GENETIC STUDIES AND HIGH THROUGHPUT PHENOTYPING FOR NUTRITIONAL QUALITY TRAITS IN *UROCHLOA* AND ITS IMPLICATIONS FOR FORAGE BREEDING

BY

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DECLARATION

I hereby declare that except for references to works of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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ABSTRACT

Urochloa species, widely cultivated in the tropics as animal feed, present strong potential for integration into Uganda's pasture-based livestock systems. Despite their importance, improvement and selection efforts in Sub-Saharan Africa remain constrained by limited and poorly characterized genetic diversity, inadequate high-throughput phenotyping tools, and insufficient genomic information resulting from the species' reproductive complexities, including apomixis and variable polyploidy. These challenges have slowed the development of improved Urochloa varieties with enhanced productivity and forage quality. To address these gaps, the study aimed to: (i) assess genetic diversity and population structure of Ugandan Urochloa collections, (ii) develop and validate Near-Infrared Spectroscopy (NIRS) predictive models for rapid forage quality phenotyping, and (iii) identify genome-wide single nucleotide polymorphisms (SNPs) associated with key forage quality traits.

Results from objective one unraveled significant morphological variability among genotypes, displaying diverse growth habits (decumbent to prostrate), leaf textures (hairy or hairless), and stem colors (purple or green). Ploidy levels ranged from diploid to hexaploidy. Genotyping 188 accessions using the DArTseq platform produced 19,668 quality SNPs with moderate polymorphism (PIC = 0.01–0.38). Population structure analysis identified six genetically distinct subpopulations, driven mainly by genetic rather than geographic differences, showing high gene flow and low differentiation (PhiPT = 0.00108), with 99% of variation occurring within subpopulations.

Results from objective two demonstrated that near infrared spectrometry as an effective, high-throughput phenotyping tool for forage quality evaluation. Using a 350–2500 nm spectral range and partial least squares regression (PLSR), the Acid Detergent Fiber (ADF) model showed the best predictive accuracy (R^2cv = 0.93, RMSEP = 1.34, RPD = 3.6), followed by the

Metabolizable Energy model (R^2cv = 0.91, RMSEP = 0.12, RPD = 3.29). Other models such ash (R^2cv = 0.92, RPD = 2.94), in vitro organic matter digestibility (R^2cv = 0.87, RPD = 2.73), and Crude Protein (R^2cv = 0.85, RPD = 2.57) all exceeded an RPD value of 2.5, confirming their reliability for routine phenotyping.

Objective three generated genome-wide SNP data (19,668 DArTseq SNPs) with phenotypic data from 188 accessions across three environments. Using multi-locus models (Bayesian Information and Linkage Disequilibrium Iteratively Nested Keyway and Multiple Loci Mixed Linear Model) in GAPIT software, 32 SNPs associated with forage nutritional quality traits were detected. Five marker-trait associations (MTAs) were consistent across models, including one on chromosome 5 linked to in vitro digestibility, Neutral Detergent Fiber (NDF), and ADF, suggesting pleiotropic effects. Candidate genes identified included translation initiation factor 2 (protein synthesis regulation), ATP-binding protein kinases (stress response regulation), and domains such as IBR and kinase inducible domains.

In conclusion, substantial genetic diversity exists within Ugandan *Urochloa* germplasm, and forage quality traits are shaped by both genetic and environmental factors. The identified SNPs, once converted into Kompetitive Allele-Specific PCR (KASP) markers, can support marker-assisted selection for enhanced forage quality in *Urochloa* and related tropical grasses.

DEDICATION

To God Almighty and my family

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LIST OF ABBREVIATION

FAO	Food and Agriculture Organization
CIAT	International Centre for Tropical Agriculture
ANOVA	Analysis of Variance
AEZ	Agroecological zone
PCA	Principal component Analysis
SP	Subpopulation
DArT	Diversity Arrays technology
NIRS	Near-infrared spectroscopy
NGS	Next generation Sequencing
PLS	Partial least squares
MSC	Multiplicative Scatter Correction
SNV+ baseline	Standard normal variate + baseline
SNV+GAP	Standard Normal variate+ GAP
SNV+SG	Standard normal variate + Savitzky-Golay
RMSEcv	Root means square errors of cross-validation.
RMSEP	Root means square errors of prediction.
SEP	Standard errors of performance
OM	Organic matter
IVDMD	In vitro dry matter digestibility
NDF	Neutral detergent fiber
N	Nitrogen
MSC	Multiplicative scatter correction;
DM	Dry matter
CP	Crude protein
Ash	Mineral matter
ADL	Acid detergent lignin
ADF	Acid detergent fiber
SNP	Single nucleotide polymorphism
GAPIT	Genome Association and Prediction Integrated Tool
GWAS	Genome-wide association mapping studies
BLINK	Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway
MLMM	Multi-loci Mixed Linear Model
LD	Linkage disequilibrium

CHAPTER ONE

GENERAL INTRODUCTION

Agriculture remains a cornerstone of Uganda's economy, contributing 24.1% to the national GDP, with the livestock subsector being the fastest growing,_accounting for 17.4% of agricultural GDP and supporting nearly 78% of households (UBOS, 2022). Despite its potential, livestock productivity is constrained by poor animal health, genetics, and nutrition. Rapid land-use changes have reduced grazing areas, leading to severe pasture shortages, particularly during dry seasons. Consequently, livestock performance remains low, with exponential rise in demand for meat and milk. The increase in meat and milk is mainly attributed to herd expansion rather than improved productivity per animal. The limited biomass yield and poor quality of natural pastures fail to meet the nutritional needs of animals, resulting in low milk yields, delayed growth, poor carcass quality, and in severe cases, animal mortality.

Urochloa species are ideal candidate grasses to narrow the feed gap especially during dry seasons (Beloni *et al.,* 2018; Cheruiyot *et al.,* 2018), and can be selected for both rangeland improvement, the cut and carry systems. Native to Africa, *Urochloa* grasses have gained global significance with potential for sustainable improvement and conservation of grasslands. Through the Swedish International Development Cooperation Agency funded livestock development project known as 'Climate-Smart *Brachiaria* Program', improved *Urochloa* cultivars were introduced and first evaluated for adaptation, agronomy, and livestock performance in East Africa, exhibiting excellent performance in yield and drought tolerance. Smallholder farmers in the region have been able to cope with feed shortages, demonstrating their significance in addressing livestock feed challenges (Djikeng *et al.,* 2014; Ghimire *et al.,* 2016; Maass *et al.,* 2015; Tesfai *et al.,* 2019; Worku *et al.,* 2022). Furthermore, the international

center for Tropical Agriculture (CIAT) and Brazilian Agricultural Research Corporation (EMBRAPA) continue to spear head the genetic improvement and selection of *Urochloa* and *Megathyrsus* species since the 1980s, underscoring the importance of long-term investment in breeding and selection of the grass species (Ferreira *et al.,* 2021; Simeão *et al.,* 2021b).

The utilization of *Urochloa* grasses has shown other additional benefits in the agroecosystem such as enhancement of soil fertility and acting as cover crops; enhancement of soil water retention (Gichangi *et al.,* 2021; Mwendia *et al.,* 2022; Paul, *et al.,* 2020; Schiek *et al.,* 2018; Tesfai *et al.,* 2019; Baptistella *et al.,* 2020; Almeida *et al.,* 2022). Other desirable benefits associated with *Urochloa* grasses include carbon sequestration abilities, inhibition of biological nitrification impeding nitrogen losses (Byrnes *et al.,* 2017; Subbarao *et al.,* 2009; Teutscherová *et al.,* 2022), coupled with reduction of greenhouse gas emissions and reduction of pollution of groundwater (Ruggieri *et al.,* 2020).

While significant advances in the breeding and selection of tropical forage grasses have been achieved by CIAT and EMBRAPA, the lack of an active forage improvement program in the East African region has resulted in a persistent shortage of both the quantity and quality of forages available for ruminant production systems, a problem that becomes more severe during the dry season (Ruggieri *et al.,* 2020). Nutrition in Uganda is largely dependent on natural pasture and crop residues (Katongole *et al.,* 2021; Matovu & Alçiçek, 2023; Nampanzira *et al.,* 2015), resulting in low livestock productivity coupled with high environmental impact. Employing superior forage cultivars not only enhances animal nutrition but also delivers multiple production and environmental benefits. A recent study on the adoption of improved forages in Eastern Africa reported that these cultivars produced 2.6 times higher biomass yields than natural pastures used as controls. Additionally, they led to a 25% increase in average dry

matter intake, resulting in a 39% average rise in milk yield compared to local controls, while manure production increased by 24% (Baltenweck *et al.,* 2020; Paul *et al.,* 2020). These findings underscore the significant potential of improved forages to boost livestock productivity and resource-use efficiency in the region.

However, the *Urochloa* cultivars currently used on the farm are imported cumulating into high seed costs that limit access, affordability and hinders adoption (Clémence-Aggy *et al.,* 2021; Paredes *et al.,* 2023). The natural existence of *Urochloa* species within Ugandan grasslands (Namaganda *et al.,* 2004; Namazzi *et al.,* 2020), presents a germplasm resource that can be harnessed for improvement through selection and breeding. Pasture improvement through breeding or selection for improved forage quality promises better animal nutrition coupled with a reduced environmental footprint (Kingston-Smith *et al.,* 2013) and remains the most cost-effective strategy for availing adequate animal nutrition (Capstaff *et al.,* 2018). Employing superior forage cultivars not only enhances animal nutrition but also delivers multiple production and environmental benefits. A recent study on the adoption of improved forages in Eastern Africa reported that these cultivars produced 2.6 times higher biomass yields than natural pastures used as controls. Additionally, they led to a 25% increase in average dry matter intake, resulting in a 39% average rise in milk yield compared to local controls, while manure production increased by 24% (Baltenweck *et al.,* 2020; Paul *et al.,* 2020). These findings underscore the significant potential of improved forages to boost livestock productivity and resource-use efficiency in the region.

Genetic characterization is among the initial steps towards developing an effective breeding strategy either through selection or hybridization. The extent of genetic diversity within *Urochloa* ecotypes in Uganda based on single nucleotide polymorphisms is nonexistent, yet

very vital in aiding parental or superior ecotype selection. Since *Urochloa* species are polyploidy and reproduce through apomixis, ploidy variation and reproductive modes render development of an effective hybridization strategy to improve *Urochloa* species in Uganda difficult.

Furthermore, hard-to-measure traits, for instance forage quality traits, remain a bottleneck when implementing large-scale genomic studies in forage crops that require large populations (Biswas *et al.,* 2020; Pereira *et al.,* 2018; Walter *et al.,* 2012). Conventional analytical methods, such as wet chemistry used for proximate analysis of nutritional quality traits, remain the standard analytical methods in forage quality assessment. However, they are extremely time-consuming, very labor-intensive, requiring sample preparation and analysis of one trait after the other, using hazardous chemicals that require experienced technical expertise (Mazabel *et al.,* 2020; Monrroy *et al.,* 2017). Wet chemistry is also unfeasible for large populations usually involved in a typical forage breeding program. Advanced technologies such as near-infrared spectroscopy (NIRS) can provide a faster, more accurate, and cost-effective alternative to traditional wet chemistry methods, making routine phenotyping forage quality at various breeding stages feasible even in large populations.

Genetic and genomic information in *Urochloa* grass species remains limited. Previous efforts geared towards development of apomictic markers (Worthington *et al.,* 2016, 2019) that aid selection based on reproductive mode (Moraes *et al.,* 2021). Genetic marker characterization studies using microsatellites have been published (Namazzi *et al.,* 2020), contributing to understanding the genetic diversity and population structure within *Urochloa* species found in Uganda. *Urochloa* improvement has primarily been achieved through conventional methods with limited genetic gains lagging behind most temperate forages, for most of the economic traits. However, Ferreira *et al.,* 2021 emphasize the need to develop molecular markers linked to traits of economic importance and tailor hybrids suitable for varied production systems. The

growing demand for more productive cultivars requires a combination of phenotypic and genotypic data to identify markers /genes that aid genomic selection and shorten the length of a selection cycle. (Biswas *et al.,* 2020; Simeão *et al.,* 2021b). Moreover, developing genotyping tools continues to advance as sequencing costs reduce, thus enabling *Urochloa* breeders to pursue large-scale precision breeding (Ferreira *et al.,* 2021; Simeão *et al.,* 2021).

The absence of a comprehensive reference genome has also limited trait mapping studies. In-depth sequencing of whole genomes in large, diverse populations coupled with high throughput phenotyping has been used to unravel genes underlying complex traits at an unprecedented rate in several crops compared to forage grasses(Ferreira *et al.,* 2021; Simeão *et al.,* 2021a). GWAS overcomes the limitations of using bi-parental-based QTL mapping methods to identify alleles, which are often challenging to develop in apomictic grasses such as *Urochloa.* GWAS offers a high resolution, high allelic richness, without the tedious need to create a bi-parental mapping population.

Multiple loci-controlled forage quality traits exhibit quantitative variation and are thus suitable for GWAS, as this approach allows detection of SNPs linked to traits of importance based on linkage disequilibrium. GWAS has been employed in unravelling several quantitative trait loci linked to nutritional quality traits in many forage grasses such as Napier (Habte *et al.,* 2020; Muktar *et al.,* 2022a; Rocha *et al.,* 2019) and in legumes including alfalfa (Jia *et al.,* 2017; X. Li *et al.,* 2014; Lin *et al.,* 2020, 2021; Yang *et al.,* 2021). Notably, there is only one published GWAS study that has identified genes underlying nutritional quality traits in *Urochloa* species by Matias *et al* (2019).

The effectiveness of combining high throughput phenotyping within genome-wide association studies (GWAS) has been demonstrated in various crops, including forage grasses. (Muktar *et al.,* 2022a). Similarly, GWAS has been employed to unravel single nucleotide polymorphisms

underlying in Buffel grass (Negawo *et al.,* 2024) and *Urochloa* (Matias *et al.,* 2019). Single nucleotide polymorphism (SNP) markers have emerged as valuable in molecular breeding, aiding genome mapping, diversity analysis, and association studies (Dwiningsih *et al.,* 2020).

Previous research collectively emphasizes the significance of characterization of genetic diversity, the power of incorporating high through put phenotyping and the effectiveness of GWAS in uncovering the genomic markers linked to traits of economic importance such as forage nutritional quality traits in diverse grass species. Integrating high-throughput phenotyping in genome-wide association mapping studies holds significant potential for advancing breeding efforts in tropical forages. By leveraging genomic information and advanced phenotyping techniques, researchers can understand genetic basis of complex traits, ultimately enhancing the efficiency and precision in a breeding program. This research study aimed to contribute towards developing high-quality *Urochloa* grass varieties in Uganda to increase animal productivity. The study was conducted with specific objectives to,

I. assess the genetic diversity and population structure of the native collection of *Urochloa* accessions of Uganda.

II. develop and validate near-infrared reflectance spectroscopy (NIRS) predictive models for forage nutritional quality evaluation.

III. identify genomic regions associated with forage quality traits that could be exploited for marker-assisted selection (MAS).

CHAPTER TWO

LITERATURE REVIEW

2.1 *Urochloa* species

2.1.1 Origin and distribution of *Urochloa* species

Urochloa grasses belong to the *Urochloa* genus that comprises of over 135 species with seven perennial species of African origin widely cultivated in tropical agroecosystems as forage (Keller-Grein *et al.,* 1996b). The seven species include *Urochloa humidicola, Urochloa brizantha, Urochloa decumbens and Urochloa ruziziensis*, in addition to *Urochloa mutica*, *Urochloa arrecta, Urochloa dictyoneura.* The first four species have been significantly prioritized for improvement through breeding and selection (Valle & Pagliarini, 2009)

Urochloa brizantha is commonly found in southern Africa whereas *Urochloa humidicola*, *ruziziensis* and *decumbens* are predominately found along the East African equator (Keller-Grein *et al.,* 1996a). Over time, several *Urochloa* species have been introduced and adapted in different regions, including the Americas, Asia, Australia, and Africa (Holmann *et al.,* 2004). These introductions have been primarily for livestock feeding purposes. Today, *Urochloa* grasses are extensively cultivated in many tropical and subtropical regions, including Latin America and Australia (Jank *et al.,* 2014), Southeast Asia (Hare *et al.,* 2009) and in Africa (Maass *et al.,* 2015), as a feed resource while creating sustainable livestock production systems.

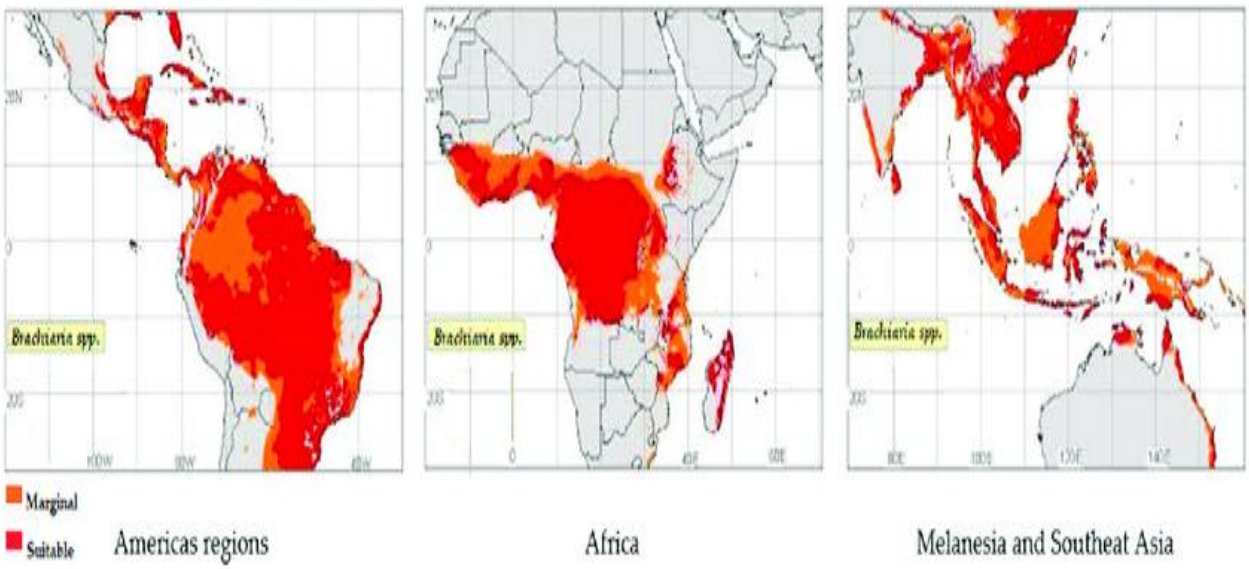


Figure 2. 1: Global *Urochloa* distribution (Source from (da Silva Cardoso *et al.*, 2020)

2.1.2 Taxonomy and biology

Urochloa taxa belong to the *Poaceae family,* also known as the grass family, in the *Panicoideae* subfamily, classified under the tribe of *Paniceae* tribe and *Melinidinae* subtribe(Soreng *et al.,* 2017). The *Urochloa* genus is large comprising of over 135 species (Miles *et al.,* 2004) previously classified as brachiaria and Megathyrsus (Ferreira *et al.,* 2021; Jank *et al.,* 2014; Salariato *et al.,* 2010). Four of the species have been widely used for cultivar development namely *Urochloa decumbens, brizantha, ruziziensis, and humidicola*, due to their high agronomic and economic value(Ferreira *et al.,* 2021; Jank *et al.,* 2014).

Taxonomically, the *Urochloa* genus has undergone several taxonomic revisions over the years based on phylogenetic analyses. Initially, Trinius (1826) placed these species in the Panicum genus, earlier described as *Brachiaria* characterized by racemose primary branches. *Brachiaria* constantly segregated from Panicum in later studies, indicating a need for reclassification(Giussani *et al.,* 2001).

Previous genetic diversity studies classified *Urochloa* population into nine groups based on inflorescence and panicle morphology, assigning *U. brizantha, U. decumbens and U. ruziziensis* into one taxonomic group (group 6) while *U. dictyoneura* and *U. humidicola* were assigned into another group (group 5). The other little-known species were assigned into the other 6 remaining groups. *Urochloa* previously referred to as *Brachiaria* was distinguished from other plant genus by its racemose main branches and spikelet adaxial orientation(Torres González & Morton, 2005).

Further studies re-classified of *Urochloa* based on presences of disarticulation at the base of the upper anthecium and a smooth and shiny muticous upper anthecium (Cook & Schultze-Kraft, 2015). Throughout this thesis, these species will be referred to as *Urochloa* species.

Molecular data has revealed that *U. brizantha*, *decumbens* and *ruziziensis* were closely related with shared evolution, however divergent lineages have been found for *U. brizantha* and *U. ruziziensis* whereas diploids of *U decumbens* were more closely related to *U. ruziziensis* while tetraploids of *U decumbens* align with *U. brizantha* (Ferreira *et al.,* 2021; Higgins *et al.,* 2022; Triviño *et al.,* 2017) .

Urochloa grasses are perennial usually propagated using both vegetative splits and seed, possess genetic flexibility that allows them to adjust to various soil and climatic conditions, thus thrive in multiple habitats, including light forest shades(Casanova-Lugo *et al.,* 2022), semi-deserts, and savannas. These species have a significant capacity to regenerate and maintain their presence in severe or regular grazing environments with the ability to withstand considerable pressure from large animals trampling. These grasses exhibit high growth rates and large biomass production due to the C4 photosynthetic pathway, which incorporates many biochemical and anatomical adaptations that enable the plants to accumulate more CO2 than C3 photosynthesis, as reported by Batistoti *et al*. in 2012.

2.1.3 *Urochloa* species of economic significance

With over 135 species in the *Urochloa* genus, only four are of economic and breeding significance enabling interspecific and intraspecific hybridization strategies of *Urochloa* (Ferreira *et al.,* 2021). *U. brizantha*, decumbens*, humidicola* are predominantly polyploids while *U. ruziziensis* is a diploid and sexual enabling interspecific hybridization(Pereira *et al.,* 2005; Risso-Pascotto *et al.,* 2005). Facultative sexuality in the previously apomixis species has enabled intraspecific hybridization(Simion & Valle, 2009; Mateus *et al.,* 2013)

U decumbens, commonly known as signal grass, is a hardy, perennial grass characterized by rhizomes and stolons that enable the species to spread and form dense mats. The signal grass typically grows to 120 cm in height with a prostrate creeping growth habit. The plant may be 160 cm in height, and stems emerge from the stolons, as noted by Loch in 1977. According to Bogda (1977), the leaves are bright green, hairy or smooth. The leaf blades are lanceolate; the leaf length can range from 10 cm to 30 cm long, whereas the leaf width ranges from 0.8 to 2.7cm wide. The inflorescence hangs on a panicle whose length varies between 16 to 25 cm with 2-7 slightly curved racemes whose height ranges from 2-5 cm and harbors the spikelets arranged in pairs along the rachis (Cook *et al*. 2005; Husson *et al*. (2008), the spikelet is hairy, 4-5 mm long, and arranged in two rows along the rachis. Signal grass has a broad ecological adaptation of soils, including acidic infertile soils. The grass grows best in areas with annual rainfall of 800mm to 1500mm and can tolerate water logging conditions. Optimal growth temperature ranges between 30 – 35°C. With a robust root system, signal grass can efficiently absorb phosphorus and nitrogen from the soil. This attribute makes it resilient to both drought and low soil fertility.

Urochloa brizantha, also known as palisade grass, is a tufted perennial grass whose plant height ranges between 80 cm to 150 cm that does not spread by stolons or rhizomes as *Urochloa ducumbens* (Renvoize *et al.,* 1996). The species' leaves are arranged alternately, are smooth,

have a linear-lanceolate shape, and have lengths ranging from 20 to 50cm and 1 to 3.8cm leaf width. The stems are erect, robust, able to withstand heavy grazing and become lignified with age. The leaf sheath encloses the stems, which can be hairy or smooth. The panicle inflorescence is usually 10 - 25 cm long, harboring several racemes arranged alternately on the central axis (Cook *et al.,* 2005). The spikelets are arranged in a single row and are hairy. It is a versatile forage option known for its adaptability to infertile acidic soils with a pH below 5.5 (Merlin *et al.,* 2015). This ability of these species to thrive in challenging soil conditions - makes it a valuable choice for livestock production systems, particularly in regions with poor soil fertility. Studies have highlighted the excellent adaptation of palisade grass to infertile soils, emphasizing its resilience and productivity in such environments (Merlin *et al.,* 2015). The grass thrives best in tropical and subtropical climates with an annual rainfall of 900 to 1500 nm and is well adapted to various soil types, including sandy soils.

Urochloa ruziziensis, commonly known as Congo grass, is an obligate sexual species with a short lifespan (Husson *et al.,* 2008). The plant has a tufted semi-erect growth habit with a fibrous root system that forms a dense mat with roots capable of reaching a depth of 1.8 m (Husson *et al.,* 2008). The plant originates from the nodes of the rhizomes and develops into a compact and leafy canopy (Cook *et al.,* 2005; Urio *et al.,* 1988). The plant height ranges from 30 to 120 cm, is light green, has a smooth texture, and is covered in fine hairs on both surfaces. They are lanceolate, measuring up to 25 cm in length and 1 to 2.5 cm in width. The inflorescence is composed of 3-9 racemes that are quite long, measuring 4-10 cm. These racemes include spikelet arranged in one or two rows on one side of a broad, flattened, and winged rachis (Cook *et al.,* 2005). The spikelet exhibits pilosity and measures 5 millimeters in length. According to Husson *et al*. (2008), the mass of 1000 grains is about 4 grams. Congo grass like signal grass is frequently misidentified (Cook *et al.,* 2005) and can be crossbred with *Urochloa brizantha* and *Urochloa decumbens*, resulting into a widely adopted cultivars such

as Mulato I and II (Argel *et al.,* 2007). The species are common in grasslands that are above 2000 mas sea level in the humid tropics of Africa and up to 1200nm in Panama (FAO, 2015). The plant thrives in regions with a minimum annual rainfall of 1200 mm and can tolerate a 3 to 4 months of drought (Schultze-Kraft *et al.,* 2020). The temperature may vary between 19°C and 33°C, but the best conditions for growth occur when the day and night temperatures of 28°C and 33°C, respectively. Congo grass can tolerate shade tolerance; thrives in well-drained, nutrient-rich soils, particularly with a ph. range between 5 and 6.8.

Urochloa humidicola also known as koronivia grass, is a perennial grass with prostrate to semi erect growth habit. It spreads through stolons and sometimes through rhizomes, can grow up to one meter with alternate leaves with lanceolate blades whose leaf length can range between 10cm to 20cm and width of 1 to 3 cm. The creeping habit distinguishes U *humidicola* from other species, such as *Urochloa dictyoneura*, which is sometimes similar(Cook *et al.,* 2005) Characterized by a high degree of root compaction, that forms a tightly packed turf. The culms persist in a prostrate position and occasionally roots develop from their lower nodes. The inflorescence has a 10 to 20 cm long open panicle with spikelets clustered along the primary branches each measuring 3 to 4mm long (FAO, 2010; Clayton *et al.,* 2006; Cook *et al.,* 2005). The *Urochloa humidicola* grasses are well adapted to poor, acidic and waterlogged soils. They are also tolerant to heavy grazing pressure due to their high regeneration ability. These species' ideally grow well between an annual rainfall between 600 and 2800 mm in their natural habitat and between 1000 and 4000 mm in other habitats (Cook *et al.,* 2005), with typical day temperatures ranging from 32 to 35°C. These grasses exhibit remarkable flood tolerance due to its extensive adventitious root system that becomes prominent during periods of excessive flooding (Jiménez *et al.,* 2021). This grass is recognized for its ability to withstand temporary waterlogging, making it suitable for cultivation in waterlogging-prone soils (Cardoso *et al.,*

2013; Jiménez *et al.,* 2019). Studies have shown that *U. humidicola*'s tolerance to waterlogging is associated with specific root traits that enhance internal aeration, such as increased aerenchyma development and reduced stele proportion to the cortex (Pereira *et al.,* 2021). Furthermore, *Urochloa humidicola* is known for its adaptability to acidic and nutrient-poor soils, making it a valuable option for areas with such soil conditions (Jiménez *et al.,* 2019).

The among and within species variability can therefore be harnessed through plant breeding to develop superior cultivars through inter-specific and intra specific hybridization respectively To overcome reproductive barriers of apomixis and varying ploidy levels, naturally occurring sexual diploids (*Urochloa ruziziensis*) undergo polyploidization to enable compatibility with male apomictic progenitors (Pessoa-Filho et al. 2017). Production of interspecific hybrids, such as those obtained by crossing tetraploidized *U. ruziziensis* with apomictic of *U. brizantha* and *U. decumbens* have been developed for use as livestock feed (Matias *et al.,* 2018).

Both intra and inter-species hybridization in *Urochloa* has been instrumental in developing hybrids with enhanced characteristics for forage production, livestock feed quality, and environmental adaptation. For instance, *U decumbens* grows well in acidic and infertile soils, yet highly susceptible to spittle bugs whereas *U. brizantha* exhibits better tolerance to spittlebug but cannot withstand waterlogged soils. *U humidicola* on the other hand withstands waterlogged soils but with low nutritional value and seed yield. In contrast, *U. ruziziensis* exhibits superior nutritional value but is susceptible to spittlebug infestation and cannot survive in acidic soils or prolonged drought. By combining different species within the *Urochloa* genus, breeding programs have successfully created hybrids that exhibit improved traits, contributing to sustainable agriculture and livestock management practices.

2.1.4 Significance of *Urochloa* Cultivars in East Africa and Adoption Challenges

Like most Sub-Saharan African regions, East Africa is under population growth pressure as projections indicate a doubling of the population by 2050(Weber *et al.,* 2020), coupled with increased urbanization (Hassen & Dawid, 2021), consequently reducing land available for livestock grazing. Therefore, the need to enhance livestock productivity to feed the growing population sustainably amidst increasing climate change effects underscores the importance of adopting sustainable practices to mitigate variations in feed quality and quantity in livestock systems(Adhikari *et al.,* 2022; Hassen & Dawid, 2021; Janssens *et al.,* 2022).

Urochloa grasses play a significant role in transformation of the integrated crop-livestock production systems in East Africa, mainly supporting the livestock industry (Maass *et al.,* 2015; Paul, Groot, *et al.,* 2020). The steadily growing market demand for *Urochloa* hybrids in East Africa underscores their usage and importance (Paredes *et al.,* 2023), rending multiple benefits ranging from soil physical characteristic improvement to being used as livestock feed(Baptistella *et al.,* 2020; Paul *et al.,* 2020) .The grasses were re-introduced in Africa back in the early 2000s to improve livestock nutrition but also promoted for use in the push-pull pest management system employed in the control of the stalk borers and lastly has been used as a cover crop in conservation agriculture (Cheruiyot *et al.,* 2018; Maass *et al.,* 2015). Furthermore, their abundant root system increases their ability to improve soil physical characteristics. enhance water infiltration, soil aggregation and aeration(Horrocks *et al.,* 2019). The grass also increases nutrient cycling, which is essential in nutrient use efficiency and reduces nutrient loss(Baptistella *et al.,* 2020). *Urochloa* grasses, when intercropped with food crops, act as a cover crop, supporting higher dry matter production and playing a role in soil phosphorous availability(Almeida *et al.,* 2018)

The agronomy, adaptation and livestock performance studies by the climate-smart *Urochloa* program from 2012 to 2016, revealed the grasses' potential to address inadequate feed quality

and quantity in the region (Njarui *et al.,* 2016). Despite farmers' interest in growing *Urochloa* to support livestock performance, developing cultivars for African environments remains a significant challenge (Kuwi *et al.,* 2018, Clémence-Aggy *et al.,* 2021). Seed access and its availability in East Africa remain the region's most limiting factor of *Urochloa* adoption. Seed access through importation consequently leads to higher average seed prices, affecting affordability. As a result, African farmers depend mainly on rooted splits for planting, which is not feasible for large-scale establishments as vegetative splits are bulky and laborious. (ABC and CIAT., 2022; Clémence-Aggy *et al.,* 2021; De Oto *et al.,* 2019; Fuglie *et al.,* 2021; Paredes *et al.,* 2023).

2.2 Inherent Breeding Barriers and Breeding Methods among Urochloa species.

2.2.1 Apomixis

Apomixis is the most predominant reproductive mode in *Urochloa* species, hampering its improvement through breeding(Ferreira *et al.,* 2021). Apomixis, the asexual reproduction through seeds, is a complex phenomenon involving multiple processes of apomeiosis, parthenogenesis and endosperm development with significant implications to plant breeding and genetic diversity in *Urochloa* (Higgins *et al.,* 2022). In *Urochloa* species, both obligate and facultative forms of apomixis have been reported, influencing their reproductive behavior and utility in breeding programs. Most *Urochloa* species exhibit facultative apomixis, allowing both sexual and asexual seed formation to occur simultaneously within the same species, which enables genetic recombination while maintaining clonal propagation of desirable genotypes (Valle & Savidan, 1996; Worthington *et al.,* 2016). For example, *Urochloa decumbens*, *U. brizantha*, and *U. humidicola* are predominantly facultative apomicts, whereas some accessions, particularly of *U. humidicola*, tend toward obligate apomixis, producing progeny entirely without fertilization (Jank *et al.,* 2014; Moraes *et al.,* 2021). This reproductive flexibility has been critical in breeding efforts, as facultative apomixis permits occasional

recombination useful for generating novel genetic variation while maintaining elite forage traits in stable apomictic lines (Worthington *et al.,* 2019; Simioni & Valle, 2009).

Apomixis is regulated by interrelated pathways, including epigenetic regulation, cell-cycle control, hormonal pathways, and signal transduction processes (Schmidt, 2020). While early genetic studies proposed a simple genetic control for apomixis, recent molecular evidence suggests a more intricate inheritance system directing the process

Like in most tropical grasses, previous studies have identified critical genetic elements associated with apomixis in *Urochloa* species, such as the apomixis-specific genomic region located near the centromere of chromosome 5, indicating potential chromosomal translocations within the *Urochloa* genus (Worthington *et al.,* 2016). Additionally, transcriptomic analyses have revealed candidate genes linked to aposporous apomixis in *Urochloa brizantha*, highlighting the genetic complexity underlying this reproductive mode (Siena *et al.,* 2014). Furthermore, the genetic control of apomixis in plants like *Urochloa brizantha* involves by passing meiosis and fertilization, leading to asexual seed formation (Hand & Koltunow, 2014). Identifying differentially expressed genes in the ovaries of sexual and apomictic plants of *Urochloa brizantha* has provided insights into the molecular mechanisms governing apomixis in this species (Rodrigues *et al.,* 2003). The genetic basis of apomixis in *Urochloa brizantha* was further supported by physical mapping studies, which collaborate the allopolyploid origin of apomictic traits (Nielen *et al.,* 2009). Additionally, the expression analyses of genes encoding ribosomal proteins during the development of ovaries and anthers in *Urochloa brizantha* have shed light on the molecular processes associated with apomixis (Lacerda *et al.,* 2012). Miles (1997) and Araujo *et al.* (2005) both provide evidence of sexual reproduction in *Urochloa* genius, which opened the possibility of hybridization to create genetic diversity. For more than four decades, apomixis and variations in ploidy among progenitors imposed innate limitations on recombination and crossing (Baldissera *et al.,* 2020; Morais *et al.,* 2018). As a

result, the improvement of *Urochloa* was solely dependent on selecting superior genotypes from the natural diversity among African grasslands. Obtaining sexual tetraploids to generate new hybrids only started in the late 1980s through tetraploidization of identified diploid sexual genotypes within and among species that had been previously known to be obligate apomictic (Avila *et al.,* 2007; Miles *et al.,* 1997; Miles, 2007; Miles *et al.,* 2004).

2.2.2 Polyploidy

The *Urochloa* genus exhibits significant ploidy variation, ranging from 2x to 9x (Tomaszewska *et al.,* 2021; Vigna *et al.,* 2016). This variation is particularly pronounced among the three species in the agamic complex that includes *U. decumbens, U. brizantha, and U. humidicola* (Tomaszewska *et al.,* 2021) influencing hybridization events within the genus. The presence of polyploidy in these species has been linked to the evolution of their genomes and the development of diverse ecotypes (Higgins *et al.,* 2022). Most polyploids in *Urochloa* (formerly *Brachiaria*) are allopolyploids, meaning their multiple chromosome sets originate from different but related species rather than from duplication within a single species. Cytogenetic and molecular studies have shown that many cultivated *Urochloa* species such as *U. brizantha*, *U. decumbens*, and *U. ruziziensis* form part of an agamic complex with both sexual diploids and apomictic allopolyploid forms (Valle & Savidan, 1996; Risso-Pascotto *et al.,* 2006; Worthington *et al.,* 2019). For example, *U. ruziziensis* is diploid and sexual, while *U. decumbens* and *U. brizantha* are predominantly apomictic tetraploids derived from hybridization and subsequent chromosome doubling classic indicators of allopolyploidy (Mendes-Bonato *et al.,* 2002; Jank *et al.,* 2014).

Allopolyploidy in *Urochloa* contributes to greater genetic diversity and heterosis through the combination of divergent genomes, enhancing adaptability and resilience to environmental stress. However, it also complicates breeding because pairing between homoeologous chromosomes can be irregular, making controlled recombination difficult (Worthington *et al.,*

2016). The presence of apomixis in allopolyploid forms enables the fixation of heterosis, allowing breeders to perpetuate superior hybrid genotypes through clonal seed reproduction (Simioni & Valle, 2009). The existence of sexual diploid species, such as *U. ruziziensis*, is crucial since they serve as bridges for introgression sexual forms generate novel variability, and desirable hybrids can later be chromosome-doubled to restore fertility and combine with apomictic allopolyploids. In contrast, if *Urochloa* polyploids were autopolyploids, where chromosome sets originate from a single species, breeding would be simpler due to regular chromosome pairing, but the resulting genetic variability would be lower, limiting long-term improvement potential.

Urochloa polyploids are predominantly allopolyploids, a feature that provides broad genetic diversity and allows apomixis to stabilize elite genotypes, but also poses challenges for hybridization and recombination-based breeding strategies (Valle & Savidan, 1996). Polyploidy complicates the genome causing varying inheritance patterns, inconsistent chromosomal behavior, heterozygosity of genomes with repetitive Deoxyribonucleic Acid (DNA) sites among and within species limiting breeding advance. It is crucial to comprehend these challenges to producing viable hybrids through intra- and interspecific crossings towards developing new cultivars (Ferreira *et al.,* 2021).

Moreover, the allopolyploid origin of *Urochloa* species, resulting from hybridization between distinct genomes, underscores the evolutionary importance of polyploidy in shaping the genetic composition and diversity within the genus (Vigna *et al.,* 2016). The genomic constitution and chromosomal rearrangements associated with polyploidy contribute to the adaptive potential and resilience of *Urochloa* hybrids (Vigna *et al.,* 2016). Quarín et al. (2001) highlighted the relationship between ploidy levels and the induction of apomixis in Paspalum notatum, emphasizing the importance of understanding cytogenetics and ploidy levels for

comprehensive studies of apomixis in related genera like *Urochloa*. Timbó *et al*. (2014) focused on determining DNA content and chromosome numbers to characterize ploidy levels, providing valuable insights into the genetic makeup of *Urochloa* grasses. Boldrini et al. (2009) discussed the origin of polyploid accessions in Brachiaria humidicola, noting that while polyploidy is common in the genus, high ploidy levels are not widespread. Vigna et al. (2011) reported limitations in crossing *Urochloa* grasses with different ploidies, underscoring the challenges posed by varying ploidy levels in breeding programs. Studies by Ishigaki et al. (2010) and Santos *et al*. (2015) shed light on the relationship between ploidy levels, chromosome morphology, and genome size. The number of ribosomal DNA loci found to correspond to ploidy levels revealed a clear association between genetic markers and ploidy status in these grasses. Furthermore, a significant correlation between polyploidy and irregularities during meiosis in *Urochloa* species, revealed insights into the impact of polyploidy on microsporogenesis in *Urochloa* grasses and its implications for seed yield in *Urochloa* sexual polyploid hybrids(Ragalzi *et al.,* 2021). Polyploidy in *Urochloa* hybrids can lead to the production of unbalanced gametes, resulting in aneuploid progenies or hybrids with modified genetic characteristics (Paula *et al.,* 2017). These abnormalities, characteristic of polyploids, influence the reproductive behavior and genetic stability of *Urochloa* hybrids, affecting their performance and adaptability (Paula *et al.,* 2017). By investigating ploidy-related traits, researchers can elucidate the genetic characteristics, reproductive mechanisms, and evolutionary dynamics of ploidy grasses to enhance breeding programs, conservation efforts, and genetic studies aimed at improving the agronomic and quality traits.

2.2.3 *Urochloa* Breeding methods

Breeding in *Urochloa* species primarily relies on understanding their unique reproductive biology, which includes the coexistence of sexual diploid and apomictic polyploid forms. The main approach involves exploiting the sexual diploid species, such as *U. ruziziensis*, as a

genetic bridge for recombination and variability. These sexual diploids are cross-compatible with apomictic polyploids like *U. brizantha* and *U. decumbens* once chromosome doubling is induced through colchicine treatment to produce fertile tetraploid sexual forms. This enables hybridization at the same ploidy level, facilitating the transfer of desirable traits such as pest and disease resistance, drought tolerance, and improved forage yield and quality (Jank *et al.,* 2014; Worthington *et al.,* 2016).

A second major breeding method involves the development and selection of hybrids between sexual and apomictic accessions. In this approach, sexual tetraploids are crossed with apomictic tetraploids, and the resulting hybrid progenies are evaluated for agronomic performance, stress tolerance, and forage quality. Hybrids exhibiting superior performance and apomictic are selected, as apomixis allows the fixation of hybrid vigor in subsequent generations through clonal seed propagation. This strategy has been successfully applied in Brazil by EMBRAPA to develop widely adopted cultivars such as *Urochloa brizantha* cv. BRS Paiaguás (Simioni & Valle, 2009; Jank *et al.,* 2014).

Additionally, molecular and genomic-assisted breeding is increasingly being integrated into *Urochloa* improvement programs. The use of molecular markers, genomic selection, and transcriptomic analyses has enhanced the identification of genes associated with apomixis, stress tolerance, and nutritional quality (Worthington *et al.,* 2019; Moraes *et al.,* 2021). These modern tools complement conventional hybridization by accelerating the selection process and improving accuracy in identifying superior genotypes. Combining classical breeding with genomic tools thus provides an efficient pathway for developing resilient, high-yielding, and nutritionally superior *Urochloa* cultivars adapted to diverse tropical environments.

According to Miles *et al*. (2006), sexual synthetic tetraploid genotypes developed from *Urochloa ruziziensis* which is sexual in nature were used as female progenitors. To enhance

limiting traits in the sexual *Urochloa* breeding population, the apomictic elites are utilized as pollen donors (Jank *et al.,* 2014; Miles, 2007) and through cycles of recurrent selection desirable alleles are accumulated (Barrios *et al.,* 2013; Worthington & Miles, 2015). Several studies have demonstrated the effectiveness of recurrent selection in apomictic grasses, such as recurrent selection based on combining ability and recurrent phenotypic selection (RPS) (Barrios *et al.,* 2013; Worthington & Miles, 2015).

To accelerate `genetic gain, a modified reciprocal full-sib recurrent selection method using the apomixis marker to discriminate the reproduction modes of hybrids was proposed (Barrios *et al.,* 2013; Worthington & Miles, 2015). Recurrent selection, however, is characterized by low genetic gains per unit of time (Hayes *et al.,* 2013; Pereira *et al.,* 2018; Resende *et al.,* 2013, 2014), whereas phenotypic selection is costly and time-consuming, requiring up to10-12 years to release a new cultivar (Miles *et al.,* 2006; Resende *et al.,* 2013)

2.3 Germplasm resources and genetic diversity

2.3.1 Germplasm

The collection of grasses and legumes germplasm from the wild dates back from between 1971 and 1972 in Eastern and southern Africa spearheaded by commonwealth scientific and industrial research organization (Maass & Pengelly, 2019). Later between 1984 and 1985 an *Urochloa* targeted collection was held by International Center for Tropical Agriculture (CIAT) in collaboration with the international livestock center for Africa (ILCA, now the international livestock research institute (ILRI)(Keller-grein *et al.,* 1996b)

Three research institutions of Brazilian Agricultural research corporation (EMBRAPA) in Brazil, International Center for Tropical Agriculture (CIAT) Cali, Colombia and the international livestock research institute (ILRI) in Ethiopia house the largest germplasm banks of *Urochloa* grass species (Keller-Grein *et al*. 1996).

Several *Urochloa* germplasm collections now exist globally and are maintained in various gene banks. By 1996, approximately nine hundred eighty-seven accessions representing thirty-three species can be found at various institutions, including five hundred and twenty accessions at International Livestock Research Institute (ILRI) (Keller-Grein *et al.,* 1996), six hundred and one accessions at CIAT (Triviño *et al.,* 2017), four hundred fifty-five accessions at EMBRAPA Valle *et al.,* 2008, one hundred seventy-seven accessions at Australian Tropical Forages Genetic Resource Centre (ATFGRC) of the Commonwealth Scientific and Industrial Research Organisation (CSIRO), ninety accessions at United States Department of Agriculture (USDA), fifty one accession National GenBank of Kenya, and thirty nine accessions at Range and Forage Germplasm Institute (RGI) of the Agricultural Research Council (ARC). Ninety-nine accessions have also been previously assembled from the natural grasslands of Uganda and maintained at the National livestock Resources Research Institute (Namazzi *et al.,* 2020)

2.3.2 Genetic diversity

The extensive natural variability within and between *Urochloa* species is vital to enhance the utilization and improvement of these species and is largely influenced by factors such as apomixis, varying ploidy levels, morphological differences and hybridization(Masters *et al.,* 2024).

Morphological markers based on vegetative, reproductive and pubescence characteristics have been used to differentiate *Urochloa* species emphasizing the importance of these traits in species identification (Assis GML *et al.,* 2003). Furthermore, Salariato et al. (2009) detailed morphological description revealing Disarticulation of the diaspore below the lower glume, rugose upper anthecium, apex features as the key discriminating features that aided the taxonomic classification of *Urochloa* species. Moreover, Ferreira et al. (2021) highlighted the significance of morphological traits in distinguishing *Urochloa* species commonly used in

pastures, including *U. brizantha, U. decumbens, U. humidicola*, and *U. ruziziensis*. This underscores the importance of morphological characterization in breeding and conservation programs for *Urochloa* species.

Although, morphological characterization remains essential in plant taxonomy and identification, its limitations in capturing genetic diversity have been recognized in various studies. For instance, Roberts et al. (2016) emphasized the constraints of taxonomy solely based on classical descriptions of test morphology, highlighting the need for integrating molecular and morphological systematics for a comprehensive understanding of biodiversity. Similarly, Khadari et al. (2004) pointed out the limited usefulness of isozyme systems in molecular characterization due to low polymorphism and environmental influences, underscoring the challenges faced in relying solely on morphological markers.

The limitations of using only morphological markers for genetic diversity studies have led to the integration of molecular markers to address these challenges. Molecular markers provide advantages such as simplicity, informativeness, cost-effectiveness, efficiency, and the ability to offer detailed genetic information without prior sequence information (Xiong *et al.,* 2011).

The molecular-level diversity among *Urochloa* species using random amplified polymorphic DNA markers, revealed a genetic dissimilarity range of 0.49 to 0.87 among four commonly used species (Chiari *et al.,* 2008) where *U. brizantha, U. decumbens, and U. ruziziensis* were more closely related genetically than *U. humidicola*. Later studies using SSR markers, such as those conducted by Jungmann *et al*. (2009a), Silva *et al*. (2013), Santos *et al*. (2015a), Ferreira *et al*. (2016), Trivino *et al*. (2017), Pessoa-Filho *et al*. (2017), and Souza *et al*. (2018), confirmed the previous findings. Ambiel *et al*. in 2010 found similar results of dissimilarity among six *Urochloa* species namely *U. decumbens, U. ruziziensis, U. nigropedata, U.*

humidicola, U. jubata and *U. brizantha* from the EMBRAPA. The dissimilarity indexes ranged from 0.262 to 0.90710 and clustered into four distinct groups.

Additional diversity studies later used microsatellites or simple sequence repeats (SSRs). These markers are more abundant in plant genomes and provide more information than RAPD markers. Genetic diversity and population structure in *Urochloa* grasses have been the focus of several recent studies aimed at understanding the variability and relationships within different species and breeding populations. Triviño *et al.,* 2017 conducted a comprehensive analysis based on 701 SSR bands among 14 *Urochloa* species, providing insights into existing extensive variation and confirmations to previous traditional classifications based on inflorescence similarities and panicle morphology.

