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Integrating molecular insights on stress responses and agronomic traits for breeding in forage grasses

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ABSTRACT

In Latin America, pasture-based systems form the backbone of cattle production, with tropical forage grasses providing the primary and most economical feed source for ruminants. These grasses are cultivated across millions of hectares because of their high biomass yield, adaptability to poor soils, and suitability for extensive grazing. However, shifting land-use patterns and climate change are pushing forage systems into more marginal environments, where water scarcity and declining soil fertility compromise productivity and nutritional quality. Despite their critical role in regional and global livestock supply chains, tropical forage grasses have historically received limited breeding attention, largely owing to biological constraints such as apomixis, polyploidy, and self-incompatibility. This review explores the current understanding of the molecular mechanisms regulating key morphophysiological traits in tropical forage species, focusing on plant responses to abiotic (e.g. drought, heat) and biotic (e.g. pests, pathogens) stress. We highlight progress in building genomic resources, with emphasis on quantitative trait locus (QTL) mapping, transcriptomics, and genome-wide association studies (GWAS), which are uncovering trait-linked markers that can inform selection strategies. Additionally, we examine how transgenic technologies and gene editing tools such as CRISPR-Cas can be employed to circumvent reproductive barriers and accelerate the development of improved cultivars. By integrating conventional breeding with cutting-edge molecular tools, we propose a roadmap for developing climate-resilient, nutritionally enhanced tropical forages to support the long-term sustainability of grass-based livestock systems.

Keywords: breeding, climate-resilient, gene editing, genomic, livestock, physiology, ruminants, stress, transgenic, tropical forage grasses.

Introduction

Grasslands cover approximately 40% of the global land surface and, when well-managed, serve as robust carbon sinks. Moreover, they provide the forage, which constitutes the primary component of the diet of ruminant livestock and the more economical alternative in ruminal feeding (Jank *et al.* 2011; Buisson *et al.* 2022; Sustek-Sánchez *et al.* 2023). In many major cattle-producing regions, particularly in South America, livestock rely almost entirely on extensive pasture as the sole source of feed (Pereira *et al.* 2018). In tropical and subtropical regions, these pastures are dominated by megathermal C_4 grasses, which are characterized by high biomass production, a seasonal growth pattern, and morphological and developmental traits that enable them to thrive in hot tropical and dry temperate ecosystems (Burns *et al.* 2004; Pardo and VanBuren 2021; Teixeira *et al.* 2025). A striking example of their importance in Latin America livestock production is the vast extension of land, ~125 million hectares, occupied by individual species or hybrids of *Urochloa* (Jank *et al.* 2011; Baptistella *et al.* 2020).

Grasses are typically cultivated on less fertile land compared to other crops. Recently, in major livestock-producing regions in South America, the expansion of agriculture into areas previously used for animal husbandry has displaced cattle production into more marginal regions, such as those experiencing more severe rainfall shortage, which hampers the productivity and nutritional quality of forage grasses (Chand *et al.* 2022; Milán *et al.* 2024;

Ribeiro *et al.* 2025). Furthermore, climate change, driven by the continuous accumulation of greenhouse gases, has led to rising temperature and increased frequency and intensity of drought episodes (Rivero *et al.* 2022). This combination of factors highlights a critical need to develop grass crops that are resilient to more adverse climate conditions, thereby ensuring the long-term sustainability of grazing systems.

Another critical determinant of success in the livestock industry is the consistent availability of high-quality forage throughout the year. However, breeding efforts have predominantly prioritized biomass yield, often treating nutritional quality as a secondary trait (Battenfield *et al.* 2016; Maheswari *et al.* 2017). Besides its nutritional values, many other growth habits and morphological traits should be taken into account to build the 'ideal ideotype of forage grass'. Among several features we could highlight are a delayed flowering, increased tillering capacity, high leaf area and photosynthetic capacity, high leaf:stem ratio, erect leaf angle, limited leaf pubescent, lower chlorophyll degradation, deep rooting, increased root length density and root hair proliferation, high grazing tolerance, and enhanced pathogen defense responses (Chand *et al.* 2022).

Despite their significant economic and ecological importance, breeding efforts in forage grasses have historically been limited. This is partly due to their complex reproductive biology, including frequent apomixis, self-incompatibility, and polyploidy, which pose obstacles for conventional breeding strategies. Consequently, genetic improvement in these species has not progressed as rapidly as it has in major crops such as cereals, where well-established breeding programs and genomic resources have driven substantial advances in yield, pathogen resistance and resilience. This disparity emphasizes the critical need for further research into innovative breeding approaches tailored to the unique biological features of forage grasses (Ferreira *et al.* 2021).

In this review, we explore the state-of-the-art understanding of the molecular basis underlying key morphophysiological traits in forage species, including responses to abiotic and biotic stresses and cell wall composition, an essential factor for improving digestibility. Although significant advances have been achieved in model and temperate grass species, this knowledge remains limited in tropical grasses, where fundamental insights are still lagging behind. Through a translational science approach, deciphering these molecular mechanisms can be effectively harnessed and applied in breeding programs. We further proposed an integration of genomic resources, multi-omics and genetic studies to accelerate the development of high-yielding, climate-resilient, pathogen-resistant and nutritionally enhanced forage cultivars.

Adaptative strategies to drought and heat stress

The greatest challenge in agriculture today is to develop plants that are resilient to adverse environmental conditions while maintaining sufficient yield to support sustainable production systems. In the case of perennial forage grasses, yield depends on the repeated harvesting of herbage over multiple years. Therefore, it is essential to identify forage grass genotypes with enhanced survival and growth under abiotic stress conditions (Sustek-Sánchez *et al.* 2023).

C_4 species are naturally resilient to heat, salinity and drought, allowing them to perform well under climate stress. Major C_4 grasses, including the annuals maize and sorghum, and the perennial sugarcane, combine high productivity with efficient water and nutrient use, owing to a C_4 photosynthetic pathway, shaped by anatomical and biochemical adaptations, that concentrates CO_2 around RuBisCO and markedly reduces photorespiration, a key limitation of the C_3 pathway. Studies have shown that the temperature at which C_4 plants begin to outperform C_3 species increases with elevated CO_2, indicating a complex interaction among climate variables. Nonetheless, under the hotter and drier conditions expected in many regions, C_4 plants are likely to play a vital role in sustaining productivity. Despite this, their responses to future climate scenarios remain uncertain, as key aspects of C_4 metabolism are still not fully understood, and many C_4 species have been reported to still be vulnerable to especially the combination of persistent drought and high temperatures (Lopes *et al.* 2011; Pau *et al.* 2013; Taylor *et al.* 2014; Heckman *et al.* 2024). Interestingly, Hoover *et al.* (2014) found that perennial C_4 grasses *Andropogon gerardii* and *Sorghastrum nutans* showed significant declines in photosynthesis, leaf water potential and productivity under drought, but minimal response to heat waves. This is likely because, when soil moisture is low, severe water stress already reduces photosynthetic rates to minimal levels, leaving little scope for heat waves to cause further decline (Hoover *et al.* 2017). Hence, understanding the combined physiological responses to heat and drought is essential for accurately predicting tropical forage grass resilience under increasing climate extremes.

Regarding drought and heat stress mechanisms, several key genes have been characterized for the forage C_3 species ryegrass, *Lolium perenne*, widely cultivated in temperate regions with mild warming trends and in selected subtropical areas. These molecular pathways involve delay in chlorophyll catabolism and leaf senescence, reactive oxygen species (ROS)-scavenging and antioxidant enzymes, maintenance of photosystems integrity, osmotic balance and heat shock proteins (Yu *et al.* 2013; Zhang *et al.* 2019*b*; Rahman *et al.* 2022; Yu *et al.* 2022; Wang *et al.* 2024*a*; Xie *et al.* 2024) (Table 1).

Water-use efficiency (WUE), the ratio of carbon gain to water loss, is a critical but complex trait in crop production and has been a key target for crop improvement for at least a century. It is influenced by factors such as photosynthesis, stomatal conductance, and canopy structure. Although the understanding of the physiological mechanisms regulating WUE has advanced considerably, significant knowledge gaps remain, particularly in tropical forage grasses (Leakey *et al.* 2019).

Table 1. Molecular determinants and breeding-relevant targets in tropical forage grasses.

