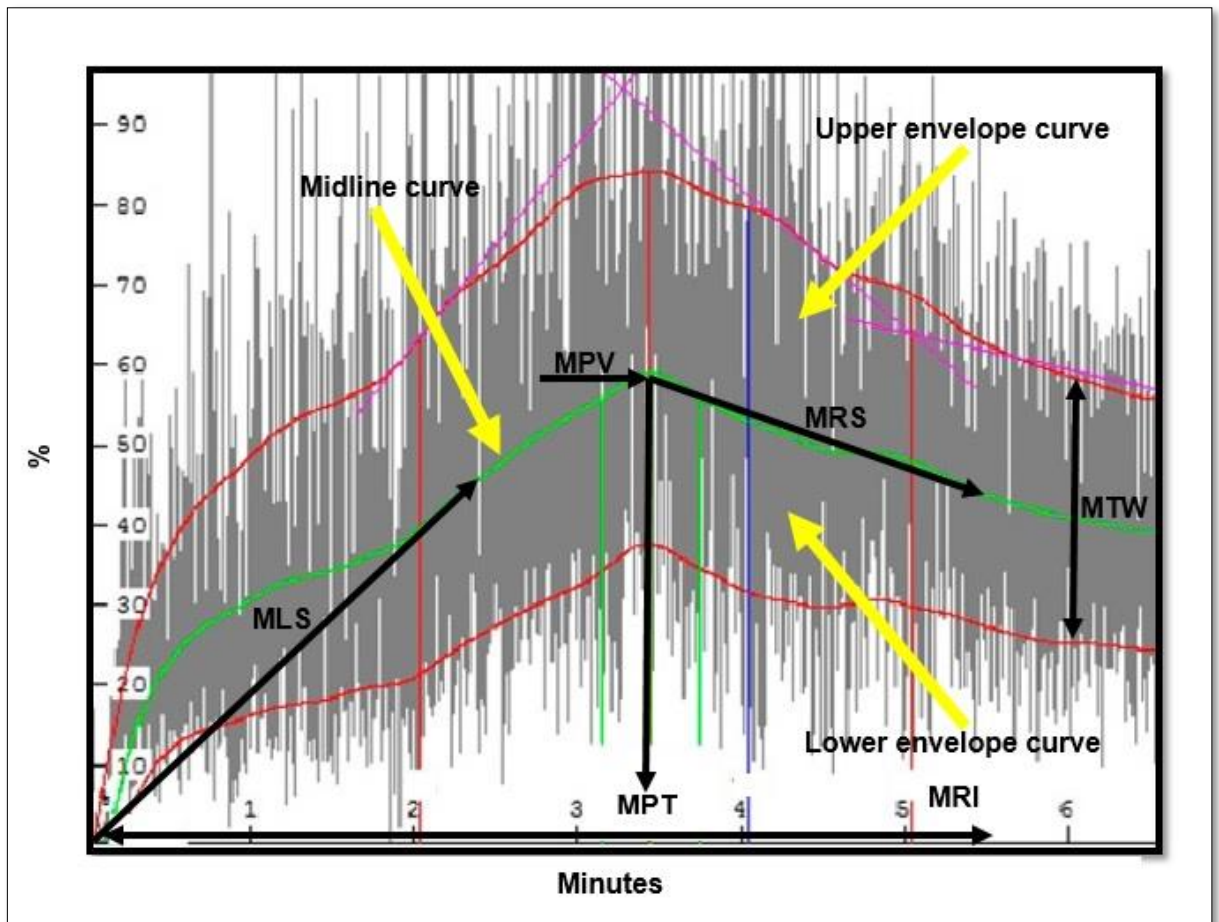


Figure 3.1 Selected Mixsmart parameters on a mixogram

### ***3.3.4 Correlations between the selected Mixsmart parameters and primary wheat classification characteristics***

Highly significant positive correlations ( $P \leq 0.001$ ) were observed between MLS and MPV with GPC and FPC (Table 3.5) and significant correlations ( $P \leq 0.01$ ) were observed between MLS and MPV with FLN. MLS was the only parameter that correlated positively with FLY and LFV12%. MRS and MPT showed positive correlations with P/L and MPT, MPV and MRI exhibited high correlations with KJ76.

**Table 3.5 Correlations between the selected Mixsmart parameters and primary quality characteristics**

Parameter	HLM	FLN	GPC	FPC	FLY	KJ76	FABS	P/L	STR	LFV	LFV12%
<b>MLS</b>	ns	0.21**	0.45***	0.40***	0.37***	Ns	ns	-0.42***	0.17**	0.42***	0.23***
<b>MRS</b>	0.13*	ns	-0.50***	-0.52***	-0.45***	Ns	-0.25***	0.41***	-0.35***	-0.55***	-0.21**
<b>MPT</b>	ns	ns	Ns	ns	-0.42***	0.46***	-0.46***	0.24***	ns	-0.28***	-0.14*
<b>MPV</b>	-0.13*	0.19**	0.40***	0.31***	ns	0.27***	-0.25***	-0.28***	0.15*	0.19**	ns
<b>MRI</b>	-0.18*	0.18**	0.16*	ns	-0.32***	0.52***	-0.54***	ns	ns	-0.17**	ns
<b>MTW</b>	ns	0.16*	-0.21**	-0.28***	-0.50***	0.33***	-0.33***	0.32***	ns	-0.33***	ns

\* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ , ns = not significant, MLS = midline left slope, MRS = midline right slope, MPT = midline peak time, MPV = midline peak value, MRI = midline right integral, MTW = midline tail width HLM = Hectolitre mass, FLN = Falling number, GPC = Grain protein content, FPC = Flour protein content, FLY = Flour yield, KJ76 = Corrected flour colour, FABS = Farinograph water absorption, P/L = Alveograph stability/distensibility ratio, STR = Alveograph dough strength, LFV = Loaf volume, LFV12% = Corrected loaf volume

### **3.3.5 Correlations between the selected Mixsmart parameters**

MLS and MPV were highly significantly ( $P \leq 0.001$ ) positively correlated (Table 3.6). Highly significant ( $P \leq 0.001$ ) positive correlations also existed between MRS and MTW. MPT positively correlated highly significantly with three of the other selected parameters, namely MRS, MRI and MTW. MPV showed a highly significant positive correlation with MRI and MRI showed highly significant positive correlations with MPT, MPV and MTW. MTW also had highly significant positive correlations with MRS and MPT respectively.

**Table 3.6 Correlations between the selected Mixsmart parameters**

Parameter	MLS	MRS	MPT	MPV	MRI
<b>MRS</b>	-0.75***				
<b>MPT</b>	-0.37***	0.30***			
<b>MPV</b>	0.71***	-0.63***	ns		
<b>MRI</b>	ns	ns	0.76***	0.59***	
<b>MTW</b>	-0.46***	0.64***	0.57***	-0.15*	0.44***

\* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ , ns = not significant, MLS = midline left slope, MRS = midline right slope, MPT = midline peak time, MPV = midline peak value, MRI = midline right integral, MTW = midline tail width

### 3.4 Discussion

Although growing conditions due to low rainfall were unfavourable before planting in the first season and during the second growing season (Table 3.2), the harvested material's quality was acceptable (Table 3.4) as indicated by HLM ( $> 76 \text{ kg} \cdot \text{hl}^{-1}$ ), protein content ( $\geq 11\%$ , except for Ladybrand 2<sup>nd</sup> season) and FLN ( $> 250 \text{ s}$ ). According to Nel et al. (1998), Koekemoer (2003) and SAGL (2013), HLM values of above  $74.00 \text{ kg hl}^{-1}$  is required for bread making purposes and values higher than  $76.00 \text{ kg hl}^{-1}$  are required when potential breeding lines are evaluated for cultivar classification purposes.

Except for a few parameters, rankings for the different parameters were inconsistent (Table 3.5) for  $h^2$ , CV,  $R^2$  and G-share. Parameters were, therefore, not selected based solely on ranking, but also taking into account the adherence of the parameters to the four selection criteria ( $h^2$ , CV,  $R^2$  and G-share). Parameters that adhered to all four selection criteria or those that exhibited higher adherence to the criteria were selected, ensuring that only one parameter describing each individual part of the dough development process, as shown on a mixogram (Figure 3.1), was selected.

The selected parameters represented the whole dough mixing process and showed highly significant positive correlations with primary criteria utilised during the commercial cultivar classification process in South Africa. MLS had highly significant ( $P \leq 0.001$ ) positive correlations with GPC and FPC, FLY and LfV12%

respectively (Table 3.6). This was in agreement with Chung et al. (2001) and Miles et al. (2012; 2014) who also reported correlations between MLS and protein content. Miles et al. (2014) reported correlations between MLS and FABS, STR and LFV. MRS, MPT, MTW and MRI exhibited highly significant positive correlations with P/L, also reported by Labuschagne et al. (2016). MPT correlated with KJ76, but did not correlate with GPC or FPC; the latter was in agreement with Khatkar et al. (1996). MPV correlated with GPC, FPC and KJ76, where elsewhere correlations between MPV and LFV (Khatkar et al., 1996; Miles et al., 2014; Labuschagne et al., 2016), MPV and protein content (Finney and Shogren, 1972; Lang et al., 1992; Khatkar et al., 1996; Martinant et al., 1998; Békés et al., 2001; Dobraszczyk and Schofield, 2002; Miles et al., 2012; 2014; Labuschagne and Moloi, 2015; Labuschagne et al., 2016), MPV and FABS (Miles et al., 2014) and MPV and STR (Miles et al., 2014; Labuschagne et al., 2016) were reported. MRI and KJ76 correlated highly significantly and Labuschagne et al. (2016) reported correlations between MRI and STR, FPC and LFV. No significant correlation was observed between MPV and LFV12%, although Khatkar et al. (1996) reported a highly significant correlation between these parameters. A negative relationship was observed between MPT and LFV12%, where Dong et al. (1992) and Preston et al. (1992) reported no relationship between these two parameters.

Correlations between several of the selected parameters existed. Highly significant positive correlations ( $P \leq 0.001$ ) were observed between MLS and MRI, compared to a study conducted by Miles et al. (2012; 2014) where correlations were reported between MLS and MPV as well as between MRI, MTW and MPT respectively. MRS, MPT and MTW also exhibited highly significant positive correlations among each other as was also reported by Miles et al. (2013; 2014). Highly significant positive correlations were also observed between MPV, MLS and MRI where Martinant et al. (1998) reported correlations between MPV and MPW. MRI, MPT, MPV and MTW also exhibited highly significant positive correlations with one another (Table 3.7). MRI correlated negatively with both slope parameters (MLS and MRS), MPT correlated negatively with MLS and MPV correlated negatively with MRS and MTW.

### 3.5 Conclusions

Combination of the four criteria ( $h^2$ , CV%,  $R^2$  and G-share) for selecting Mixsmart parameters resulted in choosing parameters which ranked below the tenth position for  $h^2$  and  $R^2$ , below 11<sup>th</sup> for G-share and below 33<sup>rd</sup> for CV. Breeders should not select parameters solely based on CV, since rankings were high for four of the selected parameters (MLS, MRS, MPR and MTW) for CV. A combination with any or with all three of the other criteria, is recommended. The importance of  $h^2$ ,  $R^2$  and G-share are closely linked, since from a breeder's perspective a selected parameter should be highly repeatable (heritable), it should be less affected by environment and higher  $R^2$  values indicate that the selected model provides sufficient description of the variability observed in the selected parameter, as can be observed in this study. The selected parameters (MLS, MRS, MPT, MPV, MRI and MTW) are representative of the whole dough mixing process, therefore bread wheat breeders should be able to apply them successfully as indicators of the quality parameters that they need to be improved.

### 3.6 References

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## **CHAPTER 4**

### **MIXSMART PARAMETERS AS POSSIBLE INDICATORS FOR BREAD MAKING QUALITY IN THE EASTERN DRY LAND SUMMER RAINFALL REGION OF SOUTH AFRICA**

#### **Abstract**

Six Mixsmart parameters, midline left slope (MLS), midline right slope (MRS), midline peak time (MPT), midline peak value (MPV), midline right integral (MRI) and midline tail width (MTW) , which were identified in the previous chapter, were selected and evaluated to determine whether they would be indicative of good bread making quality characteristics. Bread wheat cultivars of the summer rainfall region of South Africa trials, which formed part of the National Cultivar evaluation, were planted at three locations during two seasons. The 11 primary quality characteristics included hectolitre mass, falling number, grain protein content, flour protein content, flour yield, corrected flour colour, farinograph water absorption, alveograph, alveograph stability/distensibility ratio value, alveograph dough strength, loaf volume and corrected loaf volume. MLS and MPV were the only parameters correlating significantly with loaf volume, the final test for bread making potential. MLS also correlated with falling number (FLN), grain protein content (GPC), flour protein content (FPC), flour yield (FLY), alveograph dough strength (STR) and corrected loaf volume (LFV12%). MPV also correlated positively with FLN, GPC, FPC, corrected flour colour (KJ76) and STR. All six Mixsmart parameters showed highly significant positive correlations with the primary quality characteristics, confirming that they could be good indicators of primary quality characteristics for this region.

#### **4.1 Introduction**

The dry land summer rainfall region (Free State) produced 308 460 tons of bread wheat during the last season, which was the second highest figure nationally, with the Western Cape (winter rainfall region) having the highest produce (1 098 200 tons) and the irrigation areas with the lowest produce of 266 000 tons

(SAGL, 2017). Depending on the rainfall, producers have a choice to cultivate winter and/or facultative wheat types in the dry land summer rainfall region. Wheat cultivated in this region usually produces flour with high protein content, longer mixing times and acceptable LFV (SAGL, 2017).

A mixograph is a rheological apparatus where flour and water are mixed and the resistance of the gluten to the mixing action is registered as a curve known as the mixogram. Mixsmart software® analyses a mixogram, resulting in 44 parameters (Walker and Walker, 1992; Dobraszczyk and Schofield, 2002). These parameters are measurements made at different heights, widths, areas, times and slopes on a mixogram (Pon et al., 1989). The software divides the mixogram into two envelope curves with a midline. Twenty-two parameters each are measured on the envelope and midline curve respectively. In South Africa, peak time is the only mixograph parameter taken into account when potential breeding lines are evaluated for bread making quality (SAGL, 2013), although several reports exist stating that peak time is not a good indicator of good bread making quality, since it is poorly correlated with LFV (Dong et al., 1992; Preston et al., 1992; Khatkar et al., 1996; Martinant et al., 1998; Labuschagne et al., 2016). Advantages of using a mixograph includes that small sample sizes are required, results are available within six minutes, the technique is easy to perform and the analysis is cheap.

The aim of this study was to determine the relationships between six selected mixograph parameters (MLS, MRS, MPT, MPV, MRI and MTW) as measured by Mixsmart® software, and 11 primary quality criteria (HLM, FLN, GPC, FPC, FLY, KJ76, FABS, alveograph, P/L value, alveograph dough strength, LFV and LFV12% used during the cultivar classification process in South Africa, in the high potential eastern part of the dry land summer rainfall production region.

## **4.2 Materials and methods**

### ***4.2.1 Field trials***

As discussed in Chapter 3, section 3.2.1.

## 4.2.2 Laboratory analyses

As discussed in Chapter 3, section 3.2.2.

## 4.2.3 Selection of Mixsmart parameters

As discussed in Chapter 3, section 3.3.3. The selected parameters can be viewed in Chapter 3, Figure 3.1.

A description of the selected parameters, the abbreviation and unit of measurement can be seen in Table 4.1.

**Table 4.1 Mixsmart parameters, descriptions, abbreviations and units of measurement**

Mixsmart parameter	Description	Abbreviation	Unit
Midline left slope	Midline curve slope from beginning until 1 min before midline peak time	MLS	%/min
Midline peak time	Time where midline curve reaches a peak – optimum dough development	MPT	min
Midline peak value	Midline curve height at midline peak time	MPV	%
Midline right slope	Slope measured from midline peak time until 2 min after midline peak time	MRS	%/min
Midline right integral	Midline area under curve from beginning until 2 min after midline peak time	MRI	%Torque* min
Midline tail width	Midline curve-width at end of mixing process	MTW	%

## 4.2.4 Statistical analyses

A Shapiro-Wilk test for normality, Levene's test, ANOVA and Pearson correlation coefficients were determined as discussed in Chapter 3, section 3.2.3

## 4.3 Results

### 4.3.1 Analysis of variance for the selected Mixsmart parameters

ANOVA indicated that genotype effect was highly significant ( $P \leq 0.001$ ) for all six selected Mixsmart parameters (Table 4.2), indicating large differences between the cultivars for the selected Mixsmart parameters.