Jungmann *et al*. (2010) used SSR markers to analyze within and among diversity among 58 and two *U humidicola* cultivars from Embrapa Beef Cattle institute which clustered into four main groups based on their coefficients of dissimilarity range from 0.02 to 0.89. the sexual *U humidicola* accession was divergent from other accessions suggesting a high level of genetic divergent. Vigna *et al.,* 2011 developed new polymorphic microsatellite markers from *U. humidicola,* which were found to be transferable to other *Urochloa* species. Similarly, (Souza *et al.,* 2018) identified genic microsatellite markers in *U. decumbens*, with potential for use in breeding programs. (Ferreira *et al.,* 2016) isolated microsatellite loci in *U. decumbens* and demonstrated their cross-amplification in other *Urochloa* species. Kuwi *et al.,*2018 found 407 alleles in 36 Tanzanian accessions and six commercially grown varieties using 24 SSR markers revealing a high variability within genotypes (92%), low differences between populations (fixation index of 0.05), and a high genetic exchange between groups (gene flow estimate of 4.77). The Ethiopian *U. brizantha* accession assembled from nine diverse sites in Ethiopia were found genetically divergent based on 23 SSR markers revealing three distinct clusters with varying gene pools(Tegegn *et al.,* 2019) Nitthaisong *et al.,* 2016 conducted a study on

eleven species of *Urochloa*, using ten ISSR markers to evaluate their genetic diversity. They found high variability among the genotypes and clustered them into three clusters. Polyploid (apomictic) plants dominated one cluster, while the other two contained mostly diploid (sexual) plants.

The Kenyan collection was also analyzed using microsatellite markers to assess the level of genetic diversity revealed an average genetic diversity value of 0.63 and three population structures were identified (Ondabu *et al.,* 2017).Another study conducted by Trivino *et al*.2017 also used SSR markers to study the molecular genotypes of *Urochloa* spp. from CIAT germplasm. One cluster contained the majority of *U. decumbens* and *U. ruziziensis* genotypes, consistent with their morphological similarities. Interestingly, there was more genetic variation (57%) within taxonomic groups than between groups (42%). Namazzi *et al*. (2020) utilized 24 SSR markers to assess genetic diversity among nighty nine accessions assembled from Uganda. The markers were found to be highly declinatory as observed by the average polymorphism information content of 0.89. Three diverse genetic pools were identified signifying high genetic diversity within the collection. The above studies collectively illustrate presences of significant genetic diversity within and between *Urochloa* species and accessions.

2.4 Forage Nutritional quality components

Nutritional quality in forages particularly in *Urochloa* species is defined by traits that influence their value as feed including fiber composition, crude protein (CP) content, digestibility and mineral content which are all critical for optimizing animal performance and health. Crude protein is a primary indicator of forge quality as it directly affects the ruminal functionality and over all digestibility of a diet. A minimum protein requirement of 8% is necessary for maintenance of a functional rumen in bovines(Carvajal-Tapia *et al.,* 2021). Higher crude protein levels are associated with better animal performance, as they provide essential amino acids necessary for growth and milk production(Garcia *et al.,* 2019). For instance, *Urochloa*

grasses can exhibit significant CP content depending on management practices and environmental conditions with some improved cultivars showing double protein levels than that of natural grass (Garcia *et al.,* 2019).

The composition of neutral detergent fiber (NDF) and acid detergent fiber (ADF) is crucial in determining the digestibility of forage. Forage with high NDF content tends to be less digestible which can limit feed intake(Quintero-Anzueta *et al.,* 2021) .*Urochloa* species typically have NDF values ranging from 600 to 800g kg^{-1} dry matter which can vary with the age of the pasture and management practices(Mwendia *et al.,* 2021; Quintero-Anzueta *et al.,* 2021).As the plant matures, the fiber content generally increases, leading to a decline in overall nutritional quality(Roy *et al.,* 2021)

The digestibility of forage is a critical trait that influences how effectively livestock can utilize the nutrients present in grass. Factors sch as the stage of growth, environmental conditions and management practices significantly affect digestibility. For example, younger regrowth of *Urochloa* grasses tends to have higher digestibility compared to the older, more mature plants(Godina-Rodríguez *et al.,* 2024), Digestibility is essential in maximizing feed efficiency and animal productivity.

The mineral composition of forage, including essential elements sch as calcium, phosphorous and magnesium, are vital for animal health and productivity. Deficiencies in these minerals can lead to health issues in livestock, affecting growth rates and reproductive performance(Feitosa *et al.,* 2021). *Urochloa* grasses can vary in mineral content based on soil fertility and management practices, making it important to monitor and manage these factors to ensure optimal forage quality(Alvarez *et al.,* 2023)

The structural characteristics of *Urochloa* grasses such as leaf area index and canopy height play a role in their nutritional quality. These morphological traits can influence light

interception and photosynthetic efficiency which in turn affect growth rates and nutrient accumulation (Rezende *et al.,* 2021). For instance, optimal canopy structure can enhance biomass production and improve the overall nutritional profile of the forage(Villegas *et al.,* 2023). The nutritional quality of *Urochloa* grass is determined by a combination of crude protein, digestibility, mineral content and morphological traits as discussed above.

2.4.1 Classical forage quality phenotyping (Standard wet chemistry methods)

Conventional phenotyping for nutritional quality traits in forages often involves traditional wet-chemistry procedures (Jeong, 2024). These procedures involve laborious and time-consuming processes from sample preparation to analysis. Wet chemistry analysis typically includes methods such as acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein (CP), and other nutritional parameters essential for assessing forage quality (Mazabel *et al.,* 2020). While wet chemistry procedures are known for their accuracy, they can be slow and costly, requiring specialized equipment and expertise (Corson *et al.,* 1999). Wet chemistry analysis presents several disadvantages that limit its efficiency and practicality in a typical recurrent selection population. Other drawbacks of wet chemistry for forage analysis include: the need for specialized laboratory equipment, reagents, and skilled personnel to perform the analyses accurately (Jeong, 2024). Wet chemistry procedures are often not suitable for high-throughput analysis, especially when dealing with many samples, as they are time-intensive and may not be scalable. The laborious and time-consuming nature of wet chemistry procedures can make them impractical for routine commercial forage quality evaluations, where quick and efficient analyses are essential (Arnold *et al.,* 2019). Consequently, this leads to slow turnaround time of the breeding cycle due to delays in obtaining results, which may hinder real-time decision-making at selection, such as harvest decisions based on forage quality (Arnold *et al.,* 2019).

Biomass yield and forage quality are key farmer preferred traits in pasture grasses and require continuous monitoring and assessment across seasons and years(Gouveia *et al.,* 2020). However, conventional forage quality assessment procedures are slow and time-consuming as they rely mostly on destructive harvesting, chopping, drying, and weighing of samples before subjecting them to wet chemistry analysis protocols. This can hinder the pace of new cultivar development.

2.4.2 High throughput phenotyping using NIRS.

In response to the above limitations, alternative methods such as near-infrared reflectance spectroscopy (NIRS) have been explored as faster, non-destructive, and cost-effective alternatives to wet chemistry for forage analysis (Corson *et al.,* 1999; Jeong, 2024). These advancements aim to address the challenges associated with wet chemistry and improve the efficiency and accuracy of forage quality assessments.

Estimating forage traits using NIR spectroscopy offers high through put analytics in the laboratory after minimum processing or in the field using the handheld(Cherney *et al.,* 2021; Rukundo *et al.,* 2021). NIRS has been described as a non-destructive and environmentally benign tool that is lately widely employed in analysis of materials in diverse domains including agriculture and pharmaceuticals (López *et al.,* 2013; Sarin *et al.,* 2019).

It is beneficial for forage improvement programs that require quick analysis of large numbers of samples(Cherney *et al.,* 2021). Additionally, NIR spectroscopy can be used for any plant improvement program that needs rapid nutritional data. This versatile technique can be applied to samples of varying sizes, from milled dry samples to forage hay and fresh grass.

The near infrared region includes the electromagnetic spectrum covering wavelengths 780 – 2500 nm which interacts with matter, resulting into differential vibration of molecules based on their specific frequencies, to create unique spectral bands that contain information about the

sample. This interaction follows Beer-Lambert's law, making it possible to relate band intensity to composition in the sample (López *et al.,* 2013). Most of the bands observed in NIR are majorly overtones and they vary in intensity depending on the composition of the material. The most intense overtones are usually associated with hydrogen atom binding to carbon, nitrogen and oxygen, commonly found in food constituents such as protein, lipids, carbohydrates and fiber.

For accurate data acquisition, NIRS must be calibrated for several components or elements in a sample, a process that normally involves spectra data acquisition followed by wet chemistry analysis of the same sample and subsequent calibration model development using regression methods such as partial least squares regression (PLS) and modified partial least square regression (MPLS) (Alamu *et al.,* 2020).

Near-Infrared Spectroscopy (NIRS) has found a valuable tool for quantitative and qualitative assessments in agriculture (Wang *et al.,* 2022) with applications in soil health and nutrient monitoring(Andrade & Ramires, 2022) , crop quality monitoring(Amankwaah *et al.,* 2024) , crop growth monitoring(Qiu *et al.,* 2022) plant disease detection seed quality evaluation(Reddy *et al.,* 2022),maturity determination in fruits and vegetables(Anjali *et al.,* 2024)

In forage breeding, NIRS is revolutionizing the acquisition of forage quality data through increased speed of analysis, flexibility for on-field data collection, minimization of error, ease of use, reduction of drudgery and reducing cost of phenotyping. For instance, (Mazabel *et al.,* 2020) demonstrated that a FOSS could predict forage quality parameters with high prediction accuracy characterized by high coefficients of determination (R2) and a lower standard errors of cross validation (SECV) suggesting a good correlation between reference-lab and NIRS-predicted values as evidenced in the results IVDM, (R^2_c = 92%, R^2_{cv} = 93%, RPD = 3.6 and **SECV =1.55**) and NDF (R^2_c = 95%, R^2_{cv} = 92%, RPD = 3.62 and SECV = 1.41 ADF (R^2_c =

96%, R^2_{cv} = 95%, RPD = 4.4 and SECV = 0.79 CP(R^2_c = 99%, R^2_{cv} = 87%, RPD = 2.56 and SECV = 0.91) when modified partial least square (MPLS) regression model were used presenting suitability for use in *Urochloa* breeding.

2.4.2.1 Spectral data pre-processing method

Spectral pre-processing methods are essential in Near-Infrared Spectroscopy (NIRS) for forage studies, enhancing the quality of spectral data and improving the accuracy of predictive models. The type of NIRS pre-processing methods used may include, savitzky-Golay smoothing which smoothens the spectral data by fitting low degree polynomials to the data within a moving window(Fu *et al.,* 2013; Pahlawan *et al.,* 2022). Differentiation can also be used for calculating the rate of change of spectral data to enhance the spectral features. Multiplicative effects and baseline shifts in spectral data can be corrected by using standard normal variate (SNV) which adjusts the mean and standard deviation of the targeted spectral data (Cen *et al.,* 2006; Chen *et al.,* 2022). When scattering effects are observed in the spectral data, multiplicative scatter correction (MSC) corrects the spectral data by dividing each spectrum by a reference spectrum. On the other hand, the first derivative technique calculates the rate of change of spectral data to enhance spectral feature (Cen *et al.,* 2006; Chen *et al.,* 2022) whereas the second derivative calculates the second order of change of spectral data(Cen *et al.,* 2006). When spectral data is required to be scaled to a common range or distribution, normalization is used.

The MSC method was developed by Geladi *et al.,* 1985 and aims to separate the chemical light absorption from the physical light scatter in near-infrared reflectance spectra of meat samples and as a multi-wavelength concept for optical correction to achieve this separation. The Standard Normal Variate (SNV) transformation was developed for the preprocessing of Near-Infrared Diffuse Reflectance Spectra (Barnes *et al.,* 1989). The Savitzky-Golay method was developed by Savitzky and Golay in 1964. This method is utilized for applying a least-squares

fitting of a polynomial over a specified window to smooth data, reduce noise, and preserve important features in signals or spectra. It can be applied in various fields such as signal processing, data denoising, and spectral analysis to enhance the signal-to-noise ratio without significantly distorting the original signal.

Different spectral pre-processing methods can be utilized based on the specific requirements of the study and the nature of the spectral data being analyzed. Researchers have employed various pre-processing techniques in different applications to enhance the quality of spectral data and improve model performance. For example, Chen *et al.,* 2023 applied four commonly used pre-processing methods to NIR spectral data, showcasing the flexibility in selecting pre-processing techniques based on the specific characteristics of the samples. Xu *et al.,* 2022 highlighted the effectiveness of multiple scattering correction (MSC) and standard normal variate (SNV) as a widely used pre-processing techniques for NIR data in geographical traceability studies.

Generally the choice of what pre-processing technique to use is determined by sample characteristics such as its composition, homogeneity and physical form(Escuredo *et al.,* 2021; Heil & Schmidhalter, 2021).Spectral characteristics such as baseline drift, noise scattering effects(Araújo *et al.,* 2020; Qiu *et al.,* 2022) and chemometric model employed to ensure compatibility with the modelling algorithm are some of the key determinants of the choice of pre-processing technique to use(Esteban-Díez *et al.,* 2004; H. Lin & Ying, 2009).

In developing NIRS predictive models in *Urochloa*, the common pre-processing methods used have been SNV, SND and detrend (Andrade Ribeiro *et al.,* 2023); SNVD, Deritive, SNV and Detrend(Mazabel *et al.,* 2020) and whereas most recently MSC, SNV, derivatives, smoothing and normalization were all used to assess NIRS reliability and repeatability to predict lignin methods (Guimarães *et al.,* 2023)

2.4.2.2 Calibration methods

For accurate and reliable predictions, the process of calibration of NIRS is key, which usually involves generation of both spectral and wet chemistry data regressed using methods such as multiple linear regression (MLR); partial least squares (PLS) and modified partial least squares (MPLS). The robustness of the calibration model, including its ability to handle variations in the data and predict new samples accurately. MLR establishes a linear relationship between NIR spectral data and target variables. Its advantage over the other two is that it is simple, interpretable, and computationally efficient has been applied in predicting glucose and citric acid concentrations in blood anticoagulant solutions Yano *et al*. (2001), carbon and nitrogen content in compost (Suehara *et al.,* 2001). On the other hand, Principal Component Regression (PCR) involves transforming NIR spectral data into principal components and then regressing these components against target variables. Unlike MLR, it can handle multicollinearity and can reduce dimensionality. It has been applied in the prediction of hemicellulose content (Yao *et al.,* 2010), total terpene content (Champagne *et al.,* 2020). Partial Least Squares (PLS) captures the maximum covariance between NIR spectra and target variables. It handles multicollinearity, noise, and small sample sizes effectively. It has widely been used in various fields like agriculture, food, and biomedicine (Feng *et al.,* 2020).

In summary, MLR is straightforward and interpretable, PCR is useful for handling multicollinearity, while PLS is versatile and effective in dealing with noise and small sample sizes, making it a popular choice in NIR calibration across diverse applications.

Diverse applications of MLR, PCR, and PLS in fields such as environmental science, agriculture, and food analysis, showcasing the versatility and effectiveness of these calibration methods in NIRS analysis. In forage quality determination,

2.4.2.3 Validation Methods

The validation of NIRS methods is essential to guarantee the accuracy and reliability of results obtained through calibration. By following established validation protocols, comparing NIRS results with reference methods, and utilizing cross-validation techniques, the validity of NIRS predictions can be ensured across various applications.

Some of the common types of Near-Infrared Spectroscopy (NIRS) cross-validation techniques include: first, the Leave-One-Out Cross-Validation (LOOCV) which involves training the model on all samples except one, which is then used as a validation sample. This process is repeated for each sample in the dataset(Chia & Tan, 2017; Parrini *et al.,* 2023; Wulandari *et al.,* 2020). The other is known as the Five-Fold Cross-Validation which entails division of the dataset into five subsets, and the model trained on four subsets and validated on the fifth subset. This process is repeated five times, with each subset used as the validation set once (Fodor *et al.,* 2023). The Sevenfold Cross-Validation set on the other hand is similar to five-fold cross-validation, but the dataset is divided into seven subsets for training and validation(Rácz *et al.,* 2016) . The Internal Cross-Validation involves using a portion of the dataset for training and another portion for validation within the same dataset to assess model performance (Shukla *et al.,* 2021). The Full Cross-Validation method is a comprehensive method that evaluates the model's performance using all available data points for training and validation (Rehman *et al.,* 2019). The Leave-One-Cow-Out Cross-Validation is a specialized cross-validation technique where one cow's data is left out for validation while the model is trained on the remaining data points(Tsenkova *et al.,* 2009). Lastly the Two-Fold Cross-Validation involves division of the dataset into two subsets, with one subset used for training and the other for validation.

2.5 Forage Quality and Livestock Performance

The quality of forage significantly impacts livestock performance and productivity. The nutritional composition of forage, including its protein, fiber, digestibility, and mineral content, have a direct influence on an animal's growth, reproduction, and production. Forage quality indirectly affects nutrient intake, growth, and body Condition, supporting muscle development and promoting efficient weight gain in growing animals. Good body condition, achieved through proper nutrition, enhances reproductive performance and fertility in breeding animals.

Adequate nutrition derived from high-quality forage is vital for reproductive Performance and success in livestock. Proper nutrient intake promotes the development of reproductive organs, regular oestrous cycles, and successful conception. It also enhances fertility, pregnancy maintenance, and the overall health of the offspring. In dairy cows, increased milk production has been reported from high-quality forage. Forages with higher protein content, digestibility, and energy content provide the necessary nutrients for milk synthesis. Good-quality forage ensures high milk yields and supports the overall health of lactating cows

Nutritional deficiencies or imbalances in forage can compromise the animal's immune system and health rendering them more susceptible to diseases and infections. High-quality forage supports the immune system, reduces the risk of nutrient deficiencies, and contributes to overall animal health and disease resistance. Forage quality influences feed efficiency, which is the animal's ability to convert consumed feed into desired outputs such as weight gain or milk production. Animals fed high-quality forage with optimal nutrient content and digestibility tend to have higher feed efficiency and require less supplemental feed to achieve desired production outcomes.

Forage quality affects rumen function and digestion in ruminant animals. High-quality forage with good fiber digestibility and optimal protein content promotes healthy rumen microbial

activity, leading to efficient fermentation and nutrient utilization. It helps prevent digestive disorders and ensures optimal nutrient absorption. It is important to note that while forage quality is crucial in livestock performance, other factors such as animal genetics, management practices, environmental conditions, and access to clean water contribute to overall animal productivity and health. An integrated approach that considers forage quality and animal management necessary for achieving optimal livestock performance and sustainable production systems.

2.6 Breeding *Urochloa* for high forage quality

Plant breeding remains a most effective pathway towards availing nutrient enhanced food and forage varieties. High-quality forage is characterized by its digestibility, nutrient content, and palatability directly impacts animal growth, reproduction, and the quality of animal products such as milk and meat (Kang *et al.,* 2016; Yang *et al.,* 2021). The nutritional value of forage is determined by its chemical composition, including crude protein (CP) and fiber content, which are essential indicators of forage quality (Yang *et al.,* 2021; Bai *et al.,* 2022).

Breeding for improved forage quality is essential for several reasons. First, it addresses the increasing demand for high-quality feed as livestock production intensifies globally. The genetic improvement of forage crops can lead to enhanced digestibility and nutrient availability, which are critical for optimizing animal performance (Ponnaiah *et al.,* 2019)(Govintharaj *et al.,* 2021; Kulkarni *et al.,* 2018; Chen, 2024). Moreover, breeding programs that focus on forage quality traits can help mitigate the environmental impact of livestock farming by reducing methane emissions associated with low-quality forage consumption (Kingston-Smith *et al.,* 2013).

The contribution of additive and dominance effects on nutritional quality traits in *Urochloa* facilitates the identification of quantitative trait loci (QTL) associated with forage quality and

is crucial particularly in the context of breeding programs aimed at improving forage quality and agronomic performance. Additive genetic effects refer to the cumulative impact of individual allele on a trait while dominance effects arise when the interaction between alleles at a locus influences the phenotype. Understanding these genetic contributions is essential for effective selection and breeding strategies.

Research indicates that additive effects often predominate in the genetic variance of *Urochloa* species, particularly in traits related to agronomy and nutrition. For instance, Figueiredo et al. (2019) demonstrated that in a diallel analysis involving various *Urochloa* species, the predominance of additive effects was evident, particularly in traits such as leaf dry matter, crude protein and neutral detergent fiber. This finding is supported by Matias et al 2019 who noted that the genetic control of forage traits varies significantly among intra and inter specific populations of *Urochloa*, suggesting that the breeding strategies should focus on exploiting additive genetic variance for trait improvement (Matias *et al.,* 2020).

Furthermore, the work by Gouveia et al. (2021) emphasized the importance of combining ability in selecting the high nutritional value traits, although they did not report a clear predominance of additive or dominance effects for these traits. In contrast, dominance effects can also play a significant role, particularly in specific traits where hybrid vigor (heterosis) is observed.

For example, the specific hybrids of *Urochloa* species have shown varying degrees of dominance effects which can enhance certain desirable traits such as biomass yield and nutritional quality(Matias *et al.,* 2018).This complexity is further illustrated by the findings of Nitthaisong et al. (2019) highlighted that apomictic and sexual hybrids exhibit different genetic architectures which can influence the expression of traits related to forage quality. The

interplay between additive and dominance effects necessitates an elaborate breeding strategy as both types of genetic variance can contribute.

Nutritional quality traits are influenced by various traits and the leaf to stem ratio has been highly correlated to digestibility and crude protein content, with leaves generally being of higher quality compared to stems(Moore & Jung, 2001). Additionally, the degree of stem lignification negatively impacts forage quality, with increased lignification leading to reduced quality, especially as the plant progresses through the reproductive stage (Ball *et al.,* 2001)

Genetic diversity plays a crucial role in forage quality traits. Allele mining presents a pathway to effective improvement of forage quality while reducing the environmental (Hanley *et al.,* 2021). Previous studies found that a small increase of 1% in dry matter digestibility (DMD) resulted in a 3.2% increase in the daily gain of beef cattle (Casler & Vogel, 1999). In contrast, Oba and Allen. (1999) reported that a 1% enhancement in the digestibility of fiber resulted in a corresponding rise of 0.25 kg in the production of 4% fat-corrected milk. *U. ruziziensis* exhibited high digestibility with slightly low dry matter yields when evaluated with other *Urochloa* species whereas cultivars such as *U. decumbens* cv. basilisk was of higher dry matter yields couple with low invitro dry matter digestibility(Thaikua *et al.,* 2015). Based on the above, interspecific crosses could potentially present a pathway for improving forage quality.

To enhance forage quality, various strategies can be implemented based on the available literature. Agronomic interventions such as timely and adequate tillage operations, optimal nutrient management, sowing at the right time with adequate seed rates, intercropping systems, proper water management, weed control, and harvesting at the right stage have been identified as effective practices to enhance forage quality Kumar et al. (2018). Additionally, altering cutting height and stage of development can significantly impact forage quality, with higher cutting heights at vegetative and early bud stages leading to improved quality (Borreani *et al.,*

2006). Integration of legumes in grasses to create grass legume mixtures is an alternative pathway for improving the forage nutritive content (Salama & Zeid, 2016), however a more cost-effective method that is sustainable in African production systems where grass-legume mixtures are least adopted is the development of highly nutritious *Urochloa* cultivars. Furthermore, genetic manipulation, such as overexpression of miR156, has shown promise in decreasing lignin content, which can contribute to improved forage quality (Zheng *et al.,* 2016). In conclusion, a combination of agronomic practices, genetic manipulation, microbial interventions, and intercropping strategies can collectively contribute to achieving an improvement in forage quality. By implementing these approaches, it is possible to enhance the nutritional value, digestibility, and overall quality of forage, thereby benefiting livestock production systems and ensuring better animal health and performance.

2.6.1 Genotype x Environmental interaction

Forage quality varies significantly due to genotypic and environmental differences including maturity, season, management, and the presence of anti-nutritional components. Genetic diversity plays a significant role, with distinct groups and high variability among genotypes (Moreira *et al.,* 2018) . Genotypic differences in chemical composition and digestibility among *Urochloa* species has been reported, with *U. ruziziensis* showing higher nutritional values (Brito *et al.,* 2003; Lopes *et al.,* 2010) and can be used as a donor in Interspecific crosses. Environmental factors such as soil physical attributes (Pariz *et al.,* 2011), climatic conditions (Silva *et al.,* 2019), and management also contribute to variability within and among species. The lower heritability of forage quality traits in *Urochloa* further underscores the complex interplay of genetic and environmental factors (de Figueiredo *et al.,* 2012; Matias *et al.,* 2019).

2.6.2 Effect of grass maturity on Nutritional quality

Plant maturity significantly determines fodder quality in forage grasses (Mauri *et al.,* 2018). Accumulation and allocation of nutrients vary among leaves, sheaths, and stems across the

maturity stages (Hernández Álvarez *et al.,* 2024; Mganga *et al.,* 2021; Osafo *et al.,* 2023). In *Urochloa* grasses for example, leaves are of higher quality than sheaths which are of higher quality than stems whereas the dead leaves are often of lowest quality (Onjai-uea *et al.,* 2022; Santana *et al.,* 2022). This could possibly be explained by an increase in the proportion of stems as a percentage of the plant weight during the reproductive stage thus anatomical variations.

The lignified xylem cells are primarily responsible for maintaining structural integrity but also provide a significant obstacle to the degradation of stems in the rumen (Bhandari *et al.,* 2023; Ferreira *et al.,* 2023; Hao *et al.,* 2021). Cell-wall structure plays an important role in the quality of organs and their maturity. It is crucial for digestibility, as indigestible cell walls accumulate during leaf lifespan. Support tissues, such as sclerenchyma and xylem, limit forage digestibility. As plant maturity increases, the number of lignification sites decreases, affecting plant digestibility. The correlation between morphological traits and digestibility have been assessed by Thaikua *et al.,* 2015 and could be useful during selection.

2.7 Genome-wide association mapping for forage quality traits

Genome mapping continues to emerge as a robust tool for unravelling genotype–phenotype associations underlying economic traits in plants including forages (Scherer *et al.,* 2016; Tam *et al.,* 2019; Tibbs Cortes *et al.,* 2021) . Several genetic loci and potential genes associated with complex traits of economic importance, including forage quality traits in several fodder crops have been revealed through genome wide association studies (Li *et al.,* 2018a; Lin *et al.,* 2021; Somegowda *et al.,* 2021). These discoveries present an opportunity to enhance forage adaptability, yield, and quality through effective breeding strategies.

In-depth sequencing of whole genomes in large, diverse populations coupled with high throughput phenotyping has been used to unravel genes underlying complex traits at an unprecedented rate in several crops compared to forage grasses. GWAS overcomes the

limitations of using bi-parental-based QTL mapping methods to identify alleles, which are often tedious to develop in apomictic grasses such as *Urochloa*.

Pereira et al (2018) proposed genome-wide association studies to capture the complexity in the *Urochloa* polyploidy genome using appropriate ploidy-based models and tailored software that determine allele dosage and varied gene actions (Oliveira *et al.,* 2019; Dufresne *et al.,* 2014; Ferrão *et al.,* 2021; Gerard *et al.,* 2018). *Urochloa* species are characterized with large genome sizes that necessitate increasing sequencing depth to call large numbers of sequence variants (Bourke *et al.,* 2018; Gerard *et al.,* 2018) .

Matias *et al. (2019)* conducted association mapping in an interspecific segmental allotetraploid *Urochloa* spp. panel to investigate SNP underlying forage traits. By incorporating allele dosage into the analysis, seven genomic regions associated with invitro organic matter digestibility and neutral detergent fiber were identified. The same study highlighted the importance of considering allele dosage in polyploid forages such as *Urochloa* in precision to pinpoint significant SNP-trait associations in *Urochloa* spp., a complex interspecific segmental allotetraploid species. This study revealed insights into same genomic regions to be identified under diploid and tetraploid ploidy configurations, though with a possible risk of masking allele substitution effect.

2.7.1 Genotyping by sequencing

Genotyping by sequencing (GBS) has emerged as a powerful tool for understanding the genetic diversity and population structure of *Urochloa* grasses which is crucial for tropical forage systems(Ferreira *et al.,* 2021; Matias *et al.,* 2019; M. Muktar *et al.,* 2022). GBS allows for the identification of single nucleotide polymorphisms (SNP) across the genome, facilitating the study of genetic relationships, diversity and traits of interest in these species

The application of GBS in *Urochloa* has been highlighted in several studies, demonstrating its effective ness in characterizing genetic variation. For instance, Worthington et al. (2020) discussed how GBS can be utilized to identify genes associated with aluminum tolerance which is vital for adaptation of *Urochloa* species to acidic soils(Worthington *et al.,* 2020). This study underscores the importance of GBS in linking genetic markers with phenotypic traits, thereby enhancing breeding strategies aimed at improving forage quality and resilience

More over Kuwi et al. (2018) emphasized the use of SSR markers derived from GBS to assess genetic diversity among *Urochloa* accessions from Tanzania. The ability to transfer microsatellite markers across different *Urochloa* species as demonstrated further enhances the utility of GBS

Santos *et al.,*2015 further enhances the utility of GBS in comparative genetic studies and breeding applications. In addition to genetic assessments, GBS has been instrumental in understanding the evolutionary relationships with *Urochloa* species. Tomaszewska et al 2021

focused on the genomic composition of polyploid and hybrid species within the *Urochloa* group, providing insights into their evolutionary pathways and the genetic basis of apomixis, a reproductive strategy that is prevalent in these grasses (Tomaszewska *et al.,* 2021). This understanding is crucial for developing effective breeding programs that leverage the unique genetic attributes of *Urochloa* species. GBS has been employed in Napier (M. S. Muktar *et al.,* 2022a) , in Buffel grass (Negawo *et al.,* 2024), Rhodes grass (Negawo *et al.,* 2021) and in *Urochloa species (Matias et al., 2019; Worthington et al., 2016)*

Furthermore, the integration of GBS with other genomic tools, as discussed by Ferreira et al. in their overview of *Urochloa* genetics, highlights the potential for genomic selection and gene editing to accelerate breeding efforts (Ferreira *et al.,* 2021). The combination of high-throughput sequencing technologies with traditional breeding methods can significantly reduce

the time and costs associated with developing new cultivars that meet the demands of sustainable agriculture.

In summary, genotyping by sequencing represents a transformative approach in the study of *Urochloa* grasses, enabling researchers to unravel the genetic complexities of these important forage species. The insights gained from GBS not only enhance our understanding of genetic diversity and evolutionary relationships but also pave the way for more efficient breeding strategies aimed at improving the nutritional quality and resilience of *Urochloa* grasses in tropical agroecosystems.

2.7.2 Mapping Studies for forage quality traits in *Urochloa* species

Mapping nutritional quality traits in *Urochloa* species is essential for enhancing the breeding and selection processes aimed at improving forage quality and livestock productivity. The nutritional quality of *Urochloa* grasses, which are widely used in tropical pastures, is influenced by various genetic and environmental factors. Understanding the genetic architecture of these traits through quantitative trait locus (QTL) mapping can provide insights into the underlying genetic mechanisms and facilitate the development of superior cultivars.

Mapping studies in the genus *Urochloa* have for a long time been towards developing an apomictic marker(Thaikua *et al.,* 2009; Worthington *et al.,* 2016) spittlebug resistance (Ferreira *et al.,* 2019) , aluminum tolerance(Worthington *et al.,* 2020) and on forage quality traits (Matias *et al.,* 2019) GWAS has in the recent times unraveled markers governing forage quality traits in several forage crops , including buffel grass (Negawo *et al.,* 2024), in Napier grass (Habte *et al.,* 2020; Muktar *et al.,* 2022a), in alfalfa (Bhandari *et al.,* 2023; Lin *et al.,* 2021).

Recent studies have highlighted the importance of genetic parameters in evaluating nutritional quality traits in *Urochloa*. For instance, Figueiredo et al. 2019 demonstrated that significant genetic variation exists for traits such as crude protein (CP), neutral detergent fiber (NDF), and

lignin content (LIG) in *Urochloa* hybrids, indicating the potential for selection based on these traits Figueiredo et al. (2019). The authors emphasized the need for early selection strategies to enhance the efficiency of breeding programs, which can be particularly beneficial in the context of *Urochloa*, where a large number of genotypes are evaluated over multiple years.

Moreover, the work by Matias et al. provided a comprehensive analysis of the genetic parameters associated with nutritional traits in *Urochloa* spp., revealing that additive genetic variance plays a significant role in the expression of these traits (Matias *et al.,* 2020). This finding aligns with the research conducted by Ferreira *et al.,* 2019 which explored the genetics and genomics of *Urochloa* species, highlighting the importance of genetic diversity in improving forage quality (Ferreira *et al.,* 2021). The integration of genetic information with agronomic practices can enhance the nutritional profile of *Urochloa*, ultimately benefiting livestock production.

In addition to genetic evaluations, the influence of environmental factors on nutritional quality traits cannot be overlooked. Dias noted that seasonal variations, particularly increased lignification during certain periods, can negatively impact forage intake and digestibility, thereby affecting the overall nutritional quality of *Urochloa* (Dias, 2023). This underscores the necessity of considering both genetic and environmental interactions when mapping nutritional quality traits.

Furthermore, the application of advanced genomic techniques, such as genotyping by sequencing (GBS), has revolutionized the mapping of nutritional traits in *Urochloa*. GBS allows for the identification of SNPs linked to specific traits, providing a robust framework for QTL mapping (Ferreira *et al.,* 2021). This approach has been successfully employed in other crops to enhance nutritional quality, as seen in the work of Chen *et al.,* who identified candidate genes associated with protein content in rice through genome-wide association studies (Chen

et al., 2018). The methodologies developed in these studies can be adapted for *Urochloa* to facilitate the identification of genetic markers linked to nutritional quality traits.

In conclusion, mapping nutritional quality traits in *Urochloa* is a multifaceted endeavor that requires a comprehensive understanding of genetic variation, environmental influences, and advanced genomic techniques. By leveraging these insights, breeding programs can be optimized to develop *Urochloa* cultivars with enhanced nutritional profiles, ultimately contributing to improved livestock productivity and sustainability in tropical agroecosystems.

3.7.3 Diversity Arrays Technology

DArTseq is one of the commonly used genotyping-by-sequencing platforms that offers a cheap and efficient genome-wide genotyping. The utilization of DArTseq technology has significantly advanced genetic diversity assessment in polyploid crops including forage grasses. This technology combines the high-throughput nature of DArT arrays with the sequencing capabilities of next-generation sequencing (NGS), has enabled researchers to explore genetic variations in complex genomes effectively (Baloch *et al.,* 2017).

Several studies have successfully employed DArTseq for diversity, population structure genome wide mapping studies and genetic successfully employed in polyploids such as wheat(De Zutter *et al.,* 2024) barley including tetraploid and hexaploidy varieties(Dziurdziak *et al.,* 2021) Furthermore, recent research has highlighted the application of DArTseq in uncovering unexplored genetic loci in both landraces of buffel grass, demonstrating its efficacy in studying genetic diversity in polyploid forage crops (Negawo *et al.,* 2024). The DArTseq platforms facilitate the discovery of high-density SNP markers with cost-effective genotyping, contributing to the acceleration of gene discovery in climate-resilient and nutrient-rich millets (Gandhimeyyan *et al.,* 2023). The application of DArTseq technology has proven instrumental

in genotyping samples efficiently, as demonstrated in studies involving the assessment of genetic diversity in various plant species (Di *et al.,* 2022).

Diversity Arrays Technology sequencing (DArTseq) generates two main types of markers: dominant silico-DArT markers and codominant SNP markers. The silico-DArT markers are dominant and more numerous, while the SNP markers are codominant and more informative Okoń et al. (2018). The DArT software automatically computes quality parameters for each DArTseq and SNP marker, such as call rate, polymorphic information content (PIC), and reproducibility of both markers (Baloch *et al.,* 2017). DArTseq technology has been used to generate a substantial number of SNP markers in various crops, such as barley, durum wheat, strawberry, oat, and maize, which have been instrumental in genetic diversity assessment, linkage mapping, and QTL analysis (Wenzl *et al.,* 2004; Sánchez-Sevilla *et al.,* 2015; Koech *et al.,* 2019; Nadeem *et al.,* 2019; Curtolo *et al.,* 2017; Adewale *et al.,* 2023). These markers have been utilized for applications like genetic diversity studies, population structure analysis, association mapping, and QTL identification in different plant species (Negawo *et al.,* 2022; Fufa *et al.,* 2022; Negawo *et al.,* 2020; Tomkowiak *et al.,* 2021). Additionally, DArTseq markers have been employed in identifying disease resistance genes, enhancing fruit quality traits, and improving stress tolerance in crops like barley, citrus, and maize (Curtolo *et al.,* 2017; Adewale *et al.,* 2023; Piechota *et al.,* 2019). The markers generated through DArTseq technology have proven to be valuable tools for advancing plant breeding and genetic research by providing insights into genetic diversity, trait mapping, and marker-assisted selection in various crop species. In this thesis, DArTseq genotyping was used to analyze the *Urochloa* accessions for genetic diversity and genome wide association mapping studies.

CHAPTER THREE

GENETIC DIVERSITY AND POPULATION STRUCTURE ANALYSIS OF NATIVE *UROCHLOA* ACCESSIONS BASED ON PHENOTYPIC AND DArTseq MARKERS

3.1 Introduction

There are more than 100 species of *Urochloa* grasses in the *poaceae* family (Renvoize *et al.,* 1996). According to Keller-Grein *et al*. (1996), seven of which are commonly used as livestock feed include, *Urochloa ruziziensis Urochloa arrecta*, *Urochloa humidicola*, *Urochloa dictyoneura*, *Urochloa brizantha, Urochloa mutica* and *Urochloa decumbens*. Of the above, *Urochloa decumbens*, *brizantha, ruziziensis, and humidicola* are the most often used and improved species through breeding and/or selection. Most of the species are predominately tetraploid while *Urochloa ruziziensis* is largely diploid and used in hybridization.

According to Hojsgaard & Hörandl (2019), *Urochloa* grasses have been widely adopted in non-native environments, improving livestock industries globally and providing benefits to millions of people by increasing livestock productivity in tropical regions, particularly in Latin America where commercial monoculture production is practiced on a large scale(Jank *et al.,* 2014) and in smallholder mixed production systems of sub-Saharan Africa (Maass *et al.,* 2015).

Although *Urochloa* grasses are native to Africa, their improvement through breeding and selection, utilization, economic importance and adoption remain low compared to its economic impact registered in Latin America (Ghimire *et al.,* 2015; Paredes *et al.,* 2023). Information on *Urochloa* genetic diversity based on morphological and SNP markers; existing ploidy variation, and population structure is limited. With no systematic breeding and selection of *Urochloa* in Uganda, there is very scanty information on *Urochloa* morphological diversity,

ploidy variation and genetic diversity based on SNPs that could guide further grass improvement initiatives or even direct selection from natural populations. Furthermore, uncharacterized genetic diversity limit breeding and conservation efforts (Namazzi *et al.,* 2020). Collecting and assembling germplasm resources is crucial for starting breeding programs. The wild resources of *Urochloa* are particularly important because the first commercial cultivars in south America were selections from natural germplasm resources sourced from African grasslands (Jank *et al.,* 2014).

Previous genetic diversity studies among *Urochloa* in country collections in Uganda, Kenya, and Tanzania reveal, East Africa as a home to high variability with diverse gene pools (Kuwi *et al.,* 2018; Namazzi *et al.,* 2020; Ondabu *et al.,* 2017; Tegegn *et al.,* 2019) . In addition, performance of improved *Urochloa* cultivars introduced into East African countries through developmental projects such as Mulato II hybrid, Piata, xaraes, and Cobra, camello present excellent potential to improve productivity (Ghimire *et al.,* 2015; Maass *et al.,* 2015; Njarui *et al.,* 2021). Previous phylogenetic studies in *Urochloa* have used several markers ranging from RAPD marker (Ambiel *et al.,* 2010), ISSR (Nitthaisong *et al.,* 2016) to simple sequence repeat markers (Kuwi *et al.,* 2018; Ondabu *et al.,* 2017; Tegegn *et al.,* 2019). None of the diversity studies have employed SNP markers, yet they are known to be more abundant, uniformly distributed across the genome, providing a more comprehensive genome coverage compared to microsatellites (Zimmerman *et al.,* 2020).

With advances in genotyping by sequencing based on Diversity Array Technology (DArTseq), a whole genome technique can be used that allows sequencing minus preceding sequence information (Elshire *et al.,* 2011) to generate two types of markers single nucleotide polymorphisms and SilicoDArT markers. The technology has successfully been employed in genetic diversity and analysis of population structure in several tropical forages such as buffelgrass (Negawo *et al.,* 2020) Rhodes (Negawo *et al.,* 2021) and sesbania (Negawo *et al.,*

2023). Currently, there is no study on the application of these markers in *Urochloa* species. The only diversity study on part of the native Ugandan collection by Namazzi *et al* 2020 stratified the Ugandan indigenous *Urochloa* into three gene pools based on SSR markers.

Combining molecular classifications of accessions based on SNPS with ploidy variation and morphological classification reveals functional distinct clusters useful in choosing superior parents to be crossed for cultivar development, but also present opportunity to select superior ecotypes that can be promoted for adoption. Therefore, this study sought to examine the extent of agro-morphological diversity and genetic variations among of assembled *Urochloa* germplasm.

3.2 Materials and methods

3.2.1 Phenotypic Evaluation

3.2.1.1 Genetic materials

A total of two hundred and twenty-four *Urochloa* accessions including 99 ecotypes used in Namazzi *et al* 2020, and the additional one hundred and five accessions collected from eight agroecological zones of Uganda where livestock is main livelihood activity. The composition of the population used was as follows: Collected landraces (208), gene bank accession (10) and elite cultivars (6). Details are in the table of study material (Appendix 3.1).

3.2.1.2 Study site and Field layout

The collected landraces alongside the gene bank accessions and elite cultivars were established in 2020 and assessed for two cuttings (wet and dry) in 2020 and 2021 at the National Livestock Resources Research Institute, Wakiso, Uganda located at latitude 0.513404, longitude 32.638475, at 3722 ft above the sea level. Germplasm was planted in an augmented design of 24 entries x 11blocks with four checks replicated randomly in each of the eleven blocks planted

with ten plants per plot using four splits per hill. The four checks included *Urochloa* brizantha cv. Mulato II(S8), *Urochloa* decumbens cv. Basilisk(S4), *Urochloa* brizantha cv. Piata (S6), *Urochloa* brizantha cv. Xaraes (S5). Plants were planted at a spacing of one meter between plants and between rows, 1.5 meters between the plots and 2 meters between the blocks.

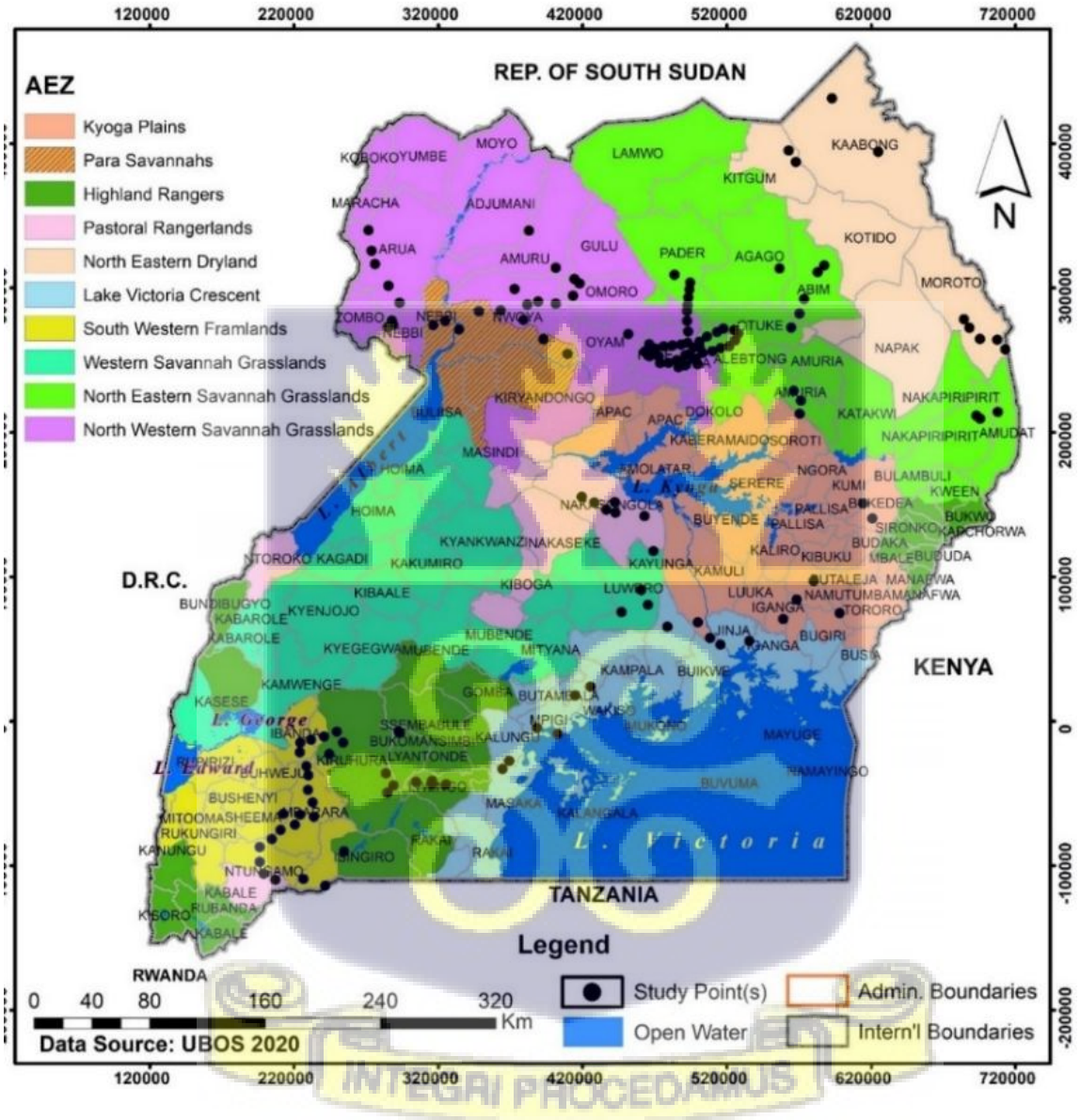


Figure 3. 1: Map showing collection sites of the native materials used in the study.

3.2.1.3 Data collection

The study materials were characterized by using the descriptors in Table 3.1. A total of 14 agro-morphological traits were evaluated. Two hundred and twenty-four accessions (220 test accessions and 4 checks). Fourteen morphological traits of which five were qualitative and nine quantitative were assessed according to the descriptor used in Njarui *et al*. (2016). The five qualitative traits were recorded as observed in the field and included growth habit, leaf hairiness, leaf color and stem color were expressed as percentages.

Quantitative traits such as days to 50% flowering, plant height, leafiness, stem thickness, leaf length, leaf width, plant spread, fresh weight single plant, leaf size, Dry matter yield-single plant were basis for evaluation of phenotypic diversity. Days to 50% flowering were recorded for days from sowing to when 50% of the plants in each accession had produced flowers. leaf length and leaf width were measured using a 30 cm ruler while plant height was measured using a meter rod and expressed in centimeters (cm).

Ploidy variation was determined by using the contents of DNA nucleus of single plant leaves were analyzed by flow cytometry to estimate the ploidy levels if each accession following procedures described by Doležel & Bartoš (2005).

The cell nuclei were isolated by chopping the midrib tissue from young leaves with a sharp razor blade in cold OTTO I buffer (0.1M citric acid monohydrate, 0.5% Tween 20). The suspension of nuclei was filtered through a 50 µm nylon mesh to remove large cellular material. The nuclei were stained in OTTO II buffer (0.4 M Na 2 HPO4, supplemented with 4 µg/ml DAPI,6-diamidino-2-phenylindole). The fluorescence of the nuclei was analyzed with a Partec flow cytometer (Partec GmbH, Munster, Germany), at a rate of 50-60 nuclei per sec.

Table 3. 1: Descriptors used for agro-morphological characterization of *Urochloa* germplasm.

	Character	Codes	Description	Observations
i.	Plant Height	PH	height from ground to flag leaf at full flowering	10 plants
ii.	Dry matter content	DMSP	Weight of harvested material after drying to constant weight	Single plant
iii.	Fresh biomass		Weight of harvested material after cutting	Single plant
iv.	Stem (Culm) thickness	ST	Thickness of the stem was measured at the second internode from the ground	10 plants
v.	Leaf length	LL	Length from ligule to tip of leaf (second leaf from flag leaf) at full flowering	10 plants
vi.	Leaf width	LW	Width of leaf of widest point (second leaf from flag leaf) at full flowering	10 plants
vii.	Leaf size	LS	Leaf length divided by leaf width	Calculated
viii.	Spread	SPD	length of widest point from center	10 plants
ix.	Leaf hairiness	LHad	Hairiness of behind surface of the leaf (0-glabrous 1 = moderately hairy and 3 Bristled)	10 plants
x.	Stem hairiness	LSH	Hairiness of the leaf sheath of the leaf (0-glabrous ,1 – hairy, 2- Bristled)	10 plants
xi.	Leafiness	LFN	Number of leaves (1= no leaves, 10= very leafy) at full flowering	Full plot
xii.	Growth habit	GH	Record at maturity after standard cut 1= Erect, 2=Decumbent ,3 = Procumbent	Full plot
xiii.	Leaf color	LC	Leaf color was scored at 50% flowering (Light green, Green, deep green)	Full plot
xiv.	Stem color	SC	color of stem at 50% flowering	Full plot

3.2.1.4 Phenotypic statistical analysis

Descriptive statistics and analysis of variance to determine the differences among genotypes were obtained using the augmented RCBD function in R package "*agricolae*" DAU test function (Mendiburu de Felipe, 2023) Adjusted means for the different genotypes for the two cropping seasons were obtained and used for further analysis. The qualitative scores were presented in bar charts for the eight agro-ecological zones (AEZ) where the genotypes were sourced. Principal component analysis (PCA) was run using f "*factoextra*" and "*vegan*" packages in R. There after eigenvalues and loading coefficients were extracted from the PCA output using the package functions. The contribution of each trait to the observed variability was determined using the first two principal components as described by Peres-Neto *et al.,*

2003. With the use of the first two principal components, a biplot plot was generated using the "*ggbiplot*" package in R.