Section	Trait/process	Species/model	Gene/quantitative trait loci (QTL)/pathway	Function/mechanism	Reference
Abiotic stress (drought/heat) mechanisms	Drought tolerance (osmotic adjustment, wax, proline)	*Cynodon dactylon*	*CER1; P5CS*	Wax and proline biosynthesis up-regulated in tolerant accessions	Ajtahed *et al.* (2021), Jiang *et al.* (2023) and Luo *et al.* (2024)
	Drought tolerance (antioxidants, dehydrin)	*C. dactylon*	*cdDHN4; Cu/ZnSOD; APX*	↑ Antioxidant activity, membrane stability, osmotic adjustment	Noor *et al.* (2024)
	Heat stress metabolism vs C_3	*C. transvaalensis* × *C. dactylon* vs *Poa pratensis*	– (metabolites)	Higher sugars/organic acids/amino acids linked to heat tolerance	Du *et al.* (2011)
	Abscisic acid (ABA) treatment improves drought tolerance	*Agrostis stolonifera*	ABA pathway (exogenous ABA)	↓ Electrolyte leakage; tricarboxylic acid cycle (TCA) organic acids ↑	Li *et al.* (2017)
	Crown root regulation under drought	*Setaria viridis*	Crown root program	Local water sensing suppresses crown roots; re-initiated after rewatering	Sebastian *et al.* (2016)
	Stomatal regulator and biomass	*Brachypodium distachyon*	*BdRFS*	↓ Density, ↑ size; ↑ biomass and leaf water content	Ying and Scheible (2023)
	Contrasting drought strategies	*Pennisetum purpureum*; *Urochloa* 'Mulato II'	–	Maintain assimilation via gs vs conserve water via lower stomata conductance	Cardoso *et al.* (2015)
	CO_2/temperature acclimation and trade-offs	*Megathyrsus maximus*	Physio/anatomy pathways	eCO_2 → ↓ stomatal density/index/conductance; warming → ↑ biomass; combined → quality trade-offs (↑ fiber/lignin, ↓ CP, ↓ digestibility)	Driscoll *et al.* (2006), Habermann *et al.* (2019, 2022, 2024) and Wedow *et al.* (2019)
	Thermo-memory under recurrent heat (epigenetic)	(*Festuca arundinacea*)	H3K4me3; *FaHSP17.8-CII*	Histone H3K4me3 marks support transcriptional memory; *FaHSP17.8-CII* maintains thermo-memory for days → faster/stronger responses; preserves ROS balance, chloroplast integrity and PSII activity	Bi *et al.* (2021)
	Drought/heat tolerance modules	*Lolium perenne*	Delayed chlorophyll catabolism and senescence; ROS-scavenging enzymes; photosystem maintenance; osmotic balance; heat-shock proteins (HSPs) (e.g. *LpY3IP1*, *LpCYP72A15*; *TaMBF1c* OE context)	Integrated pathways improve drought/heat tolerance: ↑ antioxidants and membrane stability; ↓ chlorophyll degradation; support PSI/PSII; osmoprotection	Yu *et al.* (2013), Zhang *et al.* (2019*b*), Yu *et al.* (2022), Rahman *et al.* (2022), Wang *et al.* (2024*a*) and Xie *et al.* (2024)
Immune responses in major tropical grasses	Brassinosteroids (BRs) signaling and basal resistance	*B. distachyon; Hordeum*	*BRI1/BZR1/WRKY*	*bri1* mutants heighten immunity via de-repressed signaling	Goddard *et al.* (2014)
	Detoxification and host-induced gene silencing (HIGS)	*B. distachyon*	UDP-glycosyltransferases; HIGS	Detoxify mycotoxins; reduce *Fusarium* disease	An *et al.* (2016) and Lyons and Scholthof (2015)
	Non-host resistance loci	*B. distachyon*	*Yrr1; Yrr2*	Dominant loci to wheat stripe rust	Gilbert *et al.* (2018)
	Salicylic acid (SA)/Jasmonic acid (JA)/ethylene (ET) crosstalk	Grasses	Hormone pathways	Balance growth–defense; systemic acquired resistance (SAR)/ISR	Mishra *et al.* (2021) and Shyu and Brutnell (2015)
	Beneficial microbes prime induced systemic resistance (ISR)	*Urochloa brizantha*	*Pseudomonas fluorescens*; *Burkholderia pyrrocinia*	Growth improvement; drought resilience via ISR	dos Santos Lopes *et al.* (2018)

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Table 1. (*Continued*).

Section	Trait/process	Species/model	Gene/quantitative trait loci (QTL)/pathway	Function/mechanism	Reference
	Genome-wide association studies (GWAS)/QTL for defense and quality	*Cenchrus ciliaris*	PAL-like; U-box ligases; markers for crude protein (CP)/neutral detergent fiber (NDF)/acid detergent fiber (ADF)/total digestible nutrients (TDN)	Phenylpropanoids and proteasome linked to defense/feed traits	Negawo *et al.* (2024)
	Stress/defense DEGs	*Urochloa* hybrids	Apoplastic peroxidases; wall remodeling	Strengthen structural barriers; stress modulation	Jones *et al.* (2021)
Morphological and developmental adaptation for yield	Regrowth after cutting	Sorghum × sudangrass	QTL chr 3/6/7/10	Sudangrass alleles (7,10) enhance regrowth	Yonemaru *et al.* (2022)
	Sugar remobilization in regrowth	*Lolium perenne*	*LpSUT1;* invertases*/FT/FEH*	Sucrose transport and fructan turnover; MeJA ↑ LpSUT1	Meuriot *et al.* (2022) and Liu *et al.* (2020)
	Flowering activators (C_4)	*Panicum virgatum*	*PvFT1; PvAP1-3*	Overexpression → extremely early flowering	Niu *et al.* (2016)
	Master regulator of *FLOWERING LOCUS T*-Like (*FT*-like) genes	*B. distachyon*	*BdID1* → *FT1/2; FTL9/12/13;* represses *FTL4*	Coordinates flowering timing	Liu *et al.* (2023)
	Light signaling impacts	*B. distachyon*	*BdPIL1/BdPIL3* (PIF-like)	RNAi delays flowering; alters height/chlorophyll	Hoang *et al.* (2021)
	Biomass via N metabolism	*P. virgatum*	*PvNAC2*	↑ Aboveground biomass; up-regulates N-metabolism genes (leaf) and transporter genes (root)	Yang *et al.* (2015)
	Cytokinin-delayed senescence	*Zoysia sinica*	*IPT* (senescence-inducible)	Delays senescence; ↑ yield potential	Guo and Gan (2014)
	Cytokinin homeostasis and growth	Switchgrass/rice/*Brachypodium*	*STF* → represses *CKX*	↑ Cytokinin; wider leaves, thicker stems; ↑ biomass and sugar release	Wang *et al.* (2017)
Forage quality and cell wall composition	Tradeoffs of lignin suppression	*B. distachyon*	PAL RNAi	↓ Lignin/ferulates; ↑ sugar release; growth delay; ↑ pathogen susceptibility	Cass *et al.* (2015)
	Lignin reduction in warm-season forage	*Paspalum dilatatum*	CCR down-regulation	↓ Lignin; altered composition	Giordano *et al.* (2014)
	Cinnamyl alcohol dehydrogenase (CAD) suppression and digestibility	*Paspalum notatum*	CAD RNAi	↑ *In vitro* digestibility; some growth penalties	Muguerza *et al.* (2014)
	Caffeic acid O-methyltransferase (COMT) attenuation with stable defense	*P. virgatum*	COMT down-regulation	8–16% ↓ lignin; 15–50% ↑ sugar release; no ↑ rust	Baxter *et al.* (2016)
	Forage quality QTL (near-infrared spectroscopy (NIRS))	*P. virgatum*	37 QTL (CP, NDF, ADF, etc.)	Pleiotropic/linked regions for quality	Razar *et al.* (2021)
	Heritability and correlations (quality)	*Urochloa ruziziensis*	Quantitative genetics	h^2 0.26–0.42; quality gains without digestibility loss	Santos *et al.* (2024)
	Climate impacts on nutritive value	*Megathyrsus maximus*	eCO_2 + warming	↑ Biomass; ↓ CP; ↑ fiber/lignin; ↓ digestibility	Habermann *et al.* (2022, 2024)
Reproduction barriers, genomics and editing	Apomixis locus and candidate	*Pennisetum squamulatum*; *Urochloa*; *Megathyrsus*	*ASGR; PsASGR-BBML*	Parthenogenesis; non-recombining, hemizygous region; ~1:1 segregation	Conner *et al.* (2015), Worthington *et al.* (2016) and Deo *et al.* (2020)

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Table 1. (*Continued*).

Section	Trait/process	Species/model	Gene/quantitative trait loci (QTL)/pathway	Function/mechanism	Reference
	Chromosome doubling for interploid crosses	*Urochloa* spp.	Colchicine	Enables interploid hybridization and cultivar development	Ferreira *et al.* (2021)
	Genomic prediction and candidate pathways	*U. ruziziensis*	Genotyping-by-sequencing (GBS) + Machine learning (ML) + co-expression	Auxin, lignin, stress, circadian genes ↔ agronomic traits	Martins *et al.* (2023)
	Genome resources (assemblies)	*Megathyrsus maximus*; *U. ruziziensis*; *Paspalum notatum*	Reference genomes	Enable gene discovery and breeding tools	Jauregui *et al.* (2024), Pessoa-Filho *et al.* (2019), Worthington *et al.* (2021) and Vega *et al.* (2024)
	Transformation and regeneration bottleneck	Tropical forage grasses	Embryogenic callus protocols	Genotype-dependent, low efficiency; recent advances	Bellido *et al.* (2021), Cabral *et al.* (2011), Carrion Pereira *et al.* (2019) and Maybery-Reupert *et al.* (2025)
	CRISPR editing for architecture/quality	*Eragrostis tef*; *P. virgatum*; *P. notatum*	*SD-1 KO; Pv4CL1 KO; MgCh* edits	Semi-dwarf; ↓ lignin and ↑ sugar release; efficient multi-allelic edits	Beyene *et al.* (2022), Park *et al.* (2017) and May *et al.* (2023)
	Transgenic stress and architecture	*P. notatum*; switchgrass; *Cynodon dactylon*; *Lolium perenne*	*DREB1A; AtLOV1; PIF4 OE; TaMBFIc OE*	↑ Drought/salinity; altered leaf angle/lignin/flowering; ↑ cold/heat tolerance	James *et al.* (2008), Xu *et al.* (2012), Xu *et al.* (2025) and Huang *et al.* (2022)

In a comparison among C_4 grasses, Carmo-Silva *et al.* (2009) found that *Zoysia japonica* exhibited greater drought tolerance than did *Paspalum dilatatum* and *Cynodon dactylon* (bermudagrass). It developed more xeromorphic leaf anatomy, characterized by higher leaf dry-matter content and thicker tissues, and maintained lower leaf water potential at a similar relative water content. This was accompanied by stronger osmotic adjustment through the accumulation of amino acids such as proline, valine, leucine, and isoleucine.

Similarly, water stress in the C_4 forage grasses *Digitaria commutata* and *Cenchrus ciliaris* decreases chlorophyll but increases carotenoid concentrations, whereas PSII efficiency remains stable. Both species show elevated oxidative damage markers and enhanced antioxidant enzyme activities, alongside significant accumulation of osmo-protectants such as proline and soluble sugars (Amari and Abdelly 2021). Moreover, tolerant and sensitive accessions of *Cynodon dactylon* exhibited distinct physiological responses to drought. The first group showed greater up-regulation of two stress-related genes, namely, *CER1* (*Eceriferum1*), involved in very long-chain fatty acid and wax production, and *P5CS* (*Delta 1-pyrroline-5-carboxylate synthase*), a key enzyme in proline biosynthesis. In contrast, sensitive accessions displayed higher antioxidant enzymes activity and accumulated more soluble sugars (Ajtahed *et al.* 2021; Jiang *et al.* 2023; Luo *et al.* 2024). Likewise, Noor *et al.* (2024) reported that under drought stress, tolerant bermudagrass genotypes showed strong expression of *cdDHN4* (dehydrin), *Cu/ZnSOD*, and *APX* (both antioxidant) genes, enhancing antioxidant activity, membrane stability, and osmotic adjustment, whereas sensitive genotypes showed no gene expression. These genes support photosynthesis and growth through improved WUE and stress resilience. Thus, gene expression is closely linked to drought tolerance in bermudagrass. Consistently, higher concentrations of sugars, organic acids, and amino acids have been associated with the greater heat tolerance of the hybrid *Cynodon transvaalensis* × *Cynodon dactylon* 'Tifdwarf' than of the C_3 species *Poa pratensis* (Kentucky bluegrass) (Du *et al.* 2011). Basharat *et al.* (2024) reported structural and functional modification along with accumulation of organic osmolyte and beneficial ions as key for turgor maintenance and biomass production in *Cenchrus* species under abiotic stresses.