**Table 4.2 Analysis of variance for the six selected Mixsmart parameters for the eastern dry land summer rainfall region**

Parameter	G	E	Y	G X E	G X Y	E X Y
MLS	136.40***	292.89***	185.22***	21.02***	24.73***	13.47**
MPT	5.53***	0.57***	23.11***	0.36***	1.62***	0.33**
MPV	417.03***	273.78***	5038.62***	107.50***	60.42***	210.99***
MRS	50.28***	177.41***	0.40 <sup>ns</sup>	4.74***	7.59***	12.51***
MRI	11885.13***	322.98*	209219.64***	883.46***	3599.04***	3312.31***
MTW	138.71***	132.73***	464.09***	10.21***	37.49***	30.17***

\* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ , ns = not significant, G = Genotype, E = Environment, Y = Year, G X E = Genotype environment interaction, G X Y = Genotype year interaction, E X Y = Environment year interaction, MLS = midline left slope, MPT = midline peak time, MPV = midline peak value, MRS = midline right slope, MRI = midline right integral, MTW = midline tail width

The same trend was observed for environment effect, indicating that the environment had large effects on the selected parameters, although MRI was less affected ( $P \leq 0.05$ ) than the other characteristics. Year effect was not significant for the Mixsmart parameter MRS, indicating that the season did not affect MRS, although the other Mixsmart parameters were significantly affected by the season. Interactions between genotype and environment as well as between genotype and year were also highly significant ( $P \leq 0.001$ ), indicating that the genotypes did not react similarly for the different environments as well as for the two seasons regarding the selected Mixsmart parameters. The interaction between environment and year also indicated that the environments did not respond the same for the two seasons.

#### ***4.3.2 Mixsmart parameters and primary quality characteristics***

Overall, Ladybrand 2009 exhibited the lowest slope values for both MLS and MRS, resulting in flatter curves (Table 4.3), as can be confirmed by the MPV values (curve heights) for this locality.

Scientists have recorded MPV to be a better indicator of LFV than MPT (Khatkar et al., 1996; Martinant et al., 1998; Labuschagne et al., 2016) which can also be seen here as for both seasons Ladybrand had lower MPV values and lower LFV. MPT values were higher during the first season compared to the second season, also being confirmed by higher MRI values for 2007 compared to 2009. Grain characteristics, HLM and FLN, confirmed that the kernels were well-filled and that pre-harvest sprouting did not occur. Flour colour (KJ76) for all the samples were acceptable. For both seasons, Ladybrand exhibited higher P/L values, indicating less dough distensibility, resulting in lower LFV. Lower FPC were also observed at Ladybrand for both seasons.

**Table 4.3 Selected Mixsmart parameters and primary quality characteristics for the three environments over two seasons**

Parameter	First season			Second season		
	Bethlehem	Clarens	Ladybrand	Bethlehem	Clarens	Ladybrand
<b>MLS</b>	9.96 ± 3.80	8.60 ± 3.47	7.02 ± 3.08	8.74 ± 4.44	7.92 ± 3.41	4.13 ± 2.68
<b>MPT</b>	3.92 ± 0.91	3.73 ± 0.70	3.87 ± 0.66	3.13 ± 0.50	3.14 ± 0.47	3.36 ± 0.64
<b>MPV</b>	60.52 ± 6.28	58.89 ± 7.72	58.56 ± 5.42	51.32 ± 7.50	53.63 ± 5.29	46.94 ± 5.43
<b>MRS</b>	-4.39 ± 2.22	-5.20 ± 2.71	-2.61 ± 1.88	-5.40 ± 2.35	-5.19 ± 1.91	-2.53 ± 1.19
<b>MRI</b>	238.20 ± 36.69	226.80 ± 41.66	239.00 ± 33.81	169.60 ± 18.20	183.10 ± 16.83	176.20 ± 21.17
<b>MTW</b>	10.08 ± 4.32	9.08 ± 4.29	10.59 ± 3.35	6.04 ± 2.56	5.96 ± 2.45	9.340 ± 2.55
<b>HLM</b>	79.31 ± 1.96	76.90 ± 4.03	77.13 ± 1.01	79.33 ± 0.80	76.29 ± 1.45	78.94 ± 1.24
<b>FLN</b>	523.00 ± 75.13	432 ± 16.74	452.00 ± 26.55	471.00 ± 44.57	362.00 ± 72.47	394 ± 41.67
<b>FLY</b>	73.22 ± 1.02	74.21 ± 0.99	72.73 ± 1.51	74.59 ± 0.73	73.40 ± 1.02	72.33 ± 0.71
<b>KJ76</b>	-1.71 ± 0.87	-1.20 ± 1.01	-0.90 ± 1.15	-2.18 ± 0.80	-3.07 ± 0.82	-3.01 ± 0.73
<b>GPC</b>	12.58 ± 0.50	13.16 ± 0.90	12.16 ± 0.88	13.12 ± 0.58	12.86 ± 0.74	10.08 ± 0.63
<b>FPC</b>	11.12 ± 0.63	12.40 ± 1.03	10.87 ± 0.88	12.41 ± 0.60	11.98 ± 0.81	9.17 ± 0.76
<b>FABS</b>	57.74 ± 1.21	60.35 ± 1.63	58.82 ± 1.87	64.44 ± 2.08	62.32 ± 1.52	62.77 ± 2.12
<b>P/L</b>	0.69 ± 0.28	0.83 ± 0.33	1.22 ± 0.55	0.70 ± 0.18	0.81 ± 0.21	2.03 ± 0.88
<b>STR</b>	42.65 ± 5.79	54.83 ± 11.14	45.95 ± 6.95	58.68 ± 7.31	53.71 ± 7.34	41.42 ± 6.48
<b>LFV</b>	888.10 ± 46.78	894.38 ± 52.11	808.56 ± 48.20	950.50 ± 44.75	952.13 ± 53.78	793.75 ± 52.57
<b>LFV12%</b>	921.30 ± 39.55	876.10 ± 38.18	855.60 ± 36.76	891.30 ± 54.33	907.00 ± 55.87	878.00 ± 43.72

Values ± standard deviation. MLS = midline left slope, MPT = midline peak time, MPV = midline peak value, MRS = midline right slope, MRI = midline right integral, MTW = midline tail width, HLM = Hectolitre mass, FLN = Falling number, FLY= Flour yield, KJ76 = Corrected flour colour, GPC = Grain protein content, FPC = Flour protein content FABS = Farinograph water absorption, P/L = Alveograph stability/distensibility ratio, STR = Alveograph dough strength, LFV = Loaf volume, LFV12% = Corrected loaf volume

### **4.3.3 Correlations between the selected Mixsmart parameters and primary wheat quality characteristics**

Mixsmart parameters were very good predictors of the primary quality criteria, since many highly significant ( $P \leq 0.001$ ) positive correlations were observed (Table 4.4). MLS was positively correlated with grain and FPC, LFV as well as with LFV12%. Interestingly, the correlation was much higher with LFV ( $r = 0.42$ ) compared to LFV12% ( $r = 0.23$ ). MPT and MTW correlated positively with KJ76 and P/L. MPV correlated with grain and FPC and KJ76. MRS only correlated with P/L and MRI with KJ76.

## **4.4 Discussion**

Chung et al. (2001) and Miles et al. (2012; 2014) reported highly significant ( $P \leq 0.001$ ) positive correlations between MLS and protein content, as was also observed in this study. MPT, MTW and MRI showed highly significant ( $P \leq 0.001$ ) positive correlations with P/L, also reported by Labuschagne et al. (2016), confirmed by this study, except for MRI where no significant correlations was reported with P/L. In agreement with Khatkar et al. (1996), MPT showed no correlations with protein content. MPV showed correlations with GPC and FPC, in agreement with Finney and Shogren (1972), Lang et al. (1992), Khatkar et al. (1996), Martinant et al. (1998), Békés et al. (2001), Dobraszczyk and Schofield (2002), Miles et al. (2012; 2014), Labuschagne and Moloï (2015) and Labuschagne et al. (2016).

Alveograph dough strength is determined by dividing the W-value with a constant, namely 6.54 (AACC, 2000). In a study conducted by Labuschagne et al. (2016), they reported highly significant positive correlations ( $P \leq 0.001$ ) between alveograph W-value and MPV as well as between alveograph W-value and MRI for the summer rainfall area in South Africa.

**Table 4.4 Correlations between the selected Mixsmart parameters and primary quality characteristics during cultivar classification**

Parameter	HLM	FLN	GPC	FPC	FLY	KJ76	FABS	P/L	STR	LFV	LFV12%
<b>MLS</b>	ns	0.21**	0.45***	0.40***	0.37***	ns	ns	-0.42***	0.17**	0.42***	0.23***
<b>MPT</b>	ns	ns	ns	ns	-0.42***	0.46***	-0.46***	0.24***	ns	-0.28***	-0.14*
<b>MPV</b>	-0.13*	0.19**	0.40***	0.31***	ns	0.27***	-0.25***	-0.28***	0.15*	0.19**	ns
<b>MRS</b>	0.13*	ns	-0.50***	-0.52***	-0.45***	ns	-0.25***	0.41***	-0.35***	-0.55***	-0.21**
<b>MRI</b>	-0.18*	0.18**	0.16*	ns	-0.32***	0.52***	-0.54***	ns	ns	-0.17**	ns
<b>MTW</b>	ns	0.16*	-0.21**	-0.28***	-0.50***	0.33***	-0.33***	0.32***	ns	-0.33***	ns

\*P ≤ 0.05, \*\*P ≤ 0.01, \*\*\*P ≤ 0.001, MLS = midline left slope, MPT = midline peak time, MPV = midline peak value, MRS = midline right slope, MRI = midline right integral, MTW = midline tail width, HLM = Hectolitre mass, FLN = Falling number, GPC = Grain protein content, FPC = Flour protein content, FLY = Flour yield, KJ76 = Corrected flour colour, FABS = Farinograph water absorption, P/L = Alveograph stability/distensibility ratio, STR = Alveograph dough strength, LFV = Loaf volume, LFV12% = Corrected loaf volume

In this study no significant correlation was observed between alveograph dough strength and MRI, although a significant correlation was observed between dough strength and MPV ( $P \leq 0.05$ ). MLS was the best predictor for LFV ( $r = 0.42$ ) and GPC ( $r = 0.45$ ). MRI was the best predictor for KJ76 ( $r = 0.52$ ) and MPV was the best predictor for GPC ( $r = 0.40$ ). MTW (at 6 min) was a good predictor of P/L ( $r = 0.32$ ) where Labuschagne et al. (2016) confirmed midline TimeX width (also at 6 min) a good predictor of P/L.

Negative correlations were observed between MPT and LFV as well as LFV12%, confirming reports by Dong et al. (1992), Preston et al. (1992), Khatkar et al. (1996), Martinant et al. (1998) and Labuschagne et al. (2016), where no significant positive correlations were reported between MPT and LFV. However in this study, a positive correlation between MPV and LFV was observed, as was also reported by Khatkar et al. (1996), Martinant et al. (1998), Miles et al. (2014) and Labuschagne et al. (2016), confirming that MPV was a better predictor of LFV than MPT.

In this study, MLS was the only Mixsmart parameter showing highly significant positive correlations with both LFV parameters. Interesting is the higher correlation that was observed between MLS and LFV ( $r = 0.42$ ) compared to the lower correlation between MLS and LFV12% ( $r = 0.23$ ). Wikström and Bohlin (1996) also reported positive correlations between MLS and LFV.

#### **4.5 Conclusions**

All six selected Mixsmart parameters (MLS, MRS, MPT, MPV, MRI and MTW) showed highly significant positive correlations with the 11 primary quality characteristics selected, indicating that they are useful as indicators of these characteristics in the summer rainfall region of South Africa. With LFV being the final test, a potential breeding line should have good bread making potential, therefore it would be important that the selected Mixsmart parameters have to exhibit strong positive correlations with LFV. MLS was the only parameter with highly significant positive correlations with both LFV characteristics and it was also the parameter having the most significant correlations with the primary

quality characteristics, being correlated positively with seven primary quality characteristics in total. MPV was the other Mixsmart parameter worthy of taking notice of, since it exhibited six positive correlations with primary quality characteristics, including LFV. It is risky to use a single parameter to indicate acceptable bread making quality, therefore it would be more useful to use all six Mixsmart parameters as indicators of superior bread making quality.

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## CHAPTER 5

### AMMI AND GGE-BIPLLOT ANALYSIS FOR STABILITY OF GENOTYPES FOR SELECTED MIXSMART PARAMETERS

#### Abstract

Stability of six selected Mixsmart parameters for ten South African bread wheat genotypes, grown in six environments, was investigated by means of Additive Main Effects and Multiplicative Interaction (AMMI) and Genotype main effects plus genotype environment interaction (GGE-biplots). The Mixsmart parameters were midline left slope (MLS), midline right slope (MRS), midline peak time (MPT), midline peak value (MPV), midline right integral (MRI) and midline tail width (MTW). Genotypes varied for stability in the Mixsmart parameters as indicated by AMMI and GGE-biplots. Both models agreed on the same genotypes as being most stable for MPV and MRI. For only two of the Mixsmart parameters (MPT and MTW), the most stable genotype was also the ideal genotype as indicated by the GGE-biplot. According to the AMMI analysis, PAN3377, Caledon, PAN3118, Matlabas, Gariep and Elands repeatedly ranked in the four top positions for the six Mixsmart parameters. The percentage variation explained by PC1 and PC2 of the GGE-biplots were 81.66%, 91.13%, 88.61%, 75.54%, 88.91% and 84.91% for MLS, MRS, MPT, MPV, MRI and MTW respectively.

#### 5.1 Introduction

South African wheat breeders use mixograph midline peak time (MPT) as a selection tool for acceptable bread making quality for potential breeding lines (SAGL, 2013). Mixsmart® software enables researchers to investigate more parameters on a mixogram than just the currently used MPT. The overall shape of the mixogram gives information on the dough rheological and functional properties (Walker and Hazelton, 1996). Curve heights (indicated as value parameters) give information about dough consistency, curve widths (indicated as width parameters) and slopes (indicated as slope parameters) give

information about mixing tolerance. Areas (indicated as integral parameters) under the curve, give information about dough strength and time parameters give information about the time it takes the flour to develop up to a certain point (Walker and Hazelton, 1996; Walker et al., 1997).

It is well known that quality traits are affected by genotype, environment and their interaction (G X E). Stability of quality characteristics over environments and years is, therefore, an important focus for any wheat breeding programme, since the milling and baking industry requires a constant raw quality product from producers (Grausgruber et al., 2000; Barić et al., 2004; SAGL, 2017). LFV is the final quality test a potential breeding line must pass before it can be commercially released in South Africa (SAGL, 2013). G X E influences the performance of genotypes, usually resulting in multi-environment trials to determine G X E and to identify the genotypes that are suitable to satisfy the clients' needs (Kaya et al., 2006; Mut et al., 2010; Mitrovic et al., 2012).

Fluctuations in daily temperatures (Borghini et al., 1995), precipitation distribution (Salinger et al., 1995; Smith and Gooding, 1999) and fertiliser application, especially nitrogen, during the growing season (Anderson et al., 1998; Kettlewell et al., 1998; Monaghan et al., 2001), influence wheat quality characteristics. Other environmental conditions affecting quality characteristics include humidity and temperature, especially during the grain filling stage (Blumenthal et al., 1993; Peterson et al., 1998), as well as grain fill duration (Stone and Savin, 1999), planting date and sowing rate (Anderson et al., 1998), growing season temperature (Smith and Gooding, 1999) and drought (Guttieri et al., 2001).

Researchers use the Additive Main Effects and Multiplicative Interaction (AMMI) model, which includes G X E (Gauch et al., 2008) and the genotype main effects plus genotype environment interaction (GGE) model, which includes G + G X E (Yan et al., 2007) to simplify and visualise G X E effects (Booyse, 2014). Purchase et al. (2000) developed the AMMI stability value (ASV), which is a single value used to rank genotypes and environments for stability. A genotype is regarded as stable when different environments do not affect the performance of the genotype (Becker and Léon, 1988; Grausgruber et al., 2000), in other

words when genotypes make a low contribution to G X E. An ideal genotype is a genotype that is stable and has the highest mean performance (Mohammadi and Amri, 2012). The ideal is therefore to be able to predict in advance how a genotype will perform under certain conditions (Grausgruber et al., 2000).

The aim of this study was to investigate ten commercial cultivars by means of the stability of six selected Mixsmart parameters to assist breeders in selecting stable breeding lines exhibiting superior quality.

## **5.2 Materials and methods**

### **5.2.1 *Field trials***

As discussed in Chapter 3, section 3.2.1.

### **5.2.2 *Laboratory analyses***

As discussed in Chapter 3, section 3.2.2.

### **5.2.3 *Selection of Mixsmart parameters***

As discussed in Chapter 3, section 3.3.3. The selected parameters can be viewed in Chapter 3, Figure 3.1.

A description of the selected parameters, the abbreviations and units of measurement can be seen in Chapter 4, Table 4.3.

