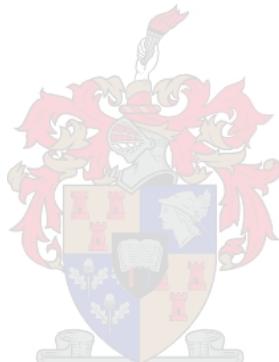


**Breeding for improved biomass yield in triticale**

**by**

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*Thesis presented in partial fulfilment of the requirements for the degree of Master of  
Science in the Faculty of Sciences at Stellenbosch University*



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**March 2020**

## **Declaration**

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## Opsomming

Korog ( $x$  *Triticosecale* Wittmack ex. A. Camus, AABBRR,  $2n = 6x = 42$ ) was die eerste mensgemaakte kleingraan wat ontwikkel is deur middel van verbastering tussen rog (*Secale cereale*, RR,  $2n = 2x = 14$ ) en broodkoring (*Triticum aestivum*, AABBDD,  $2n = 6x = 42$ ). Korog is die kombinasie van sy ouers se eienskappe wat 'n hoë mate van plaag- en siektebestandheid het asook die potensiaal om hoë opbrengste te gee wanneer verbou word onder droogte en suur grondtoestande. Korog word grotendeels bestudeer vir sy dubbele funksionaliteit omdat dit vir graanproduksie gebruik kan word asook as 'n weidingsgewas kan dien, deurdat dit biomassa-opbrengste produseer wat die gewas baie meer mededingend maak in vergelyking met ander gewasse. Korog is ryk aan vesel en kan groot hoeveelhede biomassa produseer. Die gewas benodig 'n beperkte hoeveelheid aandag en het hoë potensiaal om as 'n alternatiewe gewas ingesluit te word in wisselbou. 'n Multi-lokateit navorsingstudie was oor twee jaar uitgevoer, waartydens geselekteerde korokultivars vergelyk was met 'n seleksie van gars, hawer, rog en gevorderde korogteelmateriaal. Monsterneming was gedoen in twee groefases waartyends die planthoogte 30 cm (GS29-31) was en in die sagte deeg/melkstadium (GS69-71). Statistiese ontledings was uitgevoer via Agrobase-sagteware, waarin 'n algemene lineêre modelbenadering toegepas was op 'n ewekansige volledige blokontwerp. 'n "Nearest Neighbour Analysis" (NNA) was ook uitgevoer om die akkuraatheid van die statistiese ontleding te verhoog. Korog inskrywings wat in die 2016 proef die beste biomassa opbrengs gelewer het, was ingesluit in die proef van 2017. Die inskrywings wat verder goeie opbrengs geproduseer het, was geselekteer vir verdere evaluasie. Gedurende die eerste sny het korog 'n gemiddeld van  $1\ 000 \text{ kg.ha}^{-1}$  biomassa opbrengs gelewer, wat 'n aanduiding was van die gewas se potensiaal om as 'n weidingsgewas te dien omdat dit in die meeste gevalle beter gedoen het as sy mededingers. In die sagte deegstadium het die biomassa-opbrengste  $12\ 000 \text{ kg.ha}^{-1}$  oorskry. Tydens die proewe was die oorerflikheid vir die eienskap droëmateriaalopbrengs gemeet, met oorerflikheidswaardes wat gewissel het tussen 0,14 tot 0,88 oor die tydperk van die sagte deeg stadium. Die KV van die proewe het gewissel van 7,95% tot 24,8%. Aan die einde van die tweede jaar van die studie was vyf hawer inskrywings, een gars en agt korog geselekteer om die voedingswaardes te evalueer. Die voedingsparameters waarvolgens die voedingswaarde geëvalueer was, was proteïen, vesel, vet, vog en droëmateriaal. Sekere inskrywings was onder die top-inskrywings wat biomassa-opbrengs sowel as voedingswaarde betref. Deur hierdie studie word die hipotese aanvaar dat korog 'n hoë potensiaal het om as alternatiewe gewas te dien in gewasrotasies. Die inskrywings "AgBeacon", "Snel", "US2017",

“US2018” en “17USTRITEL020” is geselekteer om as moontlike ouers ingesluit te word in 'n formele teelprogram wat daarop gemik is om die biomassa-opbrengs te verbeter. Uit hierdie studie blyk die behoefte aan verdere navorsing vir verdere verbetering van korog. Verdere navorsing is nodig wat gefokus is op die verhoging van die biomassa-opbrengs van korog sowel as verbetering van voedingswaarde, wat benutting van die gewas in die bedryf sal aanhelp.

## Abstract

Triticale (*x Triticosecale* Wittmack ex. A. Camus, AABBRR, 2n=6x=42) is the first manmade wheat developed through distant hybridization between rye [*Secale cereale*, RR, 2n=2x=14] and bread wheat (*Triticum aestivum*, AABBDD, 2n=6x=42). Triticale combines the qualities of its parents such as high degree of pest and disease resistance and potential to produce high yield gains when cultivated under drought and acid soil conditions. These and other characteristics sparked the attention paid to the crop. Triticale is being largely studied for its dual functionality, which includes its ability to serve as a grazing crop by producing biomass yields which are highly competitive with other forages and its ability to be used for grain production. Believed to be rich in fibre, producing large quantities of biomass material and requiring reduced amounts of management, this crop has the potential to be regarded as a high potential alternative to be included into crop rotations to supplement feedstocks. A multilocation research study was conducted over two years of which selected triticale entries were evaluated with a selection of barley, oats and rye crops as well as evaluated against advanced breeding triticale entries. A two-stage biomass sampling approach was utilised, sampling at 30 cm plant height (GS29-31) or milk stage and during the soft dough growth stage (GS69-71). Statistical analyses were conducted via Agrobase software, in which a General linear model approach was done with a randomised complete block design. A Nearest Neighbour Analysis (NNA) was conducted as well for increased accuracy. Triticale entries that were ranked among the best producers in terms of biomass yield during 2016 were included in 2017, whilst entries that were ranked the best during 2017 were further evaluated. Triticale on average produced a 1 000 kg ha<sup>-1</sup> of biomass yield during the first cutting, indicative of its potential to offer grazing material and being superior to its other competitors in most cases. Within the soft dough stage biomass yields exceeded 12 000 kg ha<sup>-1</sup>. Heritability was measured among the trials for the trait of dry matter yield, with heritability values ranging from 0.14 to 0.88 across the trials during soft dough. The CV reported for the trials ranged from 7.95% to 24.8%. Among the top performing entries in terms of biomass yield at the end of the second year of the study, five oats, one barley and eight triticale entries were selected and analysed for nutritional value. The nutritional parameters analysed included ash, crude protein, fibre, fat, moisture and dry matter content. Certain triticale entries were among the top ranked entries in terms of both biomass yield as well as nutritional value. Through this study the hypothesis which considered triticale having high potential for consideration as an alternative crop to be

included on rotations, is accepted. The entries “AgBeacon”, “Snel”, “US2017”, “US2018” and “17USTRITEL020” have been selected potential crossing parents to be included into a formal breeding programme aimed at improving biomass yield. The need for further research to be conducted for further improvement of triticale is clear from this study. Further research aimed at increasing biomass yield of triticale as well as nutritional value will aid a greater uptake and utilization of the crop in the industry.

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## List of abbreviations

%	Percentage
µL	microliter
ADF	Acid detergent fibre
a.m.	Before midday
ANOVA	Analysis of Variance
ARC	Agricultural Research Council
AOAC	Association of Official Agricultural Chemists (est. 1884)
bp	Base pairs
BMY	Biomass yield
CGIAR	Consultative Group on International Agriculture Research
CIMMYT	The International Maize and Wheat Improvement Centre
°C	Degrees Celsius
cm	centimetre
CV	Coefficient of variation
CuSO <sub>4</sub>	Copper Sulphate
dH <sub>2</sub> O	Distilled water
DNA	Deoxyribonucleic Acid
DMY	Dry matter yield

DW	dry weight
e.g.	For example
Eq	Equation
Etc.	Including the rest
EW	East-West Direction
F <sub>1</sub>	First generation
F <sub>2</sub>	Second generation
FAO	Food and Agriculture Organization
Fig	Figure
g	Gram
GLM	General Linear Model
GMO	Genetically modified organism
GM	Genetically modified
GY	Grain Yield
h	Hour
HCl	Hydrogen chloride
H <sup>2</sup> <sub>b</sub>	Heritability
H <sub>3</sub> BO <sub>3</sub>	Boric Acid
H <sub>2</sub> O	Water
H <sub>2</sub> SO <sub>4</sub>	Hydrogen Sulphate
ha	Hectare
HI	Harvest index
K <sub>2</sub> SO <sub>4</sub>	Potassium Sulphate

kg	Kilogram
kg ha <sup>-1</sup>	Kilograms per hectare
L	Litre
LGW	Langgewens Research Farm
LSD	Least significance difference
m	Metre
MAS	Marker-Assisted Selection
min	minute
mL	millilitre
mm	Millimetre
N	Nitrogen
NaCl	Sodium chloride
NaOH	Sodium Hydroxide
NDF	Neutral detergent fibre
NDVI	Normalised difference in vegetation index
NH <sub>3</sub>	Ammonia
NIR	Near Infrared
NNA	Nearest Neighbour Analysis
No.	Number
NPN	Non-protein nitrogen
NS	North-South Direction
P	Phosphorous

PHS	Preharvest sprouting
Pty Ltd	Proprietary Limited
QTL	Quantitative trait loci
RCBD	Randomised complete block design
R <sup>2</sup>	Coefficient of determination
RGB	Red, Green and Blue
RSA	Republic of South Africa
RUE	Radiation use efficiency
SAGIS	South African Grain Information Service
s	Seconds
SA	South Africa
S.E. D	Standard error of a difference
SU-PBL	Stellenbosch University Plant Breeding Lab
<i>t</i>	t-Statistic
t ha <sup>-1</sup>	Tonnes per hectare
TKW	Thousand kernel weight
Tx.	Maximum temperature
UAV	Unmanned aerial vehicle
UV	Ultraviolet
v	version
WCG DoA	Western Cape Government Department of Agriculture
WWF-SA	World Wildlife Fund South Africa
Yr	Yellow/Stripe rust resistance

## Table of contents

Declaration .....	i
Abstrakte .....	Error! Bookmark not defined.
Abstract.....	iv
Acknowledgements .....	vi
List of abbreviations .....	vii
Table of contents .....	xi
List of Figures.....	xiii
List of Tables .....	xiv
Chapter 1: Introduction .....	1
Chapter 2: Literature review.....	6
2.1 Crop production in the Western Cape Province in South Africa .....	6
2.2 Triticale .....	7
2.3 The utilisation of triticale .....	7
2.4 The importance of triticale in animal nutrition .....	8
2.5 Nutritional characteristics .....	12
2.6 Digestibility as a feed source .....	14
2.7 Biomass characteristics.....	14
2.8 Resistance characteristics of Triticale.....	15
2.9 Harvest index as a breeding tool .....	16
2.10 Factors limiting crop production .....	18
2.11 Abiotic stress.....	18
2.12 Biotic stress.....	18
2.13 Climatic aspects in relation to biomass production .....	19
2.14 Threat analysis on rust disease affecting triticale .....	22
2.15 Measurement of quantitative traits .....	23
2.16 Plant physiology.....	24
2.17 Breeding for physiological traits.....	25
Chapter 3: Materials and methods.....	27
3.1 Genetic material .....	27

3.1.1 Location and cultivars .....	28
3.2. Experimental layout.....	31
3.3 Agronomic practices .....	32
3.4 Data collection.....	35
3.5 Biomass sampling procedure.....	37
3.6 Nutrient components .....	38
3.7 Statistical analysis .....	38
Chapter 4:     Results and discussion .....	41
4.1 Biomass yield data during the 2016 season .....	41
4.2 Mariendahl dry matter yield measured across both phases during 2016.....	42
4.3 Langgewens dry matter yield measured across both phases during 2016. ....	46
4.4 Riversdale dry matter yield measured across both phases during 2016. ....	51
4.5 Summary of statistical analysis of phase 1 .....	55
4.6 Welgevallen dry matter yield during 2017 .....	56
4.7 Langgewens dry matter yield during 2017 .....	58
4.8 Roodebloem dry matter yield during 2017 .....	61
4.9 Statistical analysis .....	64
4.10 Nutritional parameters .....	65
4.11 Statistical analyses on nutritional components.....	69
4.11.1 Ash content .....	69
4.11.2 Crude protein content .....	72
4.11.3 Dry matter yield .....	75
4.11.4 Fat content.....	77
4.11.5 Fibre content.....	79
4.11.6 Moisture content .....	82
Chapter 5:     Conclusion .....	88
5.1 Triticale versus small grain cereal entries in terms of BMY.....	89
5.2 Triticale versus advanced breeding line triticale entries in terms of BMY .....	90
5.3 Nutritional characteristics of top performing entries .....	91
References .....	93

## List of Figures

Figure 1: The hybridisation of Triticale from wheat and rye (Adapted from Ammar et al., 2004).	10
Figure 2: The food chain (Adapted from Reynolds et al., 2012)	11
Figure 3: A map of the Western Cape Province as well as the index of dryland potential (generated by CapeFarmMapper v 2.0.1.3).	21
Figure 4: An illustration on the process of how the pre-breeding approach fits into developing improved crop varieties (Adapted from Sharma et al., 2013)	26
Figure 5: Flow diagram of the structure and summary of the project workflow.	27
Figure 6: Illustration of the in-field experimental trial location and layout at Mariendahl in 2016 displayed by drone footage (Photo taken by Willem Botes).	31
Figure 7: Illustration of physical field layout of a forage trial taken with an unmanned aerial vehicle (UAV) displaying randomised block design in 2017 (Photo taken by Pieter Lombard).	32
Figure 8: Representation of the Zadoks cereal development scale with optimal stages in reference to biomass sampling (Onda et al., 2015)	36
Figure 9: Cereal plant developmental scale (Zadoks) and the stages sampled (Reynolds et al., 2012).	36
Figure 10: Schematic illustration of methodologies applied to this research study	40
Figure 11: Degree of ryegrass and grass weeds growing between and within rows (Photos taken by Naweed Mohamed)	49
Figure 12: Mean biomass yield of the advanced breeding line genotypes during the milk stage at the Welgevallen site during 2017	56
Figure 13: Mean biomass yield of the advanced breeding line genotypes during the soft dough stage at the Welgevallen site during 2017	57
Figure 14: Mean biomass yield of the mixed forage genotypes during the first cut at the Langgewens site during 2017	59
Figure 15: Mean biomass yield of the mixed forage genotypes during the second cut at the Langgewens site during 2017	60
Figure 16: Mean biomass yield of the mixed forage genotypes during the first cut at the Roodebloem site during 2017	61
Figure 17: Mean biomass yield of the mixed forage genotypes during the second cut at the Roodebloem site during 2017	62

## List of Tables

Table 1: Description of nutritional parameters (Adapted from McClements, 2015).....	13
Table 2: List of cultivars planted in 2016.....	29
Table 3: List of cultivars planted in 2017.....	30
Table 4: Summary of agronomic practices used to manage the experimental trials during 2016 and 2017.....	33
Table 5: The five best performing forage genotypes in terms of biomass yield (BMY) sampled at 30 cm and soft dough cutting stages during 2016 at Mariendahl.....	43
Table 6: Statistics for Biomass yield measurements (kg ha <sup>-1</sup> ) for selected triticale cultivars (US2014, AgBeacon and Snel) and other advanced breeding line triticale.....	43
Table 7: Biomass yield measurements (kg ha <sup>-1</sup> ) for selected triticale cultivars (US2014, AgBeacon and Snel) and other advanced breeding line triticale.....	44
Table 8: The five best performing forage genotypes in terms of biomass yield (BMY) sampled at 30 cm and soft dough cutting stages during 2016 at Langgewens.....	47
Table 9: Statistics for Biomass yield measurements (kg ha <sup>-1</sup> ) for selected triticale cultivars (US2014, AgBeacon and Snel) and other forage crops in Langgewens.....	49
Table 10: Biomass yield measurements (kg ha <sup>-1</sup> ) for selected triticale cultivars (US2014, AgBeacon and Snel) and other forages during 2016 in Langgewens.....	50
Table 11: The five best performing forage genotypes in terms of biomass yield (BMY) sampled at 30 cm and soft dough cutting stages during 2016 at Riversdal.....	52
Table 12: Statistics for Biomass yield measurements (kg ha <sup>-1</sup> ) for selected triticale cultivars (US2014, AgBeacon and Snel) and other forage crops in Riversdale.....	53
Table 13: Biomass yield measurements (kg ha <sup>-1</sup> ) for forages planted in Riversdale during 2016.....	54
Table 14: Agrobase CV and yield ranking outputs (ascending) for the 14 entries analysed for ash content.....	72
Table 15: Agrobase CV and yield ranking outputs (ascending) for the 14 entries analysed for crude protein.....	74
Table 16: Agrobase CV and yield ranking outputs (ascending) for the 14 entries analysed for DMY.....	76
Table 17: Agrobase CV and yield ranking outputs (ascending) for the 14 entries analysed for fat content.....	79
Table 18: Agrobase CV and yield ranking outputs (ascending) for the 14 entries analysed for fibre content.....	82

Table 19: Agrobase CV and yield ranking outputs (ascending) for the 14 entries analysed for moisture content.....	85
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## Chapter 1: Introduction

There is a growing concern regarding global food security, environmental effects and the increased demand for sustainable use of natural resources. There is a need to optimise agricultural gains in order to mitigate the effects threatening food security. The economic stability and sustainability of the agricultural sector are regarded as core components in addressing many of the concerns threatening food security such as climate change and the rapid rate of population growth. There is a major market for livestock production as the demands are exceeding the supply thereof. Achieving some balance between the supply and demand lies within the increase of grain and forage production with sustainable quantities to be used for animal feed (Takeda *et al.*, 2008).

Multiple factors exist that exert limitations on adequate grain production. Abiotic and biotic stresses are the main factors that have a significantly negative impact on the nutritional quality of the grain and more importantly has major yield limiting effects. Various measures are being applied to contribute to the mitigation of these issues to ensure a high level of productivity. Cultivar selection, which is the selection of entries with favourable characteristics, will have a major influence on productivity and adaptability by providing a greater degree of resistance to stresses within the plants (Reynolds *et al.*, 2012).

Plant breeding methodologies are being incorporated to ensure more accurate agricultural practices are applied, through combining knowledge about multiple disciplines including plant genetics, physiology and the environment. The improvement of cultivars in forage grain provides opportunities for livestock and crop production operations, ensuring more economically stable and sustainable agricultural production (Reynolds *et al.*, 2012).

Among the major small grain species that are utilised for forage production or silage in Western Cape are rye, wheat, oats, barley and triticale. Like all other crop species, these smallgrains perform differently to one another largely due to the different genetic make-ups (McGoverin *et al.*, 2011). Of particular interest to this project is the performance of triticale against various other species of small grain which includes rye, oats and barley.

Triticale (*x Triticosecale Wittmack ex. A. Camus*,  $2n = 6x = 42$ ) is a synthetically developed crop that has a high yield potential and a higher stress tolerance than other small grains (Albeit *et al.* 2014). The name triticale was derived by combining the scientific name of the two genera involved in developing triticale (Mergoum *et al.* 2009). Hybridisation between bread wheat and

rye (RR) was attempted for the first time in 1875 by Wilson (1875) who reported it to the Botanical Society in Edinburgh.

The first commercially available triticale cultivars, “Triticale no. 57” and “Triticale no. 64” were released in 1968 from a Hungarian breeding program (Ammar *et al.* 2004). A year after their release, 40 000 hectares of agricultural lands were cultivated with triticale cultivars in Hungary (Ammar *et al.* 2004). Triticale is mainly utilised as animal feed and forage, but small amounts do enter the human food chain through either the intentional or unintentional mixing with other small grain (Hills *et al.* 2007). In 2014 it was estimated that triticale was grown on over 4 million hectares worldwide, with an average yield of 4.09 t ha<sup>-1</sup> (FAOSTAT, 2017).

Furthermore, triticale also offers a renewable source of material used for biofuel production whilst alleviating the impacts of the effects of greenhouse gases due to its minimal requirements for environmentally invasive management. Most of these gas emissions are produced via the production and utilisation of agricultural inputs, machinery, soil interruption, management and irrigation (Losert *et al.*, 2016).

Thus, smarter agricultural practices contribute directly to mitigating the effects of climate change. Current Triticale cultivars are displaying significant variations to its predecessors with higher yields, increased biomass and enhanced resistance to abiotic stresses (Losert *et al.*, 2016). Triticale lacks the D-genome from its parent wheat which is associated with bread making qualities. In correlation with this and the low gluten levels of Triticale, it is mainly used as a source of animal feed (mainly for pigs and poultry), as well as forage for livestock in the form of silage, fodder, grazing, and hay (McGoverin *et al.*, 2011).

The key objectives for improvement initiatives relate to reducing the production risks and costs thereof, while improving the economic returns per hectare. Production risks are comprised of losses due to various diseases and pests, and environmental factors such as weather-related damage [i.e. winterkill lodging, shattering, late-maturity, and pre-harvest sprouting (PHS)]. The cost of production is subjective to levels of weed competitiveness, water and nutrient use efficiency, and resistance to various abiotic stresses (e.g., salinity, acid soils, drought and heat) and biotic stresses (Vern *et al.*, 2015). One of the fastest adopted innovations in agriculture is genetically modified organisms (GMOs) or transgenic crops. There are many benefits offered to farmers by the many innovations in transgenic crops, but these innovations also pose uncertain risks to society (Wree and Sauer 2015).

South Africa (SA) is one of the few countries within Africa that has introduced GM crops, and has been growing first generation GM crops since 1997 (Gouse *et al.* 2005). Currently there are no GM wheat or triticale varieties available for commercial production, but there are transgenic wheat and triticale varieties that are currently being successfully developed and field tested (Doshi *et al.* 2007; Kavanagh *et al.* 2012; Loureiro *et al.* 2012; Wree & Sauer 2015).

Before these transgenic crops can be commercially used, the environmental and economic risks have to be evaluated (Kavanagh *et al.* 2012). Environmental concerns include the risk of the GM crops becoming agricultural weeds, becoming invasive, or outcrossing with wild and weedy relatives resulting in more invasive and weedy hybrids (Warwick *et al.* 2009).

Ultimately, the returns per hectare are established by the net yield (for both grain and biomass) and the price for the end-use quality offered to the marketplace. The numerous progresses that are required have directed long-term breeding objectives toward concurrent improvement of agronomic performance, resistance to various biotic and abiotic stresses, and end-use quality features. Among the agronomic traits, higher grain and biomass yield, plant height, reduced awn, enhanced straw strength, earlier maturity, higher volume mass, improved nutrient and water use efficiency, and tolerance to various stresses are of major focal points to producers (Busemeyer *et al.*, 2013).

From a grain end-use quality standpoint, improvements in protein concentration and gluten strength, nutrient content, digestibility, and energy value (for livestock feed) are vital considerations for improvement. For industrial applications, increases in grain starch content for bio-ethanol production as well as amylose content for bio-plastic production, are often more desirable (Wree & Sauer 2015).

Enhancements in lignin and cellulose contents of the straw for uses in packaging materials and straw board are also of value (Wree & Sauer 2015). The project centres its focus on evaluating breeding parameters in triticale for increasing biomass production used in animal feed and silage (Meale *et al.*, 2015). The ultimate aim of the study is to identify suitable crossing parents for inclusion into a breeding programme aimed at improving biomass yield in triticale as well as to identify whether triticale is a viable alternative crop in comparison to other forages.

The ultimate aim of the study is to identify suitable crossing triticale parents in order for it to be included into a mainstream breeding programme at SU-PBL aimed at breeding for increased biomass potential in triticale. In addition to this, evaluate its level of performance against other

high biomass yield producing forage crops and identify whether triticale can be considered an economically and sustainably viable alternative crop for use in forage and animal feed.

Through the successful achievement of the aim mentioned above, the level of performance in terms of biomass yield as well as in nutritional value in selected triticale entries will be explored. Furthermore, comparing triticale to other advanced breeding triticale genotypes as well as with selected barley, oats and rye crops, would determine the degree of viability of triticale as an alternative crop to include in rotations. In order to ensure that the aim of the study was met, a set of objectives outlining the structure of the study was to be established.

Advanced breeding line triticale genotypes were planted along with three selected triticale entries (“AgBeacon”, “Snel” and “US2014”) during 2016 in order to compare triticale against other triticale entries in terms of biomass yield potential. In collaboration with this, two mixed trials composed of the selected triticale entries and other forage plant types were planted to assess biomass yield potential across the individual entries during 2016. The purpose of these trials was to identify whether any entries had a lower performance than the selected triticale entries or whether they were significantly better or not significantly different in terms of biomass yield potential.

The entries had a lower performance than the selected triticale was excluded from further analysis during the study in 2017, and were replanted as a replication to obtain more accurate results. Furthermore, the entries that were significantly better from the advanced breeding line trial were further included in the forage trials during 2017. Entries that were not significantly different were left in the study or were replaced by newer entries as were made available by the seed companies. The names of these entries are mentioned under the results and discussion section of this study.

The second phase involved repeating the process of the planting and assessing of trials, which included an advanced breeding line triticale trial and mixed forage trials in more than one location. The trials included all the entries planted during 2016 with particular interest shown to entries that were significantly better (“US2016” and “US2017”). The objective of this phase was to evaluate biomass yield potential in triticale in relation to advanced breeding line triticale and against other forages.