3.2.2 Molecular characterization

3.2.2.1 DNA extraction and SNP discovery by DArTseq™ technology

The young leaves of each of the one hundred eighty-eight were collected from plants on station at a tender age of 45 days. The dried leaf samples of each genotype were sent to SEQART AFRICA at the International Livestock Research Institute (ILRI) in Nairobi for genotyping. The process of DNA extraction was performed utilizing the Nucleomag Plant DNA extraction kit, specifically the Mag-Bind® Plant DNA DS 96 Kit. The isolated genomic DNA had a concentration ranging from 50 to 100 ng/µl. The quality and amount of DNA were assessed using 0.8% agarose gel. The libraries were generated utilizing the DArTSeq complexity reduction methodology (Kilian *et al.,* 2016) by the digestion of genomic DNA using PstI and MseI enzymes. Subsequently, the barcoded adapters and common adapters were joined together, and then the resulting fragments were amplified by PCR. The libraries were subsequently subjected to single-read sequencing runs, with each run generating sequences of seventy-seven bases.

The Hiseq2500 platform was utilized for next-generation sequencing (Kilian *et al.,* 2016). The SEQART AFRICA platform utilizes genotyping by sequencing (GBS) DArTseq™ technology, which allows for rapid, high-quality, and cost-efficient genome profiling of complex polyploid genomes. The scoring of DArTseq markers was accomplished using DArTsoft14, an internal pipeline for marker scoring developed by Kilian *et al.,* 2016. The SilicoDArT markers and SNP markers were assessed using a binary scoring system. A value of 1 was assigned if the restriction fragment containing the marker sequence was present in the genomic representation of the sample, and a value of 0 was assigned if it was absent.

The SilicoDArT markers and SNP markers were mapped to the *Setaria italica* (GenBank: GCF_000263155.2) to determine their placements on the chromosomes. The *Setaria italica* genome was used as a reference for mapping SilicoDArT and SNP markers in *Urochloa* because it is one of the most closely related grass species with a fully sequenced and well-annotated reference genome available. Both *Setaria* and *Urochloa* belong to the Panicoideae subfamily within the Poaceae family and share a common evolutionary lineage with other C_4 tropical grasses such as *Panicum*, *Pennisetum*, and *Cenchrus* (Zhao *et al.,* 2012; Worthington *et al.,* 2016).

Comparative genomic analyses have shown high levels of synteny and conserved gene order between *Setaria italica* and other Panicoid grasses, including *Urochloa*, making it a suitable proxy for marker alignment and chromosomal localization (Bennetzen *et al.,* 2012; Zhang *et al.,* 2012). Because the *Urochloa* genome is large, complex, and highly polyploid—with limited public reference assemblies available using the *Setaria* genome facilitates the anchoring of SilicoDArT and SNP markers onto a well-characterized genomic framework.

This comparative approach allows for approximate positioning of loci, detection of homoeologous regions, and identification of candidate genes associated with key agronomic and nutritional traits, while also enhancing cross-species marker transferability within the Panicoideae group.

3.2.2.2 Analysis of genetic diversity and population structure

The process of ensuring data quality and removing unwanted data was carried out using TASSEL (v5.2.52) (Bradbury *et al.,* 2007). SNP markers exhibiting more than 20% missing data, a minor allele frequency (MAF) below 0.05, and places on the genome that are unknown were excluded. The SNP data underwent further imputation using the k-nearest neighbor

genotype imputation approach, as described by Bradbury *et al.,* 2007. In the end, a grand total of 19,668 SNPs were retained for subsequent analysis.

The "*snpReady"* package, popgen function in R (R Core Team, 2023) was used to generate the observed and expected heterozygosity, gene diversity and the polymorphism information content. Minor allele frequencies were calculated using TASSEL (v5.2.52) (Bradbury *et al.,* 2007).

STRUCTURE software (v2.3.4) was used to assess the population structure (Evanno *et al.,* 2005) that adhered to the Hardy–Weinberg equilibrium and its correlated allele frequencies, with a burn-in period of 10,000 Markov–chain Monte Carlo iterations and a 10,000-run duration. For every value of the number of clusters, which varied from 1 to 10, ten separate runs were made. Structure harvester was used to examine the structure outputs to determine the best K value as the distinct peak in the change of likelihood (Earl & vonHoldt, 2012).

For cluster analysis, a distance matrix was constructed using the Euclidean distance under the "*stat*" package in R The genetic distances calculated were used to construct a "*ward.D*" clustering algorithms to generate a hierarchical dendrogram and visualized using factoextra in R. POPPR package in R was used to run the Analysis of Molecular Variance (Kamvar *et al.,* 2014). The genotypes of *Urochloa* were categorized according to their place of origin. Prior to AMOVA, A = 1, C = 2, G = 3, and T = 4 were the number codes assigned to the marker datasets (Blyton & Flanagan, 2006). 999 permutations of AMOVA were applied to numerically coded data.

3.3 Results

3.3.1 Phenotypic Diversity.

3.3.1.1 Qualitative trait diversity

Qualitative characteristics varied in leaf color, stem pigmentation, leaf /stem hairiness and growth habit. 70% of the accessions had green leaf color, 10% light green, and 20% dark green. 25% of the accessions had leaves with no hair (glabrous), 62% had moderately hairy leaves, and 13% had hairy leaves. Accessions differed in stem hairiness, 43% were glabrous stems, 57% hairy, and none having bristled stems. Stem pigmentation was either green (82%) or purple (18%) coloration. The growth habit among the Ugandan accessions was predominantly decumbent (57%), erect accessions accounted for 28%, and procumbent (15%). The shape of the leaves varied, ranging from long and narrow blades, long in leaf length with narrow leaf blades, long leaf length with wide leaf blades to short and extremely thin leaf blades as shown in the pictorial presentation.

Table 3. 2: Morphological variability based on qualitative traits.

Observed trait	Variant	Counts	Percentages
Leaf colour	Light green 1	22	10
	Green-2	157	70
	Deep Green-3	45	20
Stem colour	Purple-1	40	18
	Green-2	184	82
Leaf hairiness	Glabrous-0	56	25
	moderate hairiness 1	139	62
	hairy-2	29	13
Stem hairiness	Glabrous-0	96	43
	Hairy-1	128	57
	Bristled-2	0	0
Growth habit	Procumbent-1	34	15
	Decumbent-2	127	57
	Erect-3	63	28
Pliody	Diploid	155	85.1
	Triploid	17	9.04
	Polyploid	11	5.85
	Pentaploid	3	1.59
	Hexaploidy	2	1.06

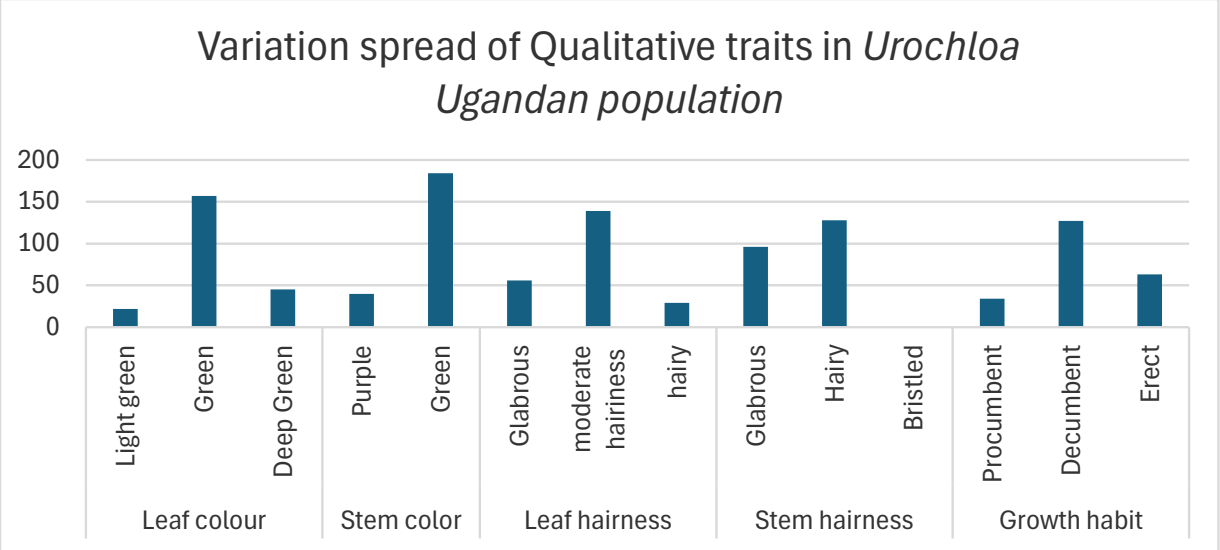


Figure 3 1 : Summary of the range of the key five qualitative morphological traits.

3.3.1.2 Ploidy variation

The genotyped pane was submitted for ploidy analysis revealed varying ploidy levels ranging from diploid to hexaploidy. The bulk of the genotypes (155, or 85.1%) were observed as diploid, whereas 11 genotypes (5.85%) were found to be polyploid. Apart from the two commonly recognized ploidy levels, seventeen genotypes were observed as triploid, three were pentaploid, and two were hexaploidy.

Figure 3. 2*:* Pictorial presentation of the variations in morphological characters of *Urochloa*.

3.3.1.3 Descriptive statistics and Analysis of quantitative traits

The mean performance of the accessions showed that average plant height was 84.5cm where the shortest accession was 24.76 cm and the tallest was 217cm. The stem thickness ranged from 4.4 to 20.42 with an average of 10.48 mm. The leaf length ranged from 6.67cm to 51.41cm and an average of 22.88 cm. Leaf width on the other hand ranged from 0.74 to 3.47cm. The average plant spread was 124cm wide, leaf. Fresh biomass yield per plant ranged from 0.2kg to 4.87kg with average biomass weight of 1.83kg and lastly dry matter yield single plant varied from 0.04kg to 0.67 kg per single plant The combined analysis of variance for the 8 quantitative traits studied is shown in. Analysis of variance revealed significant differences ($p < 0.01$) among accessions for Plant height (PH), Plant spread, fresh biomass single plant and dry matter yield single plant (Table 3.2). There were significant differences among seasons for all the evaluated traits except for the days to flowering, leaf length (LL), leaf width (LW), and stem thickness (ST). The accession × season (AxS) interaction displayed significant differences ($p<0.05$ and $p < 0.01$). The table of means is presented in appendix 2

Table 3. 3: Mean squares for phenotypic quantitative traits

SoV	df	LL	LS	LW	PH	PS	ST	dmsp	SP
Accessions	223	31.67ns	187.12ns	0.08*	572.38**	804.26 **	0.09ns	850.89**	783.52**
Seasons	3	251.64**	1442.9**	0.23*	2905.76*	177.31ns	1.02**	1947.63**	4.77**
Season*accession	1	134.45ns	1777.37*	0.40*	110.66*	2593.14**	2.87**	935.82**	114.97**
Block	10	54.08 ns	331.18 ns	0.02 ns	213.59 ns	524.45 *	0.11 ns	354.16 **	2.68 **
Residuals	30	45.47	307.87	0.06	245.65	191.71	0.1	94.69	0.42
Mean		**22.38**	**38.32**	**1.7**	**84.05**	**124.38**	**1.48**	**0.67**	**3.68**
Standard error		**2.88**	**7.48**	**0.11**	**6.68**	**5.9**	**0.13**	**4.15**	**0.28**
cv (%)		**29.74**	**44.59**	**14.65**	**18.59**	**11.24**	**20.8**	**27.59**	**19.31**

NB: SoV = Sources of variation; df = Degrees of freedom; LHAb = Leaf hairiness abaxial; Lhad = Leaf hairiness adaxial; LL = Leaf length; LS = Leaf size; LW = leaf width; PH = Plant height; PS =Plant spread; ST = stem thickness; dmsp = dry matter content-single plant; SP = single plant fresh weight.

3.3.1.4 Contribution of traits to total phenotypic variation

Principal component analysis (PCA) was used to examine patterns of variation and the relative importance of each phenotypic trait in explaining the observed variability in the population. The first six principal components (PC) accounted for about 71% of the overall variation (Table 3.4). The first principal component accounted for 21% of the total variation, in which leaf color, leaf hairiness, and stem hairiness contributed most to the variation. The second principal component accounted for 14% of the overall variance in which leaf length and leaf size were the main contributors. Plant height and plant spread contributed to the third principal component. The total variation in PC_4 was explained by dry weight of single plant, and growth habit whereas dry weight of single plant was primarily responsible for the variation in PC_5. Typically, components with eigenvalues of at least 1.0 must be considered. Considering, the first five components accounted for about 65% of the total observed variation. Overall, nine characteristics were important for differentiating the *Urochloa* genotypes.

Table 3. 4: The proportion of the morphological variation and traits contribution explained by the first six principal components.

Variable	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6
Leaf length	-0.75	**0.39**	0.08	-0.07	0.03	-0.06
Leaf size	-0.91	**0.32**	-0.07	0.00	-0.01	-0.07
Leaf width	-0.72	0.09	-0.28	0.13	-0.05	-0.08
Plant height	-0.12	0.15	**0.72**	0.20	0.09	0.07
Plant spread	-0.03	0.16	**0.74**	-0.05	-0.11	-0.32
Stem thickness	-0.58	-0.18	-0.31	-0.02	-0.14	0.10
Dry matter-single plant	0.17	0.10	-0.22	**0.35**	**0.72**	0.17
Fresh weight- Single plant	0.21	-0.13	-0.10	**0.36**	-0.68	0.18
Growth habit	-0.13	-0.10	0.15	**0.68**	0.07	-0.09
Stem color	-0.05	0.29	0.22	-0.55	0.01	0.60
Leaf colour	**0.35**	0.23	-0.26	-0.44	0.04	-0.51
Leaf hairiness	**0.30**	**0.78**	-0.27	0.18	-0.14	-0.01
Stem hairiness	**0.33**	**0.81**	-0.03	0.24	-0.10	0.08
Eigenvalue	**2.69**	**1.79**	**1.52**	**1.37**	**1.07**	**0.83**
Variance (%)	**20.69**	**13.76**	**11.71**	**10.54**	**8.23**	**6.38**
Cumulative (%)	**20.69**	**34.45**	**46.16**	**56.69**	**64.92**	**71.3**

To assess the clustering/grouping of individual genotypes, the scores of each genotype on the first two principal components, PC1 and PC2, were plotted (Figure 3.3). The genotypes were colored based on the geographical source categorized based on the agro-ecologies in Uganda. While most of the genotypes clustered around the center of the graph, others were widely scattered along the PC axes. Despite a large amount of overlap between agroecological zones, the dispersion pattern generally separated the genotypes based on the measured morphological traits (Figure 3.3). Notably, the improved introductions clustered at different quadrants with overlaps with ecotypes from Uganda.

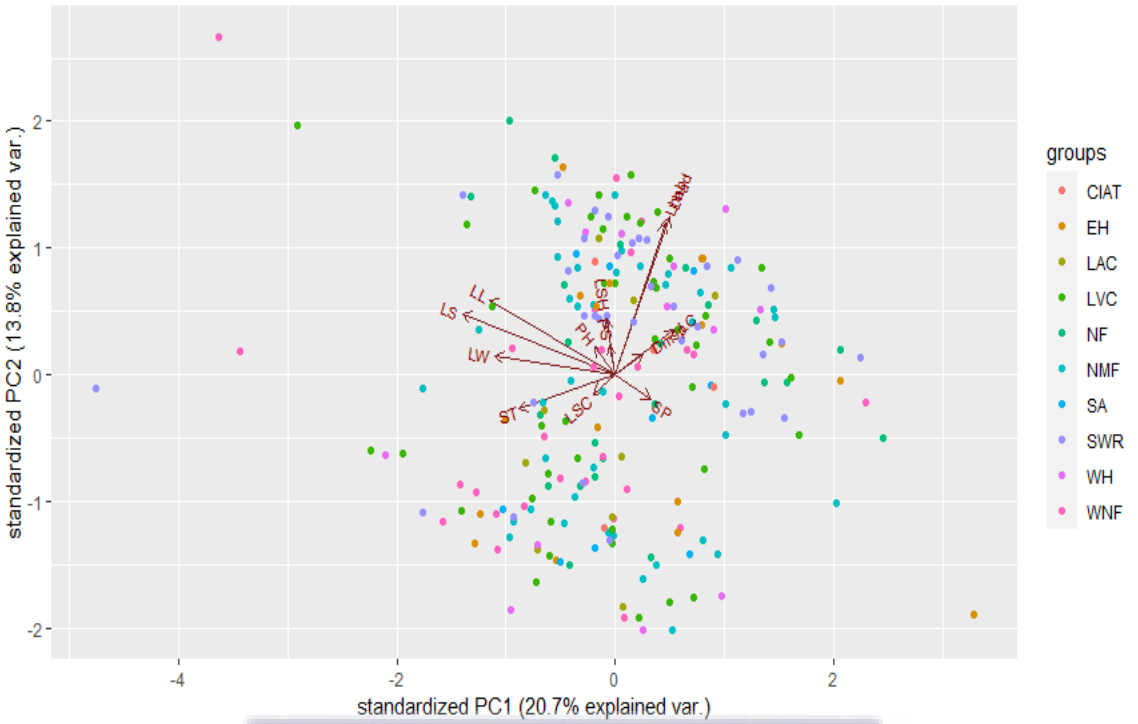


Figure 3. 3: The two-dimensional plot of the first two principal components (PC1 and PC2).

3.3.1.5 Cluster analysis and genetic relatedness among *Urochloa* genotypes based on morphological traits.

Dendrogram for the 224 *Urochloa* genotypes based on phenotypic traits is presented in Fig. 3.5 revealed eight clusters with Significant divergence attributed to the agro-morphological characteristics examined. Cluster 1 comprised 48 accessions with diverse agroecological origins. It included 10 accessions (20.83%) from the Northern farmland (Lira district), 10 accessions (20.83%) from the Lake Victoria Crescent, 14 accessions (29.17%) from southwestern rangelands, and 4 accessions (8.33%) from the eastern region. The commercial cultivar *U. brizantha* cv. MG4. Cluster 2 consisted of 33 accessions, originating from southwestern rangelands (42.42%), 18.18% from the Northern farmland, 18.18% from the Lake Victoria Crescent zone, and 18. % from southwestern rangelands. All accession within this clustered with cultivar *Urochloa humidicola cv. humidicola*. This cultivated variety is recognized for its ability to flourish on soils with poor drainage and high acidity, while

demonstrating a lower nutritional content in comparison to other species in the *Urochloa* genus. The accessions within this group also exhibit traits such as moderate plant height, leaf size, and a procumbent growth pattern. Cluster 3 consisted of 23 accessions, with 21.74% originating from the Lake Victoria Crescent, 13.04% from the Lake Albertine region, 8.70% from the southern highlands, and 52.17% from West Nile. All these accessions clustered with *U.decumbens* cv Basilisk. This variety is known for its sprawling growth pattern, huge leaf blades, and capacity to thrive in acidic soils. Cluster 4 consisted of 9 accessions, with 33.33% derived from the Northern cropland and 55.56% from the West Nile agroecological zone. Cluster 5 consists of 21 accessions, with 4.76% from western rangelands, 9.52% from West Nile, 9.52% from western highlands, 38.10% from southwestern rangelands, 23.81% from the Northern farmland, and 14.29% from the Lake Victoria Crescent zone. Cluster 6 consists of 60 accessions, with an equal distribution of 16.67% from Northern farmland, Lake Victoria Crescent, southwestern rangelands, and West Nile farmlands, 8.33% from western rangelands and 13.33% from western highlands. The commercial check Mulato II clustered with accessions of cluster 6. Cluster 7 consists of a total of 15 accessions, with 6.67% originating from western rangelands, 13.33% from West Nile, 20% from Northern cropland, 13.33% from the Lake Albertine Crescent, 13.33% from southwestern rangelands, and 26.67% from the Lake Victoria Crescent. All members clustered commercial variety of *Urochloa decumbens cv.* Piata. Group 8 consists of 17 accessions in total, with 31.25% originating from western rangelands, 12.5% from Northern cropland, and 50% from southwestern rangelands. This group has a close association with *Urochloa brizantha cv.*Xaraes (Figure 3.4).

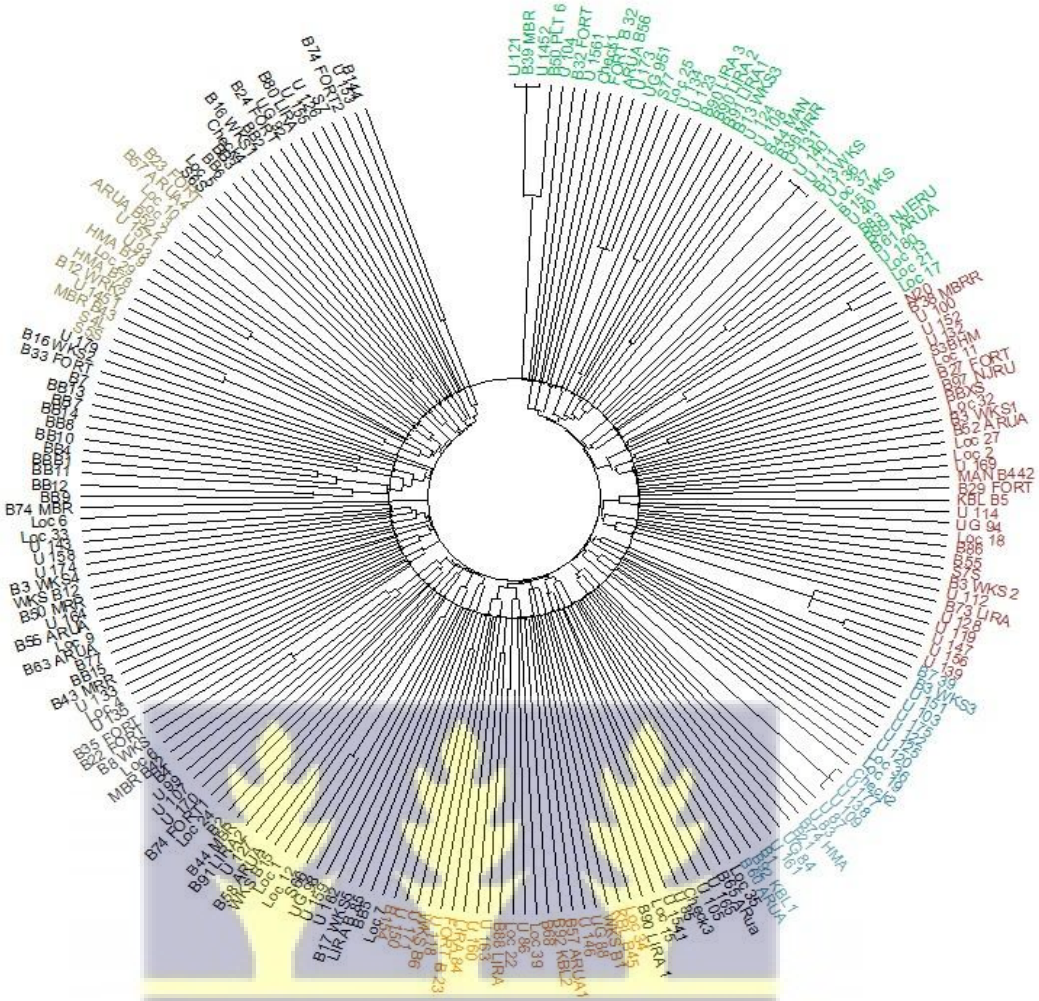


Figure 3. 4: Dendrogram showing diversity among 224 *Urochloa* genotypes based on phenotypic traits.

3.3.2 Genetic diversity and population structure based on DArTseq-SNPs

3.3.2.1 Informativeness and Diversity of the DArTseq-SNPs Markers

A total of 537,230 markers were produced, comprising 400,000 silicoDArT markers and 137,230 SNP markers. Quality control filtering retained 19,667 SNPs (14.3%) that were used for subsequent analysis. There were uneven Marker densities across chromosomes with the highest number of SNPs (3,112 SNPs, or 15.8%) mapped on Chromosome 1, followed by chromosome 2 with 2,702 SNPs (13.7%), chromosome 5 with 2,559 SNPs (13.01%).

Chromosome 3 had the least SNPs, with 1,365 SNPs, representing 6.9%. Figure 3.5 shows full distribution of the SNPs among all the 9 chromosomes.

SNP repeatability ranged from 90% to 100% with an average of 99%, while the call rate of the SNP markers varied between 38–100% with an average of 76%. The ratio of transitions (Ts, i.e., A/G or T/C substitutions) versus transversions (TV; i.e., A/T, A/C, T/G or C/G substitutions) approached 0.5 in the majority of the 24 distinct nucleotide substitution models**.** This further confirmed the quality of marker calling. With a ratio of 2.80, where transition SNPs (a total of 14995 SNPs, or 76.2% of the sample) were more common than transversions (4673 SNPs, or 23.73% of the sample). Of the six SNP combinations, A/G transitions (37.24%) had the highest frequency while G/C transversions (4.47%) had the lowest frequency. The frequencies of the four transversion types ranked as follows: A/T 8.04%, A/C 5.96%, G/T 6.83%, and G/C 4.47%. The frequencies of the two transition types were identical, namely A/G 37.24% and C/T 36.45%.

Figure 3. 5: Distribution of 19,668 SNPs across nine *Urochloa*

3.2.2.2 Genetic diversity and polymorphic information content

The average PIC value was 0.23 with only 1.64% of the markers having values less than 0.1, 44% of the markers were within the range of o.11 to 0.2, while 25% belonged to 0.21-0.3 PIC range and 30% had a PIC above 0.31. The populations general observed heterozygosity (Ho) which represents the percentage of individuals possessing distinct alleles was 0.25. The expected percentage of heterozygotes in the population represented by the average expected heterozygosity (He), was 0.28 and an average MAF of 0.19. Based on Agroecological zones, MAF range was between 0.16 to 0.20. The observed heterozygosity, genetic diversity, and PIC were highest in accessions from Southwestern rangeland and Northern farmland. Accessions from exhibited from lake Albert crescent zone exhibited the low polymorphism informative content (0.19), minor allele frequency (0.16), gene diversity (0.24). Accessions from NF, WH, LVC and SWR were more diverse than the introduced cultivars coupled with gene bank materials grouped as introduced germplasm (Table 3.5).

Table 3. 4: Diversity statistics of *Urochloa* germplasm based on DArTseq-SNP markers based on geographical origin

AEZ	NO. Acc	GD	PIC	MAF	HO	F	Ne	Va	Vd
EF	16	0.25	0.21	0.17	0.23	0.1	83	4978	1631
NF	30	0.29	0.23	0.2	0.28	0.02	619	5637	1976
WNY	22	0.26	0.21	0.17	0.22	0.14	76	5034	1671
WH	29	0.28	0.23	0.19	0.26	0.05	301	5410	1904
LVC	33	0.27	0.22	0.19	0.23	0.14	122	5291	1818
LAC	8	0.24	0.19	0.16	0.22	0.08	47	4671	1647
SWR	34	0.29	0.24	0.2	0.28	0.06	287	5731	1999
INT	16	0.26	0.21	0.18	0.24	0.07	109	5132	1827
Full Pop mean	188	0.28	0.23	0.19	0.25	0.1	986	5493	1860
Minimum		0.01	0.01	0.01	0.07	-2.3			
Maximum		0.5	0.38	0.5	0.92	0.74			

AEZ-Agroecological zone; No. Acc =Number of accessions GD= gene diversity, PIC= polymorphic information content, MAF= minor allele frequency, Ho= observed heterozygosity, F= Fixation index, EF=Eastern farmlands, NF=Northern farmlands, WNY=West Nile, WH=western highlands, LVC= Lake Victoria crescent, LAC-Lake Albert Crescent, SWR=southwestern rangelands, INT=introductions

3.3.3 Population differentiation, genetic relationships between and among accessions

The kinship correlation among of *Urochloa* accessions were between -0.043 to 0.86. In total, 62% of the 188 *Urochloa* accessions pairs had kinship values of 0.54. Two clearly delineated groups resulted from cluster analysis (Figure 3.4), and subsequently subdivided into 8 clusters

. Heterozygosity based on source/origin among the in 188 *Urochloa* accessions based on19,667 SNP marker lied between 0.07 and 0.92, with an average of 0.25 (Table 3.5). A genetic distance matrix was calculated to determine the most comparable pairings of accessions.

Figure 3.6 Panel (a) shows that **individual heterozygosity** peaks between **0.2 and 0.3**, indicating moderate genetic variation among accessions. Panel (b) reveals that **marker heterozygosity** is mostly below **0.3**, suggesting that most loci are moderately polymorphic. Panel (c) indicates that **minor allele frequency (MAF)** is skewed toward low values (<0.1), showing a high proportion of **rare alleles** within the population. Overall, the results suggest **moderate genetic diversity** across individuals and loci, with the presence of rare alleles reflecting both limited recombination and diverse accession origins.

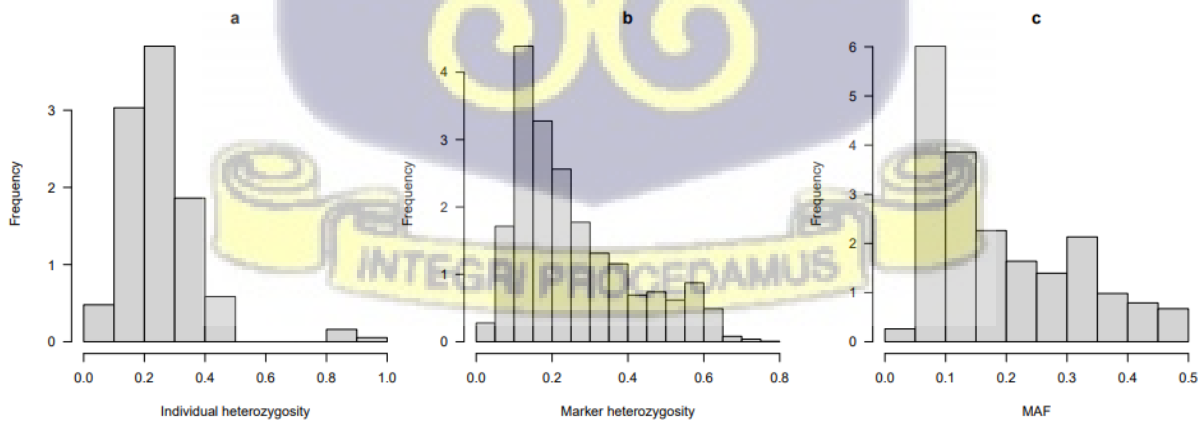


Figure 3. 6: a) Frequency of heterozygous genotypes and b) heterozygosity of 19,667 SNP markers and c) minor allele frequency of markers generated using DArTseq platform across 188 *Urochloa* genotypes.

A heatmap with hierarchical clustering generated to visualizes a pairwise genetic distance or similarity among accessions which reveals genetic relationships and sub-structuring among the genotypes was generated. Each block indicates a group of accessions that share a high level of similarity. Yellow regions suggest high similarity (low genetic distance within-group homogeneity, while orange-to-red blocks between them suggest high genetic differentiation among clusters.

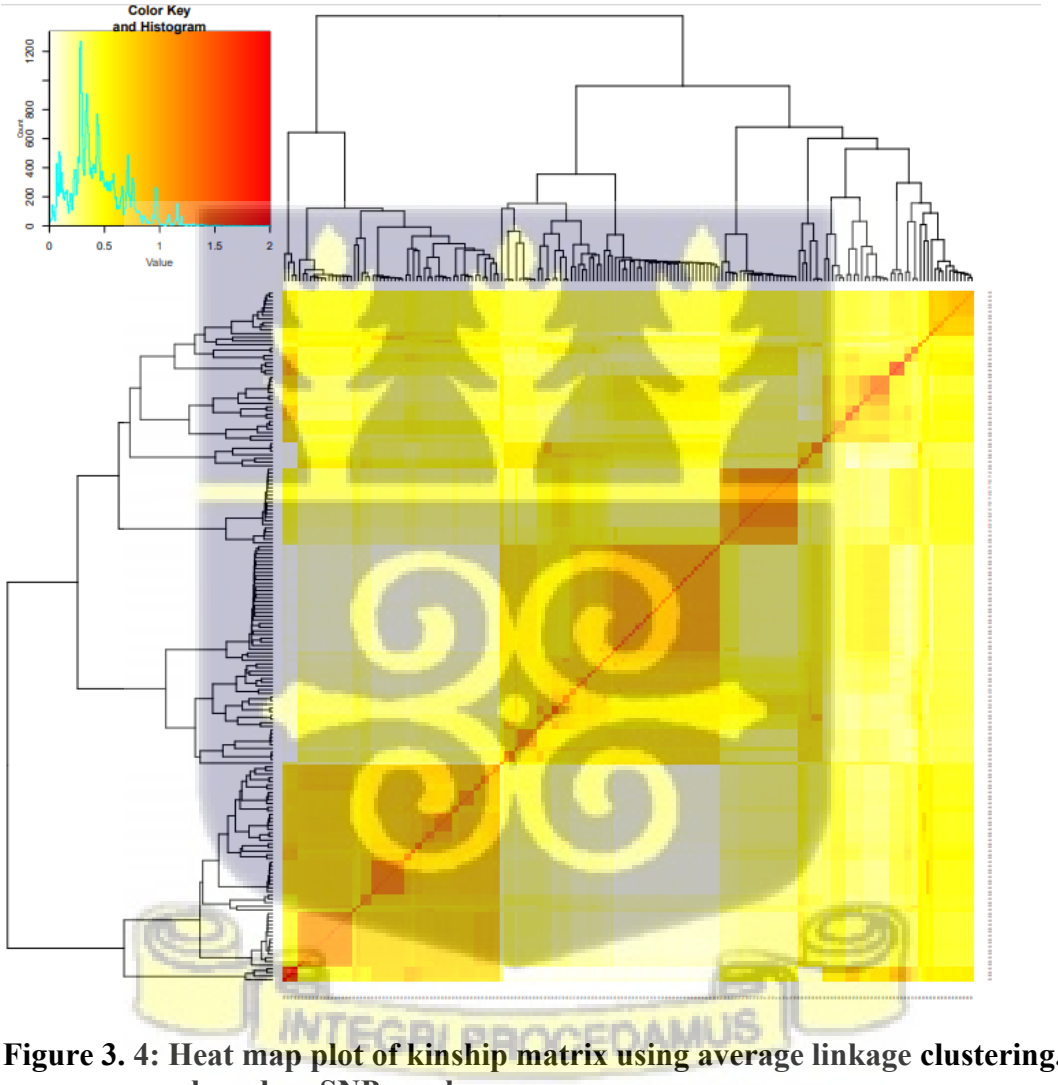


Figure 3. 4: Heat map plot of kinship matrix using average linkage clustering. based on SNP markers

3.3.4 Principal component analysis and population structure

Principal Component Analysis was used to reduce the large dataset of DArT markers into a few key dimensions that explain the main patterns of genetic variation. Dimension 1 explained 23.7% while dimension 2 explained 10.9% and together explained 34.6% of the total genetic variation among individuals (Fig. 3.7). The axes (Dim1 and Dim2) represent gradients of allelic differences genotypes that plotted close together were genetically similar, while those far apart were genetically divergent. There was no clear stratification based on geographical source of accessions.

Several clusters of points can be observed, indicating distinct genetic groups or subpopulations. Some groups overlap, while others are clearly separated, suggesting both shared ancestry and differentiation. For example: The EF, NF, and SWR groups seem closely related, as their points partially overlap near the origin. LAC and LVC individuals are scattered, indicating high within-group diversity or possible admixture with other clusters. WH and WNF accessions appear partially distinct, forming smaller groups, suggesting unique genetic composition or adaptation to different environments. The INT group shows moderate dispersion possibly representing intermediate or hybrid genotypes bridging other clusters.

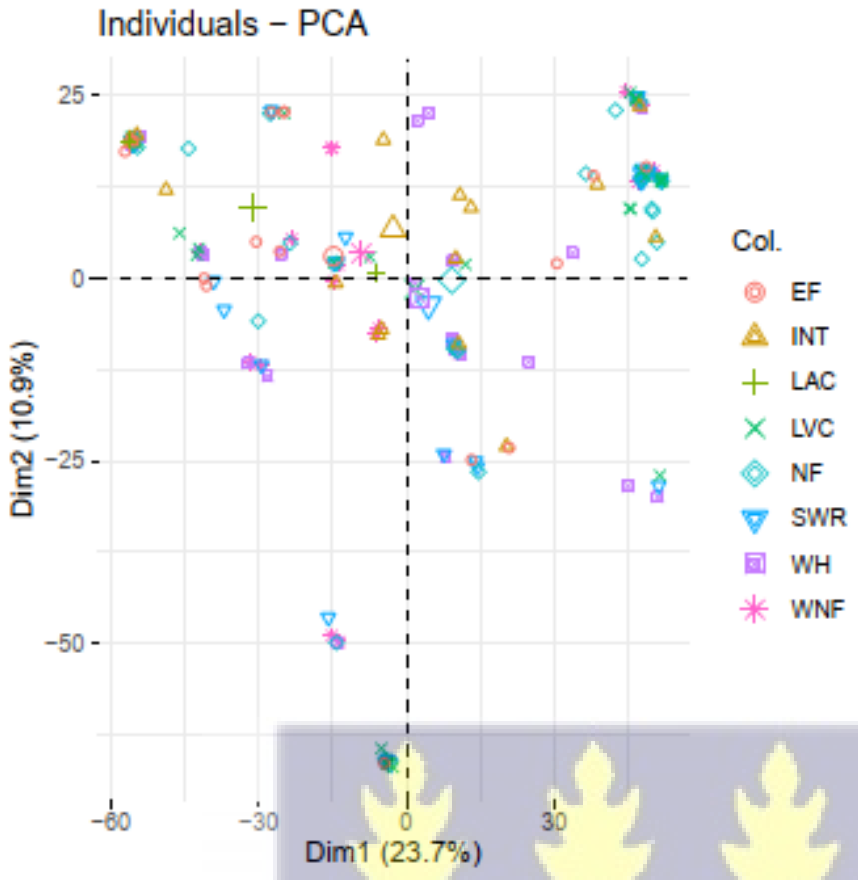


Figure 3.7 Principal Component Analysis (PCA) used to explain the genetic diversity across *Urochloa* germplasm based on DArTseq SNP data set

For population structure, a continuous-gradual increase was observed in the assessed log likelihood [LnP(D)] with the increase of K (Figure 3.8B) and the optimum grouping based on the delta K. The Delta K plot had y-axis shows ΔK, the rate of change in log-likelihood between successive K values (Evanno *et al.,* 2005) whereas the x-axis shows different possible numbers of clusters (*K*). The peak at K = 6 indicates the most likely number of genetic clusters or subpopulations in the population is six (6).

Each vertical bar represents an individual genotype while each color represents one of the six inferred genetic clusters (POP I–POP VI). The height of each color segment in a bar indicates the proportion of the individual's genome assigned to that cluster. The distinct blocks of color show genetically distinct populations while Mixed colors indicate admixture, suggesting gene

flow or shared ancestry between populations. Sub population 1 comprised 23 individuals accounting for 12.23%, sub population 2 had 19 individuals (10.10%). Subpopulation 3 was the largest with 71 individuals accounting for 37.76% whereas subpopulation 4 accounted for 6.9% with 13 individuals. Subpopulation 5 had the least membership of 6 individuals (3.19%) and finally subpopulation 6 comprised of 56 individuals representing 29.7% of the total population. Genotypes derived from several agro ecological zones were clustered in the same subpopulation.

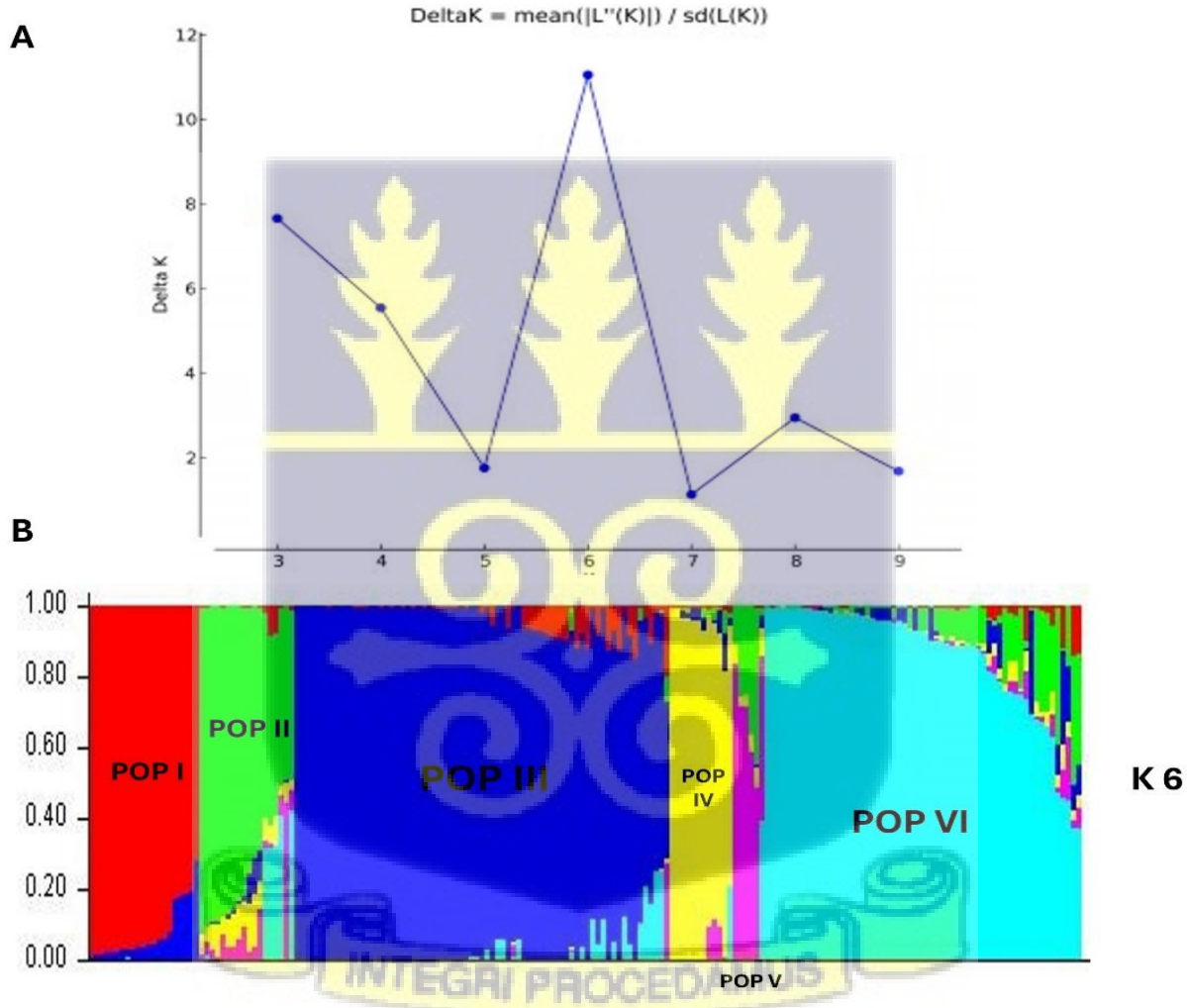


Figure 3. 8: (A) Delta (Δ)K for differing numbers of subpopulations (k), (B) estimated population structure of 188 *Urochloa* genotypes on (k = 6), each colour denotes a different subpopulation using STRUCTURE

Based on population genetics summary in table 3.6, the average inter-population distances ranged from 0.035 (SP2–SP5) to 0.295 (SP1–SP3). The lowest divergence (0.035) between SP2 and SP5 was an indication of close genetic relationship with possible recent common ancestry or gene flow. The highest divergence (0.295) between SP1 and SP3 indicated high genetic differentiation, likely representing distinct species, ploidy levels, or geographically isolated gene pools.

The fixation index (Fst) informs of the overall genetic divergence of the sub populations based on population structure. A Fst value above 0.25 indicates very strong differentiation (Wright, 1978). SP1, SP3, SP4, and SP6 show high genetic differentiation, consistent with their distinct STRUCTURE clusters. SP5 (FST = 0.01) is genetically similar to others possibly a hybrid group or recently diverged population.

Table 3. 5: STRUCTURE analysis of 188 *Urochloa* genotypes for net nucleotide distance, fixation index (Fst) (significant divergences) and average distances (expected heterozygosity) of genotypes in each subpopulation

Allele-freq. divergence among pops (Net nucleotide distance)							%Membership	FST	He
	SP1	**SP2**	**SP3**	**SP4**	**SP5**	**SP6**			
SP1	-	0.246	0.295	0.272	0.185	0.278	0.12	0.86	0.05
SP2	0.245	-	0.165	0.16	0.035	0.122	0.102	0.4	0.21
SP3	0.295	0.166	-	0.264	0.118	0.222	0.375	0.57	0.18
SP4	0.271	0.16	0.2637	-	0.101	0.18	0.071	0.65	0.14
SP5	0.184	0.035	0.1176	0.101	-	0.079	0.034	0.01	0.35
SP6	0.277	0.122	0.2222	0.18	0.079	-	0.298	0.56	0.17

SP1- Sub population 1; SP2 - Sub population 2; SP3 – Sub population 3; SP4 – Sub population 4; SP5- Sub population 5; SP6- Sub population6; FST=Fixation index, He = heterozygosity

The Euclidean distance matrix was employed for cluster analysis, leading to the categorization of the 188 genotypes into eight separate clusters determined by the silhouette method (Figure

3.9). This was similar to the clustering based on phenotypic traits. Each cluster is represented by a unique color. Cluster 1(red) consisted of 35 genotypes, which accounted for 12.7% of the total accessions clustered. This cluster included 8 genotypes from Lake Victoria Crescent, 4 from West Nile, 5 from Northern Farmland, and 3 from Southwestern Rangelands. All these genotypes were grouped together with the commercial cultivar *Urochloa* brizantha cv. *Marandu*. Cluster 2 (blue)consisted of 32 genotypes, which accounted for 14.8% of the total accessions. Among these genotypes, 9 were from Northern Farmland, 5 from Southwestern Rangelands, 5 from Western Rangelands, 2 from West Nile, and 2 from Lake Albertine Crescent. All these genotypes were clustered together with the commercial cultivar *Urochloa* brizantha cv. Xaraes. Cluster 3(green) had the largest membership of 42 genotypes, accounting for 13.8% of the total accessions. Among these genotypes, 10 were from Northern Farmland, 4 from Lake Victoria Crescent, 3 from Western, 2 from Lake Albert Crescent, and 6 from Southwestern Rangelands. These genotypes clustered with *Urochloa* hybrid called Mulato, which was introduced by CIAT popular for its high agronomic performance. Cluster 4(black) consisted of 26 genotypes, which accounted for 11.2% of the total. Among these genotypes, 7 were from Northern Farmland, 3 were from West Nile, 4 were from Southwestern Rangelands, 2 were from Western Rangelands, 3 were from Southwestern Rangelands, and one was from Eastern. These genotypes clustered along with Brachiaria brizantha cv Piata. Cluster 5(cyan) consisted of 12 genotypes, accounting for 22.9%, Cluster 6(Mahenta) consisted of 16 genotypes, which accounted for 24.5% of the total. Among these, 14 genotypes were from Lake Victoria Crescent, 13 from Northern Farmlands, 5 from West Nile, 7 from Southwestern Rangelands, 3 from Western Rangelands, and 3 from Western Highlands. Ultimately, these genotypes clustered together with the cobra hybrid. Cluster 7(orange) comprised of 4 accessions and lastly Cluster 8(dark blue) consisted of 21 accessions

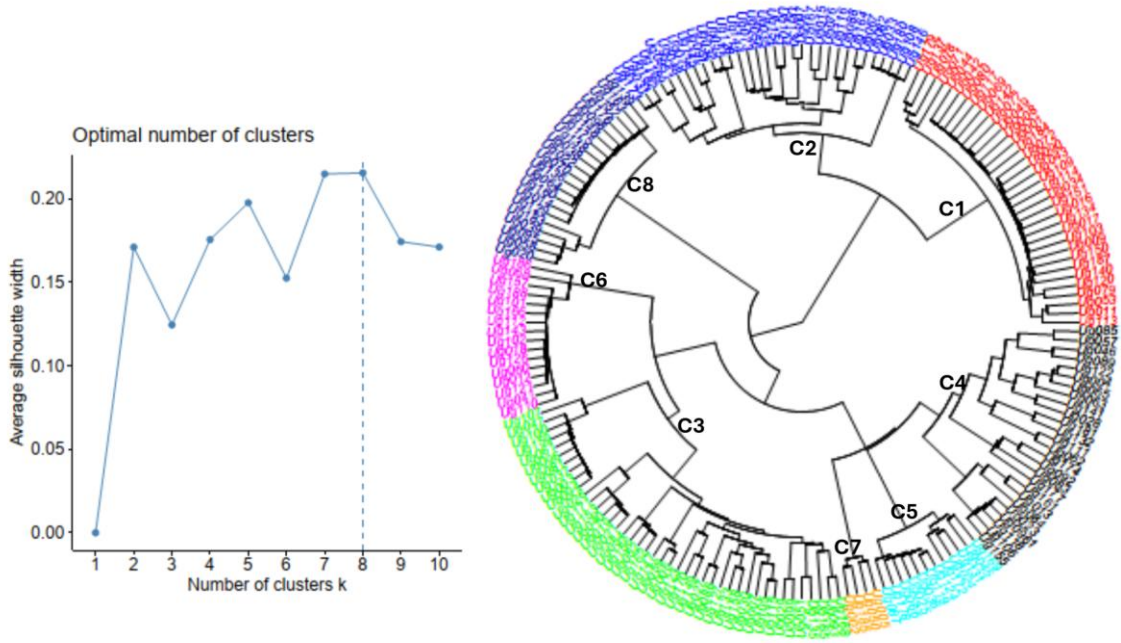


Figure 3.9: Hierarchical clustering of 188 *Urochloa* genotypes based on 19667 SNPS.

