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Review

Opportunities for Napier Grass (*Pennisetum purpureum*) Improvement Using Molecular Genetics

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Abstract: Napier grass (*Pennisetum purpureum* Schumach.) is a fast-growing perennial grass native to Sub-Saharan Africa that is widely grown across the tropical and subtropical regions of the world. It is a multipurpose forage crop, primarily used to feed cattle in cut and carry feeding systems. Characterization and diversity studies on a small collection of Napier grasses have identified a moderate level of genetic variation and highlighted the availability of some good agronomic traits, particularly high biomass production, as a forage crop. However, very little information exists on precise phenotyping, genotyping and the application of molecular technologies to Napier grass improvement using modern genomic tools which have been applied in advancing the selection and breeding of important food crops. In this review paper, existing information on genetic resources, molecular diversity, yield and nutritional quality of Napier grass will be discussed. Recent findings on characterizing disease resistance and abiotic stress (drought) tolerance will also be highlighted. Finally, opportunities and future prospects for better conservation and use arising from the application of modern genomic tools in Napier grass phenotyping and genotyping will be discussed.

Keywords: Napier grass; elephant grass; Uganda grass; *Pennisetum purpureum*; diversity analysis; characterization; phenotype; genotype

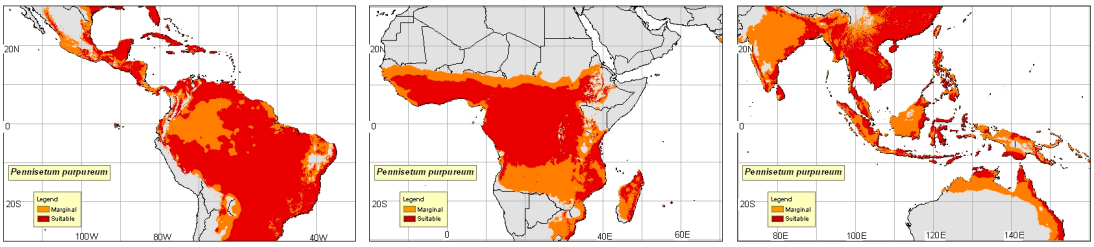
1. Introduction

Sustainable livestock production is highly dependent on the availability of quality feed and forage resources. Napier grass, also known as elephant or Uganda grass, is one of the most important tropical forage crops. It is widely used in cut and carry feeding systems [1–3] and is of growing importance in other agricultural systems. Napier grass possesses many desirable characteristics, including high yield per unit area, tolerance to intermittent drought and high water use efficiency [2], making it a forage of choice. It has the ability to withstand repeated cutting and will rapidly regenerate, producing palatable leafy shoots [4]. Consequently, enhancing the knowledge-based use and conservation of the available Napier grass resources promises to substantially benefit livestock value chains.

1.1. Origin, Propagation and Distribution

Napier grass is a monocotyledonous flowering plant belonging to the family Poaceae (the family of grasses) and the genus *Pennisetum* [5,6]. *Pennisetum* is a highly diverse genus consisting of a heterogeneous group of approximately 140 species [7–9] with different basic chromosome numbers of 5, 7, 8 or 9, a range of ploidy levels from diploid to octoploid, both sexual and apomictic reproductive

behaviours and life cycles of an annual, biennial or perennial nature [10]. Napier grass is a perennial C4 grass species [11,12] native to Sub-Saharan Africa from where it is believed to have been distributed to other tropical and subtropical regions around the world. It has been reported to be adapted to grow across a wide range of soil conditions and agro-ecologies, from sea level to 2100 m, and it can withstand minor dry spells, although it grows best in areas where the annual rainfall is between 750 and 2500 mm [6]. Given its wide agro-ecological adaptation, Napier grass has been naturalized in areas of Central and South America, tropical parts of Asia, Australia, the Middle East and the Pacific islands [6,13]. As a result, today it is widely grown in tropical and subtropical regions of the world, for use predominantly as animal fodder (Figure 1). Napier grass can be more commonly distributed by vegetative cuttings and tillers [6], since the grass cannot produce many seeds and those that are produced are normally very small, light, of poor quality and the spikelets are prone to shattering [6,14]. Consequently, the seeds are considered inappropriate for propagation as they produce weak seedlings and, as Napier grass is an open pollinated crop, the seedlings are also highly heterozygous [6,14]. Therefore, propagation by stem cuttings is currently the dominant practice for the distribution of Napier grass propagules [6,15].



Caribbean, Central and Southern America regions

Africa

Melanesia, Southeast Asia and surrounding regions

Figure 1. Regional distribution of Napier grass around the world (reproduced from [13]).

1.2. Economic Importance

A range of grass species are used as fodder crops by farmers in Africa, Asia and other tropical/subtropical regions of the world. Napier grass is one of the most important fodder crops, particularly in Eastern and Central African smallholder farming communities [1,2]. It is mainly used to feed livestock in cut and carry feeding systems [3,16,17]. It is a multipurpose forage crop that can be grazed directly, or made into silage or hay [18] and there have also been reports of using Napier grass as fish food, for example for feeding grass carp and tilapia in Nepal [19,20] and Bangladesh [21]. A recent report from Nigeria also indicated that young shoots of Napier grass were used as a cooked vegetable [22]. These varied uses provide an indication of the diversity of roles that Napier grass could contribute to the reduction of poverty and nutritional insecurity.

In addition to its value as a forage crop, Napier grass can also be used to make fences, as a windbreak, to demarcate boundaries among neighbouring farmers, and the dried material can be used as a fuel source [18]. In crop land management systems, it is used as a mulch to control weed infestation and soil erosion [2] and as a trap plant in the push–pull strategy, a pest management practice which uses repellent intercrop 'push' plants and attractant trap 'pull' plants [23] for insect pest control in Africa, particularly for the maize stem borer [24,25]. Plants are also used to scavenge pollutants, such as heavy metals, and Napier grass has been used in phytoremediation strategies, for example for the cleanup of cadmium-affected soil, reducing the concentration of cadmium to a depth of 15 cm in soil [17].