Possible crossing parents were to be identified that would be suitable for inclusion into a breeding programme for improving biomass yield in triticale. The genotypes with the best biomass yield potential across the plant types were identified. A select few of these top forming genotypes were analysed for nutritional value for usage as animal feed. Of particular interest were protein content, fat, ash, moisture and fibre content.

To achieve the aim of the study the study focused on following specific objectives:

- To evaluate selected triticale cultivar varieties against oats, barley and rye with regards to biomass yield potential.
- To evaluate selected triticale cultivar varieties with advanced breeding triticale varieties for biomass yield production.
- To evaluate nutritional value of selected entries ranked among the best in terms of biomass yield potential
- Ultimately incorporate the information and make selections of the potentially best suited crossing parents for inclusion into a breeding programme for increasing biomass yield in triticale.
- Furthermore, the study established whether triticale is a good alternative crop to be included into crop rotations to supplement feedstocks.

It is hypothesised that triticale entries would represent to be good alternatives to be included into a crop rotation, with the ability to produce biomass yields competitive to that of other forage crops in the study but may be lacking in nutritional value in comparison to that of the other crops.

## Chapter 2: Literature review

### 2.1 Crop production in the Western Cape Province in South Africa.

South Africa (SA) has diverse agro-ecologies associated with the various land types, climates, biodiversity and agricultural methodologies. The core factor limiting sustainable crop production in SA is water scarcity. Less than 20% of arable land is receiving sufficient irrigation for crop production, classifying only a small proportion of the country's land as high value land for cultivation of vegetation (Scotcher *et al.*, 2010; WWF-SA, 2016).

South Africa has a high diversity in terms of fauna and flora, mainly with regard to its types of vegetation, variable agro-climatic zones, plant and animal biodiversity. However, only 12% of the country's land is appropriate for rain-fed cropping. The majority of the remaining land (69%) is better suited for grazing (DAFF, 2017). In 2016 the Western Cape Province's population was estimated at 6 293 000. The province is regarded the fourth largest in SA in respect of its land space of 2 454 800 hectares (STATS SA, 2017).

The Western Cape is categorised into five district municipalities which include the, Central Karoo, Eden, Overberg and the West Coast as well as Cape Metropole (City of Cape Town) and Cape Winelands. Furthermore, the province is primarily a winter rainfall region with most of the cropping conducted in the Western Cape being rain-fed crops, particularly in the Swartland (West Coast district), Overberg and the Garden Route districts (DAFF, 2017; GreenCape, 2017).

The current global population is estimated at 7.4 billion and is constantly increasing at 1.13% annually. South Africa accounts for about 55 million people of the global population and is expected to reach 84 million by the year 2035 (DAFF, 2017). The need for more sustainable agriculture has been highlighted with the increasing pressures experienced by the rising population levels, elevated food prices, and the stability of the economy and the depletion of our resources (STATSSA, 2017).

Majority of South Africa's land is more suited and utilised for grazing and livestock farming, creating a massive market for forage grain cultivation. Forage grains and crops grown for animal feed evidently indirectly contributes to the nutrient contents consumed through products like meat, milk, eggs etc. The incorporation of more sustainable and productive measures would

provide stability to a declining economy, but furthermore allow for greater agricultural and genetic gains through the utilisation of fewer resources. Numerous applications have been introduced to facilitate more precise agricultural approaches using recent biotechnological tools such as Marker-Assisted selection (Wessels *et al.*, 2014).

## **2.2 Triticale**

Triticale (*x Triticosecale Wittmack ex. A. Camus*,  $2n = 6x = 42$ ) is a synthetically developed crop that has a high yield potential and a higher stress tolerance than other small grains (Edwards *et al.*, 2012). The name triticale was derived by combining the scientific names of the two genera involved in developing triticale (Niedziela *et al.*, 2015).

Hybridisation between bread wheat (Genomes: AABBDD) and rye (Genomes: RR) was attempted for the first time in 1875 by Wilson (1875) who reported it to the Botanical Society in Edinburgh. The first commercially available triticale (AABBRR) cultivars, “Triticale no. 57” and “Triticale no.64” were released in 1968 from a Hungarian breeding programme (Niedziela *et al.*, 2015). A year after their release, 40 000 hectares were grown on Hungarian farmers’ fields (Ammar *et al.*, 2004; Niedziela *et al.*, 2016).

## **2.3 The utilisation of triticale**

Triticale is mainly utilised as animal feed and forage, but small amounts do enter the human food chain through either the intentional or unintentional mixing with other small grain (Niedziela *et al.*, 2015). Hexaploid triticale cultivar improvement has been pursued since the 1970s within South Africa by the Stellenbosch University’s Plant Breeding Laboratory (SUPBL) and their research teams (Roux *et al.* 2006). The focus of this breeding programme has largely been on the development of cultivars for grazing, hay and silage.

This anthropogenic cereal crop was engineered to incorporate the desired characteristics of its parents, namely wheat and rye (Losert *et al.*, 2017). It has been bred to incorporate highyielding and protein quality attributes from its female parent (wheat) and the enhanced abiotic stress tolerance and disease resistance from its male parent (rye) in a single plant (Niedziela *et al.*, 2015).

These characteristics make it more suitable for the production in marginal areas (acidic, saline, or soils with heavy metal toxicity). It also offers a renewable source of material used for biofuel production whilst alleviating the impacts of the effects of greenhouse gasses (Mazid *et al.*, 2013).

Current Triticale cultivars are displaying significant variations to its predecessors with higher yields, increased biomass and enhanced resistance to abiotic stresses (Losert *et al.*, 2017). Triticale lacks the D-genome from its parent wheat which is associated with bread making qualities. In correlation with this and the low gluten levels of triticale, it is mainly used as a source of animal feed (mainly for pigs and poultry), as well as forage for livestock in the form of silage, fodder, grazing, and hay. (McGoverin *et al.*, 2011). The hybridisation of triticale is depicted in Figure 1 and displays the transfer of genetic material from its parent plants and the collaboration thereof to create this stable hexaploid variety.

## 2.4 The importance of triticale in animal nutrition

When consumed and assimilated, food is used in the body to maintain and repair body tissues, promote health and growth, sustain life, provide energy, for reproduction and other vital body processes through the release of its nutrients. Essentially, the basic nutritive components of food are carbohydrates, proteins, fats, minerals, vitamins and water, which are absorbed in the body in various usable forms. Food given to food-producing animals, whether made up of single or multiple materials, are generally referred to as feed or feedstuff, and could be fed raw, semi-processed or processed (Losert *et al.*, 2017).

The cultivation of triticale has steadily grown since its introduction. There have been significant advances in plant breeding which have made triticale a more viable crop around the world (Blum *et al.*, 2014; Randhawa *et al.*, 2015; Liu *et al.*, 2017). Interest in triticale as a feed grain was created due to its higher protein concentrations and better amino acid balance as compared to other feed grains (Losert *et al.*, 2017). Thus, Triticale's ability to produce good yields of plant biomass, nutritional characteristics and its level of resistance are characteristics that are highly favourable for animal feed production (Liu *et al.*, 2017).

Animal feeds are either classified as fodder, forage, or mixed feeds. Fodders could be classified as roughages (fresh cut forage, hay or dry forage, straw, root crops, stover and silage) and concentrates such as grains, legumes and by-products of processing. Plant materials consumed by grazing animals either directly as pasture, crop residue and immature cereal crops are referred to as forage. However, forage materials cut as fodder, particularly fresh, hay, and silage are sometimes loosely referred to as forage (Liu *et al.*, 2017).

Mixed feeds are produced from several feed ingredients combined in different proportions to achieve a particular nutritional quality. Feed ingredients, including additives, may or may not add any nutritional value to the mixed feed and comprises of components originating from plant, animal, or aquatic sources, which could be organic or inorganic in nature (Iqbal, 2016).

Animal feeds are important, not only to the feed manufacturers and animal producers, but also to the regulators, policy makers, processors and the final consumers of the end-products. This is because animal feed is an integral part of the food supply chain and it is critical to the efficient and profitable production of quality and safe food. Thus, feed safety is critical to food safety.

Every step from primary production to final consumption, that is, from farm to fork, makes up the food chain. Feed production plays significant role in the production of food of animal origin and it is, therefore, a critical aspect of the food chain (Figure 2). Therefore, all key actors on every node of the food chain are responsible for the production of safe, healthy and nutritious feeds.

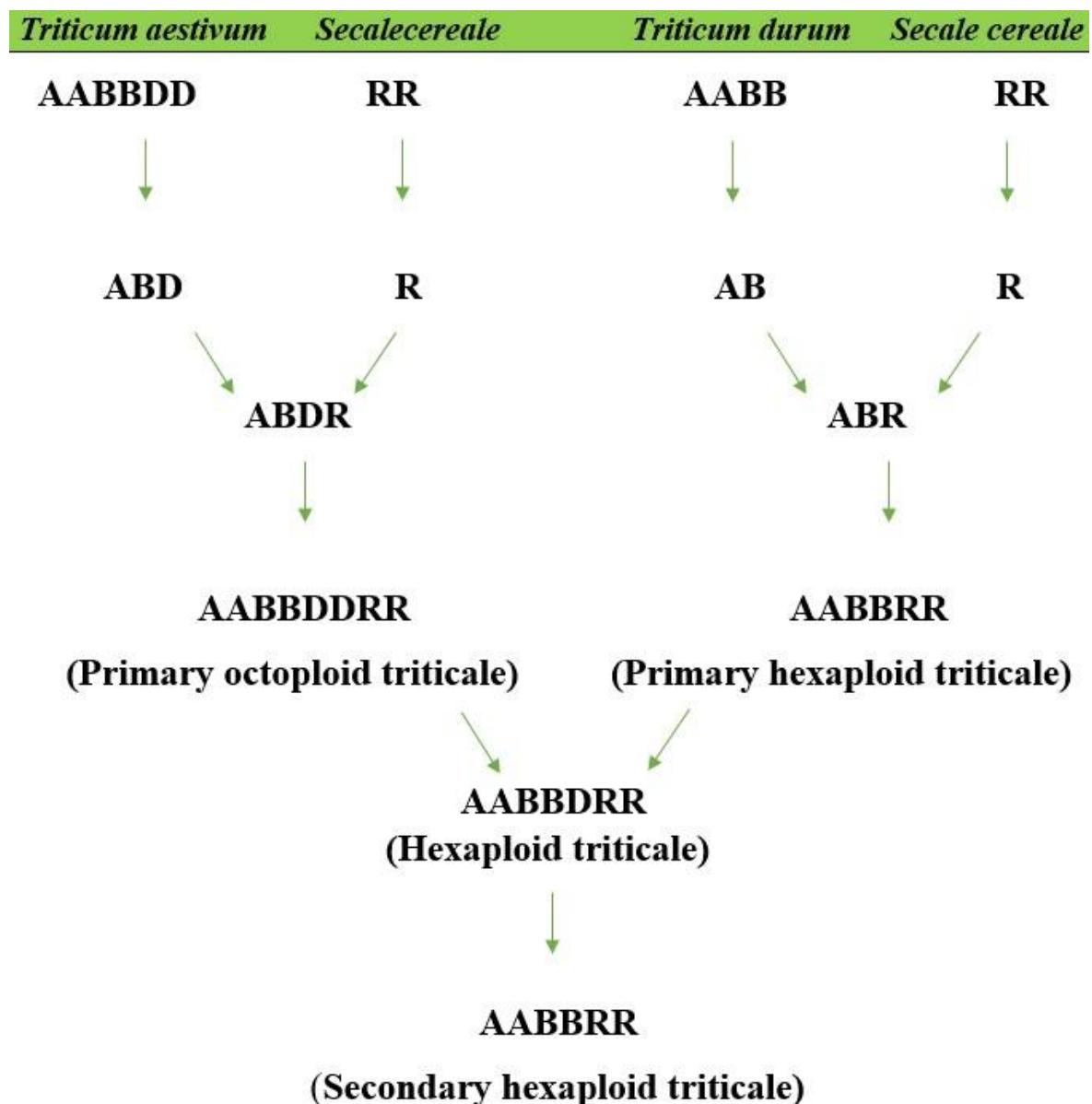


Figure 1: The hybridisation of Triticale from wheat and rye (Adapted from Ammar *et al.*, 2004).

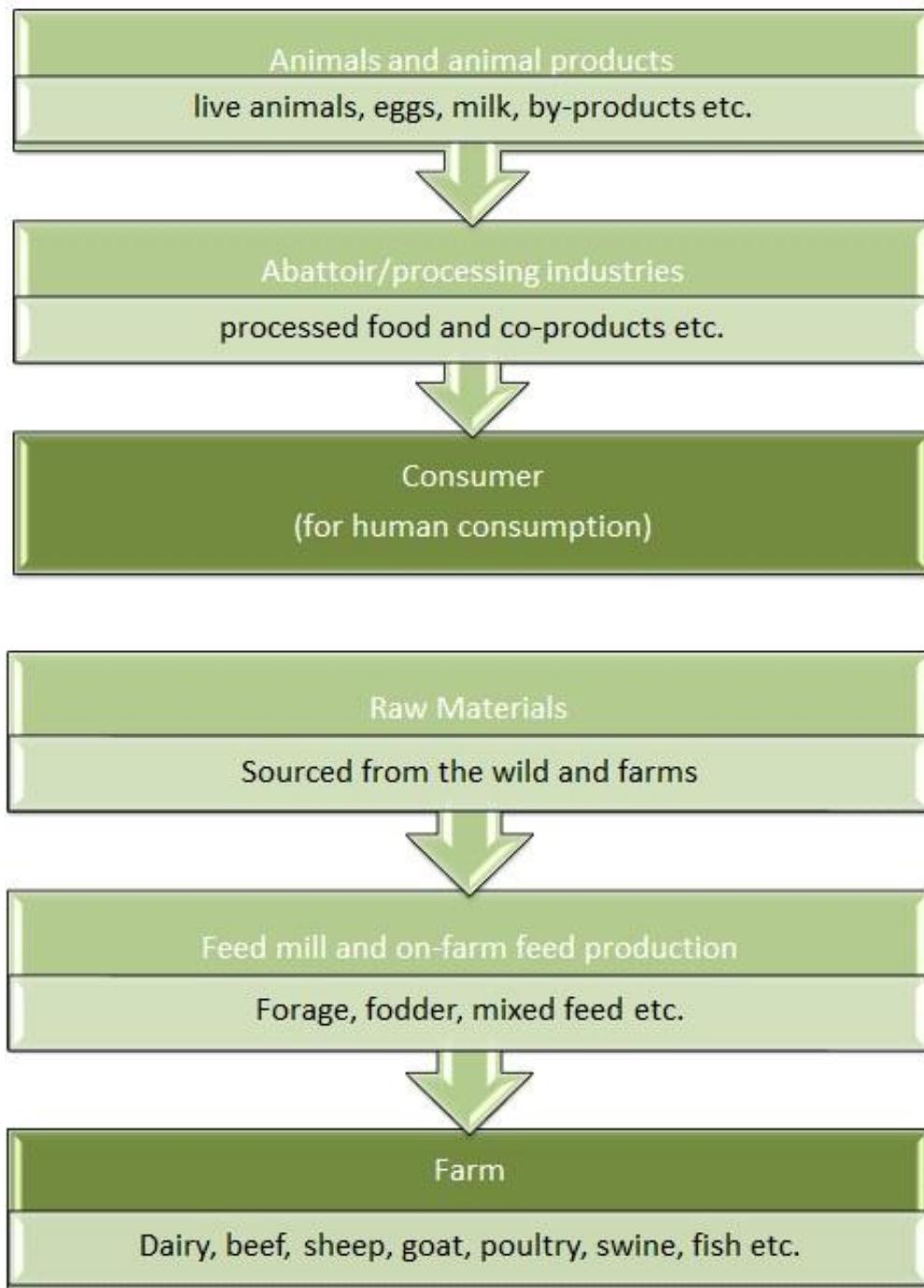


Figure 2: The food chain (Adapted from Reynolds *et al.*, 2012).

## 2.5 Nutritional characteristics

Plant nutrition is one of the most fundamental factors affecting crop production and it plays an essential role in guaranteeing the right performance of forages. In fact, plant nutrition management is one of the main strategies for increasing crop yield. In the nutrition process, nitrogen and phosphorus have great relevance because they are the nutritive elements with the highest transcendence in yield as well as in the quality attributes of green forage (Iqbal, 2016).

Evolution of triticale varieties, which included increasing the plumpness of grain of modern triticale varieties, resulted in higher starch content and consequently added energy in comparison to the older, shrivel-seeded, light-weight varieties (McGoverin *et al.*, 2011). However, with varieties possessing improved starch content, these varieties developed lower protein concentrations than that of the older varieties. Protein content and quality, nonetheless, remain superior to most of the other cereal feed grains (Mapiye *et al.*, 2011).

The nutrient composition of modern triticale is higher than maize in protein and essential amino acids, such as lysine for example. Modern, high-yielding triticale cultivar grain is similar to or slightly lower than wheat in protein, however, lysine and threonine concentrations, as a percentage of the protein, are typically higher (Reynolds *et al.*, 2012; Mapiye *et al.*, 2011). The higher concentrations of essential amino acids, specifically lysine and threonine, allow reduced usage of an additional protein source, such as soybean meal, when using triticale as opposed to maize in formulating diets for pigs and poultry (Mazid *et al.*, 2013).

Nutritional parameters can include Dry Matter Yield (DMY), Crude Ash (ASH), and Crude Protein, Crude fat, Fibre analysis, starch and more. These are further described in individual detail in table 1 below.

**Table 1: Description of nutritional parameters (Adapted from McClements, 2015).**

Nutritional Parameters	Description
Dry matter	Part of the sample that remains after drying at 103°C.
Crude ash	Remaining sample after incineration @ 550°C.
Ash (insoluble in acid sand)	Ash that remains after boiling in strong acid.
Crude protein	Total nitrogen content and to calculate the protein content by multiplying the nitrogen content by an appropriate conversion factor (usually $\times 6.25$ ). Kjeldahl method (Nitrogen is converted into ammonia which is absorbed in boric acid and titrated against a standard acid)
Crude fat	Non-polar extractable fraction of the sample. The extraction can be performed with or without prior acid hydrolysis, both being complementary methods.
Fibre analysis	Digestion of feed directly in the detergent solution and filtration using crucibles (official standard method). Digestion of sample whilst in a nylon bag and then washing the bag containing the digested sample to make it detergent free.
Starch	Starch can be measured by the classical Ewers method with an enzymatic method. The enzymatic method can be used for all sample types and is therefore preferable.
Gross energy	Gross energy represents the total energy value of the sample and is measured by bomb calorimeter
Minerals	Minerals are generally measured by spectrometric methods following incineration and hydrolysis.

## 2.6 Digestibility as a feed source

Protein and amino acid digestibility in triticale proves to be similar or even better than that of its parent crop wheat. Concentrations of various minerals in triticale grain are similar to those of wheat. With modern triticale, various anti-nutritional factors, such as non-starch polysaccharides (pentosanes) and protease inhibitors, while higher than in most other cereal grains, seem to have no effect on the growth performance of livestock consuming diets containing triticale grain (Mapiye *et al.*, 2011). The possible exception is the anti-nutritional effect of pentosanes in poultry nutrition. Poultry are rather sensitive to the anti-nutritional effects of these compounds. Pentosanes are also present in wheat and rye. Pentosanes in wheat and rye are known to interfere with digestion and absorption of various nutrients (Nikkhah, 2012).

## 2.7 Biomass characteristics

Dry-matter yield (DMY) of forage for triticale generally compares favourably to other small grain forage cereals in multiple studies around the world. Research conducted on triticale as forage for ruminants compares well with other forages in terms of nutritional characteristics (Grassini *et al.*, 2011; Reynolds *et al.*, 2012; Liu *et al.*, 2017).

In order to respond to the growing demand of forage and being able to supplement animal feed supplies, it has been necessary to establish large acreages for these forages in terms of biomass yield production (Iqbal, 2016). The farming of these small grains plant types for forage production constitutes a fast way of obtaining high dry matter production rates of good quality for animal feed when it is administered either as fresh forage or as silage (Grassini *et al.*, 2013).

In addition to this, although triticale is mainly used as animal feed, it shows great potential for utilisation as a bioenergy crop as a result of its biomass accumulation characteristics (Mapiye *et al.*, 2011; Grassini *et al.*, 2011). In relation to this, crops that are classified as good feed varieties are often also good bioenergy sources and vice versa. Significant amounts of research have taken off evaluating the potential of triticale in terms of biomass production and its functionality as an alternative renewable source of energy (Busenmeyer *et al.*, 2013; Grassini *et al.*, 2013).

Plant breeding therefore plays a vital role in driving research forward in terms of breeding for larger plant biomass (which includes attention on the intensification of photosynthetic activity or radiation use efficiency (RUE) to achieve greater agricultural and genetic gains (Reynolds *et al.*, 2009; Reynolds *et al.*, 2012).

## 2.8 Resistance characteristics of Triticale

Since the first commercial triticale cultivars were released in 1969, many studies have been carried out on the development of triticale. Triticale demonstrates many agronomic advantages, including winter hardiness, drought and disease tolerance and excellent productivity potential. However, they exhibit a wide variation in nutrient content between the various varieties (Widodo *et al.*, 2015).

Thousands of triticale varieties exist globally. These varieties vary in multiple aspects such as the phenology, levels of resistance and agricultural productivity. Triticale cultivars, grown for forage as well as for grain, can be classified into three categories in terms of the variations in growth habit: spring, winter and intermediate (Reynolds *et al.*, 2012). Spring types are varieties that do not require vernalisation to go from vegetative to reproductive developmental stages.

These types are planted during the spring. However, it is possible for them to be planted in other seasons within milder climate conditions. These spring types display stable upward growth with limited tillering and produce good amounts of forage within the early growth stages. Furthermore, they also display insensitivity to photoperiods (Mazid *et al.*, 2013).

Winter types, contrastingly requires vernalisation to go from vegetative to reproductive phases. Winter types are commonly planted within the autumn season. Generally, these types have greater forage yields than spring types primarily due to their extended growth cycle. Intermediate types, as the name implies, are intermediate to spring and winter types.

Like that of spring types, these also require vernalisation to develop from vegetative to reproductive stages. Thus, a good alternative to other spring cereals, such as barley and oats, would be spring type triticale. These types display greater tolerance to drought in comparison to other spring cereals that have been previously evaluated (Alheit *et al.*, 2012; Mazid *et al.*, 2013).

## 2.9 Harvest index as a breeding tool

The extensive progress in breeding for higher yields is accomplished primarily through manmade selection forces for the harvest index (HI), that is, improved plant capacity to distribute biomass (assimilates) into the developed reproductive parts (Gutam, 2011; Mazid *et al.*, 2013). The HI is further generally known as a measure for the efficiency of the interrelation of multiple plant processes (Reynolds *et al.*, 2012).

During the period from 1900 to 1980, the increase in HI accounted for the majority of the substantial improvement in yield potential of crops such as barley and wheat. Therefore, greater understanding of the genetic factors associated to the HI is fundamental for identifying approaches to improve agriculturally crucial characteristics such as yield (Aisawi *et al.*, 2010; Fischer, 2011).

The HI is an integrative measure which includes the total effects of all physiological processes within the crop cycle and is including the net effects of all physiological processes during the crop cycle, and is connected to various yield determining traits. Furthermore, it is thus evident that the phenotypic expression of HI is influenced by underlying genetic factors that influence yield-related traits (Wang *et al.*, 2012). Harvest index, the proportion of plant biomass allocated into grains (seeds), is the primary known measure for the efficiency of plant development (Reynolds *et al.*, 2012).

Morpho-physiological assessments conducted in the 80's by Austin *et al.*, (1980) in a modern wheat collection suggested that the improved partitioning of the dry matter into grains attained its physiologically justified limit (HI of approximately 0.6). Reynolds *et al.* (2009; 2012) confirmed that the deductions made by the researchers in the 1980s are indeed still valid. It remains evident that there remains a dire need to pay close consideration to different methods to enhance the efficiency of plant yield.

In cereals and other crops, the constant enhancements in agricultural gains are associated with several endo- and exogenous factors. Numerous interconnected morpho-physiological mechanisms contributing to better allocations of plant biomass to physiological plant parts are vital. This characteristic of the plant has been well exploited in numerous breeding programmes around the world, with its adjustments leading to various patterns of allocation of dry matter between crop cultivars (Mazid *et al.*, 2013).

Austin *et al.* (1980) conducted experiments in a modern wheat collection which indicated increased likelihood that the improved partitioning of the dry matter into grains and/or at that stage reached its physiological limit with the HI value being around 0.6. The deductions made by the researchers in the 80s do indeed remain valid. However, there is a high certainty that alternative approaches need to be explored to continue increasing agricultural potential of plants (Reynolds *et al.*, 2012).

It can be hypothesised that the range of genotypic variation in HI between modern cultivars would seem limited and that the observed variation of the particular trait is affected by multiple environmental contributors more than ever. In modern breeding methodologies, it is believed that breeders tend to direct the breeding process not only toward the HI and/or biomass, but rather biomass itself (Aisawi *et al.*, 2010; Fischer, 2011). The Harvest Index, along with its association with grain and biomass yield can be described by the following equations:

**Grain yield / Biomass yield (BMY)..... Equation 1**

**Grain yield = BMY x HI..... Equation 2**

Grain yield and biomass yield can be measured directly whilst the HI-value for the specific experimental unit can be determined on the basis of equation 1. This equation (1) has been adapted using the multiplicative model as described by Kozak *et al.*, 2007 and illustrated in equation 2. This model suggests that the final trait is a product of its constituents (Wang *et al.*, 2012).