3.3.5 Genetic differentiation of populations

The Analysis of Molecular Variance AMOVA (table 3.7) partitioned total genetic variation into components of among populations and within populations, based on molecular data. The total molecular variance (1,483,226) was largely contributed by within-population variation (99.9%), while only 0.11% of the total variation was attributed to differences among subpopulations. The Phi statistic (Phi= 0.00108), analogous to Wright's FST indicates low genetic differentiation among subpopulations. This suggests that most genetic diversity is contained within populations (99.8%). not between them

Table 3. 6: Analysis of molecular variance within and among the 188 genotypes assessed. with 19,668 SNP markers.

Sources of variation	Df	Sum Sq	Mean Sq	Sigma	%	Phi
Between sub populations	5	40795	8159.1	8.6	0.11	0.00108
Within sub populations	182	1442431	7925.4	7925.4	99.9	
Total	187	1483226	7931.7	7934.1	100	

3.4 Discussion

The availability of diverse genetic resources is crucial for designing effective breeding and conservation strategies (Fowler & Hodgkin, 2004; Hoisington *et al.,* 1999). This study examined the morphological, ploidy and genetic diversity of *Urochloa* genotypes phenotypically and based on SNPs which is an indispensable approach for differentiating germplasm to provides valuable criteria for the selection of materials that possess desirable characteristics for breeding and conservation purposes(Balehegn, Ayantunde, *et al.,* 2022; Maass & Pengelly, 2019).

The evaluated *Urochloa* accessions exhibited considerable variation across both vegetative and reproductive traits, an indicative of a broad genetic base and adaptive diversity within the collection.The wide variability observed in morphological variation was characterized by structural differences among genotypes in color of leaves, hairiness of the leaves, stem pigmentation, and growth habit could be attributed to inherent genetic factors but also due to environmental influence and management practices.

The green leaf color (70%) was the predominant, followed by deep green (20%) and light green (10%). The prevalence of green and deep-green phenotypes suggests that most accessions possess high chlorophyll content and vigorous photosynthetic capacity, traits often associated with high forage productivity and adaptation to fertile soils (Jank *et al.,* 2014). The presence of lighter green variants may indicate nutrient limitation, environmental stress, or genotypic differences influencing pigment concentration. Comparable patterns were reported by Pessoa-Filho et al. (2017) and Vigna et al. (2016), that noted strong morphological differentiation among *Urochloa* accessions based on pigmentation and leaf hue. Green stems (82%) dominated the population, while purple pigmentation (18%) occurred less frequently. The purple coloration has been typically linked to anthocyanin accumulation, which may confer tolerance to abiotic stresses such as drought or high light intensity (Souza *et al.,* 2018). The

predominance of green-stemmed types reflects common traits in cultivated *Urochloa* varieties used for pasture establishment in tropical regions. The majority of accessions showed moderate hairiness (62%), with glabrous (25%) and hairy (13%) types being less frequent.

Moderate trichome density can improve resistance to pests and desiccation, while glabrous forms are typically associated with faster regrowth and higher palatability to livestock (Valle *et al.,* 2009). Variation in hairiness indicates adaptive plasticity, possibly reflecting responses to differing microclimates and grazing pressures.

Hairy stems (57%) were slightly more common than glabrous stems (43%), while bristled stems were absent. The presence of hairy stems enhances mechanical protection and water retention, particularly under dry or windy conditions. The coexistence of both glabrous and hairy types implies selection under diverse environmental pressures, a feature also observed in *Urochloa brizantha* and *U. decumbens* (Valle *et al.,* 2009; Vigna *et al.,* 2016).

The ability of *Urochloa* grasses to adapt morphologically to varying water conditions is vital for their survival and productivity in different environments. Most of the accessions were found with hairy leaves, a trait well known to serve as a defense mechanism against various environmental stresses, both biotic and abiotic (Mahjoob *et al.,* 2022). In forage crops, pubescence can also act as a physical barrier, providing a better photosynthetic environment for leaves, resulting in higher chlorophyll content, rubisco activity, and photosynthetic rates (An *et al.,* 2023) consequently higher yielding.

The decumbent growth form (57%) was the most common, followed by erect (28%) and procumbent (15%) types. Decumbent forms are advantageous in pasture persistence and soil cover, reducing erosion and improving forage regrowth after grazing (Jank *et al.,* 2014). Erect accessions, though fewer, are often high-yielding and better suited for mechanized harvesting.

The distribution of growth habits indicates that the germplasm comprises both grazing-adapted and cut-and-carry forage types.

Most of the accessions were predominantly of decumbent growth habit. Growth habit significantly influences response to grazing management (Mullenix *et al.,* 2016). Moreover, in mixed pastures, adjusting stubble height based on the growth habit of plants can impact herbage responses under grazing conditions (Santos *et al.,* 2018). Maintaining diverse plant species with different growth habits and phonologies in pastures is crucial for offering a healthy forage mix for livestock (Campbell *et al.,* 2013) and improve soil health in tropical regions (Horrocks *et al.,* 2019).

Ploidy Level variation varied with diploids (85.1%) constituted the majority of accessions, with triploids (9.04%), polyploids (5.85%), pentaploid (1.59%), and hexaploidy (1.06%) occurring at lower frequencies. The predominance of diploid accessions suggests that the collection includes a large proportion of sexual genotypes, which are crucial for recombination and breeding. Polyploid accessions, though fewer, are important sources of apomictic reproduction, stress tolerance, and vigorous growth (Mendes-Bonato *et al.,* 2002; Worthington *et al.,* 2019). Mixed ploidy levels within *Urochloa* are typical, reflecting its complex evolutionary history involving hybridization and genome duplication (Pessoa-Filho *et al.,* 2017). Ploidy variation in the collection ranged from diploid to hexaploidy with diploids being the majority. Similar levels of ploidy were reported by (Higgins *et al.,* 2022; Nitthaisong *et al.,* 2016, 2019; Risso-Pascotto *et al.,* 2009; Tomaszewska *et al.,* 2021). Variation in ploidy is closely linked to reproduction strategies in breeding *Urochloa*, to genetic diversity and ecological adaptability (Moghe & Shiu, 2014). According to Paul et al 2017, *Urochloa* species have basic chromosome numbers of X = 6,7,8 and 9 with X = 9 being the most common. This diversity in chromosomes

contributes to genetic variability observed within and among species allowing for a wide range of morphological and physiological adaptations.

The presences od both diploid and polyploid forms within the same species can lead to significant differences in traits such as drought tolerance, forage quality and growth rates (Beloni *et al.,* 2018). While polyploidy has been shown to contribute to increased genetic diversity in some grass species like Cynodon dactylon, the relationship between ploidy levels and genetic diversity in Brachiaria/*Urochloa* grasses is complex (Higgins *et al.,* 2022; Tomaszewska *et al.,* 2021).

The Significant genotypic differences for biomass and reproductive traits confirmed the rich genetic base of *Urochloa* germplasm (Pessoa-Filho *et al.,* 2017). Traits like plant height and dry matter yield are critical for forage productivity, and their strong genetic control suggests potential for selection and hybridization. The seasonal effects highlighted *Urochloa*'s phenotypic plasticity and adaptation to tropical climates with alternating wet and dry periods. The significant season × accession interactions underscore the need for multi-location, multi-season trials in breeding programs (Valle *et al.,* 2009; Jank *et al.,* 2014). Similar results have been reported in *Urochloa* in Ethiopia (Aleme *et al.,* 2023), and in Bermuda grass (Gouveia *et al.,* 2020). Significant diversity also indicates there is potential to advance through selection.

The principal component analysis (PCA) revealed six major axes explaining 71.3% of the total morphological variation among *Urochloa* accessions. PC1 explained 20.69% of the total variation with major contributors associated with vegetative size and leaf morphology traits. It distinguished accessions with large, thick leaves and stems from those with smaller, slender foliage. Larger-leaf types showed vigorous growth and higher biomass potential (*Jank et al., 2014; Vigna et al., 2016*). PC2 explained 13.76% mainly contributed to hairiness and surface

traits. Glabrous hairy, pigmented, stress-tolerant accessions from smooth, light-colored, more palatable ones. Hairiness and pigmentation confer drought and pest resistance (*Valle et al., 2009; Souza et al., 2018*). PC3 (11.71%) mainly attributed to plant stature and spread. Differentiated tall, erect, wide-spreading types from compact, decumbent forms. Variation reflects plant architecture influencing grazing tolerance and biomass yield (*Jank et al., 2014; Pessoa-Filho et al., 2017*). PC4 (10.54%) was associated with growth habit and color contrast. Distinguished erect or decumbent, light-colored plants from prostrate, dark-pigmented ones, reflecting eco-morphological adaptation to light and soil conditions (*Vigna et al., 2016*). PC5 accounted for 8.23% characterized by Biomass partitioning. Contrasted dry matter accumulation with moisture content. Accessions with higher dry matter are more water-efficient and suitable for silage or high-fiber forage (*Worthington et al., 2019*). PC6 accounted for 6.38 total variation associated with Secondary pigmentation traits**.** Differentiated accessions with strong stem pigmentation and lighter leaves from uniformly green ones, reflecting pigment-based stress responses and genotypic variation. (Leite *et al.,* 2021) (Ochola *et al.,* 2024)

The Principal Component Analysis (PCA) plot revealed Several clusters of points can be observed, indicating distinct genetic groups or subpopulations. Some groups overlap, while others are clearly separated, suggesting both shared ancestry and differentiation. The eastern farm land (EF), northern farmland (NF), and (SWR) groups seem closely related, as their points partially overlap near the origin. Lake Albert crescent (LAC) and Lake Victoria crescent (LVC) individuals are scattered, indicating high within-group diversity or possible admixture with other clusters. Western highlands (WH) and west Nile farmlands (WNF) accessions appear partially distinct, forming smaller groups, suggesting unique genetic composition or adaptation to different environments. The introductions (INT) group shows moderate dispersion representing hybrid genotypes bridging other clusters. Clusters that overlap (EF, NF, LVC)

indicate gene flow or shared ancestry, while isolated clusters (e.g., WH, WNF) reflect distinct genetic lineages. Genotypes located far apart on the PCA axes are genetically divergent therefore ideal for crossing programs aiming to maximize heterosis. Genotypes clustering together may represent similar genetic backgrounds, useful for maintaining uniformity or purity in seed production.

Whereas several molecular markers including SSRs have been employed in *Urochloa* to evaluate and ascertain the differences within and between populations (Ferreira *et al.,* 2016; Kuwi *et al.,* 2018; Namazzi *et al.,* 2020; Santos *et al.,* 2015; Souza *et al.,* 2018; Vigna *et al.,* 2011), no study has used SNPs markers to assess the genetic diversity and population structure. This study employed 19668 SNP markers from the Diversity Array Technology (DArT) platform to investigate the extent of diversity within and among the Ugandan *Urochloa* germplasm collection. The efficacy of DArT technology in assessing genetic diversity has been demonstrated in Rhodes grass (Negawo *et al.,* 2021), Cenchrus (Negawo *et al.,* 2020), and Sesbania (Negawo *et al.,* 2023).

The DArT SNPs were moderately informative, with an average PIC value of 0.23, indicating polymorphism within *Urochloa* accessions. Botstein *et al*. conducted a study in 1980 and categorized polymorphic information content value of 0.5 and above as being highly informative were highly informative. The PIC values in this study were significantly greater than those previously reported in other populations of tropical grass species, such as Rhodes grass, where the average PIC values for SNP markers were 0.18 (Negawo *et al.,* 2021) and 0.153 in Sesbania (Negawo *et al.,* 2023). Nevertheless, when SSRs were used, previous studies conducted in Uganda (Namazzi *et al.,* 2020) and Tanzania (Kuwi *et al.,* 2018) found higher PIC values of 0.89 and 0.79, respectively but based on simple sequence repeats (SSR) markers in *Urochloa*. The mismatch arises from the fact that SNPs are bi-allelic, meaning they only

have two possible alleles, which limits the polymorphic information content (PIC) values to a maximum of 0.5 when the two alleles have the same frequencies though with great genetic resolution (Helyar *et al.,* 2011; Suvi *et al.,* 2020). In contrast, multi-allelic markers like SSRs typically have PIC values more than 0.5. Therefore, the PIC value of 0.23 suggests the SNP markers were highly informative with a good ability to detect differences between genotypes, thereby aiding in the characterization of genetic diversity within *Urochloa* populations. The He range lied between 0.01 to 0.5, with an average of 0.28 indicating a moderately high genetic diversity of the collection.

Structure analysis revealed 6 subpopulations where POP I (red) was genetically distinct, with minimal admixture, representing a unique gene pool, possibly geographically or reproductively isolated. POP II (green) was characterized by moderate admixture, possibly indicating introgression or shared ancestry with neighboring groups. POP III (blue)was a large, homogenous cluster, possibly representing the dominant or most widespread genetic group. POP IV (yellow) was a narrow representation which may correspond to rare or localized genotypes. POP V (multicolor mix) exhibited high admixture suggesting intermediate individuals or hybridization events and lastly POP VI (cyan) was clearly distinct possibly represent different species or ploidy level. The identification of six genetic clusters aligns with previous studies that reported significant genetic sub-structuring within *Urochloa* species complexes.

Furthermore, Worthington et al. (2016) used DArT markers and found distinct genetic groups corresponding to species but not based on geographic origins, supporting the idea that *Urochloa* gene pools are structured taxonomically but not geographically. The admixture observed between POP II, III, and V suggests introgression and hybrid origin, a common feature in *Urochloa* due to its apomictic and polyploid nature (Mendes-Bonato *et al.,* 2002; Vigna *et al.,*

2011). *Urochloa* species often show interspecific hybridization, facilitated by reduced reproductive barriers (Worthington *et al.,* 2019). This pattern has been documented in tropical grasses such as *Panicum maximum* (Guinea grass) and *Cenchrus ciliaris* (buffel grass), where mixed ploidy levels contribute to complex population structure (Anderson *et al.,* 2010; Tomaszewska *et al.,* 2021).

The percentage membership values indicate varying levels of genetic purity and admixture among the six *Urochloa* subpopulations. SP1 (0.12) exhibited low cluster purity, suggesting it may represent a transitional or admixed group between distinct genetic clusters. SP2 (0.102) also showed high admixture, likely reflecting a hybrid origin resulting from interspecific gene flow. In contrast, SP3 (0.375) displayed moderate membership, indicating some level of gene exchange with other populations but still retaining partial genetic identity. SP4 (0.071) recorded very low membership, implying it could be a small or highly admixed group with limited distinctiveness. Similarly, SP5 (0.034) showed minimal membership, suggesting a high degree of admixture or a recently diverged lineage. On the other hand, SP6 (0.298) had relatively high membership, denoting a more genetically pure and distinct cluster that may represent a stable and well-differentiated gene pool within the *Urochloa* collection.

According to Wright, (1978) FST > 0.25 represents very strong differentiation. SP1, SP3, SP4, and SP6 show high genetic differentiation, consistent with their distinct STRUCTURE clusters. SP5 (FST = 0.01) is genetically similar to others possibly a hybrid group or recently diverged population. Such differentiation levels are typical of polyploid, apomictic grasses, where clonal reproduction maintains distinct lineages while occasional hybridization creates admixture (Vigna *et al.,* 2016; Worthington *et al.,* 2019).

Genetic structure and differentiation is demonstrated by the clear divergence among SP1, SP3, SP4, and SP6 (high FST and distance) supports that *Urochloa* accessions represent multiple

taxonomic groups or cytotypes. Studies show *Urochloa brizantha*, *U. decumbens*, and *U. ruziziensis* form distinct but related clusters, reflecting species boundaries and ploidy levels (Vigna *et al.,* 2011; Worthington *et al.,* 2016). The low FST and high He in SP5 reflect introgression and gene flow among species. Frequent interspecific hybridization is reported in *Urochloa* due to partially compatible genomes and apomixis-driven retention of hybrids (Mendes-Bonato *et al.,* 2002; Worthington *et al.,* 2019). Similar patterns occur in other tropical grasses like *Megathyrsus maximus* (Guinea grass) and *Cenchrus ciliaris*, where polyploidy and mixed mating systems create complex genetic structures (Anderson *et al.,* 2010; Tomaszewska *et al.,* 2021).

The AMOVA analysis indicates a genetic differentiation was highest within *Urochloa* populations, rather than among populations formed according to structure. The results of our analysis on the genetic variation partitions collaborate with earlier studies conducted by Kuwi *et al.,* (2018). Namazzi *et al.,* (2020). Hence, the main contributor to the genetic variety observed in *Urochloa* is the variance among individuals, rather than differences attributed to geographical origin. Additional studies in other related tropical grasses, such as buffelgrass (Negawo *et al.,* 2020), Rhodes grass (Negawo *et al.,* 2021), and Sesbania (Negawo *et al.,* 2023), have also demonstrated the absence of grouping individuals based on their geographic origin.

Employing informative SNP markers to analyze the population structure revealed 6 sub populations aligning with earlier studies by Negawo *et al* 2022,2024 who reported the existence of 6 subpopulation among sesbania and Buffel grass respectively and in contrast to Namazzi *et al* 2020 and Kuwi et al 2018 who reported 3 sub populations based on SSR markers. Like previous studies, there was no correlation between the population structure and geographical origin (Jungmann *et al.,* 2010) or the ploidy (Higgins *et al.,* 2022).

The genetic variations among the populations were confirmed by a substantial fixation index (Fst) value, which quantifies the differentiation of populations based on genetic structure, of six sub populations ranged from least divergent Fst of 0.01 (sub population 5) to very divergent Fst of 0.86 (sub population 1). Gene diversity based on expected heterozygosity and net nucleotide distances are two indicative measures of divergence among sub populations. Net nucleotide distances as a standard measure of divergence between heterogeneous populations ranged from 0.03 to 0.3 On the other hand, GD (or He) between subpopulations ranged from 0.05 to 0.35 suggesting diversity.

Arora *et al*. (2014) showed that the limited diversity observed among subpopulations can be attributed to a significant influx of genes. Wright's 1965 research findings suggested that a Nm value (haploid) below one indicated a restricted level of gene flow between subpopulations. Nevertheless, the Nm value in the present study was remarkably elevated at 5.621, indicating a substantial extent of genetic interchange between subpopulations. Consequently, there was a limited degree of genetic divergence among subpopulations.The existing population structure mostly resulted from the processes of selection and gene flow. The significant gene flow due to the shared ancestry of individuals from several geographic regions. A potential explanation for the infrequent occurrence of sexual reproduction and recombination in facultative apomicts could be attributed to their low genetic diversity.

3.5 Conclusion

In summary, the study revealed insights into the genetic diversity of *Urochloa*, shedding light on the range of vegetative morphological traits where majority of the accessions had green hairy leaves and stems, predominately of decumbent growth habit with varying ploidy levels ranging from diploid to nanoploid. The population was moderately diverse characterized by a gene diversity of 0.28 and polymorphic information content of 0.23. By assessing genetic

diversity using morphological and molecular markers, the study identified distinct eight clusters based on hierarchical clustering with specific genotypes maintain their cluster grouping under both phenotypic and molecular clustering. Structure analysis revealed six subpopulations whose Fst value ranged from 0.01 to 0.86. Most of the diversity was found within these sub populations (99%) compared to among sub populations. Based on genetic differences and the valuable population structure a hybridization scheme to improve the target traits of importance can be developed. The high level of genetic admixture within the population indicated high gene flow and genetic exchange among individuals.

The moderate genetic diversity in forage species can influence their adaptability and resilience to environmental stresses. Studies have shown that a broad genetic base in populations can serve as a valuable resource for improving forage quality traits, such as digestibility and nutrient content. However, the genetic variation observed in breeding programs often remains limited, as evidenced by the low coefficients of genetic variation reported. This limitation suggests that while moderate genetic diversity can be beneficial, it may not be sufficient to fully exploit the potential for improving forage quality traits without targeted breeding strategies that enhance genetic variability. Based on the genetic relationships among individuals that were not correlated to geographical origin, conservation strategies for maintaining genetic variability and preserving unique genetic traits within the population will be done from an informed perspective.

The predominance of diploid forms reveals significant insights into the evolutionary dynamics and ecological adaptations of these grasses. This pattern is indicative of a complex evolutionary history marked by polyploidization events that have shaped the genetic diversity and adaptability of these species. polyploidy can confer various ecological advantages, such as increased phenotypic plasticity and adaptability to diverse environmental conditions. the importance of diploid forms as foundational genetic resources for breeding programs aimed at

improving forage quality and resilience. The diploid species serve as a reservoir of genetic traits that can be harnessed in the development of new cultivars, particularly in the context of climate change and shifting agricultural demands. In *Urochloa*, the presence of both diploid and polyploid forms allows for a broader ecological niche, enabling these species to thrive in a variety of environments, from tropical grasslands to disturbed areas

The distinct genetic clusters (especially POP I, III, and VI) may represent core gene pools useful for breeding and germplasm conservation. The admixed populations (POP II, V) offer potential for heterosis and novel trait combinations important for traits like drought tolerance, biomass yield, and forage quality. Genetic differentiation also reflects adaptive divergence to different agroecological zones.

Overall, this study sets a foundation for advance through selection, trait-marker associations, and evolutionary dynamics of *Urochloa*, thereby contributing to advancements in breeding, conservation, and genetic research.

CHAPTER FOUR

DEVELOPING NEAR-INFRARED SPECTROSCOPIC CALIBRATIONS TO PREDICT FORAGE QUALITY IN *UROCHLOA* GRASSES

4.1 Introduction

The nutrient composition of *Urochloa* grasses is the primary determinant of palatability and nutritive value to livestock, which influences the amount of feed intake, its efficiency in the rumen, subsequently, the rates of body weight gain in beef (Euclides *et al.,* 2016), the quality and milk volumes (Mwendia *et al.,* 2022; Pereira *et al.,* 2005; Ruiz-Llontop *et al.,* 2022), quality and reproductive success (Delgado-Pertíñez & Horcada, 2021; Zhu *et al.,* 2013). Forage quality in *Urochloa* is mainly defined by total digestible nutrients, fiber content that can be categorized as acid detergent fiber (ADF) or neutral detergent fiber (NDF), crude protein content (CP), invitro organic matter digestibility (IVDMD) and crude fiber (CF) (Capstaff *et al.,* 2018; Chand *et al.,* 2022; Weiss & Hall, 2020).

Conventionally, these parameters are measured using laboratory wet chemistry method that requires preprocessing of the samples, use of reagents, skilled technicians, often taking hours of analysis and digestion. This method is labor intensive, expensive, are liable to pollution, and are generally unfeasible for use on large forage breeding populations. In addition, previous studies have highlighted that the grass chemical composition of *Urochloa* is generally influenced by phenological stages (Peralta *et al.,* 2020), species composition /genotype, type of management system, climate variability(Abdalla *et al.,* 2020; Mwendia *et al.,* 2021) and soil fertility (Guerra *et al.,* 2019; Melo *et al.,* 2022; Moore *et al.,* 2020). This necessitates frequent assessment across seasons, years, environments, and management systems, requiring routine screening and a high throughput analytical tool.

Selection for accessions that are highly digestive without efficient phenotyping methods, render assessment of forage quality traits in early selection stages, often not feasible, leading to postponement for evaluation at later stages of the breeding cycle when few genotypes are left. However, this practice causes a loss of vital genetic diversity at the early breeding stages.

Near Infrared Spectroscopy (NIRS) is an alternative method for phenotyping that is accurate, fast, and cost-effective for phenotyping nutritive qualities in a typical forage breeding program (cite). NIRs depend on infrared absorption and reflection when interacting with organic compounds in an analyzed sample. Absorption of photon energy and the stimulation of molecular overtones and combined vibrations are typical of chemical groups that primarily consist of hydrogen (N–H, O–H and C–H) (Jones *et al.,* 1987; Sandorfy *et al.,* 2006).

The phenotyping bottleneck for forage quality in pastures has in the past limited the selection efficiency, NIRS presents an alternative that is rapid, accurate and nondestructive(Corson *et al.,* 1999). NIR has been successfully employed to predict forage quality in pasture(Lobos *et al.,* 2013; Paz *et al.,* 2019; Rukundo *et al.,* 2021), existing in both pure and mixed swards (Catunda *et al.,* 2022; Parrini *et al.,* 2018a, 2021) with limited or no sample pre-processing and used in regular real-time pasture management (Bell *et al.,* 2018). Employment of NIRS in estimation of *Urochloa* chemical composition has previously been evaluated in *Urochloa* humidicola hybrids revealing high coefficient of determination and prediction accuracy (Mazabel *et al.,* 2020) suggesting the effectiveness of NIRS in phenotyping nutritional quality traits.

Integration of NIRs enables breeders to optimize the selection strategy by selecting for the rather expensive-to-evaluate and hard to screen forage quality traits early in the breeding cycle instead of evaluating for quality traits in the later generations when genotype numbers have reduced. Earlier calibration studies in *Urochloa* have all used partial least square analytical

techniques to generate accurate and reliable models (Guimarães *et al.,* 2023; Monrroy *et al.,* 2017). To date, NIRS usage to assess forage nutritional value continues to become accepted globally with advances in creating handheld and mobile versions of the spectrometers (Acosta *et al.,* 2020; Cherney *et al.,* 2021; Mendes de Oliveira *et al.,* 2024; Rukundo *et al.,* 2021). Although several laboratories have acquired NIRS systems in sub-Saharan Africa, only a few are operational for forage quality and feed evaluation, mainly due to the unavailability of comprehensive wet chemistry databases for tropical grasses required to formulate and validate reliable and precise predictive models(Balehegn *et al.,* 2022).

Therefore, this study was done to assess the i) NIR prediction accuracy for *Urochloa* forage quality traits and ii) to establish the optimal spectra preprocessing method that gave the most accurate models.

4.2 Materials and methods

4.2.1 Sample description and processing

About 387 samples of *Urochloa* grass from a multilocational trial 188 genotypes diverse genotypes were used in this study. This population comprised 184 landraces and four introductions from Latin America, namely *Urochloa brizantha* cultivars of *Marandú*, *Xaraés*, *Piatã* and *Urochloa decumbens cv. Basilisk* was established in three different agroecological zones (AEZ) of Uganda in wet season 2021A, running up to wet season 2022B. The study involved using mixed *Urochloa* species informed by varying ploidy levels.

The three AEZs used included the Lake Victoria crescent site at the National Livestock Resources Research Institute (NaLIRRI) located at a latitude of 0.513404, Longitude 32.638475 and elevation of 3717 ft above sea level. The southwestern rangelands site was at Mbarara Zonal Agricultural Research and Development Institute (MbaZARDI), located at latitude 0°.36’7.20’’S and longitude: 30°36’34.89°E, and the Northern farmlands site at Ngetta

Zonal Agricultural Research Institute (NgettaZARDI) located at N02°17.903′ E032°55.007 and elevation of 3549M above the sea level. The average temperature was 25 °C, and the average annual precipitation was 1,250 mm. Sampling dates were November 2021, January 2022, March 2022, and May 2022, corresponding to two wet and two dry season cuts.

In the field, plants were established using an augmented design with 11 blocks and 19 accessions per block experimental design. Each accession was planted in 2-row plots for each genotype (3M wide by 5 M long). Plants were left to grow for three months, and a standardization cut was three months after planting. Harvesting was done on a full plot basis at a cutting interval of 45 days, during which a 300 – 350-gram sample was randomly sub-sampled from each location using a machete at a cutting height of 8cm from the ground. The sample was chopped into 2 cm pieces, after harvesting placed in a paper bag, and dried in a conventional oven at 60°C for 48 hours. Dried samples were then weighed and ground in a wiley mill (make of the mill) with a 1mm sieve size screen, stored in ziplock plastic bags and labelled for subsequent evaluation of chemical composition and generation of spectral data.

4.2.2 Generation of reference data

The chemical analyses were performed at the National livestock Resources Research Institute nutrition laboratory. The NDF and ADF chemical concentrations were measured sequentially, according to operating instructions, using fiber tec 2010 & M6 AN 384 methods. A FOSS Kjeltec 8100 (Foss Company) was used to determine crude protein according to the guidelines of the Association of Official Analytical Chemists AOAC, Method 2001.11 (AOAC International, 2002). An IVOMD estimation was done using the method described by Tilley and Terry (1963). All parameters were analyzed in duplicate, and mean values were used to construct the calibration models. Forage quality parameters such as dry matter (DM), crude protein content (CP) and ash of samples were analyzed according to standards methods; 967.03,

984.13and 942.05, of the Association of Official Analytical Chemists (AOAC 1990). Metabolizable energy was calculated using the equation below

ME(Kcal/kgDM) = 35*CP+85*EE+35*NFE

NFE = 100 – (CP + EE+CF+ moisture + ash)

Where CP= crude protein, EE = ether extract, CF = crude fiber, NFE= Nitrogen free extract.

4.2.3 Spectra acquisition

Five grams of milled powder were scanned using a benchtop near-infrared spectrometer (DS2500, Denmark). Each sample was randomly partitioned into three portions, and each sub-sample was loaded into a small sample cut accessory for the DS2500 NIRS and scanned to generate a spectrum. Ground sample was loaded in the cup in such a way to ensure uniform packing to minimize light scattering. Three spectra were generated per sample (representing a plot) and a total of 1,161 samples were collected. All spectra were collected using a 0.5 nm resolution from 400 to 2500 nm, and each spectrum generated was an average of 50 sub-spectra collected over 60 seconds.

4.2.4 Pre-processing of spectral data

Spectral data was first pre-treated prior to development of the model. Data transformation to log (1/R) was done after which outlier detection and removal was done based on Mahalanobis distance according to Hershberger *et al.,* 2022. For better model performance, spectra variability unrelated to the parameter were minimized through pre-processing treatment and spectra data was corrected to achieve more accurate and robust prediction results (Rinnan *et al.,* 2009). Based on sample characteristics and sample measurement protocol, five correction methods were employed either singly or in combination, to increase the signal-to-noise ratio before they were used in the calibration process. The effect of two light-scatter correction

methods - Standard Normal Variate (SNV) and Multiplicative Scatter Correction (MSC) were tested on several derivative and smoothing options.

The standard normal variate (SNV) was used to correct for light scatter effects on spectra variability that could have arisen from differences in sample preparation, physical variations where the different samples were sourced, particle size and distribution and general sample heterogeneity.

Derivatization using gap segments generated derivatives with options arranged as (m,w,s,delta) where m represents order of derivative, w = the spacing of data points during derivatization denoted as gap size, s = segment size and delta indicated in the sampling interval

Baseline correction was also applied according to Rinnan et al. (2009) to correct any base line drifts among the samples. Combinations of the spectral pre-processing techniques were formulated as treatments and their effects on the NIRS predictions were compared to no treatment

For SNV correction, the aim was to minimize light scattering differences among samples of a data set and for the heterogeneity among the powdery samples or differences in particle sizes among samples. SNV was applied first to correct the effects of the multiplicative interferences of scatter and particle size differences by removing the mean and scaling to unit variance. This correction was given by:

$Si = \frac{So - Sv}{Sd}$, where *Si* = corrected spectrum, *So* = original individual spectrum measured by the NIR device, *Sv* = average value of the sample spectrum to be corrected and *Sd* = standard deviation of the sample spectrum.

In Rstudio, SNV correction of spectral data was applied from the *prospectr* package, using the function known as the *standardNormalvariate* (Stevens *et al.,* 2015).

The Multiplicative Scatter Correction (MSC) uses the regression of each spectrum to a reference sample spectrum (an average spectrum of the training set) as it addresses spectral errors attributed to differences in particle size. The slope and offset of the sample spectra are adjusted to the ideal average spectra to give the MSC corrected spectrum. The process of MSC correction, considering the average spectrum as the reference, was done according to Ikeogu et al. (2017) as follows;

a. Reference spectrum calculation: $\dot{s}_j = \sum_{i=1}^{n}(Si, j)/n$

b. Using spectral responses in each spectrum to calculate a linear regression against the corresponding points in the reference spectrum: $S_i = a_i\dot{s} + b_i$

c. Subtracting the slope from the regression on the original spectrum and dividing with the offset values to obtain MSC corrected spectrum: $S_{i(MSC)} = (S_j - b_i)/a_i$, where S = spectral responses for all the wavelengths; ṡ = average responses of all the training set spectra at each wavelength; Si = responses for a single spectrum in the training set; n = number of training spectra; ai and bi = slope and offset coefficients of the linear regression of the mean spectrum vector versus Sj spectrum. In Rstudio, the msc function from the prospectr package was used (Stevens *et al.,* 2015).

Derivatives and Smoothing*:* Derivatization attempts to remove both additive and multiplicative effects in form of vertical offsets and linearly sloping baselines from spectra (Rinnan *et al.,* 2009). The basic method of derivation is finite difference where: the first-order derivation takes the difference between two values with a given gap size while second order derivative is then estimated by calculating the difference between two successive points of the first-order derivative spectra. In place of the basic derivative which is usually not feasible for most real measurements due to noise inflation, the modified smoothing and derivative of the Norris-Williams approach is usually the preferred option:

a. Smooth the spectra. Average over a given number of points according to Savitsky, A., & Golay, M. J. E. (1964);

$$\text{X smooth, i} = \frac{\sum_j^m = -mX\text{org, i} + \text{j}}{2m + 1}$$

where *m* is the radius of the smoothing window centered on the current measurement point *i*.

b. Derive at each wavelength. For the first derivative take the difference between two smoothed values at a given gap distance and for the second-order derivative, take twice the smoothed value at point i and the smoothed value at a gap distance on either side:

$X_i` = X_{smooth,i + gap} - X_{smooth,i - gap}$

$X_i`` = X_{smooth,i - gap} - 2.X_{smooth,i} + X_{smooth,i + gap}$

Importantly, derivatization should be done cautiously since it has a propensity for increasing noise in the transformed spectra and falsely improving the correlation between spectra and reference data during calibration development (Faculty *et al.,* 2010). Derivatization was done using the *gapDer* function of *prospectr* package in R statistical software (R-studio, 2022).

SavitzkyGolay smoothing

This was done to lower the likely noise inflation accruing from the previous derivatization (Basile *et al.,* 2021). This was implemented using the *savitzkyGolay* function of *prospectr* package in R. The magnitude of the polynomial order, frame size, and the derivative order were altered to find the right combination that did not inflate noise while retaining as much spectral variance as possible.

Principle component Analysis (PCA)

Pre-treated spectral data were examined for outliers using PCA. Owing to the large size of the spectral data, PCA served to reduce the dimensionality of the data by tracing fewer linear combinations of variables that contribute more to making samples different from each other (Shao *et al.,* 2009). PCA was done using scaled data with *prcomp* function in R. Principle components explaining at least 99% of the total variance were retained and k-means clustering was applied to the principal component scores (Ikeogu *et al.,* 2017); the number of clusters was equal to the desired number of calibration individuals. The calibration set was constituted by picking individuals from the center of each cluster and leaving the rest for validation. This systematic approach of sampling ensured that the calibration set was truly representative of the population.

Spectra filtering with Mahalanobis distances

This parameter, calculated based on a principal components matrix derived from a principal component analysis (PCA) of the spectral matrix, is a powerful tool for defining sample boundaries and similarity indices between spectra. Mahalanobis distance was used as a spectrum outlier tool to detect instrumental error, sample contamination and differences in sample handling (Basile *et al.,* 2021).

Sample set division

After the pretreatment, the remaining spectra and corresponding reference data were divided into calibration (train set) and validation (test set) sets using Kennard-Stone sampling algorithm (*kenStone*) in propectr package in R to achieve the an 80:20 ratio such that the calibration set contained 80% of the original observations and the validation set comprised 20% of the original observations. To partition a data set, this method selects a subset of samples (selection set) from the original, which is representative of the range and distribution of the original set and includes samples on the boundary of the original.

The method starts by tracing two samples from the original set which are farthest apart using geometric distance. To add another sample to the selection set, the algorithm selects from the remaining samples another individual who has the greatest separation distance from the selected samples. The separation distance of a candidate sample from the selected set is the distance from the candidate to its closest selected sample. This most separated sample is then added to the selection set and the process is repeated until the required number of samples, k, have been added to the selection set (Kennard & Stone, 1969).

4.2.5 Developing calibrations and Data analysis

Calibrations were developed using the most commonly used linear regression approach known as the partial least squares (PLS) which assume a linear relationship of the modelled sample characteristic (e.g. proximate composition) as a function of infrared spectra data variations (Munawar *et al.,* 2020).

PLS. This regression approach utilizes latent variables also called score vector to reduce the dimensionality of the spectra data and model the relationship between input and response variables (Bai *et al.,* 2022). In this work, the regression first generated latent variables from spectra data set and then used them as new predictor variables for the various responses (traits). Prior to training the PLS model, tuning hyper parameters were set to a maximum of tune length of 20 principal components and a 10-fold cross validation scheme (partition the training set into ten parts and use 9 parts to train the model and the tenth for testing).

The cross validation done during training of PLS was important for internal assessment of model performance using statistics such as the coefficient of determination (R^2_p), the root mean square error of prediction ($RMSE_p$) and the mean absolute error (MAE). The PLS model was fit for each trait using the *train* function of *caret* package in Rstudio (Kuhn *et al.,* 2023).

External validation of models

This was done to assess the robustness of trained NIR models and their relevance with respect to the standard assays for texture and water absorption. The optimal trained models were used to predict the test set spectra and the coefficient of cross validation and (R^2_{cv}), root mean square error of cross validation ($RMSE_{cv}$) and ratio of performance to deviation (RPD) were used to assess reliability of quantitative predictions. The R^2_{cv} was determined as the correlation coefficient between the NIR predicted and actual measurements of a trait. The RPD is a measure of the predictive ability of a model whereby values greater than 3 show that a model is excellent for making accurate and robust predictions but when less than 1.5, the model is deemed unusable (Li *et al.,* 2022). It was measured as the standard deviation of predicted samples divided by the root mean square error of prediction ($RMSE_p$).

4.3 Results

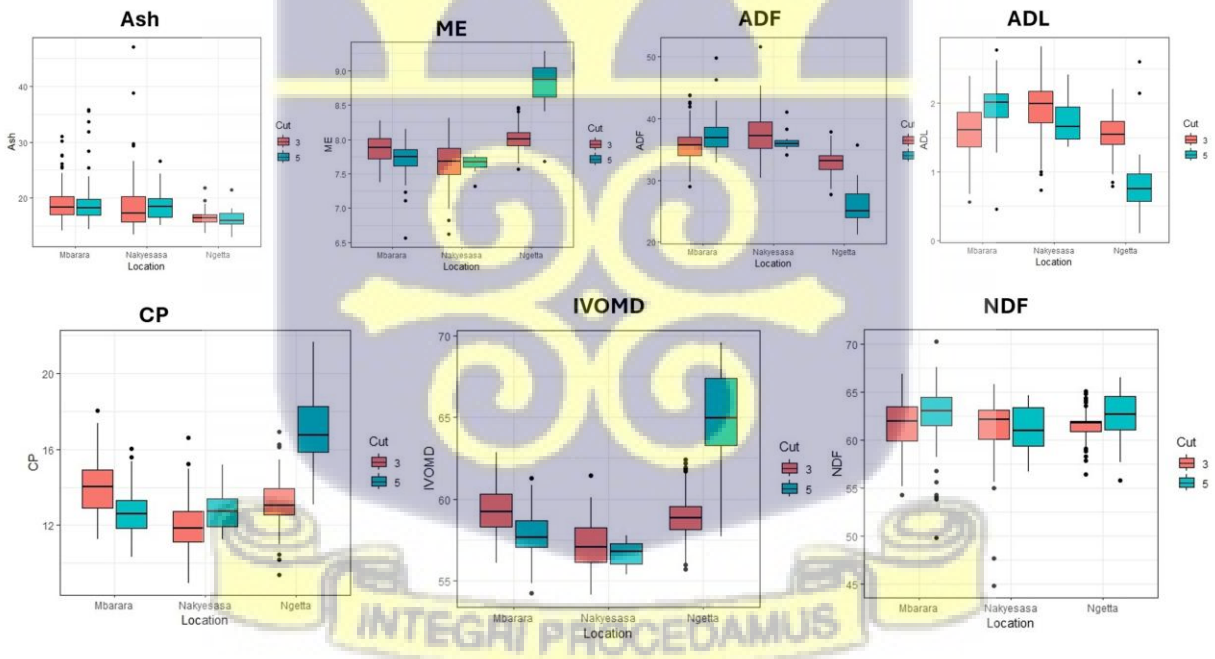
4.3.1 Variation in Feed Quality Traits of *Urochloa* Grass Accessions

Summary statistics for the chemical concentrations are presented in table 4.1. The minimum value of crude protein content was 8.96% while the maximum crude protein content was 21.65% with a coefficient of variation of 10%, NDF ranged from 44.86 to 70.2% of dry matter with a CV of 5%. IVOMD ranged from 54.14 to 69.57%, whereas ADF was between 21.89 to 49.84, and ME ranged from 6.57 to 9.26 MJ/kg DM. The calibration and cross-validation sets had similar ranges and mean values across parameter. The standard deviation ranged from as low as 0.47 for oven dry sample to 4.2 for neutral detergent fiber in the calibration set while in the cross-validation set, SD ranged from 0.54 to 5.55 for Ash. The coefficients of variation (CV) in the calibration set varied from 0.01 for oven sample dry matter to 0.30 for acid detergent lignin. The train set comprised 311 samples, while the test set comprised 76 representing a ratio of 80 to 20%.

Table 4. 1: Descriptive statistics of calibration (n = 311) and validation (n = 76) data sets

	Calibration set (n=311)					Validation set (n = 76)				
Trait	**Min**	**Max**	**Mean**	**SD**	**CV**	**Min**	**Max**	**Mean**	**SD**	**CV**
ODS	89.72	92.57	91.46	0.47	0.01	90.02	93.15	91.42	0.54	0.01
CP	8.96	19.32	13.30	1.92	0.14	9.11	21.65	13.30	1.99	0.15
NDF	49.86	70.29	62.07	2.41	0.04	44.89	66.75	62.20	2.82	0.05
ADF	21.89	49.84	35.10	4.20	0.12	21.69	51.65	35.66	4.73	0.13
IVOMD	54.14	68.20	58.75	2.65	0.05	55.29	69.57	59.12	2.74	0.05
ADL	0.60	2.82	1.69	0.51	0.30	1.06	2.62	1.57	0.58	0.37
Me	6.57	9.26	7.89	0.39	0.05	6.62	9.23	7.86	0.39	0.05
Ash	13.81	35.82	18.16	3.10	0.17	13.38	47.09	19.11	5.55	0.29
N	1.43	3.09	2.13	0.31	0.14	1.46	3.46	2.13	0.32	0.15

Min=minimum value. Max = maximum value, SD = standard deviation, CV = coefficient of variation, CP = crude protein, NDF = neutral detergent fiber, ADF= Acidified detergent fiber, IVOMD = invitro organic matter digestibility, ADL = Acid detergent lignin, ME = Metabolizable energy, N = Nitrogen



ME=Metabolizable energy, ADF = Acid Detergen, ADL=Acid detergent ligin, CP= Crude protein, IVOMD =Invitro organic matter digestibility, Fibre, NDF = Neutral detergent fibre,

Figure 4. 1 : Box plots for Ash, Metabolizable energy, acid detergent lignin, Crude protein, Invitro organic matter digestibility, Fibre Neutral Detergent Fibre,

The PCA score plot of the spectral data revealed that the variables that contributed most to the variation clustered along PC1, which explained 99.7% of the spectral variation (Figure 4.3). There was no distinct clustering based on environment, and the PCA scores displayed an evenly mixed distribution among the two principal components.