Moreover, several tropical forage grasses employ distinct, yet overlapping, strategies to cope with drought stress. Cardoso *et al.* (2015) showed that *Pennisetum purpureum* maintains carbon assimilation under water deficit through sustained stomatal regulation and photosynthetic efficiency, whereas the *Urochloa* hybrid Mulato II reduces stomatal conductance to conserve water. Similarly, Ochola *et al.* (2024) identified drought-tolerant *Urochloa* ecotypes based on traits such as leaf water content, relative WUE, root:shoot ratio, and biomass retention.

In the cool-season C_3 forage and turfgrass tall fescue (*Festuca arundinacea* Schreb), epigenetic modification via H3K4me3 has been reported under heat stress. The transcriptional memory gene *FaHSP17.8-CII*, which regulates ROS accumulation, chloroplast integrity and PSII activity during recurring heat stress, maintains thermo-memory for several days, enabling faster and stronger stress responses (Bi *et al.* 2021).

Drought and other osmotic stresses, such as salinity and cold, trigger the production of abscisic acid (ABA) in plants.

Whereas the early sensing and signaling mechanism of osmotic stress are not fully understood, recent insights into ABA receptors and signaling pathways have opened new avenues for improving abiotic stress tolerance (Bailey-Serres *et al.* 2019). Exogenous application of ABA enhanced drought tolerance in the C_3 creeping grass *Agrostis stolonifera*, as indicated by reduced electrolyte leakage and higher water content under drought stress. Metabolic profiling showed that ABA mainly promoted the accumulation of organic acids associated with tricarboxylic acid cycle (TCA), suggesting an enhancement of respiration-related metabolism to support energy production under stress conditions (Li *et al.* 2017). Concordantly, modulation of endogenous ABA concentrations via overexpression of maize *XERICO* genes, which encode E_3 ubiquitin ligases regulating ABA homeostasis, enhances drought resilience. *ZmXERICO*-overexpressing plants exhibit elevated ABA concentrations and WUE, resulting in improved growth and yield under drought, without compromising performance under well-watered conditions (Brugière *et al.* 2017).

In maize, WRKY transcription factors play key roles in abiotic stress regulation. *ZmWRKY79* is up-regulated under drought, and its overexpression in *Arabidopsis* improved the survival rate under drought. These plants show elevated ABA concentrations and up-regulation of ABA biosynthetic genes, with *ZmAAO3* encoding a key enzyme in the final step of ABA synthesis, identified as a direct *ZmWRKY79* target (Gulzar *et al.* 2021).

Root architecture is crucial for drought tolerance, with deeper rooting enhancing water uptake from lower soil layers. *ZmDRO1* (*Deeper rooting 1*), the maize homolog of a quantitative trait locus (QTL) mapping identified gene from an upland rice cultivar and key regulator of root angle, responds more strongly to ABA and drought in *Zea mays* ssp. *mexicana* than in cultivated maize (B73), contributing to steeper root growth and improved drought avoidance. Driving *ZmDRO1* expression with a synthetic ABA-inducible promoter in maize increased root downward growth and grain yield by over 40% under drought (Guseman *et al.* 2017; Feng *et al.* 2022).

Several traits such as root length, number, angle and volume determine the root architecture, adding to its complexity. Beside *DRO1*, only a few QTLs for these traits have been cloned (Viana *et al.* 2022). The *qSOR1* gene, a homolog of *DRO1*, was identified as a key regulator of root growth angle in rice, through an interplay with auxin. A loss-of-function allele of *qSOR1* promotes shallow, soil-surface rooting, enhancing grain yield under saline and waterlogged conditions. These findings demonstrated that modulating root angles by targeting homologous genes within the same family enables breeders to tailor root systems for different stresses (Kitomi *et al.* 2020).

Anatomical adaptations also enhance drought tolerance in grasses (Viana *et al.* 2022). In maize, root cortical aerenchyma reduces the metabolic cost of soil exploration, promoting deeper roots and greater shoot biomass under drought (Zhu *et al.* 2010; Chimungu *et al.* 2014*a*, 2024*b*). Complementary traits such as fewer cortical cell files and larger cortical cells further improve performance. Similarly, in rice, crown root stele lignification supports water transport and minimizes leakage during drought (Hazman and Brown 2018).

In the *Poaceae*, root systems comprise distinct types defined by their origin and function (Atkinson *et al.* 2014). Crown roots, initiated post-embryonically from basal shoot nodes, are the main channels for water uptake and are strongly regulated by water availability. In the C_4 model grass *Setaria viridis*, local water deficit suppresses crown root growth, whereas rewatering induces *de novo* formation, restoring a crown root-dominated system (Sebastian *et al.* 2016). In maize, fewer crown roots promote deeper rooting and enhanced water acquisition, leading to greater stomatal conductance, biomass, and yield under drought highlighting crown root number as a key trait for drought tolerance (Gao and Lynch 2016).

Elevated temperature, combined with adequate water availability, increases biomass production in *Megathyrsus maximus* and promotes the formation of thinner leaves with thicker cuticles. Under elevated CO_2, this species exhibits reduced stomatal density, index, and conductance, acclimation responses similar to those observed in maize, which lower transpiration, conserve soil moisture, and enhance drought resilience. The interaction between elevated temperature and CO_2 elicits coordinated anatomical, physiological, and molecular adjustments, including altered sugar and amino acid metabolism and transcriptomic reprogramming of protein synthesis and secondary metabolism, resulting in greater leaf expansion, biomass accumulation, and stress tolerance under future climate scenarios (Driscoll *et al.* 2006; Habermann *et al.* 2019; Wedow *et al.* 2019). Nonetheless, several studies have shown that elevated CO_2 may aggravate drought stress by reducing stomatal conductance and limiting transpiration-driven cooling, potentially exposing C_4 leaves to damaging temperature, suggesting that C_4 grasses might exhibit unpredicted responses to global climate changes (Leakey 2009; Jagadish *et al.* 2021).

Furthermore, in *Megathyrsus maximus*, drought treatment enlarges bulliform cells, enhancing leaf rolling, a key water conservation mechanism in grasses (Kadioglu and Terzi 2007). Another group of epidermal cells essential for plant water relations is the stomata, which regulate transpiration and gas exchange. Therefore, manipulation of genes controlling stomatal patterning has been a common strategy to improve WUE in several crops (Zuch *et al.* 2022). However, there are no known reports of transgenic approaches targeting these genes in C_4 forage grasses (Heckman *et al.* 2024).

Overexpression of *EPF1* (*Epidermal patterning factor 1*), which regulates asymmetric cell division in stomatal precursor cells, reduces stomatal density, leading to improved WUE and enhanced drought and heat tolerance in rice (Caine *et al.* 2019). Similarly, in maize, overexpression of *SDD1* (*Stomatal density and distribution 1-1*), a subtilisin-like serine protease that mediated cell-to-cell signaling during stomatal development, causes a 30% reduction in stomata number and a much higher

survival rate owing to a reduced stomatal conductance and transpiration rate on drought treatment (Liu *et al.* 2015).

In the temperate grass model *Brachypodium distachyon*, the *Regulator of flowering and stress* (*BdRFS*) gene, previously associated with flowering time and drought tolerance, was identified as a modulator of stomatal development. Overexpression of *BdRFS* leads to reduced stomatal density and increased stomatal size, regardless of soil moisture, resulting in higher biomass and leaf water content. Conversely, CRISPR-Cas9 knockout lines exhibit the opposite stomatal traits and decreased biomass. Thus, *BdRFS* represents a novel regulator of stomatal adjustment, with potential for engineering drought-resilient grasses (Ying and Scheible 2023).

Abiotic stresses, such as drought and high temperature, often occur simultaneously in nature, leading to more severe effects on plant performance than when each stress acts alone. Climate change is expected to further increase the frequency and intensity of these abiotic stresses, while also altering the distribution and behavior of different insects and pathogens, compounding biomass yield losses (Phophi *et al.* 2020; Zandalinas *et al.* 2021). Importantly, numerous studies have shown that plant responses to combined stresses are unique and not merely additive of individual stress responses (Balfagón *et al.* 2019; Shaar-Moshe *et al.* 2019; Zandalinas *et al.* 2021; Rivero *et al.* 2022). This brings to light the need to understand the molecular basis underlying grass responses to multiple concurrent stresses.

C_4 grasses possess inherent heat and drought tolerance, yet the molecular understanding of these mechanisms in tropical forages is comparatively underexplored relative to temperate grasses and major grass crops. Key adaptations include osmotic adjustment, antioxidant defense, ABA signaling, and root and leaf epidermis modifications enhancing WUE. Advancing molecular insights in tropical forages is pivotal for improving stress resilience and must be integrated into breeding programs targeting climate-change adaptation.

Immune responses in major tropical grasses

Major tropical grasses and other cereals possess sophisticated immune systems adapted to intense pathogen pressures of tropical environments (Azizi *et al.* 2016). Core defenses are constitutive barriers (lignin-enriched cell walls, waxy cuticles, and silica deposits) that impede pathogen entry (Boddy 2016). These preformed structures limit initial loads of invading bacteria, fungi, and viruses but are not impenetrable; on recognition, plants deploy dynamic, rapidly inducible responses that reinforce basal resistance and prime specialized immunity (Azizi *et al.* 2016). Transcriptomic work in *Urochloa* hybrids has indicated modulation of apoplastic peroxidases and cell-wall-remodeling enzymes under abiotic stress, further strengthening these physical defenses (Jones *et al.* 2021).