HI is considered an integrative trait which is highly associated with a number of yield-related traits in important crop species. This interrelated relationship generally observes that an increase in an individual constituent can result in a decrease or an increase within the other constituent. Substantial advancements in breeding for greater yields have already been achieved primarily through man-made selection factors for the HI, which represents the improved plant capacity to allocate biomass to the developed reproductive parts (Gutam, 2011; Mazid *et al.*, 2013). Thus, evaluating breeding parameters such as increased plant biomass yield is an essential component to multiple disciplines of plant physiology, biology, genetics and breeding.

## 2.10 Factors limiting crop production

Substantial amounts of research and funding is being used to identify better parameters to increase agricultural gain (Atkinson *et al.*, 2012). Ultimately, the increasing of both yield and biomass are of particular interest when it comes to attempting to partially mitigate the effects of the external pressures demanding better agricultural approaches. Unfortunately, forage grains, like other crops are susceptible to multiple yield and biomass yield limiting factors which include abiotic and biotic stress (Finnan and Spink, 2016).

## 2.11 Abiotic stress

Abiotic stress is considered to be stress induced by non-living organisms and includes mainly the environmental impacts on crops. Drought, severe heat, freezing and salinity are among the types of abiotic stresses that can greatly impact crop production levels. Abiotic stress has major yield-limiting affects with the ability of reducing yield potential by more than 50 percent (Atkinson *et al.*, 2012). Methods to mitigate the effects of abiotic stress can include proper irrigation, selection of soil types but mainly the effects can be addressed through better selection of cultivars with enhanced tolerance and adaptability (Krasensky and Jonak, 2012).

## 2.12 Biotic stress

Biotic stress is induced by living organisms and includes diseases, pathogens and pests. These stressors have detrimental effects on crop production and often is facilitated or enhanced by variable abiotic stresses. Proper agricultural management is essential in limiting the effects of these stresses. Various chemicals (i.e. pesticides, insecticides, herbicides etc.) exist which particularly target these specific stresses (Krasensky and Jonak, 2012).

However, it is of paramount importance to evaluate the compatibility of these chemical sprays on the specific crops prior to the application thereof. Incorrect dosages, chemical combinations and application time could further result in damage to the crops and may limit yield or deform plant development significantly (Reynolds *et al.*, 2012). Both abiotic and biotic stress may

commonly occur in a symbiotic relationship and be exposed to the crop simultaneously. The degree of exposure toward both stress types at crucial plant developmental stages could result in irreversible loss of crops (Krasensky and Jonak, 2012). In-season damage to the crop may occur as a consequence of adverse weather, environmental conditions as well as pest and/or disease effects. In each case it is important to maintain a concise record of damage to the crop in order help explain potentially confounding effects on data.

Negative consequences on yield depend on the timing of the event and/or the organ(s) affected. However, the exposure of the plant to multiple stress factor simultaneously at key developmental stages is what results in the largest reduction in agricultural gain. It is therefore essential to conduct cultivar evaluation that may aid the selection of cultivars with enhanced resistance to these stressors to increase the odds of successful higher-yielding harvests (Reynolds *et al.*, 2012).

## **2.13 Climatic aspects in relation to biomass production**

South Africa has nine provinces of which the Western Cape is situated on the southernmost tip. The Western Cape Province, displayed in Figure 3, is the fourth largest province in South Africa (Ziervogel *et al.*, 2014; Botai *et al.*, 2017). The province border is surrounded by both the Atlantic Ocean on the western side and the Indian Ocean on the southern side.

The province is categorised as having a warm temperate Mediterranean climate type on the coast, experiencing hot and dry summer periods as well as cold and wet winter periods, with the inland region reaching temperature below freezing point. Temperatures between 15°C to 27°C were reported as average temperatures during the summer season. During the winter season temperatures averaged between 5°C and 22°C (Ziervogel *et al.*, 2014; Botai *et al.*, 2017).

The level of precipitation across Western Cape Province varies significantly and can be categorised into three distinctive rainfall zones. These include: the winter, late summer and constant rainfall zones. The Boland region is known to experience hot and dry summer periods along with winter rainfall. The coastal areas along the south experience rainfall all year round. The levels of precipitation are relatively inconsistent across the province with annual

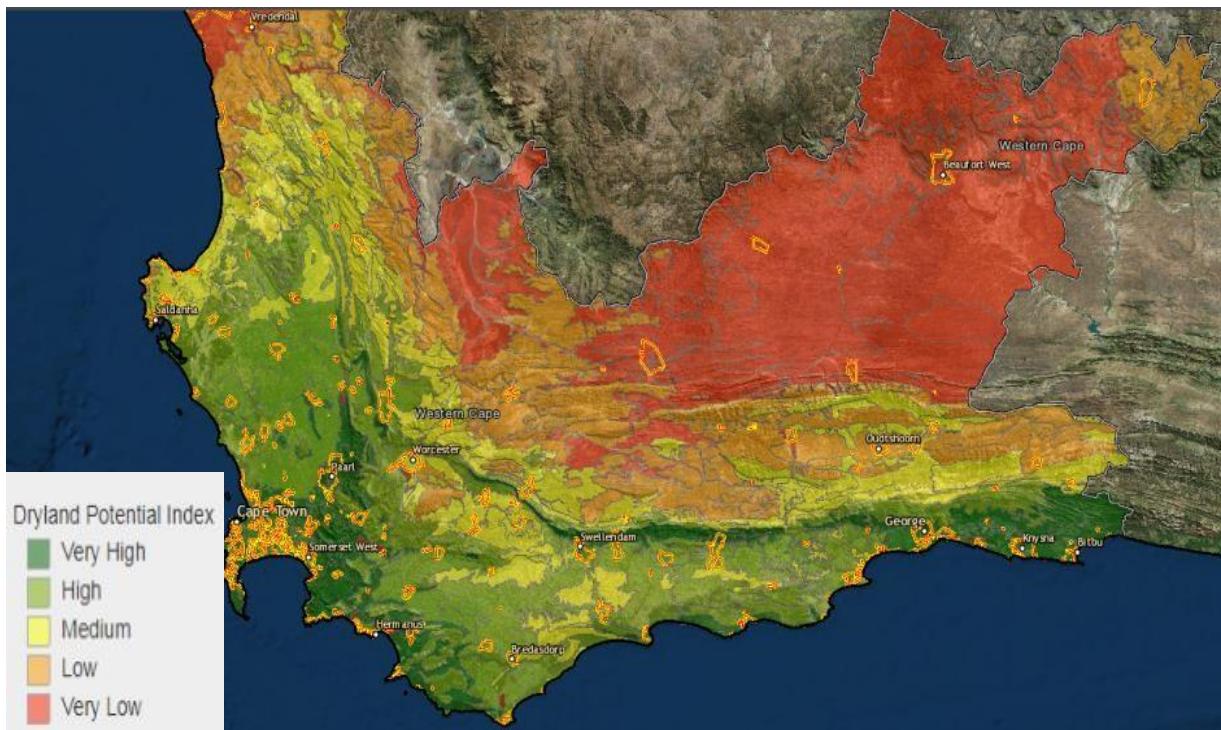
precipitation within the Western Cape ranging from +/- 300 – 900 mm. There are, however, areas within the province that receive as little as 60 mm precipitation and regions receiving precipitation of more than 3000 mm (Ziervogel *et al.*, 2014).

From 2014, the province experienced the effects of a severe drought and was declared a disaster area. This drought is at the time of writing still ongoing, however precipitation during 2018 have significantly improved dam levels. This drought is the worst experienced by the Western Cape since 1904, with areas along the west coast and central Karoo experiencing major effects on its agricultural productivity.

Cereal crops are mainly dryland crops and do not rely on anything but natural irrigation from rainfall in the Western Cape. The province has the best dryland potential along the coasts. On the Atlantic side it is mainly the Boland and Swartland regions, while the Overberg district is situated on the Indian Ocean side (Botai *et al.*, 2017).

Climatic forecast modelling foresees not only the significant increases in temperatures and greater inconsistency with precipitation, but further projects more frequent episodes of heat waves and drought conditions. Heat stress is mainly regarded as plant exposure to short periods of very high and/or severe temperatures and it has a major negative impact on crop production (Ziervogel *et al.*, 2014).

This increase in frequency and levels of intensity of climate conditions is regarded as a major limiting factor toward plant development and directly limits yield and biomass yield production (Lesjak *et al.*, 2017; Atkinson *et al.*, 2012). Crops are most vulnerable to significant shifts in temperature and environmental conditions during the plant reproductive stage as opposed to the plant vegetative stage. Plants experience the effects of water and heat stress under drought conditions, and the effects of this could be colossal particularly when plants are exposed to this kind of abiotic stress during key developmental plant developmental stages (Rezaei *et al.*, 2015).



**Figure 3: A map of the Western Cape Province as well as the index of dryland potential (generated by CapeFarmMapper v 2.0.1.3).**

High temperatures influence photosynthesis, respiration, transpiration, development rate, reproductive development) and root growth are all among the different processes that are influenced by high temperatures (Rezaei *et al.*, 2015; Lesjak *et al.*, 2017). Heat stress mainly during the plant developmental stage anthesis may significantly decrease grain number and ultimately the total yield. After the anthesis stage, grain mass may primarily be influenced due to the rate of the process of leaf senescence increasing during the grain filling period.

A great sense of variability in the degrees of sensitivity to different stressors among the different cereal crops was explained in a study by Rattalino Edreira *et al.* (2011). The critical temperature threshold for heat stress at anthesis was reported to be between 27°C - 31°C for C3 Plants and between 32°C - 38°C for C4 Plants (Rattalino *et al.*, 2011).

In addition to heat stress, drought conditions will add even more stresses to a growing plant. During drought, evapotranspiration, low water availability in the root zone as well as dry and hard soil will all increase significantly. These stressors decrease the plant's ability to grow

optimally and directly reduce the potential biomass yield and transpiration rate (Rattalino *et al.*, 2013).

When mean temperatures surpass the optimal threshold for photosynthesis to occur optimally, biomass production ability is reduced. Furthermore, when the grain filling period is reduced as a result of temperatures being surpassed for thermal time accumulation, the biomass production ability is also reduced. Both of these limiting factors to biomass assimilation relate to decreased kernel numbers (Lesjak *et al.*, 2017).

In numerous studies it is suggested that there is a dire need for farmers to shift to more sustainable and accurate farming methodologies that are better adapted for optimal agricultural gains to be obtained. The evaluation and assessment of cultivars prior to planting can therefore play a pivotal role, as identifying cultivars with better resistance, greater thermal requirements and later plant maturity result in plants which produce greater agricultural gain (Rezaei *et al.*, 2015).

The complex variability of plant sensitivity to stresses as well as the plant response to stresses between different cereal grain plants are yet to be further studied to a greater extent. It suggests a complex gene-environmental interaction and this should be exploited to achieve better and more adaptable crops with favourable production abilities.

## **2.14 Threat analysis on rust disease affecting triticale**

Wheat (*Triticum aestivum*) and triticale (*x Triticosecale Wittmack*) rust pathogens which continue to cause catastrophic damages to crop production are considered to be one of the most important pathogens to study (Fischer *et al.*, 2007; Ali *et al.*, 2014). There are three rust species infecting wheat which are dispersed globally. Yellow/stripe rust which is caused by *Puccinia striiformis*, leaf rust caused by *P. tritici* and stem rust caused by *P. graminis* f.sp. *tritici* (Berlin *et al.*, 2013). These rust pathogens have had major yield limiting effects in a variation of geographical locations across various environmental conditions (Thach *et al.*, 2015).

Yellow rust (Yr) particularly, is a growing concern, with increased reports of disease invasions around the world very likely due to the combined effects of long distant migration capacities,

high rates of virulence through mutation and genetic recombination (Hovmøller *et al.*, 2016; Walter *et al.*, 2016).

Globally, yellow rust has been reported to have resulted in 5.5 million tons per year crop losses (Beddow *et al.*, 2015). There has been a substantial number of outbreaks of yellow rust epidemics reported over the last decade in East and West Africa as well as in Asia. The wellknown *P. striiformis* population has been significantly substituted from 2011 by distinctive new lineages, named Kranich, and Warrior, triggering more epidemics on various wheat varieties (Ali *et al.*, 2017), with a distinct lineage linked to epidemics experienced by triticale during 2009 and 2010 (Thach *et al.*, 2015).

Understanding the yellow rust population genetic structure to a greater extent has become a core focus for many breeders to assist with breeding of more resistance or crop varieties with reduced susceptibility and the improvement of disease management approaches centred on host resistance (Ali *et al.*, 2017).

Warrior and Kranich are two new races that were discovered in 2011 on wheat and triticale in Europe. The isolates of these races differed from the common isolates of other European varieties. These isolates are reported to cause more diseases on adult plants of wheat genotypes possessing good long-term adult plant resistance and less disease on others, which includes genotypes that were susceptible (Ali *et al.*, 2017; Hovmøller *et al.*, 2011). More comprehensive understanding of these new races with regards to the epidemiology thereof could identify more sustainable approaches for more effective disease management and development of varieties with better resistance (Walter *et al.*, 2016).

## 2.15 Measurement of quantitative traits

Accounting for genetic interaction is essentially important when wanting to predict phenotypes and response to selection. Quantitative traits such as yield, in-season biomass, plant height and number are important to breeders as a phenotypic prediction tool and can be quantified. Quantification of these traits provides insight into the genetic and environment interaction with biological traits of a plant (Reuzeau *et al.*, 2011).

Biomass yield is considered the absolute expression of the various distinctive physiological processes correlated with the weather and environment during the crop's growth cycle. Yield measurements serve as an accurate measure of productivity and demonstrate the geneticenvironmental relationship through the various physiological processes. The determination of grain yield and its components: spike number per meter squared, grain number per meter squared and, thousand kernel weight, is quantitative traits crucial for all breeding and physiology trial (Reynolds *et al.*, 2012).

In-season biomass evaluation facilitates gaining information on the growth and rate of growth of crops, organ size and dry mass, calculating of radiation use efficiency, and serves as the initial step for morphology assessment and nutrients or metabolite analysis such as N, P, protein, water soluble carbohydrates, etc.(Thach *et al.*, 2016 ; Walter *et al.*, 2016).

Certain environmental situations, such as severe heat stress and limited water availability during key developmental stages, may significantly limit biomass production, subsequently reducing the capability of the crop to intercept solar radiation, slowing down the process of photosynthesis and radiation use efficiency. The reduction in biomass production further decreases the quantity of photosynthates accessible for remobilisation during grain-filling (White *et al.*, 2012; Reynolds *et al.*, 2012).

## **2.16 Plant physiology**

Understanding the physiological aspects related to plant growth and development is necessary to efficiently conduct experimental programmes. Several scales exist that categorise and is aimed at defining the plant developmental stages (growth stages; GS). The Zadoks scale is a non-destructive and simple scale which is the most common among the rest. It is constructed using ten major stages to define the different key developmental stages of the plant (Barber *et al.*, 2015).

Accurately defining the growth stages is of paramount importance as key physiological stages (emergence (GS10), terminal spikelet (GS30), first node at 1cm above tillering node (GS31), heading (GS51), anthesis (GS61), and maturity (GS87) represents the main variations in the crop's life cycle (Sukumaran *et al.*, 2014; Reynolds *et al.*, 2012).

Knowledge about the multiple components such as plant physiology, genetics, environmental conditions, biochemistry and agricultural management is essential to understanding and incorporating methodologies to increase sustainability and productivity. Cultivar evaluation serves as an effective tool which incorporates multiple components and allows simpler identification of better adapted lines for breeding and cultivation purposes (Sukumaran *et al.*, 2014).

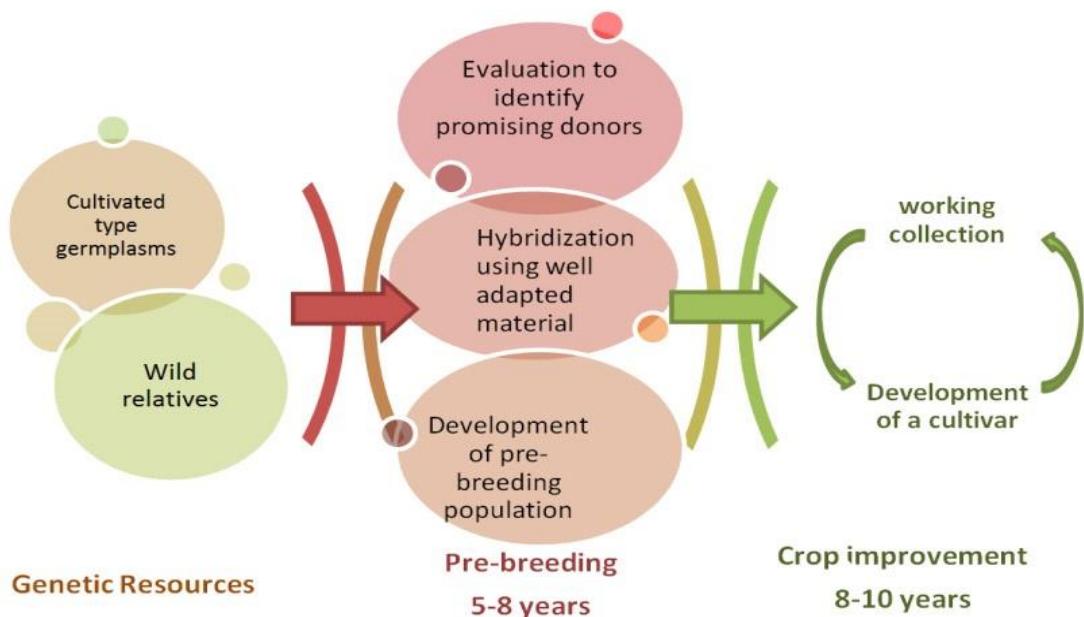
Good agricultural management involves managing levels of irrigation, pesticides, insecticide and fungicide dosage and application, fertiliser application and regimes as well as other abiotic and biotic stresses. These are closely related to the plant growth stages rather than calendar dates and this allows quantitative traits to be evaluated across the respective growth stages (Barber *et al.*, 2015; White *et al.*, 2012).

For example, measuring in-season biomass is typically measured upon stem elongation and/or booting stages as well as throughout anthesis. However, measurements can be taken throughout the other growth stages depending on the objectives that wish to be attained. Measuring yield components is routinely only done upon ripening. From this, it is clear that the identification of cultivars with enhanced resistance to abiotic and biotic stress with the ability to produce optimal yield and biomass quantities is required for the development of better adapted lines (Pask *et al.*, 2012).

## 2.17 Breeding for physiological traits

The enhancement of crop adaptation and tolerance to the various abiotic and biotic stresses with the goal of increasing the yield potential is the central focus of breeding for physiological traits. As the demands for more sustainable production around the world are evident, the incorporation of greater accuracy in agricultural practices becomes a necessity to meet the demands.

Incorporating genetic and physiological approaches with modern technological advances have provided promising results toward meeting global demands to some extent (Randhawa *et al.*, 2013). Figure 4 displays a summary of the complex integration of various processes involved in order to facilitate and create improvement of varieties.

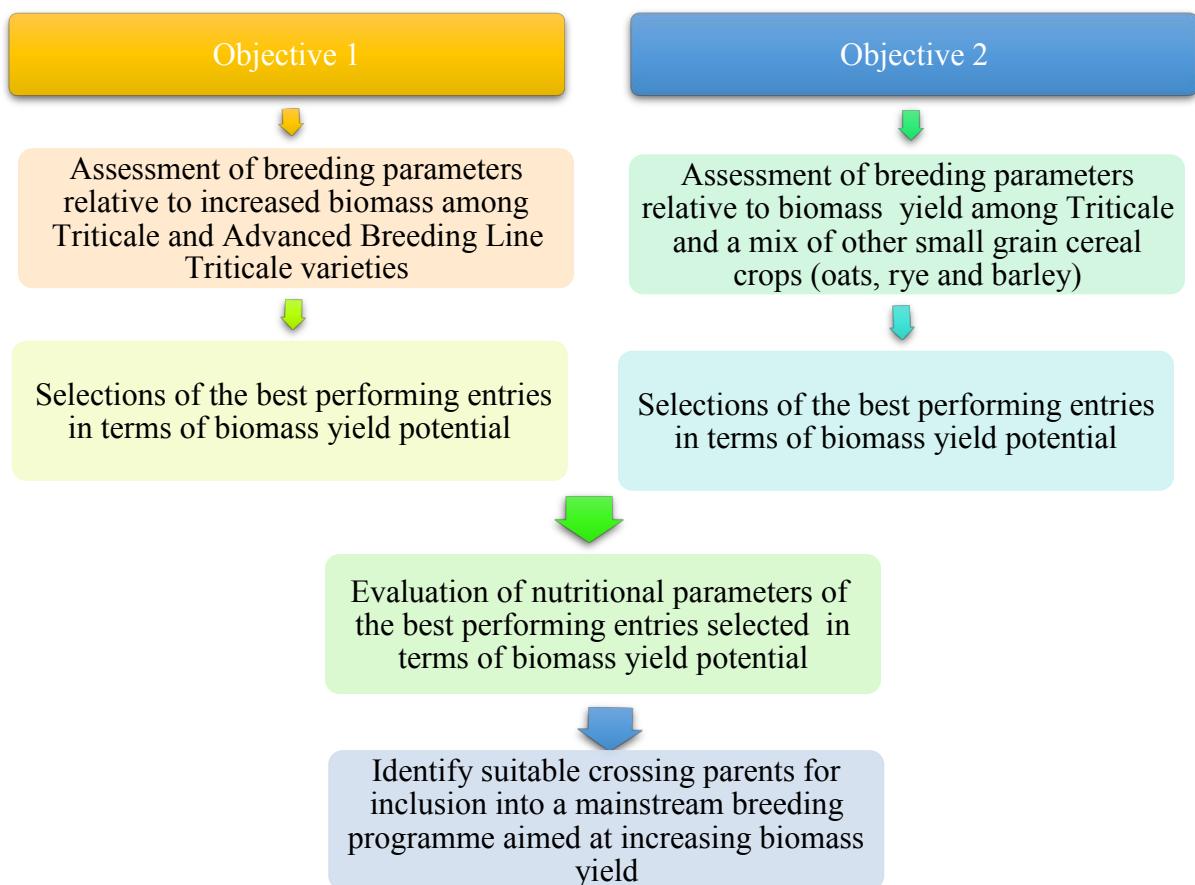


**Figure 4: An illustration on the process of how the pre-breeding approach fits into developing improved crop varieties (Adapted from Sharma *et al.*, 2013).**

## Chapter 3: Materials and methods

### 3.1 Genetic material

Planting material (untreated seed) of the various forage cultivars were obtained from the different companies with the commercial rights to disperse seed material of the specific cultivars. Triticale seed was supplied by Stellenbosch University (Stellenbosch) and the seed companies Agricol (Brackenfell, Cape Town) and Barenbrug South Africa Seeds (Pty) Ltd (Industrial Park, Cape Town). Triticale, oats, rye and barley were planted in a randomised complete block design. Figure 5 summarises the flow of the research project that are aligned to key objectives that were identified.



**Figure 5: Flow diagram of the structure and summary of the project workflow.**

### 3.1.1 Location and cultivars

The experiment was conducted at multiple locations around the Western Cape Province in South Africa. The three locations selected were Langgewens Research Farm (Moorreesburg: 33°16'34.3"S 18°42'14.9"E), Uitkyk (Riversdale: 34°01'52.4"S 21°14'09.1"E) and Mariendahl Research Farm (Stellenbosch: 33°50'20.9"S 18°49'44.6"E). During the first year (2016), 20 cultivars were tested (consisting of triticale, oats, rye and barley) at both Langgewens and Riversdale using a randomised complete block design (RCBD). The Mariendahl experimental location had 20 cultivars consisting of advanced breeding triticale lines only, planted in three replicates in a RCBD. The list of cultivars chosen for the study during 2016 is displayed in Table 2.

The experimental project, which was conducted in 2017, also consisted of three locations. The locations selected were consistent to that of Phase 1. Trials were planted in Langgewens Research Farm, (Moorreesburg: 33°16'34.3"S 18°42'14.9"E), Uitkyk (Riversdale: 34°01'52.4"S 21°14'09.1"E) and Welgevallen Research Farm (Stellenbosch: 33°56'32.8"S 18°51'59.2"E). A list of entries used in the study during 2017 is depicted in Table 3. The trials were planted on different demarcated spots from the previous year due to crop rotation systems. The total amount of plots planted where therefore 180 plots across the three locations.

The experimental trials in Langgewens and Mariendahl had 21 cultivars planted in replicates in a RCBD and in a north-south (NS) direction. The trial at Riversdale also followed a randomised complete block design and planted in an east-west (EW) direction with 20 cultivars planted. An additional trial, Roodebloem experimental farm (Caledon: 33°54'06.4"S 25°05'08.6"E) was included in the study to obtain additional data as it contained triticale entries of interest as well as other forage crops which were incorporated in the other research trials.