### **5.2.4 *Statistical analyses***

A Shapiro-Wilk test, Levene's test and ANOVA were performed as discussed in Chapter 3, section 3.2.3. The AMMI combines ANOVA and Principal Component Analysis (PCA) allowing investigation of the main effects of genotypes and environments, while G X E is explored by PCA (Gauch and Zobel, 1996). The AMMI model is given by:

$$\bar{y}_{ij.} = \mu + \tau_i + \delta_j + \sum_{k=1}^t \lambda_k \alpha_{ik} \gamma_{jk} + \bar{\varepsilon}_{ij.}$$

GGE is similar to AMMI and the GGE model is given by:

$$\bar{y}_{ij.} = \mu + \delta_j + \sum_{k=1}^t \lambda_k \alpha_{ik} \gamma_{jk} + \bar{\varepsilon}_{ij.}$$

Where:  $\bar{y}_{ij.}$  is the mean of the  $i^{\text{th}}$  cultivar in the  $j^{\text{th}}$  environments

$\mu$  is the overall mean

$\tau_i$  is the genotypic effect

$\delta_j$  is the environment effect

$\lambda_k$  ( $\lambda_1 \geq \lambda_2 \geq \dots \geq \lambda_t$ ) are scaling constants (singular values) that allow the imposition of ortho-normality constraints on the singular vectors for cultivars,  $\alpha_{ik} = (\alpha_{1k}, \dots, \alpha_{gk})$ , and environments,  $\gamma_{jk} = (\gamma_{1k}, \dots, \gamma_{ek})$ , such that

$\sum_i \alpha_{ik}^2 = \sum_j \gamma_{jk}^2 = 1$  and  $\sum_i \alpha_{ik} \alpha_{ik'} = \sum_j \gamma_{jk} \gamma_{jk'} = 0$  for  $k \neq k'$ ;  $\alpha_{ik}$  and  $\gamma_{jk}$  for  $k=1,2,3,\dots$  are called "primary," "secondary," "tertiary," . . . etc. effects of cultivars and environments, respectively;  $\bar{\varepsilon}_{ij.}$  is the residual error assumed to

be NID ( $0, \sigma^2/r$ ) (where  $\sigma^2$  is the pooled error variance and  $r$  is the number of replicates).

The GGE investigates the genotype main effect and the G X E graphically by grouping environments, which perform similar and displays which genotype performs the best within the different environment groups (Castillo et al., 2012).

## **5.3 Results**

### **5.3.1 Mean values for the six environments**

The lowest slope values (MLS and MRS) were observed for Ladybrand second season (09Lad), resulting in flatter curves (Table 5.1), which was confirmed by the lower MPV values (curve heights) for this environment. The lowest LFV were also obtained for Ladybrand both seasons. MPT and MRI values were larger during the first season compared to the second season.

### **5.3.2 Analysis of variance**

ANOVA indicated that genotype, environment and G X E effect was highly significant ( $P \leq 0.001$ ) for all six selected Mixsmart parameters (Table 5.2), indicating large differences between the cultivars, environments and their interactions for the selected Mixsmart parameters. The combined ANOVA revealed that for MLS, environment, genotype and G X E effects accounted for 22.66%, 34.89% and 26.68% of the variation respectively (Table 5.2). For MRS, 28.93%, 34.42% and 23.44% of variation were attributed by environment, genotype and G X E respectively. For MPT, 21.55%, 43.07% and 24.79% of the variation were accounted for by environment, genotype and G X E respectively and for variation in MPV, 40.36%, 25.21% and 26.41% were attributed to environment, genotype and G X E, respectively. Variation in MRI was 53.34%, 26.35% and 15.77% respectively attributed to environment, genotype and G X E. For MTW, environment, genotype and G X E effects accounted for 25.39%, 40.12% and 24.65% of the variation, respectively.

**Table 5.1 Mean values and standard deviations for the selected Mixsmart parameters and loaf volumes for the three environments over two seasons**

Parameter	First season			Second season		
	Bethlehem	Clarens	Ladybrand	Bethlehem	Clarens	Ladybrand
<b>MLS</b>	9.96 ± 3.80	8.60 ± 3.47	7.02 ± 3.08	8.74 ± 4.44	7.92 ± 3.41	4.13 ± 2.68
<b>MPT</b>	3.92 ± 0.91	3.73 ± 0.70	3.87 ± 0.66	3.13 ± 0.50	3.14 ± 0.47	3.36 ± 0.64
<b>MPV</b>	60.52 ± 6.28	58.89 ± 7.72	58.56 ± 5.42	51.32 ± 7.50	53.63 ± 5.29	46.94 ± 5.43
<b>MRS</b>	-4.39 ± 2.22	-5.20 ± 2.71	-2.61 ± 1.88	-5.40 ± 2.35	-5.19 ± 1.91	-2.53 ± 1.19
<b>MRI</b>	238.20 ± 36.69	226.80 ± 41.66	239.00 ± 33.81	169.60 ± 18.20	183.10 ± 16.83	176.20 ± 21.17
<b>MTW</b>	10.08 ± 4.32	9.08 ± 4.29	10.59 ± 3.35	6.04 ± 2.56	5.96 ± 2.45	9.34 ± 2.55
<b>LFV</b>	888.10 ± 46.78	894.38 ± 52.11	808.56 ± 48.20	950.50 ± 44.75	952.13 ± 53.78	793.75 ± 52.57
<b>LFV12%</b>	921.30 ± 39.55	876.10 ± 38.18	855.60 ± 36.76	891.30 ± 54.33	907.00 ± 55.87	878.00 ± 43.72

Mean values ± standard deviation. MLS = midline left slope, MPT = midline peak time, MPV = midline peak value, MRS = midline right slope, MRI = midline right integral, MTW = midline tail width, LFV = Loaf volume, LFV12% = Corrected loaf volume

**Table 5.2 Combined analysis of variance for ten genotypes grown in six environments**

Source	df	MS for MLS	%SST	MS for MRS	%SST	MS for MPT	%SST	MS for MPV	%SST	MS for MRI	%SST	MS for MTW	%SST
<b>Environment (E)</b>	5	159.59***	22.66	76.05***	28.93	4.98***	21.55	1201.60***	40.36	43298.00***	53.34	157.98***	25.39
<b>Rep</b>	18	7.31***	3.75	2.43***	3.32	0.08	1.30	12.30**	1.48	192.00**	0.85	2.88*	1.66
<b>Genotype (G)</b>	9	136.40***	34.89	50.28***	34.42	5.53***	43.07	417.00***	25.21	11885.00***	26.35	138.71***	40.12
<b>GXE</b>	45	20.86***	26.68	6.85***	23.44	0.64***	24.79	87.40***	26.41	1422.00***	15.77	17.04***	24.65
<b>Error</b>	162	2.61	12.02	0.80	9.89	0.07	9.29	6.00	6.54	93.00	3.69	1.57	8.18

\*P ≤ 0.05, \*\*P ≤ 0.01, \*\*\*P ≤ 0.001, MS = Mean squares, %SST = Main effect as a percentage of total sum of squares, MLS = midline left slope, MRS = midline right slope, MPT = midline peak time, MPV = midline peak value, MRI = midline right slope, MTW = midline tail width

### 5.3.3 Stability versus “which-won-where” pattern of the genotypes regarding the selected Mixsmart parameters

The best way of visualising the G X E, is through a polygon view of a biplot, which clearly shows which genotype performed the best at which environment (Farshadfar et al., 2013).

#### 5.3.3.1 AMMI and GGE-biplot results for midline left slope

PAN3377 and Gariep had the highest and lowest mean values, respectively, for MLS. According to the ASV, Elands was the most stable genotype and PAN3349 the least stable genotype for MLS (Table 5.3). According to Purchase et al. (2000), the genotype with the smallest ASV is the most stable genotype.

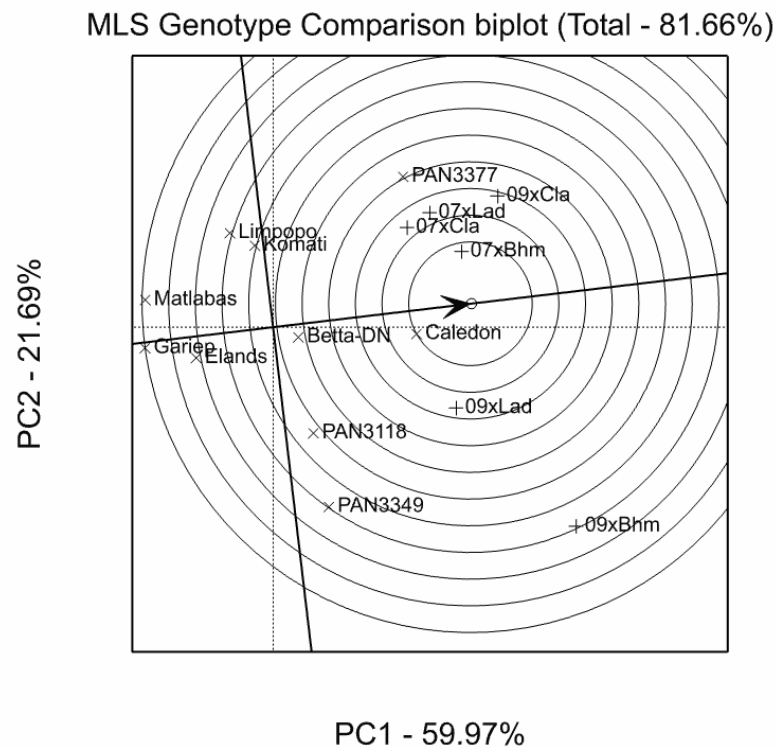
**Table 5.3 Genotype means, scores and AMMI stability value for midline left slope of ten genotypes grown in six environments over two seasons in the eastern dry land summer rainfall region of South Africa**

Genotype	Genotype mean	IPCAg[1]	IPCAg[2]	ASV
<b>Elands</b>	6.052	-0.01609	-0.74678	0.748
<b>BettaDN</b>	8.374	-0.26075	0.56169	0.928
<b>Gariep</b>	4.639	0.22648	0.77582	1.007
<b>Caledon</b>	11.56	-0.59465	0.46322	1.748
<b>Matlabas</b>	5.085	0.84296	-0.40644	2.423
<b>Komati</b>	7.741	0.95545	0.13652	2.711
<b>PAN3377</b>	11.579	1.13864	0.51496	3.268
<b>Limpopo</b>	7.115	1.17669	-0.37816	3.356
<b>PAN3118</b>	8.728	-1.27505	-1.86694	4.067
<b>PAN3349</b>	8.756	-2.19369	0.9461	6.289

IPCAg = Interaction Principal Component Analysis for genotype, ASV = AMMI stability value

The ideal genotype, according to the genotype comparison GGE-biplot (Figure 5.1), was the genotype residing closest to the centre of the concentric circles, in this case, Caledon. The GGE-biplot (Figure 5.1) and ASV (Table 5.3) therefore

agreed that PAN3349 was the least stable genotype regarding MLS, although they disagreed on the most stable genotype. The genotype with the shortest perpendicular distance on the average environmental axis (AEA), is the most stable genotype, in this case Gariep.



**Figure 5.1 GGE-biplot showing the evaluation of genotypes for midline left slope based on an ideal genotype**

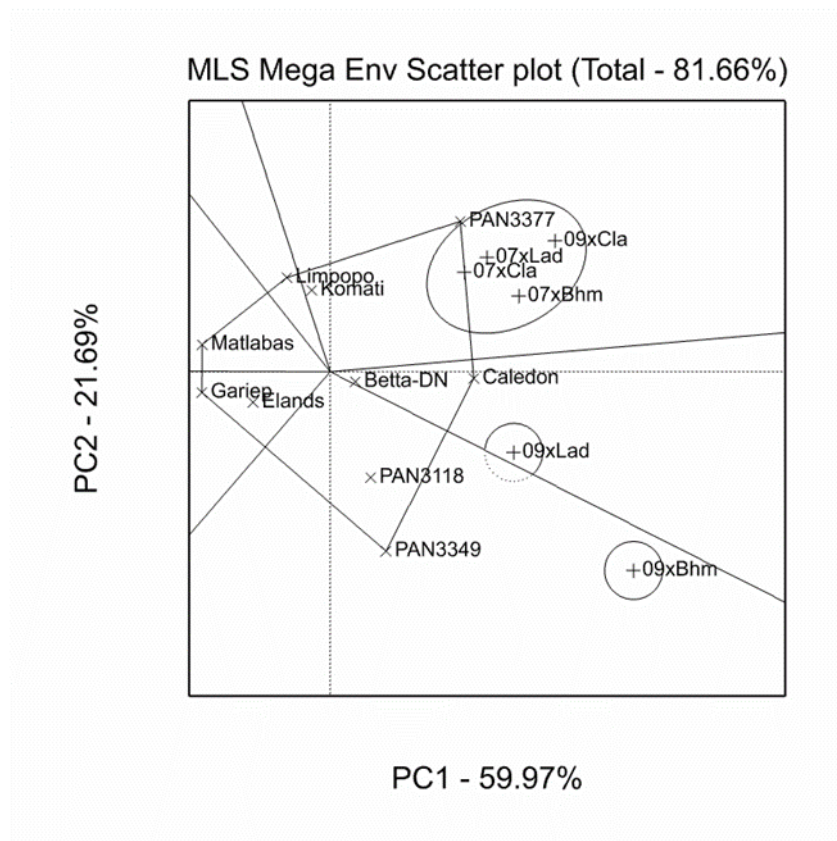
According to the AMMI (Table 5.4), PAN3377, Caledon and PAN3118 frequently ranked in the top four positions for the six environments for MLS. PAN3377 had the highest MLS values for the second season at Clarens (09Cla) and for the first season at Ladybrand (07Lad), Clarens (07Cla) and Bethlehem (07Bhm). Caledon ranked first at 09Lad and PAN3349 ranked first at 09Bhm.

**Table 5.4 AMMI selections per environment for midline left slope**

Environment	Mean	Score	1	2	3	4
<b>07Lad</b>	7.164	1.064	PAN3377	Caledon	PAN3118	Limpopo
<b>07Cla</b>	9.098	1.015	PAN3377	Caledon	PAN3118	Komati
<b>09Clar</b>	7.996	0.977	PAN3377	Caledon	Komati	BettaDN
<b>07Bhm</b>	10.262	0.533	PAN3377	Caledon	PAN3349	BettaDN
<b>09Lad</b>	4.487	-0.966	Caledon	PAN3118	PAN3349	PAN3377
<b>09Bhm</b>	8.77	-2.623	PAN3349	Caledon	PAN3118	BettaDN

07 = Environment planted during 2007, the first season, 09 = Environment planted during 2009, the second season, Bhm = Bethlehem, Cla = Clarens, Lad = Ladybrand

According to the GGE-biplot (Figure 5.2), three mega-environments formed. A mega-environment is a group of environments that consistently shares the best set of genotypes across years (Yan and Rajcan, 2002). Environments 09Cla, 07Lad, 07Cla and 07Bhm formed one mega-environment with PAN3377 as the winning genotype. Environments 09Lad and Bethlehem, second season (09Bhm) were the other two mega-environments, respectively. Caledon performed best in 09Lad and PAN3349 in 09Bhm. These results were similar in the AMMI selections (Table 5.4). Genotypes grouped into five sectors (Figure 5.2). PC1 and PC2 explained 81.66% of the total G + G X E variation, with PC1 explaining 59.97% and PC2 explaining 21.69% of the variation (Figures 5.1 and 5.2).



**Figure 5.2 Polygon view of GGE-biplot for ten genotypes grown in six environments for midline left slope**

**5.3.3.2 AMMI and GGE-biplot results for midline right slope**

PAN3377 had the largest (though negative), and Matlabas the smallest values for MRS (Table 5.5). ASV indicated Caledon to be the most stable genotype for MRS and PAN3349 to be the least stable genotype.