With growing global interest in reducing fossil fuel consumption and concerns about the impacts of climate change, the search for alternative biofuel sources has led to the promotion of large biomass plants as second- or next-generation biofuel crops. Napier grass, with its perennial nature

and fast growing characteristics, has been reported to produce a dry matter (DM) yield of up to 78 tons/ha/annum (35–41 tons/ha average) [26]. Rengsirikul et al. [27] estimated a maximum ethanol production of 350–460 L/ton DM from Napier grass varieties grown in Thailand, and an estimated ethanol yield of 329 L/ton DM. Lima et al. [28] demonstrated that this potential was 6% and 15% higher than for the tropical forages *Brachiaria brizantha* and *Panicum maximum*, respectively, around 15% higher than *Eucalyptus* bark and 17% higher than for sugarcane. Consequently, the potential exists for the use of Napier grass for phytoremediation purposes, after which the large harvest could go into processing plants for biofuel production.

1.3. Genetic Resources, Molecular Diversity and Breeding

Napier grass is considered to be a socio-economically important tropical grass species and is therefore available across most of the tropical and subtropical regions of the world. As reviewed in Sanghu et al. [5], a number of genebanks (for example: the International Center for Tropical Agriculture (CIAT); the Commonwealth Scientific and Industrial Research Organisation (CSIRO); the International Livestock Research Institute (ILRI); and the National Bureau of Plant Genetic Resources (NBPGR)) are involved in conserving a substantial amount of tropical and sub-tropical forage genetic resources. Through early exploration, Napier grass germplasm has been collected from various geographical regions and is conserved by these different institutions [5,29,30]. Consequently, over 300 accessions of Napier grass are currently being maintained in different genebanks (Table 1). Genetic resources form an essential component of agriculture and livestock production value chains where in-depth knowledge of the existing resources is required. Accurate passport, characterization and evaluation data, together with an overall understanding of the diversity of the genetic resources, are considered the primary reasons for the conservation and use of available genetic resources [5]. For example, a broad array of Napier grass accessions are currently being maintained by the ILRI forage genebank in the field at Debre Zeit and Zwai, Ethiopia with considerable diversity in growth and form (Figure 2). However, germplasm available from genebanks has so far been largely underutilized [5].

Napier grass is a cross-pollinating allotetraploid species with a chromosome number of 2n = 4x = 28 (genome A'A'BB) [11,31,32]. Although there is no clear information on the genetic origin of allotetraploidy in Napier grass, the A'A' genome has been reported to be homologous to the AA genome of pearl millet (*Pennisetum glaucum* (L.)) and the A' chromosomes are larger than the B chromosomes, which contribute genes controlling the perennial growth habit [31]. To date, Napier grass 'improvement' has mainly been based on the evaluation and selection of existing accessions for traits of interest. For example, accessions were screened for resistance to diseases, and Napier grass head smut- and stunt-resistant lines were identified from the existing collections [33,34]. Plant breeding and selection in Napier grass has primarily been aimed at improving different agronomic traits such as disease resistance, yield, nutritional quality, growth habit (dwarfing), palatability and abiotic stress tolerance [6,11,35]. Napier grass is cross-compatible with the closely related species pearl millet (*Pennisetum glaucum*) (2n = 2x = 14, genome AA) [6,15]; the resultant hybrids are triploid and sterile [6] and can only be propagated by vegetative means which, although labour intensive, ensure a true-to-type variety [15]. A number of agronomically important traits, nutritional quality and palatability for example, have been introgressed into the genome of Napier grass from pearl millet through conventional plant breeding [29] and hybrids have become a crucial part of the forage crop value chain in Africa, Asia and South America [6,36].

Table 1. Napier grass distribution and accessions in various genebanks around the world.

Native to *:	Number of Accessions at **:						
	ILRI	ICRISAT	CIAT	EMBRAPA [1]	USDA GRIN [2]	RBG [3]	Total ***
Tanzania	6	9					15
Uganda					1		1
Ethiopia	1				12 [c]		13
Malawi	1				2	1	4
Mozambique	2						2
Zimbabwe	11	5			8		24
Côte d'Ivoire					1		1
Nigeria	1				1	3	5
Cameroon		8	1				9
Sub-total	22	22	1	0	25	4	74
Collected from							
Australia					4		4
Brazil	8			39	7		54
Burkina Faso						1	1
Burundi	1						1
Central African R.		7				2	9
China					1		1
Colombia	1			5			6
Costa Rica	1			1	1		3
Cuba	2			4			6
DRC (Zaire)					4		4
Ecuador						1	1
India	2	8		3	2		15
Mexico					2		2
Namibia	1						1
Panama				1			1
South Africa					12		12
Sudan		2					2
Swaziland	6				3		9
USA	16 [a]			1	6		23
Unknown	14 [b]		2	29	44		90
Sub-total	52	17	2	83	86	4	244
Total	74	39	3	83	111	8	318

[a] Breeding lines; [b] Includes 2 cultivars 'Mott' (=PI517947) and 'Kizizi'. ILRI14983 may = PI667853; [c] 11 duplicates of ILRI accessions, not actually from Ethiopia, and none of the 12 are available; * Native distribution taken from [13]; ** Number of accessions from the forage registry, except for Brazil, United States Department of Agriculture (USDA) Germplasm Resources Information Network (GRIN), ICRISAT and RBG (Genesys [37]); *** Some of the accessions listed here are in fact duplicated between the collections (for example 20 of the ILRI collection are part of the Brazilian collection); [1] Brazilian Agricultural Research Corporation (EMBRAPA, *Empresa Brasileira de Pesquisa Agropecuária*); [2] Most USDA accessions not available; [3] Millennium Seedbank, Royal Botanic Gardens (RBG), Wakehurst Place, UK. ILRI: International Livestock Research Institute; CIAT: International Center for Tropical Agriculture.