**Table 2: List of cultivars planted in 2016.**

CULTIVAR NAME AND SPECIES	LANGGEWENS		RIVERSDAL		MARIENDAHL	
	SSH491	Oats	Sorum	Oats	US2014	Triticale
	SSH 421	Oats	PAN263	Oats	Snel	Triticale
	Sorum	Oats	Macnifico	Oats	US2012	Triticale
	PAN263	Rye	Mac blue	Rye	US2011	Triticale
	Moby	Rye	Drakensberg	Rye	AgBeacon	Triticale
	William	Barley	Moby	Barley	US2007	Triticale
	Barpower	Oats	Pallinup	Oats	Bacchus	Triticale
	Outback	Rye	Barpower	Rye	Rex	Triticale
	Majoris	Oats	Dunnet	Oats	Ibis	Triticale
	Drakensberg	Oats	US2014	Oats	Tobie	Triticale
	Simonsberg	Oats	Simonsberg	Oats	ABL1	Triticale
	ARG R1	Oats	ARG R1	Oats	ABL2	Triticale
	Pallinup	Rye	Saia	Rye	US2015	Triticale
	Mitika	Oats	SSH421	Oats	ABL3	Triticale
	Mac Blue	Oats	Mitika	Oats	ABL4	Triticale
	US 2014	Rye	Majoris	Rye	ABL5	Triticale
	Macnifico	Triticale	Outback	Triticale	ABL6	Triticale
	Dunnet	Oats	William	Oats	US2016	Triticale
	Snel	Oats	SSH405	Oats	ABL7	Triticale
	AG Beacon	Oats	SSH491	Oats	ABL8	Triticale

**Table 3: List of cultivars planted in 2017.**

CULTIVARS AND SPECIES	LANGGEWENS		RODEBLOEM		WELGEVALLEN	
	SSH 491	Oats	SSH 491	Oats	US2019	Triticale
SSH 405	Oats	SSH 405	Oats	US2018	Triticale	
SSH 421	Oats	SSH 421	Oats	US2017	Triticale	
Sorum	Rye	SSH491	Rye	US2016	Triticale	
PAN263	Rye	SSH405	Rye	US2015	Triticale	
Moby	Barley	SSH421	Barley	US2014	Triticale	
Wizard	Oats	Sorum	Oats	US2012	Triticale	
Barpower	Rye	Moby	Rye	US2011	Triticale	
Outback	Oats	Wizard	Oats	US2007	Triticale	
Majoris	Oats	Outback	Oats	Tobie	Triticale	
Letikana	Oats	Majoris	Oats	Snel	Triticale	
Simonsberg	Oats	Letikana	Oats	Rex	Triticale	
ARG R1	Rye	Simonsberg	Rye	Ibis	Triticale	
Pallinup	Oats	Pallinup	Oats	Bacchus	Triticale	
Mitika	Oats	Mac Blue	Oats	AgBeacon	Triticale	
Mac Blue	Rye	US2014	Rye	17USTRITEL016	Triticale	
US 2014	Triticale	Dunnet	Triticale	17USTRITEL017	Triticale	
Macnifico	Oats	AG Beacon	Oats	17USTRITEL018	Triticale	
Dunnart	Oats	Horsepower	Oats	17USTRITEL019	Triticale	
Snel	Triticale	US2016	Oats	17USTRITEL020	Triticale	
AG Beacon	Triticale	US2017	Triticale			
Horsepower	Oats	Snel	Triticale			
NCD Grazer	Rye					
US 2016	Triticale					
US 2017	Triticale					

### 3.2. Experimental layout



**Figure 6: Illustration of the in-field experimental trial location and layout at Mariendahl in 2016 displayed by drone footage (Photo taken by Willem Botes).**

The experimental trials in Langgewens and Riversdale form part of the cultivar evaluation trials conducted by the Western Cape Department of Agriculture. Trials on Mariendahl form part of cultivar evaluation of elite triticale conducted by SU-PBL. Cultivars/entries were planted on individual plots which were five meters in length and 0.7 meters in width each. Each cultivar/entry was replicated three times in a trial.

The agronomic management procedures that were used are discussed in more detail below. The crops were not irrigated and were grown under dryland conditions across all experimental trials. The layout involved planting of the cultivars and managing the trials accordingly to allow for sampling at the respective developmental stages aligned to the objectives of this study. Furthermore, to utilise whole plant material to obtain fresh and dry weights in order to calculate DMY. In addition to this, utilise the dried material for further analyses on nutritional components of selected entries.

The total accumulations of plots planted in 2017 differed to that of 2017. Langgewens Research Farm had 25 cultivars planted in triplicates while Roodebloem had 22 cultivars planted in

triplicate. Welgevallen Research Farm contained the same amount of entries as it did in 2016, being 20 cultivars of triticale only. The total number of plots were therefore 201 experimental plots. This amounts to a total of 381 plots that formed part of this research study over the two seasons.

As with weeding, the sampling (cutting) was done manually with scissors with the remaining material being cut down with a mechanical cutter. Cutting was conducted at two stages, with the first being at plant height of approximately 30 cm or milk stage and when plants were at the soft dough plant developmental stage. Figure 6 illustrates the geographic placement and magnitude of the trial from an aerial perspective. The physical field layout of a forage trial taken with an unmanned aerial vehicle (UAV), displays the randomised block design and designation of each plot planted in triplicate in a North-South direction using a minimal tillage planter, as shown in figure 7.



**Figure 7: Illustration of physical field layout of a forage trial taken with an unmanned aerial vehicle (UAV) displaying randomised block design in 2017 (Photo taken by Pieter Lombard).**

### 3.3 Agronomic practices

The plots were measured and marked and sprayed with a non-selective herbicide, Preeglone (active ingredient: bipyridyl), which can be used in order to control annual grasses and broad leaf weeds within the crop. However, the main purpose of applying this was to create distinguished pathways between the plots. Application of Preeglone was also conducted under dry weather conditions with further precaution being taken through using a stainless-steel guide block and dragging it along, in order to prevent the chemical from contact with the trial. Table

4 displays the details of the agronomic practises that have been used which includes dates, dosages and active ingredients.

**Table 4: Summary of agronomic practices used to manage the experimental trials during 2016 and 2017.**

PLANTED USING A 7 ROW KNIFE EDGE MINIMAL TILLAGE PLANTER						
		LGW		RIV		CAL
PLANTING DATE	2016	09-May		09-May		/
	2017	26-Apr		06-Jun		08-May
PLANTING DENSITY	2016	200 seeds/m <sup>2</sup>	200 seeds/m <sup>2</sup>	200 seeds/m <sup>2</sup>	200 seeds/m <sup>2</sup>	200 seeds/m <sup>2</sup>
	2017	200 seeds/m <sup>2</sup>	200 seeds/m <sup>2</sup>	200 seeds/m <sup>2</sup>	200 seeds/m <sup>2</sup>	200 seeds/m <sup>2</sup>
FERTILIZER DATE AND RATE	2016	09-May 17-Jun 13-Jul	24 kg N 30 kg N 30 kg N	09-May 15-Jun 11-Jul	24 kg N 30 kg N 30 kg N	/
	2017	26-Apr 31-May 29-Jun	24 kg N 30 kg N 30 kg N	06-Jun 13-Jul 16-Aug	24 kg N 30 kg N 30 kg N	24 kg N 30 kg N 30 kg N
Roundup Glyphosate 480 millilitres per hectare	2016	26-Apr		27-Apr		/
Amistar Xtra azoxystrobin and cyproconazole 700 millilitres per hectare	2016	30-May		05-Jun		/
	2017	31-May		10-Jun		31-May
DMed Dimethoate 750 ml/ha	2016	30-May		05-Jun		
	2017	31-May		10-Jun		31-May

The study made use of the standard agronomic management approach as utilised by Elsenburg, Plant Sciences technical personnel on Langgewens, Riversdale and Roodebloem experimental farms. At Mariendahl and Welgevallen the agronomic management approaches followed by the SU-PBL was utilised on the advanced breeding line triticale trials. At Langgewens, Riversdale and Roodebloem, AMISTAR® XTRA (active ingredients: azoxystrobin and cyproconazole) was used as a broad-spectrum fungicide to offer plant protection against diseases such as rust.

RoundUp (active ingredient: Glyphosate) was used as a preplanting agent and weeds were further removed by hand later in the season. The fungicide was applied to disease-free leaves to optimise the level of protection. DMed (active ingredient: dimethoate) was used to control insects and mites and was applied on the same day as AMISTAR® XTRA to the trial. The application procedure of this fungicide was largely weather dependant and was applied in dry weather conditions with minimal wind speeds to avoid drift on to other nearby trials.

Standard production protocol was applied with regards to planting date and planting densities. Post-emergence weed control, fertiliser, insect and disease control for all treatments were restricted to a minimum. Plant nutrition was applied according to recommendations received from the Elsenburg soil laboratory. The trials were planted using a Knife edged minimal tillage seven row planter which planted at a planting density of 200 seeds per square meter.

The trials were top dressed with 30 N after the first cutting at 30 cm plant height in all the mixed forage grain trials and no top ups were dispersed on the advanced breeding line triticale trials. The TKW is determined prior to planting of the seeds. A plastic tray was placed on a scale. A total of 200 seeds were counted manually and placed in the tray. The weight of the tray was subtracted and the seed weight was multiplied by five in order to gain the thousand kernel weight.

Weed management was done manually in order to be able to ensure a reduced risk of removing plant material belonging to the plot. The growth of weeds between not only the plots, but the plants as well, made manual weed management the only method which had reduced risk of incurring loss of material in each respective plot. The agronomic approaches for this study were largely dependent on manual labour as well as weather conditions to ensure reduced risk of biomass yield loss and/or influencing the results.

### 3.4 Data collection

The trials were cut at two stages, the first were conducted when plants were at +/- 30 cm height. This was categorised as being between growth stages (GS) 22-32 according to the Zadoks scale of physiological growth stages (Reynolds *et al.*, 2012). Plant developmental stages were determined phenotypically prior to each scheduled cutting. Manual screening of at least 10 different individual plants allowed for the identification of the growth stage. However, the entry was only categorised into a growth stage once more than 75% of the plot was in the same developmental growth stage. The scale used to determine growth stages is the Zadoks scale. The second cutting was conducted at soft dough stage categorised as GS 85 according to the Zadoks scale. The data that was collected was biomass yield data, obtained from cutting and weighing the fresh and dry weights from the plant material and was expressed in kg ha<sup>-1</sup>.

The expression of biomass yield during early growth stages is important when it is utilised to simulate grazing by animals. There is a second advantage by cutting the plants at 30 cm height first allowing to discern which cultivars recover best from an early grazing.

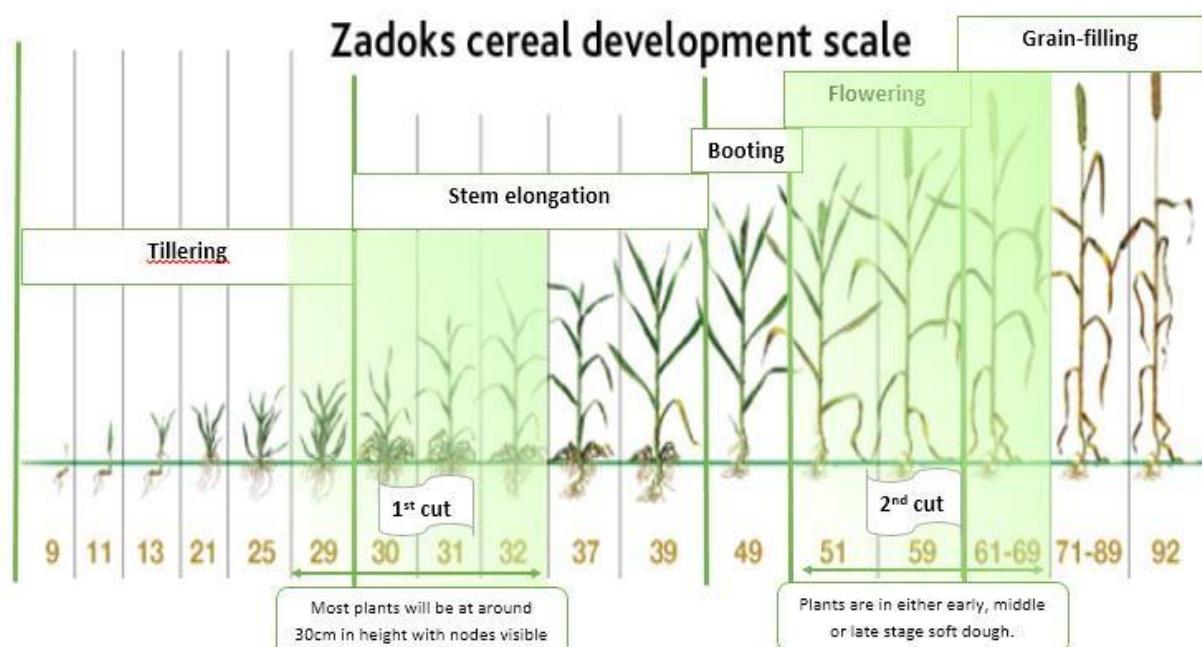
This set of data from year 1 was used to identify the poor performing cultivars for exclusion and also the inclusion of cultivars displaying significantly higher degrees of performance in comparison to other entries into the second phase. In 2017, the experiment was repeated with the inclusion and exclusion of certain cultivars based on selections made in 2016. “US2014”, “AgBeacon”, “Snel” remained in the mixed forage trials with the triticale entries “US2016” and “US2017” being included from the previous cycles advanced breeding triticale genotypes.

These entries were included as a result of its high potential to produce high BMY along with its levels of resistance against rust diseases. Samples were collected between 10 am-12 am to allow same day processing where it was possible and allow plants to dry. As illustrated in Figures 8 and 9, biomass sampling was most typically sampled during late tillering and stems elongation periods, which corresponded to the Zadoks cereal plant developmental stages GS 22-31 and GS51-70 respectively.

The harvest plots size was a plant row of 2.5 m x 0.3 m with side rows on both sides. One row of material was cut within the demarcated half using a pair of scissors and placed in empty plastic bags and thereafter labelled. When harvested at 30 cm height during GS 22-31, samples were cut approximately 5 cm above ground in order for regrowth to occur. The second cutting

was harvested between booting and flowering stages which correspond to GS50-70 stages according to the Zadoks scale.

Plants were cut as closely as possible to the ground, but above the plant growth point in order to still enable the plant's ability for regrowth once cut, plant material was collected in bags and were sealed and labelled accordingly. After harvesting the 30 cm high material and the soft dough material, the side rows were cut using an Agria 3600 BM automatic cutter to level the plot.



**Figure 8: Representation of the Zadoks cereal development scale with optimal stages in reference to biomass sampling (Onda *et al.*, 2015).**

0-11	12	21	22-29	30	31	32	37	39	45	51-59	61-69	70-79	81-89	91-99
Dry seed to first leaf emerged	2 leaves emerged	Tillering 1 main Stem + 1 tiller	Tillering 1 main Stem + 2 – 9 tillers	Stem Elongation beginning Leaf sheath becoming erect	Stem Elongation First node visible at stem base	Stem elongation Second node visible	Flag leaf just visible (Last leaf before ear emergence begins)	Flag leaf ligule just visible	Booting Ear swollen in stem just under Flag leaf	Heading i.e. ear emerging	Anthesis i.e. flowering	Clear liquid – Milk Stages (early, mid, late)	Dough Stages (early, soft, hard)	Ripening stage
Vegetative Stages														
Heading/Flowering										Grain Formation Stages				

**Figure 9: Cereal plant developmental scale (Zadoks) and the stages sampled (Reynolds *et al.*, 2012).**

During 2016 and 2017, selected triticale entries in the project were evaluated with advanced breeding triticale cultivars and other cereal and forage crops. These entries included “US2014”, “US2016”, “US2017”, “AgBeacon” and “Snel”, they were compared in terms of biomass yield and other nutritional components that relate to the protein value for use as animal feeds such as moisture content, crude fat content , ash, neutral detergent fibre (NDF) and protein content.

Nutritional components were only evaluated on a select few cultivars proven to display superior characteristic relating to increased biomass and overall improved resistance and performance. From this, it was anticipated that suitable crossing parents could be selected for inclusion into a breeding programme for increased biomass production. Furthermore, climatic data was accumulated for the geographical locations in which the field experiments were conducted from the year 2015 to the year 2017 on a daily basis. Raw data was received from the Agricultural Research Council (ARC) Agro-climatology division and was summarised to depict the relevant components in relation to the aims and objectives of this study.

The climatic data was filtered for the monthly averages per trial for components such as precipitation, maximum temperature, minimum temperatures and humidity values. The data was only collected up to November 2017 since it was the end of the growing season and plants were harvested thereafter.

### **3.5 Biomass sampling procedure**

A row of the sample was cut manually with a pair of scissors and placed in a new clean plastic bag which represented one replication. Samples were weighed to determine the fresh weight of the entire sample taken from the two rows. A sub-sample was taken from the total freshly sampled material weighing between 150 g – 200 g and placed in labelled brown paper bags before having weights recorded.

The sub-sample material was dried at 55°C - 60°C for approximately three to five days before being weighed again to determine the dry weight of the samples. Calculating the difference between fresh weight and dry weights provides an indication of the moisture content in the material. By using the dry weight value, biomass dry matter yield was calculated in kilograms

per hectare ( $\text{kg ha}^{-1}$ ). The dry whole plant material is further milled to provide sample material for nutritional analyses conducted on selected entries.

### 3.6 Nutrient components

The methodologies that were incorporated as well as the lab protocols used for the various nutritional components being analysed, differed among animal nutritionists. Analyses were conducted on the dry sub-sample of a selected few samples of mainly triticale, three oats entries and one rye entry, through Quantum Labs in Malmesbury, Swartland district. At least 30 g of dry material was required for analysis. The following lab protocols were implemented for analysing the different nutritional components respectively using standardised methods from The Association of Official Analytical Chemists (AOAC, 2000).

The nutritional analysis conducted on these small grain cereals correspond to specific methodologies. Ash content was analysed using the AACC method 08-01. Moisture was analysed using the AACC method 44-01 and Fat content via AACC method 30-20. Fibre content was analysed using the AACC method 32-15 and starch with method 76-10. Protein content for cereal grains was analysed using the standard methodology applied to cereal grains according to the AOAC protocols (AOAC, 2000).

### 3.7 Statistical analysis

The physical biomass yield data for the genotypes planted in the trials were compiled into an excel sheet adhering to the format requirements for the input file to be statistically analysed in Agrobase Generation II v. 34.4.18 (Agronomix Software, Winnipeg, Canada). The test for normality and heteroscedasticity was done by the Agrobase software using ANOVA generated by a randomised complete block design (RCBD) and nearest neighbour analysis (NNA), which is commonly utilised in the field of research for the evaluation of various genotypes for desirable traits. In this case the analysis evaluates a series of different cereal plant genotypes for the trait of biomass potential. The RCBD ANOVA produced a general linear model of representation which generalises a normal linear regression and depicts variability with error distribution models other than a normal distribution (Campbell *et al.*, 2012).

The nearest neighbour analysis (NNA) was also analysed on Agrobase, as this analysis accounts for spatial variability (Campbell & Bauer, 2007) and thus, adjusts outputs accordingly. The significant alpha value selected for these statistical analyses was 0.05 (5%). A NNA was conducted in addition to the RCBD ANOVA which factors in and accounts for multi-dimensional variability. Thus, statistical outputs often include adjusted figures as a result of factoring in variability. The statistical analysis conducted had the significant alpha value parameter set at 0.05 corresponding with 5%. The NNA and General linear model (GLM) analysis, heritability, regression and lowest significant differences were computed. Ultimately, the determination of the breeding values is essential to evaluate the response to selection.

Furthermore, the normal default parameters were selected to conduct an NNA and GLM analysis. Based on the output file of the software, using the LSD generated from the biomass yield data, and comparing it against the other entries, entries were separated in three categories. These include; significantly better performers, significantly worse performers and those that did not differ significantly to the selected entry.

The raw data which contained the weights of dry biomass during the two different cutting stages, were converted from grams or kilogram units to kilograms per hectare. This was done to give a better representation of biomass yield potential on a more logistical scale. The conversion includes dividing the size of the plots in meters by 1 hectare in order to get a factor which is multiplied with the sampled weight to produce the conversion figure. These average weights in terms of  $\text{kg ha}^{-1}$  per genotype were included into the input file used on Agrobase.

In order to distinguish the significant difference between the results obtained, the LSD was added to the mean value of the respective entry to obtain a threshold. Entries with a value higher than this calculated threshold were significantly better than the respective entry evaluated for the nutritional parameter. In contrast, the difference between the LSD and the mean of the entry produced a lower threshold of which entries with mean values below this threshold were regarded as significantly worse in terms of the parameter in question. Evidently, the entries with means not higher than the upper threshold and not lower than the lower threshold were closely clustered and could be considered as not being significantly better or worse in this regard. Therefore, on the basis of the statistical results from the output files generated, a clear indication of the degree of heritability of the trait and the degree of gene interaction was evident. A complete summary of the workflow utilised for this study is displayed in Figure 10.

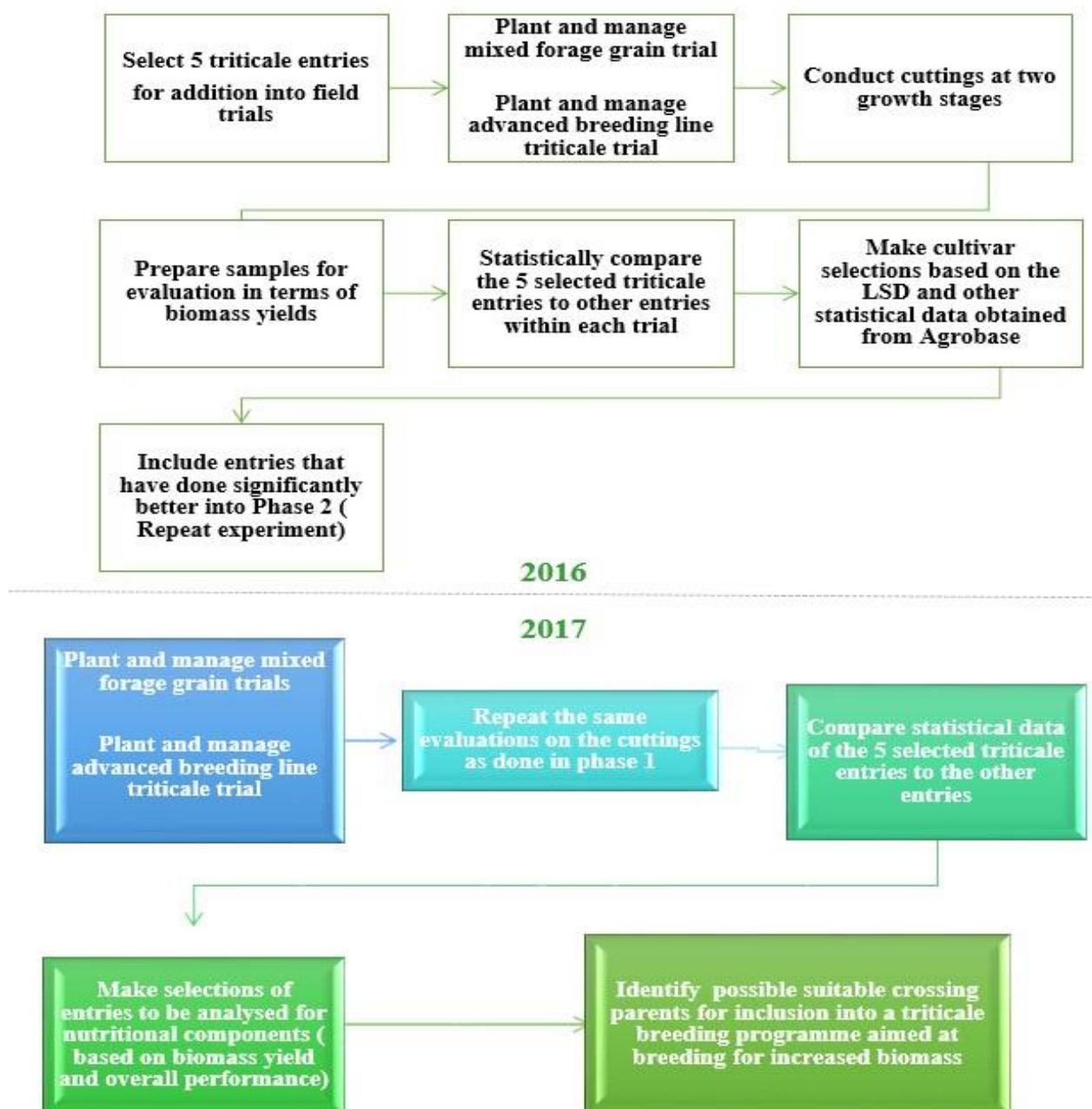


Figure 10: Schematic illustration of methodologies applied to this research study.

## Chapter 4: Results and discussion

### 4.1 Biomass yield data during the 2016 season

The two-phase study conducted across multiple locations, evaluated a number of biomass and nutritional characteristics to identify the best prospective triticale crossing parents to breed for improved biomass yield. Dry matter yield (DMY) and nutritional measurements provides an indication of the value of the particular crop as an animal feed source and provide an indication of the viability of breeding for improved biomass yields for breeders. The biomass yield and nutritional properties of triticale highly was reported by Demissew *et al.* (2017) to be highly competitive when compared to other crops and thus its abilities are further evaluated through research of this nature.

Entries that were regarded as being significantly better than a particular entry, had DMY measurements that were equal or higher than the sum of the grand mean for the entry and the LSD for DMY. The entries were evaluated for BMY and had the results reported for each entry. The top five entries were selected and summarised for ease of identifying and selecting the best suitable crossing parents in terms of BMY. In cases where entries, that were not among the top five, yielded similar BMY as the top five, they were taken into further consideration for making of selections.