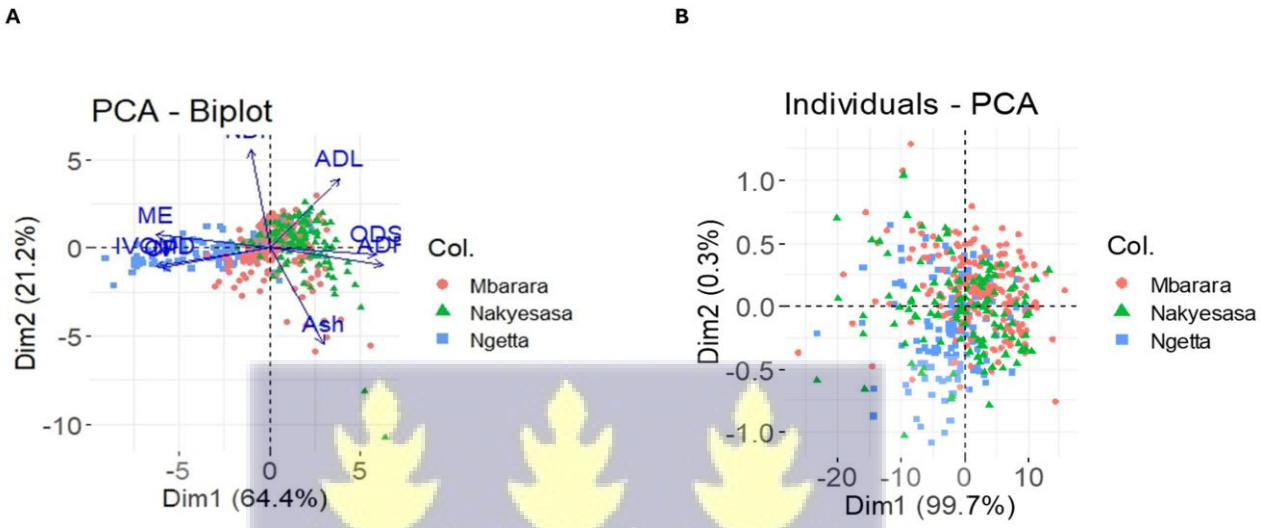


Figure 4. 2: PCA of chemical composition (A) and (B)PCA of spectra both showing variability of the first two principal components

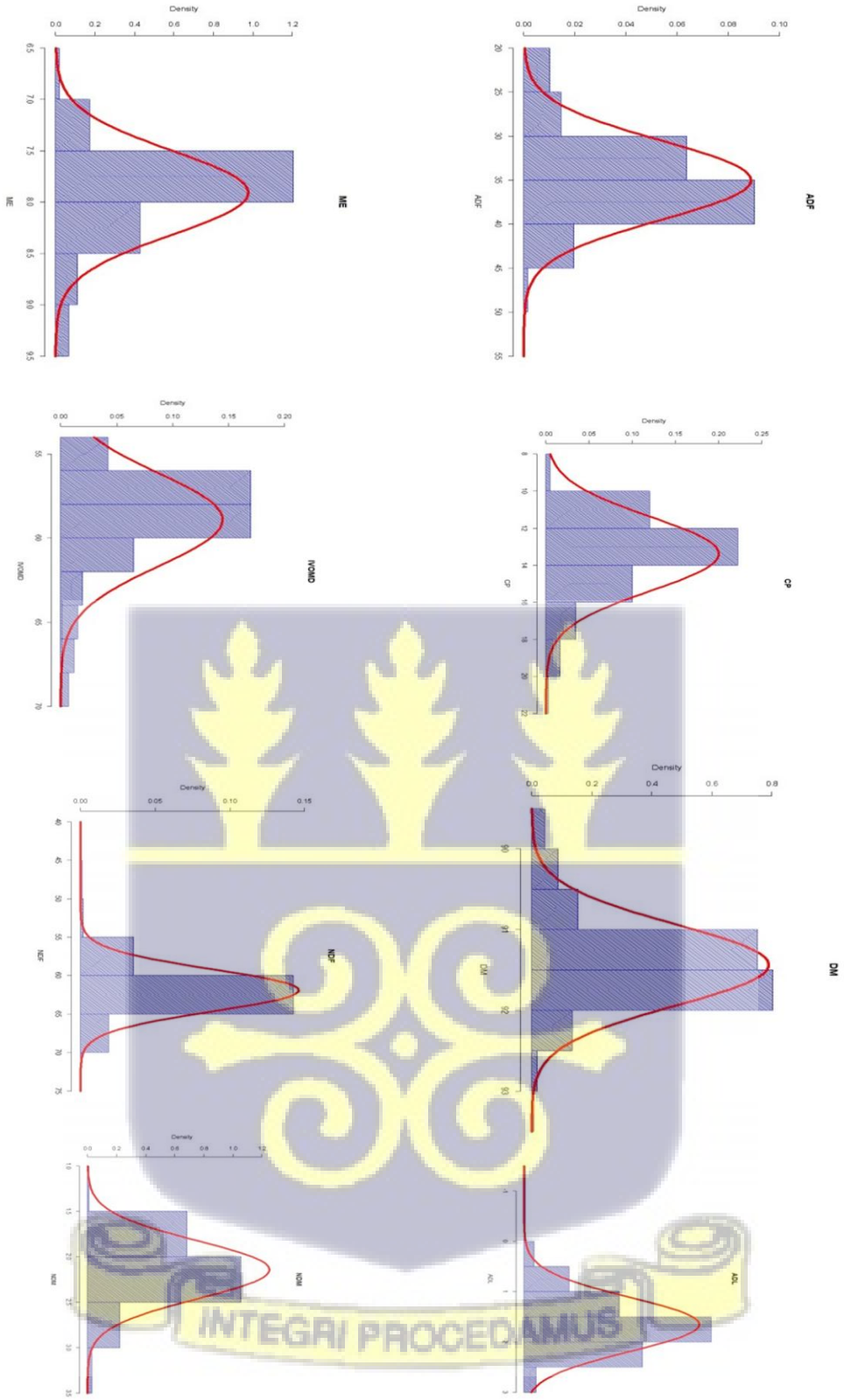


Figure 4.3:Histograms showing the variation of forage traits

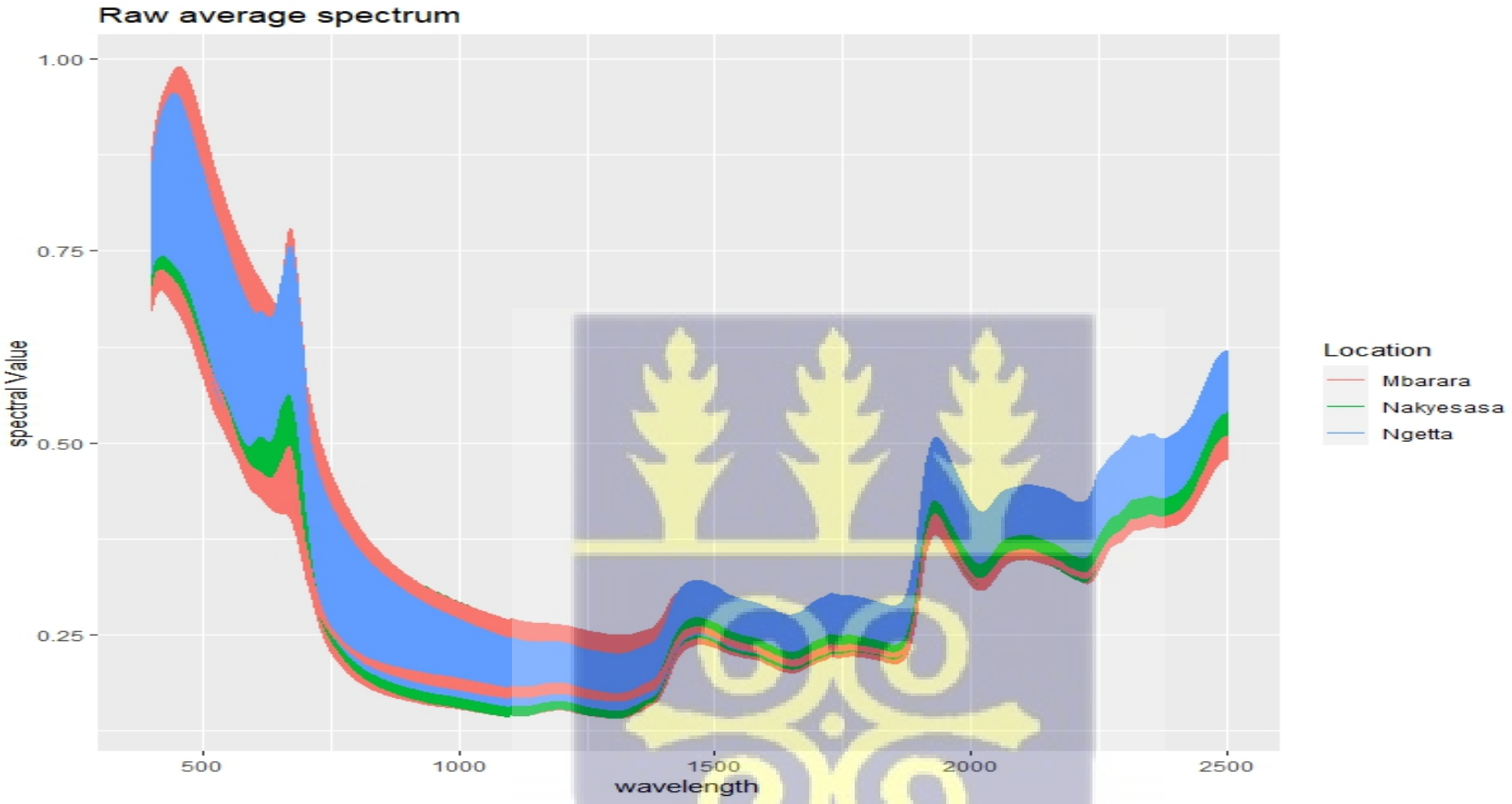


Figure 4. 4: An average NIRS reflectance spectrum of *Urochloa* after 32 scans with 6 significant bands corresponding to vibrations due to respective functional groups.

The spectra across the different preprocessing techniques Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC), SNV with Savitzky–Golay (SNVSG), SNV Gap Derivative (SNVGap), SNV Baseline, and SG + GAP + SNV in figure 4.5 showing distinct transformations in baseline correction, noise reduction, and feature enhancement. SNV and MSC effectively corrected baseline shifts and minimized light-scattering effects, producing smoother and more consistent spectra across all locations. SNVSG and SNVGap enhanced fine spectral features, particularly in the first derivative region (around 1400–1900 nm), associated with O–H, C–H, and N–H molecular vibrations, which are key indicators of organic compounds such as protein and fiber. SNVbaseline adjustment improved uniformity among spectra by centering data around zero absorbance, reducing bias from instrument drift. The combined SG + GAP + SNV approach produced flattened, noise-free spectra with well-defined absorption peaks, indicating optimal removal of scatter effects and environmental noise across all locations. Spectral patterns were consistent among samples from Mbarara, Nakyesasa, and Ngetta, showing no major site-based distortions after preprocessing.

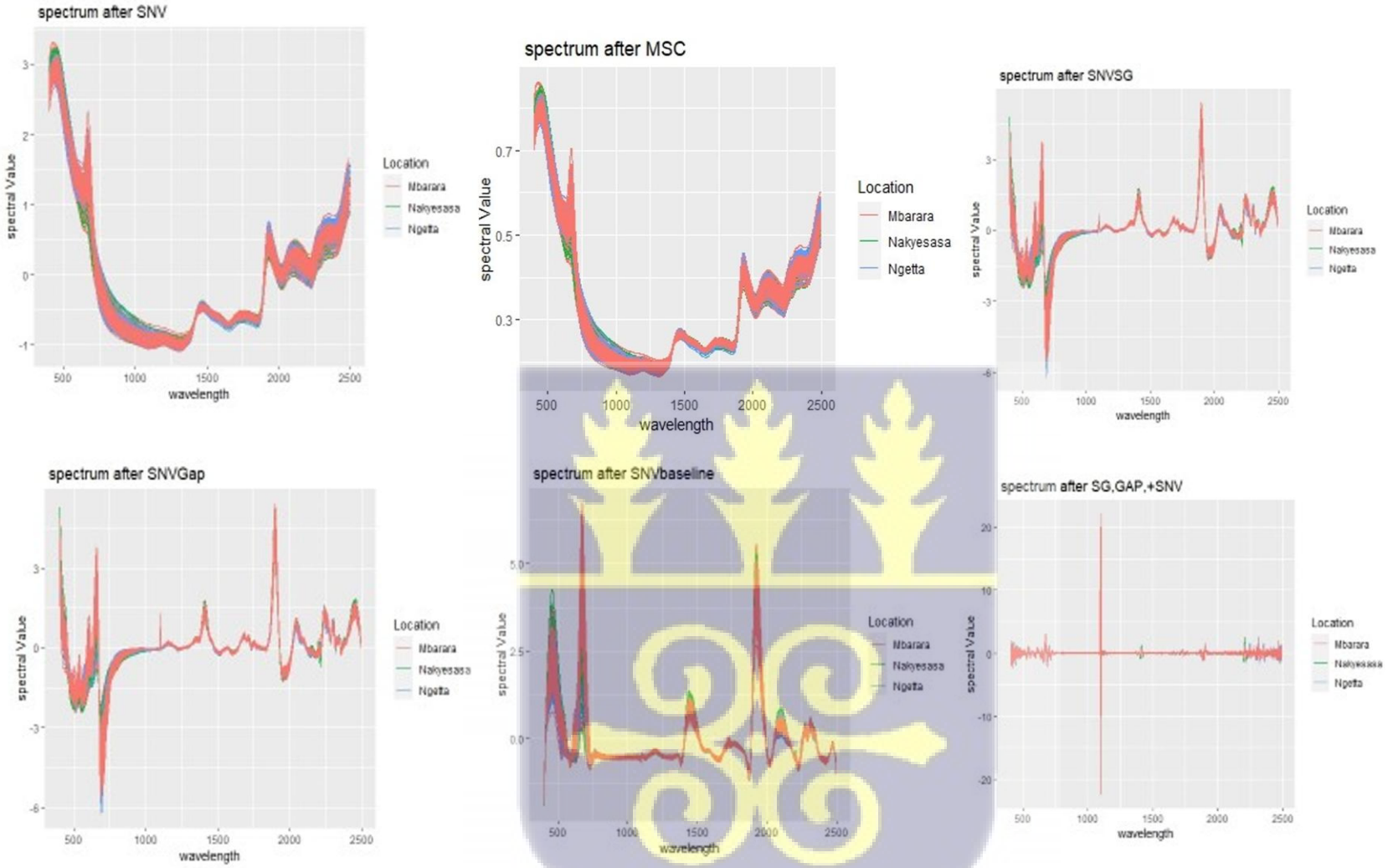


Figure 4. 5: Average treated spectra using the various pre-treatments used in the study

4A

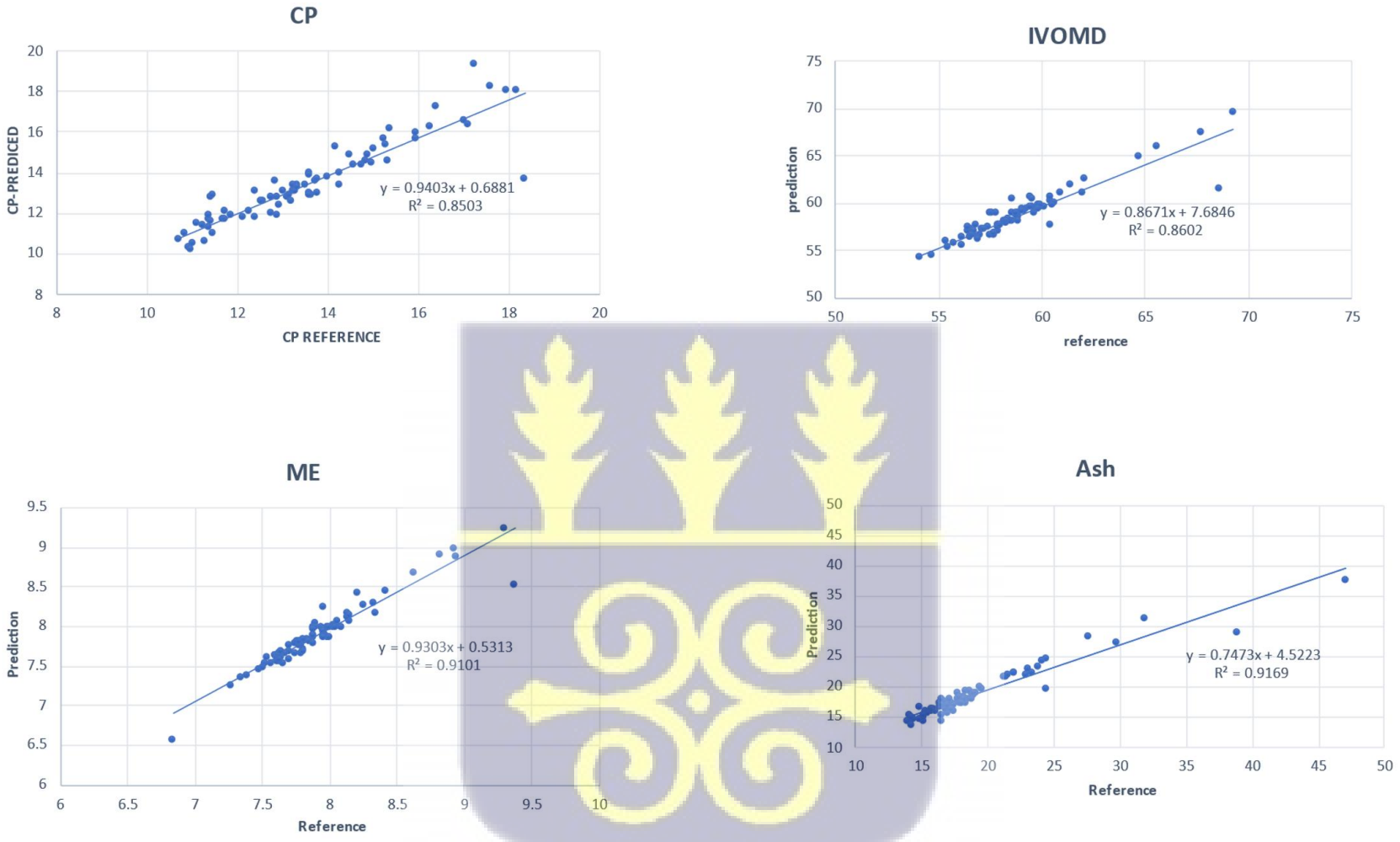


Figure 4. 6: **Scatter plots of reference and predicted values of (A) crude protein, (B) IVOMD, (C) ME (D) Ash, the key represents a linear equation with RSQ values for each trait.**

The NIRS calibration models demonstrated strong predictive relationships between reference values and NIRS-predicted values across all traits.

The model for Crude Protein (CP) showed a high coefficient of determination ($R^2 = 0.85$) with a regression slope close to unity ($y = 0.9403x + 0.6881$), indicating a strong correlation and minimal bias between predicted and reference values. The IVOMD model achieved the highest fit ($R^2 = 0.86$), demonstrating excellent predictive capacity with good alignment between observed and predicted values ($y = 0.8671x + 7.6846$). The ME model exhibited the strongest predictive accuracy ($R^2 = 0.91$) and a regression slope near 1 ($y = 0.9303x + 0.5313$), suggesting robust reliability for estimating energy content. The Ash model also performed well ($R^2 = 0.92$) but displayed a slightly lower slope ($y = 0.7473x + 4.5223$), suggesting minor underestimation at higher concentrations. Overall, all models displayed high R^2 values (0.85–0.92), confirming the strong predictive ability of NIRS for these key forage quality parameters.

The NIRS calibration models showed strong predictive performance for most fiber-related traits (figure 4.7): ADF exhibited the highest accuracy ($R^2 = 0.93$, $y = 0.9738x + 1.172$), indicating a nearly perfect alignment between predicted and reference values. NDF recorded a moderate predictive strength ($R^2 = 0.80$, $y = 0.9536x + 2.886$), suggesting good estimation capability though with slightly higher data dispersion. ADL displayed acceptable accuracy ($R^2 = 0.80$, $y = 0.9402x + 0.08$), reflecting reliable prediction of lignin concentration. Overall, the models demonstrated strong predictive ability ($R^2 = 0.66$–0.93), particularly for fiber fractions, confirming that NIRS effectively captures spectral variations associated with cell wall components.

4B

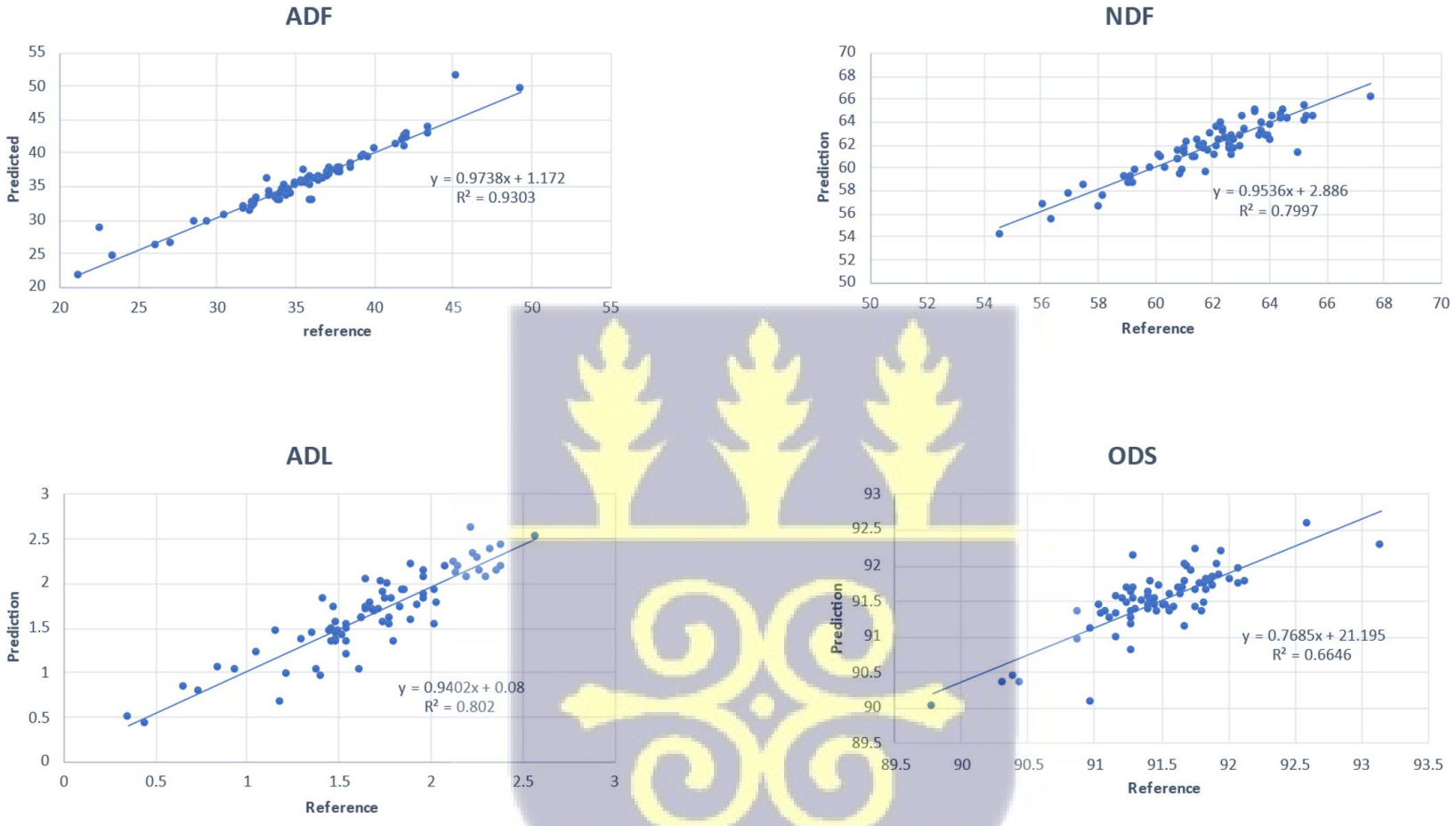


Figure 4. 7: Scatter plots of reference and predicted values of (A) Acidified Detergent Fiber, (B) Neutral Detergent Fiber, (C) Acidified detergent lignin, (D) oven sample Lab dry matter,

4.3.2 Calibration

The calibration and cross-validation results in figure 4.2 revealed strong predictive performance across most forage quality traits. The neutral detergent fiber models using combined preprocessing methods of SNV+SG and SNV+Gap achieved the best predictive performance, with R^2cv = 0.80, RPD = 2.24–2.21, and CCC = 0.89, indicating excellent reliability and minimal bias (–0.02). SNV and MSC alone yielded slightly lower predictive strength (R^2cv = 0.76–0.77). The acid detergent fiber models showed the highest prediction accuracy among all traits, with R^2cv values between 0.89 and 0.93 and RPD values up to 3.6. The SNV preprocessing produced the most robust model (R^2cv = 0.93; RPD = 3.6; CCC = 0.96), confirming a very high predictive capability suitable for routine phenotyping. The ADL models performed moderately well across all preprocessing methods, with R^2cv = 0.77–0.80 and RPD = 2.08–2.22, showing good predictive capacity. The SNV and MSC models yielded slightly better performance (CCC = 0.89) and minimal bias (~0.03).

The NIRS calibration and cross-validation results demonstrated excellent predictive accuracy for key forage quality traits, particularly for CP and ME, with consistent performance across preprocessing methods in figure 4.3. The Crude Protein models showed high predictive strength across all preprocessing treatments, with R^2cv = 0.84–0.85 and RPD = 2.38–2.57. The MSC and SNV+Gap treatments achieved the best balance of accuracy and stability (R^2cv = 0.85; RPD = 2.56–2.57; CCC = 0.92). Low RMSE values (0.77–0.84) and minimal bias (<0.13) indicate high reliability and minimal systematic error. The IVOMD models performed well, with R^2cv = 0.79–0.87 and RPD = 1.89–2.73, showing good predictive capacity. The MSC preprocessing yielded the best model (R^2cv = 0.87; RPD = 2.73; CCC = 0.92), suggesting that multiplicative scatter correction effectively enhanced spectral signal stability for digestibility prediction. The ME models exhibited the strongest performance among all traits, with R^2cv = 0.87–0.91, RPD = 2.58–3.29, and CCC = 0.93–0.95, indicating excellent predictive precision.

The SNV and SNV+Gap treatments achieved the highest accuracy (R^2cv = 0.91; RPD = 3.26–3.29) with negligible bias, confirming robust estimation of energy content.

4.3.3 Cross-validation of calibration models

Cross-validation model performances estimate how best a model would perform when applied to unknown samples. The best prediction models were obtained for ADF (R^2_P = 0.93; RPD = 3.6), ME (R^2_P = 0.91; RPD = 3.29), and Ash (R^2_P = 0.91; RPD = 2.93), whose accuracy indicates a reasonable estimation of the reference values; the calibration model with accuracy below 0.90 was obtained for CP (R^2_P=0.84; RPD = 2.44), IVOMD (R^2_P=0.86; RPD = 2.72), NDF (R^2_P=0.79; RPD = 2.23), ADL (R^2_P=0.79; RPD = 2.22). The RMSECV values were all low ranging from 0.11 to 1.14. The specifics for ME, N, ADL, OSD, CP, NDF, IVOMD, ADF, Ash, ADL and CP prediction equations were 0.11, 0.11, 0.22, 0.28, 0.76, 1.14, 1.14, 0.79, respectively. The bias observed was lower that RMSECV of all the selected models, ranging from 0.01 to 0.3.

The R^2cv ranged from 0.79 for ADL to 0.93 for ADF, Specific R^2cv obtained for Ash (R^2cv = .91), ADF (R^2cv = .93), ME (R^2cv = .91), IVDMD (R^2cv = .86), N (R^2cv = .86), CP (R^2cv = .84) were above 0.80. For NDF (R^2cv = .79), ADL (R^2cv = .79) and ODS (R^2cv = .69) the R^2cv obtained were relatively lower. Additionally, predictive accuracy was based on RMSECV, ME and N had the lowest RMSEcv of 0.11, followed by ADL with 0.22, OSD (0.28), CP (0.76), whereas NDF (1.14), IVOMD (1.18) and ADF (1.34) revealed high RMSECV. The concordance correlation coefficient (CCC) of the cross-validation models ranged from 0.81 (OSD prediction model) to 0.95 (ADF prediction model), indicating substantial to accurate predictive abilities.

The best spectra transformation to improve models' predictive ability varied among forage quality traits. The effect of all the various mathematical t pretreatment (SNV, MSC, SNV+SG,

SNV + Gap, and SNV + baseline), applied for developing the prediction models, which included on the model performance was generally significant, with the coefficient of determination of calibration for NDF ranging from 0.72 to 0.77. The combination of SNV+ SG resulted in the highest coefficient of cross-validation of 0.8, whereas for ADF and ADL, SNV resulted in the highest coefficient of cross-validation of 0.93 and 0.8, respectively.

Table 4. 2: **Summary of calibration with corresponding cross validation for Neutral detergent fiber , Acidified detergent fiber and Acid detergent ligin**

		Calibration					Cross validation						
Trait	**Treat**	**Spectra**	**ncomp**	**RMSE**	**R^2p**	**MAE**	**RMSE**	**R^2cv**	**RPD**	**RPIQ**	**CCC**	**Bias**	**SE**
NDF	SNV	400 -2499	22	1.38	0.72	1.01	1.24	0.77	2.08	2.25	0.88	-0.15	1.25
NDF	MSC	400 -2499	21	1.38	0.72	1.01	1.26	0.76	2.04	2.21	0.87	-0.13	1.27
NDF	SNV+SG	404 -2495	19	1.36	0.73	0.99	1.15	0.8	2.24	2.42	0.89	-0.02	1.16
NDF	SNV+ Gap	402 -2497	19	1.37	0.73	1	1.16	0.8	2.21	2.4	0.89	-0.02	1.17
NDF	SNV+ baseline	400 -2499	20	1.34	0.77	0.96	1.19	0.79	2.06	2.38	0.88	0.24	1.2
ADF	SNV	400 -2499	17	1.31	0.9	0.9	1.34	0.93	3.6	3.2	0.96	-0.15	1.35
ADF	MSC	400 -2499	17	1.22	0.91	0.81	1.58	0.9	3.03	2.8	0.94	-0.38	1.59
ADF	SNV+SG	404 -2495	12	1.18	0.92	0.81	1.57	0.9	3.05	2.81	0.95	-0.36	1.58
ADF	SNV+ Gap	402 -2497	12	1.19	0.92	0.81	1.57	0.9	3.05	2.81	0.95	-0.37	1.58
ADF	SNV+ baseline	400 -2499	21	1.14	0.93	0.77	1.63	0.89	2.94	2.71	0.94	-0.46	1.64
ADL	SNV	400 -2499	24	0.32	0.65	0.24	0.22	0.8	2.22	2.74	0.89	0.03	0.23
ADL	MSC	400 -2499	25	0.32	0.66	0.24	0.22	0.8	2.22	2.73	0.89	0.03	0.23
ADL	SNV+SG	404 -2495	18	0.31	0.66	0.24	0.23	0.79	2.19	2.69	0.89	0.02	0.23
ADL	SNV+ Gap	402 -2497	18	0.32	0.66	0.24	0.23	0.79	2.2	2.71	0.89	0.02	0.23
ADL	SNV+ baseline	400 -2499	20	0.31	0.67	0.23	0.24	0.77	2.08	2.56	0.87	0.04	0.24

There were no observable differences between the effect of SNV alone and its combinations with SG, GAP, and BASELINE SNV on the coefficient of determination of the calibration of crude protein, which ranged from 0.81 to 0.83. The best coefficient of determination cross-validation yielded from MSC of 0.85 with an RMSE of 0.77 and RPD of 2.57. The spectra pre-treatments resulted in a varied coefficient of determination of the calibration for invitro digestibility ranging from 0.78 to 0.84. MSC pre-treatment resulted in the highest coefficient of determination for cross-validation of 0.87 with a relatively high RMSE of 1.18. Both SNV + GAP and SNV resulted in a coefficient of determination for cross-validation of 0.91 and the same RMSCV OF 0.12 for metabolizable energy. However, the number of factors corresponding with SNV +GAP was 9, which was much lower than those corresponding to SNV.

Among the pre-treatments tested for Ash, SNV in combination with baseline resulted in the highest coefficient of determination for cross-validation, 0.92, with an RMSECV of 1.81 and a prediction efficiency of 2.94, which is considered adequate. Nitrogen and OSD had much lower coefficients of determination for cross-validation. Specifically, for OSD, the coefficient of determination for cross-validation was generally lower than that of calibration.

Among the pre-treatments tested for Ash, SNV in combination with baseline resulted into the highest coefficient of determination for cross validation of 0.92 with an RMSECV of 1.81 and a prediction efficiency of 2.94 which is considered adequate. Nitrogen and OSD had a much lower coefficient of determination for cross validation. Specifically, for OSD, generally the coefficient of determination for cross validation was lower than that of calibration.

Table 4. 3: **Summary of calibration with corresponding cross validation for Crude Protein , invitro organic matter digestibility and Metabolizable energy**

		Calibration					Cross validation						
Trait	**Treat**	**Spectra**	**ncomp**	**RMSE**	**R^2p**	**MAE**	**RMSE**	**R^2cv**	**RPD**	**RPIQ**	**CCC**	**Bias**	**SE**
CP	SNV	400 -2499	18	0.63	0.83	0.46	0.84	0.84	2.45	3.22	0.9	-0.04	0.85
CP	MSC	400 -2499	17	0.81	0.82	0.56	0.77	0.85	2.57	3.27	0.92	0.12	0.77
CP	SNV+SG	404 -2495	15	0.86	0.81	0.56	0.78	0.85	2.51	3.2	0.92	0.12	0.79
CP	SNV+ Gap	402 -2497	15	0.82	0.83	0.57	0.77	0.85	2.56	3.26	0.92	0.13	0.78
CP	SNV+ baseline	400 -2499	21	0.84	0.81	0.57	0.79	0.85	2.38	3.15	0.92	0.09	0.8
IV	SNV	400 -2499	20	1.04	0.84	0.76	1.39	0.79	1.89	1.8	0.88	0.1	1.4
IV	MSC	400 -2499	16	1.13	0.78	0.81	1.18	0.87	2.73	2.08	0.92	0.01	1.19
IV	SNV+SG	404 -2495	8	1.13	0.82	0.84	1.14	0.83	2.37	2.07	0.89	-0.14	1.15
IV	SNV+ Gap	402 -2497	8	1.15	0.78	0.83	1.23	0.86	2.62	2	0.91	0.01	1.24
IV	SNV+ baseline	400 -2499	17	1.07	0.83	0.8	1.23	0.86	2.62	2	0.91	-0.06	1.24
ME	SNV	400 -2499	21	0.14	0.86	0.1	0.12	0.91	3.29	2.96	0.95	0.01	0.12
ME	MSC	400 -2499	23	0.14	0.87	0.1	0.16	0.87	2.58	2.21	0.93	0.03	0.16
ME	SNV+SG	404 -2495	20	0.14	0.87	0.1	0.16	0.88	2.59	2.21	0.93	0.02	0.16
ME	SNV+ Gap	402 -2497	9	0.15	0.85	0.11	0.12	0.91	3.26	2.93	0.95	0	0.12
ME	SNV+ baseline	400 -2499	24	0.14	0.87	0.1	0.15	0.89	2.73	2.33	0.94	0.02	0.15

Table 4. 4: Summary of calibration with corresponding cross validation for Ash, Nitrogen and oven dry sample

		Calibration					Cross validation						
Trait	**Treat**	**Spectra**	**ncomp**	**RMSE**	**R^2p**	**MAE**	**RMSE**	**R^2cv**	**RPD**	**RPIQ**	**CCC**	**Bias**	**SE**
Ash	SNV	400 -2499	25	1.24	0.85	0.87	1.97	0.9	2.7	1.4	0.91	-0.32	1.98
Ash	MSC	400 -2499	24	1.24	0.86	0.87	1.94	0.9	2.74	1.42	0.92	-0.3	1.95
Ash	SNV+SG	404 -2495	15	1.12	0.88	0.78	1.96	0.9	2.71	1.41	0.91	-0.35	1.97
Ash	SNV+ Gap	402 -2497	15	1.12	0.88	0.79	1.97	0.9	2.7	1.4	0.91	-0.35	1.98
Ash	SNV+ baseline	400 -2499	21	1.15	0.87	0.82	1.81	0.92	2.94	1.52	0.93	-0.28	1.82
N	SNV	400 -2499	17	0.14	0.8	0.09	0.12	0.85	2.54	3.23	0.92	0.02	0.13
N	MSC	400 -2499	15	0.14	0.8	0.09	0.12	0.87	2.77	3.27	0.93	-0.01	0.12
N	SNV+SG	404 -2495	15	0.14	0.81	0.09	0.13	0.84	2.49	3.18	0.92	0.01	0.13
N	SNV+ Gap	402 -2497	14	0.14	0.81	0.09	0.12	0.85	2.57	3.28	0.92	0.01	0.12
N	SNV+ baseline	400 -2499	19	0.13	0.81	0.09	0.13	0.84	2.43	3.1	0.91	0.02	0.13
OSD	SNV	400 -2499	14	0.25	0.71	0.2	0.3	0.65	1.62	1.66	0.8	0.03	0.3
OSD	MSC	400 -2499	14	0.25	0.71	0.2	0.29	0.65	1.63	1.68	0.81	0.04	0.3
OSD	SNV+SG	404 -2495	16	0.26	0.69	0.21	0.29	0.66	1.69	1.73	0.81	0.01	0.29
OSD	SNV+ Gap	402 -2497	16	0.26	0.69	0.21	0.28	0.66	1.7	1.74	0.81	0.01	0.29
OSD	SNV+ baseline	400 -2499	14	0.26	0.7	0.2	0.31	0.63	1.56	1.61	0.79	0.03	0.31

4.4 Discussion

The utility of NIRS in predicting forage chemical concentration, such as CP, ADF and NDF, in forages has been demonstrated in several studies (Norman *et al.,* 2020; Oluk *et al.,* 2022; Paz *et al.,* 2019). The initial variability of the calibration data and its representativeness are key determinants of prediction performance and robustness in future predictions in an external population (Parrini *et al.,* 2018). The average crude protein content and ADF in this study were higher than that reported for *Urochloa* species by Monrroy *et al.,* 2017 but lower than the ranges reported by Mazabel *et al*. 2020 in *Urochloa humidicola* hybrids. The results were in agreement with IVOMD, Ash, ME, and ADL ranges reported for tropical forages (Jayasinghe *et al.,* 2022).

The variation in chemical composition can be attributed to the population variability in terms of species composition, influence of genotype by environment interactions (Aleme *et al.,* 2023). The influence of cut/harvest season was significant with high variability seen in the dry season cuts similar to previous studies that also reported a significant effect of genotype by cut interaction of forage quality (Mwendia, Ohmstedt, *et al.,* 2021; Rodrigues *et al.,* 2023). Melo *et al*. 2022 has also reported the influence of climatic variability and soil fertility on pasture forage quality in *Urochloa*.

The result of PCA demonstrated that the spectrum of *Urochloa* samples displayed greater variation, suggesting that the spectra would be more conducive to modelling. Overall, no distinct distribution was observed among the *Urochloa* samples, indicating these samples could be utilized for NIRS modelling, similar result was reported by Tang *et al.* (2023).

The method of partitioning of sample subsets and the choice of spectral range affect precision and reliability of the calibrations (. Ferreira *et al.,* 2022). Although previous calibration studies in *Urochloa* used random sampling (Guimarães *et al.,* 2023; Monrroy, Gutierrezi, *et al.,* 2017)

for its ease of use, it fails to guarantee that the sample subsets are representative of observed. In this study, a *createDatapartition* function of the *caret* package in R was used, which optimized partition of the data into train and test sets to enhance the reliability of the calibration and validation sets for subsequent multivariate calibrations

Furthermore, selection of wavelengths plays a pivotal role in multivariate calibration as it enhances the accuracy of predictions, guarantees the reliability of the calibration, and facilitates a more precise interpretation (Li *et al.,* 2022; Wang *et al.,* 2022). A full wavelength of 400-2500nm, was used which could have affected the accuracy of the prediction models in contrast to 1100 to 2000nm range used in studies by Monrroy *et al* 2017 and Mazabel *et al* 2020 for determining the crude protein model. This study used PLS in calibration development which was found to be effective in Brachiaria NIR prediction studies compared to Multi variate curve resolution (MCR) used in the study by Monrroy., 2017.

To measure model performance, the prediction models were assessed based on accuracy, reliability and robustness normally based on a high R^2 and RPD above 3.0, with low RMSE and Bias (Serrano *et al.,* 2021; Williams & Antoniszyn, 2019; Williams, 2004). Despite the limitation of this study like determining an appropriate spectral range for each trait, the coefficients of determination obtained in this study for the validation model for Ash (0.91), ADF (0.93) and, ME (0.91), CP (0.85), signified a stronger correlation between the predicted and wet chemistry values. In contrast, Monrroy *et al*. 2017 reported lower R^2P values of 0.90 and 0.56 for ADF and CP models in a mixed *Urochloa* population similar to what was used in the present study. Mazabel *et al* 2020 reported slightly higher values of 0.95, 0.96, and 0.99 for NDF, ADF and CP. This may have been attributed to the different pretreatments, preprocessing, and wavelength selection for each trait and in comparison, to Mazabel *et al*. 2020. The extent of variability of the chemical composition traits could also cause the disparity in prediction accuracies (Addo *et al.,* 2022).

The prediction performance for fiber fractions (NDF, ADF, and ADL) varied across spectral preprocessing methods, reflecting the importance of correcting scatter and baseline effects in NIRS calibration. For NDF, models combining standard normal variate (SNV) with Savitzky–Golay smoothing (SG) achieved the highest accuracy ($R^2cv = 0.80$; RPD = 2.24; CCC = 0.89), outperforming SNV or multiplicative scatter correction (MSC) alone. The improvement indicates that smoothing effectively reduced spectral noise while preserving relevant absorption features, enhancing the relationship between spectral variation and chemical composition (Rinnan *et al.,* 2009; Savitzky & Golay, 1964). For ADF, the SNV + baseline correction model gave the most robust performance ($R^2cv = 0.89$; RPD = 2.94), indicating strong predictive capacity suitable for quantitative applications (Williams, 2014). This preprocessing combination minimized baseline shifts caused by scattering and instrumental drift, producing consistent predictions comparable to those of SNV + SG or SNV + Gap methods.The ADL models showed moderate prediction accuracy across preprocessing methods ($R^2cv \approx 0.77–0.80$; RPD ≈ 2.1). The lower performance reflects the narrower concentration range and weaker spectral response of lignin, a common limitation in NIRS analysis of low-abundance constituents (Workman & Weyer, 2012).

Prediction accuracy for crude protein (CP), in vitro digestibility (IV), and metabolizable energy (ME) varied with spectral preprocessing, highlighting the importance of correcting light scatter and baseline shifts during NIRS calibration.

For crude protein (CP), all preprocessing methods yielded high predictive accuracy ($R^2cv = 0.84–0.85$; RPD = 2.38–2.57; CCC ≈ 0.9–0.92). The MSC and SNV + Gap models performed best, suggesting that scatter-correction techniques effectively enhanced calibration performance by minimizing particle-size and path-length effects, improving spectral linearity with chemical composition (Rinnan *et al.,* 2009). For in vitro digestibility (IV), the MSC and SNV + baseline correction treatments produced superior cross-validation results ($R^2cv = 0.86–$

0.87; RPD = 2.62–2.73; CCC ≈ 0.91–0.92). The enhanced performance under MSC demonstrates the method's efficiency in reducing multiplicative interferences, allowing more accurate estimation of digestibility traits, which are often influenced by complex spectral interactions (Williams & Norris, 2001). For metabolizable energy (ME), the SNV and SNV + Gap models provided the most robust predictions ($R^2cv = 0.91$; RPD = 3.26–3.29; CCC = 0.95), meeting the criteria for highly reliable quantitative models (Williams, 2014). The high RPD (>3) values confirm strong predictive capacity suitable for routine feed evaluation and energy estimation in forage breeding or quality monitoring programs.

Overall, results confirm that SNV- and MSC-based preprocessing, especially when combined with smoothing or baseline correction, significantly enhances NIRS model stability and accuracy. These findings align with previous reports showing that optimized preprocessing minimizes spectral noise and improves prediction of nutritional constituents in forages (Savitzky & Golay, 1964; Rinnan *et al.*, 2009; Workman & Weyer, 2012).

According to Williams 2004, the models generated in this study for Ash and ME that had had R^2P values above 0.90 with an RPD greater than 3, indicating excellent reliability and robustness. From a practical point of view, prediction models developed in the present study for NDF, ash, and ME can be considered excellent and applicable to the selection. None of our models had RPD values below 1.9, which are normally considered very poor and not recommended for forage quality assessment.

The RPD values for CP and NDF lay between the 2.0–2.4 range, which were sufficient for rough screening, whereas the RPD for IVOMD was between 2.5 and 2.9, which often offers a fair selection potential (Williams & Antoniszyn, 2019; Williams, 2004). To the best of our knowledge, no models have been developed for Ash and ME in *Urochloa* grasses.

The concordance correlation coefficient (CCC) of the crossvalidation models ranged from 0.92 (for the ash prediction model) to 0.94 (for the ME prediction model). These results were higher than the range reported in hay mixed rations whose predictive models had a CCC ranging from 0.66 to o.92(Buonaiuto *et al.,* 2021). However, similar ranges have been reported in feeds (Visentin *et al.,* 2015). The CCC obtained for all the traits investigated in the present study indicates a substantial to accurate models' predictive ability.

To my knowledge, no model for estimating metabolizable energy in *Urochloa* using NIRS has been developed. The final metabolizable energy model had an R^2CV of 91, RMSEcv 0.11, with an RPD value of 3.29 which is considered accurate based on the RMSEcv, reliable and robust. These results had a high coefficient of determination, RMSEcv and RPD than the ME model developed for corn fed to animals whose R^2CV was 0.88 with an RPD of 2.65 (Juntao, 2016).

Improving the accuracy of Near-Infrared Spectroscopy (NIRS) prediction models to enhance the reliability, begins with capturing a broader range of both spectral information and reference data. However, caution to optimize wavelength range is key. Mouazen et al. (2006) demonstrated that selecting the appropriate wavelength range can improve predictions for specific soil constituents. Fine-tuning the spectral range based on the target trait can enhance model accuracy. In addition, Local database development has been suggested by Despal et al. (2020) emphasizing the importance of creating a local database of NIRS spectra for specific applications to improve prediction accuracy. Tailoring the calibration dataset to the local context can lead to more accurate predictions.

4.5 Conclusions

The high predictive accuracies observed for CP, IVOMD, and ME confirm the strong potential of NIRS technology for rapid and non-destructive phenotyping of forage quality traits in *Urochloa* species. The results highlight that appropriate preprocessing especially SNV, MSC,

and derivative-based combinations (e.g., SNV+Gap)—significantly improves model robustness by minimizing light-scattering effects and enhancing spectral feature extraction.. Adequate variation in key traits such as crude protein, fiber fractions, and digestibility supported robust calibration, while higher variability in ADL and ash enhanced model generalizability. Overall, the data were consistent, representative, and well suited for developing accurate and stable NIRS prediction models for forage quality assessment

High predictive strength reported in the results reflect NIRS's capacity to capture the chemical bonds associated with energy-yielding organic compounds. The ME model's high RPD (>3.0) meets the Williams and Sobering (1996) criterion for "excellent prediction," making it suitable for selection, ranking, and routine evaluation in breeding programs.

Minor variation among preprocessing methods reflects spectral noise sensitivity, but all achieved high CCC values (≥0.90), confirming strong agreement between predicted and reference values. Collectively, these results validate NIRS as a high-throughput, cost-effective alternative to wet chemistry, capable of supporting large-scale screening of forage germplasm for nutritional quality improvement.

For structural fiber components (NDF and ADF), these combinations yielded strong predictive performance (R^2cv = 0.80–0.89; RPD = 2.2–2.9), suitable for quantitative applications, while ADL models achieved moderate accuracy (RPD ≈ 2.1), reflecting lignin's low spectral sensitivity. For nutritional traits, MSC, SNV + Gap, and SNV + baseline provided highly reliable predictions of crude protein (CP), in vitro digestibility (IV), and metabolizable energy (ME) (R^2cv = 0.85–0.91; RPD = 2.5–3.3).These results confirm that scatter and baseline correction, coupled with smoothing, effectively enhance signal quality, reduce noise, and strengthen the relationship between spectral and chemical data—supporting the use of optimized NIRS models for rapid, non-destructive forage evaluatio

CHAPTER FIVE

GENOME-WIDE ASSOCIATION MAPPING FOR FORAGE NUTRITIONAL QUALITY TRAITS IN *UROCHLOA* SPECIES.

5.1 Introduction

Urochloa grasses are economically valuable components of the pasture-based livestock systems in the tropics addressing feed shortages(Jank *et al.,* 2014). The grasses are grown for both open grazing systems, hay, and silage production (Arzate-Vázquez *et al.,* 2016; Nascimento *et al.,* 2022), (Leite *et al.,* 2021; Oliveira *et al.,* 2019) with a remarkable adaptability to a range of climatic and soil conditions, including tolerance to waterlogged areas (Jiménez *et al.,* 2017; 2021) with an ability to grow in acidic and low nutrient soils (Merlin *et al.,* 2016). This adaptability allows *Urochloa* to thrive in environments where other forage species may fail, making it a reliable choice for farmers in diverse ecological settings.

While *Urochloa* is generally widely adapted, *Urochloa* brizantha and other species within the genus exhibits significant variability for nutritional quality traits influenced by genotypic differences, environmental factors and their interaction effects (Gaviria-Uribe *et al.,* 2020; Rezende *et al.,* 2021; Tessema *et al.,* 2023). For instance, studies have reported different levels of crude protein and neutral detergent fiber depending on soil fertility and moisture levels(Alvarez *et al.,* 2023; Ramos *et al.,* 2022). *Urochloa* species exhibit a positive response to nitrogen and phosphorus fertilization which enhances dry matter and protein accumulation (Leite *et al.,* 2021; Oliveira *et al.,* 2019). Seasonal variations also lead to forage quality fluctuations throughout the year, studies indicate that the crude protein content values were higher during the rainy season compared to the dry season .(Leal *et al.,* 2020; 2022).The influence of grazing management practices on nutritional quality of *Urochloa* for example, the

grazing age and height determines the leaf stem ratio which is a key determinant of forage digestibility and nutritional value (Pedreira *et al.,* 2017; Santana *et al.,* 2021). Furthermore Furthermore, continuous grazing without rest periods can lead to reduced forage quality due to over grazing while rotational grazing systems allow adequate recovery time thus maintaining the forage quality (Moura *et al.,* 2021). Moreover, it has been observed that as *Urochloa* matures, the increase in structural carbohydrates and lignin can lead to a decrease in digestibility and over all nutritional value (Martínez *et al.,* 2020; Gaviria-Uribe *et al.,* 2020). This variability poses challenges for livestock producers that require consistent forage quality but also complicates selection for highly nutritious genotypes during the breeding process.

Against this background, phenotypic based selection is therefore ineffective for nutritional quality traits in forages, which are characterized by a high genotype by environment interaction. Conventional breeding strategies based on phenotypic-based recurrent selection involve repeated phenotyping in multiple locations seasons and cuts (Gouveia *et al.,* 2020b, 2021; Lascano & Euclide, 1996) resulting into increased breeding costs and time per selection cycle. Genetic selection therefore remains the most effective selection method for such traits. Genetic variance among *Urochloa* hybrids for crude protein content and fiber composition has been reported within *Urochloa decumbens* (Figueiredo *et al.,* 2019); among *Urochloa* hybrids(Gouveia *et al.,* 2022) . This variability suggests that selective breeding can be employed to effectively develop cultivars with improved nutritional profiles suitable for specific environmental conditions (Figueiredo *et al.,* 2019)

Limited genomic information however continues to constrain the development of markers for application in marker assisted selection in *Urochloa* breeding (Ferreira *et al.,* 2021; J. F. Pereira *et al.,* 2018; Simeão *et al.,* 2021b). Sequencing polyploidy involves determining the allele

dosage in heterozygotes (Bourke *et al.,* 2018) unlike in diploids. The DArTseq genotyping platform offers a cheap and easy approach that allows the generation of genome-wide SNPs using restriction enzymes and genome reduction. Recent studies have demonstrated that with advances in genotyping technologies for forage species still lacking reference genomes such as *Urochloa* coupled with high throughput phenotyping for hard to phenotype traits such as nutritional quality traits, genome wide association mapping is an effective tool for identifying genetic markers linked to nutritional quality traits. GWAS compared to traditional linkage mapping, presents allelic richness and high genetic resolution for capturing underlying marker trait associations without the tedious process of creating bi-parental mapping populations ref. Previous genome wide association studies in related tropical forage grasses for forage quality traits have been reported in Napier (Muktar *et al.,* 2022), guinea grass (Deo *et al.,* 2020)and more recently in Buffel grass (Negawo *et al.,* 2024) demonstrating the potential of GWAS in identifying significant SNPs underlying key forage quality traits, such as crude protein content, fiber content, and invitro organic matter digestibility. In *Urochloa*, Matias *et al.,* 2019 reported the first GWAS study that identified seven SNPs in candidate regions linked to biomass yield, regrowth, fiber content and digestibility using several related reference genomes in an interspecific segmental allotetraploid population panel. This study therefore, set to determine the genetic loci controlling forage nutritional quality traits of *Urochloa* species using DArTseq SNP markers in a Ugandan assembled population.