On pathogen detection, grasses activate pattern-triggered immunity (PTI) via conserved pattern recognition receptors (PRRs) sensing chitin, flagellin. In rice, the chitin-binding protein CEBiP initiates Ca^{2+} influx, mitogen-activated proterin kinase (MAPK) activation, and rapid transcriptional reprogramming (Azizi *et al.* 2016). Outputs include ROS bursts, callose deposition, and pathogenesis related protein (PR-protein) expression that constrain pathogen spread (Doughari 2015; Boddy 2016). In sorghum, pretreatment with pathogen-associated molecular patterns (PAMPs) such as flg22 and chitooctaose primes defenses and enhances basal resistance; challenge with *Herbaspirillum rubrisubalbicans* (red stripe) uncovered resistance-associated QTLs and differential expression of LRR receptors, MAPK1, calcium-binding proteins, and transcription factors (Tuleski *et al.* 2020). In maize, the same PAMPs were used to assay natural variation in ROS and nitric oxide (NO) production and defense-gene expression, showing major and minor QTLs for ROS across diverse germplasm and backgrounds, without overlap with loci controlling quantitative disease resistance (Zhang *et al.* 2017; Wang *et al.* 2021).

Although effective, PTI is often suppressed by pathogen-secreted effectors. In response, grasses evolved effector-triggered immunity (ETI) mediated by nucleotide-binding LRR (NBS-LRR) proteins sensing effectors (Azizi *et al.* 2016). In rice, R proteins such as Pik and Pi-ta trigger a hypersensitive response (HR), cell death that restricts pathogen spread (Rutherford 2013). ETI is faster and robust than is PTI, reprogramming transcription and defenses. Beyond R genes, quantitative and non-host resistance confers protection in maize/sugarcane (Bettgenhaeuser *et al.* 2014). Aphid resistance in *Cenchrus clandestinum* suggests ETI or inducible defenses (Miyasaka *et al.* 2007). Intracellular signaling after pathogen perception engages calcium-dependent kinases, GTPases (OsRac1), and WRKY, NAC, bHLH transcription factors, coordinating immune expression and rapid ROS bursts (Azizi *et al.* 2016; Herlihy *et al.* 2020). In *Urochloa*, genotype-dependent signaling underpins abiotic-stress resilience (Jones *et al.* 2021). Also, hormonal crosstalk fine-tunes immunity. Salicylic acid (SA) defends against biotrophs and promotes systemic acquired resistance, whereas jasmonic acid (JA) and JA-Ile target necrotrophs and herbivores (Shyu and Brutnell 2015; Mishra *et al.* 2021). Yet, in tropical forage grasses, metabolic modulation remains under-characterized, and metabolomic studies dissecting SA, JA, or SA–JA antagonism during infection are scarce.

A key feature reported in tropical grasses, including sugarcane, is rapid accumulation of pathogenesis-related proteins (chitinases, glucanases, thaumatin-like proteins, protease inhibitors) that degrade walls, inhibit microbial enzymes, and amplify defense signaling (Souza *et al.* 2017). Lipid-transfer proteins and defensins fortify apoplast and suppress pathogens, whereas nutrient sequestration in infection sites sustains ROS and starves invaders (Herlihy *et al.* 2020).

Building on these inducible defenses, tropical grasses exploit defense priming, whereby pathogen or elicitor exposure imprints epigenetic memory, enabling faster, stronger responses on reinfection (Rutherford 2013). This primed state underpins local resistance and systemic acquired resistance, optimizing

resource allocation under field constraints (Shyu and Brutnell 2015). Collectively, constitutive barriers, PTI/ETI, signaling networks, hormonal crosstalk, PR proteins, and priming craft a defense architecture, guiding breeding for durable resilience in tropical agroecosystems.

Brachypodium distachyon illustrates how model grasses clarify cereal immunity. With a compact genome, it deploys PTI and ETI against rusts (*Puccinia* spp.), *Fusarium* spp., and viruses *Panicum mosaic* and *Barley stripe mosaic*. Pre- and post-haustorial barriers limit rust, whereas infection up-regulates *PR1/PR2/PR5* and ethylene-response factors (Mandadi and Scholthof 2013; Gill *et al.* 2015). Antiviral defense relies on SA-driven activation with JA/ET suppression (Mandadi *et al.* 2014). Brassinosteroid signaling modulates resistance; BRI1 mutants show heightened immunity via de-repressed BZR1/WRKY factors (Goddard *et al.* 2014). Furthermore, post-transcriptional silencing targets viral RNAs, and UDP-glycosyltransferases detoxify mycotoxins (Lyons and Scholthof 2015; An *et al.* 2016).

Complementing C_3 models, *Setaria viridis* exemplifies C_4 immunity. *Brachypodium* and *Setaria* up-regulate SA-dependent PR1/PR5 after viral challenge, yet *Setaria* shows distinct timing and magnitude of JA/ET signaling and SAR, reflecting C_4-specific metabolic–environmental adaptations. These contrasts guide breeding and management for mixed C_3–C_4 pastures, optimizing growth–defense trade-offs (Mandadi *et al.* 2014).

Across all these grasses, the following themes emerge: PRR-mediated perception initiating PTI and ETI; SA, JA, and ET balancing defense with growth; secondary metabolism, via phenylalanine ammonia-lyase (PAL), tryptophan decarboxylases, and UDP-glycosyltransferases, generating antimicrobials and fortifying walls (Mandadi and Scholthof 2013; Figueroa *et al.* 2015; Negawo *et al.* 2024). Beneficial microbes such as *Pseudomonas fluorescens* prime induced systemic resistance (ISR) in *Urochloa*, indicating analogous pathways in *Brachypodium* (Gagné-Bourque *et al.* 2015; dos Santos Lopes *et al.* 2018). Nevertheless, forages need deeper genomic/transcriptomic resolution.

Enhancing yield in forage grasses through morphological and developmental adaptation

In perennial pastures, the continuous growth of leaves and tiller enables rapid leaf area recovery after grazing, thereby sustaining forage yield over time. Consequently, most yield-related traits are evaluated in the field across extended periods, often involving multiple harvests. This repeated-cutting scenario introduces variation among cuts, creating a dynamic quite distinct from that observed in annual species, where evaluations are typically limited to a single growing cycle (Simeão *et al.* 2021).

Grasses commonly experience defoliation through grazing and hay harvesting, which reduces leaf area, photosynthesis, and carbon and nitrogen uptake. To cope, they have evolved mechanisms to rapidly regrow by reallocating internal nutrient reserves (Liu *et al.* 2020). These adaptations support early recovery, yet the molecular basis regulating these responses in tropical grasses remains unknown. Reflecting this trait, sudangrass (*Sorghum bicolor ssp. drummondii*) is known for its vigorous regrowth after cutting, aided by its high tillering capacity and finer, leafier shoots than in forage sorghum. This regrowth ability supports multiple harvests and resilience in adverse climates. To elucidate the genetic basis of this trait, Yonemaru *et al.* (2022) conducted a QTL analysis using populations derived from *bmr* sorghum (described in the next section) × sudangrass crosses, and QTLs for enhanced regrowth were identified on Chromosomes 3, 6, 7, and 10, with sudangrass alleles on Chromosomes 7 and 10 positively contributing to this trait.

Reallocation of sugar reserves is critical for regrowth in grasses following defoliation, involving both mobilization and targeted transport of carbohydrates to developing tissues. These processes involve significant transcriptional changes in genes encoding key enzymes of carbohydrate metabolism, including invertases, fructosyltransferases, and fructan exohydrolases (FEHs) (Liu *et al.* 2020). In ryegrass, defoliation triggers fructan degradation and induces expression of *LpSUT1*, a sucrose transporter gene, enabling efficient redistribution of sugars (Meuriot *et al.* 2022). Moreover, nitrogen and gibberellins both enhance regrowth in perennial ryegrass, but through distinct effects on carbohydrate metabolism, gibberellins by promoting reserve mobilization, and nitrogen by improving photosynthate use and reducing fructan accumulation (Liu *et al.* 2020).

The transition to flowering is a key developmental shift in plants, and delaying it can boost biomass by extending the vegetative growth phase in forage grasses. Model species have been key to uncovering flowering time mechanisms for crop improvement, and many of these regulatory factors appear to be conserved. However, these remain poorly understood in perennial C_4 forage grasses (Nuñez and Yamada 2017).

Among the several molecular players that respond to the myriad of endogenous and external cues that regulate flowering, *Flowering locus T* (*FT*) is the most important. In switchgrass (*Panicum virgatum*), a C_4 perennial used for forage and biofuel, ectopic expression of *PvFT1* and *PvAP1-3*, a MADS-box transcription factor and downstream target of FT, induces extremely early flowering, confirming their roles as strong flowering activators in C_4 grasses (Niu *et al.* 2016). In *Brachypodium distachyon*, the monocot-specific regulator *Indeterminate1* (*BdID1*) promotes flowering by activating multiple *FT*-like genes and repressing the flowering inhibitor *FTL4*. Loss of *BdID1* delays flowering, whereas its target *FTL9* partially rescues this phenotype (Liu *et al.* 2023).

Regarding warm-season C_4 grasses, several studies in sorghum have shown conserved regulatory components in the flowering pathway when compared with *Arabidopsis* and rice. The sorghum gene *SbCO* functions as a homolog of *CONSTANS* (*CO*) in *Arabidopsis* and an ortholog of (*Heading-date 1*) *Hd1* in

rice, promoting flowering under both short- and long-day conditions. Similarly, *(Early Heading-date 1) SbEhd1* acts analogously to *Ehd1* in rice by activating downstream florigens such as *SbCN8 (CENTRORADIALIS 8)*, *SbCN12*, and *SbCN15*, which are homologous to *FT* in *Arabidopsis* and *Hd3a* in rice (Murphy *et al.* 2014; Yang *et al.* 2014; Nemoto *et al.* 2016).

Plants regulate flowering time through photoperiodism, by using the phytochrome system to sense day length and light quality (Nuñez and Yamada 2017). In *Brachypodium distachyon*, phytochrome-interacting factors (PIFs) play a key role in light-mediated developmental processes, although their functions are less studied than in dicots. Recent RNAi-based studies on *BdPIL1* and *BdPIL3*, two PIF-like genes, showed their involvement in regulating flowering time, plant height, cell number, and chlorophyll biosynthesis (Hoang *et al.* 2021).