The two analyses differed in one aspect as the NNA adjusted values were regarded more accurate. As planting practices differ in terms of its direction from either North-South (NS) OR East-west (EW), having a more precise picture of the plot's location and trends it is essential for more accurate statistical representation of these trials. Thus, the preferred results within this study were from that generated by the NNA analysis.

In-field trends were able to be factored in due to the availability of the NNA's adjacent residual method which is available on Agrobase and provides adjusted statistical values that more accurately represents the traits in question. Multiple values were computed through the Agrobase software. However, the values of particular interest and importance to this study for biomass yield potential include the coefficient of variation (CV), coefficient of determination ( $R^2$ ) and broad-sense heritability ( $h_b^2$ ) values.

These statistics are essential and it functions as a representation of the variability of the trait, the goodness-to-fit of the model and the degree of heritability of the trait by the generations that follow. The CV is regarded as a statistical quantity that explains the degree of phenotypic variation and consequently can be utilised to evaluate stability and denotes the degree of environmentally induced variation (Reynolds *et al.*, 2012).

Upon computing the biomass yield results into Agrobase software and conducting both general linear models and a nearest neighbour analysis, the results provided a clearer indication of the degree of heritability and variations within each trial. During the initial sample stage cutting of 30 cm during 2016, there were no selected entries that varied significantly from the other entries at Langgewens, Riversdale or Mariendahl. The results are based on the comparison against the least significant difference (LSD) and either adding and/or subtracting it from the general mean for the biomass yield of the selected entry.

#### **4.2 Mariendahl dry matter yield measured across both phases during 2016.**

The research trial at Mariendahl yielded an average of  $615 \text{ kg ha}^{-1}$  biomass yields during the cutting while plants were roughly 30 cm tall. The results of the five best performing entries during the 30cm and soft dough plant developmental stages in terms of biomass yield potential within the advanced triticale breeding line trial at Mariendahl is shown in Table 5.

The average biomass yield during the milk and soft dough plant developmental stages were  $9310 \text{ kg ha}^{-1}$  and  $6503 \text{ kg ha}^{-1}$  respectively. The triticale entry “US2014” had biomass yields were above the average across both cutting stages. During the 30 cm and soft dough cutting stages, yields of  $687 \text{ kg ha}^{-1}$  and  $7142 \text{ kg ha}^{-1}$  respectively, were recorded. Material collected during the late milk developmental stage were higher and weighed  $10558 \text{ kg ha}^{-1}$ . The triticale entries “Snel” and “AgBeacon” never featured in the top performing entries, but the results were comparative as can be seen in table 7 which includes the data obtained for all entries planted at Mariendahl during 2016.

**Table 5:** The five best performing forage genotypes in terms of biomass yield (BMY) sampled at 30 cm and soft dough cutting stages during 2016 at Mariendahl.

RANK	30 CM		SOFT DOUGH	
	ENTRY	BMY (kg ha <sup>-1</sup> )	ENTRY	BMY (kg ha <sup>-1</sup> )
1	Ibis	780	US2014	4775
2	US2016	770	Bacchus	4667
3	Bacchus	726	US2016	4661
4	Tobie	704	US2007	4567
5	Rex	697	US2012	4532

Biomass yield for “AgBeacon” (554 kg ha<sup>-1</sup>) as well as “Snel” (566 kg ha<sup>-1</sup>) had DMY below the grand mean of the trial (614 kg ha<sup>-1</sup>). The entries “Ibis”, “US2016” and “Bacchus” performed significantly better in comparison to the other advanced triticale breeding lines included in the trial. “Ibis” had the highest biomass yield during the 30 cm stage, followed by “US2016”, “Bacchus”, “Tobie” and “Rex”. From the samples obtained during the 30 cm stage, the top five entries from highest to lowest were “Ibis” (780 kg ha<sup>-1</sup>), “US2016” (770 kg ha<sup>-1</sup>), “Bacchus” (726 kg ha<sup>-1</sup>), “Tobie” (704 kg ha<sup>-1</sup>) and “Rex” (697 kg ha<sup>-1</sup>). Table 6 summarises the statistical results for biomass yield measurements conducted in this trial.

**Table 6:** Statistics for Biomass yield measurements (kg ha<sup>-1</sup>) for selected triticale cultivars (US2014, AgBeacon and Snel) and other advanced breeding line triticale.

	30 cm	Soft dough
Mean	615	4159
LSD (5%)	251.9	1250.1
CV (%)	24.8	18.2

**Table 7: Biomass yield measurements (kg ha<sup>-1</sup>) for selected triticale cultivars (US2014, AgBeacon and Snel) and other advanced breeding line triticale.**

<b>Mariendahl</b>		
<b>Entry</b>	<b>30 cm</b>	<b>Soft dough</b>
	<b>kg ha<sup>-1</sup></b>	<b>kg ha<sup>-1</sup></b>
<b>US2014</b>	631	4775
<b>Snel</b>	566	4281
<b>US2012</b>	671	4532
<b>US2011</b>	679	4496
<b>AgBeacon</b>	554	3208
<b>US2007</b>	687	4567
<b>Bacchus</b>	726	4667
<b>Rex</b>	697	4485
<b>Ibis</b>	780	3975
<b>Tobie</b>	704	3711
<b>ABL1</b>	527	4468
<b>ABL2</b>	638	4340
<b>US2015</b>	689	2893
<b>ABL3</b>	441	4318
<b>ABL4</b>	400	4192
<b>ABL5</b>	487	4152
<b>ABL6</b>	621	4042
<b>US2016</b>	770	4661
<b>ABL7</b>	576	3943
<b>ABL8</b>	445	3467

The “LSD per ENTRY” calculated for each of the cutting stages were 251.89 (30 cm) and 1250.10 (soft-dough). The entries within the trial were tested against the selected entries “Snel”, “AgBeacon” and “US2014” for significant differences with the use of the LSD values. During the 30 cm cutting stage, there were no entries that were significantly better in terms of DMY. However, “US2014” had the highest DMY of the three with a DMY of  $631.13 \text{ kg ha}^{-1}$ . During the soft-dough cutting stage “US2014” significantly better than both “AgBeacon” and “Snel”.

Despite the lack of significant variations within the first cutting, the second stage sampling period displayed a variety of different plant types and entries that did significantly better than the selected entries and that will serve as good selections for biomass yield production. Among the advanced breeding line triticale genotypes sampled during the soft dough stage, “US2014” was the only entry of the selected triticale entries to be among the top five performers in terms of BMY with  $7467 \text{ kg ha}^{-1}$  yield reported. Ranked from the highest to the lowest of the top five entries, the BMY recorded were as follows: “US2014” ( $4775.31 \text{ kg ha}^{-1}$ ), “Bacchus” ( $4666.67 \text{ kg ha}^{-1}$ ), “US2016” ( $4661.34 \text{ kg ha}^{-1}$ ), “US2007” ( $4567.23 \text{ kg ha}^{-1}$ ) and lastly “US2012” ( $4532.18 \text{ kg ha}^{-1}$ ).

The entries “US2016” and “Bacchus” were ranked among the five highest in the trial in both the 30cm and soft dough sampling stages in terms of BMY. The entries “Snel” had DMY measured at  $4280.83 \text{ kg ha}^{-1}$  whilst “AgBeacon” had a DMY of only  $3207.52 \text{ kg ha}^{-1}$ . With a grand mean for DMY  $4158.63 \text{ kg ha}^{-1}$ , the three selected entries (“US2014”, “Snel” and “AgBeacon”) are shown to have performed competitively. However, “AgBeacon” had a DMY which was lower than the grand mean during both cutting phases. “US2016” and “Bacchus” were consistently among the top five performing entries during both measurement phases.

Evaluation of the biomass yield across the multiple developmental stages clearly facilitates easier identification of possible crossing parents that are complementary when breeding for increased biomass yield (Ayalew *et al.*, 2018). The entries “US2014”, “Snel” and “AgBeacon”, along with the entries “US2016” and “Bacchus”, that performed significantly better than most entries in the study and are suitable candidates with desirable characteristics that could serve as suitable crossing parents to include into breeding triticale with improved biomass yield. These entries were taken forward into the next phase of the study conducted during 2017.

The grand means of the trials during the 30cm stage ( $614.52 \text{ kg ha}^{-1}$ ) and soft-dough stage ( $4159 \text{ kg ha}^{-1}$ ) particularly during the drought conditions experienced in the Western Cape, is an indication of this crop's hardiness and biomass yield potential. CV percentages can be utilised as an indication of the degree of homogeneity. The CV for this trial was 24.80% during the 30cm stage and 18.19% during that of soft-dough stage. The smaller the CV percentages are, the more homogenous the sample means would be resulting in better sampling capacity of these trials (Liu *et al.*, 2017). The CV percentages observed could be attributed to the variation of different plant genetic make-ups of each cultivar. Heritability values were lower than expected, being 0.165 (30cm) and 0.103 (Soft-dough).

The heratibility value is an indication and measure of viability for selecting for this specific trait and the potential for it to be transferred to future generations (Demissew *et al.*, 2017). However, even though the heratibility values are lower than expected, it could potentially be attributed to multiple genetic influences on variation which includes the interaction between genetics and the environment and its effects, epistasis and other influences (Losert *et al.*, 2017). Therefore, it is essential to evaluate and conduct research of this nature over multiple seasons for increased accuracy and further ensure that priority is given to proper agronomic management procedures.

#### **4.3 Langgewens dry matter yield measured across both phases during 2016.**

The entries “US2014”, “Snel” and “AgBeacon” were planted together with other forage grain entries composed of oats, barley and rye and further evaluated over different cutting stages for biomass yield. At Langgewens, “US2014” yielded  $604 \text{ kg ha}^{-1}$ , “AgBeacon”  $595 \text{ kg ha}^{-1}$  and “Snel” yielded  $459 \text{ kg ha}^{-1}$  during the first cutting stage as shown in table 8. The average biomass yield within this stage was  $556 \text{ kg ha}^{-1}$ , placing both “US2014” and “AgBeacon” above the trial average. When compared to other cereal plant types, “US2014” was ranked among the five highest biomass yields producing entries in the trial at the 30cm stage. During the first cutting at 30 cm, the trial with a grand mean for DMY of  $1058.15 \text{ kg ha}^{-1}$  yielded the following results. The highest biomass yield producing cultivar was “ARG R1” (rye), yielding  $1518 \text{ kg ha}^{-1}$ . Thereafter, “SSH421” (oats) with  $1349 \text{ kg ha}^{-1}$ , “Moby” (barley) with  $1330 \text{ kg ha}^{-1}$ , “AgBeacon” (triticale) with  $1189 \text{ kg ha}^{-1}$  and the last of the five being “SSH491” (oats) with  $1181 \text{ kg ha}^{-1}$ .

During the soft dough plant development stage, the biomass yields recorded identified “US2014”, “AgBeacon” and “Snel” as all being among the five highest biomass producing entries. “AgBeacon” had the highest biomass yield measuring  $8858 \text{ kg ha}^{-1}$ , with “US2014”  $8087 \text{ kg ha}^{-1}$  and “Moby” (barley)  $8050 \text{ kg ha}^{-1}$  following in respect of biomass yield.

**Table 8: The five best performing forage genotypes in terms of biomass yield (BMY) sampled at 30 cm and soft dough cutting stages during 2016 at Langgewens.**

RANK	30CM		SOFT DOUGH	
	ENTRY	BMY ( $\text{kg ha}^{-1}$ )	ENTRY	BMY ( $\text{kg ha}^{-1}$ )
1	ARG R1	1518	AgBeacon	8858
2	SSH421	1349	US2014	8087
3	Moby	1330	Moby	8050
4	AgBeacon	1189	Snel	7838
5	SSH491	1181	Drakensberg	7604
<b>SD = 253</b>			<b>LSD = 1679</b>	

The fourth and fifth highest biomass yield producing entries in this stage were “Snel” with  $7838 \text{ kg ha}^{-1}$  and “Drakensberg” (oats) with  $7604 \text{ kg ha}^{-1}$  respectively. The trial biomass yield average during the soft dough stage was  $5865 \text{ kg ha}^{-1}$ . All three triticale entries produced significantly higher the trial average indicating the strong ability to produce superior biomass yields despite the stress conditions experienced.

The entries were evaluated for significant differences using the “LSD for Entry” during both cutting stages. The LSD during the 30cm cutting was LSD = 253.4211 whilst the LSD during the soft-dough period was LSD = 1679. Using the LSD and the mean of each of the selected triticale entries (“US2014”, “AgBeacon” and “Snel”), all entries which had DMY which was above the sum of the LSD and the mean were regarded significantly better. The entry “Snel” during the 30cm cutting had five entries that had DMY above  $1172 \text{ kg ha}^{-1}$  and were regarded significantly better.

The entries “SSH491”, “AgBeacon”, “Moby”, “SSH421”and “Drakensberg” were all significantly better “Snel”. The entry “US2014” only had two entries that were significantly better (DMY above 1338.80 kg ha<sup>-1</sup>). These entries were “SSH421” and “Drakensberg”. The last of the selected triticale entries, “AgBeacon”, had no entries that were significantly better in terms of DMY. The results are further supported with statistical interpretation in which CV percentages for this trial had an adjusted value computed from an NNA which was CV = 14.50%, representing that a rather small proportion of variation that exists among the samples. This can be attributed to the different plant types that have been included and varying genetic make-ups of each cultivar respectively. The regression vale ( $R^2 = 0.60$ ) is indicative that the statistics fits well with the regression model.

Comparing the results across these stages, it is evident that higher biomass yields were obtained during the soft-dough developmental stage. The ability to produce over 1 t ha<sup>-1</sup> DMY during grazing and thereafter produce over 7 t ha<sup>-1</sup> during soft dough, shows that triticale has good potential and has competitive yields to that of the other forages in the trial. Despite the dry environmental conditions experienced by the drought in addition to varying degrees of stress and competition induced due to weeds and broad leave occupation within the plots, the BMY were still regarded as a good yield. Figure 11 shows the degree of ryegrass and weeds growing between and within rows of the research trial. It provides an indication of the intensity level of the competition between plants for food and sunlight. Furthermore, through manual removal of grass weeds growing between the plants, plant material of the sample plants may be removed which may affect the BMY results obtained. Furthermore, with CV values at 14.50% (30 cm) and 17.25% (soft-dough), along with high heritability values  $h_b^2 = 0.84$  (30 cm) and  $h_b^2 = 0.71$  (soft-dough), it supports the viability of being able to breed for improved biomass yield in triticale.

In research studies conducted by Demissew *et al.* (2017) and Ayalew *et al.* (2018), Triticale produced higher biomass yields than its parent plant wheat but lower DMY than oats forages. However, even though oats plants produced higher DMY than triticale, triticale entries were highly competitive and produced larger DMY content than other forages such as rye and barley (Demissew *et al.*, 2017; Ayalew *et al.*, 2018). This has evolved with increased breeding approaches to improve triticale genotypes and therefore further necessitates that further improvement be done to increase triticale cultivars’ potential to supplement animal feed stocks.



**Figure 11: Degree of ryegrass and grass weeds growing between and within rows (Photos taken by Naweed Mohamed).**

Triticale has featured among the best biomass yield producers across the research trials conducted over the three locations to this point. “US2014”, “Snel” and “AgBeacon” prove to have high potential for producing BMY and may be suitable triticale selections from this trial, considering its levels of resistance and superior ability to produce better biomass yields in comparison to the other forage plant types. The entries “AgBeacon”, “US2014” and “Snel” were therefore further included into the research trials during 2017 and showed to have desirable characteristics being suitable possible selections for biomass improvement in triticale. Table 9 summarises the statistics for BMY measurements ( $\text{kg ha}^{-1}$ ) for selected triticale cultivars (US2014, AgBeacon and Snel) and other forage crops in Langgewens. The biomass measured across the plant developmental stages are further displayed in table 10, illustrating how entries differ in terms of biomass accumulation, not only among plant types but across developmental stages as well.

**Table 9: Statistics for Biomass yield measurements ( $\text{kg ha}^{-1}$ ) for selected triticale cultivars (US2014, AgBeacon and Snel) and other forage crops in Langgewens.**

	30 cm	Soft dough
Mean	1090	5875
LSD (5%)	253.42	1398.87
CV (%)	14.50	17.25

**Table 10: Biomass yield measurements (kg ha<sup>-1</sup>) for selected triticale cultivars (US2014, AgBeacon and Snel) and other forages during 2016 in Langgewens.**

Langgewens		
Genotype	30cm	Soft dough
<b>SSH491</b>	1181	4135
<b>SSH 421</b>	1349	6586
<b>Sorum</b>	941	4841
<b>PAN263</b>	687	4182
<b>Moby</b>	1330	8050
<b>William</b>	1085	5177
<b>Barpower</b>	1048	6532
<b>Outback</b>	812	2205
<b>Majoris</b>	1086	5015
<b>Drakensberg</b>	1098	7604
<b>Simonsberg</b>	787	6503
<b>ARG R1</b>	1518	4698
<b>Pallinup</b>	1127	5462
<b>Mitika</b>	867	8087
<b>Mac Blue</b>	1146	4558
<b>US 2014</b>	1208	7421
<b>Macnifico</b>	1319	4714
<b>Dunnet</b>	1113	4674
<b>Snel</b>	919	7838
<b>AG Beacon</b>	1189	8858

#### **4.4 Riversdale dry matter yield measured across both phases during 2016.**

At the Riversdale trial, the only triticale entry included in this trial was “US2014”. During the first cut the grand mean for the trial was  $793.26 \text{ kg ha}^{-1}$ , with the only selected triticale entry planted (“US2014”) having a DMY of  $438.99 \text{ kg ha}^{-1}$  and being ranked second lowest among the other forages in terms of DMY. The LSD was 263.8 during the 30cm cutting phase with  $\text{CV} = 20.1\%$  and  $h_b^2 = 0.772\%$ .

There were 11 entries namely; “Simonsberg”, “Barpower”, “Saia”, “Mitika”, “ARG R1”, “Macnifico”, ”Dunnet”, ”William”, ”Pallinup”, ”SSH491” and ”Moby” that had a DMY above  $702 \text{ kg ha}^{-1}$  and were regarded as significantly better than “US2014” in terms of DMY. The results of the five top ranked entries in terms of DMY are tabulated in table 11.

The conditions of this trial were similar to that of Langgewens with plots experiencing a varying degree of stress from weeds, grasses and/or other broad leaf plants that have infiltrated the plot. The five best entries ranked in the order of highest to lowest include: “Moby” (barley) with  $1394 \text{ kg ha}^{-1}$ , ”SSH491” (oats) with  $1191 \text{ kg ha}^{-1}$ , ”Pallinup” (oats) with  $1131 \text{ kg ha}^{-1}$ , ”William” (oats) with  $1070 \text{ kg ha}^{-1}$  and ”Dunnet” (oats) with a biomass yield of  $1065 \text{ kg ha}^{-1}$ . ”US2014” had a biomass yield of only  $439 \text{ kg ha}^{-1}$  which was below the trial’s average.

Synchronising the biomass yield results of the three trials, the three triticale entries (“US2014”, “AgBeacon” and “Snel) performed consistently despite the stress conditions it was exposed to. The oats cultivars experienced significant yield reduction due to stem rust disease and competition from weeds. The level of management for triticale was a lot less than that required by the other forage plants. Good resistance to abiotic and biotic stresses were observed in all triticale plots.

During the second cutting at soft dough, the entry “US2014” had a BMY of  $7551.81 \text{ kg ha}^{-1}$ . The entries ”SSH405” and ”SSH491” had a BMY higher than  $9222.36 \text{ kg ha}^{-1}$ , which was the sum of the BMY of “US2014” and the LSD, was ”SSH491” and ”SSH405”. Therefore, ”SSH491” and ”SSH405” were the only entries significantly better than “US2014”, whilst there were none which were significantly better than ”SSH491”. With LSD of  $1670.55 \text{ kg ha}^{-1}$  for the trial only three entries had a BMY less than  $5881.26 \text{ kg ha}^{-1}$ . These included the cultivars ”PAN263” (rye), ”Macnifico” (oats) and ”Sorum” (rye). Table 13 represents a list of all entries and results for this trial showing how entries vary in terms of biomass accumulation across the different plant types as well as different developmental stages.

**Table 11: The five best performing forage genotypes in terms of biomass yield (BMY) sampled at 30 cm and soft dough cutting stages during 2016 at Riversdal.**

RANK	30 CM		SOFT DOUGH	
	ENTRY	BMY (kg ha <sup>-1</sup> )	ENTRY	BMY (kg ha <sup>-1</sup> )
1	Moby	1394	SSH491	10758
2	SSH491	1191	SSH405	9800
3	Pallinup	1131	William	9152
4	William	1070	Outback	8718
5	Dunnet	1065	Majoris	8593
	<b>LSD = 264</b>		<b>LSD = 1671</b>	

The trial average for biomass yield during the soft dough stage was 7540 kg ha<sup>-1</sup>. The five highest biomass producers ranked from highest to lowest were “SSH491” (oats) with 10758 kg ha<sup>-1</sup>, “SSH405” (oats) with 9800 kg ha<sup>-1</sup>, “William” (oats) with 9152 kg ha<sup>-1</sup>, “Outback” (oats) with 8718 kg ha<sup>-1</sup> and lastly “Majoris” (oats) with 8593 kg ha<sup>-1</sup>.

The R<sup>2</sup> value was 0.713, indicating that the results show a goodness of fit to the regression model. Heritability was measured h<sub>b</sub><sup>2</sup> = 0.833%, further supporting the view that this trait is easily transferable to future generations and it is indeed viable to breed triticale for improved biomass. Table 12 summarises the statistical results for biomass yield measurements conducted in this trial.

Consulting the data generated from the three trials, it was decided to exclude cultivars that performed statistically lower than these entries. Those that performed statistically better, were included into phase 2 of the project during 2017. The selected triticale entries “US2014”, “AgBeacon” and “Snel” remained within the research trial conducted during 2017. No exclusions were made, however, due to the availability of seed from the different companies that contributed seed to this study. Further additions were included into the forage trial. In 2017, the entries that were included as per the statistical results and rankings achieved in “2016” were “US2016” and “US2017”.

These entries were present in the advanced breeding line triticale trial during 2016 and were maintained in the trial planted at Welgevallen during 2017. Not only was the BMY statistics considered, but the cultivars rust resistance characteristics were also considered in comparison to other good performing entries within the trial.

The quantitative and qualitative phenotypic evaluations conducted during 2016 served as a vital role in fast tracking the preselecting of possible crossing parents for further inclusion into a breeding programme aimed at breeding for increased biomass production. The phenotypic evaluation in the degrees of environmental stress the plants had exposure to, displays that triticale is indeed a viable alternative with greater resistance and capabilities for producing sustainable amounts of biomass yields. These characteristics make triticale an attractive dual-purpose crop.

**Table 12: Statistics for Biomass yield measurements (kg ha<sup>-1</sup>) for selected triticale cultivars (US2014, AgBeacon and Snel) and other forage crops in Riversdale.**

	30 cm	Soft dough
<b>Mean</b>	793	7540
<b>LSD (5%)</b>	264	1671
<b>CV (%)</b>	20.1	13.4

**Table 13: Biomass yield measurements (kg ha<sup>-1</sup>) for forages planted in Riversdale during 2016.**

<b>Riversdale</b>		
	<b>30cm</b> <b>kg ha<sup>-1</sup></b>	<b>Soft dough</b> <b>kg ha<sup>-1</sup></b>
<b>Sorum</b>	183	4775
<b>PAN263</b>	662	4826
<b>Macnifico</b>	933	5812
<b>Mac blue</b>	493	6143
<b>Drakensberg</b>	677	6291
<b>Moby</b>	1394	6305
<b>Pallinup</b>	1131	6523
<b>Barpower</b>	608	7073
<b>Dunnet</b>	1065	7440
<b>US2014</b>	439	7552
<b>Simonsberg</b>	769	7602
<b>ARG R1</b>	933	8274
<b>Saia</b>	895	8333
<b>SSH421</b>	485	8407
<b>Mitika</b>	924	8456
<b>Majoris</b>	692	8593
<b>Outback</b>	495	8718
<b>William</b>	1070	9152
<b>SSH405</b>	586	9800
<b>SSH491</b>	1191	10758

#### 4.5 Summary of statistical analysis of phase 1

The results generated by Agrobase through general linear model analysis display. The coefficient of variance (CV) ranged between 14.50% and 24.8% during the 30 cm stage cutting and between 13.39% and 18.19% during the second cutting at soft dough across the multiple trials. The high CV can be attributed to the high degree of variability within the trials considering the fact that the trials were composed of different plant types. Furthermore, it serves as an indication that there is a greater sense of variation within each trial rather than variation across trials.

The LSDs reported for the trials in 2016 ranged from 284.55 to 516.61 in the 30 cm sampling stage while in the soft dough stage LSD amounts were between 1679.02 and 1954.83. The LSD is a measure of variability within the test possibly due to factors such as population density variations, environmental or experimental errors. The higher LSDs are in line with what was expected due to a greater biomass yield during the soft dough sample cutting. This was also evident in the exponentially higher grand means reported for the biomass yields in the three respective experimental locations.