Figure 2. Partial view of the Napier grass field genebank in Debre Zeit (Ethiopia).

Since the early 1990s there have been a number of reports characterizing the genetic diversity of Napier grass. Tcacenco and Lance [38] evaluated the usefulness of morphological characteristics for the characterization of Napier grass and were able to differentiate nine accessions based on leaf, stem and inflorescence characteristics. Van De Wouw et al. [39] also studied a collection of Napier grass using morphological and agronomic characters where the collection was clustered into six groups. Smith and co-workers [40] were the first group to develop and use molecular restriction fragment length polymorphism (RFLP) and random amplification of polymorphic DNA (RAPD), markers in Napier grass studies and they were able to link quantitative trait loci to several plant traits. A diversity analysis, based on RAPD molecular markers, revealed a moderate level of diversity with clear differentiation of Napier grass accessions from pearl millet and its hybrids, and the accessions were clustered into groups according to their geographical origin [4,41]. However, the difficulty of differentiating some of the accessions based on their RAPD profile was also acknowledged [4]. Bhandari and co-workers [42] were able to differentiate 64 accessions of Napier grass based on polymorphisms in isozymes and total proteins and reported the availability of a wide range of genetic diversity. They suggested that the markers could be used to efficiently complement the morphological traits for diversity assessment and varietal identification of Napier grass accessions.

Harris et al. [11] were able to study the genetic relationship among 89 nursery accessions using amplified fragment length polymorphism (AFLP) markers and the results revealed a moderate to high degree of genetic relatedness among the accessions. In addition, clustering of the accessions into five groups in line with geographical origin was observed, which was a similar result to that observed using RAPD markers [11]. However, in another study using AFLP markers, Napier grass accessions of different geographical background obtained from research centres in Botswana, Mozambique, Ghana, South Africa, and Ethiopia (ILRI forage genebank collection) came together into different groups, with no clear evidence of clustering according to geographical origin [43]. Recent studies using AFLP markers [44] provided an indication that there was little to moderate within population diversity and a clustering of two groups in the Napier grass collection held at the ILRI forage genebank together with some additional accessions collected from Kenya, Tanzania and Uganda. Interestingly, these results also did not reveal the clustering of different accessions according to their geographical origin, which was demonstrated by the previous morphological, agronomic and RAPD marker studies. Other types of molecular markers, such as inter-sequential simple repeat (ISSR) markers [41,45] and sequence-related amplified polymorphisms (SRAPs) [46] have also been used for the characterization and identification of Napier grass clones.

Finally, a number of microsatellite, or simple sequence repeat (SSR), markers from pearl millet genetic studies have been demonstrated to be transferable to Napier grass [47–51]. The transferrable markers were successfully used in diversity analyses and clone identification of Napier grass accessions [12,52]. Expressed sequence tag (EST)-based SSR markers have been successfully used to identify pearl millet-Napier grass hybrids (the majority of hybrid Napier grass varieties under cultivation have been developed using pearl millet as maternal parents and Napier grass as paternal parents) [53]. Napier grass collections from Kenya and Uganda [54], the United States Department of Agriculture-Agricultural Research Service (USDA-ARS, Tifton nursery) [12] and the ILRI forage genebank (unpublished data) have also been characterized using SSR markers. Results from the SSR analyses demonstrated the availability of a broad array of genetic diversity in Napier grass germplasm while some duplicates were also identified in the collections. Kawube and his colleagues [54] also reported the allelic uniqueness of Napier grass from Uganda when compared with some of the accessions from the ILRI forage genebank. This array of outcomes highlights the need for the integration of modern molecular tools (for example, genotyping by sequencing) for the establishment and management of core collections in order to better capture the available genetic diversity.

2. Current Status

Due to the fact that most smallholder livestock producers predominantly own small and fragmented pieces of land, grasses such as Napier grass offer a best-fit alternative to other feed options, as these are high yielding forages which require a minimum amount of inputs and acreage. Napier grass possesses a number of attributes including: high biomass yield [55,56]; rapid re-growth potential and ease of propagation [57]; attributes that help with the control of soil erosion [58]; resistance to a broad spectrum of pests and diseases [59]; and suitability for cellulosic biofuel production [60]. The growth rate and biomass production of Napier grass surpasses other tropical grasses including Johnson grass (*Sorghum halepense*), switchgrass (*Panicum virgatum*), maize (*Zea mays*) and sugarcane (*Saccharum officinarum*) [61]. In addition, by following best management practices (regular cutting between 60 to 90 days and keeping soil moisture level at an optimum level) and applying fertilizer when required, harvesting of Napier grass can be maintained for decades [39].

The aforementioned qualities of Napier grass make it an attractive option for livestock production systems. However, the adoption and utilization of Napier as an alternative forage crop has not been totally successful due to the limited amount of research and attention given to this crop [62]. At present, only a handful of molecular characterization studies have been reported and its genome is yet to be sequenced. Therefore, in order to increase the utility of Napier grass and advance its breeding initiatives, genotyping by sequencing (GBS) of the Napier grass collection held at ILRI is currently underway. This characterization will be of great importance to assess the available diversity within ILRI collections. Furthermore, GBS characterization will also help develop sufficient SNP markers for marker-assisted breeding of Napier grass.