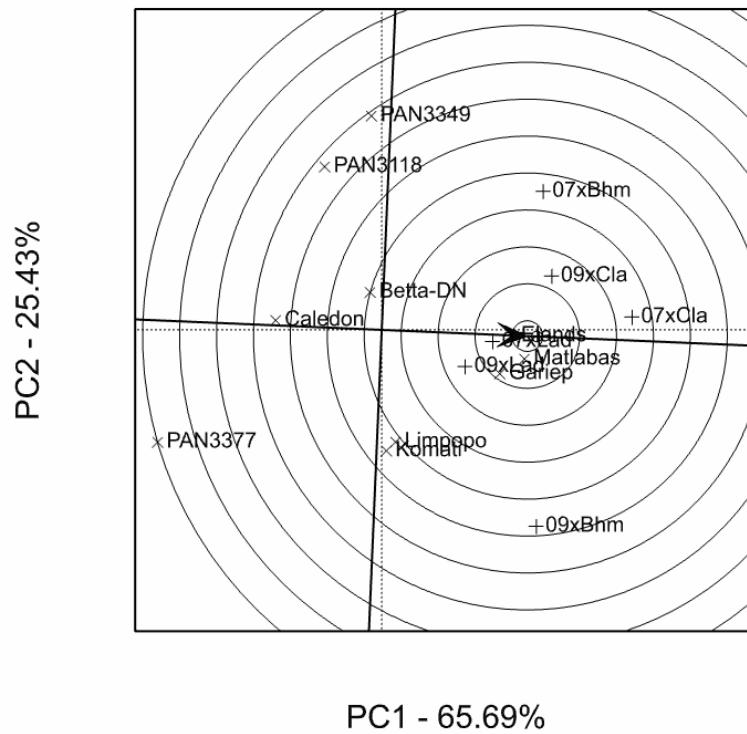
**Table 5.5 Genotype means, scores and AMMI stability value for midline right slope of ten genotypes grown in six environments over two seasons in the eastern dry land summer rainfall region of South Africa**

<b>Genotype</b>	<b>Genotype mean</b>	<b>IPCAg[1]</b>	<b>IPCAg[2]</b>	<b>ASV</b>
<b>Caledon</b>	-5.797	0.02171	-0.06325	0.083
<b>Matlabas</b>	-2.363	0.09762	0.08141	0.254
<b>Elands</b>	-2.746	-0.10028	0.82101	0.857
<b>Gariep</b>	-2.89	0.20161	0.7608	0.909
<b>BettaDN</b>	-4.3	-0.24382	-0.82517	1.021
<b>Limpopo</b>	-4.169	0.83552	0.34247	2.088
<b>Komati</b>	-4.284	0.91015	0.49915	2.298
<b>PAN3377</b>	-7.074	1.10658	-1.20802	2.983
<b>PAN3118</b>	-5.062	-1.17634	-0.75033	2.995
<b>PAN3349</b>	-4.727	-1.65274	0.34194	4.088

IPCAg = Interaction Principal Component Analysis for genotype, ASV = AMMI stability value

The GGE-biplot (Figure 5.3) indicated Elands to be the ideal genotype and Caledon and Elands to be the most stable genotypes and PAN3349 to be the least stable genotype for MRS. ASV and the GGE-biplot agreed regarding the most stable genotype.

MRS Genotype Comparison biplot (Total - 91.13%)



**Figure 5.3 GGE-biplot showing the evaluation of genotypes for midline right slope based on an ideal genotype**

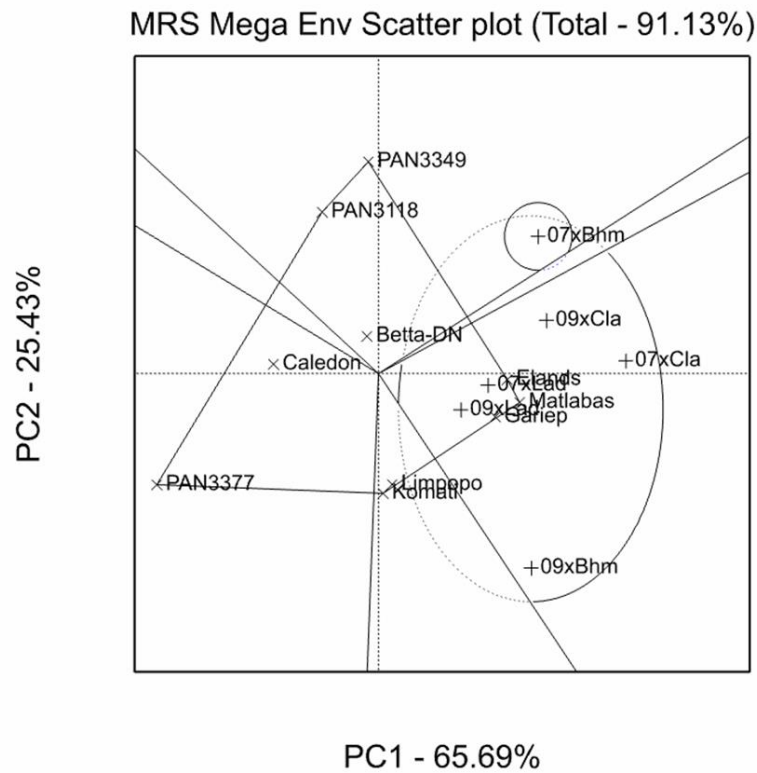
Matlabas ranked first for MRS values in 09Bhm, 09Lad, 07Lad and 09Cla, PAN3349 ranked first at 07Bhm and Elands ranked first at 07Cla (Table 5.6). Matlabas, Gariep and Elands ranked frequently under the top four positions for the six environments for MRS (Table 5.6).

**Table 5.6 AMMI selections per environment for midline right slope**

Environment	Mean	Score	1	2	3	4
<b>09Bhm</b>	-5.421	1.9839	Matlabas	Gariep	Komati	Limpopo
<b>09Lad</b>	-2.463	0.4274	Matlabas	BettaDN	Gariep	Elands
<b>07Lad</b>	-2.835	0.117	Matlabas	Elands	Gariep	BettaDN
<b>07Cla</b>	-5.64	-0.3599	Elands	Gariep	Matlabas	PAN3349
<b>09Cla</b>	-5.263	-0.652	Matlabas	Elands	Gariep	PAN3349
<b>07Bhm</b>	-4.426	-1.5165	PAN3349	Matlabas	PAN3118	Elands

07 = Environment planted during 2007, the first season, 09 = Environment planted during 2009, the second season, Bhm = Bethlehem, Cla = Clarens, Lad = Ladybrand

Two mega-environments were formed (Figure 5.4), with 07Bhm grouping by itself and the other five environments forming the second mega-environment. PC1 and PC2 explained 91.93% of the total G + G X E variation, with PC1 and PC2 explaining 65.69% and 25.43% respectively (Figures 5.3 and 5.4).



**Figure 5.4 Polygon view of GGE-biplot for ten genotypes grown in six environments for midline right slope**

### 5.3.3.3 AMMI and GGE-biplot results for midline peak time

Gariiep had the smallest ASV, being the most stable genotype and PAN3118 was the least stable genotype according to ASV (Table 5.7). PAN3118 exhibited MPT values of longer than 4 min and Caledon had the shortest MPT values.

**Table 5.7 Genotype means, scores and AMMI stability value for midline peak time of ten genotypes grown in six environments over two seasons in the eastern dry land summer rainfall region of South Africa**

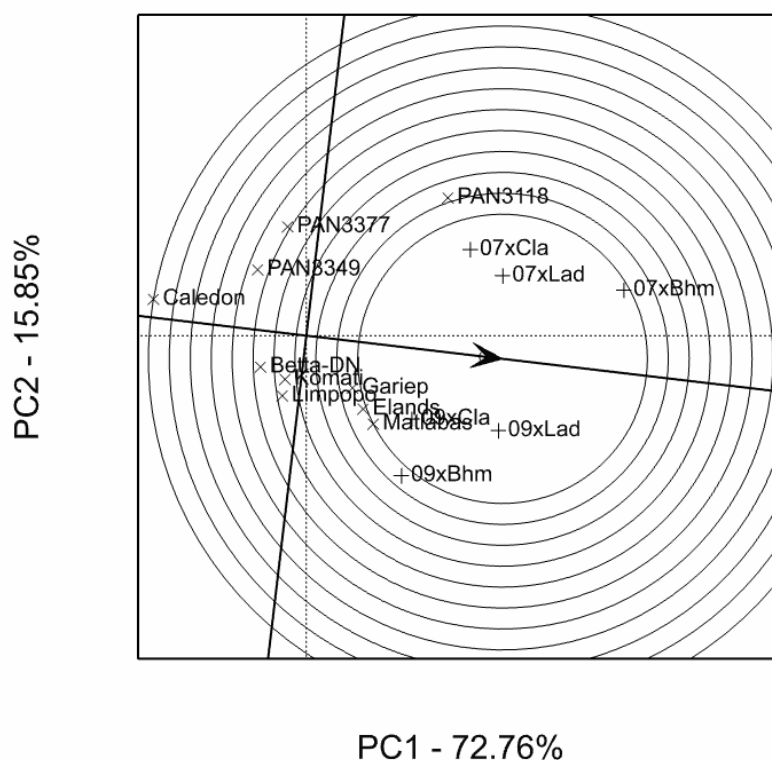
<b>Genotype</b>	<b>Genotype mean</b>	<b>IPCAg[1]</b>	<b>IPCAg[2]</b>	<b>ASV</b>
<b>Gariep</b>	3.808	0.09391	0.10033	0.2444
<b>Elands</b>	3.919	0.20669	0.08272	0.4974
<b>Komati</b>	3.393	0.27225	0.02437	0.6465
<b>PAN3349</b>	3.208	-0.01673	-0.66176	0.663
<b>Caledon</b>	2.516	0.28286	-0.0699	0.6749
<b>Matlabas</b>	3.901	0.06876	0.66975	0.6893
<b>BettaDN</b>	3.236	0.29348	0.01227	0.6966
<b>Limpopo</b>	3.398	0.36479	0.13493	0.8761
<b>PAN3377</b>	3.339	-0.35634	-0.55946	1.0139
<b>PAN3118</b>	4.188	-1.20968	0.26675	2.8831

IPCAg = Interaction Principal Component Analysis for genotype, ASV = AMMI stability value

According to the biplot (Figure 5.5), Caledon was the most stable and PAN3118 the least stable genotype. The ASV and GGE-biplot agreed on these findings. Gariep was indicated to be the ideal genotype (Figure 5.5).

PAN3118 ranked first at all three environments planted during the first season (Table 5.8). Matlabas ranked first at two of the three environments planted during the second season (Bhm and Lad), Elands ranked first at Clarens during the second season. Elands, Gariep, Matlabas and PAN3118 frequently ranked under the top four positions for MPT (Table 5.8).

MPT Genotype Comparison biplot (Total - 88.61%)



**Figure 5.5 GGE-biplot showing the evaluation of genotypes for midline peak time based on an ideal genotype**

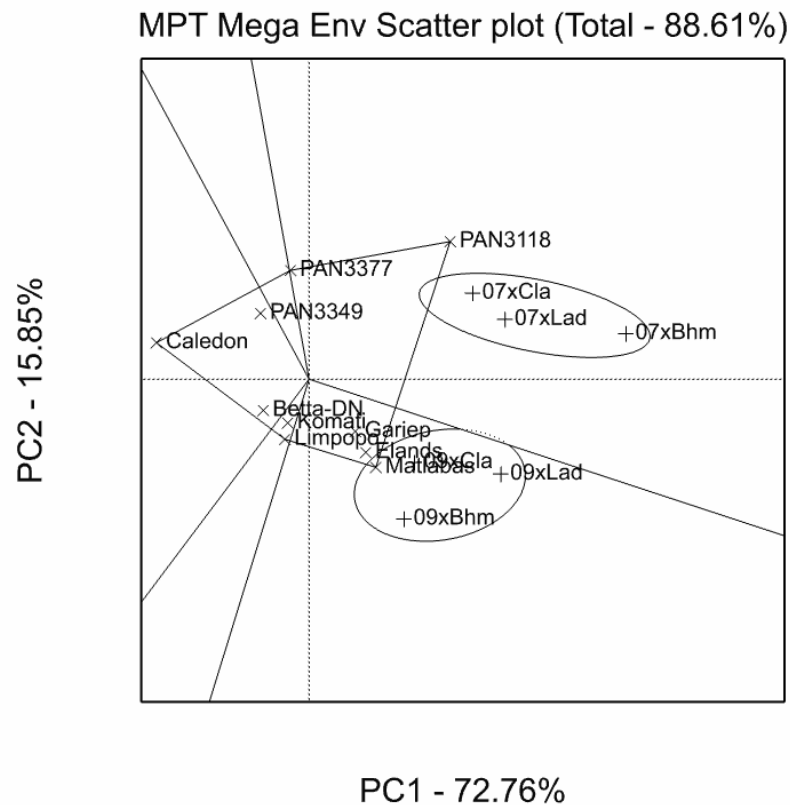
**Table 5.8 AMMI selections per environment for midline peak time**

Environment	Mean	Score	1	2	3	4
09Bhm	3.11	0.792	Matlabas	Elands	Gariep	Limpopo
09Clar	3.119	0.5493	Elands	Matlabas	Gariep	Limpopo
09Lad	3.312	0.2695	Matlabas	Elands	PAN3118	Gariep
07Clar	3.691	-0.3737	PAN3118	PAN3377	Elands	Gariep
07Lad	3.835	-0.3799	PAN3118	Elands	Gariep	PAN3377
07Bhm	3.877	-0.8571	PAN3118	Matlabas	Elands	Gariep

07 = Environment planted during 2007, the first season, 09 = Environment planted during 2009, the second season, Bhm = Bethlehem, Cla = Clarens, Lad = Ladybrand

Environments formed two mega-environments (Figure 5.6). The environments for the first and second season formed separate mega-environments. PAN3118 was the most responsive genotype at the first season environments and Matlabas at the second season environments. PC1 and PC2 explained 88.61% of the total G + G X E variation, with PC1 and PC2 explaining 72.67% and

15.85% respectively (Figures 5.5 and 5.6).



**Figure 5.6 Polygon view of GGE-biplot for ten genotypes grown in six environments for midline peak time**

#### **5.3.3.4 AMMI and GGE-biplot results for midline peak value**

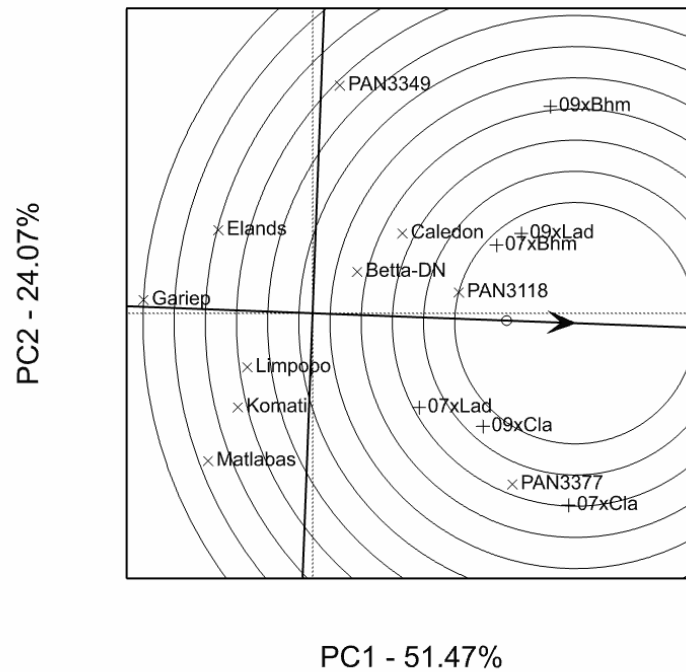
According to ASV for MPV (Table 5.9), and the GGE-biplot (Figure 5.7), Gariep was the most stable genotype and PAN3349 was the least stable genotype for MPV. Gariep had the lowest MPV values and PAN3377 had the highest. The GGE-biplot (Figure 5.7) indicated PAN3118 to be the ideal genotype.

**Table 5.9 Genotype means, scores and AMMI stability value for midline peak value of ten genotypes grown in six environments over two seasons in the eastern dry land summer rainfall region of South Africa**

<b>Genotype</b>	<b>Genotype mean</b>	<b>IPCAg[1]</b>	<b>IPCAg[2]</b>	<b>ASV</b>
<b>Gariep</b>	49.46	0.02717	1.07822	1.079
<b>Caledon</b>	58.37	-1.11837	0.33137	1.841
<b>BettaDN</b>	56.39	-0.61776	-1.56352	1.856
<b>Elands</b>	52.14	-0.94085	1.27808	1.989
<b>Limpopo</b>	53.87	0.81297	1.57267	2.051
<b>Komati</b>	53.01	1.29477	0.1988	2.106
<b>PAN3118</b>	60.40	-0.44762	-2.10622	2.227
<b>PAN3377</b>	62.53	1.97673	1.02686	3.362
<b>Matlabas</b>	51.32	1.9655	-1.88962	3.702
<b>PAN3349</b>	55.87	-2.95254	0.07335	4.782

IPCAg = Interaction Principal Component Analysis for genotype, ASV = AMMI stability value

MPV Genotype Comparison biplot (Total - 75.54%)



**Figure 5.7 GGE-biplot showing the evaluation of genotypes for midline peak value based on an ideal genotype**

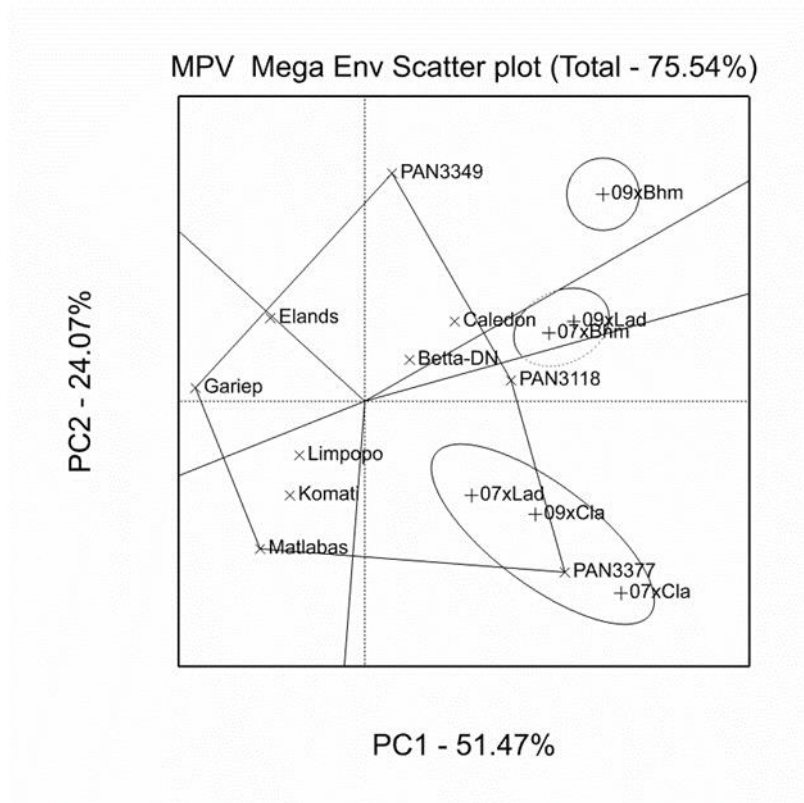
PAN3377 ranked first in five of the six environments (Table 5.10). PAN3377, PAN3118 and Caledon frequently ranked under the top four positions for MPV.