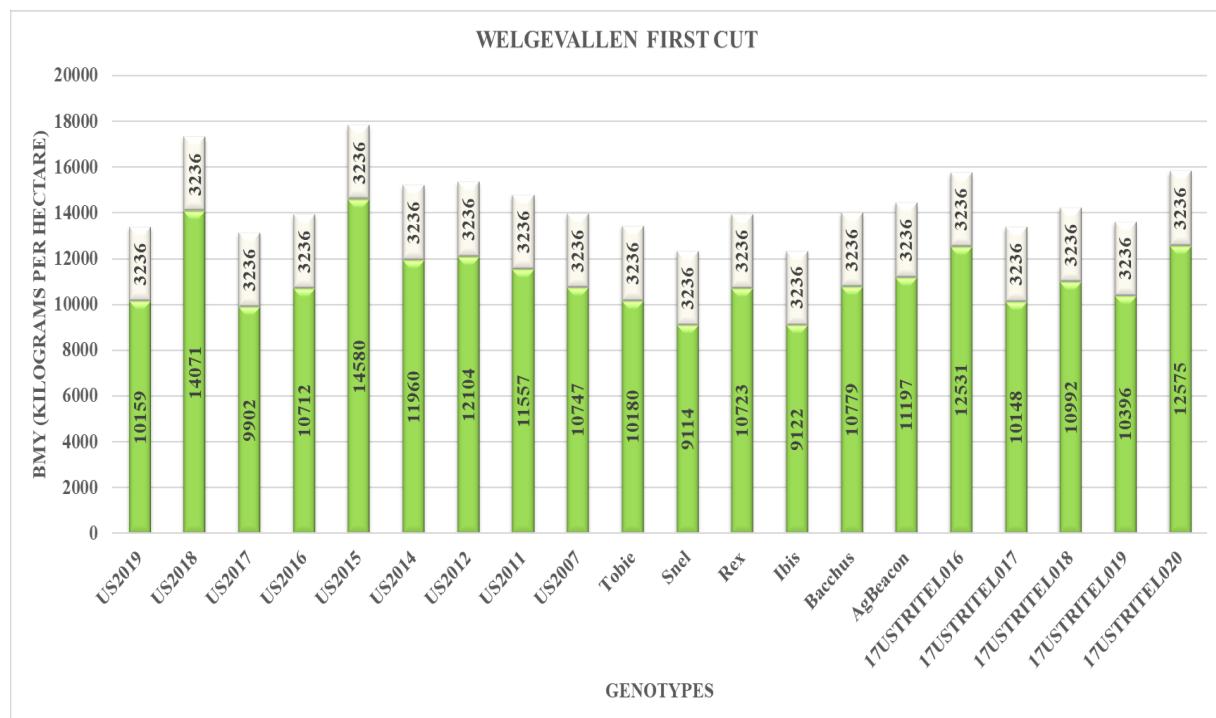
Furthermore, based on the LSD values, and considering a significance level of 0.1( $P > 0.05$ ), it can be deduced with confidence (alpha = 5%) that during the 30 cm sampling period, LGW performed significantly better in biomass yield than the other two trials. However, during soft dough, although biomass yields were optimal during this plant development stage, no individual trial did significantly better or worse and the results were relatively uniform across the three different experimental locations.

Within each trial however, the identification of entries that did significantly better using the reported LSD values was done in such a manner that the differences in terms of biomass yield needed to exceed the respective LSD value for the trial in order for it to be considered significantly different with 90% certainty.

#### 4.6 Welgevallen dry matter yield during 2017

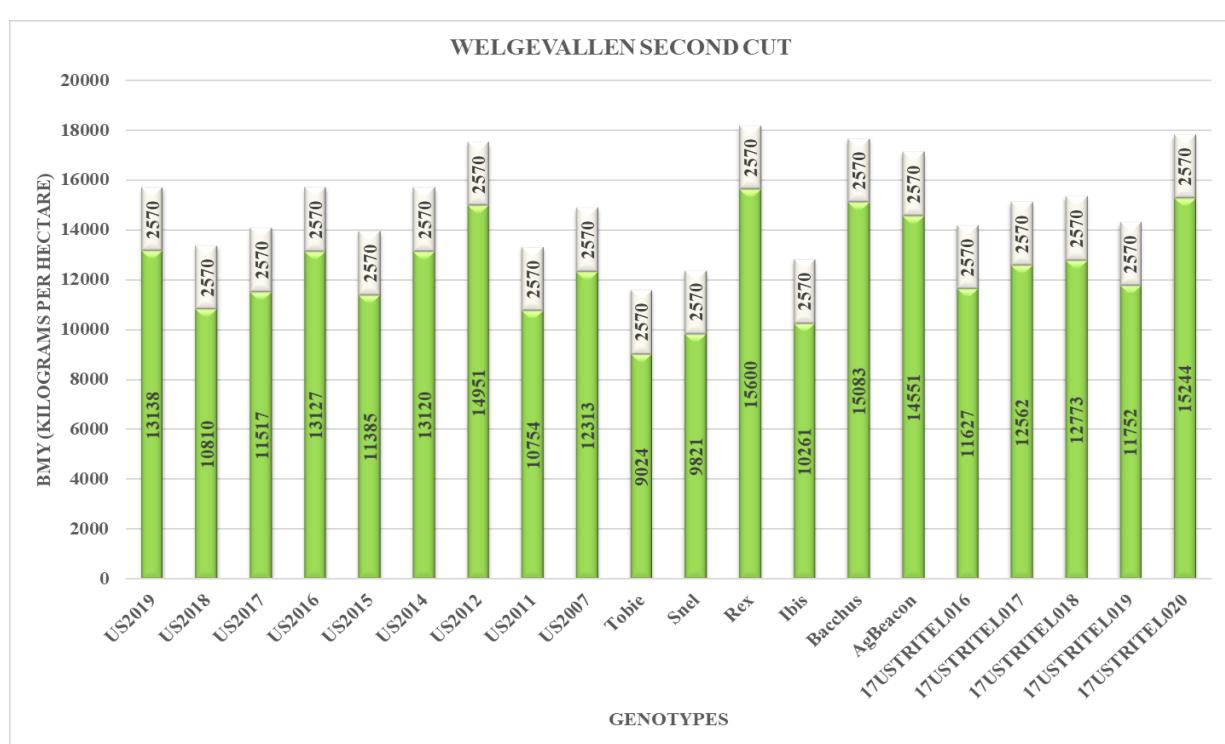
The research sites for 2017 included Welgevallen, Langgewens and Roodebloem (Caledon) Research Farms, planted by the Western Cape Government Department of Agriculture (WCG DoA) and Stellenbosch Plant Breeding Laboratory (SU-PBL).

At Welgevallen, all five of the selected entries “US2016”, “US2017”, “US2014”, “Snel” and “AgBeacon” were included in the trial. Figure 12 shows the BMY results of the first cut at Welgevallen and gives an indication of the variations in BMY between the advanced triticale breeding lines, cultivars and the five selected triticale (“AgBeacon”, “US2014”, “US2016”, “US2017” and “Snel”) during 2017. The entries “US2015”, “US2018”, “17USTRITEL017” had the highest BMY weighing  $14580 \text{ kg ha}^{-1}$ ,  $14071 \text{ kg ha}^{-1}$  and  $12531 \text{ kg ha}^{-1}$  respectively. The trial had an LSD for Entry of  $3235.55 \text{ kg ha}^{-1}$  and an  $R^2 = 0.38$ . The CV that was reported was  $\text{CV} = 21.03\%$  with heritability at 0.14, which was lower than expected.



**Figure 12: Mean biomass yield of the advanced breeding line genotypes during the milk stage at the Welgevallen site during 2017.**

In Figure 13 the BMY results of the second soft dough cutting stage conducted at Welgevallen, is shown as well as an indication of the variations in BMY between the advanced breeding line triticale cultivars and the five selected triticale (AgBeacon, “US2014”, US2016, US2017 and Snel) during 2017. The trial had LSD of  $2570 \text{ kg ha}^{-1}$  and a grand mean of  $12470.61 \text{ kg ha}^{-1}$ . The CV for the trial was  $\text{CV} = 14.97\%$  with the  $R^2 = 0.63$ .



**Figure 13: Mean biomass yield of the advanced breeding line genotypes during the soft dough stage at the Welgevallen site during 2017.**

During the soft dough stage, “Rex” was recorded to have the highest BMY ( $15600 \text{ kg ha}^{-1}$ ) at Welgevallen. The entries that followed with the highest BMY included “17USTRITEL020” with  $15244 \text{ kg ha}^{-1}$ , “Bacchus” with  $15083 \text{ kg ha}^{-1}$ , “US2012” with  $14951 \text{ kg ha}^{-1}$  and lastly “AgBeacon” with  $14551 \text{ kg ha}^{-1}$  biomass yield. The entries “Rex”, “AgBeacon”, “US2012” and “USTRITEL020” were significantly better than “US2014” and “Snel”, of the selected entries having a BMY which is higher than the combination of the BMY and the LSD for the entry.

The modern cultivars displayed a trend which showed higher BMY and was significantly better than the selected entries in terms of BMY. However, “AgBeacon” displayed consistency in being among the best performing genotypes in terms of BMY. This further indicates that the modern cultivars are

perhaps better adapted and have more favourable characteristics for selection to improve biomass production abilities within triticale.

“AgBeacon” was the only entry of the selected triticale that featured in the top five during this sampling stage. However, of the five entries, four had BMY above 15000 kg ha<sup>-1</sup>. Using the LSD and the mean of the individual entry, entries that were significantly better than the selected entries were identified. “17USTRITEL020” and “AgBeacon” had no entries that were significantly better having DMY which is higher than the sum of the entry mean and the LSD). The entries “17USTRITEL020” and “AgBeacon” were significantly better than the remaining three within the top five entries, with a DMY higher than 17000 kg ha<sup>-1</sup>.

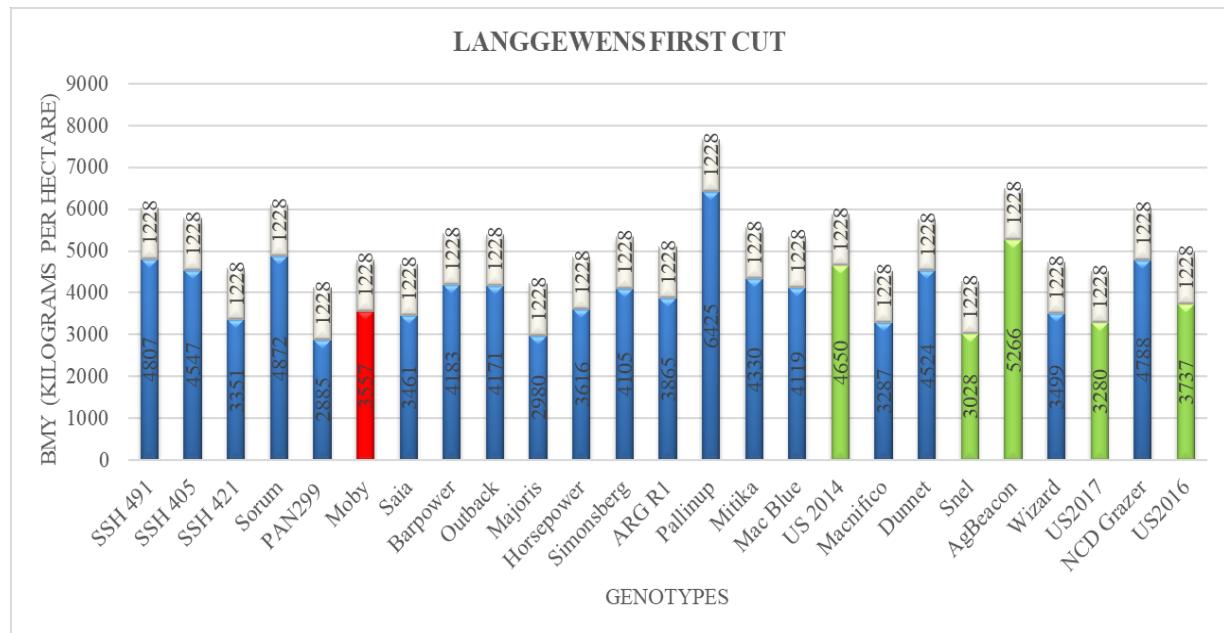
The results further show a much larger yield during 2017 being measured. Furthermore, the entries among the newer advanced breeding line material have shown to produce favourable characteristics in terms of its ability to produce biomass yield. “AgBeacon” has consistently also been found among the best performing entries in terms of biomass yield across the various trials to this point.

#### **4.7 Langgewens dry matter yield during 2017**

With the statistical analysis conducted in 2016, the CV, LSD, R<sup>2</sup> and heritability values are of main interest for the selection of possible crossing parents for further inclusion into a mainstream breeding trial aimed at increasing biomass yield in triticale. R<sup>2</sup> values ranged from R<sup>2</sup> = 0.3843 for the trial in Welgevallen to R<sup>2</sup> = 0.7868 in Caledon and will be further discussed. The results of the R<sup>2</sup> values indicate that the model displays a high degree of goodness of fit to the linear regression. This further illustrates that the model accounts for more than 70% of the variability within the trials at LGW and Caledon. The CV values generated by a RCBD analyses ranged from 18.12% to 21.03% during the milk stage and 13.27% to 23.25 % during the soft dough stage cutting.

During 2017, the trial planted at Langgewens yielded the following BMY measurements during the first cutting phase. Figure 14 displays these results graphically. The highest performing entry was “Pallinup” yielding 6125 kg ha<sup>-1</sup>. “AgBeacon” followed with 5266 kg

$\text{ha}^{-1}$  BMY. The third highest BMY was recorded in the entry “Sorum” with “SSH491” and “US2014” following it with  $4872 \text{ kg ha}^{-1}$  and  $4650 \text{ kg ha}^{-1}$  respectively.



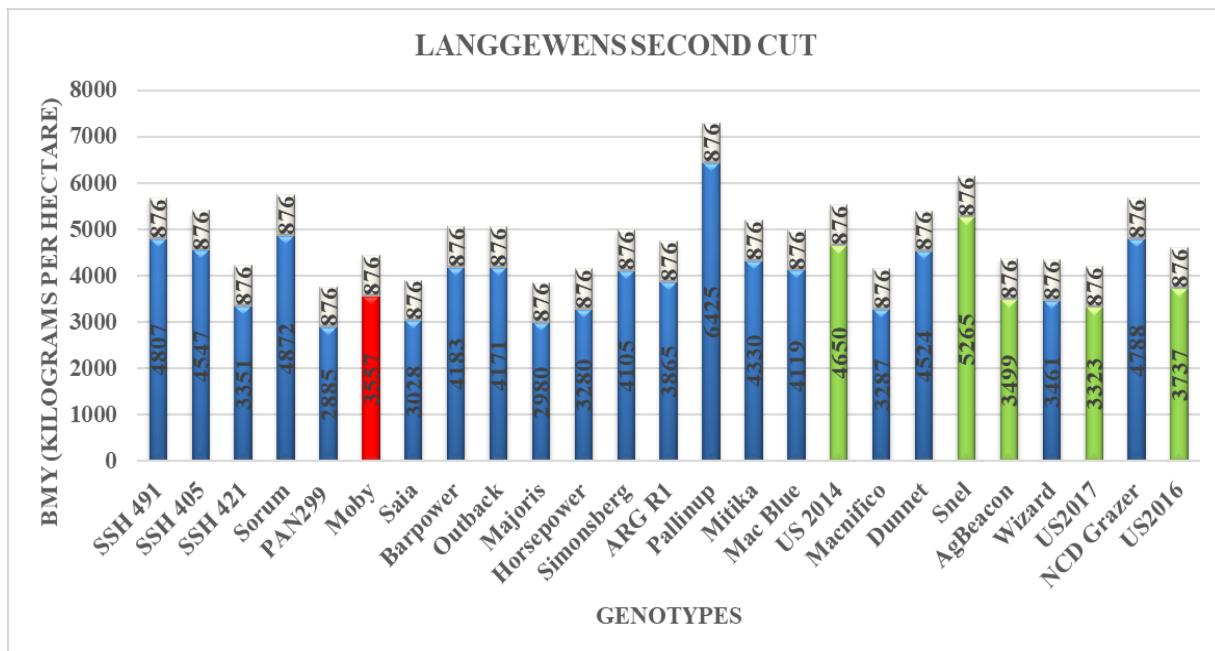
**Figure 14: Mean biomass yield of the mixed forage genotypes during the first cut at the Langgewens site during 2017.**

The trial had grand mean of  $3980.38 \text{ kg ha}^{-1}$  with the coefficient of variance being  $\text{CV} = 18.12\%$ . The LSD for Entry was  $1228 \text{ kg ha}^{-1}$  with the  $R^2$  value being 0.71. Although “AgBeacon” and “US2014” was the only triticale among the top five performers, the remaining triticale entries were competitive in terms of BMY.

The remaining triticale entries yielded the following results; “Snel” ( $3028 \text{ kg ha}^{-1}$ ), “US2016” ( $3737 \text{ kg ha}^{-1}$ ), “US2017” ( $3280 \text{ kg ha}^{-1}$ ) and “AgBeacon” with  $5266 \text{ kg ha}^{-1}$ . Similarly, to the other trials conducted in 2016 and 2017, this trial also experienced stress due to extensive weed growth between and within the plots. Through extensive weed management on all trials the effect of the stress was reduced as best possible.

The results obtained for the BMY at the soft dough stage at Langgewens during 2017 are shown in Figure 15. “Pallinup” was the highest performing entry in terms of its BMY yielding  $6425 \text{ kg ha}^{-1}$ . “Snel” had the second highest BMY ( $5266 \text{ kg ha}^{-1}$ ). “Sorum”, “SSH491” and “NCD Grazer” followed with  $4872 \text{ kg ha}^{-1}$ ,  $4807 \text{ kg ha}^{-1}$  and  $4788 \text{ kg ha}^{-1}$  BMY respectively.

The LSD reported was 876 kg ha<sup>-1</sup> whilst the grand mean for the trial was measured to be 4025 kg ha<sup>-1</sup> in DMY with a CV of 13.27%. Heratibility values were calculated to be 0.669. The entry “Pallinup” was the only entry that was significantly better than the two entries (“Snel” and “US2014”) in terms of DMY, which featured among the five selected triticale entries. “Snel” and “US2014” had a DMY of 5265 kg ha<sup>-1</sup> and 4650 kg ha<sup>-1</sup> respectively.



**Figure 15: Mean biomass yield of the mixed forage genotypes during the second cut at the Langgewens site during 2017.**

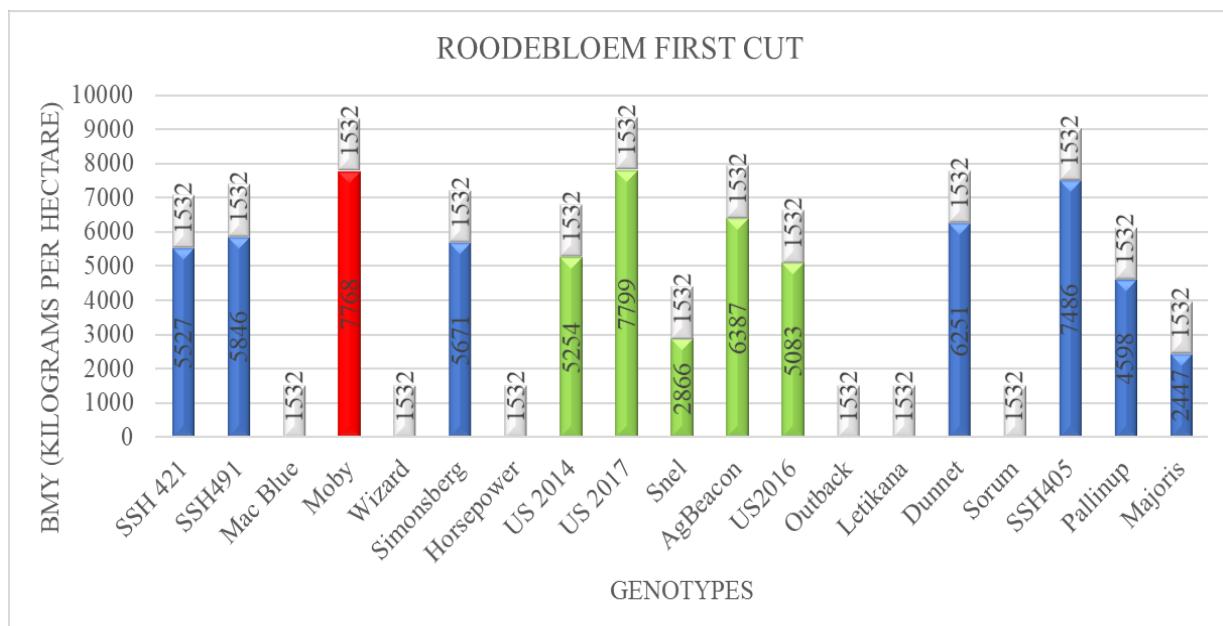
For “US2016”, all entries with a DMY above 4437.4 kg ha<sup>-1</sup> were considered significantly better. The entries that were significantly better than “US2016” were “Pallinup”, “SSH491”, “SSH405”, “Dunnet”, “Sorum”, “NCD Grazer”, “Snel” and “US2014”. The coefficient of variance (CV) was 7.95% with heritability being 0.88. This represents not only a small proportion of variation among the samples and high heritability, but also further supports the significance of these results and the idea that breeding for improved biomass in triticale is indeed viable.

#### 4.8 Roodebloem dry matter yield during 2017

The research sites for 2017 initially included Welgevallen, Langgewens and Riversdale. The trial at Riversdale was considered unfit for the purpose of this study as a result of the plot being planted over by a third party, and was replaced with an existing trial in Roodebloem (Caledon). Due to the fact that a third party planted oats seed on the same allocated trial as this, being able to distinguish the differences and identifying each cultivar (particularly in the early stages) would not have been possible.

The trial at Roodebloem was taken on later in the season in order to obtain mainly soft-dough DMY measurements. The results of the BMY during the first cutting for each entry are shown in Figure 16. The trial had a grand mean of  $5587 \text{ kg ha}^{-1}$  and an LSD for entry which was  $1532 \text{ kg ha}^{-1}$ . The coefficient of variance was 19.63% while heritability was calculated to be 0.68% and the  $R^2 = 0.787$

The top five performing cultivars were ranked from highest as follows; “US2017” ( $7799 \text{ kg ha}^{-1}$ ), “Moby” ( $7768 \text{ kg ha}^{-1}$ ), “SSH405” ( $7486 \text{ kg ha}^{-1}$ ), “AgBeacon” ( $6387 \text{ kg ha}^{-1}$ ) and lastly “Dunnet” with  $6251 \text{ kg ha}^{-1}$  BMY.

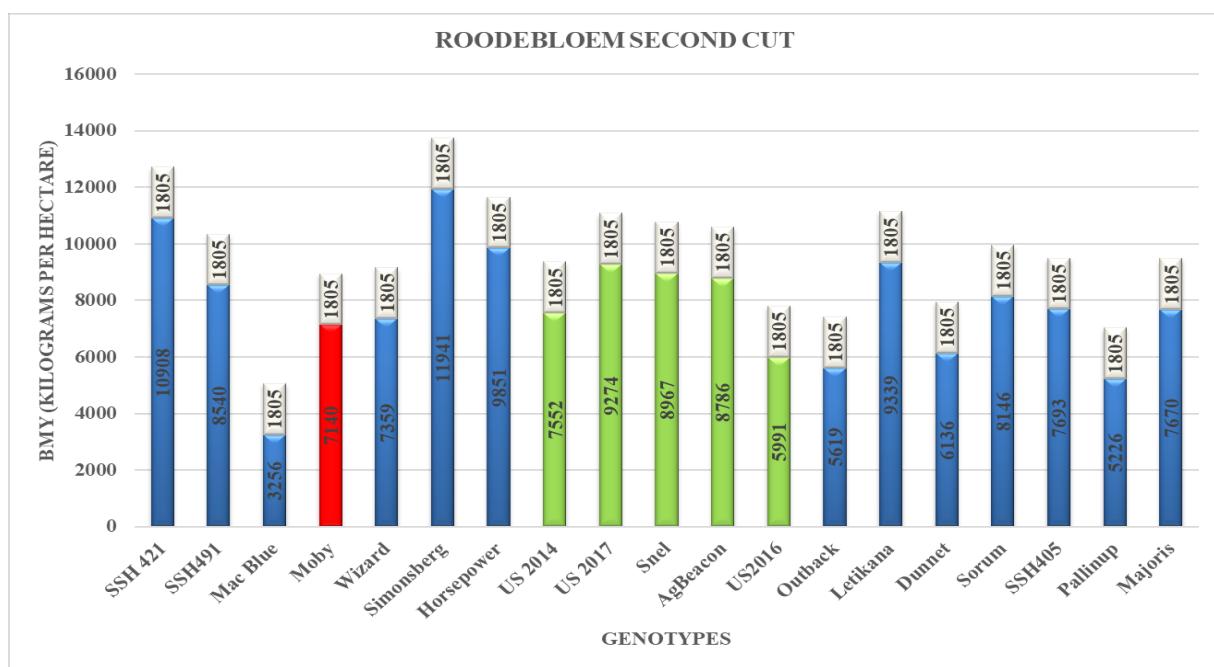


**Figure 16: Mean biomass yield of the mixed forage genotypes during the first cut at the Roodebloem site during 2017.**

Of the five selected triticale, “AgBeacon” and “US2017” were the only triticale entries that made top five with a DMY. However, “US2014” and “US2016” did moderately well in comparison to other forages yielding 5254 kg ha<sup>-1</sup> and 5083 kg ha<sup>-1</sup> respectively. “Snel” was the lowest performing entry in terms of biomass yield (2866 kg ha<sup>-1</sup>). The entries displayed in figure 16, whereby there are no BMY data recorded, was as a result of the plot being completely taken over with foreign plant material which resulted in the exclusion of those plots.