2.1. Yield and Morphology

Napier grass cultivars have been reported to yield around 60 tonnes dry matter/ha/year, with some studies indicating significantly higher yields [26,27]. The yield of Napier grass mainly depends on the type of cultivar used which in turn is influenced by both the environment and management practices employed. Nevertheless, there are two major categories of Napier grass cultivars based on their morphology, the normal or tall (up to 4–7 m) varieties (for example 'Australiano', 'Bana' and 'French Cameroon') and the dwarf or semi-dwarf (<2 m) varieties (for example 'Mott') [27]. The normal varieties have been reported to produce up to twice as much yield as the dwarf ones [27,63]. However, dwarf varieties also have a number of positive attributes, including enhanced overwintering capacity in the border areas between subtropics and temperate zones, better nutritive value, and ease of management and harvesting [64,65]. Therefore, different cultivars of Napier grass can be adopted by farmers depending on their situation and ultimate use of the crop.

The performance and yield of Napier grass is heavily influenced by agro-ecology, climatic conditions, management practices and other edaphic factors [27,66]. According to Kebede et al. [67], the most significant factor affecting DM production of Napier grass is the environment, followed by genotype by environment interactions and then the genotype. However, the genotype is still important and the DM yield of Napier grass has been demonstrated to be superior to other tropical forages including Guinea grass (*Megathysus maximus*) and Rhodes grass (*Chloris gayana*) [68]. Table 2 provides a summary of the dry matter yield, and other important forage quality attributes, obtained in different studies conducted on Napier grass.

Table 2. Yield and nutritional qualities of Napier grass accessions across different studies.

Country	DM (t/ha/year)	CP (%)	NDF (%)	ADF (%)	No. of Accessions Evaluated	Ref.
Bangladesh	14.9–16.5	10.3–11.4	NA	29.9–45.9	4	[69]
Brazil	14.9–78	NA	NA	NA	85	[26]
Ethiopia	4.6–20.5	7.5–15.7	52–64.6	28.8–36.6	9	[70]
Kenya	12.1–19	NA	NA	NA	8	[71]
Malaysia	43.7–65.9	10–12	60–70	35–40	9	[64]
Mexico	NA	9.2–9.9	65.2–69.7	42.2–44.7	3	[72]
Thailand	27.1–58.4	NA	NA	NA	8	[27]
USA	25.3–28.2	12.42–15.68	62.7–66.8	37.2–39.6	2	[57]
Zimbabwe	90.2	5.35	56.8	39.2	2	[73]

DM: dry matter production; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; NA: 'Not available' is given when that particular component was not measured in that study.

2.2. Nutritional Qualities

Significant variation in dry matter production (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and other nutritional qualities have been reported across different studies and accessions of Napier grass (Table 2). Nutritional quality is strongly influenced by management practices and age at harvest but, on average, Napier grass is considered to contain 9% CP, 20% DM, 70% NDF, 50% ADF, 9% ash and 6% lignin in samples taken from 10–15 week old plants [74,75]. Apart from genetics, the nutritional qualities of forages are influenced by many factors including the climate [76], soil nutrition [77], season and grazing pressure [78], management [65,79,80] and fertilizer application [81]. Consequently, great care should be taken to determine the optimum time when planning to harvest or graze Napier grass in order to maximize both yield and nutritional qualities [82].

An important aspect for most forages is that cutting treatments and interval can have a significant impact on both yield and nutritional qualities [80,83]. For example, the CP content of Napier grass has been demonstrated to decrease significantly from 28.2% at a 40-day cutting interval to 8.8% at an 80-day interval [56,84]. In addition, the CP content of Napier grass has been shown to be negatively impacted over recurrent cuttings, although the possibility exists to increase both DM and protein content through increased fertilizer application [85]. On the other hand, DM production has been shown to increase significantly over consecutive cuttings from the first to the third [69]. Although cultivar and environment specific, Wanghchuck et al. [56] recommended a 60-day cutting interval in the optimum growing season to maintain a high yield without compromising nutrient composition too much.

Forages, when harvested during the early stages of their development, are considered to possess relatively higher crude protein content [76,86]. On the other hand, plant structural components (NDF, ADF and lignin) increase during later harvests, resulting in decreased dry matter digestibility [76,86]. Lignin, an integral component of the plant secondary cell wall [87], is the primary factor limiting organic matter digestibility and nutrient availability in forages [88,89] by interfering (as a physical barrier) with microbial enzymatic activity [88,90]. Despite many desirable characteristics, Napier grass is generally considered to be of inferior nutritional quality depending on management (particularly in terms of metabolisable energy, digestion kinetics and percentage crude protein) and palatability when compared to other forage crops [16,64,84]. Napier grass is reported to possess around 50% NDF, which is higher than the recommended NDF content for forage grasses [64]. Feeding experiments using Friesian cows revealed a loss of body weight and reduced milk yield when solely fed Napier grass varieties [71,74]. Lactating cows that were producing 10 kg/day milk yield, when fed on Rhodes grass (*Chlolris gayana*), produced only 6–8 kg/day, which was accompanied by a loss of body weight, when fed solely on Napier grass [74]. Therefore, when using Napier grass as a main forage supplement, it is recommended that a portion of the ration needs to be substituted with high energy/protein feed

to prevent reduced rumen microbial activity and depressed digestibility [74]. On the other hand, dwarf and semi-dwarf Napier grass varieties have been shown to contain a higher CP content and lower ADF and hence are considered to be more suited as a forage for dairy farming applications [64]. In general, in areas where supplementary feed is scarce, intercropping Napier grass with legumes is considered to be a better option and such an approach could be the best alternative for smallholders who cannot supplement their milking cows with additional protein sources. In addition, using an increased amount of fertilizers can enhance organic matter content of Napier grass and subsequently enhance its nutritive quality as a feed [80].