Dense, uniform canopies in tropical grasses often cause self-shading, reducing carbon fixation in lower leaves and overall light-use efficiency. Selecting for more upright leaf angles improves canopy light distribution, photosynthesis, and potentially WUE, by optimizing carbon gain relative to water loss (Drewry *et al.* 2014). In sorghum, a non-functional *DWARF-3* allele, encoding a P-glycoprotein for auxin transport, decreases leaf angle by up to 34%, enhancing light penetration and increasing predicted biomass yield by at least 3% (Truong *et al.* 2015). Similarly, in maize, *ZmCLA4*, the *LAZY1* ortholog and auxin transport repressor, also regulates leaf angle (Zhang *et al.* 2014).

As seen in perennial grasses, high biomass yield is a key forage trait. Breeding efforts focus on extending vegetative growth and delaying flowering, with emphasis on functional *stay-green* traits that prolong leaf photosynthesis and boosts biomass. Moreover, delayed senescence and nitrogen remobilization also help retain leaf protein, improving forage quality at harvest (Yang and Udvardi 2018). In grasses, transcription factors of the NAC family have been implicated directly through genetic studies in the regulation of senescence and N remobilization from leaves. It was reported in switchgrass that overexpressing *pvNAC2* plants exhibit increased above-ground biomass, linked to up-regulated nitrogen metabolism genes in leaves and transporter genes in roots (Yang *et al.* 2015). In maize, a novel QTL controlling functional *stay-green* was mapped to *NAC7*. Transcriptome analyses showed that NAC7 regulates genes involved in photosynthesis, chlorophyll degradation, and protein turnover, positioning it as a key negative regulator of *stay-green* and a promising target for yield improvement (Zhang *et al.* 2019*a*).

Moreover, hormone metabolism is also a key regulator of organ senescence, and ectopic expression of *Isopentenyl transferase* (*IPT*) gene, which encodes a key enzyme in cytokinin biosynthesis, driven by a senescence-inducible promoter, delays leaf senescence in *Zoysia sinica* (Guo and Gan 2014). Concordantly, overexpression of the transcription factor *Stenofolia* (*STF*), a *WOX* family regulator, enhances biomass yield by modulating cytokinin metabolism (Wang *et al.* 2017).

Collectively, in perennial subtropical forage grasses, the coordination of carbohydrate and nitrogen metabolism, hormonal signaling, and flowering regulation, within a conserved molecular framework for photoperiodic control shared across grass species, drives regrowth and productivity. Still, the molecular basis of these traits is largely unknown, limiting progress in genetic improvement.

Key factors influencing nutritional quality in forage grasses

Forage biomass consists primarily of plant cell walls, which are composed of complex polymers including the structural polysaccharides cellulose, hemicellulose and pectin, as well as the polyphenol lignin (Loqué *et al.* 2015). The plant cell wall is the main sources of carbohydrates and dietary fiber for ruminants, serving as a substrate for the rumen microbial ecosystem. Cellulose, hemicellulose and pectin comprise 90% of the dry matter (DM) of cell walls, and their contents are negatively associated with DM intake, DM digestibility and animal performance, with lignin acting as the primary factor limiting cell wall digestion (Li 2021).

Even though many C_4 forage grasses species exhibit higher biomass productivity and growth rates than their temperate counterparts, this advantage often comes at the cost of reduced digestibility. The rapid growth and high photosynthetic efficiency of these grasses are supported by specialized anatomical features such as the presence of lignin deposits in the thick-walled parenchyma bundle-sheaths around each vascular bundle, which act as a physical barrier to microbial enzymes, reducing cellulose and hemicellulose breakdown and limiting digestible energy for ruminants (Wilson 1994; Giordano *et al.* 2014; Heckman *et al.* 2024; Monson *et al.* 2025). Hence, manipulation of the lignin biosynthetic pathway is a key focus of breeding programs.

Gaining genetic control of cell wall composition and architecture is fundamental to improving forage digestibility, reducing greenhouse emissions and advancing sustainable livestock production (Tedeschi *et al.* 2023). Maize breeders aim to improve quality by increasing the digestible sugar content, often by reducing lignin concentration. Brown midrib (*bm*) mutants have significantly reduced lignin content and enhanced cell wall digestibility (Barrière *et al.* 2004). Six *bm* loci have been identified to date. The *bm1*, *bm2*, *bm3* and *bm4* encode cinnamyl alcohol dehydrogenase (CAD), methylenetetrahydrofolate reductase (MTHFR), caffeic acid O-methyltransferase (COMT) and folylpolyglutamate synthase (FPGS) respectively. The *bm5* locus encodes 4-coumarate: coenzyme A ligase (4CL), *bm6* has been localized to a 180-kb region on Chromosome 2, although the underlying gene has yet to be identified. Among these, *bm3* has been widely utilized in commercial forage hybrids, owing to its consistent improvement in silage intake and milk production (Li *et al.* 2022).

The challenge lies in reducing lignin and other cell wall components to enhance digestibility without compromising

plant growth or survival. In maize, *bm* lines and hybrids often exhibit reduced vegetative vigor, increased stalk breakage and lower grain and stover yields. Similar yield penalties have been observed in *bm* hybrids of Sudangrass and sorghum. Contrary, polymorphisms in lignin biosynthetic genes, specifically those encoding 10 enzymes in the monolignol pathway, have been associated not only with cell wall digestibility but also with key agronomic traits in maize, including plant height, DM yield, and flowering time. The absence of adverse pleiotropic effects suggests that selecting favorable alleles can simultaneously improve both forage quality and crop performance (Chen *et al.* 2010).

However, altering the phenylpropanoid pathway, essential for lignin biosynthesis, can boost digestibility yet impose costs on growth and defense. In *Brachypodium distachyon*, RNAi suppression of PAL reduced lignin (~43%) and wall-bound ferulates (~57%), nearly doubling released carbohydrates and improving digestibility, but also delayed development, curtailed root growth, and heightened susceptibility to fungi such as *Fusarium culmorum* and *Magnaporthe oryzae* (Cass *et al.* 2015; Matušinsky *et al.* 2022). Tropical forages show analogous trade-offs; *Cinnamoyl1-CoA reductase* (*CCR*) gene down-regulation in *Paspalum dilatatum* lowered lignin and altered composition, and CAD suppression in *Paspalum notatum* increased *in vitro* digestibility while producing undesirable growth phenotypes in some lines (Giordano *et al.* 2014; Muguerza *et al.* 2014). In switchgrass, COMT-attenuated lines reduced recalcitrance without increasing field rust (*Puccinia emaculata*) susceptibility. Over two seasons, transgenics had 8–16% less lignin, a 23–41% lower syringyl-to-guaiacyl ratios from the reproductive stage onward, and 15–50% greater sugar release with growth and yield than the controls; rust damage did not differ at any sampling (Baxter *et al.* 2016).

Complementing this, in a comprehensive QTL study examining dynamic changes in cell-wall composition and digestibility traits across five developmental stages after silking, 72 QTLs were identified, including six major hotspots in bins 1.08, 2.04, 2.07, 7.03, 8.05, and 9.03 (Li *et al.* 2022). Notably, within the pleiotropic QTL region in bin 1.08, six candidate genes involved in cell-wall biosynthesis were found, including three lignin-related genes and three cellulose synthase genes (Li *et al.* 2022). Similarly, Lopez-Malvar *et al.* (2021) identified several *MYB* transcription factors within QTL regions linked to forage digestibility in maize. *MYB51* and *MYB22*, associated with neutral detergent fiber (NDF), belong to functional groups related to cell-wall development and thickening, suggesting roles in fiber accumulation. *MYB38*, located within a QTL for digestibility of organic matter, was previously shown to be down-regulated in brown midrib (*bmr1* and *bmr2*) mutants with enhanced digestibility.

Razar *et al.* (2021) conducted QTL mapping in an F_1 switchgrass population to evaluate biomass composition and forage quality by using near-infrared spectroscopy (NIRS). They measured traits including crude protein (CP), NDF, acid detergent fiber (ADF), hemicellulose, cellulose, and Klason lignin. A total of 37 significant QTL were identified, with individual loci explaining 4.9–8.6% of the phenotypic variance. Several chromosomal regions harbored co-localized QTL for multiple traits, suggesting pleiotropy or tight linkage. Moreover, in a study on 178 half-sib families of *Urochloa ruziziensis*, narrow-sense heritability estimates ranged from 0.26 for DM yield to 0.42 for *in vitro* DM digestibility, indicating moderate genetic control over these traits. Genetic correlations showed that selecting for higher DM yield may inadvertently reduce CP content, while selection for low fiber or high CP could enhance forage quality without compromising digestibility (Santos *et al.* 2024).

The study of *Megathyrsus maximus* genotypes conducted by Roy *et al.* (2020) showed substantial genetic variation among the evaluated germplasm, with distinct clustering patterns correlating with differences in nutritional attributes such as crude protein, fiber content, and digestibility. Multivariate and cluster analyses identified groups of genotypes exhibiting superior forage quality, highlighting the potential of certain clusters for targeted improvement.

The concentration of atmospheric CO_2 and temperature are pivotal drivers of ecosystem productivity, carbon balance, and food security. In *Megathyrsus maximus cv. Mombasa*, elevated CO_2 (0.0245 mol/m^3 ppm) enhanced photosynthesis, reduced stomatal conductance, and improved WUE and plant carbon content. However, stem biomass increased without a corresponding rise in leaf biomass, reducing the leaf:stem ratio and negatively affecting forage nutritional value by lowering leaf nitrogen and increasing fiber content. Elevated temperature (+2°C) increased photosynthesis and biomass but reduced phosphorus (P) and potassium (K) concentrations, forage quality and digestibility. Under combined eCO_2 and warming, warming offset CO_2-induced improvements in water relations, while further exacerbating declines in nutritive value, resulting in 12% higher ADF, 28% higher lignin and a 19% decrease in CP, causing a 15% reduction in digestibility. These declines are driven primarily by changes in leaf chemical composition rather than tissue proportions, being consistent with patterns observed in both C_4 and C_3 grasses under warming and drought, and may compromise livestock performance and increase methane emissions (Habermann *et al.* 2022, 2024). This highlights the need for multifactorial field studies to assess forage responses under future climate scenarios.