**Table 5.10 AMMI selections per environment for midline peak value**

Environment	Mean	Score	1	2	3	4
07Clar	59.49	2.43	PAN3377	PAN3118	Matlabas	BettaDN
09Clar	53.73	1.499	PAN3377	PAN3118	Caledon	Limpopo
07Lad	59.32	1.312	PAN3377	PAN3118	Caledon	BettaDN
07Bhm	60.94	-1.007	PAN3377	Caledon	PAN3349	Limpopo
09Lad	47.1	-1.198	PAN3377	Caledon	PAN3349	PAN3118
09Bhm	51.43	-3.036	PAN3118	PAN3349	BettaDN	Caledon

07 = Environment planted during 2007, the first season, 09 = Environment planted during 2009, the second season, Bhm = Bethlehem, Cla = Clarens, Lad = Ladybrand

Environments formed three mega-environments (Figure 5.8). PC1 and PC2 explained 75.54% of the total G + G X E variation, with PC1 and PC2 explaining 51.47% and 24.07% respectively (Figures 5.7 and 5.8).



**Figure 5.8 Polygon view of GGE-biplot for ten genotypes grown in six environments for midline peak value**

**5.3.3.5 AMMI and GGE-biplot results for midline right integral**

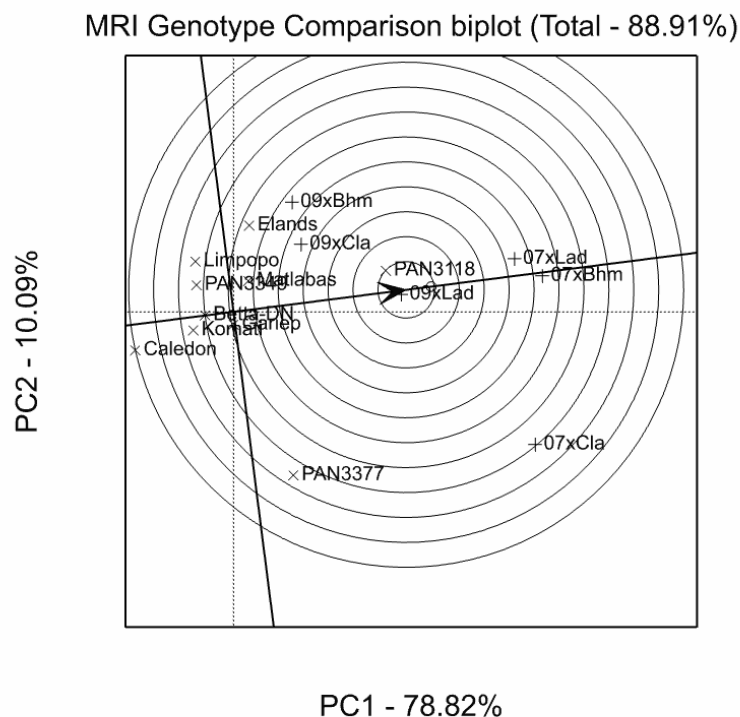
Caledon had the lowest and PAN3118 had the highest MRI values, respectively (Table 5.11). ASV indicated Gariep as the most stable and PAN3118 the least stable genotype for MRI values.

The GGE-biplot (Figure 5.9) showed PAN3118 as the ideal genotype but indicated that Betta-DN was the most stable and PAN3377 the least stable genotypes.

**Table 5.11 Genotype means, scores and AMMI stability value for midline right integral of ten genotypes grown in six environments over two seasons in the eastern dry land summer rainfall region of South Africa**

Genotype	Genotype mean	IPCAg[1]	IPCAg[2]	ASV
Gariep	203.3	-0.82781	-1.1227	3.201
Matlabas	213.9	1.06851	1.17985	4.045
Komati	191.2	1.1795	1.0073	4.388
BettaDN	197.3	1.63041	1.32683	6.051
Elands	214.4	1.56212	-2.2182	6.076
PAN3349	193.3	1.80959	-0.87283	6.61
Caledon	169.7	2.37459	0.1976	8.601
Limpopo	196.1	3.17013	0.11723	11.48
PAN3377	218.8	-5.87657	4.7058	21.793
PAN3118	253.8	-6.09046	-4.32089	22.473

IPCAg = Interaction Principal Component Analysis for genotype, ASV = AMMI stability value



**Figure 5.9 GGE-biplot showing the evaluation of genotypes for midline right integral based on an ideal genotype**

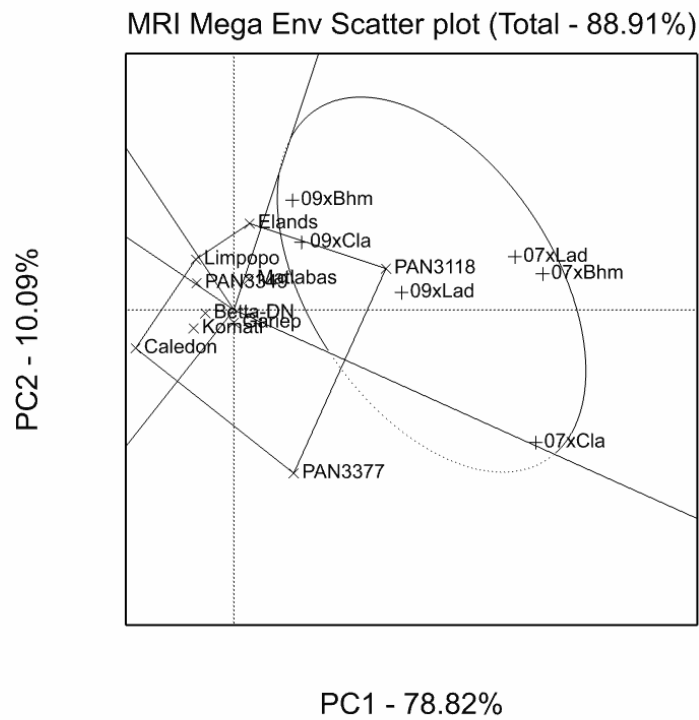
AMMI selections (Table 5.12) ranked PAN3118 first at three locations (09Lad, 07Lad and 07Bhm). PAN3377 ranked first at 07Cla and second at Ladybrand2 en Bethlehem1, Elands ranked first at 09Bhm and Matlabas was the genotype with the highest MRI values at 09Cla. PAN3118, Elands, Matlabas and PAN3377 frequently ranked under the top four positions for MRI in the six environments (Table 5.12).

**Table 5.12 AMMI selections per environment for midline right integral**

Environment	Mean	Score	1	2	3	4
<b>09Bhm</b>	169.2	5.527	Elands	PAN3118	Matlabas	Limpopo
<b>09Clar</b>	182.6	4.809	Matlabas	Elands	PAN3118	Limpopo
<b>09Lad</b>	175.1	0.607	PAN3118	PAN3377	Elands	Matlabas
<b>07Lad</b>	239.6	-2.234	PAN3118	Elands	PAN3377	Gariep
<b>07Bhm</b>	237.5	-3.365	PAN3118	PAN3377	Elands	Gariep
<b>07Clar</b>	227	-5.344	PAN3377	PAN3118	Matlabas	Gariep

07 = Environment planted during 2007, the first season, 09 = Environment planted during 2009, the second season, Bhm = Bethlehem, Cla = Clarens, Lad = Ladybrand

Environments all formed one mega-environment (Figure 5.10). PC1 and PC2 explained 88.91% of the total G + G X E variation, with PC1 and PC2 explaining 78.82% and 10.09% respectively (Figures 5.9 and 5.10).



**Figure 5.10 Polygon view of GGE-biplot for ten genotypes grown in six environments for midline right integral**

**5.3.3.6 AMMI and GGE-biplot results for midline tail width**

Matlabas had the highest MTW values and PAN3349 the lowest (Table 5.13). ASV indicated Matlabas and PAN3118 as the most and least stable genotypes, respectively.

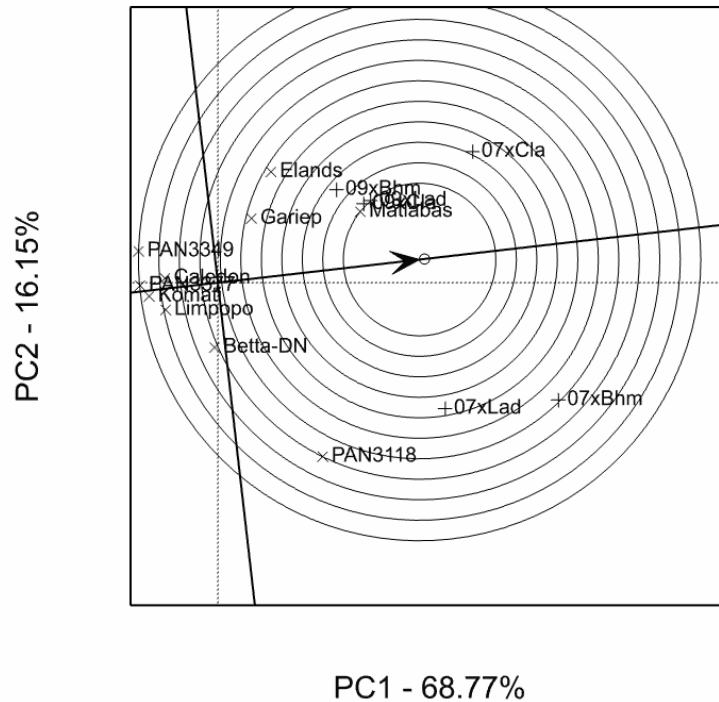
**Table 5.13 Genotype means, scores and AMMI stability value for midline tail width of ten genotypes grown in six environments over two seasons in the eastern dry land summer rainfall region of South Africa**

<b>Genotype</b>	<b>Genotype mean</b>	<b>IPCAg[1]</b>	<b>IPCAg[2]</b>	<b>ASV</b>
<b>Matlabas</b>	13.072	0.01771	0.09241	0.097
<b>Caledon</b>	6.708	0.40498	0.05468	0.672
<b>PAN3377</b>	5.92	0.495	0.13478	0.83
<b>BettaDN</b>	8.153	-0.41863	0.94326	1.17
<b>PAN3349</b>	5.743	0.59518	-0.63965	1.174
<b>Komati</b>	6.405	0.58726	0.75929	1.233
<b>Gariep</b>	9.318	0.10525	-1.32567	1.337
<b>Elands</b>	10.081	0.49524	-1.4234	1.642
<b>Limpopo</b>	7.052	0.58947	1.47822	1.771
<b>PAN3118</b>	10.565	-2.87147	-0.07392	4.75

IPCAg = Interaction Principal Component Analysis for genotype, ASV = AMMI stability value

The GGE-biplot (Figure 5.11) indicated Matlabas as the ideal genotype, and Caledon, Komati, PAN3377 and Limpopo as the most stable genotypes and PAN3118 as the least stable genotype for MTW.

MTW Genotype Comparison biplot (Total - 84.91%)



**Figure 5.11 GGE-biplot showing the evaluation of genotypes for midline tail width based on an ideal genotype**

AMMI selections (Table 5.14) ranked Matlabas first and second during the second and first seasons respectively, for all three environments. Matlabas, Elands, Gariep and PAN3118 ranked frequently under the top four positions regarding MTW in the six environments.

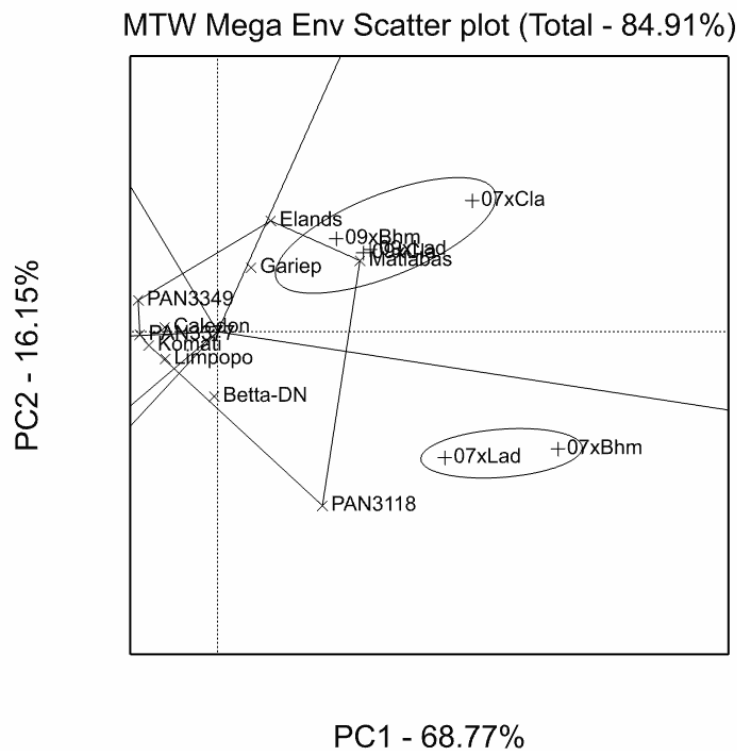
**Table 5.14 AMMI selections per environment for midline tail width**

Environment	Mean	Score	1	2	3	4
09Bhm	5.914	1.37	Matlabas	Elands	Limpopo	Gariep
09Clad	5.802	1.053	Matlabas	Elands	Gariep	Limpopo
09Lad	9.018	0.895	Matlabas	Elands	Gariep	PAN3118
07Clad	8.776	0.121	Elands	Matlabas	Gariep	PAN3118
07Lad	10.486	-1.263	PAN3118	Matlabas	BettaDN	Limpopo
07Bhm	9.815	-2.176	PAN3118	Matlabas	Gariep	Elands

07 = Environment planted during 2007, the first season, 09 = Environment planted during 2009, the second season, Bhm = Bethlehem, Cla = Clarens, Lad = Ladybrand

Two mega-environments formed (Figure 5.12), with 07Bhm, 07Lad forming one

mega-environment, and the other four environments forming the second mega-environment.



**Figure 5.12 Polygon view of GGE-biplot for ten genotypes grown in six environments for midline tail width**

PC1 and PC2 explained 84.91% of the total G + G X E variation, with PC1 and PC2 explaining 68.77% and 16.15% respectively (Figures 5.11 and 5.12).

#### 5.4 Discussion

South African wheat breeders employ MPT as a tool to indicate whether potential breeding lines possess acceptable bread making quality (SAGL, 2013). Acceptable MPT values for bread making purposes, may vary from 2.5 to 4.5 min. Longer MPT values were obtained for the first season compared to the second season, indicating that the dough took longer to develop and this was confirmed by the higher MRI (area) values obtained during the first season.

Acceptable MPV values are  $\geq 60\%$  for hard red wheat, indicating acceptable bread making quality. MPV values were lower during both growing seasons for Ladybrand, indicating lower LFV for this locality. The findings were therefore, in agreement with Khatkar et al. (1996), Martinant et al. (1998) and Labuschagne et al. (2016) who reported MPV being a better indicator of LFV than MPT.

A challenge to wheat breeders is that quality traits are influenced by G X E, making it very difficult to identify which genotypes are superior as well as stable performers (Khazratkulova et al., 2015). Several studies on quality traits showed that the G X E contribution to variation was significant but smaller than the genetic contribution (Baenziger et al., 1985; Basset et al., 1989; Lukow and McVetty, 1991; Peterson et al., 1992; Robert and Denis, 1996). In this study the G X E contribution for MPV was slightly higher compared to the genotypic contribution for the six mixogram parameters selected. Although the contribution of genotype to variation was one of the selection criteria when selecting the six Mixsmart parameters (see Chapter 3), the contribution of environment to variation was larger than the contribution of genotype for only two of the six Mixsmart parameters namely MPV and MRI. Genotype made the largest contribution to variation in MPT and MTW (Table 5.2).