2.3. Water Use Efficiency

A number of traits, including high dry matter production, ease of establishment and regeneration, persistence, and enhanced water use efficiency make Napier grass the primary forage of choice in the regions of Eastern and Central Africa where smallholder dairy farmers and pastoralists suffer from sporadic droughts and possess limited irrigation infrastructure [71]. Grasses which possess a C4 photosynthetic pathway are considered to have a competitive edge over C3 grass species when grown in tropical and subtropical regions [91]. Napier grass is a C4 grass species that also has the capacity to reduce shoot dry matter and maximize carbon assimilation during times of water stress, making it a desirable forage crop in areas subjected to intermittent droughts [92]. Napier grass undergoes changes in its morphology including leaf rolling, reduced stomatal conductance and enhanced water use efficiency when subjected to water stress conditions [93]. Since Napier grass is a perennial crop, it is expected to face rainfall fluctuations which would induce water stress at some point during the year and cultivars have been reported to lose as much as 20% of their yield potential when grown under water-deficient conditions in comparison to a control environment [93]. Hence, the development of cultivars that can withstand and produce during short periods of drought is considered to be useful for areas without irrigation, particularly as the effects of climate change are expected to impact on a growing number of regions.

Successful forage cultivation is influenced by the ability to minimize the trade-off between DM production and yield potential when grown under stress conditions such as drought. Biomass yield loss in Napier grass has been demonstrated to be less severe than in Guinea grass when grown under water stress conditions [93]. However, due to the fact that Napier grass has so far received little attention in terms of research investment, its advancement through breeding is considered to be minimal and lags behind other forages [62]. In addition, the lack of available genomic tools for Napier grass has hampered breeding initiatives. If an appropriate genomic toolbox is established and physiological responses to water stress are well understood, cultivars that can cope with intermittent drought should be able to be developed in the foreseeable future. The water use efficiency of a range of accessions from the ILRI forage genebank are currently being evaluated in irrigated and non-irrigated blocks, which will help further our understanding of their drought response mechanisms and provide the basis for the development of more drought tolerant Napier grass cultivars.

2.4. Pests and Diseases

Napier grass has been shown to be affected by many insects and other pests, bacteria, viruses, fungi and phytoplasmas, although most of them do not produce severe disease symptoms [3]. There have been numerous records of insect infestation on Napier grass. Farrell et al. [3] listed seventy-two different insects and mites that infect the species although for most of them, Napier grass acts as a reservoir in which the insect can survive between the growing seasons of other crops. To date, there has only been a single report of a bacterial infection (*Xanthomonas albilineans*), the causal agent of scald disease in sugarcane, in Napier grass and in this case the disease symptoms were unclear [94]. Potyviruses are considered to be the emerging problem for Napier grass but at the moment the two most significant threats to its productivity are the diseases smut and stunt which are caused by a fungus and a phytoplasma, respectively.

2.4.1. Viral Diseases

There have only been nine reported cases of viral infection of Napier grass worldwide. These viruses belong to the genera *Mastrevirus*, *Potyvirus* and *Sobemovirus* [3]. A geminivirus was the first virus reported to naturally infect Napier grass, described in Zimbabwe [94]. Subsequently, sugarcane mosaic virus [95], sugarcane chlorotic streak [96], maize mosaic (stripe disease) [97], die-back virus [98], maize streak geminivirus [99], elephant grass mosaic virus [100], a member of the potyviruses [101] and Johnson grass mosaic virus [3,95] have been reported. However, none of these were reported to produce serious disease symptoms or any significant productivity loss. The only report of symptoms was by Mih and Hanson [101], who reported that infection by one of the potyvirus isolates (Is16840), among three potyviruses isolated from the ILRI field genebank at Debre Zeit in Ethiopia in 1994–1996, produced severe mosaic and stunting symptoms in Napier grass that would cause productivity losses. Apart from this, an unclassified insect-borne virus was suggested to cause stunt disease in Napier grass in Uganda [102], that may also cause productivity loss.

2.4.2. Fungal Diseases

There have been as many as seventy-one different fungi reported to infect Napier grass [3]. Among them, only three diseases, namely eyespot, snow mould and head smut, have been addressed by researchers, mainly because the other fungi do not appear to have a significant effect on plant growth and productivity. The eyespot disease, caused by the fungus *Helminthosporium* spp., was first reported in the Caribbean in 1938 [96]. Although Burton [97] later reported that a Napier grass variety, 'Merkeron', was resistant to this disease (as the fungus did not cause a severe disease outbreak) there has been no significant effort to further any studies on this disease. The snow mould disease, also known as white mould disease, caused by the fungus *Beniowskia sphaeroidea*, was first reported to affect Napier grass in Kenya [98,99] and was later discovered in Malawi, Tanzania, Mauritius, Uganda and Zimbabwe [100,101]. The disease symptoms only appear during heavy rains and there is limited damage, restricted to during that season; it also does not appear to affect the vigour of the plants and livestock feeding on the diseased leaves do not appear to suffer any adverse effects. However, efforts have been made to introduce resistance to this disease and a resistant variety, 'Clone 13', was developed by conventional plant breeding in French Cameroon in the early 1970s [103].