Besides cell wall composition, anti-nutritional factors such as plant secondary compounds can affect the forage crop palatability and intake. In sorghum, for instance, the cyanogenic glucoside dhurrin can release toxic hydrogen cyanide (HCN) on tissue disruption, posing a risk to animal health and reducing acceptability (Wang *et al.* 2024*b*). To mitigate this issue, transgenic approaches have targeted *CYP79A1*, the key gene in dhurrin biosynthesis that encodes a cytochrome P450 enzyme. Antisense-mediated down-regulation of *CYP79A1* has been shown to significantly reduce HCN concentrations in sorghum leaves and stems, thereby

enhancing its palatability and suitability as a forage crop (Pandey *et al.* 2019).

Overall, these studies have emphasized that genetic variation in forage grasses influences key nutritional traits, including fiber composition, digestibility, and protein content. Transcriptional regulators and specific genomic regions play central roles in controlling cell wall biosynthesis, offering potential targets for improving forage quality. Nonetheless, breeding strategies need to balance enhanced digestibility with maintaining plant growth, and abiotic and biotic stress tolerance. Compared with research on bioenergy crops, research on C_4 perennial forage species remains limited, highlighting the importance of leveraging existing germplasm to guide future improvement programs.

Genetic resources and genomic tools in tropical forage grasses

Through traditional grass forage breeding, several elite cultivars have been developed over the past decades; however, genetic gains have remained limited and molecular breeding efforts are still lacking due to the scarcity of genomic resources for these species, because their genomic complexity hinders advances in molecular approaches to genetic improvement. In contrast to major cereals such wheat and rice, where genomic and genetic resources are well developed, only a few chromosome-scale genome assemblies of tropical grasses are publicly available, including; *Megathyrsus maximus* cv. Mombasa (Jauregui *et al.* 2024), two diploid genotypes of *Urochloa ruziziensis* (Pessoa-Filho *et al.* 2019; Worthington *et al.* 2021) and *Paspalum notatum* Flüggé var. *saurae* (Vega *et al.* 2024). Maximizing the future impact of livestock breeding programs requires the integration of genotyping, phenotyping and envirotyping approaches, which are critical for enhancing genetic gain.

Genetic variability is pivotal for breeding, as it fuels selection and long-term improvement. Institutions such as EMBRAPA, CIAT, IBERS, ICARDA, ILRI, ARDI and USDA preserve this variability in their germplasm banks for different species of *Megathyrsus*, *Urochloa* and *Cenchrus*, providing genetic resources adapted to a wide range of edaphoclimatic conditions (Simeão *et al.* 2021). In fact, germplasm introduction remains a key strategy in forage breeding, involving the evaluation and selection of accessions as a fundamental step in cultivar development (Jank *et al.* 2011; Paul *et al.* 2020). Moreover, in grasses, phenotypic identification based on traditional morphological traits is often challenging due to their high plasticity and susceptibility to environmental and developmental influences. As a result, morphological characterization alone may not provide reliable intraspecific differentiation. In this context, molecular markers offer a valuable tool for uncovering genetic variability and accurately distinguishing genotypes, independent of external factors (Jesus *et al.* 2024).

For better use and understanding of these available resources, in-depth molecular and phenotypic characterization is necessary. Molecular markers are widely used in this context to assess genetic diversity and structure within and among germplasm collections. In *Urochloa* species, numerous studies have applied random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), and especially simple sequence repeat (SSR) markers. These have uncovered substantial genetic variation both within and among species, often grouping accessions into distinct clusters. SSR markers, in particular, stand out for their high discriminatory power and cross-species transferability, supporting their continued use in genetic resource characterization (Azevedo *et al.* 2011; Vigna *et al.* 2011; Tegegn *et al.* 2019; Namazzi *et al.* 2020).

Another widely used tool to define genomic regions responsible for relevant agronomic traits is genetic mapping. This approach has been used as the main primary source of genetic data for non-model species, such as tropical forage grasses. The elaboration of dense linkage maps helps uncover genome structure and evolution, support mapping of both simple and complex traits, and contribute to enhancing genome assembly (Flint-Garcia *et al.* 2003). Deo *et al.* (2020) mapped 10 QTLs for agronomic traits and 36 QTLs for nutritional traits in guinea grass (*Megathyrsus maximus*). They identified 23 QTL-rich regions associated with these traits. Due to the lack of a reference genome for guinea grass, genotyping-by-sequencing (GBS) reads were aligned to the *Panicum virgatum* genome, with comparative analysis using *Arabidopsis thaliana* and *Oryza sativa*. This approach showed 55 gene families related to hormone signaling, metabolism, and cell wall biosynthesis, candidate genes that represent valuable targets for marker-assisted selection in tropical forage breeding programs.

To evaluate agronomic traits through a genome-wide approach, GBS was applied in *Urochloa ruziziensis* under contrasting environmental conditions. Predictive models that integrated conventional genomic prediction with machine learning and feature selection were used to improve accuracy and reduce marker complexity. Tree-based analyses identified single-nucleotide polymorphisms (SNPs) linked to QTL regions, whereas multi-omic strategy integrating transcriptome data and gene co-expression networks showed candidate genes involved in auxin transport, lignin biosynthesis, stress response, and circadian regulation, key processes determining agronomic traits. Heritabilities ranged from 0.44 to 0.92, with a mean predictive ability of 0.762, highlighting the potential of integrative genomic tools in tropical forage breeding (Martins *et al.* 2023).

Transcriptome studies enable the identification of the molecular components at cell and tissue levels, providing insight into functional elements of the genome and the molecular mechanisms involved in diverse biological processes, including responses to biotic and abiotic stresses, as well as during key developmental stages. These studies help elucidate gene regulatory networks that drive plant growth, differentiation

and adaptation. In this context, two transcriptomic studies of the *Urochloa* species were conducted to investigate aluminum and drought tolerance. These analyses identified differentially expressed genes (DEGs) between genotypes classified as tolerant and sensitive to aluminum and drought stress respectively, highlighting the utility of transcriptomic approaches in uncovering candidate genes potentially associated to traits of agronomic and economic interest (Salgado *et al.* 2017; Jones *et al.* 2021; Worthington *et al.* 2021).

Genome wide association (GWAS) has been extensively applied for rapid mining and identification of key genes for yield, nutritional value and stress resistance traits. In *Lollium perenne*, GWAS study identified DNA polymorphisms significantly associated with reduced leaf growth under water stress, located near genes encoding phytochrome B and the MYB41 transcription factor, two genes previously described as key regulators of drought and osmotic stress responses (Lippold *et al.* 2009; Liu *et al.* 2012; Kosma *et al.* 2014; Pearce *et al.* 2016; Jaškūnė *et al.* 2020).

Altogether, genetic resources in forage grasses harbor valuable variation for key agronomic traits, offering opportunities for targeted improvement. For instance, in *Megathyrsus maximus*, multi-variate and multi-harvesting trials identified accessions with consistently high plant height, green forage weight, and DM yield across environments, providing key material for breeding resilient, high-performing cultivars (Carvajal-Tapia *et al.* 2022).

Most tropical forage grasses, including *Urochloa* species, are natural polyploids, and differences in ploidy levels act as reproductive barriers that limit hybridization (Fig. 1). Colchicine-induced chromosome doubling allows the production of interploid *Urochloa* hybrids, enabling the development of new cultivars with improved traits. However, polyploidy increases genomic complexity, generating multiple alleles per locus, high heterozygosity, and complex segregation patterns, which complicates genomic studies (Ferreira *et al.* 2021; Schaart *et al.* 2021). In contrast to forage grasses, where apomixis is prevalent, apomixis is largely absent in major crops, although it is found in their wild relatives. Transferring this trait to crops to fix heterosis has been shown to be difficult, mainly because of limited understanding of its underlying mechanisms. In recent years, several candidate genes have been identified and functionally analyzed in model species such as *Arabidopsis* and rice (Bellido *et al.* 2021).

Artificial hybridization and controlled crosses in grasses are often challenging because of aposporous apomixis, an asexual mode of reproduction, in which seeds develop without fertilization, resulting in progeny that are genetic clones of the female parent. Molecularly, this trait is governed by the apospory-specific genomic region (ASGR), which is highly conserved among forage grasses species, and typically follow a 1:1 Mendelian segregation, suggesting monogenic inheritance. In both *Urochloa* and *Megathyrsus*, the ASGR has been mapped to non-recombinant, hemizygous regions through QTL and linkage analyses, reinforcing its stable association with apomixis (Worthington *et al.* 2016; Deo *et al.* 2020). In *Pennisetum squamulatum*, the *PsASGR-BBML* (*Baby boom-like*) gene, located within the ASGR, is a candidate regulator of parthenogenesis. Functional studies using RNAi knockdown and transgenic approaches in sexual pearl millet demonstrated that *BBML* controls the formation of asexual embryos. F_1 hybrids inheriting both the ASGR and the RNAi construct showed reduced parthenogenetic embryo sacs, confirming the gene's role in enabling parthenogenesis and its potential to transfer this trait via hybridization (Conner *et al.* 2015). Consequently, the transfer of one of the *BBM-like* genes into other sexual grasses, such as rice and maize, is sufficient to trigger embryo formation without fertilization (Wang and Underwood 2023) (Fig. 1).

Despite its reproductive constraints, apospory enables breeders to capture, fix and propagate the hybrid vigor in superior heterozygous genotypes via seed, resulting in uniform and high-quality cultivars (Ferreira *et al.* 2021). Last, synthetic apomixis has been successfully engineered to obtain clonal seeds from sexual plants like *Arabidopsis* and rice (Wang and Underwood 2023).

Albeit the recent expansion of genomic resources in tropical grasses, several critical aspects of their immune systems remain poorly understood. In particular, antiviral defenses in monocots are less well characterized than are those in dicots, underscoring the need for deeper investigation in model grasses such as *Brachypodium* and tropical grasses (Mandadi and Scholthof 2013). Integrating multi-omics approaches, including transcriptomics, proteomics and metabolomics, under field conditions is essential to unravel the complex defense networks involved (Guo *et al.* 2019). Moreover, high-resolution mapping of quantitative resistance loci and genomic regions linked with non-host resistance will support the development of molecular markers for breeding (Bettgenhaeuser *et al.* 2014). Equally important is elucidating how abiotic stresses intersect with immune signaling pathways, as tropical grasses are frequently exposed to multiple environmental challenges simultaneously (Devi *et al.* 2017). Addressing these knowledge gaps will not only enrich our basic understanding of plant immunity, but will also inform strategies for engineering crops resilient to evolving pathogens (García-Guzmán and Heil 2014).