MPV and MRI were the only two parameters where ASV and the GGE-biplot agreed on the most stable genotype, being Gariep. In both cases PAN3118 was indicated as the ideal genotype. In the case of the two parameters having genotype as the larger contributor to variation (MPT and MTW), both ASV and the GGE-biplot indicated that the most stable genotypes (also being the ideal genotypes) were Gariep for MPT and Matlabas for MTW. ASV also indicated, in both cases, that PAN3118 was the most unstable genotype. Regarding the two slope parameters (MLS and MRS), the least stable genotype in both cases was PAN3349, indicated by ASV and the GGE-biplot. For MLS, Elands was the most stable genotype and Caledon the ideal, and the opposite was indicated for MRS where Elands was the ideal genotype and Caledon the most stable genotype.

It is clear that genotypes differed for stability or for being the ideal genotype for the measured characteristics, indicating yet again the large effect that G X E has

on bread dough quality traits as measured on a mixograph. It was also seen that the most stable genotype might not be the ideal genotype. Six of the genotypes (PAN3377, Caledon, PAN3118, Matlabas, Gariep and Elands) consistently ranked in the top four positions according to AMMI selections for the six Mixsmart parameters, indicating that these genotypes might contribute to stability when using Mixsmart parameters as indicators of acceptable bread making quality. Breeders must keep in mind that the sets of genotypes and environments used, influence the assessment of stability (Robert and Denis, 1996).

## **5.5 Conclusions**

The milling and baking industry regards stability of wheat quality characteristics important, since they require consistency in raw material to establish optimal use of their processing lines (Grausgruber et al., 2000; Barić et al., 2004; SAGL, 2017). Wheat breeders have to adhere to the domestic industry's requirements when they release cultivars commercially. With the growing population, yield has become the focus point of breeding programmes for most crops, but it is general knowledge that yield and quality are often negatively correlated. It can be wrongly decided that stability is an undesirable selection criteria, because the best yielders may not be the most stable genotypes. Breeders should therefore focus on selecting or crossing stable, high yielding breeding lines having superior quality. The ultimate goal should be to locate the genes responsible for processing quality attributes to enable breeders to select for processing quality independently of G X E (Williams et al., 2008).

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## CHAPTER 6

### THE INFLUENCE OF DIFFERENT MIXING TIMES OF MIXSMART PARAMETERS ON WHEAT QUALITY AND THEIR RELATIONSHIPS WITH PRIMARY QUALITY CRITERIA

#### Abstract

During the cultivar classification process, potential breeding lines are compared to a biological standard. Currently, a total mixing time of 6 min for mixograph analysis is sufficient, but biological standards are changed from time to time, which may imply that longer mixing times are required for mixograph analysis. Since breeders use mixograph analysis as a selection tool to be indicative of primary quality criteria, they need to be informed if relationships change when longer mixing times are applied. Mixograph analyses applying Mixsmart® software were performed on ten South African bread wheat cultivars, grown in six environments for 6.5, 8.5 and 10.5 min, where timeX was indicated as 6.0, 8.0 and 10.0 min respectively. ANOVA indicated envelope timeX slope (ETXS), envelope timeX width (ETXW), envelope timeX integral (ETXI), envelope timeX value (ETXV), midline timeX slope (MTXS), midline timeX width (MTXW), midline timeX integral (MTXI) and midline timeX value (MTXV) to show variation (causing differences at the different time intervals), but when these parameters were eliminated from linear discriminant analyses, there were no variations in mixing time interval values for the three timeX times. Correlations did not differ significantly between the primary quality criteria and Mixsmart parameters at the different time intervals, indicating that relationships between Mixsmart parameters and primary quality criteria remained constant at the three timeX times.

#### 6.1 Introduction

The mixograph is an important tool in wheat breeding programmes, assisting breeders in assessing the breeding lines for acceptable mixing properties, especially during early generation selections. Breeders expect the mixograph to

indicate desirable dough properties like high water absorption, acceptable mixing times, strong gluten properties and acceptable dough tolerance. Breeding lines exhibiting extreme mixing times (longer than 5 min), poor mixing tolerance, weak gluten properties and low water absorption, are eliminated early from the breeding programme. Mixsmart<sup>®</sup> software allows breeders to utilise 44 measured parameters on a mixogram. The software divides the mixogram into two envelope curves and a midline curve. Measurements are made at different heights, times, widths and areas on the mixogram and slope measurements are also performed (Pon et al., 1989; Walker and Walker, 1992; Martinant et al., 1998; Dobraszczyk and Schofield, 2002; Labuschagne et al., 2016). Mixing time is the only mixogram parameter used in South Africa when new potential breeding lines are proposed for cultivar classification (SAGL, 2013).

When potential breeding lines are evaluated during the cultivar release process, a biological wheat quality standard is used, to which the potential breeding line is compared regarding primary and secondary quality criteria. The potential breeding line is allowed to deviate from the quality standard according to fixed tolerances regarding primary criteria, which is non-negotiable (SAGL, 2013). Primary criteria include HLM, FLN, GPC, FPC, FLY, KJ76, FABS, P/L, alveograph dough strength (STR), LFV and LFV12%. Since mixograph analysis is easy to perform, requiring small sample sizes and is relatively cheaper than other rheological analyses (alveograph and farinograph analyses), breeders use it extensively to evaluate their breeding lines. In South Africa, mixing times between 2.5 to 4.5 min, are required for bread making purposes (SAGL, 2013), therefore, currently the mixograph analysis is stopped after a running time of 6.0 min, enabling breeders to effectively select potential breeding lines. Biological quality standards are changed from time to time, which may require for mixograph analysis to commence beyond 6 min, therefore it is necessary to investigate if relationships with primary quality criteria will remain unchanged if the analysis is 8 or 10 min. It is clear from literature that scientists also perform mixograph analyses for 8 or 10 min (Branlard et al., 1991; Khatkar et al., 1996; Wikström and Bohlin, 1996; Martinant et al., 1998; Chung et al., 2001).

The aim of this study was to determine if the 6 min timeX value currently used in the South African wheat industry is optimal and whether extended timeX values (8 and 10 min) would lead to different relationships between the Mixsmart values and the primary quality criteria.

## **6.2 Materials and methods**

### **6.2.1 *Field trials***

As discussed in Chapter 3, section 3.2.1.

### **6.2.2 *Laboratory analyses***

As discussed in Chapter 3, section 3.2.2.

### **6.2.3 *Selection of Mixsmart parameters***

As discussed in Chapter 3, section 3.3.3. The selected parameters can be viewed in Chapter 3, Figure 3.1.

A description of the selected parameters, the abbreviations and units of measurement can be seen in Chapter 4, Table 4.3.

### **6.2.4 *Statistical analyses***

A Shapiro-Wilk test, Levene's test and ANOVA were performed as discussed in Chapter 3, section 3.2.3.

Discriminant function analysis is a statistical analysis where a categorical dependant variable is predicted (called a grouping variable) by one or more continuous or binary independent variables (called predictor variables). LDA is multivariate and has two functions, namely to classify groups and to separate groups. The method is simple, mathematically robust and produces models with accuracy comparable to more complex methods. The separation method aims to

separate two or more known groups using a set or subset of measured variables, where in this study this method was used to investigate time interval separation. New variable sets are formed by constructing linear combinations using the original variables, and are thus indicated as vectors of loading for the original variables. A set of directions is obtained in such a way that the ratio of the between-group variability to the within-group variability in each direction is maximised. Horizontal separation, indicated on the x-axis, represents the one set of canonical variates (indicated as F1 on the plots) and the vertical separation, indicated on the y-axis, represents the second set of canonical variates (indicated as F2 on the plots). F1 and F2 account for the highest and second highest variation in the data set respectively. The LDA's were constructed using XLStat software (2012).

Pearson product-moment correlation coefficients were determined as discussed in Chapter 3, section 3.2.3 .

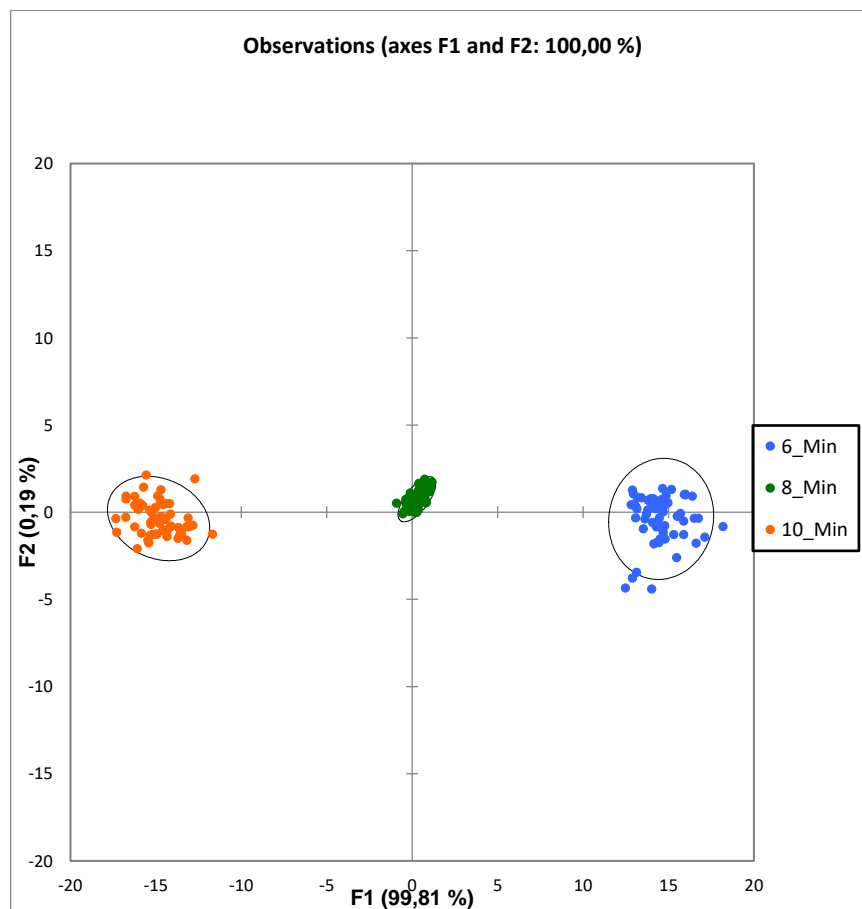
### **6.3 Results**

ANOVA (results not shown) indicated eight of the 44 Mixsmart parameters showing variation within the time intervals. These parameters were all related to timeX values, as was expected. They included envelope timeX slope (ETXS), envelope timeX width (ETXW), envelope timeX integral (ETXI), envelope timeX value (ETXV), midline timeX slope (MTXS), midline timeX width (MTXW), midline timeX integral (MTXI) and midline timeX value (MTXV) (Table 6.1).

**Table 6.1 Mixsmart timeX parameters, descriptions, abbreviations and units of measurement**

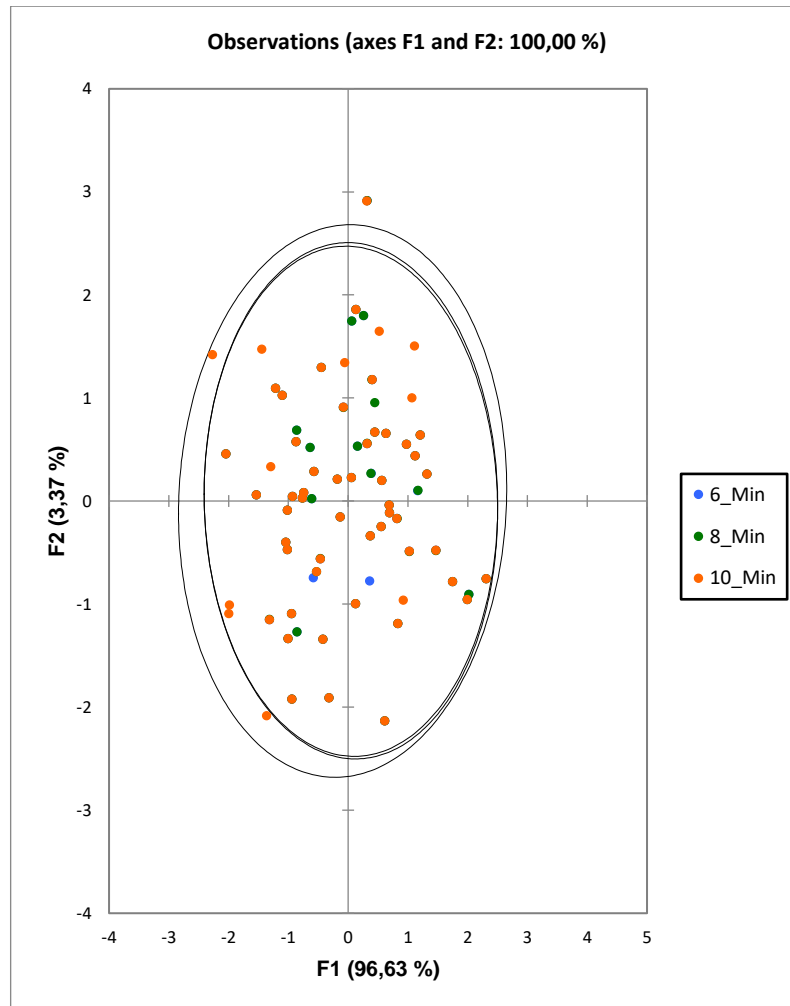
Envelope timeX value	Envelope curve height at 6, 8 or 10 min	ETXV	%
Envelope timeX slope	Envelope slope from envelope peak time until 6, 8 or 10 min measured on envelope curve	ETXS	%/min
Envelope timeX width	Envelope curve-width at 6, 8 or 10 min	ETXW	%
Envelope timeX integral	Envelope area under envelope curve from starting point until 6, 8 or 10 min	ETXI	%Torque*min
Midline timex value	Midline curve height at 6, 8 or 10 min	MTXV	%
Midline timex slope	Slope from midline peak time till 6, 8 or 10 min measured on midline curve	MTXS	%/min
Midline timex width	Midline curve-width at 6, 8 or 10 min	MTXW	%
Midline timex integral	Midline area under curve from beginning until 6, 8 or 10 min	MTXI	%Torque*min

LDA involving all 44 Mixsmart parameters resulted in the time intervals clearly separating (Figure 6.1).



**Figure 6.1 Linear discriminant biplot of the minute intervals with all 44 variables (Mixsmart parameters)**

When using all 44 parameters, F1 accounted for 99.81% of the variation. Another LDA was performed where the eight parameters responsible for the variations, as indicated by ANOVA, were eliminated. The remaining 36 variables grouped the three time intervals together, showing no differences for the remaining 36 variables for the different time intervals (Figure 6.2).



**Figure 6.2 Linear discriminant biplot of the minute intervals with the remainder of the 36 variables**

Differences in the correlation intensity at different timeX values occurred for ETXV with FLN, ETXW with GPC, ETXI with GPC, P/L and STR, MTXV with FLN and FLY, MTXW with GPC and MTXI with FLN and P/L (Table 6.2, differences indicated in red).