The fungus responsible for head smut disease caused a severe disease outbreak with huge productivity losses of Napier grass in Kenya [18,95,102]. The causal agent of this disease is a fungus from the genus *Ustilago*, which was initially named as the species "*kamerunensis*" based on the place 'Cameroon' from where it was first isolated [104] and then later described as *Ustilago kamerunensis*" by Sydow and Sydow in 1911 [105]. The fungus appears to be slowly spreading from West Africa to the eastern parts of Africa as the disease was first reported in Cameroon [104], and subsequently in Uganda [106], Congo [107], Rwanda, Tanzania and then Kenya [108]. However, head smut occurs only in Africa as it has not been reported elsewhere outside the continent so far [95,109]. The mode of transmission of this disease is either by wind-borne spores or infected planting materials [110]. The spores of the smut-causing fungus are very light and have been reported to be able to spread by wind over large distances [111]. However, during a survey of the smut-infected districts of Kenya it was found that the disease spreads mainly by infected planting materials, as the farmers were completely unaware of this disease and the possible symptoms [110]. Although the smut disease of Napier grass has been recorded in many African countries, Kenya was the first country to be threatened by a potential epidemic. This could be because the strain identified in central Kenya causes the greatest yield losses when compared with the strains reported in other African countries [95,109,110]. The disease is widely spread across the central regions of Kenya and has been reported to cause 25–46% loss of biomass production [18,95,102]. The infected plants have thinner and shorter stems, a reduced numbers of leaves, and suffer from slower re-growth after cutting. The continual spread of the disease to other parts of the country, including the Rift valley and lower eastern region has also been recorded [1], which raises concerns about the possible future spread of this disease. Fungicide treatment is not currently an

option to control the disease, especially for vegetatively propagated cuttings. Following diagnosis of infected planting material, destroying these materials by burning is currently the only option to control this disease. Efforts to select and breed resistant accessions or varieties have led to the identification of two resistant varieties, namely 'Kakamega 1' and 'Kakamega 2' [95,112] and the Muguga South branch of the Kenya Agricultural and Livestock Research Organization (KALRO) is promoting and distributing planting materials of these varieties to farmers in the affected regions in order to minimize the effect of this disease in the country. Co-evolutionary modification of some African accessions, particularly from Southern Africa, has also resulted in the development of resistance to the disease over time [113,114] and further focus is currently being placed on developing resistant plant material to manage the disease in the future. Many Napier grass clones have been collected from various sites across the world and are currently under trial in an attempt to discover whether they exhibit any selection bias related to their geographic origin which may have developed due to a co-evolutionary cycle of selection [109,112]. However, this co-evolutionary process in the induction of resistance may also lead to the selection of more virulent strains of the pathogen *U. kamerunensis*, in case of widespread use of selected resistant accessions [114]. Therefore, it is considered advisable to adopt a strategy which promotes the planting of varieties of mixed origin and resistance levels that could slow down the likely natural selection of the pathogen into a more virulent strain [33].

2.4.3. Phytoplasma (Stunt) Disease

Napier grass stunt disease is by far the most devastating disease of Napier grass as the infected plant material shows severe stunting symptoms, resulting in eventual death of the plant [115–117]. The disease was first observed in western Kenya in 1997 and has been reported to spread quickly, causing serious economic losses [118]. It has been demonstrated that the disease is associated with the 16SrXI phytoplasma (*Candidatus* (*Ca*.) Phytoplasma oryzae) group [118,119]. Subsequently, the disease has been reported to occur in Ethiopia [120], Uganda [116] and Tanzania and Rwanda [2]. The phytoplasma responsible for the stunt disease in Uganda was discovered to be similar to the Kenyan strain; however, in Ethiopia the strain was found to be a member of the 16SrIII group, '*Ca*. Phytoplasma pruni' or X-disease [116,121], which caused symptoms similar to those observed for stunt disease in Uganda and Kenya but without the severe stunting [117]. The disease symptoms include yellowing of foliar material, smaller leaves, a proliferation of tillers, yellow to purple streaking and shortening of internodes to the extent that clumps appear severely stunted, resulting in a low biomass yield and eventual death of the plant, although this only occurs after cutting or grazing the grass [118]. However, the level of expression of the symptoms in phytoplasma-infected plants partly depends on the virulence of the strain, strain interference and phytoplasma concentration [122] and the abundance of insect vectors and phytoplasma-infested host plants [123]. The primary mode of transmission of the disease is by vegetative propagation of infected planting material or by phloem-feeding insects belonging to the families Cicadellidae (leafhoppers), Delphacidae (planthoppers) and some psyllids (Psylloidea) [124,125]. Obura et al. [126] identified *Maiestas banda* Kramer (Hemiptera:Cicadellidae) as a vector for Napier grass stunt disease phytoplasma in Kenya and *Leptodelphax dymas* and *Exitianus* spp. have been recorded in Ethiopia [127]. However, so far no vector has been identified in Uganda [126]. The vector–phytoplasma–host plant three-way interaction plays an important role in determining the spread of the disease [128]. There is the possible involvement of other phytoplasma susceptible food crops and grasses which could act as a reservoir, providing a source of inoculum for the spread of the disease [127] which would present a challenge to the development and implementation of management strategies for the disease. Two stunt-resistant varieties, 'Ouma 2' and 'South Africa', were selected by the International Centre of Insect Physiology and Ecology (icipe) in collaboration with KALRO and Rothamsted Research (UK) [129]. However, despite the efforts made to date to develop resistant varieties by national research organizations at various locations in western Kenya, many selected accessions have ultimately been found to be susceptible [130]. Consequently, the only guaranteed way to control the disease is through removal of the infected plants [131–133].

3. Future Prospects

Through years of effort, a number of cultivars have been selected and are currently in production in different regions of the world [6]. In addition, active breeding programs have been established to generate and capture greater diversity for both animal production and biofuel applications [134]. However, despite the efforts made so far, the production and use of Napier grass remains constrained by many factors. Nutritional quality, palatability, and propagation by seed or vegetative organs are currently the main limitations, and the diseases Napier grass stunt and head smut are significantly challenging its production in some regions of Africa. Moreover, enhancing the crop's water use efficiency is another key area of research which will allow for production and use in areas with annual rainfall below its optimal range (<750 mm), and maintenance of current areas under the threat of climate change. In this review, we have compiled an extensive amount of evaluation and characterisation data which has been derived from various collections over the past few decades and demonstrated that significant diversity exists in these traits of interest which have the potential to be captured [39,44,54]. Consequently, by integrating modern molecular approaches into improvement strategies, some of the constraints in Napier grass production and use could now be efficiently addressed [44].