However, a possible reason for this could be that the designated plot was among many plots that were significantly put under pressure by weeds, rye and other unwanted plants. The numbers of triticale plants within the plot were also significantly lower. These could account to some extent for the low BMY recorded in some entries which were expected to yield higher DMY as was produced in other research trials conducted.

A correlation between this trial and the other trials show that plants can produce on average over 3 t ha<sup>-1</sup> DMY during this stage and further go on to regrow and produce good yields at a later developmental stage.



**Figure 17: Mean biomass yield of the mixed forage genotypes during the second cut at the Roodebloem site during 2017.**

In Figure 17 the DMY of entries planted at Caledon displays the variation between forages and triticale in terms of BMY recorded during the soft dough developmental stage. “Mac Blue” experienced the yield limiting effects under the pressure of weeds and also sustained yield loss due to wind damage. In addition to this, plant material was growing not only between the lines but between the plants as well. As a result, a strong possibility for this degree of DMY reduction could be due to some of the plant material of “Mac Blue” being mistook and removed as part of weeding. The number of plants within a row within the designated plot for this entry was therefore significantly low.

The trial had an LSD of 1804.9 kg ha<sup>-1</sup> and a grand mean of 7862.81 kg ha<sup>-1</sup> in DMY. The selected triticale entries “US2017”, “AgBeacon”, “Snel”, “US2016” and “US2014” had DMY which ranged from 5991 kg ha<sup>-1</sup> to 9274 kg ha<sup>-1</sup>. The coefficient of variance (CV) was 13.85% with heritability at 0.82%.

All of the remaining triticale entries in the trial were significantly better than “US2016” with BMY higher than the addition of BMY for “US2016” and the LSD for Entry. The highest yielding triticale entry was “US2017” with a BMY of 7274 kg ha<sup>-1</sup>. The only entry significantly better than “US2017” with a BMY higher than the LSD for Entry added to the BMY of “US2017”, is an oats cultivar “Simonsberg” with a BMY of 11941 kg ha<sup>-1</sup>. A correlation exists in which triticale on average produces better DMY than barley and rye, but remains to be superseded by oats varieties.

However, although selected oats entries tend to do better in terms of DMY than triticale, it is not always the case with the two plant types producing competitive amounts of DMY. Similar findings were reported in studies and reviews conducted on cultivar evaluation among forages (Ayalew *et al.*, 2018).

The top five performing entries ranked from the highest to the lowest BMY include: “Simonsberg” (11941 kg ha<sup>-1</sup>), “SSH421” (10908 kg ha<sup>-1</sup>), “Horsepower” (9851 kg ha<sup>-1</sup>), “Letikana” (9339 kg ha<sup>-1</sup>) and “US2017” (9274 kg ha<sup>-1</sup>) BMY. Although “US2017” was the only triticale entry to be ranked among the top 5, the triticale entries “Snel” and “AgBeacon” performed well with BMY results of 8967 kg ha<sup>-1</sup> and 8786 kg ha<sup>-1</sup> BMY respectively, being competitively close to that of “US2017” in terms of BMY. However, triticale plants’ BMY were all over 5000 kg ha<sup>-1</sup>, with “US2017” having the highest among the triticale entries yielding 9274 kg ha<sup>-1</sup> BMY.

The environmental conditions at this experimental location seems to be more optimal for the growth of forages. The maximum temperature ( $T_x$ ) averages were proportionally lower when compared to maximum temperature averages for Langgewens and Riversdale with a higher occurrence of precipitation (rain) throughout the year.

The minimum temperature averages ranged from  $7.09^{\circ}\text{C}$  to  $19.34^{\circ}\text{C}$  during the three-year period 2015 to 2017 for which data was recorded. Spring type triticale is more susceptible to displaying reduced hardiness in winter conditions. Furthermore, the later maturity of these triticale plants as opposed to the other forage plant types contributes to lower BMY observed.

#### 4.9 Statistical analysis

An NNA generated adjusted values that were better fit as it takes into better consideration the field plan, layout and other factors that will influence the statistical output. The CV percentages represents the degree of variation among entries Higher CVs are expected in a case where multiple different plant types and cultivars are planted within a single trial as each entry has a different level of variation to the next (Reynolds *et al.*, 2012). The adjusted CV percentage for Welgevallen for example, during the milk stage, was 21.03%. Within the soft dough stage cutting period, the adjusted CV values now range from 7.95% in LGW and 13.85% in Caledon, with the Welgevallen trial presenting an adjusted CV of 14.97%.

However, in comparison to the CV values obtained in the first phase of the study, these CV values were significantly lower, making the results more favourable simply because it illustrates that there was less variation between triticale and other forage type entries in terms of its biomass yield potentials. This further supports the selection of triticale for an alternative crop in supplementing forage and animal feed stocks.

The heritability values obtained for the trials by RCBD analysis ranged from 0.05 to 0.68 for the BMY obtained during the milk stage cutting. However, the NNA adjusted values showed a significantly much better and more accurate fit with heritability values during milk stage. The significantly high values recorded support the hypothesis that biomass yield potential is a highly heritable trait and can be selected for with a high degree of heritability in future

generations. The results across the trials conducted over both phase 1 and phase 2 of this study illustrate the degree of comparison among triticale entries and other forage cultivars.

It is evident that the trend that is visible supports the fact that the dual-purpose capabilities of triticale can be exploited even further by farmers as it offers additional flexibility for other small grains in winter and spring for forage production. The high H values as well as lower CV values and well fitted model supports the need to propose further breeding of triticale varieties for increased biomass yield potentials for use as animal feed. Furthermore, the excellent level of resistance across different geographical locations and in various environmental conditions, along with favourable characteristics shown in its ability for regrowth and biomass yield potential makes this crop a good alternative for supplementing animal feed stocks during the season.

#### **4.10 Nutritional parameters**

The nutrient composition of grains is affected by many factors, including grain variety (genetic factors), growing location (agronomic conditions) and the season or environmental factors (Alheit *et al.*, 2012). However, in this study, nutrient composition was similar between the triticale cultivars in two consecutive harvesting years. Such similarities between grain batches have been reported by researchers working at different locations on different cultivars of triticale (Reynolds *et al.*, 2012).

Triticale appears to have genetic uniformity between the cultivars, although breeding programmes are still keen to improve their quality. The DM content of the triticale entries in the current study was comparative to that to results reported in other studies. Variations, particularly lower DM content, may be the result of differences in varieties, drought conditions as experienced in the Western Cape during the trial season, as well as the growth location of the triticale used in the studies. The triticale cultivars used in the current study were the spring varieties, a variety widely suited to the regions in the WC which these trials have been set up.

Animal feed, particularly fodder and silage, aims to have high protein and good fibre compositions. These triticale entries serve part of the first selection as possible crossing parents that can be further included into a formal breeding programme that is aimed at breeding for increased biomass yield. Furthermore, these varieties can further be included into breeding for

better nutritional compositions to provide an even better protein and fibre source for animal consumption.

The relationship between crude protein, ash, fat and fibre is clearly illustrated among the different forage plant types (triticale, oats and barley). The reason that no rye entries were included in the analysis was due to the fact that rye never reached the soft dough plant developmental stage. For the purpose of this study we focused on the “As is” percentage values and not that of dry Matter.

Among the list of selected entries, eight were triticale (“USTRITEL020”, “US2018”, “US2017”, “US2016”, “US2014”, “US2011”, “Snel” and “AgBeacon”). There were four oats cultivars namely “SSH421”, “Simonsberg”, “Pallinup” and “Majoris” with only a single barley entry (“Moby”). “USTRITEL020”, “US2018” and “SSH421” only had two out of three samples that were analysed as the analysis of the third replicate was not possible as a result of sample contamination.

It is important to have some idea of the dry matter (DM) content of the respective feed because cattle usually consume a fairly predictable quantity of dry matter per day, if feed is readily available. Cattle has been reported to consume DM equivalent to two to three percent of its body weight per day (Botai *et al.*, 2017). Other animals may however consume more silage (between 20% and 30% DM content) than hay (between 80% to 90% DM) daily, despite the fact that feeds may be similar in the amount of energy and protein content in terms of DMY. The DMY reported in previous studies were on average above 90% (Botai *et al.*, 2017).

The average DM content is significantly high among all the entries selected for nutritional analysis. “SSH421” (oats) had the highest DM content with “Simonsberg” and “Pallinup” following with 94.29% and 94.17% respectively. These three entries that were ranked top in terms of DM content were all oats cultivars. This was in line with what was hypothesised considering the fact that oats plants yield more green feed or plant material per unit area than most other cereal or forage crops.

Despite the higher DM content reported in oats cultivars, triticale entries yielded highly competitive amounts of dry matter. A DM content of 94.03% is reported in “US2016”. The DM content for the triticale entries ranged from 93.32% and 94.03%. The percentage of crude fibre among the 14 entries ranged from 17.99% to 29.24%. “Simonsberg” had the highest crude

fibre content with “US2014” having the lowest. All the oats cultivars along with the individual barley cultivar (“Moby”) were substantially higher (over 23.94%) than that of the triticale entries. The oats cultivars’ crude fibre percentage ranged from 26.69% to 29.24% which constitutes a third of the total nutritional makeup of the plant. Triticale entries had a crude fibre percentage that ranged from 17.99% to 23.86% constituting roughly a fifth of the total nutritional composition of the plant.

Although triticale cultivars were significantly lower in terms of the crude fibre percentage in contrast to the oats and barley entries, the crude fibre percentage remains competitively comparable. The important component to consider is the ratio of crude protein percentage and crude fibre percentages. Previous studies have displayed variable results in this regard and could be a result of different environmental conditions, genetic make-up or a combination of multiple components that may affect the nutritional parameters recorded.

In this study, the ratio of the crude protein content: crude fibre content was comparative to that reported in previous studies. A positive correlation between the ratio of crude protein content: crude fibre content was seen in some cases but not in others. It was hypothesised that with low crude protein content the opposite would be evident in crude fibre content. However, looking into the cultivars with the highest crude protein content namely “Pallinup”, “USTRITEL020” and “Snel”, the crude fibre content corresponded differently.

The average ratio of crude protein: crude fibre percentage for “Pallinup” is reported as 10.88%: 26.69% with “USTRITEL020” and “Snel” with average ratio of crude protein: crude fibre content of 9.41%: 20.86% and 8.46%: 19.41% respectively. Taking into consideration the three top entries, a relationship that is directly proportional is observed. The higher the crude protein content within the plant, the higher the crude fibre content within that entry. However, this was not true for all entries as there were entries that displayed an inverse relationship between the ratios of crude protein content: crude fibre content.

The percentage of crude fat content reported as “As is” only made up a small proportion of the total nutritional components of the plants. The crude fat content for the entries selected ranged between 1.14% and 3.66%. The entry with the highest crude fat content was “Pallinup”. “SSH421”, “SSH491” and “Simonsberg” followed thereafter with crude fat content of 2.72%, 2.19% and 2.14% respectively. All of these were oats cultivars. Triticale had a very similar

nutritional composition as the oats cultivars in terms of the percentage of crude fat content. Triticale had a crude fat content slightly lower than that reported within the oats cultivars, but also slightly higher than that of “Moby” (barley). “AgBeacon” followed with crude fat content of 1.83%. “USTRITEL020” and “US2014” both had crude fat content of 1.73%.

The protein requirements of cattle vary according to the mass and type of animal, as well as the level of production (growth, reproduction and lactation). It is important to know the protein contents of various feeds so that feeding management can match the protein available in an animal’s diet with the animal’s needs. Crude protein values give a good indication of whether or not a particular feed will satisfy the protein needs of an animal, but two points should be considered:

The crude protein content reported as “As is” was comparative to other studies and ranged from 5.04% to 10.88%. “Pallinup”, an oats cultivar, had the highest crude protein content (10.88%) with the lowest also being an oats cultivar, “SSH421” with a crude protein content of 5.04% being reported. Triticale entries followed immediately after “Pallinup”, with “USTRITEL020” having a crude protein content of 9.41% and “Snel” with a crude protein content of 8.46% which is relatively higher than hypothesised. “Moby” (Barley) had a crude protein percentage of 8.33%. However, the crude protein content varied considerably within the oats cultivars as opposed to the relatively consistent uniformity observed in the crude protein content among the different triticale cultivars.

From these results it can be deduced that oats provided the best biomass yield in terms of quantity, but further have good nutritional parameters particularly in crude protein and crude fat contents. Although the oats varieties were expected to perform in this manner, the triticale entries have just fell short of a similar degree of performance.

It can therefore be deduced that “USTRITEL020”, “Snel”, “AgBeacon” and “US2014” are the best suited triticale entries during phase 1 in terms of dry matter yield and the nutritional value with regards to the ratio between crude protein content and crude fat content. These cultivars have displayed superior characteristics in comparison to other plant types included in the study as well as other triticale cultivars. The triticale entries were highly competitive with the oat’s cultivars within the study, but were superior to the other plant types such as rye and barley used within the study in terms of its dry matter yield potential and other nutritional characteristics.

## 4.11 Statistical analyses on nutritional components

### 4.11.1 Ash content

The results of ash content produced through Agrobase are depicted in Table 14. From the ANOVA conducted, the grand mean was calculated to be 4.41%, with an R-squared value of 0.88. Furthermore, the overall test had a CV of 11.88% with a value of 0.794. Utilising the LSD value against the mean of each entry for ash content it is possible to distinguish the degree of statistically significant differences between the entries and identify which are significantly better and/or worse.

The R-squared value (0.879) indicates how close the data fits the regression line. It indicates that the model explains 87.94% of the variability of the response data around its mean. The coefficient of variation (CV) was created to measure population variability. The CV can be used to measure variability in genetic populations, to determine the best plot size in uniformity trials, to measure stability of phenotypes, or measure variation in other individual or population attributes. The CV for the statistical analyses for ash content is 11.88%, depicting that there is a low degree of variability among the sample population.

Taking into consideration the aims and objectives of breeding, the lowest CV values are commonly what would be desired. The heritability was 0.794, which explains that a large percentage (79.4%) is due to genetic variation between individuals in this sample population. High heritability further denotes that the traits are transferable to future generations. Looking at the reported results with regard to Ash content within the 14 entries analysed, “SSH421”, “Pallinup”, “Simonsberg”, “Moby” and “SSH491” were among the top five entries. Ash content values among the top 5 ranged from 6.27% to 5.27%.

Ash content values for the entire set of entries ranged from 6.27% to 3.15%. Four of the five top ranked entries were oats cultivars. In terms of the LSD calculated from the ANOVA, entries with the ash content below 5.54% differed with statistical significance to “SSH421” (ash = 6.27%).

The entries that were significantly lower included Moby, “SSH491”, “Majoris”, “AgBeacon”, “Snel”, “US2016”, “US2014”, “US2011”, “US2018”, “USTRITEL020” and “US2017”. “US2017” has a reported average of 3.15% with a CV of 14.1. Based on the LSD calculated in the ANOVA, a number of entries differ with statistical significance. “Snel”, “AgBeacon”, “Majoris”, “SSH491”, “Moby”, “Simonsberg”, “Pallinup” and “SSH421” were all statistically and significantly better than “US2017” in terms of Ash content. For the triticale entry “AgBeacon”, the LSD added to the mean is equal to 4.78%.

From this deduction entries “Majoris”, “SSH491”, “Moby”, “Simonsberg”, “Pallinup” and “SSH421” had means above this value and could be considered statistically as good and significantly better than “AgBeacon”. The difference between the entry mean and the LSD is equal to 3.32%. The entries “US2018”, “USTRITEL” and “US2017” were the only three entries that had mean values lower than this and thus were characterised as being significantly worse in terms of ash content when compared to the “AgBeacon” entry.

The barley entry (“Moby”) had a mean of 5.37%. The mean in addition to the LSD was calculated to be 6.10%. An oats entry (“SSH421”) was the only entry that had a higher mean than 6.10% and could be classified as being significantly better than “Moby” in terms of ash content. The difference between the mean and the LSD was calculated to be 4.64%. The entries “AgBeacon”, “Snel”, “US2016”, “US2014”, “US2011”, “US2018”, “USTRITEL020” and “US2017” all had means below this and thus were significantly worse than the “Moby” entry in terms of ash content.

The oats entry (“Simonsberg”) had a mean of 5.78%. The LSD and mean combined equated to 6.52%. There are no entries that had a higher mean than the calculated amount and therefore none were considered to be significantly better than the “Simonsberg” entry. However, the difference between the mean and LSD calculated was 5.05%. The entries “Majoris”, “AgBeacon”, “Snel”, “US2016”, “US2014”, “US2011”, “US2018”, “USTRITEL020” and “US2017” had mean values below the calculated amount and were therefore regarded as being significantly worse than the “Simonsberg” entry in terms of ash content.

The three triticale entries “Snel”, “US2011” and “US2016”, had individual means of 3.90%, 3.45% and 3.79% respectively. For “Snel” the mean and the LSD combined was calculated to be 4.63047%. For “US2011” and “US2016”, the same combination was equal to 4.18% and 4.52% respectively. The entries “Majoris”, “SSH491”, “Moby”, “Simonsberg”, “Pallinup” and “SSH421” had individual means above the calculated amounts in respect to each of the three triticale entries mentioned above.

For this reason, they were considered to be significantly better than “Snel”, “US2011” and “US2016” in terms of ash content. The differences between the individual mean of the entry and the LSD for “Snel”, “US2011” and “US2016” were calculated to be 3.16%, 2.72% and 3.05% respectively. In each instance there were no entries that had individual means below the calculated amount and were therefore not regarded as being significantly worse in terms of ash content than the three entries. From these deductions a trend can be observed in terms of ash content.

The oats entries were significantly better than the triticale entries within the trial. Furthermore, the barley entry was comparable to that of the top performing oats entries and was significantly better than the triticale entries in terms of ash content. However, ash content within the triticale entries were observed to be more uniform ranging from 3.15% and 4.05% in comparison to the oats cultivars which displayed more variability between the entries with a range between 4.89% and 6.27%. Table 14 represents all the entries analysed for ash content and the respective rankings in an ascending order. The entries are clustered into three groups namely the lower, middle and upper clusters which correspond in an ascending order from lowest to highest ash content per entry.

**Table 14: Agrobase CV and yield ranking outputs (ascending) for the 14 entries analysed for ash content.**

Rank	Averages (%)	
	--- Y ---	CV
1 US2017	3.1500	14.1
2 UStritel020	3.2030	10.3
3 US2018	3.3030	10.0
4 US2011	3.4500	8.40
5 US2014	3.4900	9.40
6 US2016	3.7867	18.3
7 Snel	3.8967	19.1
8 AgBeacon	4.0500	17.1
9 Majoris	4.8900	8.70
10 SSH491	5.2680	8.50
11 Moby	5.3700	14.7
12 Simonsberg	5.7833	10.8
13 Pallinup	5.8700	1.50
14 SSH421	6.2700	10.9
LSD = 0.74		

#### 4.11.2 Crude protein content

The Agrobase output results for the Crude protein content analysis were evaluated with the same entries being analysed for ash content and the other nutritional parameters. Protein was one of the core components of interest when it came to analysing animal feed. Triticale entries “USTRITEL020”, “AgBeacon” and “Snel” had crude protein contents which ranged between 8.01% and 9.55% and were categorised in the middle-upper ranked entries. The oats cultivar “Majoris” ranked the lowest for crude protein content, with the entry mean of 5.04% as displayed in Table 15.

There were no entries that were significantly poorer than “Majoris”. All entries with an entry mean for crude protein content of 5.98% and above were classified as being significantly better than “Majoris” in terms of crude protein. These were “SSH491”, “US2016”, “SSH421”, “Simonsberg”, “US2018”, “US2017”, “US2011”, “US2014”, “AgBeacon”, “Moby”, “Snel”, “USTRITEL020” and “Pallinup”. The oats entry “Pallinup” had the highest amount of crude protein content with an entry mean of 10.8767% and thus there were no entries considered significantly better than “Pallinup” in terms of crude protein.

The difference between the mean and the LSD was calculated and all entries below 9.94% were significantly lower than “Pallinup”. The entries which had means below 9.94% were “USTRITEL020”, “Snel”, “Moby”, “AgBeacon”, “US2014”, “US2011”, “US2017”, “US2018”, “Simonsberg”, “SSH421”, “US2016”, “SSH491” and “Majoris”. “Pallinup” was the only entry that did significantly better than all the other entries in terms of crude protein content. “SSH491” (oats) had an entry mean of 6.46% with the second lowest mean among the entries.

The difference between the mean and the LSD was 5.52%. Since no entries were below this threshold, none were significantly worse than “SSH491”. The upper threshold was calculated to be 7.4% with entries “US2018”, “US2017”, “US2011”, “US2014”, “AgBeacon”, “Moby”, “Snel”, “USTRITEL020” and “Pallinup” performing significantly better than “SSH491” in this regard.

“Moby” (barley) was ranked among the top five highest in terms of crude protein content. The lower threshold calculated by the difference between the LSD and entry mean for “Moby” was 7.40%. “SSH421”, “US2016”, “SSH491” and “Majoris” were the only entries that were considered to be significantly worse than “Moby”. The upper threshold was calculated to be 9.27% with “USTRITEL020” and “Pallinup” being above this threshold once again. Upon the evaluation of the triticale entries, a trend was observable. “Snel” (Triticale) had a lower threshold calculated (7.52%). The entries US2018,

“Simonsberg”, “SSH421”, “US2016”, “SSH491” and “Majoris” performed poorer than “Snel” in terms of crude protein content. The upper threshold calculated using the LSD and the entry mean was 9.40%.

“USTRITEL020” and “Pallinup” were the only two entries that were significantly better than “Snel”. “US2014” and “AgBeacon” (Triticale) had lower thresholds of 7.07% and 7.07% respectively. “SSH491” and “Majoris” were significantly lower than both “US2014” and “AgBeacon”. The upper thresholds of “US2014” and “AgBeacon” were 8.95% and 8.954% respectively. “USTRITEL020” and “Pallinup” were significantly better than both these triticale entries. A trend that seems to be observable is that both “Pallinup” and “USTRITEL020” were the dominant entries in the sample population analysed for crude protein content. “Pallinup” was the only oats entry among the top ranked along with the barley cultivar “Moby”. However, triticale entries had more of a uniform trend with results for crude protein content ranging from 7.10% to 9.56%. The triticale entries were ranked from middle to top performers and did significantly better than the oats varieties within the study concerning crude protein content.

**Table 15: Agrobase CV and yield ranking outputs (ascending) for the 14 entries analysed for crude protein.**

Rank	Averages (%)	
	--- Y ---	CV
1 Majoris	5.0400	13.5
2 SSH491	6.4602	12.7
3 US2016	7.0967	16.1
4 SSH421	7.2667	6.40
5 Simonsberg	7.3200	14.9
6 US2018	7.4852	10.2
7 US2017	7.6533	6.70
8 US2011	7.9267	9.90
9 US2014	8.0067	7.20
10 AgBeacon	8.0133	8.20
11 Moby	8.3333	2.40
12 Snel	8.4567	1.50
13 UStritel020	9.5552	6.30
14 Pallinup	10.8767	6.80
LSD = 0.94		

### 4.11.3 Dry matter yield

Dry matter yield (DMY) was analysed through a statistical package in Agrobase as well. The results generated a grand mean of 93.83 with ‘LSD for Entry’ of 0.38. Furthermore, an  $R^2$  value of 0.65 indicated that approximately 65% of the generated outputs fit well with the regression model. Table 16 displays the entries analysed for DMY and their respective rankings.

A CV value of only 0.29% accounted for a very small percentage of variability, which was further supported with entry means ranging between 93.28 and 94.32. Heritability for this nutritional characteristic was 0.40% which suggested that approximately 40% could be attributed to genetics.

The “US2018” triticale entry had no entries that were regarded as significantly worse, as this was the lowest in terms of the DMY. The upper threshold calculated was 93.66% and the entries “AgBeacon”, “US2011”, “Snel”, “Moby”, “SSH421”, “Majoris”, “US2016”, “Pallinup”, “Simonsberg” and “SSH491” were all considered to be significantly better performers, having DMY values above this threshold.

“AgBeacon” and “Snel”, both triticale entries, had an entry mean for DMY of 93.69% and 93.79% respectively. The lower thresholds calculated for “AgBeacon” and “Snel” were 93.31% and 93.40%. Both these entries were ranked among the middle group and the only entry that was below the lower threshold was “US2018”. “US2018” was therefore significantly worse than both “AgBeacon” and “Snel” in terms of DMY. The upper threshold for the two (“AgBeacon” and “Snel”) were 94.07% and 94.14% respectively. Regarding both these cultivars, the entries “Pallinup”, “Simonsberg” and “SSH491” were the only entries with DMY means above this and was therefore regarded as significantly better than both. “US2016” (triticale) had a DMY mean of 94.03% and was ranked the fourth highest.

The lower threshold was 93.66% in terms of the DMY with the entries “US2014”, “USTRITEL020” and “US2018” being above this threshold. Thus, these entries were significantly worse than “US2016”. The upper threshold computed was 94.41% with no entries observed to have been above this threshold. Therefore, no entries were significantly better than “US2016”.