**Table 6.2 The relationship of three Mixsmart timeX values with primary wheat quality characteristics over six environments**

Parameter (mixing time)	HLM	FLN	GPC	FPC	FLY	KJ76	FABS	P/L	STR	LFV	LFV12%
ETXV 6	ns	0.21**	ns	ns	-0.37***	0.45***	-0.51***	ns	ns	-0.24**	ns
ETXV 8	ns	0.24**	ns	ns	-0.41***	0.45***	-0.52***	ns	ns	-0.22**	ns
ETXV 10	ns	0.30***	ns	ns	-0.37***	0.42***	-0.51***	ns	ns	-0.21**	ns
ETXS 6	ns	ns	ns	ns	ns	-0.14*	0.21**	ns	ns	ns	ns
ETXS 8	ns	ns	ns	ns	ns	-0.15*	0.23**	ns	ns	ns	ns
ETXS 10	ns	ns	ns	ns	ns	-0.14*	0.23**	ns	ns	ns	ns
ETXW 6	ns	ns	-0.30***	-0.36***	-0.53***	0.34***	-0.37***	0.41***	ns	-0.45***	ns
ETXW 8	ns	ns	-0.31***	-0.38***	-0.56***	0.34***	-0.32***	0.40***	ns	-0.43***	ns
ETXW 10	ns	ns	-0.21**	-0.29***	-0.51***	0.33***	-0.35***	0.29***	ns	-0.33***	ns
ETXI 6	ns	0.13*	-0.21**	-0.31***	-0.39***	0.40***	-0.49***	0.23**	-0.16*	-0.45***	ns
ETXI 8	ns	0.13*	-0.26***	-0.36***	-0.46***	0.41***	-0.50***	0.29***	-0.17**	-0.48***	ns
ETXI 10	ns	0.13*	-0.22**	-0.32***	-0.51***	0.40***	-0.43***	0.28***	-0.14*	-0.42***	ns
MTXV 6	ns	0.21**	0.18**	ns	-0.23**	0.44***	-0.50***	ns	ns	ns	ns
MTXV 8	ns	0.26***	0.19**	ns	-0.25***	0.42***	-0.50***	ns	ns	ns	ns
MTXV 10	ns	0.29***	0.17**	ns	-0.24**	0.38***	-0.49***	ns	ns	ns	ns
MTXS 6	ns	ns	ns	ns	ns	-0.27***	0.28***	ns	ns	ns	ns
MTXS 8	ns	ns	ns	ns	ns	-0.39***	0.42***	ns	ns	ns	ns
MTXS 10	ns	ns	ns	ns	ns	-0.38***	0.38***	ns	ns	ns	ns
MTXW 6	ns	ns	-0.30***	-0.36***	-0.53***	0.34***	-0.37***	0.41***	ns	-0.45***	ns
MTXW 8	ns	ns	-0.31***	-0.38***	-0.56***	0.34***	-0.35***	0.40***	ns	-0.43***	ns
MTXW 10	ns	ns	-0.22**	-0.29***	-0.51***	0.33***	-0.35***	0.29***	ns	-0.33***	ns
MTXI 6	ns	0.15*	0.24**	ns	ns	0.25***	-0.32***	-0.21**	ns	ns	ns
MTXI 8	ns	0.18**	0.23**	ns	ns	0.31***	-0.38***	-0.17**	ns	ns	ns
MTXI 10	ns	0.21**	0.21**	ns	ns	0.33***	-0.41***	-0.13*	ns	ns	ns

ns = not significant, \*P ≤ 0.05, \*\*P ≤ 0.01, \*\*\*P ≤ 0.001, HLM = Hectolitre mass, FLN = Falling number, GPC = Grain protein content, FPC = Flour protein content, FLY = Flour yield, KJ76 = Corrected flour colour, FABS = Farinograph water absorption, P/L = Alveograph stability/distensibility ratio, STR = Alveograph dough strength, LFV = Loaf volume, LFV12% = Corrected loaf volume, 6, 8, 10 = 6 min, 8 min, 10 min, ETXV = envelope timeX value, ETXS = envelope timeX slope, ETXW = envelope timeX width, ETXI = envelope timeX integral, MTXV = midline timeX value, MTXS = midline timeX slope, MTXW = midline timeX width, MTXI = midline timeX integral

Small differences in correlation intensity (\*P ≤ 0.05, \*\*P ≤ 0.01, \*\*\*P ≤ 0.001) were also observed for the selected Mixsmart parameters at the different timeX values; MLS 6, MLS 8 and MLS 10 for FLN, MPT 6, MPT 8 and MPT 10 for P/L and MRI 6, MRI 8 and MRI 10 for HLM (Table 6.3, differences indicated in red).

**Table 6.3 The relationship of the six selected Mixsmart parameters with primary wheat quality characteristics over six environments**

Parameter (mixing time)	HLM	FLN	GPC	FPC	FLY	KJ76	FABS	P/L	STR	LFV	LFV12%
<b>MLS 6</b>	ns	0.18**	0.44***	0.42***	0.36***	ns	ns	-0.42***	0.17**	0.43***	0.23**
<b>MLS 8</b>	ns	0.18**	0.45***	0.44***	0.36***	ns	ns	-0.42***	0.17**	0.44***	0.23**
<b>MLS 10</b>	ns	0.16*	0.47***	0.46***	0.36***	ns	ns	-0.41***	0.18**	0.43***	0.23**
<b>MRS 6</b>	ns	ns	-0.48***	-0.51***	-0.42***	ns	-0.27***	0.39***	-0.30***	-0.54***	-0.21**
<b>MRS 8</b>	ns	ns	-0.47***	-0.49***	-0.42***	ns	-0.27***	0.39***	-0.30***	-0.53***	-0.21**
<b>MRS 10</b>	ns	ns	-0.45***	-0.49***	-0.44***	ns	-0.27***	0.37***	-0.30***	-0.53***	-0.22**
<b>MPT 6</b>	ns	0.17**	ns	ns	-0.43***	0.45***	-0.44***	0.25***	ns	-0.25***	ns
<b>MPT 8</b>	ns	0.17**	ns	ns	-0.43***	0.45***	-0.44***	0.25***	ns	-0.25***	ns
<b>MPT 10</b>	ns	0.18**	ns	ns	-0.40***	0.43***	-0.45***	0.22**	ns	-0.26***	ns
<b>MPV 6</b>	ns	0.15*	0.39***	0.31***	ns	0.23**	-0.21**	-0.30***	0.16*	0.22**	0.15*
<b>MPV 8</b>	ns	0.14*	0.39***	0.31***	ns	0.23**	-0.21**	-0.30***	0.16*	0.22**	0.13*
<b>MPV 10</b>	ns	0.15*	0.37***	0.29***	ns	0.22**	-0.22**	-0.28***	0.14*	0.20**	0.13*
<b>MRI 6</b>	-0.17**	0.19**	0.14*	ns	-0.34***	0.51***	-0.52***	ns	ns	-0.15*	ns
<b>MRI 8</b>	-0.18**	0.19**	0.14*	ns	-0.34***	0.51***	-0.52***	ns	ns	-0.15*	ns
<b>MRI 10</b>	-0.16*	0.21**	0.14*	ns	-0.32***	0.49***	-0.53***	ns	ns	-0.16*	ns
<b>MTW 6</b>	ns	0.17**	-0.21**	-0.28***	-0.48***	0.32***	-0.32***	0.32***	ns	-0.33***	ns
<b>MTW 8</b>	ns	0.17**	-0.21**	-0.28***	-0.48***	0.32***	-0.32***	0.32***	ns	-0.33***	ns
<b>MTW 10</b>	ns	0.20**	-0.19**	-0.27***	-0.47***	0.32***	-0.33***	0.29***	ns	-0.32***	ns

ns = not significant, \*P ≤ 0.05, \*\*P ≤ 0.01, \*\*\*P ≤ 0.001, HLM = Hectolitre mass, FLN = Falling number, GPC = Grain protein content, FPC = Flour protein content, FLY = Flour yield, KJ76 = Corrected flour colour, FABS = Farinograph water absorption, P/L = Alveograph stability/distensibility ratio, STR = Alveograph dough strength, LFV = Loaf volume, LFV12% = Corrected loaf volume, 6, 8, 10 = 6 min, 8 min, 10 min, MLS = midline left slope, MRS = midline right slope, MPT = midline peak time, MPV = midline peak value, MRI = midline right integral, MTW = midline tail width

## **6.4 Discussion**

ANOVA indicated that eight Mixsmart parameters (the timeX parameters) varied with the different sources of variation (data not shown) and when these parameters were eliminated, the mixograms for the different time intervals grouped together (Figure 6.2). As expected, differences occurred when different timeX values were involved and this was confirmed when the different time intervals grouped separately (Figure 6.1). Correlations between the timeX values and the six selected Mixsmart parameters and the primary quality criteria were consistent, meaning that highly significant correlations with the primary quality criteria ( $P \leq 0.001$ ) were similar at the different time intervals (Tables 6.2 and 6.3).

## **6.5 Conclusions**

Dough mixing properties are important to breeders, especially during the early stages of breeding programmes to enable them to eliminate breeding lines exhibiting unacceptable dough characteristics. Dough breakdown behaviour is indicated on a mixogram after peak time has been reached and it is referred to as dough mixing tolerance. Since desirable mixing times in South Africa is between 2.5 and 4.5 min, a total running time for mixograph analyses of 6 min is sufficient for breeders to assess acceptable dough tolerance. TimeX on a mixogram can be selected by Mixsmart<sup>®</sup> software, most frequently towards the tail of the mixogram curve. Slopes, integrals, heights and widths of the mixogram are therefore evaluated at different stages on the mixogram depending on what timeX was selected. Since the relationships between the Mixsmart parameters (at the different time intervals) and the primary criteria remained constant, based on the specific required quality, breeders can choose whether they want mixograph analyses to be stopped after 6, 8 or 10 min of mixing.

## 6.6 References

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## CHAPTER 7

### THE INFLUENCE OF DIFFERENT MILLING PROCEDURES ON MIXSMART PARAMETERS AND THEIR RELATIONSHIPS WITH PRIMARY QUALITY CRITERIA

#### Abstract

Superior bread making quality is required before breeding lines can be commercially classified. The Southern African Grain Laboratory (SAGL) is responsible for performing wheat quality analysis for the purpose of cultivar classification. The best representative samples, together with a quality standard, from five environments must be submitted for quality analysis to SAGL and this must be done for three years in a row before a breeding line can be commercially classified. Breeders depend on falling number (FLN) and mixograph analysis to indicate which breeding lines must be selected for submission to SAGL. Depending on time availability, either whole meal or white flour is used for this quick screening process. During the classification process by the SAGL, the lines have to adhere to primary quality criteria where a fixed deviation from the quality standard is allowed. Primary criteria include; hectolitre mass (HLM), falling number (FLN), grain protein content (GPC), flour protein content (FPC), flour yield (FLY), corrected flour colour (KJ76), farinograph water absorption (FABS), alveograph stability/distensibility ratio (P/L), alveograph dough strength (STR), loaf volume (LFV) and corrected loaf volume (LFV12%). It is important that relationships between Mixsmart analysis and primary criteria stay unchanged whether whole meal or white flour is utilised. Ten genotypes from two environments, planted during 2009, were milled on a falling number hammer mill, a Junior Quadrumat mill as well as a Bühler-mill to obtain flour from three different milling procedures. Mixograms were done on all these samples and six Mixsmart parameters were selected which showed significant correlations with the primary quality criteria. Linear discriminant analysis confirmed that no differences existed between the white flour and whole meal used for mixograph analysis, as indicators of quality.

## 7.1 Introduction

The focus of bread wheat breeding programmes is to have superior bread making quality, for which traditionally milled white flour is utilised for measuring quality characteristics (Bruckner et al., 2001). The Southern African Grain Laboratory (SAGL) is responsible for conducting wheat quality analysis on potential breeding lines that are submitted by breeding companies for commercial classification in South Africa (SAGL, 2013). For this purpose, samples are milled on a Bühler-mill. South African breeding companies have limited time from harvesting (November) until submission in January to identify the most suitable lines for commercial classification submission, to enable SAGL to perform the quality analysis for discussion during the first week of April each year.

Therefore, breeders have to obtain a quick indication of the potential lines' quality to enable them to select only the best for submission to SAGL. Depending on available sample sizes and time availability for quick screening at each breeding company's own quality laboratory, samples are milled on a falling number hammer mill, resulting in whole meal. Otherwise, samples are milled on a Junior Quadrumat or a Bühler-mill, resulting in white flour, although the Bühler-mill is used at this stage only when uncertainty around flour yield potential exist. Protein content, FLN and mixograph analyses are then conducted on the samples.

At SAGL, breeding lines have to adhere to primary and secondary criteria during the classification process (SAGL, 2013). The lines are evaluated against a quality standard, which was planted (together with the breeding lines) at five environments for three years. The breeding lines are allowed to deviate from the quality standard regarding the primary and secondary criteria, although primary criteria are fixed and non-negotiable. Primary criteria include HLM, FLN, GPC, FPC, FLY, KJ76, FABS, P/L, STR, LFV and LFV12%.

The aim of this study was to determine if differences occur when using six selected Mixsmart parameters as indicators of the primary classification criteria

when flour obtained from three different milling procedures are used for mixograph analyses.

## 7.2 Materials and methods

### 7.2.1 Field trials

Ten genotypes with four replicates from the National Cultivar Trials, planted during 2009 in two localities (Bethlehem, Bultfontein) in the eastern part of the summer rainfall region in South Africa were used for this research. Planting occurred in April and June for Bultfontein and Bethlehem respectively (Table 7.1).

**Table 7.1 Information on the environments, planting and harvesting dates in the eastern part of the summer rainfall region**

Locality	GPS coordinates	Altitude	Planting dates	Harvesting dates
<b>Bethlehem</b>	28° 09' 17 25"S 28° 17' 45 15" E	1721	22-06-2009	14-12-2009
<b>Bultfontein</b>	28° 17' 25 92" S 26° 28' 19 50" E	1302	29-04-2009	26-11-2009

Trials were planted with a precision planter according to a randomised complete block design with four replicates. Plots consisted of five rows, five metres in length and inter-row spacing was 45 cm. Seeds were planted 5 cm apart and fertiliser, 6:2:1 (31) was applied. Total N, P and K were 50 kg, 17 kg and 9 kg respectively. To eliminate side row effect, only the three middle rows were harvested.

Trials were harvested during November and December for the two environments respectively (Table 7.1). Sufficient rainfall occurred before planting, although Bultfontein had less rain before and during the whole growth cycle of the plants. Bultfontein also exhibited higher temperatures during the last three months before harvesting (Table 7.2).

**Table 7.2 Information on rainfall and temperatures for the two environments in the eastern part of the summer rainfall region**

Month	2009 Bethlehem				Month	2009 Bultfontein			
	Min °C	Max °C	Days >30°C	Rainfall (mm)		Min °C	Max °C	Days >30°C	Rainfall (mm)
				315.40 <sup>(a)</sup>					168.1 <sup>(a)</sup>
					<b>April<sup>(b)</sup></b>	9.75	26.12	0	13.70
					<b>May</b>	5.73	21.19	0	26.90
<b>June<sup>(b)</sup></b>	2.49	14.90	0	58.70	<b>June</b>	3.48	17.77	0	47.00
<b>July</b>	0.43	15.06	0	0.00	<b>July</b>	-0.56	16.49	0	7.10
<b>Aug</b>	1.74	18.41	0	20.10	<b>Aug</b>	3.24	21.29	0	1.80
<b>Sept</b>	6.61	23.29	0	8.40	<b>Sept</b>	6.08	26.38	3	6.10
<b>Oct</b>	9.13	22.58	0	42.40	<b>Oct</b>	11.89	26.80	6	88.90
<b>Nov</b>	9.95	23.39	0	74.80	<b>26 Nov</b>	12.27	26.93	7	31.80
<b>14 Dec</b>	12.51	26.26	0	37.10					
				241.50 <sup>(c)</sup>					223.20 <sup>(c)</sup>

Min=average minimum temperature, Max=average maximum temperature, <sup>a</sup>=measured rainfall from January till planting month, <sup>b</sup>= planting month, <sup>c</sup>= measured rainfall from planting month till harvesting date

### 7.2.2 Laboratory analyses

Milling on the Junior Quadrumat mill was done according to AACC method 26–50 using a Brabender Junior Quadrumat (Brabender GmbH & Co KG). Whole meal was obtained by milling on a falling number hammer mill (Perten Instruments, model LM 3100 hammer mill), fitted with a 0.8 mm sieve. Bühler-milling and other quality analyses were discussed in Chapter 3, section 3.2.2.

### 7.2.3 Selection of Mixsmart parameters

The selection of six Mixsmart parameters was discussed in Chapter 3, section 3.3.3 and a description thereof can be found in Table 3.3 in Chapter 3.

#### **7.2.4 Statistical analyses**

A Shapiro-Wilk test, Levene's test and Pearson product-moment correlation coefficients were determined as discussed in Chapter 3, section 3.2.3. Linear discriminant analysis was performed as discussed in Chapter 6, section 6.2.4.

#### **7.3 Results**

Mean values for mixing times (MPT) as well as width values (MTW) obtained from whole meal (WM) samples were higher for both environments (Table 7.3). Peak values (MPV) and integral values (MRI) were higher for samples obtained from the Bühler-mill's flour (Table 7.3).