5.2 Materials and Methods

5.2.1 Source of germplasm

A total of one hundred and eighty-eight (188) diverse *Urochloa* accessions were locally sourced from a pool of 224 accessions previously assembled from 8 Ugandan Agroecological zones used in morphological characterization in objective one. Sixteen introduced cultivars were

introduced from the Brazilian Agricultural Research corporation (EMBRAPA) and the International Center of Tropical Agriculture (CIAT). The introductions were included for their high biomass and forage quality while the Ugandan accessions selected were those with an average agronomic performance in terms of yield and regrowth, with enough planting material for multi-location establishment.

5.2.2 Experimental and site layout

The experiments were arranged in 11 × 19 augmented design with four checks. Each plot 2 row plot was 2 X 5 M long, planted at a spacing of 1M X 1M inter and intra-row, respectively resulting into 10 plants per accession. The plots were 1.5 meters apart whereas the blocks were 2 meters apart. The four checks used included *Urochloa brizantha cv*. Mulato II (S8), *Urochloa decumbens cv*. Basilisk(S4), *Urochloa brizantha cv.* Piata (S6), *Urochloa brizantha cv*. Xaraes (S5).

The accessions were evaluated in three locations established at the National Livestock Resources Research Institute, Nakyesasa (located at 0.513404, 32.638475, the elevation of 3717 ft above sea level) representing the Lake Victoria crescent agroecological zone, Mbarara Zonal Agricultural Research and Development Institute (0° 36'7.20"S,30°36'34.89"E) representing southwestern rangelands and Ngetta zonal Agricultural research and development institute (N02° 4.903 E082° 55.007, elevation 3549) representing the Northern farmlands during the 2021 to 2022 cropping seasons covering 2 wet and 2 dry harvests/cuts. Accessions were planted in a 2-row plot (10 m long) at a density of 10,000 plants/ha. No fertilizer or herbicide was applied throughout the phenotypic evaluation. Hand weeding was done to control the weeds every month.

5.2.3 Phenotyping for forage quality traits

The plants were left to establish, and a standardization cut back was done after three months at a stubble of 8cm from the ground. At a cutting interval of 45 days (Mwendia *et al.,* 2021), data was collected on fresh biomass weight at a full plot basis. After measuring fresh weight, a 200-gram sample were subsampled for determination of dry matter yield. The samples were chopped into 2mm pieces, oven dried at 60°C for 48 hours and weighed. Then samples were ground in a wiley mill (Thomas Scientific, US) before the final grinding in cyclotec1093 sampling mill through a 1mm screen. Powder samples were collected and scanned using NIRS™ DS2500 Foss feed analyzer (FOSS Hillroad Denmark) in the spectral range from 400 to 2500nm. Eight feed constituents based on dry matter basis were determined which included crude protein, ash, nitrogen, neutral detergent fibre, acid detergent fibre, acid detergent lignin, metabolizable energy and in-vitro organic matter digestibility.

5.2.4 Genotyping

Urochloa leaf samples were collected and dried before they were transported for genotyping at the SEQART AFRICA situated at the International Livestock Research Institute (ILRI), Nairobi. DNA extraction was done using Nucleomag plant tissue extraction kit (Mag-Bind® Plant DNA DS 96 Kit). The genomic DNA isolated was in the range of 50-100ng/ul. DNA quality and quantity were tested on 0.8% agarose gel. Libraries were produced using DArTSeq complexity reduction approach (Kilian *et al.,* 2012) through digestion of genomic DNA using a mix of PstI and MseI enzymes. This was followed by ligation of barcoded adapters and common adapters followed by PCR amplification of adapter-ligated fragments. Libraries were then sequenced utilizing single-read sequencing runs for 77 bases.

Next-generation sequencing was performed using Hiseq2500 (Kilian *et al.,* 2012). SEQART AFRICA platform leverages genotyping by sequencing (GBS) DArTseqTM technology, which

delivers quick, high quality, and economical genome profiling, even from the most complicated polyploid genomes. DArTseq markers scoring was done using DArTsoft14, which is an in-house marker scoring pipeline (Kilian *et al.,* 2012) Two types of DArTseq markers were scored, SilicoDArT markers and SNP markers, both scored as binary for presence/absence (1 or 0, respectively) of the restriction fragment with the marker sequence in the genomic representation of the sample.

5.2.5 Genetic diversity and population structure,

Molecular diversity and model-based population structure analysis were conducted as in chapter 3 sub section 3.3.2 of this thesis.

5.2.6 Data analysis

5.2.6.1 Phenotypic statistical analysis

Descriptive statistical analysis was conducted to summarize the phenotypic variation among breeding materials and to provide an overview of trait distribution before advanced genetic analyses. Data were first examined for completeness, accuracy, and consistency, and outliers were verified against raw records. Key statistical parameters, including the mean, range, standard deviation (SD), and coefficient of variation (CV%), were computed to describe the central tendency and dispersion of traits across genotypes. Skewness and kurtosis were assessed to evaluate data normality and suitability for subsequent parametric tests. The analyses were performed using standard biometric procedures as described by Gomez and Gomez (1984) and Singh and Chaudhary (2010).

The analysis of variance was performed in R using the augmented block design using the R forage quality-related traits data recorded on investigated traits were subjected to descriptive statistics including mean, standard deviation, range, coefficient of variation, and broad sense heritability. The analysis of variance (ANOVA), Pearson's correlation analysis, cluster

analysis and principal component analysis for recoded data were performed in R (Version 4.1.2). Frequency distribution graphs of recorded traits were developed using MS-EXCEL program. The ANOVA was carried out for the Augmented Block Design Using R Agricola package 1.4.0. Statistical analysis was conducted using analysis of variance (ANOVA) in R software (version 4.2.2) to determine the significance of the main effects and the interactions using the following model.

$$Yijkl=\mu+Gi+Bj+Tk+Cl+(Gi\times Tik\times Cikl)+\varepsilon ijkl \quad (1)$$

where Y_{ijk} is the response, μ = overall mean, G_i = effect of the *i*th *Urochloa* grass accession, B_j = effect of the *j*th Block effect, T_k = effect of the *k*th growing season due to cut, Cl is the effect of lth location, $G \times T_{ij} \times Cl$ = the interaction of *i*th genotype and *j*th growing season and the lth location and ε_{ijkl} = the residual error. The least significant difference (LSD) test for a comparison of the mean values of traits was employed to compare locations and cuts for traits with significant differences. Genetic variability parameters including genotypic coefficient of variation and phenotypic coefficient of variation were generated based the formulae below ;

$GCV = \sqrt{\sigma g^2}/GM \times 100$

$PCV = \sqrt{\sigma p^2}/GM \times 100$; where *GCV* = genotypic coefficient of variation, *PCV* = phenotypic coefficient of variation, σg^2 = genotypic variance, σp^2 = phenotypic variance and *GM*= grand mean.

R was used to determine the descriptive analysis and the analysis of variance (ANOVA).

The correlation analysis among the traits was determined using the corr function in R and visualize using Corrplot package in R software. To determine the BLUP values for each trait, a mixed linear model in the "lme4" package in the R software was followed (Henderson, 1975)

$$Y = (1|Taxa) + (1|Loc) + (1|Cut) + (1|Taxa{:}\,Loc{:}\,Cut)$$

where Y represents trait data,"1|" means groups the parenthesis indicates random effects, ":" means interactions, "Taxa" means all genetic materials, "Loc" means locations and "Cut" means cut number corresponding to the wet or dry season.

The variance components were used for broad sense heritability(h^2) for each of the traits as.

$$h^2 = \sigma^2 g/((\sigma^2 g + \frac{\sigma^2 gCut}{l} + \frac{\sigma^2 gLoc}{c} + \frac{\sigma^2 gCL}{lc}) + \sigma^2 e/lc)$$

Where $\sigma^2 g$ is genetic variance, $\sigma^2 gC$ is genotype by season due to cut interaction, $\sigma^2 gloc$ is genotype by location, $\sigma^2 gCL$is genotype by cut by location variance. The number of environments was denoted as "l" while the number of locations was denoted "l" while the number of cuts was denoted as "c"

5.2.6.2 Genome-wide association study of forage quality traits

19668 SNPs and the BLUP value were used to conduct the GWAS by using the two mixed two multi-locus tests of multiple loci mixed model (MLMM) (Segura *et al.,* 2012) and Bayesian information and linkage disequilibrium iteratively nested keyway model (BLINK) (Huang *et al.,* 2019) implemented in the GAPIT package with both K and Q matrix considered in the R software (Wang & Zhang, 2021)

The two GAPIT multi-locus models (MLMM) and BLINK) that reduce both erroneous positive significant markers and false negative significant markers were used in R using the GAPIT package (Wang & Zhang, 2021b). Both models are highly efficient with improved computational efficiency, statistical power, and control of false positives (Wang & Zhang, 2021b). These models also eliminate confounding issues arising due to population structure, kinship, multiple testing correction, etc.

5.2.7 Prediction of candidate genes

Candidate genes were predicted based on the significant SNPs and their extension regions from 100 kb up stream to 100 kb downstream (LD decay) in the genomes of *Setaria itelica* V2, *Seteria viridis* V2 (Brutnell *et al.,* 2015; Huang *et al.,* 2014) and *sorghum bicolor* V4. We used the JGI_Phytozome database, to obtain annotations of candidate genes. The bonferroni-corrected P-value threshold was set as 0.05 (-log p-value = 5.78). Quantile-quantile (Q-Q) plots were generated through MLMM and BLINK to show model fitting (account for population structure). Haplotypes were determined by interval distance between associated SNPs. Significant SNPs present within LD range (100 Kb) were considered as one haplotype.

5.3 Results

5.3.1 Phenotype analysis for forage quality traits

The summary statistics of key nutritional forage traits are presented in Table 5.1 showing the range of variability. On a population basis, crude protein content ranged from 3.65 to 22.87 with an average of 13.32, invitro organic matter digestibility varied from 51.26% to 69.57% with most accessions averaging 58.5%. The fiber component was evaluated as acidified detergent fiber which varied from 21.26 to 77.19 whereas neutral acidified acid varied from 31.83 to 76.89 with a mean of 61.59. The rest of the nutrient variation is shown in Table 5.1 while the frequency distribution curve showed that the population was normally distributed for all the nine elements Figure 5.1.

Table 5. 1:Summary statistics of nutritional quality traits

Trait	Minimum	Maximum	Mean ± SD	CV
CP	3.65	22.87	13.32 ± 2.06	15.5
IVOMD	51.26	69.47	58.50 ± 2.6	4.3
NDF	31.83	76.89	61.59 ± 3.06	5.0
ADF	21.26	77.19	35.32 ± 2.06	5.8

ADL	1.12	2.3	1.67 ± 0.83	49.7
ME	6.03	9.54	7.86 ± 2.06	26.2
DM	89.57	96.11	91.60 ± 0.55	0.6
ASH	11.34	19.11	18.65 ± 4.55	24.4
N	1.14	3.66	2.13 ± 0.33	15.5

CP=crude protein, IVOMD=invitro organic matter digestibility, NDF= neutral detergent fiber, ADF= acidified detergent fiber, ADL= Acidified detergent lignin ME=metabolizable energy,DM= dry matter,N=Nitrogen,SD=standard deviation,CV= coefficient of variation

Analysis of variance (ANOVA) revealed significant effects of genotype, location, cut and their interaction in explaining the observed variability among all the nutritional parameters ($P < 0.001$) (Table 5,2). The broad-sense heritability ($h2$, %) for the traits ranged from medium (0.44) for ADL to high (0.72) for nitrogen indicating the predominant role of both genetic and environmental factors influence of these traits. For most of the traits, there was a significant cut by location effect observed.

There was significant effect of location, and cut on crude protein, invitro digestibility, acidified detergent fibre, neutral detergent fibre, acidified detergent lignin, Ash, nitrogen and dry matter yield was observed explaining variability among traits. Cuts 1 and 5 represented the wet season/cuts while cuts 3 and 7 represented the dry season/cuts. The average crude protein was highest (13.86) in cut 5 and was lowest (12.54) in cut 7 (dry cut). By testing location, average CP was highest in the Mbarara site (13.92) and least in Nakyesasa. Invitro digestibility was highest (59.32) in cut 5 and lowest (57.21) in cut 7. The highest average digestibility was observed in Ngetta and least in Nakyesasa. Generally, across all parameters, cut 5 had higher nutritional values particularly or crude protein and invitro digestibility indicating better overall quality. The Mbarara location consistently ranked higher for crude protein and invitro digestibility whereas Nakyesasa had higher ADF and NDF compared to the other two locations (Mbarara and Ngetta).

Table 5. 2: Combined analysis of variance for the 159 *Urochloa* genotypes evaluated across three agroecological zones for four cuts.

SOV	df	Mean squares								
		ADF	ADL	ASH	CP	DM	IVOMD	ME	NDF	N
Block	10	12.7	9.1***	68.4***	13***	0.82***	18***	12.2***	12.57***	13.022***
Genotype	158	29.3***	18.24***	55.2***	20.1***	0.37***	11.6***	15.09***	34.52***	17.274***
Cut	3	864.5***	17.74***	232.1***	224.1***	21.03***	529***	10.779***	220.58***	8.081***
Loc	2	1044.2***	46.55***	476***	200.2***	68.16***	710.6***	0.22*	86.19***	4.41***
Geno: Cut	474	13.5***	6.53***	15.8***	6.3***	0.15***	5.5***	5.069***	7.72***	5.698***
Geno: Loc	316	25.4***	18***	37***	18.2***	0.27***	10.4***	14.75***	18.81***	16.825***
Cut: Loc	6	2667.1***	8.34***	1689.9***	424.2***	22.6***	623.5***	20.033***	98.06***	13.148***
Gen x Cut x Loc	944	15.6***	6.34***	19.1***	6.7***	0.17***	5.9***	5.114***	8.47***	5.71***
Residuals	594	7.6	0.17	7.1	1.8	0.09	2.5	0.05	5.13	0.046

Geno=Genotype, CP = Crude protein, IVOMD = Invitro organic matter digestibility, NDF = Neutral digestible fiber, ADF = Acidified detergent fiber, ADL= Acid detergent lignin, ME = Metabolizable energy

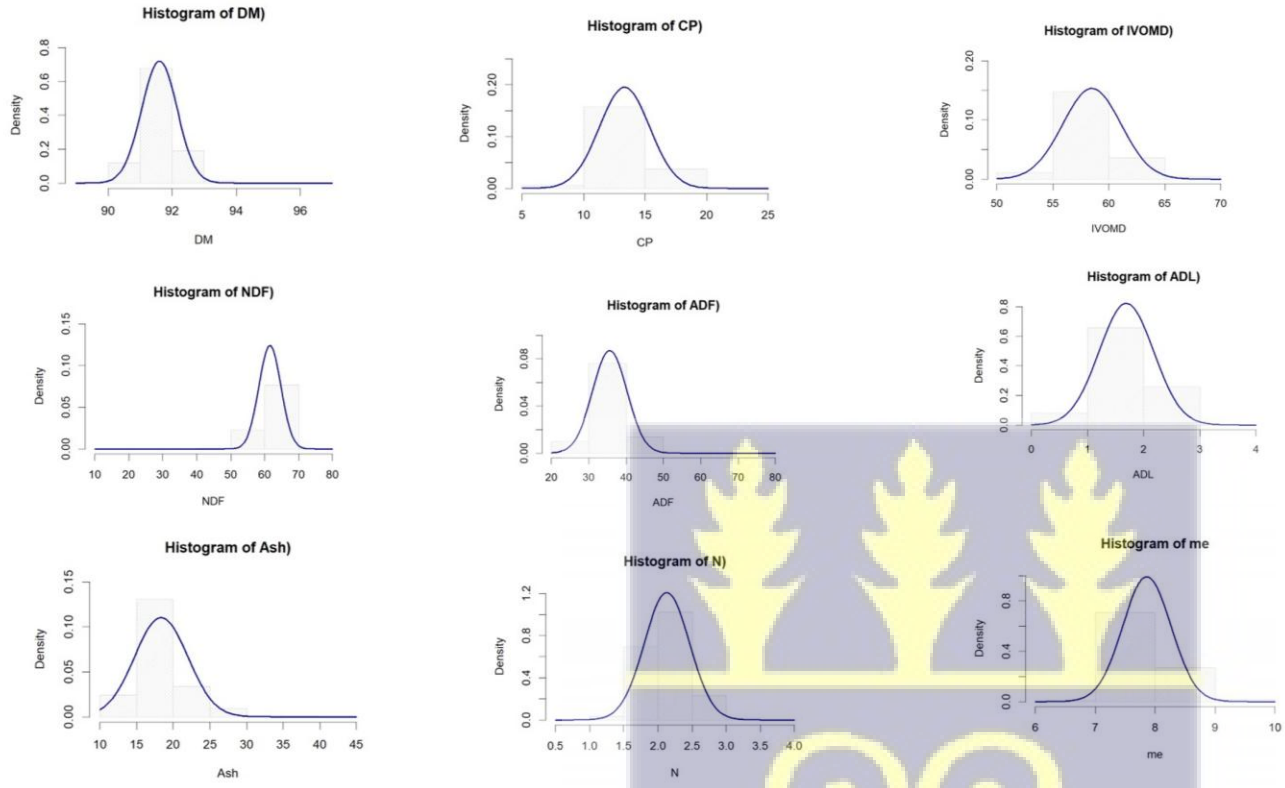


Figure 5. 1*:* Frequency distribution for all the forage quality traits in association mapping panel.

The variability parameters of the nutritional quality traits revealed moderate heritability with a potential for genetic improvement. A moderate heritability for Crude protein (0.56) showed that about 56% of its total variation was due to additive/genetic effects indicating a good potential for genetic selection to improve CP content as seen by a high genetic advance as a percentage of mean of 22.87% (GAM percentage above 20% is considered high).

IVOMD had a moderate heritability of 0. 57 suggesting that genetic selection could moderately improve digestibility, however a low GAM OF 6.135 indicates limited genetic gain per cycle of selection. Same trend was noted for metabolizable energy with a GAM of 3.68% suggesting small genetic gains limiting improvement.

NDF had a wide genetic variability and a moderate heritability of 0.55 showing high potential for improvement through genetic selection. The high GAM of 40.69% suggests a strong possibility of selection progress. Though ADF registered a much higher heritability and GAM indicating a similar trend as NDF. A low heritability and genetic advance observed for acid detergent lignin suggested a slower improvement through selection compared to other traits. On the other hand, the ash content that represents the mineral content exhibited a high heritability (0.72) and the highest GAM suggesting it can be improved through breeding and selection efforts. A similar trend was observed for nitrogen content.

Table 5. 3: Genetic Parameters and Variance components from the GWAS panel for the studied traits

Trait	GV	PV	Heritability	GCV	PCV	GA	GAM
CP	0.47	2.64	0.56	0.05	0.12	3.05	22.87
IVOMD	0.56	3.06	0.57	0.01	0.03	3.59	6.13
NDF	1.10	4.78	0.55	0.02	0.04	5.42	40.69
ADF	0.90	5.23	0.64	0.03	0.04	6.90	51.79
ADL	0.14	1.26	0.44	0.22	0.02	1.14	8.55
ME	0.07	0.46	0.52	0.03	0.01	0.49	3.68
DM	0.18	0.86	0.58	0.00	0.02	1.03	1.13
ASH	1.41	6.44	0.72	0.06	0.04	9.55	51.23
N	0.07	0.42	0.72	0.13	0.01	0.62	28.97

CP = Crude protein, IVOMD = Invitro organic matter digestibility, NDF = Neutral digestible fiber, ADF = Acidified detergent fiber, ADL= Acid detergent lignin, ME = Metabolizable energy, GV=Genetic Variance, PV=Phenotypic variance, GCV= Genotype coefficient of variation= Phenotype coefficient of variation, GA=genetic advance, GAM=genetic advance as a percentage of the mean

5.3.2 Marker distribution, Population structure and variation characteristics

A set of 19,668 high quality SNP markers were distributed across the genome with the least number of SNPs mapped on chromosome 3(1365) and the highest number of markers mapped on chromosome 1(31120). Population structure inferred by structure and structure harvest software revealed the highest peak of delta K at K = 6, indicating presences of six sub populations within the GWAS panel (Figure 5.2). Sub population 1 comprised of 67 (36%) while 55 accessions (29%) were assigned to sub population 2. Sub populations three, four, five and six comprised of twelve, twenty-nine, twenty-one and four accessions accounting for seven, fifteen, eleven, and two percent respectively. As shown in the STRUCTURE plot, some individual accessions possessed alleles inherited from multiple sub populations indicating presences of admixtures. Complementing structure analysis, was principal component analysis, using the GAPIT 3 R package within first two PCs accounted for 34.80 % revealing moderate genetic differentiation.

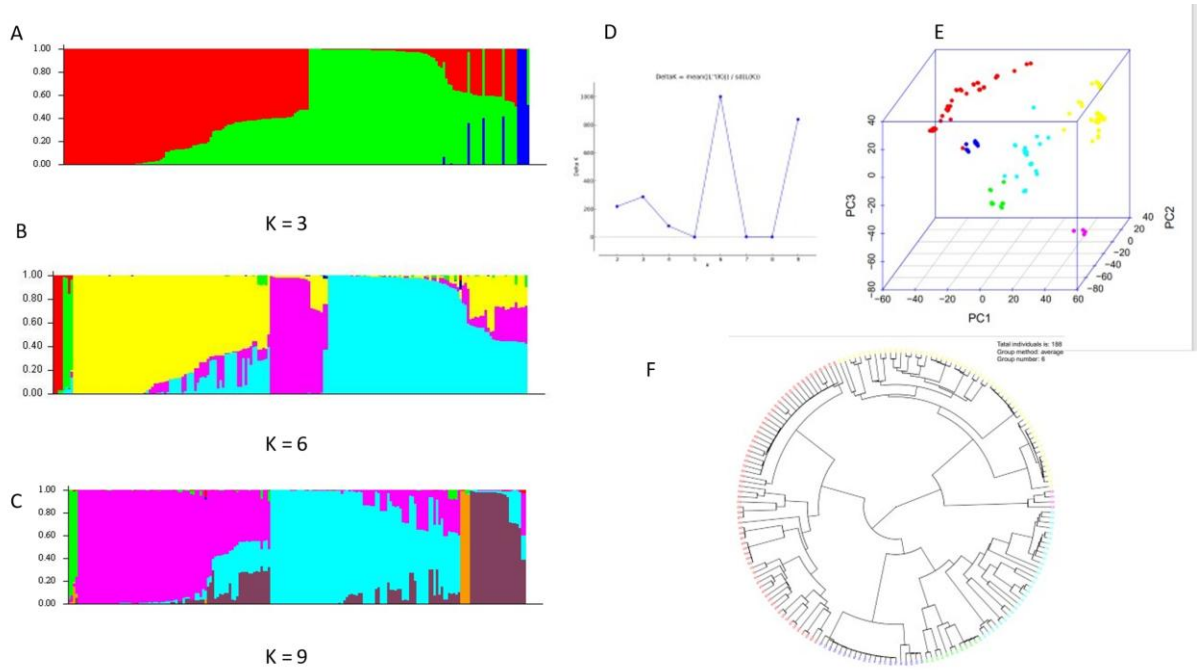


Figure 5. 2: Population structure of the association panel as revealed by structure.

The nucleotide diversity of the entire population was 0.28 suggesting high divergence. The nucleotide diversity of the SP1 SP2, SP3, SP4, SP5 and SP6 subpopulations had an Fst of 0.86, 0.4, 0.57,0.65,0.01 and 0.56 respectively, showing high differentiation genetic diversity. Comparing F_{ST} among the six subpopulations, the genetic differentiation index was largest between the SP1 and SP3 subpopulations, indicating the greatest differentiation. It is generally believed that $F_{ST} > 0.25$ indicates that there is great genetic differentiation among populations. SP1 and SP2, SP1 and SP4, SP1 and SP6 all had F_{ST} greater than 0.25 while the genetic differentiation between SP5 and all the other populations was less than 0.25 with the least fixation index of 0.01.

The degree of genetic differentiation and average distance (H_e) in each subpopulation suggested that there was highest degree of genetic differentiation detected in sub population1 (F_{st} = 0.86), whereas the lowest value was observed in subpopulation 5 (F_{st} = 0.01). On the

other hand, the highest H_e was observed in sub population 5(He=0.35) and the lowest H_e was detected in sub population 1(He =0.05)

5.3.3 Marker trait association

5.3.3.1 Marker trait association under wet and dry seasons

A total of twenty-two significant marker trait associations (MTAs) were generated for the four key forage nutritional quality-related parameters (CP, IVOMD, NDF, and ADF) across the wet and dry season using BLINK and MLMM under GAPIT models. Seven significant SNPs were detected through the BLINK GAPIT model while 15 were detected under the MLMM model. Three MTAs linked to crude protein were identified under both models on chromosome 5 (two MTAs) and one other MTA on chromosome 9. Out of the 22 MTAs, seven were identified during the wet season, including one for crude protein, two for invitro organic matter digestibility and four for neutral detergent fibre. In the dry season, nine, two and one MTAs were found linked with CP, IVOMD and NDF, respectively. No MTA was identified for NDF in the dry season. Multiple MTAs controlled CP and IVOMD.

For Crude protein, a total of 13 significant marker trait associations were identified for crude protein across the environments through either BLINK model or MLMM models (Table 5.3) widely spread on all chromosomes except for chromosomes three and seven. The phenotypic variation explained (PVE) by these MTAs ranged from 2% to 27.4%. Three of the marker trait associations on chromosome 5, 5 and 9 (Brac-5-29543232, Brac-5-5990992 and Brac-9-33109087) were validated under both models for crude protein. In the dry season, a major SNP was detected by MLMM model on chromosome 2 at position 42687944 which explained 27.4% of the phenotypic variance. In the wet season a total of 12 MTAs were distributed across the 9 chromosomes, except on chromosomes 3 and 7. Seven of the markers were identified through BLINK (two on chromosome 5 (position 5990992 and position 29543232) and one on each of the chromosome 1(position 41717043), chromosome 4 (position 11447831),

chromosome 6 (position 58658705), chromosome 8(position 37347904) and chromosome 9(position 33109087).The above MTAs ranged from 2 to 19.4%. The MLMM model detected 5 significant MTAs for crude protein, two on chromosome 5(position 5990992 and position 29543232) explaining 23.5% and 9.3% respectively. The other three SNPs were distributed on chromosome 2 (Brac-2-29667035), chromosome 6 (Brac-6-36355165) and chromosome 9 (Brac-9-33109087) explaining 15.1%, 7.8% and 9.1% of the phenotypic variance respectively

For invitro organic matter digestibility, during the wet season, two MTAs were detected using the MLMM model on chromosome 2 and 6 at position 67026907 and 45198942 explaining 17.6% and 17.3% respectively. In dry season, another two were identified on chromosomes 5 and 8 at position 35749131 and 32865539 during the dry season explaining 18.2 and 37.1% of the phenotypic variance respectively.

For fiber content (Neutral detergent fiber and acidified detergent fiber), five MTAs were identified related to fiber content using the MLMM model. Four MTAs were associated with neutral detergent fiber during the wet season, 2 on chromosome 1 (position 52223225; 57140690) whereas the other two each was on chromosome 2 (position 49788452) and 6 (position 34140690) respectively explaining 11.5% (chromosome 6) to 19 % (chromosome 1) of the phenotypic variance. No significant MTA was detected for acidified detergent fiber in the dry season. On the other hand, during the dry season only one MTA on chromosome 1, position 57887552 was detected for acidified detergent fiber explaining 38.3% of the phenotypic variance.

For acid detergent lignin a total of four significant MTAs were identified for acid detergent lignin across locations in the dry season. The MTA explained 2.6 to 42.7% of the phenotypic variance. A major SNP detected on chromosome 7 (position 52180640) explained 42.7% of the phenotypic variance.

5.3.3.2 Marker trait association under combined seasons

Using combined seasonal data, a total of twelve marker trait associations were found significant with 3, 4, 2, and 3 SNPs associated with crude protein content, IVOMD, ADF and NDF respectively (Table 5.4, Fig. 5.6). For crude protein content, three SNPs were located on chromosomes 7, 8, and 9, which individually explained 13.53%–23.16% of the phenotypic variation. For invitro digestibility, four SNPs were located on chromosomes 3, 5, 5 and 6, which individually explained 10.1%–20.5% of the phenotypic variation. For acidified detergent fiber, 2 SNPs were located on chromosomes 5 and 6, which individually explained the phenotypic variation of 14.04–46.4%. For neutral detergent fiber, three SNPs were located on chromosomes 3, 3, and 8, which individually explained the phenotypic variation of 12.4–32.3%.

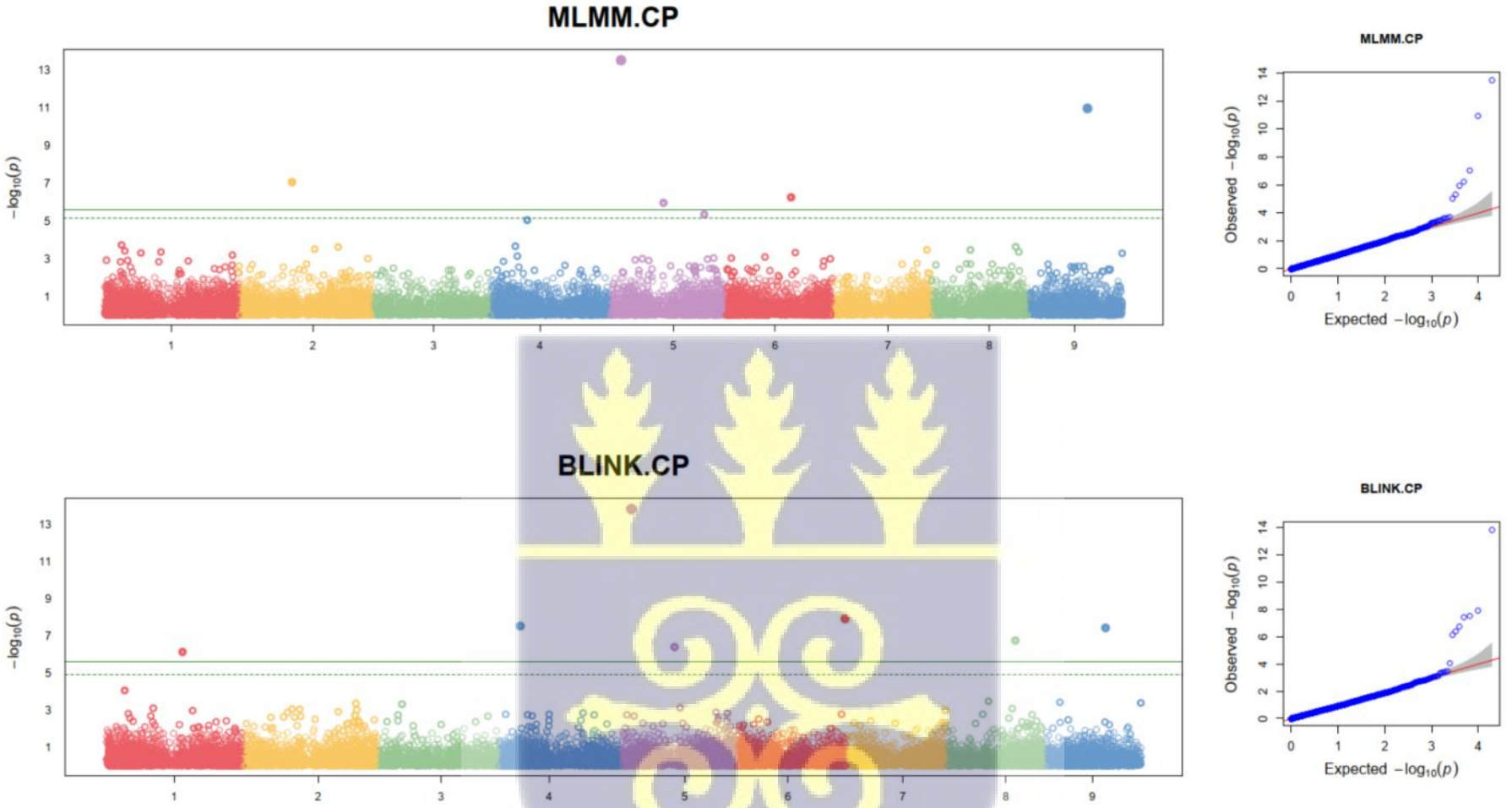


Figure 5. 3: Manhattan and Quantile-Quantile plots for genome-wide diagnosis of association signals using BLINK and MLMM models for CP during the dry season.

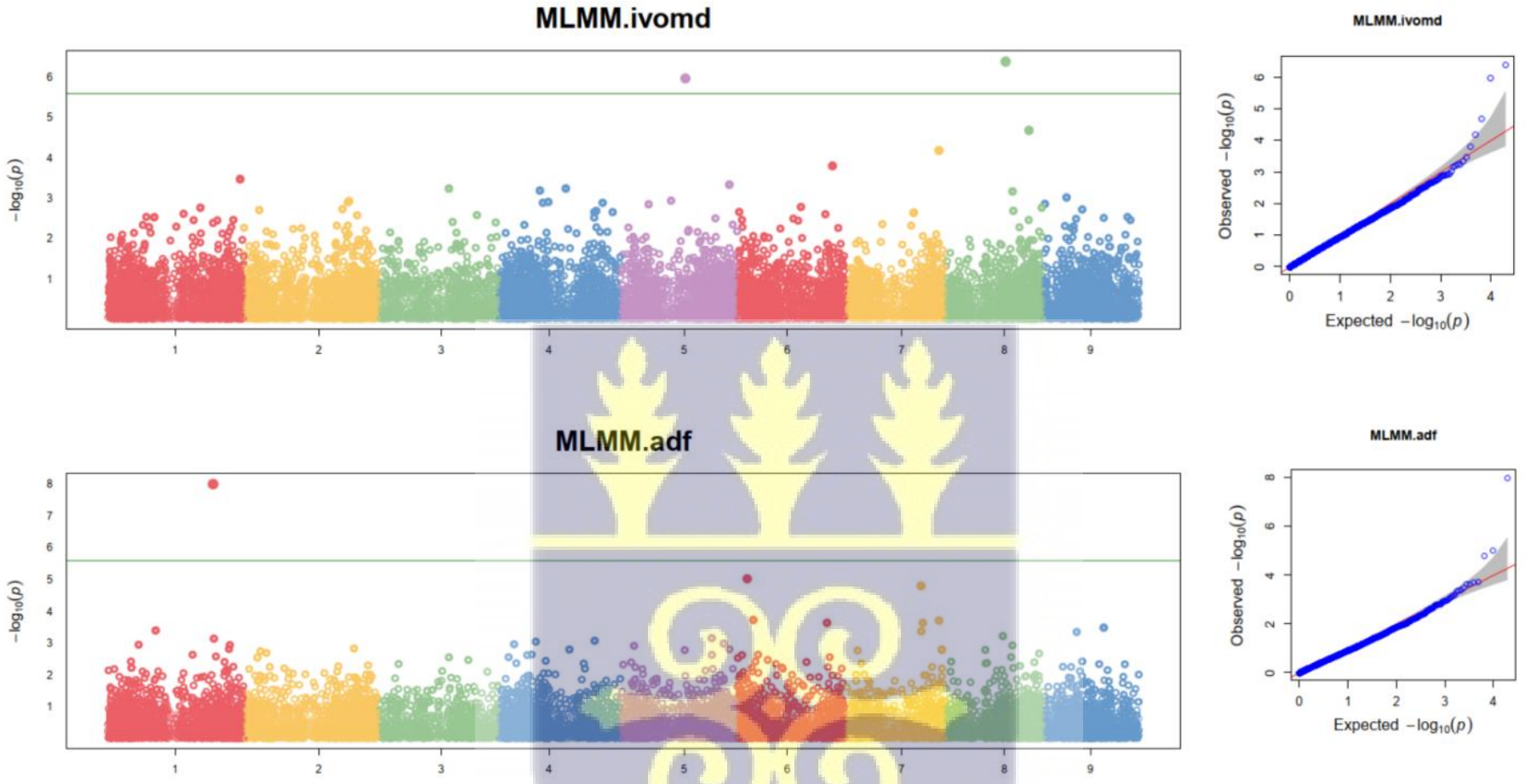


Figure 5. 4: **Manhattan and Quantile-Quantile plots for genome-wide diagnosis of association signals using MLMM model for IVOMD and ADF during the dry season.**

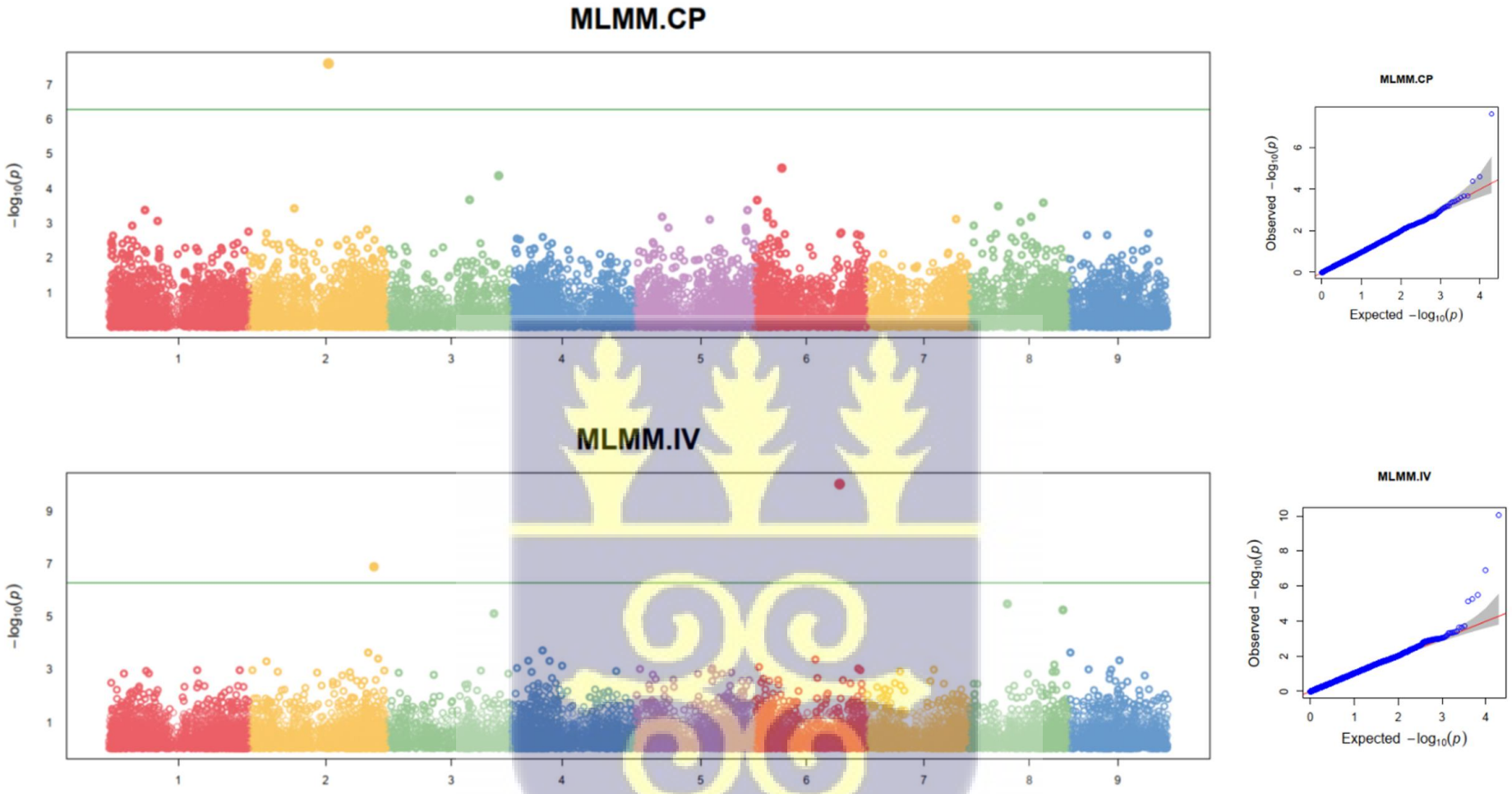


Figure 5. 5: **Manhattan and Quantile-Quantile plots for genome-wide diagnosis of association signals using MLMM model for CP and IVOMD during the wet cut.**

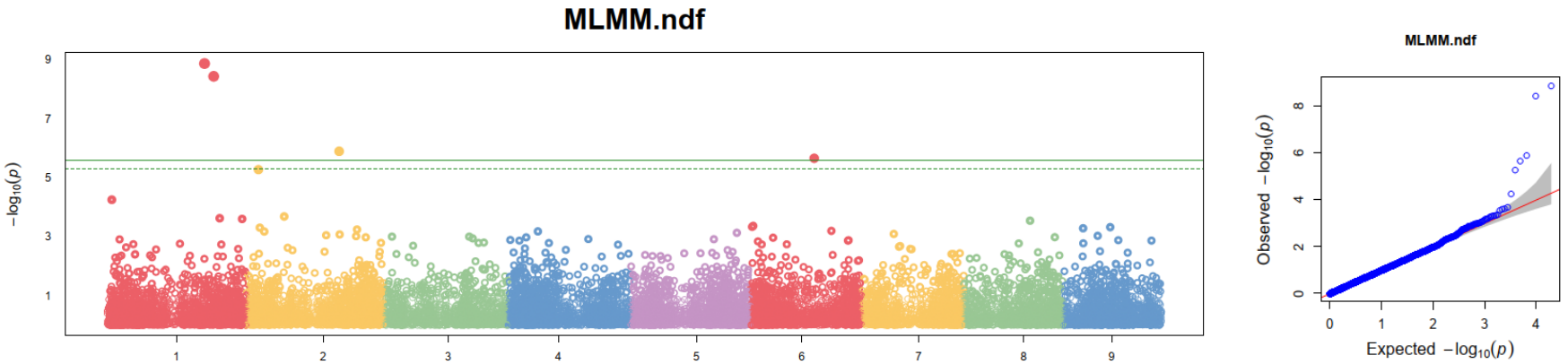
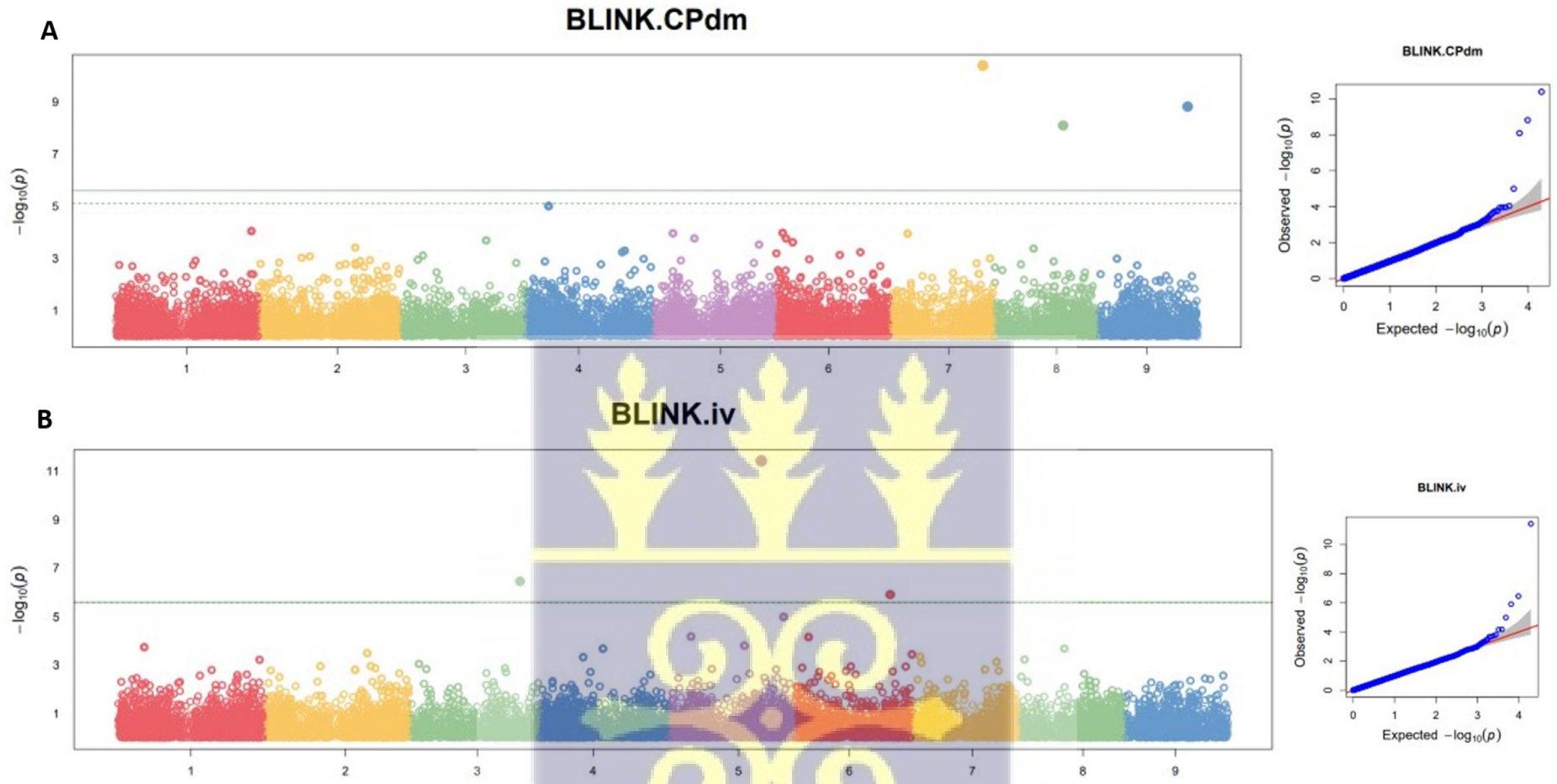
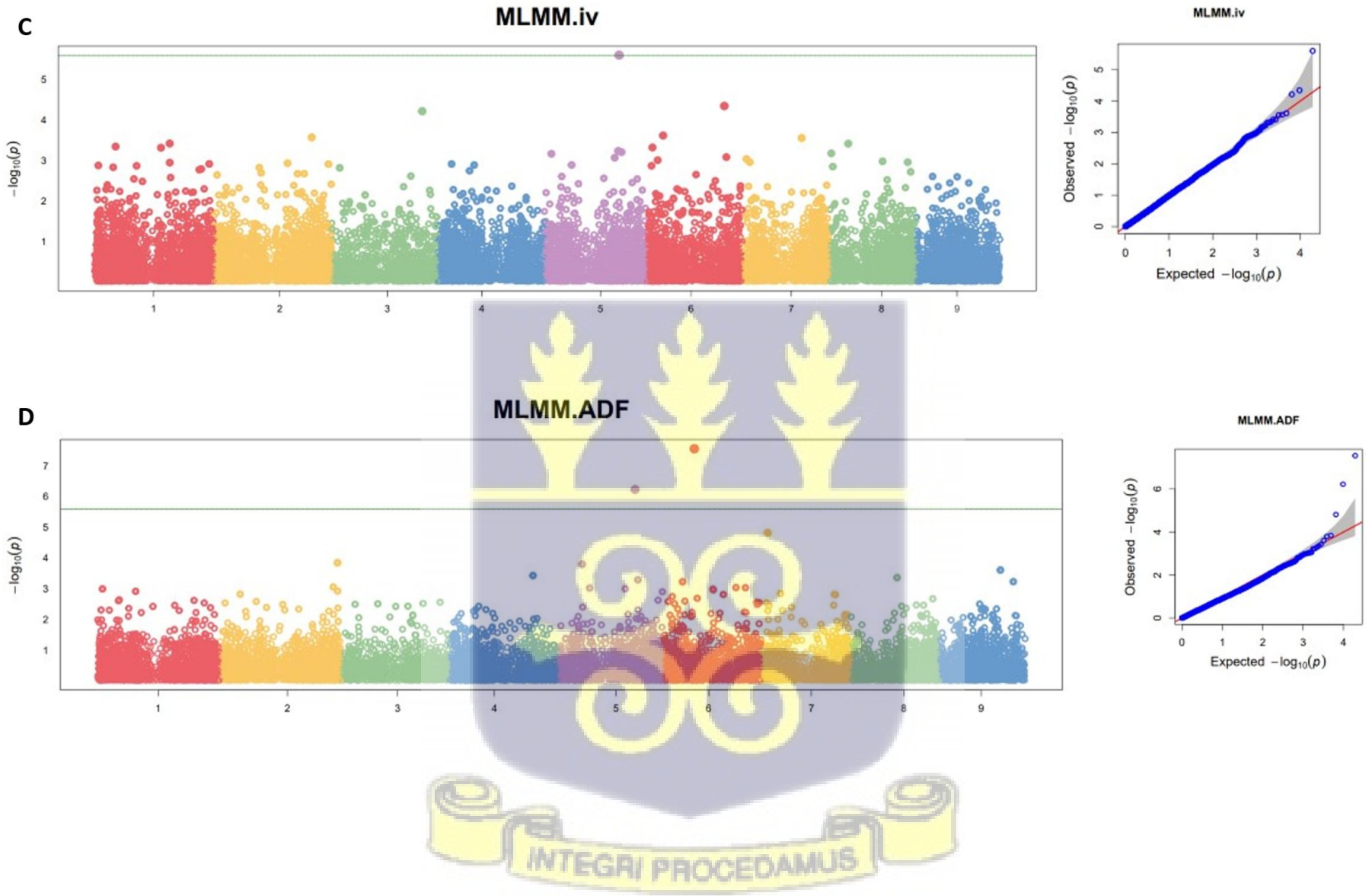


Figure 5. 6: **Manhattan and Quantile-Quantile plots for genome-wide diagnosis of association signals using MLMM model for NDF during the wet cut.**





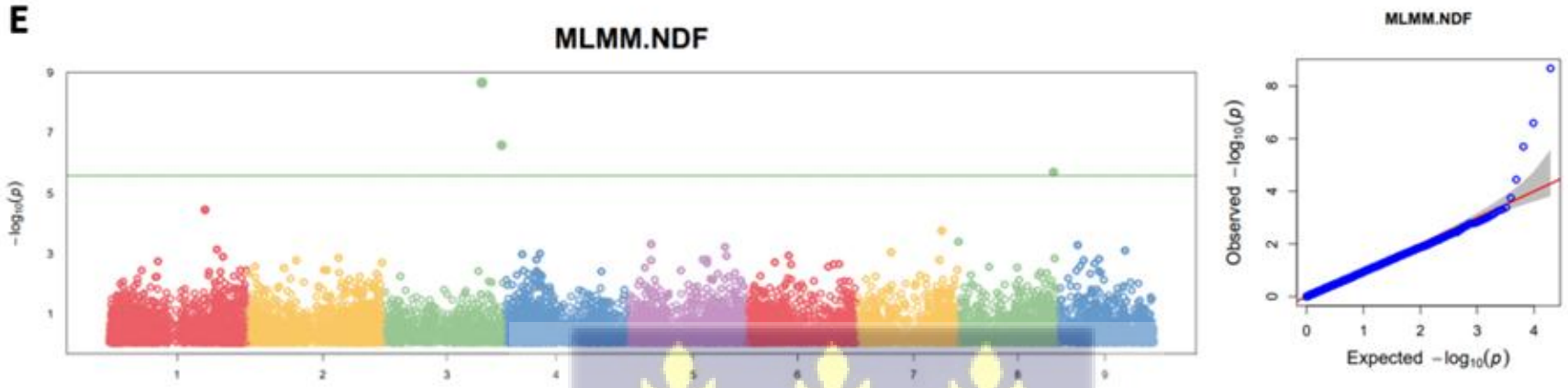


Figure 5. 7: **Manhattan and Quantile-Quantile plots for genome-wide diagnosis of association signals using A&B) BLINK model for CP and IVOMD, C&D) MLMM for IV and ADF, E) MLMM model for NDF under combined seasons.**

Table 5. 4:Significant markers associated with forage quality traits from genome-wide association analysis of 188 *Urochloa* accessions using 19,668 SNPs with MAF greater than 0.05 for wet and dry season.