The barley entry (“Moby”) and the oats entry “SSH421” had upper thresholds of 94.29% and 94.24% respectively. In both instances, the only two entries that were above the threshold were “Simonsberg” and “SSH491” and can therefore be regarded as significantly better than either “Moby” or “SSH421” in terms of DMY. “SSH491” had a lower threshold of 93.53%, with “USTRITEL020” and “US2018” falling below this threshold. Moby had a lower threshold of 93.39% with only “US2018” falling below the threshold. Thus, “USTRITEL020” and “US2018” can be regarded as significantly worse than “SSH421” with “US2018” also being significantly worse than “Moby” as well.

**Table 16: Agrobase CV and yield ranking outputs (ascending) for the 14 entries analysed for DMY.**

Rank	Averages (%)	
	--- Y ---	CV
1 US2018	93.28	0.1
2 UStritel020	93.51	0.2
3 US2014	93.56	0.4
4 US2017	93.64	0.5
5 AgBeacon	93.69	0.1
6 US2011	93.72	0.3
7 Snel	93.76	0.1
8 Moby	93.77	0.3
9 SSH421	93.91	0.2
10 Majoris	94.02	0.3
11 US2016	94.03	0.5
12 Pallinup	94.17	0.3
13 Simonsberg	94.29	0.2
14 SSH491	94.32	0.0
LSD = 0.38		

Taking the top ranked entry, “SSH491” (oats), into consideration, there were no entries that were significantly better than “SSH491”. The lower threshold was 93.94% with the entries “SSH421”, “Moby”, “Snel”, “US2011”, “AgBeacon”, “US2017”, “US2014”, “USTRITEL020” and “US2018” all regarded as significantly worse in terms of the DMY. From these deductions, the oat entries were dominant in terms of the DMY, as was expected.

The reason for this expectation was simply due to oat plants’ generating more green leaf plant material. However, triticale was highly comparable to both oats and the barley entries; with “US2016” further being ranked among the top five. Thus, triticale could be regarded as a suitable alternative yielding a similar amount of DMY as opposed to the other oat varieties tested.

#### **4.11.4 Fat content**

In terms of fat content, the same entries within the study were also assessed. The study population had a grand mean of 1.94% with an R-squared value of 0.96%. This R-squared value was significantly close to one and represented the goodness of fit of the model. In addition to this, the CV amount of 8.18% illustrated that there was significantly low percentage of variation observed among the entries. The LSD for entry was equal to 0.22 as calculated from the ANOVA conducted.

There was a high degree of heritability which equalled 0.93 which indicated that a large percentage (~93.3%) may have been attributed to the genetic makeup and influence of the entries. “Majoris” (oats) was ranked the lowest of all the entries in terms of the fat content. Therefore, there were no entries that were regarded as significantly worse than “Majoris” in terms of performance.

The upper threshold calculated for “Majoris” using the entry mean and LSD was 1.72%. The fat content for entries “US2014”, “USTRITEL020”, “AgBeacon”, “Simonsberg”, “SSH491”, “SSH421” and “Pallinup” were all above this and were considered as significantly better among the entries. “US2016”, “US2011”, “US2017” and Moby had no entries that were below the lower thresholds calculated for each of them. The LSD was a relatively small number in comparison to the intervals between the mean values per entry. With no entries below the threshold, none were considered significantly worse than these entries. “US2016”, “US2011”,

“Moby” and “US2017” had upper thresholds calculated for each which ranged between 1.76% and 1.81%. “AgBeacon”, “Simonsberg”, “SSH491”, “SSH421” and “Pallinup” were among the entries that were considered to be significantly better than each of these in terms of fat content.

The triticale entries “US2018” and “Snel”, had no entries that were below the lower thresholds calculated per entry. There were no entries significantly worse than “US2018” and “Snel”. The upper thresholds calculated for “US2018” and “Snel” were 1.89% and 1.92% respectively. In this instance the entries “Simonsberg”, “SSH491”, “SSH421” and “Pallinup” were considered significantly better than the two triticale entries in terms of fat content.

The entries “US2014” and “USTRITEL020” had a lower threshold being 1.51% and 1.51% respectively. The only entry which fell below this threshold in both instances was “Majoris”, and was therefore regarded as significantly lower than “US2014” and “USTRITEL020”. “AgBeacon” (triticale) had a lower threshold of 1.62%, while entries “US2017”, “Moby”, “US2011”, “US2016” and “Majoris” being was significantly worse than “AgBeacon”. In the case of the three triticale entries (“US2014”, “USTRITEL020” and “AgBeacon”), the upper threshold per entry were below 2.14. Therefore, the entries “Simonsberg”, “SSH491”, “SSH421” and “Pallinup” was consistently significantly better than all three triticale entries in terms of fat content.

“Pallinup” and “SSH421”, both oats, were ranked first and second respectively in terms of fat content. Although both entries had no entries that were above the upper thresholds calculated per entry, “SSH421” differed significantly from “Pallinup”. The results displayed that “Simonsberg”, “SSH491”, “SSH421” and “Pallinup” were significantly better than most entries within some of the analyses. The oats varieties produced the highest fat content consistently and can be clearly classified as higher in fat content in comparison to the triticale and barley entries. Table 17 displays the entries in respect of their rankings in terms of fat content.

**Table 17: Agrobase CV and yield ranking outputs (ascending) for the 14 entries analysed for fat content.**

	Averages (%)	
Rank	--- Y ---	CV
1 Majoris	1.4933	11.4
2 US2016	1.5400	3.20
3 US2011	1.5467	7.40
4 Moby	1.5633	3.70
5 US2017	1.5967	2.00
6 US2018	1.6665	3.80
7 Snel	1.7000	10.9
8 US2014	1.7267	3.40
9 UStritel020	1.7315	12.3
10 AgBeacon	1.8333	3.90
11 Simonsberg	2.1400	10.7
12 SSH491	2.1864	0.30
13 SSH421	2.7167	11.0
14 Pallinup	3.6633	5.10
LSD = 0.22		

#### 4.11.5 Fibre content

The results computed for fibre content generated a grand mean which was 21.84% and the Rsquared value of 0.94. This suggested that the model fits well (~94%) with the regression model. The CV was 6.02% which is significantly low. This accounted for a small percentage of variation among the samples within the study. The LSD for ENTRY was 1.8396. Using the LSD and the means of the individual entries, an upper and lower threshold is calculated to identify significant differences among the entries.

Heritability ( $h_b^2 = 0.894\%$ ), suggested a large influence which is attributed to the genetic makeup within the plants. Table 18 displays the rankings along with the individual CV for each entry. Key significant differences are discussed to establish and illustrate an observable trend in order to make further deductions.

The entry which ranked the lowest in terms of fibre content was “US2014” with an average of 16.84%. Being the lowest ranked, there were no entries that could further be significantly worse than “US2014”. The upper threshold calculated with the use of the LSD and individual entry mean was 8.94%. The entries “AgBeacon”, “US2011”, “Snel”, “US2018”, “USTRITEL020”, “US2016”, “US2017”, “Moby”, “Pallinup”, “SSH491”, “Majoris”, “SSH421” and “Simonsberg” had fibre content averages above this and were considered significantly better than “US2014”.

The triticale entry “US2018” had a fibre content average of 19.16% and was clustered between the middle-ranked entries in terms of its performance in fibre content. The lower threshold calculated was 17.32%, with entries “US2014”, “AgBeacon” and “US2011” being significantly worse than “US2018”. The upper threshold was calculated to be 20.99%, with entries “US2016”, “US2017”, “Moby”, “Pallinup”, “SSH491”, “Majoris”, “SSH421” and “Simonsberg” being significantly better than “US2018” in terms of fibre content.

Another triticale (“US2016”) also clustered within the middle in terms of its ranking and had a lower threshold of 19.28%. The entries “US2014”, “AgBeacon”, “US2011”, “Snel” and “US2018” were therefore regarded as significantly lower in fibre content than “US2016”. With an upper threshold of 22.96%, the entries “Pallinup”, “SSH491”, “Majoris”, “SSH421” and “Simonsberg”, were significantly better than “US2016” in terms of fibre content. “Pallinup”, was the lowest among the top cluster of entries with an average of 25.13%.

The lower threshold calculated for “Pallinup” was 23.29% with entries “US2014”, “AgBeacon”, “US2011”, “Snel”, “US2018”, “USTRITEL020”, “US2016”, “US2017” and “Moby” being significantly lower than “Pallinup”. The upper threshold was 26.97% with “SSH421” and “Simonsberg” being significantly better than “Pallinup”. “SSH491” had a lower threshold of 23.44% with entries “US2014”, “AgBeacon”, “US2011”, “Snel”, “US2018”,

“USTRITEL020”, “US2016”, “US2017” and “Moby” being significantly lower than “SSH491”.

The upper threshold of “SSH491” was 27.12%, leaving “SSH421” and “Simonsberg” significantly better than “SSH491” in terms of fibre content. “Majoris”, “SSH421” and “Simonsberg” had upper thresholds above the 27.57% and therefore had no entries that were significantly better in terms of fibre content. The highest ranked entry (“Simonsberg”) with an average of 27.57% for fibre content, had a lower threshold of 25.73% with entries “SSH421”, “Majoris”, “SSH491”, “Pallinup”, “Moby”, “US2017”, “US2016”, “USTRITEL020”, “US2018”, “Snel”, “US2011”, “AgBeacon” and “US2014” all significantly lower than “Simonsberg”. Triticale entries were therefore clustered within the bottom and middle cluster in terms of fibre content. The entire top cluster was composed of oats entries with “SSH421” and “Simonsberg” being the dominant two entries in this regard.

**Table 18: Agrobase CV and yield ranking outputs (ascending) for the 14 entries analysed for fibre content.**

Rank	Averages (%)	
	--- Y ---	CV
1 US2014	16.84	7.10
2 AgBeacon	17.10	8.20
3 US2011	17.20	3.80
4 Snel	17.94	6.30
5 US2018	19.16	1.70
6 UStritel020	19.67	12.2
7 US2016	21.12	7.30
8 US2017	22.34	7.10
9 Moby	22.45	10.8
10 Pallinup	25.13	3.20
11 SSH491	25.28	1.80
12 Majoris	26.69	4.00
13 SSH421	27.20	1.30
14 Simonsberg	27.57	0.30
LSD = 1.84		

#### 4.11.6 Moisture content

The final nutritional parameter assessed was moisture content among the same study samples. The grand mean for the analysis was 6.16% with an R-squared value of 0.65, suggesting a good sense of fit to the regression model. The CV of 4.44% explains a small percentage of variation within the analysis.

The LSD for entry was 0.38 which was used in conjunction with the individual averages of the entries for moisture content to calculate lower and upper thresholds similarly to that done in the above analyses. The degree that can be attributed to the genetic makeup of the plants in the

study was just below 40% as the heritability was calculated to be 0.40. Table 19 displays the entries analysed for moisture content and their respective rankings.

The lowest ranked entry in terms of moisture content was “SSH491” with an average of 5.6607% and was the first entry clustered among the lower entries. Considering the fact that this was the lowest entry, none were regarded to be significantly worse. The upper threshold was calculated to be 6.04%, with entries “SSH421”, “Moby”, “Snel”, “US2011”, “AgBeacon”, “US2017”, “US2014”, “USTRITEL020” and “US2018” all being significantly better than “SSH491”.

“Majoris”, also an oats cultivar, was in the lower cluster with an average of 5.98% for moisture content. The lower threshold was calculated to be 5.60% with no entries remaining to be classified as being significantly lower than “Majoris”. Therefore, all entries within the lower cluster namely, “SSH491”, “Simonsberg”, “Pallinup”, “US2016” and “Majoris” had no entries that were significantly worse. The upper threshold calculated for “Majoris” was 6.36%, with entries “US2017”, “US2014”, “USTRITEL020”, “AgBeacon” and “US2018” being significantly better than “Majoris” for moisture content.

“SSH421” was in the middle cluster having a lower threshold calculated which was 5.71%. The entries “Simonsberg” and “SSH491” had averages below this threshold and were therefore significantly worse than “SSH421”. The upper threshold of 6.48% resulted in the entry “US2018” being the only entry significantly better than “SSH421”. The triticale entry clustered in the middle cluster and had an average of 6.23% with a lower threshold calculated to be 5.84%. “Pallinup”, “Simonsberg” and “SSH491” were below this threshold and were significantly worse than “Moby”. With an upper threshold of 6.61%, “US2018” was again the only entry significantly better than “Moby”.

“AgBeacon” was the highest among the lower cluster with an average of 6.31% for moisture content. The lower and upper thresholds for “AgBeacon” were 5.93% and 6.70% respectively. The entries “Pallinup”, “Simonsberg” and “SSH491” were significantly worse than “AgBeacon”, whilst “US2018” again remained the only entry significantly better than “AgBeacon” in terms of moisture content. The triticale entry “US2014” was clustered in the

middle with an average of 6.44%. The lower and upper thresholds for “US2014” were 6.05% and 6.82% respectively.

The entries “Majoris”, “US2016”, “Pallinup”, “Simonsberg” and “SSH491” were significantly worse than “US2014”. However, with a threshold above the 6.70%, there were no entries that were significantly better than “US2014”. This was observable for “USTRITEL020” and “US2018” as well as the upper thresholds are above or equal to 6.70%, therefore, no entries are significantly better in this regard. However, in the instance of “US2018”, the lower threshold was 6.32% with “AgBeacon”, “US2011”, “Snel”, “Moby”, “SSH421”, “Majoris”, “US2016”, “Pallinup”, “Simonsberg” and “SSH491” all significantly lower than “US2018” in terms of moisture content.

Most of the triticale entries were clustered in the top cluster and some in the middle with only “US2016” in the lower cluster based on moisture content of the plants’. The oats entries were all ranked below the triticale entries, and thus triticale unanimously had higher moisture content than oats entries. In addition to this, from the clusters, an observable trend which displays the fact that both the oats and triticale entries had little variation between the individual entries, and clustered together in most cases where moisture content was concerned.

The low LSD and the small statistical intervals between the averages illustrate that all the plant types were highly comparable with one another and produced similar moisture contents. Through the collective observations made on the various results achieved, it can be deduced that triticale entries performed considerably consistent throughout in the respective parameters assessed. “US2018”, “USTRITEL020”, “Snel” and “AgBeacon” seemed to be among the top ranked triticale entries in the respective analyses. In addition to these observations; it was evident that the oats cultivars did really well in terms of forage production and the nutritional quality thereof. Regardless of this, triticale cultivars were highly competitive in this regard. It was hypothesised that the oats cultivars would dominate in terms of biomass yield production and furthermore provides better nutritional quality in terms of plants used for animal feed.

**Table 19: Agrobase CV and yield ranking outputs (ascending) for the 14 entries analysed for moisture content.**

Rank	Averages (%)	
	--- Y ---	CV
1 SSH491	5.661	0.1
2 Simonsberg	5.710	3.7
3 Pallinup	5.833	4.1
14 US2016	5.970	8.5
5 Majoris	5.980	5.0
6 SSH421	6.093	2.8
7 Moby	6.227	4.3
8 Snel	6.240	1.7
9 US2011	6.280	4.4
10 AgBeacon	6.313	1.5
11 US2017	6.363	6.7
12 US2014	6.437	6.2
13 UStritel020	6.466	2.9
14 US2018	6.701	1.8
LSD = 0.38		

Triticale can therefore be considered as a suitable alternative to oats. With increased breeding initiatives to optimise biomass yield potential within the plant types it could further offer an even better alternative to supplement feed stocks through the seasons. “USTRITEL020”, “US2018”, “US2017”, “Snel” and “AgBeacon” were among the highest producers of biomass yield among the triticale as well as oats and barley entries in some cases within the study. For this reason, these five entries were further shortlisted to be considered as suitable crossing parents for inclusion into a breeding programme aimed at increasing biomass yield production.

Through the assessment of the data generated from the trials over the period of 2016 and 2017, it is further hypothesised that the selection of high biomass yielding crossing parents for inclusion into breeding programs will surely lead to the successful transfer of desirable traits and produce higher biomass yielding triticale varieties with improved forage quality.

The statistical analysis on the results in this study provides more information for a more calculated approach to being able to prove such a hypothesis. Looking into the degrees of variation, heritability and ultimately the response to selection are considered to be vital when initiating breeding approaches for improved varieties (Reynolds *et al.*, 2012).

The degrees of variation reported across the trials were too an extent that which was hypothesised. There was a greater degree of variation between triticale and other the other small grain crops within the mixed trials with higher coefficients of variance reported. However, although plants differed from one another in terms of genetic make-up, the results over the two seasons were relatively consistent. A large portion of the variability within the BMY potential and nutritional composition of the plants can be associated to genetic and environmental interaction. The high degree of heritability reported in most instances represents that BMY potential is indeed a trait that can be highly transferable and can be selected for future generation plants.

The nutritional analyses results added further value to this allowing a better comparison between triticale and other forages in terms of nutritional value. Triticale entries were competing along with oats entries among the top performing plants in terms of both DMY and nutritional value. Although oats entries have proven to have greater nutritional value, particularly in crude fibre, fat and crude protein, triticale ranked just below these oats' entries in terms of these three parameters. However, triticale entries were dominant among the best performing in terms of crude protein, with only "Pallinup", an oats entry, having a higher crude protein percentage.

The study has identified numerous factors for consideration that would further improve the accuracy and replication of studies of this nature in the future. When measuring DMY, weighing of the foreign plant material and weeds and taking soil samples could have added more value. The results differed significantly across research locations and was largely

influenced by environmental influences. Further insight into the amino acid composition of the best cultivars would add much more value, however, was unable to be conducted within the scope of this study as a result of budget constraints.

## Chapter 5: Conclusion

The aim of the study was to evaluate breeding parameters aimed at increasing biomass production mainly in triticale and other crops used for animal feed and/or silage. Furthermore, to use the information to identify suitable crossing parents for inclusion into a breeding program aimed at improving biomass yield in triticale. The aim and objectives were obtained through conducting a two-stage cutting system approach, evaluating biomass yield during the 30cm plant height stage (corresponding to GS22-32 according to Zadoks) and during the soft dough developmental stage (GS70-79).

The various measurements that were taken are therefore considered to fall in line with that which was hypothesised. Rye and oats entries were hypothesised to be superior in terms of biomass production considering the substantially higher leafy content it yields as a plant (Botai *et al.*, 2017). The oats varieties in the trial did significantly well in terms of its BMY and nutritional characteristics for utilisation as silage and animal feed in general. However, it's clear that triticale produced competitively to that of oats in terms of both biomass yield production and nutritional characteristics, of which similar results were observed in previous studies (Ayalew *et al.*, 2018; Reynolds *et al.*, 2012).

Rye cultivars produced competitively within the first cutting stage (30cm), However, oats and triticale were dominant during soft dough with rye producing the lowest DMY among them in most cases. This can be associated to the fact that all rye entries matured very late and produced a lot less plant material. Rye entries also matures very late and never reached soft-dough at the end of the season and therefore had measurements taken accordingly.

Although the plots experienced stress conditions by weeds, these were mainly oat cultivars, with triticale entries displaying greater resistance to weeds and rust diseases it required agronomic management to keep the plants healthy. The results therefore clearly veer toward presenting the strong ability of triticale varieties to be considered as viable alternative crops to supplement feedstocks. Further research into the breeding parameters of this crop would facilitate the production of better suited varieties that are adjusted to meet the nutritional and yield characteristics that are desired.

A study of this nature is faced with multiple constraints, as the case in many studies. As biomass samples were physically obtained, there was increased potential for risks due to manual error and the influences of environmental factors. Furthermore, the varieties all varied in their respective levels of maturation and development and thus the readiness of samples for cutting was reliant on physical inspection and informed judgement. Therefore, further fine-tuning of the process should be considered to generate more accurate prediction models of such research investigations.

The study has identified an increased need for further research. As we move through the fourth industrial revolution, the further incorporation of precision agricultural tools and software could add significant value to these types of investigation. In addition to this, biotechnological approaches could enhance the streamlining of identification of crossing parents through the adoption of approaches such as Marker assisted selection in support of the phenotypic selection and evaluation approach.

Selected triticale entries were compared to advance breeding line triticale in terms of biomass yield potential as well as nutritional characteristics and further compared to other forage crops such as barley, oats and rye grass as well in terms of these measurements across the two stages. The combination of phenotypic, nutritional and statistical evaluation of these entries enabled the identification of best performing entries within each trial.

### **5.1 Triticale versus small grain cereal entries in terms of BMY**

The results of BMY in triticale in most instances were never among the entries classified to have the lowest biomass yield produced. The consistent trend of being ranked above average to being among the top performers, in terms of BMY, represents the biomass yield potential of triticale across various research environments in relation to other small grain crops. However, the oats entries proved to be superior varieties with regard to BMY, as was expected. The production of substantially more green leaf plant material is the reason for this higher production of BMY, as opposed to the material produced by triticale.

The regrowth rate and BMY results were substantial. The replication rows that were previously cut at 30 cm displayed a great ability of regrowth (provided they were cut above the growth point). Plants cut at 30 cm were not far from maturation when cuttings were conducted on the other rows for soft dough developmental stage. This creates possibilities for further research to be conducted, as looking into the rates and yield components through before and after regrowth could be valuable to farmers and researchers.

The key advantage for triticale therefore was observed in its ability to offer extended grazing during the early spring and autumn graze, as well as in its capacity for utilisation as silage. The special characteristics of this multi-purpose crop clearly illustrates the sustainable cropping application of triticale through its utilisation for silage, grazing material and to serve as an alternative to supplementing feed stocks of other forage crops through the season. The entries “US2014”, “Snel” and “AgBeacon”, along with the entries “US2016” and “US2017”, performed significantly better than most entries in the study during the first phase and was carried through to the next phase.

## **5.2 Triticale versus advanced breeding line triticale entries in terms of BMY**

The selected triticale entries were evaluated against other advanced breeding line entries in order to establish whether these entries and/or other advanced breeding line triticale entries could be possible crossing parents which possess the most favourable characteristics in terms of BMY potential. The entries “US2018”, “USTRITEL020”, “Snel” and “AgBeacon” seemed to be among the top ranked triticale entries in the respective analyses that were conducted. The entries “US2018” and “USTRITEL020” were among the advanced breeding line triticale entries which had better BMY potential than “US2014” and “US2016”. Thus, these triticale entries could be considered to be good candidates as possible crossing parents that are suitable when breeding for increased biomass yield in triticale.

Despite modern spring triticale entries having shown its ability to perform highly competitively in terms of biomass yield potential against the other forage crops, there lay a significant gap in the amount of information available regarding the nutritional value as animal feed and silage within triticale, considering the physical and nutritional variability between triticale and the other forage crops. Through in-field experimental analysis, Triticale entries produced greater

than average biomass yield potentials in more than 80% of the time, taking into consideration the variability among locations and environmental conditions and stresses induced this is a considerably well display of performance in terms of biomass yield.

### 5.3 Nutritional characteristics of top performing entries

The nutritional parameters evaluated included crude protein content, moisture content, ash content, crude fibre and crude fat contents. Triticale performed competitively with the likes of the oats entries in terms of the amount of content they produced. Both triticale and oats cultivars were among the top ranked among the nutritional parameters measured. In terms of both crude protein and crude fat compositions, oats varieties contained a higher content than the other small grain crops. Triticale entries were similar with slightly lower contents of crude protein and crude fat reported. However, triticale entries in general had better nutritional compositions than that of barley, and fell slightly below oats in terms of crude protein and fat.

For crude protein content the highest crude protein was reported in the entry “Pallinup” (oats). The entry “USTRITEL020” was the second highest but was not significantly lower. However, although “Snel” and “AgBeacon” also featured among the five highest in terms of crude protein content, they had significantly lower content in comparison to the oat entry “Pallinup”. As for fat content, the oats entries were significantly better than the closest triticale entry “AgBeacon”. The top five entries in terms of fat content were oat cultivars. Although the triticale entries were significantly lower, it produced greater fat content than that of rye cultivars and similar content to the remaining oat entries not ranked in the top five.

Both ash content and crude fibre displayed a similar trend, with higher compositions reported in oats entries than that of triticale, rye and barley. Triticale remains highly competitive in terms of ash and crude fibre content as well. The triticale entries “USTRITEL020”, “Snel”, “AgBeacon”, “US2016” and “US2017” were mainly among the highest of the triticale entries in terms of the nutritional parameters assessed. The general trend observed identifies oats varieties, particularly “Pallinup” to be better suited for forage and animal feed as they produce BMY and nutritional composition that are desirable. Triticale has high potential for consideration as an alternative for supplementation of feedstocks during the season as it was

highly competitive in terms of its BMY potential and nutritional compositions with the oats entries incorporated into this study.

The resultant ability of the crop identifies the need for further research initiatives to be adopted to aid the development of breeding trials aimed at producing improved varieties with an enhanced forage quality / nutritional value, and improved levels of technical transference to more potential growers and feeders to practice more precise and sustainable agricultural approaches. Further research into the variations in amino acid composition would be of great value. However, as a result of budget constraints these analyses were too costly to conduct on the number of samples.

With pressing matters such as climate change, increasing population and food demands, the need for such research initiatives is pivotal in the mitigation of the impacts of these matters. Practising more sustainably, precisely and continuing to improve varieties that are better adapted to these changing environments is the foundation to ensuring a sustainable future. It is recommended that future research initiatives that be focused on investigating the amino acid and other nutritional components, optimal time for cutting to be conducted, lengths of cuts, optimal crop management required, other physiological components that could aid better selection for biomass yield potential as well as further research in the digestibility in animals across different feeding situations.

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