The model *Brachypodium distachyon* provides valuable genomic resources and insights. Tan and Wu (2012) cataloged 126 NBS-LRR genes across four subfamilies, mapped stress-responsive promoter elements, and validated expression via expressed sequence tags (ESTs) and full-length cDNAs. Complementarily, QTL mapping by Barbieri *et al.* (2012) pinpointed loci conferring resistance to *Puccinia brachypodii* at various growth stages, and Gilbert *et al.* (2018) identified two dominant non-host resistance loci (Yrr1, Yrr2) conferring immunity to wheat stripe rust. Additionally, host-induced gene silencing (HIGS) against *Fusarium graminearum* genes reduced disease severity in *Brachypodium*, demonstrating translational potential across cereals.

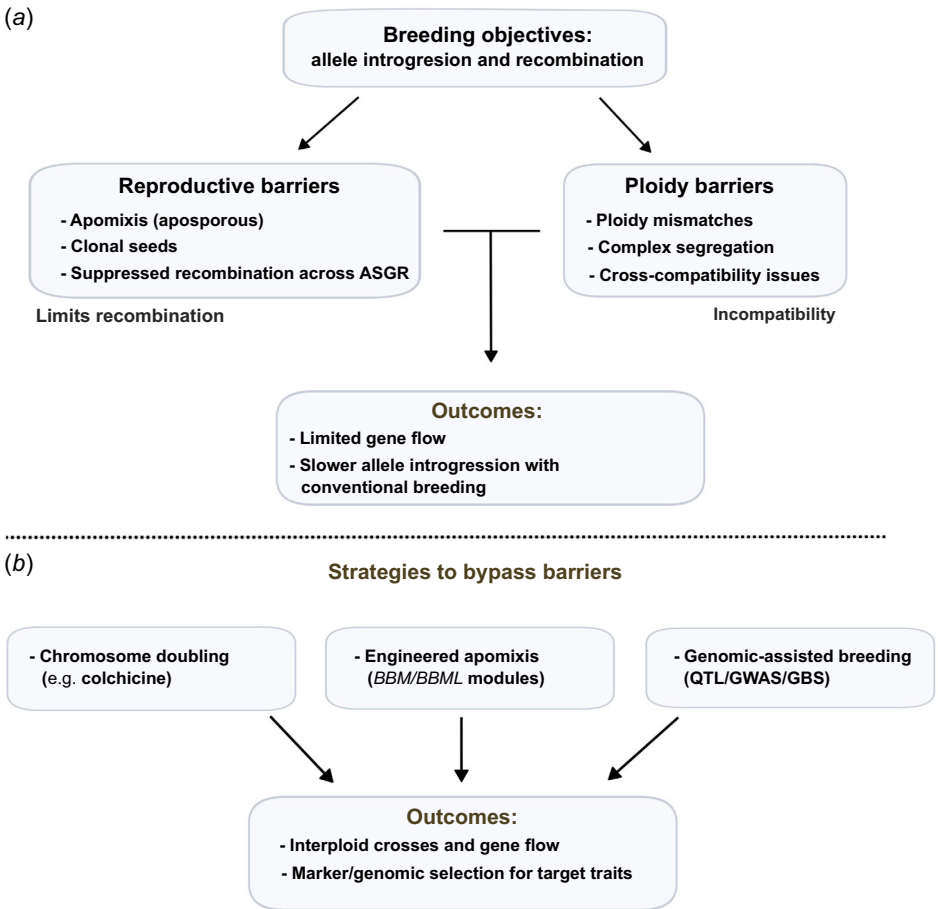

Fig. 1. Breeding barriers and strategies to enable allele introgression in tropical forage grasses. (*a*) A diagram summarizing how reproductive and ploidy barriers constrain allele introgression and reduce recombination-based breeding efficiency. Reproductive barriers: aposporous apomixis produces clonal seed and suppresses recombination across the apospory-specific genomic region (ASGR), limiting gene exchange. Ploidy barriers: mismatched ploidy levels, complex segregation, and cross-compatibility issues further restrict gene flow. (*b*) Proposed strategies to bypass barriers. Chromosome doubling (e.g. colchicine) to enable interploid crosses; engineered apomixis using BABY BOOM/BABY BOOM-like (BBM/BBML) modules (or synthetic apomixis) to manage reproduction; and genomics-assisted breeding (QTL, GWAS, GBS) to track and select target loci.

Building on insights from *Brachypodium*, recent studies have begun to unravel immune mechanisms in tropical grasses. *Urochloa* hybrids display cultivar-dependent resistance to blast (*Pyricularia* spp.) and spittlebugs. In inoculation trials; Xaraés, BRS Tupi and BRS Ipyporã exhibited low disease severity, reflecting rapid activation of both constitutive barriers, such as enhanced cell-wall lignification, and inducible defenses (Krug *et al.* 2024; Simon *et al.* 2024). Moreover, spittlebug resistance correlates with trichome density, cuticular wax composition and lignin deposition in vascular tissues, suggesting integration of structural and hormone-mediated biochemical responses, particularly via phenylpropanoid pathways (Negawo *et al.* 2024; Begnami *et al.* 2025). Recent GWAS have begun to map quantitative trait loci (QTLs) for stress and immunity traits in *Urochloa*, highlighting candidate genes encoding PAL, U-box ligases and ankyrin repeat proteins linked to resistance (Ferreira *et al.* 2021; Singh *et al.* 2024).

In arid regions, *Cenchrus ciliaris* is indispensable, and GWAS have identified defense-associated genomic regions. Crucially, a QTL adjacent to a *PAL*-like gene implicates phenylpropanoid-mediated lignin, flavonoid and SA biosynthesis in both structural and systemic resistance. Additionally, markers tethered to ubiquitin ligases suggest proteasome-mediated protein turnover as a defense regulator. Although molecular insights remain sparse, these markers offer entry points for elucidating immunity in polyploid forage grasses, with potential to strengthen phenolic compound accumulation and cell-wall fortification. In the same study, 78 significant marker–trait associations were identified for biomass yield and feed quality traits. Among these, 24 markers were linked

to CP, and multiple markers were also associated with total digestible nutrients (TDN), NDF, ADF, and DM intake. Importantly, some markers affect two or more nutritional traits, suggesting pleiotropy or close linkage, which provides opportunities for simultaneous improvement of forage quality components (Negawo *et al.* 2024).

Transgenic and gene editing in C_4 grasses

Transgenic technology complements conventional plant breeding and has played a critical role in agricultural advancement by introducing beneficial foreign genes or modifying the expression of endogenous genes to enhance existing traits or introducing novel ones absent in the given crop species (Visarada *et al.* 2009; Adegas *et al.* 2022). Moreover, this technology is also pivotal for functional genetic studies, enabling precise analysis of gene roles and regulatory mechanisms underlying stress response and development; yet, the application of this tool in tropical grass forage is scarce (Fu *et al.* 2024).

Giordano *et al.* (2014) reported decreased lignin content and altered lignin composition in *Paspalum dilatum* by silencing *CCR* gene, which catalyzes the first step in the lignin-specific branch of the phenylpropanoid pathway, making it a viable target for lignin modification. In parallel, transgenic plants of switchgrass that constitutively expressed an *Arabidopsis* NAC transcriptional factor gene *Long vegetative phase one* (*AtLOV1*) exhibit reduced leaf angle, altered lignin content and delayed flowering time (Xu *et al.* 2012).

Dehydration-responsive element binding (*DREB*) transcription factors regulate abiotic stress responses via the ABA-independent pathway by binding to the DRE/CRT (C-repeat) element in stress-inducible genes. In *Paspalum notatum*, expression of the *DREB1A* gene from xeric wild barley (*Hordeum spontaneum*) driven by the stress-inducible *HVA1s* promoter conferred tolerance to severe salinity and repeated dehydration stress (James *et al.* 2008).

Moreover, overexpression of *LpHUB1*, a ubiquitin-like modifier, enhances drought tolerance in perennial ryegrass by maintaining higher leaf water potential, chlorophyll content, and photosynthetic rate under stress, while overexpression of *LpCYP72A15*, a cytochrome P450 gene, confers tolerance by reducing ROS concentrations, enhancing antioxidant enzyme activity and increasing soluble sugar content (Patel *et al.* 2015; Xing *et al.* 2024).

Overexpression of *PIF4* in *Cynodon dactylon* enhances cold tolerance (Xu *et al.* 2025). Similarly, transgenic ryegrass ectopically expressing wheat *Multiprotein bridging factor 1*(*MBF1c*) gene, shows improved thermotolerance owing to enhanced antioxidative capacity, water retention, chlorophyll content and net photosynthetic rate under stress conditions (Huang *et al.* 2022).

Regarding plant immunity, combining PTI and ETI with defense priming and hormonal crosstalk enhances resistance without compromising yield. Transgenic overexpression of pathogenesis-related proteins such as chitinases and thaumatin-like proteins offers broad-spectrum protection, whereas modulation of WRKY transcription factors, key regulators of plant immune responses helps balance growth and defense. Insights from rice and sorghum can be translated to improve tropical forage grasses (Souza *et al.* 2017).

Until recently, genome modification in crops primarily relied on the random integration of exogenous DNA or the untargeted induction of mutations through physical or chemical mutagens. However, in recent years, targeted genome editing systems such as zinc finger nucleases (*ZFN*s), transcription activator-like effector nucleases (TALENs) and clustered interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR-Cas9), have been widely applied in various cereals and forage crops. Among these, CRISPR-Cas9 uses site-specific or guide RNA-directed nucleases to precisely clave or modify target gene sequences (Hilscher *et al.* 2017; Fu *et al.* 2024).