Season	Trait	Model	RG	SNP	Chr	Position	Ref	Alt	P.value	MAF	effect	PVE
Wet season	CP	MLMM	SV	Brac_2_42687944	2	42687944	C	A	$2.45x10^{-08}$	0.19	0.12	27.36
	IVOMD	MLMM	SB	Brac_2_67026907	2	6.7E+07	G	C	1.22E-07	0.26	0.17	17.57
		MLMM	SB	Brac_6_45198942	6	4.5E+07	G	A	8.83E-11	0.16	0.22	17.30
	NDF	MLMM	SB	Brac_1_52223225	1	5.2E+07	G	T	1.34E-09	0.22	0.50	13.29
		MLMM	SB	Brac_1_57140744	1	5.7E+07	T	A	3.65E-09	0.08	1.00	19.01
		MLMM	SV	Brac_2_49788452	2	5E+07	C	T	1.26E-06	0.07	-0.51	13.86
		MLMM	SV	Brac_6_34140690	6	3.4E+07	T	C	2.18E-06	0.49	0.48	11.45
Dry Season	CP	BLINK	SV	Brac_1_41717043	1	4.2E+07	A	G	7.35E-07	0.02	0.49	1.99
		BLINK	SB	Brac_4_11447831	4	1.1E+07	T	C	2.98E-08	0.03	0.45	4.91
		BLINK	SV	Brac_5_5990992[a]	5	5990992	G	A	1.52E-14	-0.06	0.29	19.45
		BLINK	SV	Brac_5_29543232[b]	5	3E+07	G	A	3.99E-07	-0.05	0.06	5.93
		BLINK	SB	Brac_6_58658705	6	5.9E+07	A	G	1.21E-08	0.07	0.34	24.77
		BLINK	SV	Brac_8_37347904	8	3.7E+07	T	C	1.78E-07	0.03	0.07	4.37
		BLINK	SV	Brac_9_33109087[c]	9	3.3E+07	C	T	3.72E-08	-0.04	0.19	6.99

	MLMM	SV	Brac_2_29667035	2	3E+07	G	A	8.99E-08	0.11	0.04	15.06
	MLMM	SV	Brac_5_5990992[a]	5	5990992	G	A	3.19E-14	0.29	-0.07	23.53
	MLMM	SV	Brac_5_29543232[b]	5	3E+07	G	A	1.12E-06	0.06	-0.05	9.27
	MLMM	SV	Brac_6_36355165	6	3.6E+07	C	G	5.73E-07	0.16	0.03	7.79
	MLMM	SV	Brac_9_33109087[c]	9	3.3E+07	C	T	1.13E-11	0.19	-0.06	9.11
IVOMD	MLMM	SV	Brac_5_35749131	5	3.6E+07	A	G	1.07E-06	0.15	-0.05	18.18
	MLMM	SV	Brac_8_32865539	8	3.3E+07	T	C	4.12E-07	0.49	-0.08	37.09
ADF	MLMM	SV	Brac_1_57887552	1	5.8E+07	C	A	1.02E-08	0.34	0.00	38.31

MAF = Minor allele frequency; Chr = Chromosome; PVE = Phenotypic variance explained, SNPs sharing the same superscript are the same though detected by different model

Table 5. 5: Significant markers associated with forage quality traits from genome-wide association analysis of 188 *Urochloa* accessions using 19,668 SNPs with combined data

Season	Trait	Model	SNP	CHR	Position	Ref	Alt	P.value	MAF	Effect	PVC
combined season	**CP**	BLINK	Brac_7_48604931	7	48604931	A	G	4.01E-11	0.103571	-0.20335	13.48144
		BLINK	Brac_8_35712096	8	35712096	T	C	8.03E-09	0.128571	0.152853	13.03045
		BLINK	Brac_9_47195791	9	47195791	A	G	1.51E-09	0.114286	-0.17466	16.73923
	IVOMD	MLMM	Brac_5_46754281	5	46754281	G	A	2.49E-06	0.142857	0.202905	42.12628
		BLINK	Brac_3_56547992	3	56547992	C	T	3.54E-07	0.092857	-0.2102	20.55692
		BLINK	Brac_5_46754281	5	46754281	G	A	3.84E-12	0.142857	0.233063	20.23329
		BLINK	Brac_6_48376627	6	48376627	G	A	1.24E-06	0.139286	-0.18758	10.19231
	ADF	MLMM	Brac_1_53309852	1	53309852	A	G	1.69E-08	0.042857	-0.51417	39.82485
		MLMM	Brac_5_46754281	5	46754281	G	A	3.06E-08	0.142857	-0.16718	9.364674
		MLMM	Brac_6_18825849	6	18825849	G	T	1.58E-08	0.067857	0.40027	27.76923
	NDF	MLMM	Brac_3_53589219	3	53589219	A	C	2.15E-09	0.078571	-1.19001	32.34421
		MLMM	Brac_3_64285961	3	64285961	T	C	2.56E-07	0.314286	-1.07247	19.25208
		MLMM	Brac_8_51597387	8	51597387	G	A	2.02E-06	0.071429	1.016773	12.4845

5.3.6 Delineation of putative candidate genes

Using three reference genomes of *Setaria itelica Sorghum bicolor* and *Setaria viridris*, five of the ten SNPs associated with crude protein content were positioned directly within the gene homologs (SNP Brac-2-42687944 (gene id Servir2G376500 encoded ATP binding protein kinase with protein phosphorylation, ATP-binding protein kinases are involved in various signaling pathways that regulate plant responses to environmental stresses. (Cheng *et al.,* 2011) found that protein kinases are the most common modifications that regulate the structures and functionality of cellular proteins in a range of cellular processes from cell fate to regulation of metabolism whereas they have also been link to plants responses to environmental stresses (Wang *et al.,* 2020) ; Brac-1-4171704(gene id Sevir1G387200) was associated with translation initiation factor 2 which play essential roles in protein synthesis and stress responses.(Table 5.4). Brac-1- 41717043 Sevir.1G387200 was linked a translation initiator factor (IF2) with functions in regulation of protein biosynthesis (Dutt *et al.,* 2015); Brac-4-11447831 Sobic004G113600 encoded for a protein phosphatase; Brac-5-5990992 Sevir.5G076600 encoded for a phosphodiesterase; Brac-5-29543232 Sevir.5G240800 encoded for a WRKY DNA-binding domain which is located in a superfamily of plant transcription factors that regulate numerous plant-specific physiological programs, including senescence, trichome formation, pathogen defense, and the biosynthesis of secondary metabolites. and Brac-6-58658705 sobic006G227800 was linked to NAD dependent epimerase; Brac-8-37347904. The functional characterization of genes such as serine-threonine protein kinases and WRKY transcription factors indicates their involvement in signaling pathways that regulate plant responses to environmental stresses. These pathways are crucial for maintaining forage quality under varying conditions, which is essential for sustainable livestock production (Yue *et al.,* 2016; Aamir *et al.,* 2017). The ability to manipulate these pathways through breeding or genetic engineering can lead to the development of *Urochloa* cultivars with enhanced stress tolerance.

Regarding invitro organic matter digestibility, we identified 2 candidate genes: SNP Brac-6-45198942 on chromosome six was found in the Sobic.006G075300 gene which encoded for malate transporter and Brac- 8-32865539, Sevir 8G186266, which encoded a wall associated receptor kinase galacturonan binding.

Finally for neutral detergent fiber content four SNPs on chromosomes 1, 2 and 6 were found.

Brac- 1-52223225 was found in gene Sobic.001G270800 which encoded for GTPase; Brac- 1-57140744 located close to Sobic 001G276100 that encoded an oligopeptide transporter protein, Brac-2-49788452, Sevir 6G229400 which encoded for Exo70 exocyst complex subunit C-terminal and finally Brac- 8-3414069

Table 5. 6: Gene annotation for the significant SNPs for forage quality traits under wet and dry seasons

Season	Trait	Model	RG	SNP	Gene id	PF/	Function
Wet season	CP	MLMM	SV	Brac_2_4268794	Sevir.2G376500	PF00069	ATP binding; protein kinase activity; protein phosphorylation
	IVOMD	MLMM	SB	Brac_2_6702690 7	Sobic.002G274700	PF01501	Members of this family are involved in lipopolysaccharide biosynthesis and glycogen synthesis
		MLMM	SB	Brac_6_4519894 2	Sobic.006G075300	PF13515	aluminum-activated malate transporter
	NDF	MLMM	SV	Brac_1_5222322 5	Sobic.001G263500	PF00332	hydrolase activity, hydrolyzing O-glycosyl compounds
		MLMM		Brac_1_5714074 4	Sobic.001G276100	PF00854	Oligopeptide transporter
		MLMM		Brac_2_4978845 2	Sevir.6G229400	PF03081	Exo70 interacts with phospholipids and Rho3 GTPase
		MLMM		Brac_6_3414069 0			Unknown function
Dry Season	CP	BLINK	SV	Brac_1_4171704 3	Sevir.1G387200	PF11987	Translation initiation factor (IF-2)
		BLINK	SB	Brac_4_1144783 1	Sobic.004G113600	PF00481	Protein phosphatase 2C is a Mn++
		BLINK	SV	Brac_5_5990992	Sevir.5G076500	PF00069	Represents the protein kinase domain containing the catalytic function of protein kinases
					Sevir.5G076600	PF00069	Glycerphosphodiester phosphodiesterase
		BLINK	SV	Brac_5_2954323 2	Sevir.5G240800	PF03106	WRKY DNA -binding domain; wrky transcription factor 4
		BLINK	SB	Brac_6_5865870 5	Sobic.006G227800	PF01370	NAD dependent epimerase/dehydratase family; This family of proteins utilise NAD as a cofactor. The proteins in this family use nucleotide-sugar substrates for a variety of chemical reactions.

	BLINK	SV	Brac_8_37347904			Unknown function
	BLINK	SV	Brac_9_33109087	not seen		Unknown function
	MLMM	SV	Brac_2_29667035	Sevir.2G215500	PF01485	IBR domain, a half RING-finger domain
	MLMM		Brac_5_5990992	As under Blink		As above
	MLMM		Brac_5_29543232	As under Blink		As above
	MLMM	SV	Brac_6_36355165	Sevir.6G258501	PF16987	Coactivator CBP, KIX domain; transcription coregulator activity;
	MLMM		Brac_9_33109087			Unknown function
IVOMD	MLMM	SV	Brac_5_35749131			
	MLMM	SV	Brac_8_32865539	Sevir.8G186266	PF13947	Wall-associated receptor kinase galacturonan-binding (GUB_WAK_bind)
ADF	MLMM		Brac_1_57887552			

Table 5. 7: Gene annotation for the significant SNPs for forage quality traits under combined season data

Season	Trait	Model	RG	SNP	CHR	Position	Effect	PVC	Gene ID	PF	Function
combined season	**CP**	BLINK	SB	Brac_7_48604931	7	48604931	-0.20335	13.48144	Sobic.006G101900	PF07690	MFS general substrate transporter like domains
		BLINK	SI	Brac_8_35712096	8	35712096	0.152853	13.03045	LOC_Os11g34000.1		hypothetical protein
		BLINK	SI	Brac_9_47195791	9	47195791	-0.17466	16.73923	LOC_Os03g21530.1	PF08142 /PF04950	AARP2CN domain containing protein expressed
	IVOMD	MLMM	SI	Brac_5_46754281	5	46754281	0.202905	42.12628	LOC_Os01g72930.1	PF01535	pentatricopeptide putative expressed Tetratricopeptide repeat (TPR)-like superfamily protein
		BLINK	SB	Brac_3_56547992	3	56547992	-0.2102	20.55692	Sobic.003G185800		Cytochrome c-like domain
		BLINK	SI	Brac_5_46754281	5	46754281	0.233063	20.23329	LOC_Os01g72930.1	PF01535	pentatricopeptide putative expressed
		BLINK	SB	Brac_6_48376627	6	48376627	-0.18758	10.19231	Sobic.006G099600		Ulp1 peptidase / Ulp1 protease enzyme
	ADF	MLMM	SV	Brac_1_53309852	1	53309852	-0.51417	39.82485			
		MLMM	SI	Brac_5_46754281	5	46754281	-0.16718	9.364674	LOC_Os01g72930.1	PF01535	pentatricopeptide putative expressed

	MLMM	SI	Brac_6_18825849	6	18825849	0.40027	27.76923	LOC_Os04g28820.1	PF05970	retrotransposon protein putative unclassified expressed
NDF	MLMM	SB	Brac_3_53589219	3	53589219	-1.19001	32.34421	Sobic.003G181500		Ubiquitin fusion degradation protein 1 F
	MLMM	SB	Brac_3_64285961	3	64285961	-1.07247	19.25208	Sobic.003G240700		YVTN repeat-like/Quinoprotein amine dehydrogenase
	MLMM	SB	Brac_8_51597387	8	51597387	1.016773	12.4845	Sobic.008G102100	PF00737	Photosystem II 10 kDa phosphoprotein

5.4 Discussion

The nutritive traits exhibited wide variability among accessions, indicating substantial genetic diversity suitable for forage quality improvement. Crude protein (CP) ranged from 3.65–22.87% (mean 13.32 ± 2.06%), showing sufficient levels to support rumen microbial activity (Minson, 1990). In vitro organic matter digestibility (IVOMD) varied moderately (51.26–69.47%, CV = 4.3%), suggesting generally good feed digestibility (Moore & Jung, 2001). Fiber fractions showed limited variation, with NDF (31.83–76.89%) and ADF (21.26–77.19%) averaging 61.59% and 35.32%, respectively, values consistent with tropical grasses (Van Soest, 1994). The high variability in lignin (ADL, CV = 49.7%) highlights potential for selecting low-lignin genotypes to improve digestibility (Jung *et al.,* 2012). Metabolizable energy (ME) ranged from 6.03–9.54 MJ/kg DM, aligning with values typical of *Urochloa* and *Brachiaria* forages (Jank *et al.,* 2014).

The significant variation detected among genotypes, locations, and cuts across all nutritive quality traits aligns with previous studies on *Urochloa* and related tropical grasses and confirmed that both genetic and environmental factors strongly influence forage quality. Similar to the present findings, Barrios *et al.* (2020) reported highly significant genotype effects for crude protein, fiber fractions, and digestibility among *Brachiaria brizantha* accessions, underscoring the presence of wide genetic diversity amenable to selection. The pronounced location effects observed here, particularly for ADF, ADL, and IVOMD, are consistent with results by Lascano and Euclides (1996) and Wilson (1994), who demonstrated that temperature, rainfall, and soil fertility markedly alter fiber composition and digestibility in tropical forages. Likewise, the cut effect, which showed strong significance across all traits, corroborates the findings of Santos et al. (2019), who observed that advancing plant maturity reduces crude protein and increases fiber content due to lignification.

Moderate to high heritability estimates across most nutritive traits indicate strong genetic control and promising potential for selection in *Urochloa* improvement programs (**Jank *et al.,*** 2014; Barros *et al.,* 2021). The narrow gap between GCV and PCV values across traits implies minimal environmental influence, indicating reliable expression of genotypic potential (Falconer & Mackay, 1996; Allard, 1999). High genetic advance and GAM for ADF (51.79%), ASH (51.23%), and NDF (40.69%) point to additive gene action, making these traits amenable to direct selection (Burton & DeVane, 1953), while low GAM for ME (3.68%) and DM (1.13%) suggests limited improvement through phenotypic selection alone (Barros *et al.,* 2021; Vigna *et al.,* 2016).

Population structure analysis identified six population stratifications, with significant admixtures, emphasizing the importance of considering structure and relatedness to prevent misleading associations and false positives.

This study used several genomes, such as *Setaria viridris*, *Sorghum bicolor*, and *Setaria itelica,* and discovered 22 SNPs that showed significant associations with *Urochloa* forage quality parameters. Matias et al. (2019) also used similar reference genomes to map the significant marker trait associations. The genomes of *Setaria viridis*, *Sorghum bicolor*, and *Setaria italica* were employed as reference frameworks for the *Urochloa* because of their close phylogenetic relationship, high-quality genome assemblies, and complementary genomic resources that facilitate accurate marker alignment and gene annotation in *Urochloa* species, which currently lack a complete reference genome. Comparative genomic studies (Ferreira *et al.,* 2021; Worthington *et al.,* 2016) have shown substantial synteny and conserved collinearity among these genera, allowing orthologous genes and SNP loci in *Urochloa* to be reliably anchored to these reference genomes.

The identification of multiple significant SNPs across wet and dry seasons highlights the complex and environment-dependent genetic architecture of forage quality traits in *Urochloa*. Under the wet season, significant associations for crude protein were linked to *Sevir.2G376500*, encoding a protein kinase domain (PF00069), which regulates phosphorylation processes involved in nitrogen metabolism and protein synthesis (Seok, 2021; Wang *et al.,* 2020; Zhang *et al.,* 2016). This gene family is known to mediate nutrient signaling pathways that control amino acid transport and assimilation, critical for crude protein accumulation in vegetative tissues. Protein phosphorylation is essential in post-translational activities that affect protein function and stability._For IVOMD, associated genes such as *Sobic.002G274700* (PF01501) and *Sobic.006G075300* (PF13515) encode enzymes involved in lipopolysaccharide biosynthesis and malate transport, respectively, suggesting roles in carbohydrate metabolism and energy translocation, which are central to cell wall digestibility (Wilson, 1994; Small & Peeters, 2000). The malate transporter gene is particularly relevant, as it may influence organic acid balance and fiber degradation efficiency during rumen fermentation.

In the wet season NDF associations, genes such as *Sobic.001G263500* (PF00332) and *Sobic.001G276100* (PF00854) were linked to glycosyl hydrolase and oligopeptide transporter activities, respectively, indicating involvement in cell wall modification and peptide transport. The presence of *Sevir.6G229400* (PF03081), encoding Exo70, suggests cytoskeletal remodeling and vesicle transport mechanisms that may regulate secondary cell wall deposition (Van Soest, 1994; Vigna *et al.,* 2016).

During the dry season, CP-associated loci included *Sevir.1G387200* (PF11987), a translation initiation factor, and *Sobic.004G113600* (PF00481), a protein phosphatase 2C, both of which regulate gene expression and stress response, indicating adaptive regulation of protein

synthesis under moisture stress (Barrios *et al.,* 2020; Santos *et al.,* 2019). Additional loci such as *Sevir.5G240800* (PF03106), encoding a WRKY transcription factor, are known regulators of stress-inducible genes involved in metabolic adjustment and nitrogen remobilization (Eulgem & Somssich, 2007). The detection of NAD-dependent epimerase (*Sobic.006G227800*) implies a role in carbohydrate interconversion and secondary metabolite biosynthesis influencing fiber digestibility.

The recurrence of regulatory domains such as protein kinases, WRKY transcription factors, and CBP coactivators across environments suggests that signal transduction and transcriptional regulation are key determinants of forage quality variation in *Urochloa*. The presence of several unknown-function loci also points to unexplored genomic regions potentially unique to *Urochloa*.

Brac-1-41717043, identified on chromosome 1, was associated with Sevir.1G387200, which encodes IF2, a translation initiation factor essential for protein synthesis in the initiation pathway (Kaledhonkar *et al.,* 2019). The binding involves formylmethionine-tRNA, GTP, IF1, IF3, and both ribosomal subunits (Hedegaard *et al.,* 2000). IF2 assists in binding the initiator tRNA to a specific location on the smaller ribosomal subunit and initiates the hydrolysis of GTP upon formation of the initiation complex (Hedegaard *et al.,* 2000). Marker Brac-4-11447831 on chromosome 4 is associated with a protein phosphatase 2C, a distinct type of Mg2+/Mn2+-dependent enzyme that reverses protein phosphorylation, a crucial protein modification regulating various physiological functions. Brac-5-29543232 on chromosome 5 was associated with a WRKY DNA-binding domain. These transcription factors have been previously identified as playing a role in cell wall formation in grasses. Members of the WRKY transcription factor family have the ability to inhibit lignification (Gallego-Giraldo *et al.,* 2016;

Rao and Dixon, 2018). An in-depth examination of various gene families involved in cell wall biosynthesis, such as transcription factor families, could provide insights into the control of secondary cell wall formation in *Urochloa* species.

Brac -6-58658705 on chromosome 6 is associated with the NAD dependent epimerase/dehydratase family. The Brac-2-29667035 gene on chromosome 2 is associated with the IBR domain, which is a half RING-finger domain involved in protein quality control and can indirectly influence transcription (Marín *et al.,* 2004). This system not only eliminates defective or truncated proteins for cellular upkeep but also regulates the quantity of functional proteins based on the balance between protein production and degradation (Mazzucotelli *et al.,* 2006). Brac-6-36355165 on chromosome 6 is associated with the kinase-inducible (kix) domain, which functions as a transcription coregulator.

When the seasons were combined, for CP, significant loci were detected on chromosomes **7,** 8, and 9, explaining up to 16.7% of phenotypic variation, with candidate genes including *Sobic.006G101900* (MFS substrate transporter-like domain) and *LOC_Os03g21530.1* (AARP2CN domain protein). The presence of transporter-related genes suggests a role in amino acid or nitrogen compound translocation, which may influence protein accumulation in plant tissues (Santos *et al.,* 2019; Jank *et al.,* 2014).For IVOMD, multiple associations were identified on chromosomes 3, 5, and 6, with the strongest SNP (*Brac_5_46754281*) explaining 42.1% of phenotypic variance and annotated to *LOC_Os01g72930.1*, a pentatricopeptide repeat (PPR) gene. PPR proteins are involved in organelle gene expression and energy metabolism, which can affect chloroplast function and consequently forage digestibility (Small & Peeters, 2000; Barros *et al.,* 2021). The detection of *Sobic.003G185800* (cytochrome c-like

domain) and *Sobic.006G099600* (Ulp1 peptidase) further indicates that oxidative and proteolytic processes contribute to variation in organic matter degradability.

Significant associations for ADF were found on chromosomes 1, 5, and 6, with loci explaining up to 39.8% of phenotypic variance. Notably, the repeated identification of *LOC_Os01g72930.1* across IVOMD and ADF indicates a potential pleiotropic gene influencing both digestibility and fiber synthesis. The candidate *LOC_Os04g28820.1*, encoding a retrotransposon-like protein, may be involved in regulatory genome dynamics affecting lignin biosynthesis (Vigna *et al.,* 2016).

For NDF, strong associations were detected on chromosome 3 with *Sobic.003G181500* (ubiquitin fusion degradation protein) and *Sobic.003G240700* (quinoprotein amine dehydrogenase), explaining up to 32.3% of variation. These genes are known to mediate protein turnover and redox metabolism, which influence cell wall development and structural carbohydrate accumulation (Van Soest, 1994; Wilson, 1994). The detection of *Sobic.008G102100*, encoding a photosystem II phosphoprotein, suggests an indirect link between photosynthetic efficiency and fiber deposition, supporting earlier reports of trade-offs between fiber concentration and energy capture (Minson, 1990; Barrios *et al.,* 2020).

Overall, the identification of loci across several chromosomes and multiple shared candidate genes highlights the polygenic and interconnected genetic basis of forage quality traits in *Urochloa*. The repeated detection of PPR-related and transporter-domain genes suggests that metabolic and regulatory processes are central to variation in both digestibility and nutrient content.

5.5 Conclusion

The study revealed multiple specific genomic areas containing genes associated with quality attributes in *Urochloa* germplasm. The genes discovered in this study offer insights into the

genetic foundation of the forage quality traits in the Ugandan *Urochloa* gene pool. Under contrasting wet and dry seasons GWAS identified environment-specific specifically, Protein kinases, phosphatases, and WRKY transcription factors emerged as major regulators of crude protein metabolism, while transporter and receptor kinase genes were associated with digestibility and fiber composition. The variation in gene expression patterns between wet and dry conditions highlights the plasticity and adaptability of *Urochloa* under diverse environments. When seasons were combined, GWAS revealed multiple significant SNPs associated with CP, IVOMD, ADF, and NDF, distributed across chromosomes 1, 3, 5, 6, 7, 8, and 9. High-effect loci, particularly on chromosomes 3 and 5, accounted for substantial proportions of phenotypic variance, indicating genomic regions of major influence on nutritive quality. Candidate genes identified were PPR proteins, MFS transporters, cytochrome c-like, and ubiquitin degradation proteins suggesting involvement of metabolic pathways regulating nitrogen assimilation, energy metabolism, and cell wall biosynthesis. These identified loci and candidate genes provide valuable molecular targets for marker-assisted selection and genomic breeding aimed at developing high-quality, high-digestibility *Urochloa* cultivars adapted to Ugandan environments.

The evaluated *Urochloa* accessions exhibited substantial genetic and phenotypic variability in nutritive quality traits, reflecting wide potential for genetic improvement. Significant differences ($p < 0.001$) among genotypes, cuts, and locations, as well as their interactions, demonstrated strong genetic control combined with notable environmental influence on crude protein, fiber fractions, digestibility, and energy traits. The wide range in CP (3.65–22.87%), moderate IVOMD (51.26–69.47%), and variable fiber contents (ADF, NDF, ADL) revealed clear differentiation among accessions in nutrient composition and digestibility potential. High heritability values for key traits such as ASH, N, and ADF, coupled with high genetic advance and genetic gain, indicate additive gene action and strong prospects for selection. The narrow

differences between genotypic and phenotypic coefficients of variation further confirm minimal environmental masking, suggesting reliable phenotypic expression of genotypic potential. Collectively, these results demonstrate that nutritive quality in *Urochloa* is a heritable and selectable trait complex, responsive to breeding and environment. Therefore, targeted selection for high-protein, low-fiber, and high-digestibility accessions—validated across diverse environments—offers an effective pathway to develop superior forage varieties suited for tropical livestock production systems.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 General conclusion

The morphological characterization of *Urochloa* germplasm revealed substantial variability among accessions across key qualitative traits, including leaf and stem colour, hairiness, growth habit, and ploidy level. The predominance of green leaf and stem pigmentation, moderate hairiness, and decumbent growth habit indicates adaptation to tropical environments and selection for traits conferring both vigour and persistence under variable moisture conditions.

Variation in pigmentation and trichome density suggests divergent adaptive strategies, where accessions with purple stems and higher hairiness may possess enhanced tolerance to drought and high irradiance. The coexistence of diploid, triploid, and polyploid accessions demonstrates evolutionary complexity within the germplasm, reflecting hybridization and polyploidization events typical of the *Urochloa* genus.

Generally, the observed morphological diversity provides valuable phenotypic indicators of genetic variation, complementing molecular evidence from DArTseq-SNP analysis. This diversity forms a critical resource for selection, breeding, and conservation efforts aimed at developing high-yielding, stress-resilient, and nutritionally superior tropical forage cultivars.Diploid accessions dominated (85.1%), suggesting a rich source of sexual genotypes for breeding, while the occurrence of polyploid forms reflected apomictic reproduction and hybrid origin.

The ANOVA results confirmed significant differences among accessions and across seasons for key agronomic traits such as plant height, leaf width, dry matter yield, and spike productivity, reflecting strong genotypic and environmental influences. The significant accession × season interactions highlighted genotype-by-environment (G×E) effects, emphasizing the need for multi-season and multi-location evaluations in selection programs.

The PCA analysis summarized the main axes of morphological differentiation in which PC1 captured vegetative size and leaf morphology, PC2 reflected surface traits (hairiness and pigmentation), PC3 described plant stature and spread, PC4–PC6 involved growth habit, pigmentation contrasts, and biomass partitioning. Together, these axes explained over 71% of total variation, confirming the multidimensional nature of diversity and the interplay between morphology, adaptability, and productivity.

Diversity statistics of *Urochloa* germplasm collected across different Agro-Ecological Zones (AEZs) based on DArTseq-SNP markers reflected genetic variation, heterozygosity, allele frequency, and differentiation parameters. The overall diversity (mean GD = 0.28, PIC = 0.23, HO = 0.25) confirms moderate-to-high genetic variation in the *Urochloa* germplasm collection. Accessions from the Northerm Farmland and South Western rangelands showed broad allelic diversity whereas accessions from lake albert crescent and Eastern Farmland being more genetically uniform. This pattern is consistent with the PCA and hierarchical clustering results, which identified distinct but overlapping genetic clusters, suggesting both shared ancestry and regional adaptation.

The study successfully revealed substantial genetic diversity and population structure among the evaluated *Urochloa* accessions using DArT-based SNP markers. The hierarchical clustering heatmap indicated the formation of distinct genetic groups with varying degrees of relatedness, while the PCA confirmed this structure by separating accessions along two

principal dimensions that together explained over 34% of the total molecular variance. The observed clustering patterns didnot correspond to species differentiation, ploidy variation, towards potential geographical or breeding origins, reflecting both shared ancestry and adaptive divergence within the germplasm collection. Closely clustered accessions demonstrated high within-group genetic similarity, while those positioned distantly in both analyses represented genetically distinct individuals.

Population structure analysis (STRUCTURE and FST) identified six major genetic clusters (K = 6) with varying degrees of admixture and differentiation (FST 0.01–0.86), indicating both distinct gene pools and ongoing gene flow among accessions. The low overall AMOVA Phi statistic (0.00108) confirmed that most genetic variation (99.9%) exists within populations, typical of outcrossing, polyploid tropical grasses. This pattern reflects widespread hybridization, shared ancestry, and high intra-population variability, key characteristics of the *Urochloa* complex. The clear divergence observed among SP1, SP3, SP4, and SP6, characterized by high FST and nucleotide distance values, indicates strong genetic isolation and limited gene exchange, while the low FST and high heterozygosity in SP5 highlight the presence of admixture and interspecific hybridization.

The findings demonstrate that *Urochloa* populations possess a complex genetic architecture shaped by polyploidy, apomixis, and natural hybridization, consistent with patterns observed in other tropical grasses such as *Megathyrsus maximus* and *Cenchrus ciliaris*. The null hypothesis stating that there was no selectable variation exists among Ugandan *Urochloa* germplasm was rejected.

Study two results demonstrate that Near-Infrared Spectroscopy (NIRS) is a highly effective and reliable tool for predicting major forage quality traits in *Urochloa* species. Across all traits,

the models achieved strong coefficients of determination (R^2cv = 0.77–0.93) and high ratios of performance to deviation (RPD = 2.0–3.6), confirming their robustness for both research and routine phenotyping.

Among the traits, ADF and ME exhibited the highest predictive accuracies ($R^2cv \geq 0.90$; RPD > 3.0), indicating excellent model performance suitable for precise selection and quality assessment. NDF, CP, IVOMD, and ADL models also achieved acceptable to strong predictive power (RPD ≥ 2.0), making them valuable for screening and ranking genotypes in breeding programs.

Preprocessing combinations such as SNV, MSC, and derivative-based corrections (SNV+SG, SNV+Gap) significantly improved model accuracy by minimizing spectral noise, correcting baseline shifts, and enhancing chemical signal detection.

Overall, the NIRS models provide a rapid, cost-effective, and non-destructive alternative to wet chemistry for evaluating large *Urochloa* germplasm collections. Their demonstrated precision and stability support their integration into high-throughput phenotyping pipelines for forage quality improvement, enabling accelerated selection of nutrient-dense and high-performing cultivars for tropical livestock production systems.

Using NIRS therefore offers the ability to accurately predict forage traits and significantly enhance breeding programs aimed at developing *Urochloa* cultivars with improved nutritional profiles. By integrating NIRS into breeding strategies, researchers can more efficiently select desirable traits, leading to the development of high-quality forage varieties that meet the nutritional needs of livestock. This approach can accelerate the breeding process and improve the overall efficiency of forage production systems.

The insights gained from NIRS analyses can contribute to more sustainable livestock production systems. By ensuring that livestock are fed high-quality forage, farmers can

enhance animal health and productivity, leading to better economic outcomes. Moreover, the ability to monitor forage quality throughout the growing season allows for timely adjustments in feeding strategies, which is particularly important in the context of variable climatic conditions.

The findings underscore the need for continued research into the application of NIRS for other forage species and traits. As the technology evolves, expanding its use to include a broader range of nutritional parameters and forage species can provide valuable insights into the management of tropical grasslands and their role in sustainable agriculture

The implications of the NIRS prediction models developed in this study is that farmers and agronomists can make more informed decisions about forage management, leading to improved livestock nutrition and productivity. This can ultimately contribute to enhanced food security and economic stability in regions reliant on livestock production. By utilizing NIRS for rapid assessments of forage quality, agricultural stakeholders can reduce the time and resources spent on traditional laboratory analyses. This efficiency can lead to cost savings and more effective resource allocation in forage management practices. The ability to monitor and predict forage quality supports sustainable agricultural practices by promoting the use of high-quality forages that can improve soil health and reduce the environmental impact of livestock production. This aligns with global efforts to enhance sustainability in agriculture and mitigate the effects of climate change. In summary, the moderately high NIRS prediction accuracies and reliability for forage traits in *Urochloa* grasses provide valuable tools for improving forage management, breeding programs, and sustainable livestock production practices. By leveraging these insights, agricultural stakeholders can enhance productivity while promoting environmental sustainability.in plant breeding programs.

Lastly, study three was based on the hypothesis that there are no SNP markers associated to forage quality traits in *Urochloa* grasses. The null hypothesis was rejected. The comprehensive evaluation of *Urochloa* accessions across environments revealed significant genetic and phenotypic variability for key forage nutritive traits including crude protein (CP), in vitro organic matter digestibility (IVOMD), neutral and acid detergent fiber (NDF, ADF), lignin (ADL), and metabolizable energy (ME). The wide ranges in CP (3.65–22.87%), IVOMD (51.26–69.47%), and fiber fractions demonstrated the existence of accessions with contrasting nutritional profiles suitable for selection. Analysis of variance showed highly significant ($p < 0.001$) effects of genotype, environment (location and cut), and their interactions, indicating that both genetic constitution and environmental conditions strongly influence forage nutritional quality.

Moderate to high heritability estimates (0.44–0.72) combined with high genetic advance and genetic gain in traits such as ADF, NDF, ASH, and N confirmed the predominance of additive gene action and the potential for effective selection and genetic improvement. The close correspondence between genotypic and phenotypic coefficients of variation suggested minimal environmental masking, implying that phenotypic performance is a reliable indicator of genetic potential.

Genome-wide association studies (GWAS) further identified several significant SNPs and candidate genes linked to key nutritive traits across wet, dry, and combined seasons. Notably, genes encoding protein kinases, WRKY transcription factors, pentatricopeptide repeat (PPR) proteins, malate transporters, and ubiquitin-related enzymes were associated with variation in crude protein content, digestibility, and fiber composition. These loci explained substantial proportions of phenotypic variance, emphasizing their biological importance in nitrogen metabolism, energy production, and cell wall biosynthesis. The repeated identification of

transporter and kinase-related genes across environments highlights conserved regulatory networks influencing forage nutritional quality.

Overall, the integration of phenotypic, genetic, and genomic analyses demonstrates that *Urochloa* possesses a broad genetic base for nutritive quality traits. This diversity, coupled with the identification of key genomic regions, provides a strong foundation for the development of nutritionally superior and environment-resilient cultivars suited to tropical livestock systems.

For instance, loci associated with crude protein content and fiber composition in *Urochloa* identified in this study were robust, with the integration of environmental data. This is particularly relevant in the context of climate change, where understanding the resilience of forage crops to environmental stressors is crucial for sustainable livestock production. The application of GAPIT in GWAS also enabled the use of multiple statistical models, which improve the robustness of the findings. The latest versions of GAPIT incorporated advanced methodologies that enhanced the power and accuracy of genetic predictions, making it a valuable resource for researchers and breeders. This capability is essential for developing high-quality forage varieties that meet the nutritional needs of livestock while also addressing economic and environmental sustainability concerns. The functional characterization of these genes can lead to the identification of key regulatory networks that influence forage traits. By understanding how these genes interact and contribute to phenotypic expression, researchers can target specific pathways for manipulation through breeding or genetic engineering. This targeted approach can accelerate the development of *Urochloa* cultivars with improved traits, such as higher nutritional value and better stress tolerance.

The implications to Breeding Programs revolves around the use of knowledge gained from GWAS can significantly enhance breeding programs aimed at improving *Urochloa* grasses. By

focusing on genetic markers associated with desirable traits, breeders can implement marker-assisted selection (MAS) to develop new cultivars that exhibit enhanced forage quality and resilience. This targeted breeding strategy can lead to more efficient and effective improvements in *Urochloa* grasses, ultimately benefiting livestock production systems. 5.

6.2 Recommendation

Future Research Directions need to explore the genetic mechanisms underlying ploidy variation and its relationship with ecological adaptability in *Urochloa*. Studies focusing on the interaction between ploidy levels, genetic diversity, and environmental factors will provide valuable insights for breeding programs aimed at developing resilient forage systems (Tomaszewska *et al.,* 2021).

The implications of high ploidy variation and moderate genetic diversity in *Urochloa* grasses are profound for agricultural sustainability and ecological resilience. By harnessing the genetic potential of these grasses, farmers and researchers can develop improved cultivars that not only meet the nutritional needs of livestock but also contribute to the health of the ecosystems in which they are grown. This balance between agricultural productivity and ecological integrity is essential for addressing the challenges posed by climate change and food security in tropical regions.

Notably and very important, Study one revealed majority of the accessions as diploid *Urochloa* accessions, which are automatically sexual and can be used as females in a hybridization scheme. However, the use of the sexually reproducing lines is only possible after tetraploidization. Therefore, optimization of the tetraploidization protocol can be fast-tracked to create tetraploidized females to initiate a hybridization scheme for *Urochloa* in Uganda.

Under study two, recommendations to enhance and improve the robustness of NIRS calibration models, it is essential to include a wide range of samples that represent the genetic and

environmental variability of *Urochloa* grasses. Increase the variability of the calibration set and select the most informative spectral range for each of the traits investigated while testing other pre-processing treatments to potentially reduce noise signal ratio and subsequently increase the prediction model performance of the characteristics with a lower coefficient of determination and ratio of performance.

Also, developing local databases that reflect the specific forage types and conditions in which *Urochloa* is grown can significantly enhance prediction accuracy. Asekova et al. found that using a local database improved the detection accuracy of nutrient compositions in feedstuffs (Despal *et al.,* 2020). This approach allows for more tailored calibrations that account for regional variations in forage quality. Incorporating additional physical or chemical traits into the calibration sample set can improve the robustness of NIRS models. Peiris et al. suggested that including moisture variation and other relevant traits can enhance the predictive performance of NIRS calibrations (Peiris *et al.,* 2019). This strategy is particularly important for *Urochloa*, where moisture content can significantly affect forage quality. Employing advanced statistical techniques, such as machine learning, can improve the predictive capabilities of NIRS models. Bastianelli et al. demonstrated that combining different spectral data can yield better predictions for digestibility and nutrient composition (Bastianelli *et al.,* 2015). Utilizing machine learning algorithms can help capture complex relationships between spectral data and forage traits.

Improving sample preparation and analysis techniques - Standardizing Sample Preparation Consistency in sample preparation is crucial for accurate NIRS predictions. Ensuring that samples are processed uniformly, whether as whole plants, ground material, or preserved forages, can reduce variability and improve prediction reliability (Andueza *et al.,* 2016). This standardization can help mitigate the effects of processing on the spectral data.

Continuous validation and model updating with new samples is essential to maintain their accuracy over time. As new cultivars and environmental conditions emerge, regularly updating calibration models ensures they remain relevant and effective (Parrini *et al.,* 2017). This process is crucial for adapting to changes in forage quality due to breeding advancements or shifts in agricultural practices. Lastly implementing cross-validation techniques can help assess the reliability of NIRS predictions. By dividing the dataset into training and validation subsets, researchers can evaluate the model's performance and make necessary adjustments to improve accuracy (Andueza *et al.,* 2011).

Study three first and most importantly, A significant hurdle in fostering genomic research on tropical forages is the need for a reference genome. Several reference genomes were used in this study, including *Setaria italica, Setaria Viridris and Sorghum bicolor,* to map the *Urochloa* grass DArTSeq markers created in this thesis. However, we could only map 14,2% of the total markers generated. The availability of a fully referenced *Urochloa* genome will enhance mapping resolution and enable the identification of SNPs that explain most of the existing phenotypic variation. Therefore, the key recommendation to create a reference genome to facilitate marker mapping and genome-wide association studies is paramount, as it aims to identify significant quantitative trait loci (QTL) for specific characteristics of interest with enhanced precision in the association.

To enhance the effectiveness of genome-wide association studies (GWAS) for forage traits in *Urochloa* grass, several recommendations can be made based on current research and methodologies. These recommendations focus on improving the identification of genetic markers associated with desirable traits, optimizing experimental design, and integrating environmental factors. 1) Incorporate more diverse genetic Material. It is crucial to utilize a diverse set of *Urochloa* grass cultivars in GWAS to capture a wide range of genetic variation.

Including a broad spectrum of ecotypes will enhance the robustness of the findings and allow for the identification of markers that are beneficial across different environments.

2) Comprehensive phenotypic data collection is essential for effective GWAS. Traits such as forage yield, nutritional value, and morphogenetic characteristics should be meticulously measured under controlled conditions and in field trials (Ramos *et al.,* 2022). This data will provide a solid foundation for associating genetic markers with specific phenotypic traits. 3) Incorporating environmental factors such as soil type, climate conditions, and management practices into the GWAS framework can provide insights into how these variables interact with genetic traits. Research indicates that environmental conditions significantly influence forage quality and yield. By integrating these data into the GWAS analysis, researchers can better understand the genotype-by-environment interactions, leading to more accurate predictions of forage performance under varying conditions. 4) Establishing collaborative networks among researchers, agronomists, and breeders can facilitate data sharing and resource pooling. This collaboration can lead to larger sample sizes and more comprehensive datasets, which are essential for robust GWAS. Additionally, sharing findings and methodologies can accelerate the adoption of successful breeding strategies across different regions (Baptistella *et al.,* 2020). In conclusion, implementing these recommendations can significantly improve the outcomes of GWAS for forage traits in *Urochloa* grass. By focusing on genetic diversity, thorough phenotypic characterization, environmental integration, advanced statistical modeling, key trait prioritization, and collaborative efforts, researchers can enhance the breeding of *Urochloa* grass for improved forage quality and resilience.

Continued research into the functional roles of identified genes in study 3 is essential for further elucidating their contributions to forage traits. Investigating gene interactions and their effects on phenotypic expression will provide deeper insights into the complex genetic architecture of

Urochloa grasses. Additionally, exploring the potential for gene editing technologies, such as CRISPR/Cas9, to enhance desirable traits could revolutionize forage breeding.

The implications of identifying genes associated with forage traits in *Urochloa* grasses are profound for agricultural sustainability and food security. By leveraging the genetic insights gained from study 3, breeders can develop *Urochloa* cultivars with improved nutritional profiles, contributing to better livestock health and productivity.

In conclusion, the interplay between moderate genetic diversity, advanced predictive modelling through NIRS, and the identification of key genetic determinants of forage quality presents a multifaceted approach to enhancing forage crops. This integrated strategy not only improves the nutritional value of forage but also contributes to sustainable agricultural practices by optimizing the use of genetic resources and technological advancements.

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APPENDICES

Appendix 1:Details of material used in morphological study.