**Table 7.3 Descriptive statistics of the six selected Mixsmart parameters determined on flour obtained from a hammer mill (whole meal, WM), Junior Quadrumat mill (JQ) and a Bühler-mill (BH)**

Parameter	Flour type	Env	Min	Max	Mean	Std. dev.
MLS	WM	Bhm	0.21	8.80	4.50	2.34
		Bult	-10.39	32.14	5.68	8.73
	JQ	Bhm	4.75	19.65	11.03	3.66
		Bult	-0.92	17.19	7.66	4.07
	BH	Bhm	2.47	17.39	9.96	3.80
		Bult	5.02	30.10	14.14	6.10
MPT	WM	Bhm	3.30	5.95	4.40	0.61
		Bult	0.84	6.39	3.53	1.33
	JQ	Bhm	2.41	3.88	3.09	0.41
		Bult	2.30	4.34	3.31	0.46
	BH	Bhm	2.53	5.94	3.92	0.91
		Bult	1.93	3.85	2.80	0.47
MPV	WM	Bhm	40.75	56.07	48.25	3.68
		Bult	42.40	58.41	46.82	3.60
	JQ	Bhm	42.92	56.09	48.30	3.37
		Bult	38.36	50.37	45.40	2.64
	BH	Bhm	46.39	72.72	60.52	6.28
		Bult	49.06	70.24	59.33	5.24
MRS	WM	Bhm	-7.95	-0.34	-2.86	1.89
		Bult	-9.43	8.71	-1.74	3.88
	JQ	Bhm	-9.42	-3.56	-5.28	1.59
		Bult	-6.97	0.21	-3.92	1.70
	BH	Bhm	-9.21	-0.47	-4.39	2.22
		Bult	-12.27	-1.42	-7.50	2.94
MRI	WM	Bhm	176.26	252.24	217.64	20.17
		Bult	73.30	250.80	168.74	51.74
	JQ	Bhm	127.98	182.10	149.01	13.29
		Bult	117.64	184.48	154.22	16.01
	BH	Bhm	183.08	331.74	238.16	36.69
		Bult	149.67	217.70	178.65	18.64
MTW	WM	Bhm	4.40	26.25	17.22	5.54
		Bult	5.49	64.70	18.14	10.65
	JQ	Bhm	1.05	14.73	5.94	2.41
		Bult	4.08	18.60	8.00	4.29
	BH	Bhm	4.46	20.34	10.08	4.32
		Bult	4.75	26.03	10.01	5.36

MLS = midline left slope, MPT = midline peak time, MPV = midline peak value, MRS = midline right slope, MRI = midline right integral, MTW = midline tail width, WM = Whole meal, JQ = Junior Quadrumat, BH = Bühler, Env = Environment, Bhm = Bethlehem, Bult = Bultfontein, Min = Minimum, Max = Maximum, Std.dev. = Standard deviation

When considering the flour types from the different milling procedures across both environments, JQ Mixsmart parameters correlated with HLM, STR and LFV12%, BH Mixsmart parameters correlated with HLM and STR and WM Mixsmart parameters correlated only with FABS (Table 7.4). Irrespective of the environments, the Mixsmart parameters obtained from the three different flour types (whole meal, Junior Quadrumat and Bühler) showed respectively 31, 41 and 23 correlations with the primary quality characteristics (Table 7.4).

Overall, JQ Mixsmart parameters MLS, MRS, MPV and MRI showed the most correlations with primary quality characteristics (irrespective of the environments) and when considering both environments, all six JQ Mixsmart parameters (MLS, MRS, MPT, MPV, MRI and MTW) again exhibited more correlations (Table 7.4).

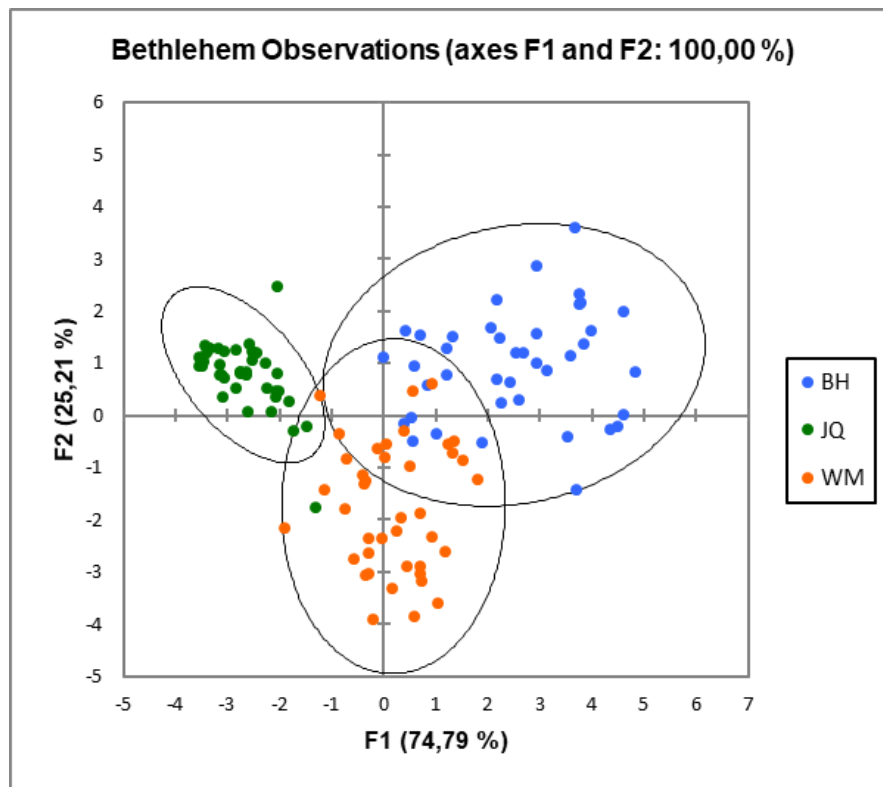
Most correlations existed with primary quality criteria HLM, followed by STR and FABS. No correlations were observed between the Mixsmart parameters and LFV, but seven correlations were observed with LFV12% (Table 7.4).

LDA was performed separately for Bethlehem and Bultfontein. LDA involving the three different flour types and the six Mixsmart parameters, resulted in grouping together of the three different flour types for both Bethlehem (Figure 7.1) and Bultfontein (Figure 7.2) respectively, indicating no differences for the different flour types used for mixograph analysis. F1 (Mixsmart parameters) accounted for 74.79% and 85.00% of the variation in Bethlehem and Bultfontein respectively and F2 (milling procedures) accounted for 25.21% and 15.00% of the variation respectively.

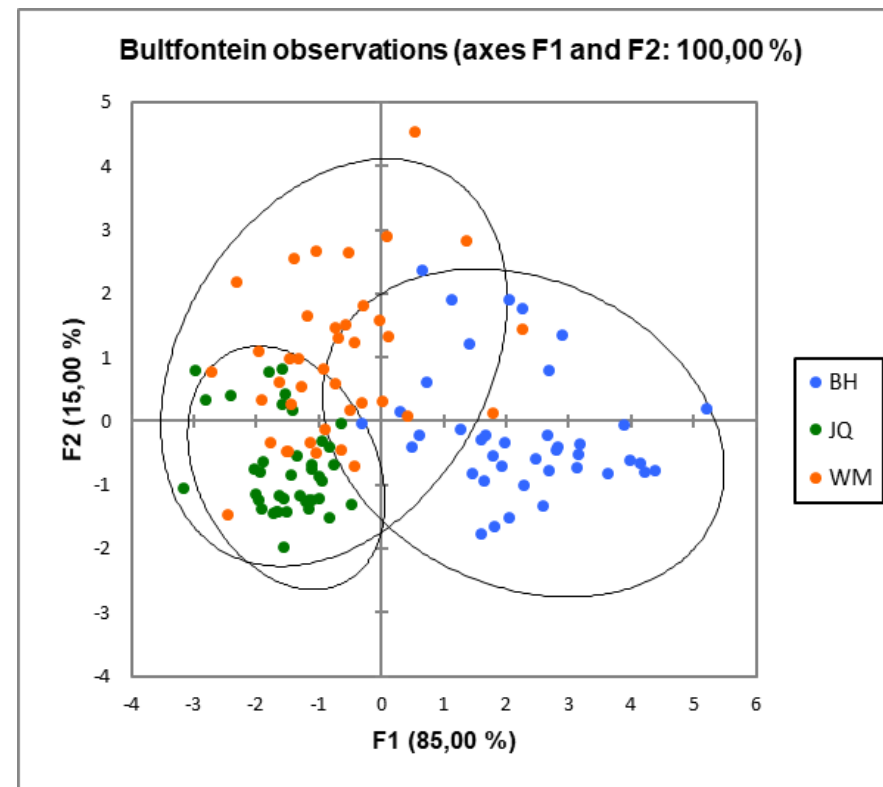
**Table 7.4 Correlations between primary quality characteristics and the six selected Mixsmart parameters obtained from flour from a hammer mill (whole meal, WM), Junior Quadrumat mill (JQ) and a Bühler-mill (BH)**

Parameter	Flour type	Env	HLM	FLN	GPC	FPC	FLY	KJ76	FABS	P/L	STR	LFV	LFV12%
MLS	WM	Bhm					0.35*	-0.34*	0.35*				
		Bult							0.39*				
	JQ	Bhm	-0.42*					0.47*	-0.69*	0.46*		-0.34*	
		Bult	-0.36*	0.41*								-0.47*	
	BH	Bhm									-0.34*	-0.33*	
		Bult	-0.46*									-0.35*	
MPT	WM	Bhm	0.33*					0.45*	-0.39*	0.33*			
		Bult					-0.34*	0.41*	-0.49*				
	JQ	Bhm	0.48*				-0.42*	0.64*	-0.55*		0.41*		
		Bult	0.56*								0.35*		
	BH	Bhm											-0.35*
		Bult	0.62*									0.33*	
MPV	WM	Bhm		-0.35*				-0.64*	0.48*				-0.34*
		Bult											
	JQ	Bhm	-0.49*	-0.37*					-0.60*	0.51*			-0.36*
		Bult	-0.38*									-0.38*	-0.32*
	BH	Bhm					0.47*				-0.47*		
		Bult	-0.42*					0.36*				-0.33*	
MRS	WM	Bhm	0.41*		-0.41*	-0.34*			-0.56*				
		Bult							0.34*				
	JQ	Bhm	0.44*				-0.49*	0.61*	-0.47*			0.51*	
		Bult	0.34*										
	BH	Bhm					-0.35*			0.38*			
		Bult	0.53*					-0.42*					0.36*
MRI	WM	Bhm		-0.31*			-0.34*			0.43*			
		Bult					-0.46*	0.68*	-0.57*				
	JQ	Bhm					-0.50*	0.41*	-0.34*	0.38*	0.47*		
		Bult	0.48*								0.32*		
	BH	Bhm					0.33*						-0.34*
		Bult	0.58*										
MTW	WM	Bhm	0.37*		-0.37*	-0.38*			-0.56*				
		Bult										0.31*	
	JQ	Bhm					-0.42*	0.37*			0.38*		
		Bult	0.65*								0.37*		
	BH	Bhm	-0.32*										
		Bult	0.60*										0.36*

\*P ≤ 0.05, MLS = midline left slope, MPT = midline peak time, MPV = midline peak value, MRS = midline right slope, MRI = midline right integral, MTW = midline tail width, WM = Whole meal, JQ = Junior Quadrumat, BH = Bühler, Env = Environment, Bhm = Bethlehem, Bult = Bultfontein, HLM = Hectolitre mass, FLN = Falling number, GPC = Grain protein content, FPC = Flour protein content, FLY = Flour yield, KJ76 = Corrected flour colour, FABS = Farinograph water absorption, P/L = Alveograph stability/distensibility ratio, STR = Alveograph dough strength, LFV = Loaf volume, LFV12% = Corrected loaf volume



**Figure 7.1** Linear discriminant biplot of the three different milling procedures' flour involving six selected Mixsmart parameters for Bethlehem



**Figure 7.2** Linear discriminant biplot of the three different milling procedures' flour involving six selected Mixsmart parameters for Bultfontein

## **7.4 Discussion**

A positive relationship between the quality of whole meal versus white flour is expected, but has not been widely studied (Bruckner et al., 2001). In this study, similar Mixsmart parameters (MLS for WM and JQ, MRS for WM and JQ, MPT for WM and JQ, MPV for WM and JQ, MRI for WM and JQ, MTW for WM and JQ) correlated, but similar correlations did not exist with BH (data not shown) for the selected Mixsmart parameters. Bruinsma et al. (1978) reported that mixograms performed on whole meal showed less strength compared to mixograms performed on white flour, while the same was not observed from these results, since mean MRI values (Table 7.3), indicative of dough strength, were similar for WM and BH flour samples. Sufficient correlations were observed between the Mixsmart parameters with the primary quality criteria, to assist breeders effectively with selection of the best potential breeding lines for submission to SAGL, whether they utilise whole meal, white flour from the Junior Quadrumat mill or flour from the Bühler-mill to perform mixograph analysis as a quick indication of bread making quality. Sedláček and Horčíčka (2014) used reomixer analysis and reported whole meal and white flour analysis to be comparable when predicting baking quality. LDA also indicated that no differences existed between Mixsmart parameters obtained by using flour from the different milling procedures.

## **7.5 Conclusions**

It is important for breeders to select the best representative samples of their potential breeding lines destined for commercial cultivar classification purposes, since lines can be easily eliminated if they do not adhere to the primary classification criteria. A reliable but quick indication of the quality is necessary to enable them to select lines exhibiting the best adherence to the primary criteria, before samples are submitted to SAGL for the final quality analysis. Samples are milled on a Bühler-mill at SAGL, but for quick screening purposes, Bühler-milling is time-consuming, labour intensive and expensive to perform. Depending on time availability before submission to SAGL, samples can be milled on a falling number hammer mill, a Junior Quadrumat mill or a Bühler-mill, resulting in whole

meal or white flour respectively. It is clear from these results, that breeders can select successfully, whether whole meal or white flour was used for mixograph analysis, since significant correlations existed between mixograms performed with the different flour types and primary quality criteria and LDA also indicated no differences between the different flour types used.

## **7.6 References**

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## CHAPTER 8

### GENERAL CONCLUSIONS AND RECOMMENDATIONS

Ten hard red wheat genotypes (Betta-DN, Caledon, Elands, Gariep, Komati, Limpopo, Matlabas, PAN3118, PAN3349 and PAN3377) from the National Cultivar trials conducted in South Africa were planted at three environments over two seasons in the eastern part of the summer rainfall area of South Africa. In order to revive the South African wheat industry (there has been a slump in the wheat industry in South Africa in the past few years due to a number of factors), breeding programmes currently have to focus on higher yield. The industry on the other hand, still expects excellent quality, which adds to the challenge, since quality and higher yield are often negatively correlated (Peña et al., 2002; Shewry, 2007; Saint Pierre et al., 2008).

The mixograph is user-friendly, quick to perform and requires small sample sizes – all characteristics of high importance to breeders to distinguish quickly between good and poor bread making quality. During the cultivar classification process, mixing time is still the only mixograph parameter taken into account, where other mixograph parameters may be able to indicate acceptable quality as indicators of primary quality criteria. By using only six mixograph parameters, their selection based upon repeatability, coefficient of variation, coefficient of determination and their genotype contribution to variation, relationships with primary quality criteria were acceptable as indicators of good bread making quality. These mixograph parameters are therefore effective in assisting breeders during early generation selections for potential breeding lines that would exhibit acceptable primary criteria during the final evaluation phases for cultivar classification.

Millers and bakers require stable quality characteristics to have access to consistently performing raw material (Grausgruber et al., 2000; Barić et al., 2004; SAGL, 2017). AMMI and GGE-biplots are effective to indicate ideal and/or stable genotypes, which can assist breeders in selection of parental material having required characteristics (superior quality, high yield and stability) for utilising in crossing blocks. It remains a challenge though, since genotypes react differently

in different environments and the best would be, as indicated by Williams et al. (2008), to locate the genes responsible for certain quality attributes to enable breeders to select independently of G X E, since the most stable genotypes for a quality parameter may not be the highest yielding genotypes.

Scientists perform mixograph analysis for a certain time and some stop the analysis after 6, 8 or 10 min. Since relationships exist between parts of the mixogram measured at the tail of the mixogram, it was investigated if the relationships with primary criteria would differ if measured “later” on the mixogram. It was found that the relationships with primary criteria did not differ significantly if measurements were done “later” on the mixogram. A total mixing time of 6 min under South African conditions is sufficient to indicate acceptable bread making properties.

A problem breeders experience is that very little time is available for testing of potential breeding lines after harvesting, in time to select the best representative lines to submit to the South African Grain Laboratory (SAGL) to conduct the analysis required for cultivar classification purposes in time for discussion at the annual meeting, held during early April. Depending on the time available for quick testing, breeders utilise whole meal or white flour. Protein and moisture content are determined and mixograph analysis and falling number are determined, which serve as criteria to pre-select for submission to SAGL. At SAGL, samples are milled on a Bühler mill and all quality analysis are therefore performed on white flour obtained from this mill. Again, no significant differences were observed between primary criteria and the six selected mixograph parameters when performed on the different types of flour used for pre-selections. Breeders may therefore utilise either whole meal or white flour for mixograph analysis to serve as a quick indication of poor or acceptable bread-making quality.

## 8.1 Questions arising from this study

- If only one of the selection criteria are applied to reduce the 44 mixograph parameters (as measured by Mixsmart software), will the same mixograph parameters be selected and would relationships with primary criteria differ?
- Could a combination of two or more Mixsmart® parameters be better indicators of primary quality criteria compared to when only a single parameter is used as an indicator?

It would be interesting to conduct research on all South African genotypes across the three production regions, including all 44 Mixsmart parameters. The ideal will be then to select respectively the best area, width, value, slope and time parameters, which could be utilised by breeders to effectively select for superior quality. Quality data from SAGL (performed on breeding lines for classification processes) can be used going forward to verify and confirm whether the best Mixsmart parameters were identified and applied as superior quality indicators.

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