Genome editing tools offer a promising solution to the limited success achieved over decades of traditional breeding in several forage grasses. Recent studies have demonstrated the rapid domestication of underutilized crops using such techniques, highlighting their potential to improve valuable agronomic traits in orphan species (Østerberg *et al.* 2017; Zsögön *et al.* 2018). Recent advances in CRISPR technologies have shown that, despite the complexity of polyploid genomes, efficient genome editing is achievable in several polyploid grass species. For instance, Beyene *et al.* (2022) reported the successful generation of semi-dwarf, lodging resistant tef (*Eragrostis tef*) lines using CRISPR-Cas9 based genome editing. This was achieved through a knock-out mutation of the tef *SD-1* gene, the orthologue of the rice *SD-1* gene, which encodes the enzyme GA-20 oxidase, a key component in gibberellin biosynthesis. Park *et al.* (2017) developed an efficient CRISPR-Cas9 system in *Panicum virgatum*, generating plants with simultaneous tetra-allelic mutations that resulted in the knock-out of the *4-Coumarate:coenzyme A ligase* (*Pv4CL1*) gene, a key enzyme in lignin biosynthesis. These plants show thinner cell walls, 8–30% less lignin, and improved saccharification efficiency, with 7–11% more glucose and 23–32% more xylose released. Moreover, multiple allelic editing was successfully achieved in *Paspalum notatum* by targeting the chlorophyll biosynthesis gene *MgCh* (*Magnesium chelatase*). High mutagenesis frequencies and heritable edits confirmed the efficiency of *CRISPR-Cas9*, demonstrating its potential as a powerful tool for breeding in tropical grasses (May *et al.* 2023).

Technologies such as CRISPR-Cas9 have also opened new avenues for enhancing disease resistance in major tropical grasses by enabling the precise modification of genes encoding PRRs, NBS-LRR proteins and key hormonal signaling regulators, thereby strengthening both PTI and ETI responses (Tyagi *et al.* 2021). Strategies such as stacking multiple resistance genes or inducing constitutive immune priming are being used to develop crop varieties with durable,

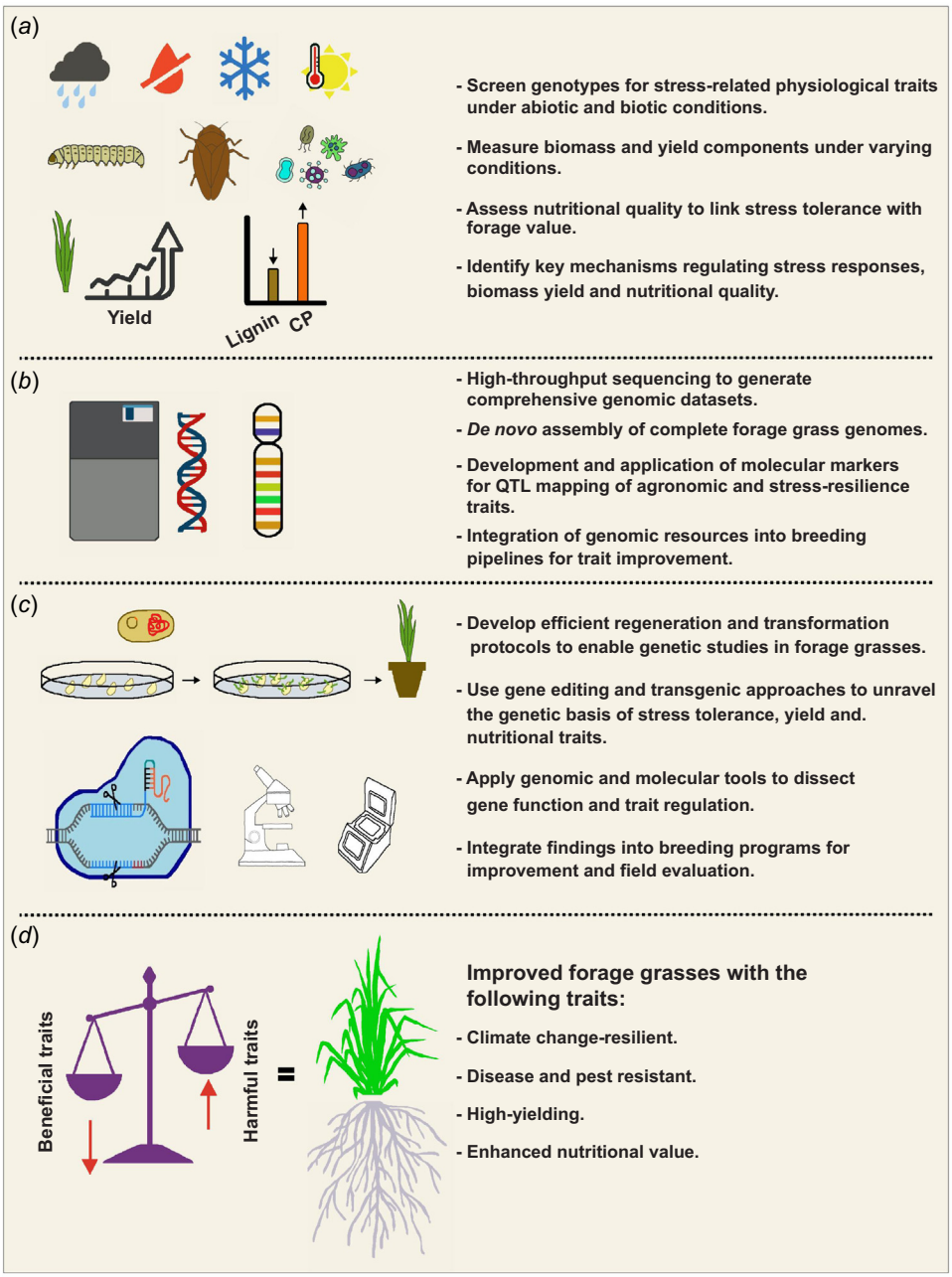

Fig. 2. A framework to bridge foundational research and genomic tools in forage grass improvement. (*a*) Climate change-driven interactions among abiotic stressors such as drought, heat, flooding and chilling stress have the potential to further reduce forage yield. Pathogens and pests also negatively affect forage yield. Moreover, climate change may shift their dynamics, intensifying pressure and altering distribution. Because plant responses to combined stresses are unique and unpredictable from single-stress studies, unraveling mechanism of stress combinations is critical to improving forage resilience. (*b*) High-throughput sequencing, integrated with multi-omics and field phenotyping, enables the discovery of genomic regions underlying agronomic traits and stress responses, supporting whole-genome analysis of tropical forage grasses and guiding marker-assisted breeding strategies. (*c*) Identifying gene-trait associations enables functional validation and the discovery of causative genes, supported by genetic studies in model systems and forage crops. Unraveling the genetic basis of key agronomic traits enables the application of gene editing and transgenic approaches. However, the development of efficient transformation and regeneration protocols remains a critical prerequisite for tropical forage grasses. (*d*) Enhanced key traits contributing to high yield, improved nutritional values and greater resilience in forage grasses, targeting both aerial parts and the root system.

broad-spectrum disease resistance (Silva *et al.* 2018). These approaches are particularly promising for crops such as rice and maize, where the rapid evolution of pathogen virulence often undermines single-gene resistances (Azizi *et al.* 2016). Additionally, integration of RNA interference (RNAi) techniques targeting pathogen virulence factors and the expression of antimicrobial peptides are being explored as complementary tools to conventional breeding (Tyagi *et al.* 2021). The successful applications of CRISPR-Cas9/Cas12a technology in polyploid grasses, as described by Bilal *et al.* (2025), have demonstrated that genome editing is feasible even in complex grass genomes. However, future research on high-quality genome assembly, gene redundancy, desired target loci, effective delivery systems and suitable vectors is still needed to fully realize its potential (Chen *et al.* 2024; Rengasamy *et al.* 2024).

Efficient transformation protocols are crucial for the effective use of genetic transformation as a tool in breeding programs. Note that few efficient protocols have been developed for inducing and regenerating embryogenic calli, enabling successful transformation in tropical forage grasses, and their outcomes are genotype-dependent and still very low (Bellido *et al.* 2021). For instance, a robust regeneration and transformation system was developed for *Chloris gayana* cv. Tolgar, achieving 2.16% transformation efficiency with the isogenic line T108. In *Urochloa brizantha*, embryogenic calluses induced using 2.4-D and picloram produced compact structures that regenerated efficiently, with sugar sources, medium pH and hormone adjustments further enhancing shoot formation (Cabral *et al.* 2011; Carrion Pereira *et al.* 2019; Maybery-Reupert *et al.* 2025).

Future perspective

In South America, major cattle-producing regions are expanding into forested and marginal areas because of agribusiness growth. In northern Mato Grosso, the south-eastern Amazon lost 59.663,51 km^2 of native forest across 20 years, with 17,047.27 km^2 being converted to pasture and 42.034,18 km^2 to agriculture. Strong correlations between deforestation and forest-to-pasture conversion (0.98), along with substantial pasture-to-crop conversion, show that agriculture is displacing cattle into frontier areas (Ribeiro *et al.* 2025). The Great Chaco exhibits a similar trend, with deforestation doubling between 2001 and 2012 versus 1987–2000 and annual losses of ~180,000 ha between 2017 and 2021, in Paraguay (Da Ponte *et al.* 2021; INFONA 2022; Milán *et al.* 2024). These patterns demonstrate how agricultural intensification and cattle expansion drive socioeconomic impacts and threaten remaining native forests, emphasizing the importance of tropical forage breeding to improve grass resilience to abiotic and biotic stresses, combined with proper grazing management, as a strategy to promote sustainable livestock production, enhance productivity, preserve soil health, and reduce pressure on forests.

Looking ahead, the sustainable improvement of tropical forage grasses will require a strategic shift towards integrating genetic studies, basic science, and translational research (Fig. 2). Despite their crucial role in livestock systems, particularly in tropical regions such as Latin America, these grasses remain underexplored compared with major crops. Bridging this gap demands the adaptation of genomic knowledge and molecular insights from model species and well-studied C_3 grasses to the complex biology of tropical C_4 forages. A key challenge lies in the development of efficient regeneration and transformation systems, which are essential to fully harness gene editing technologies such as CRISPR-Cas for precise and rapid trait improvement. As climate change intensifies, bringing more frequent droughts, heatwaves, and pathogen pressures, breeding resilient cultivars becomes not only a matter of productivity but also a crucial strategy to maintain ecosystem services and mitigate greenhouse-gas emissions from livestock production. To this end, there is an urgent need to unravel the molecular mechanisms underlying abiotic and biotic stress responses, as well as the genetic basis of key agronomic traits such as biomass yield, digestibility, and persistence under grazing. Investing in integrative, multidisciplinary approaches, combining genomics, physiology, biotechnology, and breeding, will be essential to accelerate the development of climate-resilient, high-yielding forage cultivars. Such innovations will contribute not only to agricultural sustainability but also to broader climate goals by enhancing the carbon sequestration potential and feed efficiency of grass-based systems.

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