Opportunities to help capture the genetic diversity in crops for plant breeding and crop improvement have recently been revolutionized by the integration of advances in molecular genetics and genomics, plant biotechnology and next-generation sequencing. These advances have already been widely applied to crop improvement and offer the opportunity for new approaches to enhance quality and performance traits of feeds and forages at a relatively low cost. However orphan crops, which include tropical forages in general and Napier grass in particular, have not yet substantially benefited from these advances in molecular genetics and the associated modern tools that are available. There remain few reports on characterizing the genetic diversity of Napier grass through the application of molecular markers, and genetic maps and genome sequence information is largely lacking. As a result, there is little molecular information on Napier grass, which has implications for the knowledge-based use and conservation of available genetic resources for sustainable development. This, for example, has limited the ability to locate genomic regions controlling traits of interest and gene discovery. Consequently, the potential to use Napier grass as a 'climate-smart' forage crop, with traits such as enhanced water use efficiency, disease resistance and temperature tolerance stacked in new varieties which perform well in the face of climate change, has not been fully realised. Breeding efforts are also limited in Napier grass [6], which could be due to poor quality and limited seed production. The current distribution of planting materials to farmers is considered bulky, expensive [6] and carries the potential risk of disease distribution (for example, stunt disease) to new areas. In other vegetatively propagated crops such as potato, cassava and sugarcane, the use of diseased planting materials has been demonstrated to be the main source of inoculum for disease-causing agents [135]. Therefore, improving the seed production ability (both in terms of quantity and quality) of Napier grass potentially conveys a multitude of benefits including using seeds for distribution to farmers, creating genetic variation and new hybrid varieties through crossing and reducing the risk of disease spread related to distribution of vegetative propagules.

With respect to advances in nutritional quality of Napier grass, a number of opportunities exist to leverage the knowledge and advances seen in other fodder crops to the improvement of Napier grass and the benefit of livestock productivity. The plant cell wall provides the major source of dietary fibre and the nutritional availability of forage fibre to livestock is highly dependent on its composition and structure [136]. The plant cell wall is a complex biological structure, mainly composed of cellulose, hemicellulose, protein and lignin, which varies greatly depending on developmental stage, tissue type and plant species [137]. The bioavailability of cellulose, the major structural polymer of plants and the most abundant organic polymer on Earth [138] as an energy source is restricted by the β-glucosidic linkages, making it insoluble in water in its native form [139] and the lignin complex [140]. Lignin affects the digestion of cell-wall polysaccharides by interfering (as a physical barrier) with microbial enzymatic activity [88,90] and therefore, developing low-lignin Napier grass lines could substantially

improve its digestibility and nutritional quality for enhanced livestock productivity. For example, it has been reported that a 1% increase in in vitro dry matter digestibility of forages leads to a 3.2% increase in daily weight gains of beef cattle [141]. Thus, the selection and/or development of low lignin varieties is another area of research where modern genomic tools could contribute substantially to improved feed quality in Napier grass. For example, the brown midrib mutants could offer an opportunity for selection in Napier grass. These mutants, which contain mutations in the lignin biosynthesis pathway and offer improved forage digestibility for livestock, have been selected in maize (*Zea mays*), sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*) and a similar approach could be employed on Napier grass to improve its nutritional deficiency as a forage crop [142]. Transgenic approaches have also been used to enhance the nutritional quality of forages; for example, low-lignin alfalfa lines with enhanced digestibility have been developed [143] and similar technology can also be an option for Napier grass improvement. However, it is not only the reduction but the modification of lignin structure that can be important and the incorporation of *p*-coumaric acid instead of ferulic acid can improve cell wall digestibility in grasses by reducing cross linking [144].

In addition to enhancing nutritional quality, the improvement of other attributes of Napier grass, such as resistance to pests and diseases, also requires attention in order for this species to realise its full potential. Despite evidence demonstrating that insect vectors are responsible for transmitting diseases such as stunt and viruses, information on the impact of insects on feed yield and quality in Napier grass remains largely ambiguous. By employing modern molecular technologies and tapping into the genetic diversity available, we can develop a better understanding of the potential impacts and identify accessions which are tolerant to certain pests and diseases which could be used to introgress plant-derived resistance mechanisms into modern varieties. Two accessions, 'Kakamega 1' and 'Kakamega 2', have been identified and developed as varieties which provide resistance to the smut disease [95,112]. However, the mechanism of resistance is not fully understood and molecular approaches can play a role to augment and transfer the resistance genes in to commercial varieties. In addition, more severe strains of the pathogen may develop in the future through co-evolutionary mechanisms. Consequently, a more proactive effort is required, directed towards the discovery and development of new varieties with alternative resistance mechanisms to help address this threat in the future. The same applies for stunt disease, caused by the phytoplasmas, which results in severe productivity loss of Napier grass. To date there has been limited success achieved in the development of resistant varieties to combat this disease [133]. Therefore, the primary approach towards this disease would be screening the primary and secondary gene pool of Napier grass held in global collections that could lead to the identification of disease resistance genes with different modes of action against the phytoplasma.