	Accession	Lat	Long	District
1	Ug001	0.5139	32.6192	WAKISO
2	Ug002	0.5179	32.623	WAKISO
3	Ug003	0.5199	32.6223	WAKISO
4	Ug004	0.5256	32.6189	WAKISO
5	Ug005	0.5292	32.6141	WAKISO
6	Ug006	0.523	32.6232	WAKISO
7	Ug007	0.518	32.6274	WAKISO
8	Ug008	0.5218	32.6287	WAKISO
9	Ug009	0.5222	32.63	WAKISO
10	Ug010	0.5193	32.6341	WAKISO
11	Ug011	0.5167	32.6375	WAKISO
12	Ug012	0.5047	32.6456	WAKISO
13	Ug013	0.5087	32.6478	WAKISO
14	Ug014	0.5062	32.6469	WAKISO
15	Ug015	0.5157	32.6415	WAKISO
16	Ug016	0.5169	32.6406	WAKISO
17	Ug017	0.5161	32.6395	WAKISO
18	Ug018	0.5155	32.6387	WAKISO
19	Ug019	0.416	30.2001	FPORTAL
20	Ug020	0.4168	30.1993	FPORTAL
21	Ug021	0.5645	32.651	FPORTAL
22	Ug022	0.4175	30.1991	FPORTAL
23	Ug023	0.4176	30.1987	FPORTAL
24	Ug024	0.4196	30.1981	FPORTAL
25	Ug025	0.45661	30.1891	FPORTAL
26	Ug026	0.4188	30.199	FPORTAL
27	Ug027	0.4193	30.1989	FPORTAL
28	Ug028	0.4192	30.1979	FPORTAL
29	Ug029	0.4145	30.1999	FPORTAL
30	Ug030	0.4186	30.1994	FPORTAL
31	Ug031	0.4179	30.1982	FPORTAL
32	Ug032	0.4177	30.1986	FPORTAL
33	Ug033	0.4169	30.1988	FPORTAL
34	Ug034	0.416	30.1989	FPORTAL
35	Ug035	0.425	30.1619	FPORTAL
36	Ug036	-0.6072	30.6115	MBARARA
37	Ug037	-0.6072	30.6122	MBARARA
38	Ug038	-0.608	30.6104	MBARARA
39	Ug039	-0.6073	30.6112	MBARARA
40	Ug040	-0.6072	30.6115	MBARARA
41	Ug041	-0.6076	30.6104	MBARARA

42	Ug042	-0.608	30.4105	MBARARA
43	Ug043	-0.6079	30.6104	MBARARA
44	Ug044	-0.6076	30.6104	MBARARA
45	Ug045	-0.6077	30.6091	MBARARA
46	Ug046	-0.6004	30.6141	MBARARA
47	Ug047	-0.6	30.6135	MBARARA
48	Ug048	N02.28590	E032.75592	MBARARA
49	Ug049	N02.24338	E032.76350	ARUA
50	Ug050	N02.24547	E032.81593	ARUA
51	Ug051	N02.26317	E032.86740	ARUA
52	Ug052	N02.70484	E032.94628	ARUA
53	Ug053	N02.74751	E032.95023	ARUA
54	Ug054	3.071	30.94	ARUA
55	Ug055	3.234	30.94	ARUA
56	Ug056	3.067	30.94	ARUA
57	Ug057	3.0712	30.942	ARUA
58	Ug058	3.0748	30.94	ARUA
59	Ug059	3.075	30.94	ARUA
60	Ug060	N02.37161	E032.93527	ARUA
61	Ug061	N02.44194	E032.93829	ARUA
62	Ug062	3.7619	30.947	ARUA
63	Ug063	3.0077	30.9484	ARUA
64	Ug064	3.0767	30.9494	ARUA
65	Ug065	3.076	30.949	ARUA
66	Ug066	3.076	30.9502	ARUA
67	Ug067	N02.65562	E032.93916	ARUA
68	Ug068	1.501	31.495	HOIMA
69	Ug069	1.4984	31.4951	HOIMA
70	Ug070	1.4976	31.4949	HOIMA
71	Ug071	1.4977	31.4937	HOIMA
72	Ug072	1.4979	31.4933	HOIMA
73	Ug073	1.4981	31.4933	HOIMA
74	Ug074	1.498	31.4934	HOIMA
75	Ug075		MG3	
76	Ug076	1.4974	31.4953	HOIMA
77	Ug077	1.4977	31.4976	HOIMA
78	Ug078	1.5001	31.4986	HOIMA
79	Ug079	1.4986	31.4982	HOIMA
80	Ug080	1.4989	31.4971	HOIMA
81	Ug081	1.4997	31.499	HOIMA
82	Ug082		marundu	
83	Ug083	2.2956	32.9218	LIRA
84	Ug084	2.2943	32.9214	LIRA
85	Ug085	2.2931	32.9209	LIRA
86	Ug086	2.291	32.9208	LIRA
87	Ug087	2.2935	32.9205	LIRA

88	Ug088	2.2956	32.9191	LIRA
89	Ug089	2.2958	32.9187	LIRA
90	Ug090	2.2958	32.9184	LIRA
91	Ug091	2.2959	32.9182	LIRA
92	Ug092	2.2951	32.9191	LIRA
93	Ug093	2.2963	32.9212	LIRA
94	Ug094	-1.2496	29.9373	KABALE
95	Ug095		Cobra	
96	Ug096		camillo	
97	Ug097	0.4114	33.1909	NJERU
98	Ug098	0.4137	33.1836	NJERU
99	Ug099	0.4176	33.1787	NJERU
100	Ug100	-1.2517	29.9374	KABALE
101	Ug101	0.4217	33.1793	NJERU
102	Ug102	0.4217	33.1793	ARUA
103	Ug103	3.0757	30.9436	ARUA
104	Ug104	0.694	30.3468	FPORTAL
105	Ug105	1.4977	31.4945	HOIMA
106	Ug106	1.4974	31.4949	HOIMA
107	Ug107	-1.252	29.9377	KABALE
108	Ug108	-1.2518	29.9374	KABALE
109	Ug109	1.6488	31.8357	MASINDI
110	Ug110	-0.6083	30.6099	MBARARA
111	Ug111	0.5133	32.6433	WAKISO
112	Ug112	0.524	32.6291	WAKISO
113	Ug113	0.5266	32.6278	WAKISO
114	Ug114	0.5266	32.6278	WAKISO
115	Ug115	1.652	31.8325	MASINDI
116	Ug116	S00 °04.326'	E031 °08.551	WAKISO
117	Ug117	S00 °03.785'	E031 °08.136	WAKISO
118	Ug118	S00 °26.803	E031 °03.967	WAKISO
119	Ug119	S00 °38.832'	E030 °29.445	WAKISO
120	Ug120	S00 °40.820'	E030 °24.024	WAKISO
121	Ug121	S00 °44.253'	E030 °20.793	WAKISO
122	Ug122	S00 °47.247'	E030 °16.244	WAKISO
123	Ug123	S00 °52.820'	E030 °16.230	WAKISO
124	Ug124	S00 °57.213'	E030 °17.759	WAKISO
125	Ug125	S00 °59.533'	E030 °22.050	WAKISO
126	Ug126	S00 °59.224'	E030 °32.591	WAKISO
127	Ug127	S01 °01.701'	E030 °40.720	WAKISO
128	Ug128	S00 °48.813'	E030 °47.758	WAKISO
129	Ug129	S00 °35.828'	E030 °36.455	WAKISO
130	Ug130	S00 °35.123'	E030 °31.433	WAKISO
131	Ug131	S00 °34.753'	E030 °25.007	WAKISO
132	Ug132	S00 °30.398'	E030 °35.998	WAKISO
133	Ug133	S00 °25.742'	E030 °34.265	WAKISO

134	Ug134	S00 °20.298'	E030 °34.470	FPORTAL
135	Ug135	S00 °16.861'	E030 °33.659	FPORTAL
136	Ug136	S00 °11.676'	E030 °31.242	FPORTAL
137	Ug137	S00 °08.024'	E030 °31.377	FPORTAL
138	Ug138	S00 °06.887'	E030 °35.444	FPORTAL
139	Ug139	S00 °05.720'	E030 °40.282	FPORTAL
140	Ug140	S00 °03.859'	E030 °45.044	FPORTAL
141	Ug141	S00 °07.986'	E030 °47.432	FPORTAL
142	Ug142	S00 °12.244'	E030 °42.250	FPORTAL
143	Ug143	S00 °19.511'	E031 °03.407	FPORTAL
144	Ug144	S00 °24.101'	E031 °06.217	FPORTAL
145	Ug145	S00 °22.733'	E031 °14.594	FPORTAL
146	Ug146	S00 °23.577'	E031°20.622	FPORTAL
147	Ug147	S00 °23.684'	E031 °25.848.	FPORTAL
148	Ug148	S00 °22.356'	E031 °310358.	FPORTAL
149	Ug149	S00 °17.921'	E031 °46.958.	FPORTAL
150	Ug150	S00 °14.901'	E031 °49.419.	FPORTAL
151	Ug151	S00 °02.498'	E031 °59.837.	MBARARA
152	Ug152	S00 °04.628'	E032 °07.495.	MBARARA
153	Ug153	N00 °09.722'	E032 °14.025.	MBARARA
154	Ug154	N00 °13.029'	E032 °19.808.	MBARARA
155	Ug155	N02 °31.062'	E034 °39.418.	MBARARA
156	Ug156	N02 °27.845'	E034 °41.433.	MBARARA
157	Ug157	N02 °23.673'	E034 °45.368.	MBARARA
158	Ug158	N02 °23.318'	E034 °51.885.	MBARARA
159	Ug159	N02 °19.776'	E034 °54.775.	MBARARA
160	Ug160	N02 °93.965'	E034 °54.021.	MBARARA
161	Ug161	N01 °56.272'	E034 °52.025.	MBARARA
162	Ug162	N01 °53.745'	E034 °45.471.	MBARARA
163	Ug163	N01°054.851'	E034 °44.035.	MBARARA
164	Ug164	N30 °00.441'	E034 °04.844.	MBARARA
165	Ug165	N03 °54.167'	E033 °50.129.	MBARARA
166	Ug166	N02 °51.392'	E033 °47.146.	MBARARA
167	Ug167	N02 °48.834'	E033 °44.749.	MBARARA
168	Ug168	N02 °38.742'	E033 °39.631.	MBARARA
169	Ug169	N02 °33.254'	E033 °37.883.	ARUA
170	Ug170	N02 °28.014'	E033 °34.813.	ARUA
171	Ug171	N02 °94.521'	E033 °033.925.	ARUA
172	Ug172	N02 °90.246'	E033 °36.569.	ARUA
173	Ug173	N02 °04.219'	E033 °35.760.	ARUA
174	Ug174	N02 °00.483'	E033 °38.433.	ARUA
175	Ug175	N01 °55.570'	E033 °37.975.	ARUA
176	Ug176	N01 °21.830'	E034 °01.668.	ARUA
177	Ug177	N01 °16.280'	E034 °05.117.	ARUA
178	Ug178	N00 °52.748'	E033 °43.212.	ARUA
179	Ug179	N00 °45.779'	E033 °36.821.	ARUA

180	Ug180	N00 °38.415'	E033 °31.824.	ARUA
181	Ug181	N00 °30.223'	E033 °19.126.	ARUA
182	Ug182	N00 °28.857'	E033 °08.375.	ARUA
183	Ug183	N00 °31.332'	E033 °04.484.	HOIMA
184	Ug184	N00 °37.236'	E032 °59.966.	HOIMA
185	Ug185	N00 °40.687'	E033 °52.923.	HOIMA
186	Ug186	N00 °35.524'	E032 °48.537.	HOIMA
187	Ug187	N00 °41.040'	E032 °31.409.	HOIMA
188	Ug188	N02 °18.084'	E032 °11.195.	HOIMA
189	Ug189	N02 °20.751'	E032 °60.561.	HOIMA
190	Ug190	N02 °23.661'	E032 °02.114.	HOIMA
191	Ug191	N02 °30.856'	E031 °54.6921.	HOIMA
192	Ug192	N02 °34.410'	E031 °046.161.	HOIMA
193	Ug193	N02 °34.068'	E031 °38.099.	HOIMA
194	Ug194	N02 °27.220'	E031 °30.620.	HOIMA
195	Ug195	N02 °28.793'	E031 °021.017.	HOIMA
196	Ug196	N02 °30.418'	E031 °25.490.	HOIMA
197	Ug197	N02 °37.777'	E032 °0.1745.	HOIMA
198	Ug198	N02 °37.002'	E032 °06.801.	LIRA
199	Ug199	N02 °39.942'	E032 °13.218.	LIRA
200	Ug200	N02 °44.552'	E032 °15.676.	LIRA
201	Ug201	N02 °46.281'	E032 °13.722.	LIRA
202	Ug202	N02 °50.260'	E032 °90.493.	LIRA
203	Ug203	N02 °50.437'	E032 °06.729.	LIRA
204	Ug204	N02 °47.860'	E032 °51.214.	LIRA
205	Ug205	N02 °42.465'	E031 °51.306.	LIRA
206	Ug206	N02 °36.592'	E031 °56.142.	LIRA
207	Ug207	N02 °25.537'	E031 °93.906.	LIRA
208	Ug208	N02 °29.016'	E031 °05.928.	LIRA
209	Ug209	N03 °04.426'	E031 °56.721.	KABALE
210	Ug210	N03 °04.471'	E030 °56.694.	ARUA
211	Ug211	N02 °56.742'	E030 °57.828.	ARUA
212	Ug212	N02 °51.819'	E030 °59.131.	NJERU
213	Ug213	N02 °43.639'	E031 °04.196.	NJERU
214	Ug214	N02 "37.260	E031 °08.361.	NJERU
215	Ug215	N02 °30.5431'	E031 °05.481.	KABALE
216	Ug216	N02 °28.100'	E031 °03.980.	NJERU
217	Ug217	N01 °24.382'	E032 °16.537.	NJERU
218	Ug218	N01 °22.212'	E032 °21.249.	ARUA
219	Ug219	N01 °19.509'	E032 °25.675.	FPORTAL
220	Ug220	N01 °22.358'	E032 °.28.917	HOIMA
221	Check1			CHECKS
222	Check2			CHECKS
223	Check3			CHECKS
224	Check4			CHECKS

Appendix 2: Means of quantitative traits in study one.

Acession	LL	LS	LW	PH	PS	ST	DM SP	SP
Ug001	26.64	44.27	1.64	92.57	103.19	1.52	6.717	0.9
Ug002	22.44	38.39	1.69	117.27	105.19	1.72	5.717	0.9
Ug003	17.96	28.81	1.57	56.28	106.29	1.48	8.488	0.87
Ug004	18.44	28.49	1.51	50.1	96.47	1.55	9.353	0.82
Ug005	27.64	49.85	1.79	66.27	93.19	1.12	4.059	1.37
Ug006	15.21	25.54	1.65	46.34	107.3	1.84	3.318	1.49
Ug007	18.47	32.22	1.72	75.01	150.13	1.89	4.859	1.24
Ug008	24.44	44.09	1.79	96.27	130.19	1.92	2.445	1.87
Ug009	21.42	35.04	1.61	93.18	156.19	1.82	0.666	3.51
Ug010	23.59	43.76	1.84	124.17	143.24	1.7	0.815	3.53
Ug011	24.27	34.59	1.38	45.75	114.09	1.64	1	4.54
Ug012	24.07	46.56	1.94	88.17	145.63	1.91	0.391	4.21
Ug013	22.02	38.31	1.74	71.44	190.09	1.59	0.76	3.37
Ug014	22.43	33.03	1.44	97.51	115.35	1.58	0.169	4.75
Ug015	25.24	46.44	1.83	126.84	168.19	1.8	0.568	3.52
Ug016	21.66	39.81	1.83	101.74	106.76	1.66	3.717	1.45
Ug017	21.66	39.81	1.83	101.74	117.76	1.66	4.634	1.24
Ug018	15.87	26.31	1.61	87.91	117.76	1.66	1.391	2.44
Ug019	21.34	36.92	1.71	66.27	108.19	1.92	1.391	2.45
Ug020	29.04	52.37	1.79	94.27	117.19	1.62	1.353	2.05
Ug021	31.25	47.69	1.51	111.47	184.99	1.51	1.634	2.24
Ug022	21.79	36.15	1.64	71.07	153.69	1.32	0.965	3.27
Ug023	16.3	24.62	1.44	54.52	141.99	1.29	1.731	2.1
Ug024	25.44	53.09	2.09	66.27	117.19	1.92	0.944	3.12
Ug025	23.14	43.92	1.89	76.27	115.19	2.12	1.58	2.46
Ug026	18.24	29.57	1.59	86.27	109.19	1.52	1.765	2.05
Ug027	17.52	29.13	1.71	115.17	247.29	1.31	4.937	1.12
Ug028	31.97	57.37	1.8	87.67	164.89	1.38	9.353	0.8
Ug029	25.25	56.1	3.01	75.77	139.69	1.3	4.921	1.23
Ug030	35.61	66.94	1.88	47.9	138.19	1.99	7.02	0.95
Ug031	30.79	55.52	1.79	83.82	134.19	1.34	2.536	1.91
Ug032	31.44	41.99	1.3	104.47	139.69	1.49	1.767	2.24
Ug033	32.44	55.08	1.68	106.57	176.99	1.26	1.873	2.26
Ug034	43.15	92.93	2.14	69.11	137.49	2.42	1.362	2.69
Ug035	18.23	28.83	1.55	96.27	107.19	1.29	0.455	4.12
Ug036	31.19	54.03	1.72	86.53	129.19	1.37	0.188	4.26
Ug037	25.4	45.82	1.79	72.57	169.69	1.52	2.241	1.74
Ug038	38.29	55.38	1.43	92.17	117.19	1.37	3.584	0.48
Ug039	18.84	28.79	1.49	73.27	117.19	1.38	2.753	1.37
Ug040	14.37	20.35	1.36	64.27	109.85	1.35	2.953	1.25
Ug041	29.44	48.89	1.64	69.69	151.04	1.25	0.698	3.33

Ug042	36.49	71.64	1.95	87.4	128.09	1.56	4.628	1.26
Ug043	28.26	42.46	1.48	57.33	108.39	1.62	7.252	0.95
Ug044	24.53	44.59	1.82	78.73	133.59	1.42	0.754	3.42
Ug045	26.29	50.67	1.92	53.44	109.94	1.16	2.847	1.6
Ug046	25.13	48.33	1.92	48.65	112.44	1.68	5.652	1.12
Ug047	24.82	37.48	1.45	71.63	128.19	1.3	2.725	1.66
Ug048	15.31	23.52	1.48	63.62	108.19	1.15	3.734	1.32
Ug049	21.08	26.63	1.19	44.84	142.44	1.21	2.158	2.12
Ug050	15.96	37.57	2.38	63.62	111.09	1.18	3.055	1.66
Ug051	16.87	22.82	1.28	63.78	116.2	1.37	3.173	1.52
Ug052	25.83	53.32	2.07	89.42	114.87	1.38	0.711	3.34
Ug053	19.67	36.89	1.88	56.65	131.69	1.46	5.505	1.01
Ug054	24.14	46.44	1.93	48.28	162.49	1.42	0.764	3.65
Ug055	17.61	31.83	1.79	69.37	146.39	1.27	2.561	1.68
Ug056	15.91	27.56	1.7	66.54	136.55	1.33	11.11	0.72
Ug057	19.25	28.11	1.42	52.87	118.12	1.12	2.163	1.83
Ug058	22.5	39.87	1.77	48.86	152.99	1.23	0.684	3.51
Ug059	25.44	41.09	1.59	108.27	197.19	1.12	3.353	1.4
Ug060	24.56	40.28	1.61	99.38	165.52	1.24	3.353	1.4
Ug061	23.58	38.55	1.61	58.42	129.34	1.54	3.551	1.41
Ug062	26.44	60.19	2.29	106.27	112.19	1.42	2.353	2.35
Ug063	16.69	29.81	1.77	36.41	78.5	1.46	2.535	0.79
Ug064	26.84	43.33	1.59	66.27	107.19	1.42	2.901	0.66
Ug065	28.31	49.9	1.72	58.44	123.99	1.54	4.077	1.35
Ug066	28.31	49.9	1.72	58.44	123.99	1.92	0.492	4.26
Ug067	21.94	38.46	1.73	42.05	112.59	1.33	1.478	2.26
Ug068	25.18	38.52	1.5	62.35	123.69	1.3	2.652	1.81
Ug069	16.93	27.89	1.63	61.75	109.19	1.42	1.47	2.57
Ug070	14.89	26.9	1.79	83.27	105.19	1.38	4.027	1.27
Ug071	18.93	31.72	1.63	59.27	114.39	1.24	1.486	2.53
Ug072	18.21	31.66	1.71	44.47	118.19	1.29	3.074	1.64
Ug073	27.44	46.89	1.69	96.27	107.19	1.52	4.117	1.31
Ug074	28.44	45.89	1.59	85.27	109.19	1.62	2.242	1.8
Ug075	28.77	50.16	1.73	62.12	140.48	1.6	1.329	2.48
Ug076	20.45	35.47	1.73	46.43	131.89	1.32	3.009	1.49
Ug077	24.44	37.19	1.49	56.27	104.19	1.52	2.553	1.95
Ug078	21.31	34.32	1.57	50.21	114.49	1.22	0.664	3.82
Ug079	24.44	41.79	1.69	106.27	117.19	1.32	2.639	1.75
Ug080	28.44	45.89	1.59	94.27	112.19	1.42	2.891	1.65
Ug081	18.49	37.52	2.05	81.23	89.47	1.58	6.471	1.9
Ug082	13.43	10.39	1.35	71.63	111.47	1.39	2.906	1.8
Ug083	15.51	28.46	1.95	64.23	130.07	1.26	6.15	1.3
Ug084	10.5	10.54	1.57	24.76	25.77	1.48	4.572	1.6
Ug085	23.86	50.56	2.09	49.53	58.77	1.28	4.217	1.1
Ug086	8.49	1.12	1.25	69.23	64.47	1.38	8.435	1.4
Ug087	11.49	3.22	1.15	90.23	94.47	1.48	10.864	1.8

Ug088	12.49	15.52	1.65	103.23	97.47	1.38	9.864	2.15
Ug089	14.59	36.17	2.35	82.08	115.47	1.37	8.15	1.95
Ug090	16.69	17.84	1.45	101.23	84.47	1.58	10.435	0.8
Ug091	9.17	4.29	1.38	80.23	135.51	1.21	7.158	0.95
Ug092	15.5	15.21	1.42	70.88	114.67	1.42	4.123	0.4
Ug093	13.61	11.72	1.4	111.23	105.47	1.43	3.468	0.72
Ug094	6.67	0.87	1.4	66.03	99.67	1.22	1.874	4.17
Ug095	7.1	4.64	1.56	81.73	135.27	1.13	4.203	1.6
Ug096	14.55	14.42	1.45	98.43	150.87	1.43	4.941	1.8
Ug097	14.55	14.42	1.45	51.69	150.87	1.43	3.404	0.8
Ug098	19.84	28.11	1.62	117.12	175.37	1.35	3.415	0.78
Ug099	13.39	7.83	1.24	82.48	117.77	1.15	3.141	1.16
Ug100	15.49	15.92	1.45	101.23	150.87	1.28	2.864	1.91
Ug101	23.59	37.33	1.6	111.55	88.66	1.85	4.159	0.57
Ug102	9.94	15.62	1.61	54.49	142.31	1.6	3.959	0.67
Ug103	15.87	23.57	1.53	74.93	135.53	1.68	3.222	1.43
Ug104	20.36	33.09	1.64	111.66	78.71	1.57	6.218	0.22
Ug105	12.64	19.63	1.6	67	98.15	1.59	6.218	0.22
Ug106	8.86	16.41	1.79	105.58	136.62	1.8	2.786	2.11
Ug107	21.64	40.81	1.86	111.73	180	1.81	2.606	2.41
Ug108	16.41	29.9	1.8	54.13	126.52	1.8	2.314	2.92
Ug109	23.44	44.5	1.86	81.42	112.58	1.97	2.538	2.71
Ug110	20.24	35.25	1.74	77.43	131.36	1.5	2.849	1.83
Ug111	11.48	18.08	1.62	55.37	100.21	1.92	3.136	1.57
Ug112	18.49	40.44	2.11	68.15	98.06	1.77	4.241	0.9
Ug113	17.24	32.46	1.84	65.04	120.75	1.58	2.527	2.81
Ug114	21.62	42.71	1.94	75.37	107.41	1.64	2.662	2.53
Ug115	17.43	28.48	1.65	79.29	105.26	1.77	2.662	2.53
Ug116	21.27	33.16	1.58	92.12	160.56	1.51	4.539	0.79
Ug117	22.81	43.5	1.87	68.41	72.89	1.66	5.408	0.34
Ug118	13.94	20.81	1.54	119.45	188.06	1.38	5.279	1.07
Ug119	28.84	46.93	1.64	78.75	115.76	1.55	6.489	0.87
Ug120	18.82	25.12	1.4	100.3	102.56	1.44	4.775	0.73
Check1	25.47	42.35	1.64	86.94	165.79	1.11	5.85	1.06
Check2	23.01	32.1	1.35	97.67	142.79	1.05	4.719	1.17
Check3	13.38	21.07	1.53	66.02	115.59	1.38	2.123	2.07
Check4	19.37	26.63	1.33	48.34	125.69	1.15	4.175	1.34
Ug121	19.58	31.12	1.55	68.4	135.49	1.27	12.96	0.66
Ug122	25.46	46.22	1.8	102.57	178.69	1.51	2.494	1.91
Ug123	29.44	47.21	1.58	119.27	163.89	1.26	5.085	1.14
Ug124	22.72	42.03	1.83	84.76	135.38	1.35	0.721	3.79
Ug125	16.79	29.05	1.71	72.63	155.79	1.16	2.394	1.95
Ug126	18.54	28.92	1.52	100.78	194.99	1.52	1.758	2.33
Ug127	29.44	49.45	1.66	82.67	127.19	1.29	2.46	1.91
Ug128	24.3	48.41	1.99	90.02	117.19	1.21	1.434	2.56
Ug129	28.62	51.09	1.77	91.07	118.99	1.2	29.434	2.56

Ug130	28.62	51.09	1.77	91.07	118.99	1.2	3.646	1.31
Ug131	16.79	29.05	1.71	72.63	155.79	1.16	3.646	1.31
Ug132	29.44	47.49	1.59	96.27	109.19	1.22	1.583	2.44
Ug133	28.44	51.29	1.79	76.27	138.19	1.02	3.353	1.35
Ug134	27.14	46.91	1.71	94.27	169.79	1.18	1.181	2.79
Ug135	19.42	30.38	1.53	109.17	202.69	1.15	1.181	2.79
Ug136	19.42	30.38	1.53	109.17	202.69	1.15	1.604	2.26
Ug137	24.08	38.67	1.58	72.39	123.64	1.25	1.906	2.02
Ug138	25.03	43.85	1.74	89.38	134.22	1.48	4.733	1.14
Ug139	16.42	25.72	1.54	26.3	99.36	1.27	9.58	0.48
Ug140	24.08	38.67	1.58	72.39	123.64	1.25	1.906	2.02
Ug141	24.44	43.63	1.77	66.7	98.2	1.47	6.21	1.05
Ug142	23.9	42.75	1.78	95.64	128.47	1.36	2.127	1.9
Ug143	24.08	38.67	1.58	72.39	123.64	1.25	1.906	2.02
Ug144	20.94	29.29	1.34	62.47	125.91	1.28	2.112	2.09
Ug145	26.95	51	1.88	75.36	115.79	1.25	1.381	2.52
Ug146	21.45	35.7	1.64	77.38	114.92	1.11	0.782	3.75
Ug147	16.14	29.33	1.8	48.17	99.17	1.25	0.782	3.75
Ug148	24.66	44.48	1.78	72	121.8	1.38	1.137	2.99
Ug149	25.22	41.44	1.62	103.87	187.59	1.24	3.96	1.24
Ug150	19.28	34.26	1.76	117.27	105.57	1.45	3.96	1.24
Ug151	22.6	34.77	1.5	128.27	144.16	1.31	2.517	1.63
Ug152	18.08	29.35	1.59	45	111.48	1.35	1.84	2.14
Ug153	19.28	34.26	1.76	76.18	105.57	1.45	3.96	1.24
Ug154	22.6	34.77	1.5	75.02	144.16	1.31	2.517	1.63
Ug155	51.41	117.26	2.28	93.66	120.94	1.66	6.813	0.98
Ug156	25.44	40.74	1.58	60.07	136.28	1.28	3.353	1.4
Ug157	31.49	62.18	2	125.1	139.01	1.41	3.269	1.72
Ug158	20.58	40.29	1.91	79.51	81.89	1.64	5.5	0.59
Ug159	23.9	40.8	1.65	78.35	120.48	1.5	4.057	0.98
Ug160	29.67	52.33	1.76	118	157.62	1.4	3.013	1.61
Ug161	20.58	40.29	1.91	79.51	81.89	1.64	5.5	0.59
Ug162	23.9	40.8	1.65	78.35	120.48	1.5	4.057	0.98
Ug163	33.24	55.12	1.66	79.72	104.51	1.75	10.893	0.91
Ug164	23.22	48.6	2.09	62.84	77.01	1.22	7.987	0.29
Ug165	20.58	40.29	1.91	79.51	81.89	1.64	5.5	0.59
Ug166	23.9	40.8	1.65	78.35	120.48	1.5	4.057	0.98
Ug167	23.9	40.8	1.65	78.35	120.48	1.5	4.6	0.86
Ug168	16.54	28.88	1.6	161.1	159.21	1.33	2.579	2.28
Ug169	22.74	44.72	1.94	122.6	114.51	1.44	3.996	1.01
Ug170	25.94	49.39	1.89	92.84	134.61	1.37	3.604	1.36
Ug171	29.74	62.72	2.14	99.6	86.51	1.91	3.318	1.7
Ug172	24.47	48.02	1.95	57	94.91	1.38	10.893	0.9
Ug173	23.28	43.66	1.84	77.1	94.51	1.25	4.412	0.2
Ug174	20.49	40.94	1.96	95.7	114.41	1.27	5.062	0.47
Ug175	23.28	43.66	1.84	77.1	108.51	1.25	3.893	0.72

Ug176	23.28	43.66	1.84	77.1	104.51	1.25	2.418	0.88
Ug177	23.39	39.12	1.61	95.24	122.21	1.87	4.875	1.86
Ug178	22.21	40.41	1.74	96.49	93.14	1.91	3.181	1.7
Ug179	22.21	40.41	1.74	106.49	93.14	1.89	2.781	1.7
Ug180	22.21	40.41	1.74	116.49	93.14	2.21	2.581	1.7
Ug181	22.21	40.41	1.74	86.49	93.14	1.89	6.125	1.7
Ug182	17.01	32.87	1.83	97.37	145.65	2.07	1.019	3.92
Ug183	34.01	87.04	2.64	74.9	79.05	2.36	1.019	3.92
Ug184	29.21	57.87	1.99	87.02	130.52	2.01	1.97	2.73
Ug185	24.21	39.91	1.59	67.85	126.64	2.15	3.141	2.3
Ug186	28.94	51.18	1.76	101.35	117.5	1.84	0.483	4.77
Ug187	24.22	45.16	1.82	108.12	111.14	1.97	4.293	1.91
Ug188	32.37	107.58	3.47	92.92	123.77	2.34	1.481	3.05
Ug189	24.68	45.1	1.76	73.05	110.92	1.72	4	2.04
Ug190	23.87	43.7	1.79	69.45	126.85	1.97	5.299	1.79
Ug191	19.53	37.35	1.82	88.12	137.47	2.01	1.101	3.45
Ug192	26.43	46.85	1.72	75.82	110.87	2.01	1.201	1.35
Ug193	21.73	46.75	2.12	94.82	133.27	1.91	0.971	1.35
Ug194	24.83	53.05	2.22	93.02	123.07	1.91	2.001	2.15
Ug195	25.93	57.75	2.32	96.32	120.97	1.91	2.791	3.25
Ug196	27.03	62.55	2.52	99.72	118.87	1.81	1.881	4.25
Ug197	24.73	40.65	1.64	130.47	115.26	1.66	7.081	0.58
Ug198	26.06	43.23	1.66	124.07	198.06	1.12	2.906	1.77
Ug199	14.21	20.82	1.54	94.77	181.16	0.99	0.484	0.27
Ug200	20.88	32.31	1.54	217.17	204.66	1.18	7.091	0.6
Ug201	16.92	26.07	1.54	109.47	165.76	1.19	2.437	0.83
Ug202	18.13	33.19	1.84	109.47	109.26	1.26	3.037	0.93
Ug203	24.43	32.61	1.34	114.47	119.26	1.56	0.852	4.33
Ug204	22.33	28.52	1.34	99.47	99.26	1.06	2.362	0.77
Ug205	17.33	19.07	1.14	109.47	109.26	0.96	1.641	2.33
Ug206	23.33	27.61	1.24	94.77	94.76	0.86	0.805	4.87
Ug207	20.53	22.68	1.14	129.47	109.26	0.72	2.362	0.77
Ug208	12.19	11.9	1.04	109.47	125.26	0.58	4.416	0.94
Ug209	26.67	26.32	1.04	119.47	100.26	0.44	2.647	1.13
Ug210	37.15	34.86	0.94	69.47	94.26	1.56	2.454	2.33
Ug211	24.63	46.09	1.84	109.47	109.26	1.16	1.584	1.47
Ug212	22.11	39.73	1.84	107.47	125.26	2.36	1.234	2.33
Ug213	26.59	18.96	0.74	118.47	112.26	2.06	2.454	2.33
Ug214	21.59	35.79	1.64	109.47	114.26	1.06	3.461	1.33
Ug215	24.11	38.47	1.64	119.47	109.26	1.26	4.235	1.13
Ug216	26.63	40.82	1.54	110.47	109.26	1.56	3.875	0.66
Ug217	17.3	29.14	1.63	74.65	113.1	1.52	2.51	2.04
Ug218	27.91	54.09	1.87	70.84	121.54	2.2	1.813	2.71
Ug219	26.72	52.71	1.96	91.4	113.05	1.6	3.183	1.64
Ug220	25.13	44.68	1.75	106.12	116.81	1.69	0.4917	1.15

Appendix 3:Quality of markers when grouped against across the chromosomes.

Chromosome number	No of filtered. SNPs	MAF	GD	PIC	Ho	He	PiPerBP	Theta PerBP	Tajima D
1	3112	0.18	0.42	0.37	0.25	0.31	0.28	0.16	2.2
2	2702	0.17	0.41	0.36	0.25	0.32	0.27	0.16	1.96
3	1365	0.17	0.40	0.36	0.21	0.29	0.25	0.16	1.73
4	2458	0.17	0.41	0.36	0.24	0.29	0.27	0.16	2.05
5	2559	0.17	0.41	0.37	0.23	0.30	0.27	0.16	2.09
6	2240	0.17	0.41	0.36	0.22	0.31	0.26	0.16	1.94
7	1667	0.17	0.41	0.36	0.25	0.30	0.27	0.16	2.03
8	1811	0.17	0.41	0.37	0.20	0.29	0.27	0.16	2.02
9	1754	0.17	0.40	0.36	0.21	0.29	0.26	0.16	1.83
Total / Average	19,668	0.17	0.41	0.36	0.23	0.30	0.27	0.16	1.98

Appendix 4: Means of Genotypes across Locations and cuts

Accession	ADFdm	ADLdm	ASH	CPdm	DM	IVOMD	ME	NDFdm	N
Ug001	35.8	1.4	21.4	14.0	91.3	58.7	7.8	59.3	2.2
Ug002	34.9	1.6	18.1	13.1	91.6	58.0	7.9	61.9	2.1
Ug003	34.9	1.8	17.7	13.4	91.5	58.9	7.9	62.3	2.1
Ug005	35.0	2.1	18.9	11.8	91.8	58.1	7.6	64.9	1.9
Ug006	33.2	1.5	16.2	14.1	91.7	59.1	8.0	59.7	2.3
Ug008	33.1	1.3	19.9	14.9	91.4	60.7	8.0	60.0	2.4
Ug009	35.9	1.9	18.7	12.8	91.6	58.3	7.9	62.0	2.0
Ug011	34.9	1.9	17.1	13.6	91.6	58.7	8.0	62.2	2.2
Ug012	35.5	1.8	17.9	13.3	91.6	58.2	7.8	61.6	2.1
Ug013	35.1	1.8	18.6	12.7	91.8	57.9	7.8	60.2	2.0
Ug014	35.0	1.6	16.9	13.2	91.6	58.2	7.9	63.0	2.1
Ug015	35.6	2.0	16.5	13.5	91.9	57.6	7.8	62.1	2.2
Ug016	35.1	1.9	18.0	13.5	91.7	59.0	7.9	61.9	2.2
Ug017	36.9	1.5	21.2	12.9	91.6	58.3	7.8	59.4	2.1
Ug018	35.8	1.7	19.6	13.9	91.7	58.1	7.7	59.4	2.2
Ug020	35.7	1.8	17.3	13.5	91.6	58.5	7.9	62.8	2.2
Ug021	35.9	1.6	19.5	13.5	91.6	58.7	7.9	60.7	2.2
Ug023	36.2	1.3	21.4	12.6	91.6	58.3	7.8	59.1	2.0
Ug025	35.2	1.7	17.5	13.0	91.5	58.4	7.9	62.3	2.1
Ug026	35.1	1.6	17.8	13.5	91.5	58.6	7.9	62.6	2.2
Ug027	36.2	1.6	18.4	12.9	91.6	58.3	7.9	62.2	2.1
Ug028	35.9	1.7	19.7	12.5	91.5	58.1	7.8	60.3	2.0
Ug030	33.9	1.5	16.6	14.0	91.2	59.9	8.1	63.4	2.2
Ug031	35.3	1.6	17.5	13.3	91.6	58.4	7.8	62.0	2.1
Ug032	33.8	1.4	18.5	14.3	91.4	60.3	8.1	61.3	2.3
Ug033	36.2	1.3	21.5	13.6	91.7	58.7	7.8	60.5	2.2
Ug034	34.6	1.7	20.3	14.1	91.4	59.3	8.0	60.2	2.3
Ug037	35.2	1.5	18.7	13.3	91.6	59.1	8.0	61.8	2.1
Ug038	38.0	1.7	20.3	12.5	91.7	57.6	7.8	61.9	2.0
Ug040	35.6	1.6	17.9	13.1	91.6	58.5	7.8	63.2	2.1
Ug041	35.9	1.7	18.3	13.0	91.7	58.3	7.8	62.1	2.1
Ug042	35.3	1.5	17.8	13.6	91.6	58.7	7.9	63.2	2.2
Ug043	35.0	1.3	19.7	13.6	91.5	59.3	8.0	61.0	2.2
Ug044	33.7	1.8	18.1	14.7	91.6	60.1	8.1	59.7	2.3
Ug045	35.9	1.8	18.8	13.1	91.6	58.0	7.8	61.0	2.1
Ug046	37.0	1.7	20.5	12.8	91.6	57.7	7.8	60.6	2.0
Ug047	36.4	1.5	22.4	13.9	91.7	58.9	7.8	59.4	2.2
Ug048	34.4	1.5	19.8	14.6	91.5	59.6	7.9	59.3	2.3
Ug049	34.4	1.4	20.1	14.1	91.4	59.6	8.0	60.4	2.3
Ug050	37.9	1.7	20.0	12.6	91.9	58.1	7.8	61.4	2.0
Ug052	35.5	1.5	18.5	12.8	91.6	58.6	7.8	62.1	2.1
Ug054	35.8	1.8	19.1	13.4	91.6	58.2	7.8	61.5	2.1
Ug055	36.2	1.9	19.2	12.8	91.7	58.1	7.8	60.9	2.0

Ug056	36.3	1.7	19.7	12.6	91.6	58.3	7.8	61.1	2.0
Ug057	33.5	1.3	16.9	15.0	91.3	59.7	8.1	63.0	2.4
Ug058	36.4	1.7	18.7	13.6	91.5	57.9	7.8	63.1	2.2
Ug059	33.8	1.6	16.6	14.1	91.5	58.9	7.9	61.5	2.2
Ug060	34.9	1.9	17.8	13.0	91.6	58.5	7.9	60.9	2.1
Ug061	33.4	1.5	16.7	14.0	91.3	59.2	8.0	62.9	2.2
Ug062	35.0	1.8	17.6	13.2	91.7	58.0	7.8	61.5	2.1
Ug064	35.6	1.7	18.4	13.3	91.6	59.2	8.0	62.2	2.1
Ug065	36.4	1.5	19.7	13.3	91.7	58.1	7.8	61.7	2.1
Ug067	36.9	1.5	20.6	13.0	91.6	58.2	7.8	59.8	2.1
Ug068	36.2	1.3	19.8	12.6	91.6	58.2	7.8	61.3	2.0
Ug069	36.2	1.8	19.2	13.5	91.7	58.0	7.7	62.0	2.2
Ug071	36.3	1.5	20.2	12.9	91.6	58.9	7.9	60.9	2.1
Ug072	36.3	1.7	18.7	12.3	91.6	57.9	7.8	61.7	2.0
Ug073	35.1	1.5	20.0	13.9	91.6	59.0	7.9	60.5	2.2
Ug075	34.6	1.7	18.7	13.2	91.5	58.8	7.9	60.2	2.1
Ug077	37.5	2.1	19.9	13.8	91.4	58.0	7.8	57.7	2.2
Ug078	35.8	1.4	19.9	13.7	91.7	58.7	7.8	62.3	2.2
Ug079	34.3	1.6	18.7	14.4	91.4	59.8	8.0	62.2	2.3
Ug080	36.6	1.5	23.0	14.1	91.4	59.3	7.8	59.1	2.3
Ug081	35.1	1.6	18.2	13.6	91.7	58.7	7.8	61.6	2.2
Ug083	36.1	1.9	18.3	13.3	91.7	57.3	7.8	60.3	2.1
Ug084	38.6	1.3	19.2	13.2	92.0	59.3	7.8	57.8	2.1
Ug086	34.9	1.6	17.9	13.3	91.6	58.8	7.9	61.7	2.1
Ug088	34.8	1.6	23.0	12.0	92.0	58.9	7.7	58.0	1.9
Ug089	36.2	1.8	17.4	13.1	91.6	57.9	7.8	63.5	2.1
Ug090	35.9	1.8	17.8	13.3	91.5	58.4	7.9	61.7	2.1
Ug091	33.7	1.9	17.3	14.3	91.5	59.3	8.0	60.4	2.3
Ug093	35.4	1.9	17.1	13.3	91.4	58.3	7.9	63.0	2.1
Ug094	36.2	2.1	16.2	13.4	91.7	57.6	7.8	63.0	2.1
Ug095	36.1	1.6	21.2	13.7	91.7	58.5	7.7	60.2	2.2
Ug095	34.7	1.7	21.6	13.9	91.6	59.9	7.9	60.4	2.2
Ug096	35.4	1.6	19.1	13.6	91.6	58.4	7.7	61.0	2.2
Ug097	35.4	1.7	18.2	13.4	91.6	58.8	7.9	62.6	2.1
Ug098	37.7	2.0	19.5	12.4	91.6	56.8	7.6	60.6	2.0
Ug099	35.2	1.7	23.0	14.0	91.6	61.3	7.9	61.5	1.9
Ug102	36.7	1.6	20.9	13.4	91.7	57.7	7.7	58.4	2.1
Ug104	33.7	1.4	19.7	14.8	91.3	60.2	8.0	60.1	2.4
Ug105	35.1	1.8	16.7	13.3	91.4	58.3	7.9	62.4	2.1
Ug106	34.0	1.8	17.4	13.3	91.4	58.8	7.9	61.5	2.1
Ug107	36.2	2.0	18.9	13.2	91.7	57.6	7.7	60.9	2.1
Ug108	37.9	1.5	23.0	12.6	91.8	58.1	7.6	58.7	2.0
Ug111	35.5	1.7	20.2	13.7	91.7	58.4	7.8	59.6	2.2
Ug112	35.1	1.6	19.5	13.6	91.6	58.9	7.9	59.9	2.2
Ug113	36.9	2.1	18.0	11.8	91.8	57.3	7.8	63.1	1.9
Ug114	35.8	1.6	16.8	14.7	91.1	60.5	8.1	60.4	2.4

Ug115	35.7	2.0	18.0	13.9	91.5	58.2	7.9	61.2	2.2
Ug118	36.4	1.7	18.6	13.1	91.6	57.8	7.7	62.1	2.1
Ug119	35.3	1.7	17.7	13.6	91.5	58.5	7.9	61.6	2.2
Ug120	35.2	1.8	18.1	13.7	91.6	58.6	7.9	59.9	2.2
Ug123	36.0	1.8	19.4	14.5	91.6	59.1	7.9	61.0	2.3
Ug124	34.9	1.6	17.6	13.4	91.6	58.5	7.8	62.5	2.1
Ug126	36.4	1.5	20.8	12.7	91.6	59.0	7.9	60.6	2.0
Ug129	35.4	1.8	16.4	13.1	91.5	59.0	7.9	63.5	2.1
Ug130	35.7	1.8	18.9	13.3	91.7	58.6	7.9	61.1	2.1
Ug132	35.3	2.0	16.2	13.1	91.6	58.4	7.9	63.5	2.1
Ug134	38.0	2.0	19.0	11.7	92.0	58.0	7.9	62.8	1.9
Ug135	35.7	1.8	18.1	13.3	91.7	58.3	7.8	61.5	2.1
Ug136	35.0	1.6	19.4	13.6	91.4	59.5	8.0	60.5	2.2
Ug137	34.2	1.7	18.1	13.9	91.5	59.3	8.0	62.3	2.2
Ug138	36.2	1.4	20.6	14.4	91.6	59.5	7.9	59.3	2.3
Ug139	36.9	1.8	20.6	12.9	91.5	58.3	7.7	58.7	2.1
Ug140	33.2	1.4	19.0	15.5	91.5	60.4	8.1	60.4	2.5
Ug144	34.3	1.6	18.2	13.9	91.3	59.7	8.0	62.9	2.2
Ug145	37.4	1.9	18.9	12.8	91.8	57.8	7.9	60.2	2.1
Ug147	35.2	1.6	18.3	13.3	91.6	58.4	7.8	61.2	2.1
Ug150	34.7	1.5	20.1	14.2	91.4	59.8	7.9	60.9	2.3
Ug151	35.6	1.7	18.0	12.3	91.7	58.2	7.8	62.9	2.0
Ug152	35.0	1.8	16.7	12.9	91.6	58.6	7.9	62.3	2.1
Ug153	36.1	1.9	18.1	13.6	91.7	57.6	7.7	62.2	2.2
Ug154	37.5	1.2	23.0	13.0	91.9	58.5	7.7	59.3	2.1
Ug155	36.6	1.6	19.4	12.6	91.7	58.2	7.8	61.4	2.0
Ug156	35.7	1.9	16.1	13.1	91.5	57.7	7.9	62.3	2.1
Ug158	37.4	1.9	21.8	13.2	91.8	57.7	7.6	60.6	2.1
Ug159	36.3	1.7	18.4	12.5	91.7	58.0	7.9	63.0	2.0
Ug160	34.4	1.4	17.3	14.0	91.7	58.9	7.9	62.8	2.2
Ug162	35.0	1.5	18.5	13.7	91.3	59.7	8.0	61.1	2.2
Ug163	36.8	1.6	20.0	12.5	91.6	58.2	7.8	60.8	2.0
Ug164	35.5	1.7	19.2	12.3	91.8	57.9	7.8	60.4	2.0
Ug166	34.8	1.7	17.8	13.5	91.5	58.3	7.9	60.4	2.2
Ug167	36.0	1.8	19.6	12.6	91.5	58.3	7.8	60.7	2.0
Ug168	35.4	1.9	16.1	13.0	91.6	57.9	7.9	63.8	2.1
Ug169	34.2	2.0	16.7	14.1	91.5	58.7	7.9	61.0	2.3
Ug170	36.2	1.8	19.1	12.9	91.7	58.0	7.8	60.5	2.1
Ug174	36.5	2.1	18.3	12.9	91.5	57.4	7.7	61.6	2.1
Ug175	35.1	1.4	19.2	13.5	91.7	59.8	8.0	61.1	2.2
Ug178	35.1	1.7	18.8	13.4	91.6	58.7	7.9	61.4	2.1
Ug179	34.0	1.7	17.7	13.9	91.4	59.5	8.0	62.2	2.2
Ug180	35.9	1.9	19.7	12.9	91.6	58.1	7.9	61.6	2.1
Ug182	33.9	1.9	15.3	14.1	91.7	59.0	8.0	61.1	2.3
Ug183	35.2	1.4	21.3	13.7	91.5	59.5	8.0	60.0	2.2
Ug184	33.9	1.6	17.7	14.0	91.5	59.6	8.0	61.8	2.2

Ug185	38.0	1.5	21.7	12.0	92.0	57.7	7.7	60.4	1.9
Ug187	37.5	1.5	22.3	12.9	91.8	58.9	7.8	59.6	2.1
Ug188	34.7	1.7	17.0	13.5	91.7	58.3	7.8	61.5	2.2
Ug189	34.8	1.4	18.3	13.4	91.4	59.7	8.0	60.0	2.1
Ug190	35.3	1.7	18.0	12.9	91.5	58.5	7.9	62.0	2.1
Ug191	35.3	1.7	22.7	11.9	92.3	57.3	8.0	59.9	1.9
Ug192	34.4	1.6	15.4	12.1	91.6	58.2	7.9	63.7	1.9
Ug193	36.7	1.9	17.8	12.2	91.8	57.3	7.8	61.5	2.0
Ug194	37.8	2.1	16.9	12.2	91.8	57.2	7.6	63.8	1.9
Ug195	34.9	1.6	16.9	13.2	91.5	58.3	7.9	63.7	2.1
Ug196	34.9	1.6	18.5	13.7	91.6	58.2	7.9	61.1	2.2
Ug197	36.9	1.7	22.7	11.8	92.4	58.5	7.9	60.9	1.9
Ug198	35.0	1.8	17.4	13.2	91.4	58.5	7.9	61.5	2.1
Ug199	33.9	1.5	17.6	13.6	91.5	59.0	8.0	61.2	2.2
Ug200	33.7	1.3	18.5	13.3	91.9	58.4	7.9	58.8	2.1
Ug201	34.9	1.7	17.8	12.3	91.5	58.2	7.9	61.5	2.0
Ug202	35.4	1.7	18.7	13.8	91.6	59.5	7.9	62.1	2.2
Ug203	35.6	1.7	16.3	12.1	91.8	58.0	7.8	63.7	1.9
Ug204	36.1	1.7	16.7	11.8	92.0	56.8	7.7	63.8	1.9
Ug205	36.0	1.7	21.4	13.3	91.6	58.7	7.8	58.5	2.1
Ug206	34.7	1.9	16.3	13.4	91.5	58.9	8.0	62.1	2.2
Ug207	36.3	1.8	21.2	12.7	91.5	57.9	7.7	59.0	2.0
Ug208	35.1	1.7	18.7	12.9	91.6	58.2	7.8	60.3	2.1
Ug209	34.8	1.6	19.3	14.3	92.0	58.9	7.9	60.4	2.3