The development of disease resistance in plants by introducing a gene, or a part thereof, from the pathogen is another approach which could be applied. For example, many viral diseases have been reported to infect Napier grass [3] which could have implications for both yield and quality, and the transgenic expression of viral coat proteins, replicases or other sequences from the virus genome could potentially be used to introduce resistance into the grass. Similarly, antifungal genes such as chitinases and glucanases could be introduced into the genome in order to confer resistance to fungal diseases such as the head smut disease reported to substantially affect household feed supply in Eastern and Central Africa. The use of antimicrobial genes to engineer the plant to produce antimicrobial proteins could also be considered to strengthen resistance mechanisms. Also, engineering with genes producing antibodies against a protein crucial for pathogenesis could result in a level of immunity or resistance to the pathogen. Alternatively, genetic modification or the recently developed technique of gene editing could be used in Napier grass to combat economically important insect pests. Accordingly, transgenic lines with resistance to different groups of insect pests can be generated using genes from various origins (Bt *cry* genes, the insect chitinase gene, RNA interference (RNAi) technology, plant-derived genes for proteinase inhibitors, and α-amylase inhibitors and lectins for example).

Good agricultural practices and management of diseases are currently the only option to protect against the spread of Napier grass diseases. The development and deployment of management practices to guard against any disease in a particular geographical area is guided by quantitative information on the existing levels of disease risk, definitive identification of the pathogen and a clear understanding of the factors that correlate strongly with disease/pathogen risk within a defined host population [145]. Although most of the viruses infecting Napier grass do not appear to cause any severe disease symptoms and productivity losses, there have been some reports on the effects of the potyvirus (Is 16840) identified in Ethiopia [146] and an insect-borne virus identified in Uganda [147] which need to be investigated more thoroughly, especially in terms of potential productivity loss, as they were reported to cause stunting in infected plants. There is limited information available on farmers' knowledge and understanding of Napier grass diseases, in particular, and forages in general, which need to be addressed through extension packages. Similarly, in order to manage head smut disease of Napier grass, there is a need to educate farmers on the identification of disease symptoms and implementation of management strategies. Although burning of infected material is a good option to destroy the source of infection, the development of visible symptoms can take time, which allows the disease to spread further. Therefore, efficient diagnostic tools (serological or molecular) could offer a valuable asset for the early detection and diagnosis of the disease and to monitor its spread for improved management and containment. A number of studies have been undertaken towards the identification of the pathogen, possible vectors and disease severity for an outbreak of Napier grass stunt disease in Kenya [118,126,148]. However, further studies will be required to elucidate factors involved in the plant–host–vector three-way interaction related to the spread of stunt disease in Ethiopia and Uganda. There is also a need for further research to confirm whether *Exitianus* sp., *L. dymas*, or both species, act as a vector(s) of stunt disease. Further, for the disease outbreak in Uganda, there is a lack of information regarding the possible vectors involved in the transmission of the disease. Molecular studies would provide more information about the identity of the causal agent, vectors involved in disease transmission and the factors supporting the spread of the disease, which may help in the development of an effective management tool to control/minimize its spread. Seasonal monitoring of the insect vector populations could also provide information on the spread of the disease, and should facilitate the prediction of future Napier grass stunt disease outbreaks.

In a similar manner to the approaches reported for other crops, improvements in tolerance to abiotic stresses such as drought, salinity, soil pH and extreme temperatures in Napier grass could be achieved by employing a range of modern molecular tools. Despite the successful selection of a few accessions with resistance to the diseases head smut and stunt, the introgression of stress resistance into advanced breeding lines is yet to be effectively tackled in Napier grass. Moreover, genomic regions controlling desirable characteristics such as the dwarf growth habit, smooth (hairless) leaf, water use efficiency, etc. remain to be elucidated in Napier grass. Genetically Napier grass has two different sets of genomes: A'A' and BB. The homologous nature of the A'A' genome with the AA genome of pearl millet and the contribution of the B genome to perennial growth habit offers many other opportunities for future genomic studies in Napier grass.

It is expected that research in Napier grass characterization, phenotyping, genotyping and breeding will be aided by the application of modern tools in the near future. This will facilitate clone identification, the establishment, management and exploitation of core collections, generation of sequence information, development of genetic maps and identification of high throughput marker systems such as SSRs and single-nucleotide polymorphisms (SNPs) for the localization of genomic region(s) and discovery of genes controlling traits of interest in Napier grass. Once linkages between traits of interest and known genetic markers are well established, marker-assisted selection/breeding could facilitate the selection of new clones and/or varieties with improved agronomic traits. In general, modern molecular genetics should be quickly integrated into the current conservation, use and improvement strategies to address nutritional quality and palatability concerns, and biotic and abiotic stresses in Napier grass.

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Abbreviations

The following abbreviations are used in this manuscript:

ADF	Acid Detergent Fibre
AFLP	Amplified Fragment Length Polymorphism
Bt	Bacillus thuringiensis
CIAT	International Center for Tropical Agriculture
CP	Crude Protein
CSIRO	Commonwealth Scientific and Industrial Research Organization
DM	dry Matter
DRC	Democratic Republic of Congo
EST	Expressed Sequence Tag
GBS	Genotyping by Sequencing
GRIN	Germplasm Resources Information Network
ha	hectare
icipe	International Centre of Insect Physiology and Ecology
ILRI	International Livestock Research Institute
ISSR	Inter-Sequential Simple Repeat
KALRO	Kenya Agricultural and Livestock Research Organization
L	Litre
NBPGR	National Bureau of Plant Genetic Resources, Delhi, India
NDF	Neutral Detergent Fibre
RNAi	RNA Interference
RAPD	Random Amplification of Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RBG	Royal Botanic Gardens
SNPs	single-nucleotide polymorphisms
SRAPs	Sequence-Related Amplified Polymorphisms
SSR	Simple Sequence Repeat
USDA	United States Department of Agriculture

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